Prevention of occupationally-related infections in Western Australian lobster fishermen

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The University of Western Australia

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Table of Contents

List of Tables and Figures

1998/338	**The prevention of occupationally related infections in Western rock lobster fishermen**

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OBJECTIVES:

1. To determine the role of *Erysipelothrix rhusiopathiae* plays in "crayfish poisoning".
2. To determine the distribution of *E.rhusiopathiae* in the work environment.
3. To develop interventions and strategies to reduce the incidence of "crayfish poisoning".

NON TECHNICAL SUMMARY:

OUTCOMES ACHIEVED

1. Increased knowledge of occupationally related infections amongst lobster fishermen.
2. Evidence that at a proportion of these infections are caused by the bacterium *Erysipelothrix rhusiopathiae.*
3. Dissemination of information about these infections to healthcare professionals as well as information about appropriate antibiotic therapy.
4. Inclusion of information about these infections in the WAFIC Code of Practice

The Western Australian rock lobster industry is the most valuable single species fishing industry in Australia and earns $400 million annually. "Crayfish poisoning" is the common name for a painful wound infection affecting lobster fishermen and other industry workers in Western Australia. Despite improvements in working conditions and antibiotic therapies, evidence suggests that infection continues to be a source of morbidity for workers. Although rare, life threatening severe infections can result from these skin infections. Little is known about the aetiology of these infections; however, there are some similarities with another occupationally related human infection, erysipeloid, caused by *Erysipelothrix rhusiopathiae*. The aims of the project therefore were to elucidate the cause(s) of "crayfish poisoning", with particular reference to *E. rhusiopathiae*, and to assess interventions for preventing or treating infection.

An epidemiological and microbiological investigation of "crayfish poisoning" was conducted. The potential pathogens isolated or detected from 47 suspected "crayfish poisoning" wound swabs were: *Staphylococcus aureus*, 22 (47%); *Acinetobacter* spp., 18 (38%); *Streptococcus pyogenes*, 11 (23%); *Erysipelothrix* spp., 9 (19%); *Vibrio alginolyticus*, 7 (15%); other Gram negative bacilli, 16 (34%). While *S. aureus* was the predominant organism found, *Erysipelothrix* was detected in 19 % of the samples. *Strep. pyogenes* was also frequently isolated, as was *Acinetobacter* spp. An objective of this project was to establish the role that *Erysipelothrix* plays in these skin infections. Clearly it does play a role but the natural history of this disease needs more work. Both *S. aureus* and *Strep pyogenes* are common skin pathogens. It is likely that their growth may obscure the growth of *Erysipelothrix*, hence our use of a molecular diagnosis. The other potential confounder, however, is time to presentation. *Erysipelothrix* is typically

a sub-dermal infection and the skin is not broken. When the skin does beak the opportunity exists for other organisms like *S. aureus* and *Strep pyogenes* to infect the wound. The hypothesised progression of diseases therefore is *Erysipelothrix* infection first followed by the others. However, by the time the other organisms appear, *Erysipelothrix* may have disappeared. The *Acinetobacter* spp., *Vibrio* spp. and other Gram negative bacilli are likely to be environmental contaminants. This is not to say that *S. aureus* and *Strep pyogenes* are not important in the overall problem of skin infections in fishermen. They are both potentially serious pathogens.

From the epidemiological survey carried out the following information was obtained: 68% of cases were young deckhands; 52% of infections were on the fingers; 22% on the feet, 15% on the arms, and 15% on the hands; at the time of the injury 43 % were not wearing gloves; and 20 % of cases had a previous skin breach. The presenting signs were erythema (redness), cellulitis (skin breakdown), blisters, furuncles (boils) and paronychia (inflammation of the nail); and systemic symptoms presented in 33%, fever in 29% and lymphadenitis (inflammation of the lymph nodes) in 18%. Antibiotics were given in 94% of cases, mainly flucloxacillin, 56%.

To assess the distribution of *Erysipelothrix* spp. in the aquatic environment, a survey of 19 Australasian seafoods was conducted and methodologies for detection of *Erysipelothrix* spp. evaluated. Twenty-one *Erysipelothrix* spp. were isolated from 52 seafood parts. Primary isolation of *Erysipelothrix* spp. was most efficiently achieved with broth enrichment, followed by subculture onto a selective agar containing kanamycin, neomycin and vanocmycin, after 48 h incubation. Selective broth, with 48 h incubation, was the best culture method for detection of *Erysipelothrix* spp with polymerase chain reaction (PCR). PCR was 50 % more sensitive than culture. *E. rhusiopathiae* was isolated from a variety of different fish, cephalopods and crustaceans, including Western Rock Lobster (*Panulirus cygnus*). There was no significant correlation between the origin of the seafoods tested and the distribution of *E. rhusiopathiae.* An organism indistinguishable from *E. tonsillarum* was isolated for the first time from an Australian oyster and a silver bream. The fishermen's work environment was heavily contaminated with *Erysipelothrix* spp. Overall, *Erysipelothrix* spp. was widely distributed, illustrating the potential for erysipeloid-like infections in fishermen. Additional isolates were also obtained from a survey of an abattoir.

The susceptibility of 60 *E. rhusiopathiae* strains from various sources to 13 antimicrobial agents was determined. Penicillins and cephalosporins remained active against *E. rhusiopathiae* and should continue to be recommended for treatment. Ciprofloxacin minimum inhibitory concentrations (MICs) were particularly low (MIC_{90} 0.06 mg/l), offering an alternative agent for the penicillin allergic patient. *E. rhusiopathiae* is still resistant to vancomycin (MIC_{90} 64 mg/l), highlighting the importance of early diagnosis of *E. rhusiopathiae* infection in cases of endocarditis. In addition, 31 *E. rhusiopathiae* isolates were tested against several commercially available home disinfectants. Most were effective in killing *E. rhusiopathiae* with minimum bactericidal concentrations of 0.001% for Pinocleen, and 0.03% for Domestos, Linely and the Wheelie Bin Phenyl Cleanser. These disinfectants could be used following mechanical cleaning of work environments, such as fishing boats and equipment, to reduce the risk of infection with *E. rhusiopathiae.*

KEYWORDS: **Western rock lobster, skin infections, *Erysipelothrix rhusiopathiae*.**

Acknowledgements

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Dr Qinning Wang assisted in certain aspects of the project, and Ms Josie Brooke was involved in the initial setting up phase. Their help was invaluable.

Mr Richard Stevens of WAFIC always cheerfully attempted to point the project team in the correct FRDC direction and, although this was not always successful, the project was always fun, as good science should be.

Finally, we are grateful for the technical, financial and institutional support of the Department of Microbiology, The University of Western Australia, and the Division of Microbiology and Infectious Diseases, The Western Australian Centre for Pathology and Medical Research.

Background

The Western Australian rock lobster industry is sustained by fishing one species, the Western Rock Lobster (WRL) (*Panulirus cygnus*). It is the most valuable single-species fishery in Australia earning Australia approximately 330 million dollars annually (2000-01 season figures) from live and frozen exports, mainly to Japan and the USA, and from local sales. Lobster fishing vessels have fished from Geraldton and Fremantle for over 50 years, and since then fleets have developed in numerous small towns and settlements along the western coast of Australia (Jones and Morgan 1994). "Crayfish poisoning" is the common name for a skin infection sustained by rock lobster fishermen and handlers in Western Australia. The infection seems to begin when a mild injury becomes infected. This injury is frequently caused by lobster spines, although any cut or abrasion can be involved. There is little reaction for 1-7 days while the injury appears to heal. The area around the wound then becomes erythematous, with concomitant pain and swelling (Figure 1). Fever may be experienced, and some fishermen complain of nightmares immediately prior to the development of erythema. Healing takes place over 3 weeks, or a secondary infection can occur. Anecdotally, lobster fishermen presenting with infection are treated with intramuscular penicillin.

Figure1: "Crayfish poisoning" after a spiking injury to the finger.

Only one prior investigation into these infections has been carried out (Sheard and Dicks, 1949). The investigators described the five general types of lesions which were responsible for impairing the efficiency of the lobster fishermen and other workers. Two kinds were attributed to staphylococcal infections and a third to streptococcal infection. The fourth type of lesions were salt water sores which were blamed on a poor diet and constant contact with sea water. The final form seemed clinically similar to erysipeloid, and was thought to be caused by *Erysipelothrix rhusiopathiae*. Culture was attempted from the advancing edge of the erysipeloid-like lesions without success. It was not until a biopsy was performed on a lesion which one of the investigators had sustained that an organism could be isolated. This organism shared characteristics with *E. rhusiopathiae*, but proper identification was hampered by field conditions. The

organism was reinoculated into the finger of the other hand, causing similar symptoms at the point of injury. Sheard and Dicks (1949) maintained that the clinical picture and tests were "not inconsistent with a determination that the causative organism was *E. rhusiopathiae*" however, there has never been a follow-up study. Since 1947, when this investigation took place, working conditions, available treatments and recommended preventive measures have improved. Despite these facts infections still appear to be common among lobster fisherman.

E. rhusiopathiae is a Gram positive rod-shaped bacterium which is a pathogen or saprophyte of a wide variety of mammals, birds and fish. The organism can also infect humans, causing three syndromes. The most common of these is a skin infection known as erysipeloid. Infection of humans is occupationally related, and is most prevalent in those working in association with animals and animal products (Murray *et al*. 1995).

Twenty six cases of infection associated with lobster fishing were recorded in the town of Leeman during the 1993 season, in a study conducted by the Health Department of Western Australia into the frequency of this infection. As approximately 100 lobster fishermen were working on boats from the town at this time, the attack rate was about 25%. Despite an awareness of the infection in the industry and by local medical staff, infection continues to be a cause of morbidity. A preliminary study of the microbiology of these infections was carried out during the early part of the 1995/96 season, i.e. December 1995 to March 1996. The study was limited to fishing fleets operating out of Lancelin, Ledge Point, Jurien Bay, Cervantes and Leeman, as these communities had limited access to medical facilities. The study was explained to potential participants at a series of occupational health and safety meetings held in each community prior to the season commencing. These meetings were conducted by the Western Australian Fisheries Industry Council Occupational Health and Safety consultant. At risk individuals were asked to present to their local general practitioner or nursing post when they suspected they had an episode of infection during the season. When cases presented they were assessed by medical or nursing staff and, after obtaining informed consent, swabs (or a biopsy in one case) were taken from the lesion. A total of 26 swabs and one biopsy were collected. .*E rhusiopathiae* was detected in six (23%) of the samples by either culture or molecular methods. A variety of other potential pathogens was also isolated from specimens, including *Staphylococcus aureus*, *Streptococcus pyogenes* and several environmental Gram negative bacilli. These bacteria may be responsible for other types of occupational infection. Staphylococci and streptococci can cause particularly serious infections with some significant long term sequelae. However, little is known about the role of these organisms. In addition, *E. rhusiopathiae* was detected in numerous specimens taken from WRL, as well as in environmental swabs collected from a fishing vessel. These samples included swabs of lobster pots, protective clothing, ropes and bait. This study is the first to conclusively implicate *E.rhusiopathiae* as a cause of these infections. The finding of *E.rhusiopathiae* on WRL was to be expected given that many cases occur following a spiking injury, however, the apparently high numbers and the widespread presence of *E.rhusiopathiae* in the fishing environment were not. These findings suggest that cases may occur following contamination of a previously received would, and further imply that environmental decontamination may be successful in preventing some cases. It is likely that many of the infections with staphylococci and streptococci which were noted were secondary infections occurring following a primary infection with *E.rhusiopathiae*.

Need

The incidence and severity of skin infection associated with WRL fishing does not appear to have changed vastly since 1949, despite penicillin therapy and a heightened awareness of the infection by those in the industry. Infection may result in loss of working days. Severe cases may progress to more serious conditions such as septic arthritis and endocarditis, potentially life-threatening conditions. The inappropriate and unnecessary use of antibiotics is of great concern currently as antibiotic resistant bacteria emerge world-wide. The emphasis is now on prevention of infection rather than treatment with antibiotics. However, when infection does occur it is important to have current antibiotic susceptibility data available to healthcare professionals. More advanced techniques for the recovery and detection of *E.rhusiopathiae* have been developed, including the use of selective media and a molecular method. Further study of these infections, utilizing more appropriate techniques, is warranted to determine the exact role of *E.rhusiopathiae* has. Also, the role of other organisms causing apparent infection in fishermen of all types needs to be evaluated. This will allow preventive strategies to be developed which will, in turn, result in less infection.

Objectives

1. To determine the role of *E.rhusiopathiae* plays in "crayfish poisoning".
2. To determine the distribution of *E.rhusiopathiae* in the work environment.
3. To develop interventions and strategies to reduce the incidence of "crayfish poisoning".

Methods

Background

This investigation was carried out in two phases, an epidemiological investigation and a microbiological investigation. The primary investigations were carried out from November 1999 to June 2000, during the lobster fishing season in Western Australia. The first phase of this project, conducting a survey of potential clinical cases, required access to a compliant group of lobster fishermen, a compliant group of general practitioners and a suitable transport system to enable specimens to be transported to the central laboratory in Perth. It was also important that the site chosen for the investigation had limited access to medical facilities so that all cases could be recruited. Assistance for the project was volunteered by the Mid-West Public Health Unit based in Geraldton, and a series of meetings was held with local fishermen and general practitioners to gauge support for the project. Based on the enthusiasm of the local community, Geraldton was chosen as the site for initial investigations. Transport was arranged via the Western Australian Centre for Pathology and Medical Research that has a branch laboratory in Geraldton. In addition, some advertorials were run in local newspapers. In addition, operators of all fishing vessels operating out of Fremantle were contacted and asked to fill in the questionnaire in the event that cases occur on their vessels. Arrangements were made to collect specimens from these individuals.

The epidemiological investigation was conducted using the attached questionnaire (Appendix 3). Copies of the patient information and consent form are also provided in

Appendix 3. Approval for the study was obtained from the Human Rights Committee at The University of Western Australia and all subjects gave informed consent.

1) Epidemiological investigation

The questionnaire was filled out both by the patient presenting with "crayfish poisoning", and the attending medical or nurse practitioner. Details of age, sex, occupation, date and site of injury, immediate and subsequent wound treatment, systemic symptoms and current antibiotic usage were ascertained from the patient. Medical or nursing staff provided a clinical description of the illness, including dates of injury and presentation, a description of the lesions(s), and the treatment prescribed.

A case of "crayfish poisoning" was defined as a patient presenting during the 1998-99 season with a history of injury, plus a lesion that was described as erythematous or resembling cellulitis or paronychia.

Data obtained via the questionnaire were entered into a database created in Epi-Info V5 and analysed to determine the epidemiological characteristics of infection (Dean *et al*. 1990).

2) Microbiological investigation

Clinical samples
Wound swab samples were obtained from individuals presenting with "crayfish poisoning" with Trans® tube Amies charcoal swabs (Medical Wire and Equipment Co., England). Samples were transported to Perth by courier.

Wound swabs were cultured as soon as practicable on a variety of microbiological media for the recovery and identification of all possible wound pathogens. These media included blood agar, thiosulpate citrate bile salts agar, cysteine lactose electrolyte deficient agar, colistin naladixic acid agar, chocolate agar and Maconkey agar. Specimens were also enriched in brain heart infusion broth and alkaline peptone water. Media specific for the isolation of *E. rhusiopathiae* recovery were also utilized; these were based on ESB medium (Wood 1975), with brain heart infusion broth replacing the base ingredients. The identification of all organisms recovered was based on Gram staining properties, colony morphologies and the results of various biochemical tests (Murray *et al*. 1995).

Environmental samples
Samples from the lobster fishermen's environment were collected during the investigation. WRLs, bait, water, protective gear and other equipment on fishing vessels were sampled during field trips. Water samples were collected from holding tanks on fishing vessels and in wholesale establishments, and from ocean locations.

Lobsters were dissected with alcohol flamed tools, and segments of the exoskeleton were placed in sterile sample jars and cultured in selective and non-selective liquid media. Analysis of the bacterial flora of the exoskeleton was carried out by subculture to the appropriate solid media.

The other environmental specimens were analysed for the presence of *E. rhusiopathiae* only. Swabs taken of bait and equipment on a lobster fishing vessel were eluted in liquid media. Water specimens were filtered through 0.45μm membrane filters (Millipore), and the filters cultured in liquid media. After incubation, the liquid media were treated in a similar manner to the exoskeleton samples.

Polymerase chain reaction (PCR)
In addition to cultural methods, PCR was employed to detect *E.rhusiopathiae* in clinical and environmental samples. Initially, primers were based on a genus specific sequence of the 16S rRNA gene, and the method described by Makino *et al*. (1994) was utilized, except that DNA was extracted from samples by boiling, and only 5mM dNTPs were used in the PCR reaction mixture. *E.rhusiopathiae* ATCC 19141 was used as the positive control. For swab samples, the cotton tip was eluted in a small volume of sterile distilled water, and the extracted DNA was subjected to PCR. Portions of turbid liquid media from culture of these samples were treated in the same manner. The liquid media from cultured lobster exoskeleton and water samples were analysed similarly.

PCR detection of *Erysipelothrix* spp.
DNA extraction from pure and enrichment cultures. Frozen isolates were thawed, streaked onto HBA and incubated at 37°C for 24 h. After 24 h, a few colonies were suspended in 100 µl of sterile diethylpyrocarbonate (DEPC) water (Ausubel 1995) in sterile Eppendorf tubes and then heated to 100°C for 15 min. Heated samples were centrifuged in a microcentrifuge at 10 000 x g for 2 min and the supernatant transferred to sterile Eppendorf tubes and frozen at –70°C for later use in the PCR. PCR was performed on all enrichment cultures at both 24 and 48 h. Broths were vortexed vigorously, 1.5 ml was transferred to sterile Eppendorf tubes and organisms pelleted by centrifugation at 10 000 x *g* for 3 min. The pellet was then washed by resuspension in 1.5 ml of DEPC water. After further centrifugation, the supernatant was removed and the pellet resuspended in 100 µl of sterile DEPC water. The samples were then treated in the same way as the pure cultures.

PCR primers. The primers used were MOIO1 and MOIO2 (Gibco BRL, Paisley, UK), derived from the DNA sequence coding for 16SrRNA of *Erysipelothrix* spp. (GenBank/EMBL accession no. M23728) (Makino *et al*. 1994).

DNA amplification. The PCR method was modified from that previously described (Makino *et al*. 1994). PCR was carried out in a DNA thermocycler (GeneAmp 9700; Perkin Elmer, New Jersey, USA) in 20 µl of a reaction mixture containing 1 x PE buffer II (100 mM Tris-HCL, ph 8.3, 500 mM KCl) (Perkin Elmer); 2.0 mM $MgCl_2$; 0.2 mM of each deoxyribonucleotide triphosphate (dNTP) (Boehringer Mannhem, Germany); 0.2 µM of each primer (Gibco BRL); 0.5 U of Taq DNA polymerase (Perkin Elmer) and 4.0 µl of extracted DNA sample. The amplification consisted initially of heating at 94°C for 15 min and then 45 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 45 s, followed by an additional extension step at 72°C for 7 min and cooling to 4°C.

In preliminary experiments, attempts were made to enhance the sensitivity of the PCR by including a second reaction using 0.4 µl of the first round reaction product as template. In a sample of 23 seafoods, cultured in various enrichment broths, the proportion of positive PCRs increased from 31% to 38% with a second PCR. Only one

of the 115 PCRs changed from positive to negative with a second amplification and, therefore, double amplification PCR was used throughout the study.

All broth culture samples were tested twice on separate days. If discrepant results were obtained, then those samples were repeated. If the third PCR was positive, then the overall result was considered positive. If the third PCR was negative, then the overall result was considered negative. The pure culture samples were tested once to confirm for *Erysipelothrix* spp.

Agarose gel electrophoresis. Aliquots of the amplification reactions were electrophoresed on 2% (wt/vol) agarose gels (Seakem LE agarose, FMC Bioproducts, Rockland, Maine, USA) containing ethidium bromide (0.5 µg/ml). Bands were visualised on an UV transilluminator and compared to the positive control, *E. rhusiopathiae* ATCC 19414, and a DNA Molecular Weight VIII (Boehringer Mannhem) standard. A band of 407 bp was considered diagnostic for *Erysipelothrix* spp.

3) Improved isolation and detection methods for *E.rhusiopathiae*

Control bacterial strains
E. rhusiopathiae ATCC 19414 was used as a positive control and *E. tonsillarum* ATCC 43339 was used as a negative control, for both cultural and molecular identification procedures.

Seafood
Seafood samples were purchased from three seafood markets, and direct from lobster fishing boats, in the port of Fremantle, Western Australia. Over one third of the samples (37%) had been caught in local waters and 37% were caught in waters 1000 km north of Fremantle. The remaining samples were from southern Western Australia, New Zealand, South Australia and Tasmania, and an aquaculture establishment at Fremantle. None of the seafood had been washed and lobsters were purchased alive; two directly from a lobster fishing boat and one from a live storage facility. More details of the seafoods and their origin are given in the Results section.

Media
Two types of selective enrichment broths and one non-selective broth, as well as selective and non-selective agar plates, were assessed. Brain heart infusion broth and agar (Oxoid Pty. Ltd, Basingstoke, Hampshire, UK) were prepared according to manufacturer's recommendations and sterilised by autoclaving at 121°C for 15 min. Once cooled to room temperature, non-selective brain heart infusion (BHIB) and selective brain heart infusion (BHIB/S) broths were supplemented with 5% horse serum. For BHIB/S, the following antibiotics were added: kanamycin (40 µg/ml), neomycin (50 µg/ml) and vancomycin (25 µg/ml). Selective brain heart infusion agar (BHIA/S) consisted of molten agar cooled to 50°C supplemented with serum and antibiotics as for BHIB/S. Selective trypticase soy broth (TSB/S) (Becton Dickinson & Co., Cockeysville, USA) was prepared as described previously (Shimoji *et al*. 1998) and contained tryptic soy broth (pH 7.6) supplemented with 0.3% Tris (hydroxymethyl)aminomethane, 0.1% Tween 80, 0.03% sodium azide and crystal violet (5µg/ml).

Isolation of *Erysipelothrix* spp. from seafood
Whilst handling seafood, gloves were worn at all times. After collection, seafood was placed into separate clean plastic bags and care was taken to ensure no cross contamination. The seafood was cut aseptically into small portions which were immersed in 10 ml of each of the three broths and mixed vigorously for 30 sec. All broths were incubated at 37°C. After 24 and 48 h, BHIB was subcultured onto BHIA/S, and TSB/S and BHIB/S onto horse blood agar (HBA) (Oxoid). The agar plates were incubated at 37°C and, after 24 and 48 h, examined for growth of suspected *Erysipelothrix* colonies which were small (approximately 0.1mm), convex, circular and transparent (Reboli and Farrar 1992). Suspect colonies were Gram stained, and Gram positive rods were kept for confirmation by PCR. Isolates were stored as heavy suspensions in BHIB plus 10% glycerol at –70°C.

Differentiation of *Erysipelothrix spp.*
Carbohydrate fermentation.
To differentiate *E. rhusiopathiae* from *E. tonsillarum,* fermentation of glucose and sucrose was used. The method described by White and Shuman (1961) was modified by replacing Andrade's fermentation broth with agar.

PCR detection of *E. rhusiopathiae.*
The PCR method described by Shimoji *et al*. (1998) was used with slight modifications to differentiate between *E. rhusiopathiae* and *E. tonsillarum.* The basic protocol was as for PCR detection of *Erysipelothrix* spp. from the pure and enriched cultures with alterations to the reaction mixtures and amplification cycles.
PCR Primers. The primers used were ER1 and ER2 (Gibco BRL), derived from a region possibly coding for virulence of *E. rhusiopathiae* (GenBank/EMBL accession no. D64177) (Shimoji *et al*. 1998).
DNA amplification. PCR was carried out in 20 µl of reaction mixture as before, however, the $MgCl_2$ concentration was decreased to 1.5 mM. Amplification consisted initially of heating at 94°C for 5 min and then 30 cycles of denaturation at 94°C for 1 min, annealing at 63°C for 30 s and extension at 72°C for 1 min, followed by an additional extension step at 72°C for 7 min and cooling to 4°C. A band of 937 bp was considered diagnostic of *E. rhusiopathiae.*

In preliminary experiments, the specificity of *Erysipelothrix* and *E. rhusiopathiae* specific PCR methods was tested with several marine bacteria (*Vibrio, Pseudomonas, Acinetobacter, Proteus* and *Aeromonas*). The results indicated that the primers were specific for *Erysipelothrix* spp. with MOIO1 and 2 and *E. rhusiopathiae* with ER1 and 2.

Statistical analysis. The Chi square test was used to detect statistically significant differences between proportions.

4) Typing of isolates

All stored isolates of *E. rhusiopathiae* were recovered and typed to determined relationships between strains. Typing was done using pulsed field gel electrophoresis (PFGE). For each organism tested, a subculture was made from the frozen culture, onto a blood agar plate and incubated overnight in air at 37°C. One colony of the subsequent culture was inoculated into 3ml Luria-Bertaini broth (10g Bacto-tryptone, 5g Bacto-yeast extract, 5g NaC1, per litre) (Promega Corporation), and incubated overnight at

37°C on a shaker. Seventy-five microlitres (75µl) of the overnight suspension was placed in a sterile microcentrifuge tube and centrifuged in a Hettick Mikroliter microcentrifuge for 60 sec at 10,000 to 12,000 rpm. The supernatant was carefully aspirated and the pellet placed at -70°C for at least 1h. One hundred microlitres (100µl) of TE buffer (10mM Tris-0.1M EDTA) was added to the pellet, which was resuspended by gently pipetting up and down with a wide-bore pipette, and then 100µl of 1.6% low-melt agarose (Low Melt Preparative Grade 56°C Agarose) (BioRad Laboratories, USA) was added. A 100µl aliquot of this mixture was immediately pipetted into one well of a chilled CHEF disposed plug mould (10 mm x 5 mm x 1.5 mm) (BioRad Laboratories, USA) and left to solidify for 15 to 20 min at 4°C. The plug was then pushed out of the mould into a 1.5 ml vial containing 500µl of 50mM Tris-50mM EDTA (pH 8.0), 1% N-lauryl sarcosine, to which had been added 20µl proteinase K (20 mg/ml solution) (Biotecx Laboratories, Inc., Texas). The vial was incubated for 16 to 20 h (overnight) in a 50°C water bath without agitation (over 2 nights). Several washes were carried out as detailed below, initially to remove all traces of unwanted cellular material and proteinase K mixture, and subsequently to remove EDTA salts and provide the correct buffer for restriction enzyme digestion.

Buffer*	**Volume**	**Wash time**
TE	1ml	60 min
TE	1ml	60 min
TE	1ml	60 min
TE	1ml	30 min
TE 0.1 x concentration	1ml	60 min
TE 0.1 x concentration	500µl	60 min
RE buffer *****	500µl	30 min
RE buffer	500µl	30 min

***T**he buffer supplied by the manufacturer of the restriction enzyme, and specific for that enzyme. This was supplied at 10x concentration, and was diluted before use, with sterile, distilled water (sterile water for injection, Astra).

After removal of the final wash solution, 300µl RE buffer was added, together with 5µl (50 units) of restriction enzyme – *Smal* (Promega Corporation, USA). The digest was incubated overnight at 25°. The restriction digestion reaction was stopped by aspirating the reaction mixture and adding 1ml TE buffer to the vial, which was then held at 4°C for at least 30 min to allow the plug to harden. One-quarter to one-third of the plug was used during the electrophoretic run; the remainder of the plug was stored in TE buffer at 4°C. The electrophoresis gel was poured using 100ml of 1.2% agarose (Pulsed Field Certified Agarose) (BioRad Laboratories, USA) in 0.5x TBE buffer (44.5mM Tris-borate, 1.25mM EDTA). Two methods of loading the plug samples were used. In the first method, samples were placed directly onto the teeth of the comb, which was the lowered into a cooled but still molten agar gel. When the gel had set, the comb was removed and the holes back-filled with 1.2% low-melt agarose. The second, more traditional, method was to pour the agar with the comb *in situ*, move the comb when the gel had set and then to gently push the plugs into their respective wells. The wells were then back-filled as for the first method. Lambda ladder DNA size standards (λ ladder) (BioRad Laboratories, USA) were used as molecular weight markers, and were heated

for 30 min at 56ºC before being placed in a gel. The PFGE run was performed using a CHEF Mapper, Pulsed Field Electrophoresis System (BioRad, USA) and a running buffer of 2 l of 0.5χ TBE, chilled to 4ºC. Run parameters were identical to those used for the GenePath Group 3 Reagent Kit method (BioRad, USA), that is, 200V for 17 h with pulse times ranging from 2 to 17 sec. The gel was stained for 45 min in 600ml of distilled water containing 10 drops of a 1 mg/ml solution to ethidium bromide (BioRad Laboratories, USA), destained for 1 to 2 h in fresh, distilled water and the bands visualized using a UV Transilluminator (UVP Inc., USA). Gels were photographed and analysed using a BioRad Gel-doc 2000 system and Gel-compar software.

5) Susceptibility of *E.rhusiopathiae* to antimicrobials and disinfectants

Antimicrobial agents

The antimicrobial agents tested were: penicillin (CSL Ltd), cephalothin (Eli Lilly Australia Pty Ltd), oxacillin, chloramphenicol, clindamycin, erythromycin, tetracycline and vancomycin (Sigma Chemical Co), ceftriaxone (Roche Products Pty Ltd), minocycline (Lederle Laboratories), ciprofloxacin (Bayer Pharmaceuticals Pty Ltd), fusidic acid (Leo Pharmaceuticals Pty Ltd) and rifampin (Alphapharm Pty Ltd). Stock solutions were prepared according to the instructions of the manufactures and stored at –70°C for not more than six months.

Disinfectants

The following disinfectants were purchased from the manufacturers: two chlorine-based disinfectants, Linely (Ramprie Laboratories, Western Australia) containing sodium hypochlorite with 1% available chlorine (w/v), and Domestos (Lever Rexona, NSW, Australia) containing sodium hypochlorite with 5% available chlorine (w/v); one phenolic disinfectant, Wheelie Bin Phenyl Cleanser (Recochem Inc., Auckland, New Zealand) containing 2.9% phenols; one quaternary ammonium disinfectant, Pinocleen (Reckitt & Colman, Auckland, New Zealand), containing 1.5% (w/w) benzalkonium chloride, and one disinfectant containing citric acid and vinegar, Down to Earth Toilet Cleaner (Reckitt & Colman, NSW, Australia).

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using the agar dilution technique and recommended breakpoints approved by the National Committee for Clinical Laboratory Standards (NCCLS) (1997) with some modifications. Agar plates were prepared by adding 5% horse blood to Mueller Hinton agar (MHB) (Oxoid Ltd., Basingstoke, Hampshire, UK), and then adding the antibiotics in doubling dilutions ranging from 512 to 0.008 mg/l, except for rifampin which was tested up to a maximum concentration of 8 mg/l. Control plates containing only 5% horse blood and Mueller Hinton agar were also included.

The isolates were streaked on horse blood agar (HBA) (Oxoid) and incubated at 37°C. After 24 h incubation, a few colonies were suspended in 0.85% saline to make a suspension with an opacity equivalent to that of a 0.5 McFarland turbidity standard. The suspension was then diluted in 0.85% saline to obtain approximately 10^7 CFU/ml. To confirm the inoculum size, viable counts were performed.

The plates were dried for 20 min and then inoculated with the 10^7 CFU/ml bacterial suspensions with a multipoint replicator (Mast Laboratories Ltd., Liverpool, UK). With

the replicator, 1-2 μl spots containing approximately 10^4 CFU/ml of each organism were transferred. The inoculated plates were then incubated at 37°C for 48 h. The MICs were determined after 48 h incubation as the lowest concentration of antimicrobial that completely inhibited growth, disregarding one or two colonies or a faint haze due to the inoculum. The minimum concentrations of antimicrobial that inhibited at least 50 and 90% of the isolates were defined as the MIC_{50} and MIC_{90}, respectively. Testing was performed in triplicate on separate days, and the module MIC values were recorded.

Disinfectant susceptibility testing
MICs were determined using the broth dilution technique approved by the NCCLS, with some modifications. A range of dilutions of the disinfectants was prepared in BHIB (Oxoid), plus 0.05% Tween 80 (Sigma) in 96-well microtitre trays (Falcon, Becton Dickson and Co., Lincoln Park, New Jersey, USA).

Bacterial suspensions were prepared as for the agar dilution method, but diluted in 0.85% saline so that a final concentration of approximately 5.0 x 10^5 CFU/ml was obtained in each well following inoculation of the microtitre tray. The trays were then placed in plastic bags to prevent drying and incubated at 37°C. After 48 h incubation, 10 μl of the cultures was taken from each well and spotted on BHIA (Oxoid) plates that had been previously dried. The plates were then incubated at 37°C for 48 h. The MIC was defined as the lowest concentration of disinfectant that completely inhibited growth of the organism in the wells. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of disinfectant resulting in death of 99.9% of the initial inoculum. The MIC_{50} and MIC_{90}, and MBC_{50} and MBC_{90},were also determined. Growth and sterility controls were included in every tray. Tests were performed in triplicate on separate days, and the module MIC and MBC values recorded.

6) Serological response

To determine if antibodies were produced against *E. rhusiopathiae,* blood was collected from recent and past cases of infection. *E. rhusiopathiae* cells were disrupted by sonication and the resulting protein preparation separated using SDS-PAGE. In addition, outer membrane vesicle preparations were also examined by SDS-PAGE. The gels were probed with sera from cases to determine which proteins, if any, were involved in the immune response (Sato *et al*. 1995).

Sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE)

Preparation of samples
(a) **Broth cultures**
Overnight broth cultures were used directly for the SDS-PAGE.
(b) **Sonicate**
One ml of overnight broth culture was sonicated for 30 sec and placed on ice for another 30 sec. The same procedure was repeated 4 times. After centrifugation for 5 min at 15,000 rpm in Hettich Zentrifugen Mikroliter D-7200 Tuttlingen micro-centrifuge, the supernatant was collected for use.

(c) **Outer membrane vesicles**
The method of Murphy and Loeb (1989) was used with some modification. Briefly, 10 ml of overnight broth culture was centrifuged at 10,000 g for 15 min at 4ºC. The

resulting supernatant was centrifuged again at 10,000 g for 15 min. This supernatant was filtered through a Nalgene 0.45μm filter unit (Nalgene Labware, USA) and centrifuged again at 100,000 *g* overnight at 4°C. The pellet was suspended in 10 ml of DPBS and ready for use.

SDS-PAGE

All polyacrylamide gels were prepared and run using the Bio-Rad Mini-PROTEAN II apparatus. The glass plates were washed thoroughly and dried in air. The 12% separating gel was prepared by mixing the first three components and degassing them to exclude air for approximately 15 min or until all air bubbles disappeared. Fresh ammonium persulphate solution and TEMED were then added. The mixture was swirled quickly and gently, and the gel was poured. The top of the gel was overlaid with water-saturated butanol and allowed to polymerise for at least 20 min. After the gel was set, the water was poured off and rinsed and the top of the gel was blotted dry with strips of filter paper. The 4% stacking gel was prepared in a similar manner to the separating gel and the mixture was layered over the top of the 12% gel and embedded with a comb to form sample wells. The stacking gel was then allowed to polymerise for 20-30 min.

Once the gel set, the comb was removed and the sample wells were rinsed with distilled water and blotted dry with strips of filter paper. Plates were clamped into the electrophoresis apparatus and surrounded by SDS-PAGE running buffer. Equal volumes of standards or samples were mixed with sample buffer and then boiled for 2 min to denature and linearise proteins before loading 10 μl of each into the sample wells. Gels were run at 180V for 1 h. After electrophoresis was complete the buffer was poured off and the gels carefully peeled from the glass plates. Western blotting and probing with antibody were done as described by Harlow and Lane (1988).

Results

1) Epidemiological investigation

Forty seven cases of suspected "crayfish poisoning" were identified. From the epidemiological survey carried out the following information was obtained:

- 68% of cases were young deckhands,
- sites of infection were 52% on the fingers; 22% on the feet; 15% on the arms; and 15% on the hands,
- at the time of the injury 43 % were not wearing gloves,
- 20 % of cases had a previous skin breach,
- the presenting signs were erythema; cellulitis; blisters; furuncles and paronychia,
- systemic symptoms presented in 33% ; fever in 29% and lymphadenitis.

Antibiotics were given in 94% of cases. These consisted of:

- flucloxacillin 56% of cases,
- penicillin 22%,
- cephalexin 12%, and,
- erythromycin 7%.

2) Microbiological investigation

Clinical specimens
Specimens were processed in Perth using both solid and enrichment media cultured for 48 hours at 37 degree Celsius. Media specific for marine organisms (eg. *Vibrio* spp.) were employed, such as thiosulphate citrate bile sucrose agar. In addition, a direct PCR detection method for *E.rhusiopathiae* was also used. Culture media were employed to isolate a variety of other potential pathogens in addition to *E.rhusiopathiae*. Identification of these other organisms was done using commercially-available kits. The potential pathogens isolated or detected from 47 suspected "crayfish poisoning" wound swabs are shown in Table 1.

While .*S aureus* was the predominant organism found (47%), *E. rhusiopathiae* was detected in 19% of the samples. *Strep. pyogenes* was also frequently isolated, as was *Acinetobacter* spp. Using the case definition as described, subjects from whom swabs had been taken were divided into cases and non-cases. Interestingly, all *E. rhusiopathiae* isolates came from cases, while most *Strep. pyogenes* came from non-cases. *S. aureus* isolates were spread evenly across the two groups as were various Gram –ve bacilli.

Environmental specimens
As well as looking at clinical samples, it was necessary to determine the distribution of the potential pathogen, *E. rhusiopathiae*, in the environment in which lobster fishermen worked. To achieve this, arrangements were made to take samples from two boats, one operating from Fremantle and one from Mindarie Keys. Swabs of various surfaces on the vessels, as well as from protective equipment such as gloves, boots and aprons, were collected. At this stage lobsters were also collected for processing in the laboratory. The results from 14 swabs looking for *Erysipelothrix* spp. taken on lobster boats are shown in Table 2.

Additional isolates of *E. rhusiopathiae*
In order to have *Erysipelothrix* strains for comparative purposes, a series of environmental samples was collected from local abattoirs slaughtering sheep and pigs. In addition, carcass washings from various other meat samples were investigated. The reason for this was that *Erysipelothrix* causes a similar disease (called erysipelas) in the pig industry, as well as arthritis in sheep. Human cases in association with these industries are common and several isolates from human infections were also obtained.

The characteristics of these strains can be seen in Tables 3-6. The phenotypic differentiation of *E.rhusiopathiae* from *E.tonsillarum* can be difficult and characterisation of isolates was not as straightforward as expected. All *Erysipelothrix* spp. produced hydrogen sulphide, however, *E.tonsillarum* ferments sucrose and *E.rhusiopathiae* does not. This is why the PCR-based methods were established: one species specific and the other *E.rhusiopathiae* specific. Later an *E.tonsillarum* specific PCR was developed. Several *Erysipelothrix* spp. have now been sequenced as they appear to be neither *E.rhusiopathiae* nor *E.tonsillarum*. These are most likely previously undescribed species. This part of the investigation was not central to the overall aims of the project, however, a paper arising from this work has been published and the manuscript is attached as Appendix 4.

Table 1: Recovery of potential pathogens from 47 “crayfish poisoning” wound swabs

	All swabs		Cases		Non-cases	
Potential pathogens	n= 47	(%)	n = 23	(%	)n = 24	(%)
Staphylococcus aureus	22	(47)	11	(50)	11	(50)
Acinetobacter spp.	18	(38)	6	(33)	12	(67)
Streptococcus pyogenes	11	(23)	2	(18)	9	(82)
Vibrio alginolyticus	7	(15)	7	(100)	0	-
Other Gram –ve bacilli	16	(34)	8	(50)	8	(50)
Erysipelothrix rhusiopathiae (culture plus PCR +ve)	9	(19)	9	(100)	0	-

Table 2: A comparison of PCR detection and isolation *of E. rhusiopathiae* from environmental swabs

Swab	Source	PCR	Culture
S1	Fresh salmon head	+	+
S2	Fresh salmon head	+	+
S3	Fresh mackerel	+	-
S4	Fresh mackerel	+	+
S5	Used bait	+	+
S6	Used bait	+	+
S7	Used bait	+	-
S8	Used bait	-	-
S9	Crayfish pot	+	+
S10	Crayfish pot	+	+
S11	Rope	-	-
S12	Rope	+	-
S13	Gloves	+	+
S14	Boots	-	-

+ = a 407bp band was detected by PCR, or *E. rhusiopathiae* was isolated
- = no band was detected by PCR, or no *E. rhusiopathiae* was isolated

Table 3: PCR results from pig and sheep abattoir samples and isolates

		Samples		Isolates	
Source	n	G-PCR*(+)	S-PCR**(+)	G-PCR(+)	S-PCR(+)
Joints	12	5	2	2	2
Effluent	10	5	2	3	1
Carcass surface	3	2		2	
Blood	7	4		1	
Soil	23	11		7	
Mouth	10	3			
Lungs, liver and intestine	4	1			
Slaughtering areas	21	4			
Tonsil, heart and lymph nodes	19	0			
Total	109	35(32.1%)	4(3.7%)	15(13.8%)	3(2.8%)

* Genus-specific PCR (Makino *et al.* 1994)
**Species-specific PCR (Shimoji *et al*. 1998a)

Table 4: PCR results from carcass wash samples and isolates

		Samples		Isolates	
Source	n	G-PCR*(+)	S-PCR**(+)	G-PCR(+)	S-PCR(+)
Beef	26	1			
Lamb	23	4	2		2
Pork	23	3	1		1
Mutton	11	2	1	1	1
Chicken	40	2			
Total	123	12(9.8%)	4(3.3%)	1(0.8%)	4(3.3%)

* Genus-specific PCR (Makino *et al.* 1994)
**Species-specific PCR (Shimoji *et al*. 1998a)

Table 5: Biological characteristics of isolates from meat and abattoir samples

No	Isolate	G-PCR*	S-PCR**	TSI†	GLU‡	SUC§	Source
1	M1	+	+	+	+	-	Lamb
2	M2	+	+	+	+	-	Lamb
3	M3	+	+	+	+	-	Mutton
4	M5	+	+	+	+	-	Pork
5	S16	+	+	+	+	-	Sheep arthritic joint
6	S19	+	+	+	+	-	Sheep arthritic joint
7	D14	+	+	+	+	-	Pig abattoir effluent
8	D23fbl	+	-	+	+	-	Pig Skin
9	D23yws	+	-	+	+	-	Pig Skin
10	D22fbl	+	-	+	+	-	Pig Skin
11	D22yws	+	-	+	+	+	Pig Skin
12	D11	+	-	+	+	+	Pig abattoir effluent
13	D25	+	-	+	+	+	Pig blood
14	D55	+	-	+	+	+	Pig pen soil
15	D66	+	-	+	+	+	Pig pen soil
16	D67fbl	+	-	+	+	+	Pig pen soil
17	D67yws	+	-	+	+	+	Pig pen soil
18	D13	+	-	-	+	+	Pig abattoir effluent
19	D56	+	-	-	+	+	Pig pen soil
20	D57	+	-	-	+	+	Pig pen soil
21	D63	+	-	-	+	+	Pig pen soil
22	M4fbl	+	-	-	+	+	Mutton
23	M4yws	+	-	-	+	+	Mutton
24	D65yws	+	-	-	+	+	Pig pen soil
25	D65fbl	+	-	-	+	-	Pig pen soil
	E. rhusiopathiae (ATCC 19414)	+	+	+	+	-	
	E. tonsillarum (ATCC 43339)	+	-	+	+	+	

* Genus-specific PCR (Makino *et al.* 1994)
**Species-specific PCR (Shimoji *et al*. 1998)
† Triple sugar iron agar plus 10 % horse serum
‡ Glucose fermentation
§ Sucrose fermentation

Table 6: Biological classification scheme of *Erysipelothrix* spp.

	Property					
Species	G-PCR*	S-PCR**	TSI†	GLU‡	SUC§	Isolates
E. rhusiopathiae	+	+	+	+	-	M1, M2, M3, M5, S16, S19, D14
E. tonsillarum	+	-	+	+	+	D22yws, D11, D25, D55, D66, D67fbl, D67yws
Erysipelothrix spp.	+	-	+	+	-	D23fbl, D23yws, D22fbl
Erysipelothrix spp.	+	-	-	+	+	D13, D56, D57, D63, M4fbl, M4yws, D65yws
Erysipelothrix sp.	+	-	-	+	-	D65fbl

* Genus-specific PCR (Makino *et al.* 1994)
**Species-specific PCR (Shimoji *et al*. 1998a)
† Triple sugar iron agar plus 10 % horse serum
‡ Glucose fermentation
§ Sucrose fermentation

3) Improved isolation and detection methods for *E.rhusiopathiae*

Evaluation of culture methods for the isolation of *Erysipelothrix* spp. A total of 52 different seafood parts from 19 different aquatic species was examined (Table 7). Twenty-one suspected *Erysipelothrix* spp. were isolated, some by more than one of the six culturing methods. The best recovery of *Erysipelothrix* spp. was achieved using BHIB enrichment followed by subculture to BHIA/S. After 24 and 48 h incubation, 15 (29%) and 19 (37%) of the 52 different seafood parts, respectively, were culture positive. TSB/S enrichment followed by subculture to HBA resulted in recovery of *Erysipelothrix* spp. from 8 (15%) and 11 (21%) of the 52 seafood parts after 24 and 48 h incubation, respectively. The poorest recovery of only 2 isolates (4%), at both 24 and 48 h, was achieved by enrichment in BHIB/S followed by subculture to HBA. The numbers of *Erysipelothrix* spp. isolated using the 48 h BHIB to BHIA/S protocol was significantly higher than the BHIB/S to HBA protocol, and the 24 h TSB/S to HBA protocol ($p < 0.05$).

Evaluation of PCR detection of *Erysipelothrix* spp. from different broths. The best PCR detection of *Erysipelothrix* spp. was achieved with the TSB/S 48 h enrichment culture, where 35 (67%) of the 52 seafood parts were positive. From BHIB/S 48 h and TSB/S 24 h enrichment cultures, 29 (56%) and 26 (50%), respectively, of the 52 seafood parts were positive. The BHIB 48 h enrichment cultures produced 25 positives (48%), while the lowest detection rate of 9 positives (31%) came from BHIB/S 24 h enrichment cultures. TSB/S at 48 h was significantly better than all the other combinations of

enrichment broths and incubation times ($p < 0.05$), except 24 h TSB/S and 48 h BHIB/S followed by subculture to HBA.

Table 7: Types of seafood investigated and their origin

Seafood	Common Name	Scientific Name	Origin
Fish	Blue mackerel	*Scomber australasicus*	Fremantle – WA
	Australian Herring	*Arripis georgianus*	Fremantle – WA
	Snapper	*Pagrus auratus*	North of WA
	Pilchard	*Sardinops neopilchardus*	Fremantle – WA
	Scaly mackerel	*Amblygaster postera*	Fremantle – WA
	Black bream	*Acanthopagrus butcheri*	Fremantle – WA
	Yellow tail kingfish	*Seriola lalandi*	Fremantle – WA
Other	Australian oyster	*Saccostrea commercialis*	Tasmania & SA
	Banana prawn	*Fenneropenaeus merguiensis*	North of WA
	Blue swimmer crab	*Portunus pelagicus*	Farm in Fremantle
	Coral prawn	*Metapenaeopsis* spp	Carnarvon – WA
	Cuttlefish	*Sepia officinalis*	Carnarvon – WA
	King prawn	*Melicertus latisulcatus*	North of WA
	Blue mussel	*Mytilus edulis*	South of WA
	Green mussel	*Perna canaliculus*	New Zealand
	Octopus	*Octupus australis*	North of WA
	Pacific oyster	*Crassostrea gigas*	New Zealand
	Squid	*Teuthoidea* spp.	North of WA
	Western Rock Lobster	*Panulirus cygnus*	Fremantle – WA

Four percent of enrichment cultures were PCR positive after 24 h and negative after 48 h incubation, and 23% were PCR negative after 24 h and positive after 48 h. Of 56 culture positive enrichment broths, 16 (29%) were initially PCR negative. Dilutions of these samples achieved PCR positive results in all cases.

Differentiation of *Erysipelothrix* spp. Of the 21 isolates which were positive by *Erysipelothrix* specific PCR, 19 fermented glucose but not sucrose, consistent with *E. rhusiopathiae*. The other two fermented both glucose and sucrose, suggesting, possibly, *E. tonsillarum.* The 19 glucose fermenting isolates were also positive by *E. rhusiopathiae* specific PCR.

Distribution of *Erysipelothrix* spp. in different seafoods by culture and PCR. The distribution of *Erysipelothrix* spp. in different seafood groups, using both culture and PCR results, is shown in Table 8. All isolates that were cultured were also detected by PCR, however, PCR gave additional positives. The highest isolation (culture positive) and detection (PCR positive) of *Erysipelothrix* spp. was 15 positives (29%) from all the different fish parts. Fourteen of these isolates were *E. rhusiopathiae*. In addition to the black bream being culture and PCR positive for *E. rhusiopathiae, E. tonsillarum* was cultured. *E. tonsillarum* was also cultured from the Australian oyster shell. The antennae, leg and carapace of the WRL obtained directly from the boat, and surfaces of one octopus and the squid, were all culture and PCR positive for *E. rhusiopathiae.* The highest PCR detection of *Erysipelothrix* spp. was achieved from crustaceans, with 14 positives (27%). All the prawns, various parts of the Blue swimmer crab and the other two WRLs were culture negative and PCR positive, as were, the two Pacific oyster shells and the cuttlefish. The lowest PCR detection of 2 positives (4%) was from the bivalves. *Erysipelothrix* spp. was not isolated or detected from the flesh of either the Australian and Pacific oysters, or the local and New Zealand green mussel shells.

TABLE 8: Distribution of *Erysipelothrix* spp. from different seafoods according to culture and PCR results

Seafood type	No. parts tested	Culture +ve		Culture –ve	
		PCR +ve	PCR –ve	PCR +ve	PCR -ve
Bivalves	10	1 (2) [a]	0 (0)	2 (4)	7 (13)
Cephalopods	7	2 (4)	0 (0)	3 (6)	2 (4)
Crustaceans	18	3 (6)	0 (0)	14 (27)	1 (2)
Fin fish	17	15 (29)	0 (0)	2 (4)	0 (0)

[a] % positive out of the total of 52 seafood parts

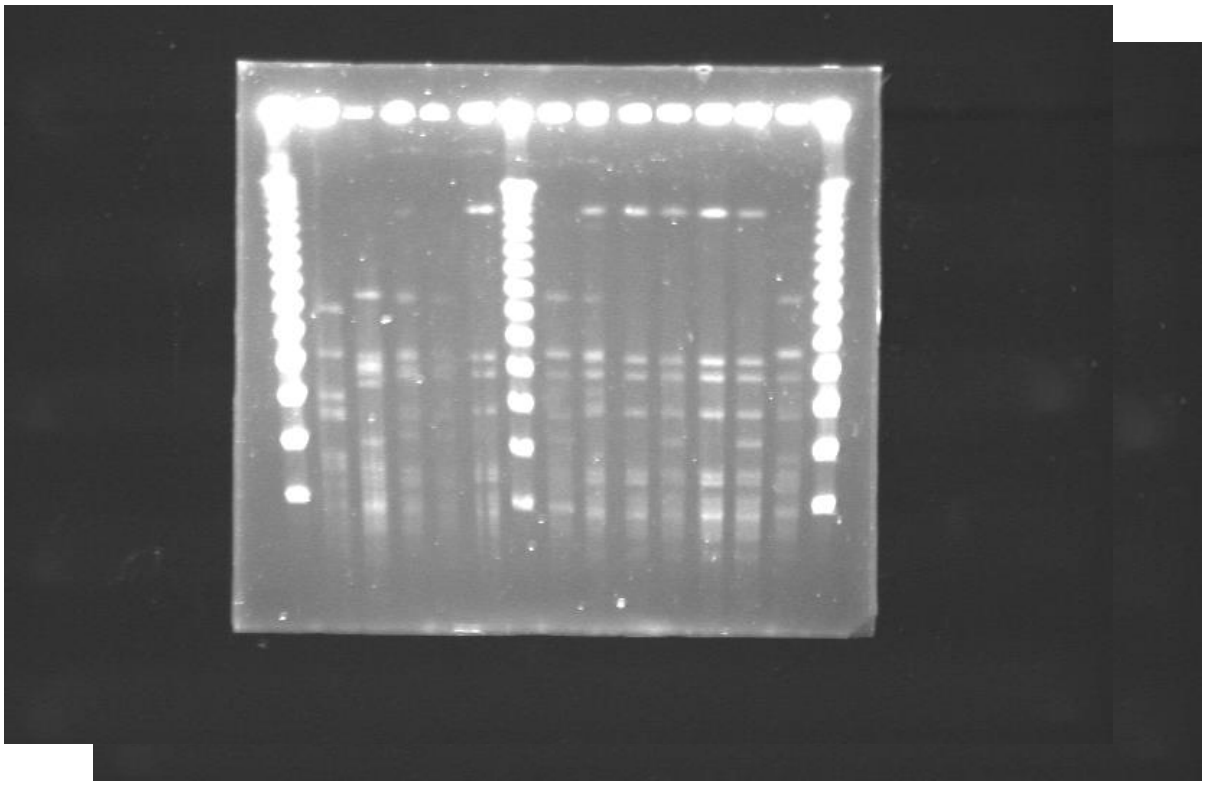
4) Typing of isolates

The aim of this work was to determine the relationship between strains by typing the collection of *Erysipelothrix* isolates, both clinical (animal and human) and environmental. It was important to know whether the organisms infecting fishermen were the same as the organisms present on the lobsters and in the environment. Pulse field gel electrophoresis is the typing method considered the standard currently. Unfortunately, this typing method took a considerable amount of time to optimise for *Erysipelothrix*, as the method has not been previously used for this organism. Also, this method requires about one week of work before the results can be obtained. Several parameters were altered to try and reduce the time required to less than one week, and to improve the intensity of the banding patterns obtained. Eleven different restriction enzymes were tested and only one gave desirable banding patterns. This one restriction enzyme, *Sma* I, was used to type a representative sample all the isolates (see Figures 2a and b for some typical PFGE patterns).

Some *Erysipelothrix* isolates produced more than one colony form and these different colony forms were difficult to isolate. The morphology of the two colonies seemed to vary between some isolates, while others were very similar. The typing of these different colony forms was also important to determine if they were the same isolate with different colony morphologies, or different isolates from the same family. It now appears from of our work with animal strains that there are some nonpathogenic species of *Erysipelothrix*.

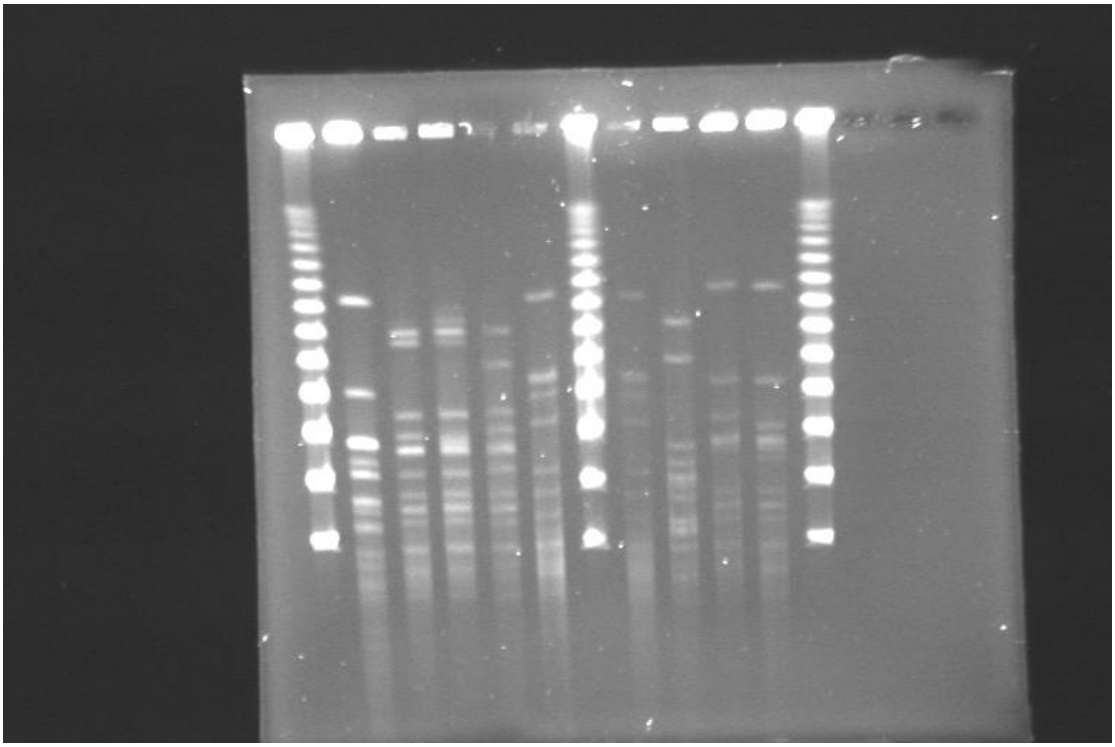
A dendrogram was constructed to show the relatedness of isolates (Figure 3). Several things are obvious from the dendrogram. First, there is significant variation between isolates of the one species, *E.rhusiopathiae*. In the middle of the dendrogram are 4 isolates (13S-1, 13S-2, 513-1 and 513-2) that are genotypically *Erysipelothrix* spp and therefore considered non-pathogens. Note that they have very similar patterns, and thus group together, but they are at some distance from *E.rhusiopathiae*. Isolate 13S came from a glove used on board a lobster fishing boat showing that workers can be contaminated with non-pathogens also. The diversity of isolates can also be seen in several groups from lobsters. Isolates 1B and 4-8B all came from the same lobster and grouped closely together, however, isolate 9B from the same lobster is at some distance, suggesting it is different and that animals may carry more than one type. B12-14 are from a different lobster and are distant from the 1B and 4-8B isolates but quite close to the 9B isolate. There were no human pathogenic isolates of *E.rhusiopathiae* that grouped with any of the fish or crustacean isolates, however, given the great diversity seen in PFGE patterns, this is not surprising and should not be taken as indicating that fish or crustacean isolates cannot infect humans. In fact, the conclusion should be the exact opposite: that all strains of *E.rhusiopathiae* are potentially capable of infecting humans. Interestingly, two isolates from infected lobster fishermen grouped closest to the non-pathogenic *E.tonsillarum*.

Figure 2a: PFGE of *E. rhusiopathiae* isolates.



Lane	Isolate	Source	Species
1.	Marker		
2.	ATCC 19414	Pig spleen	*E. rhusiopathiae*
3.	A16	Herring scales	*E. rhusiopathiae*
4.	A19	Snapper fin	*E. rhusiopathiae*
5.	A20	Snapper scales	*E. rhusiopathiae*
6.	B14	Crayfish #3 carapace	*E. rhusioipathiae*
7.	Marker		
8.	B21`	Squid tentacles	*E. rhusiopathiae*
9.	C1	Pilchard tail	*E. rhusiopathiae*
10.	C3	Yellow tail tail	*E. rhusiopathiae*
11.	C4	Yellow tail surface	*E. rhusiopathiae*
12.	C5	Scaly mackerel tail	*E. rhusiopathiae*
13.	C6	Scaly mackerel scales	*E. rhusiopathiae*
14.	C7	Blue mackerel tail	*E. rhusiopathiae*
15.	Marker		

Figure 2b: PFGE of *E. rhusiopathiae* isolates.



Lane	Isolate	Source	Species
1.	Marker		
2.	ATCC 19414	Pig spleen	*E. rhusiopathiae*
3.	153	Sheep	*E. rhusiopathiae*
4.	C8	Blue mackerel scales	*E. rhusiopathiae*
5.	M1	Lamb	*E. rhusiopathiae*
6.	M2	Lamb	*E. rhusiopathiae*
7.	Marker		
8.	M3	Pork	*E. rhusiopathiae*
9.	M5-1	Mutton	*E. rhusiopathiae*
10.	M5-2	Mutton	*E. rhusiopathiae*
11.	R965	Endocarditis (human) non-cutaneous	*E. rhusiopathiae*
12.	RA115	Cutaneous (human)	*E. rhusiopathiae*
13.	Marker		

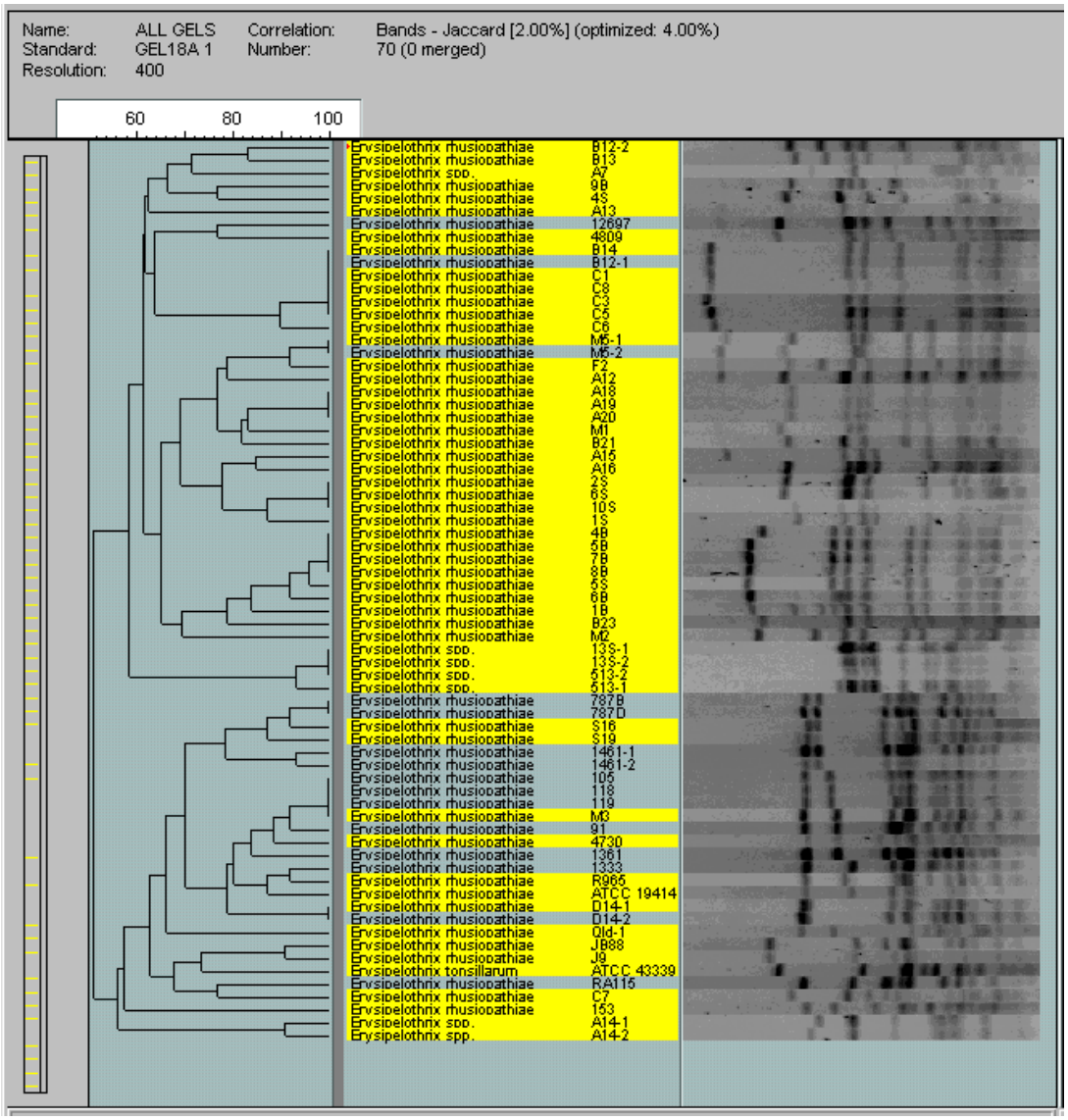


Figure 3: Dendrogram showing relatedness of *E. rhusiopathiae* isolates.

5) Antimicrobial and disinfectant susceptibility testing

A total of 60 *E. rhusiopathiae* isolates was examined from a variety of sources for their antimicrobial susceptibility. These included isolates from human infections (8), pigs (8), sheep (7), emus (2), crustaceans (10), fin fish (18), dolphins (2), cephalopods (2), and an abattoir (1) and fishing boat (2). All the isolates were confirmed as *E. rhusiopathiae* with the *E. rhusiopathiae*-specific PCR method. *E. rhusiopathiae* ATCC 19414 was used as a control for all susceptibility testing. The MIC_{50} and $_{90}$ values obtained for the 60 *E rhusiopathiae* isolates are shown in Table 9. The most active antimicrobial against *E. rhusiopathiae* was penicillin with MIC_{50} and $_{90}$ values of 0.03 mg/l, followed by ciprofloxacin with MIC_{50} and $_{90}$ values of 0.06 mg/l, and ceftriaxone with MIC_{50} and $_{90}$ values of 0.06 mg/l and 0.125 mg/l, respectively. However, all antimicrobials tested apart from vancomycin, rifampin and tetracycline showed good activity, with no evidence of any resistance development. Vancomycin with a very high MIC_{50} and $_{90}$ of 64 mg/l remained inactive against *E. rhusiopathiae*, as was rifampin (MIC_{50} and $_{90}$ >8 mg/l). The tetracycline MICs of 2-4 mg/l suggest borderline susceptibility.

Table 9: MIC values for 60 isolates of *E. rhusiopathiae* against 13 antimicrobial agents

	MIC (mg/l)		
Antimicrobial	Range	50%	90%
Penicillin	0.03	0.03	0.03
Oxacillin	0.125-0.5	0.125	0.125
Tetracycline	2-4	2	2
Minocycline	0.125-0.25	0.25	0.25
Erythromycin	0.125	0.125	0.125
Clindamycin	0.5-2	1	2
Chloramphenicol	8-16	16	16
Cephalothin	0.125-1	0.125	0.25
Ceftriaxone	0.06-0.125	0.06	0.125
Fusidic acid	0.125-1	0.25	0.25
Rifampin	>8	>8	>8
Ciprofloxacin	0.06-0.125	0.06	0.06
Vancomycin	64-128	64	64

The MIC and MBC_{50} and $_{90}$ values obtained for the 31 *E. rhusiopathiae* isolates tested against disinfectants are shown in Table 10. Pinocleen appeared to be extremely active against *E. rhusiopathiae*, and only 0.001% was required to inhibit (MIC) and kill (MBC), 50 and 90% of the isolates. The Wheelie Bin Phenyl Cleanser, Linely and Domestos disinfectants were also effective in inhibiting and killing *E. rhusiopathiae,* with MIC and MBC $_{50}$ and $_{90}$ values of 0.03%. However, the biodegradable toilet cleaner with citric acid and vinegar had poor activity, with MIC and MBC $_{90}$ values of 4.0%.

TABLE 10: MIC and MBC values for 31 *E. rhusiopathiae* against five disinfectants.

Disinfectant	MIC (%)			MBC (%)		
	Range	50%	90%	Range	50%	90%
Linely	0.03	0.03	0.03	0.03	0.03	0.03
Wheelie Bin Phenyl Cleanser	0.016-0.03	0.03	0.03	0.03-0.06	0.03	0.03
Pinocleen	0.001	0.001	0.001	0.001	0.001	0.001
Domestos	0.016-0.03	0.03	0.03	0.016-0.03	0.03	0.03
Toilet cleaner	2.0-4.0	2.0	4.0	2.0-4.0	2.0	4.0

6) Serological response

The examination of serological responses to *Erysipelothrix* infections was problematic. A number of *Erysipelothrix* isolates from various seafoods were used as potential antigens. They were cultured in brain heart infusion broth, then broken up by sonication and the preparations electrophoresed (SDS-PAGE). Western blots were performed using stored fishermen sera, however, no bands were visible. No known positive serum was available at the time and so pig sera from an abattoir was collected, as theoretically this should have had antibodies. Again Western blots were carried out with negative results.

Discussion

Human infections with *Erysipelothrix* **appear** to be rare today. While the older literature contains large series of patients with such infections (Gilchrist 1904), most recent reports about *Erysipelothrix* spp. relate to swine erysipelas. However, occasional case reports do appear and emphasise the fact that isolation and identification of *Erysipelothrix* can be difficult (Dunbar and Clarridge 2000; Robson *et al*. 1998).

The presence of *E. rhusiopathiae* in 19% of wound swab samples suggests the organism has a role in "crayfish poisoning". However, the role of *E. rhusiopathiae* is confounded by the other potential wound pathogens in the samples. Of the organisms in Table 1, *S.*

aureus, *Strep. pyogenes*, and *Acinetobacter* spp. are members of the normal human microflora, and the presence of these organisms could indicate either infection or contamination from this source. S. *aureus* (47%) and *Strep. pyogenes* (23%) in particular are major pathogens of traumatic wounds and important causes of skin and soft tissue infections (Murray *et al*. 1995). However, the fact that all *E. rhusiopathiae* isolates came from "cases" of crayfish poisoning adds weight to the argument that this is an important component of the syndrome.

An objective of this project was to establish the role that *Erysipelothrix* plays in these skin infections. Clearly it does play a role but the natural history of this disease still needs more work. It is likely that the growth of both *S. aureus* and *Strep. pyogenes* may obscure the growth of *Erysipelothrix*, hence our attempts at a molecular diagnosis. The other potential confounder, however, is time to presentation. *Erysipelothrix* is typically a sub-dermal infection and the skin is not broken. When the skin does break, the opportunity exists for other organisms like *S. aureus* and *Strep. pyogenes* to infect the wound. The even distribution of *S. aureus* between cases and non-cases supports this hypothesis.

The remaining potential pathogens can be attributed to the marine environment in which most of the injuries were sustained. *V. alginolyticus* is associated with wound and ear infections and mild cellulitis after contact with marine environments (Patterson *et al*. 1988). While generally regarded an uncommon isolate (Pezzlo *et al*. 1979), Prociv (1978) isolated the organism from 56% of wound infections on Houtman's Abrolhos. *V. alginolyticus* was isolated from 15% of wound swabs in this study. Other Gram negative bacilli such as *Aeromonas hydrophilia* can cause soft tissue infections and infections to traumatic wounds after exposure to contaminated water (Czachor 1992). *Shewanella putrefaciens,* which was also isolated, is rarely significant in clinical specimens, however, it occasionally causes soft tissue infection after exposure to fish and water (Heller *et al*. 1990)

The role of these other organisms and their relationship to *E. rhusiopathiae* in "crayfish poisoning" is still unclear. It is possible that the syndrome is caused by more than one organism; however, it is more likely that secondary infection is common. More than one potential pathogen was detected in greater than 50% of clinical samples, and more than two pathogens were found in 20% of samples. Sheard and Dicks (1949) recognized that numerous lesions were responsible for impairing workers, but their only reference to "crayfish poisoning" was anecdotal, noting that "the fishermen realised underlying factors were present and they named these, in general, 'crayfish' or 'coral' poisoning". The term has persisted to the present, without the natural history or aetiology of the infection being determined.

Despite advances in methods to detect *E. rhusiopathiae*, the number of organisms detected in this study is likely to be an underestimate, due to limitations imposed by sampling procedures. *E. rhusiopathiae* is difficult to isolate from cases of erysipeloid; it is said to live deep in the skin (Jones 1986), and a biopsy from the advancing edge of the lesion extending the entire thickness of the dermis is required. The practicalities of obtaining biopsies from lobster fishermen (this is a minor operative procedure) precluded this line of investigation. Aspirates of the lesion or associated bullae and vesicles are usually less rewarding (Conklin and Steele 1979), and swabbing will often not detect the pathogen (McClain 1990). Sneath *et al*. (1951) reported that culture from

a biopsy was not always successful also and that biopsies from cases of erysipeloid were often sterile.

In the present study, *E. rhusiopathiae* was isolated from one swab of fluid from a vesicle. This possibly reduced contamination by skin flora and a pure culture of the organism was obtained. *E. rhusiopathiae* can be difficult to detect in mixed culture because of the initial small size of colonies, the time required for larger colonies to form, and the tendency for overgrowth by more rapidly growing organisms. A PCR method was therefore essential to the success of the study, and a combination of culture and PCR is required for the best detection (see below).

The hypothesised progression of disease therefore is *Erysipelothrix* infection first followed by the others. However, by the time the other organisms appear, *Erysipelothrix* may have disappeared. The *Acinetobacter* spp., *Vibrio* spp. and other Gram negative bacilli are likely to be contaminants, most likely from the environment. This is not to say that *S. aureus* and *Strep. pyogenes* are not important in the overall problem of skin infections in fishermen as they are both potentially serious pathogens.

On the basis of the findings, the use of oral flucloxacillin in the management of these lesions could be justified. The three major potential pathogens detected, *S. aureus*, *Strep. pyogenes* and *E. rhusiopathiae*, are all susceptible to flucloxacillin. Given that some cases had systemic symptoms, and that vancomycin might be administered empirically in a case of endocarditis, it should be emphasized that all strains of *E. rhusiopathiae* are intrinsically resistant to vancomycin (Gorby and Peacock 1988).

Human infection by *E. rhusiopathiae* is based on the opportunity of exposure (Reboli and Farrar 1992), which is one reason why the infection is frequently occupationally related. *E. rhusiopathiae* was recovered from multiple sites in the lobster fishermen's environment, thus showing that there was ample opportunity for exposure to the organism on the vessel. The majority of infections were in young males employed as deckhands. While this may reflect the overall demography of workers in the lobster fishing industry, it may also be due to inexperience or lack of education about the risks of infection. No questions were asked formally about previous exposure or length of time spent in the industry which may have provided further information. Sheard and Dicks (1949) noted that a poor diet could contribute to the development of illness in fishermen. Anecdotal observations by local health practitioners support this view, noting that diet appeared to be important in the development of "crayfish poisoning", and that younger males were less careful with their diet, particularly regarding nutrition and alcohol consumption. Fingers were the most frequently injured body part, reflecting the type of work involved. This may have been prevented as there was only a moderate amount of glove usage (57%).

Many of the cases reported being spiked by lobster, so the microflora of the lobster was examined. As well as *E. rhusiopathiae*, potential human pathogens among the common isolates from lobster included *V. alginolyticus*, *Proteus* spp., streptococci, and *S. putrefaciens*. While some of these species were present among the isolates from wound swabs from crayfish poisoning, no conclusions could be drawn about their role in the infections.

Isolation of *E. rhusiopathiae* from the exoskeleton of lobster has not been previously reported, although initiation of human infection by injury with crab, lobster and crayfish claws has been documented (Wood 1975). *E. rhusiopathiae* was also detected and isolated from swabs of bait on the vessel, including fish used as bait. *E. rhusiopathiae* can be found in the exterior mucoid slime of fish, and this may enable spread of the organism to the adjacent environment (Murase *et al*. 1959; Proctor and Richardson 1954). This finding prompted a further investigation of fish, cephalopods and crustaceans as a source of *E. rhusiopathiae* (see below). On the lobster fishing boat, it was noticed that equipment and protective clothing were constantly wet and slimy. The spread of *E. rhusiopathiae* by slime could have occurred on the vessel, and could explain the presence of *E. rhusiopathiae* on swab specimens from equipment.

Ensuring protection from injury on the fishing vessel or in other environments in which lobsters are present would be the most practical method of reducing infection. The use of protective clothing such as gloves and wetsuit sleeves (as observed on the field trip) would be effective at preventing injury by scratching from the lobsters or lobster pots. "Crayfish poisoning" often occurs when individuals do not wear gloves or shoes, and infection may result after a minor injury to the unprotected extremity. It is unlikely that the apparently ubiquitous distribution of *E. rhusiopathiae* , in general or on the lobster fishing, vessel could be reduced. Most lobster fishing vessels appear to be kept in an orderly manner, and the spread of water and associated organic matter is unavoidable. The regular disinfection of contaminated sources has been reported to be important in limiting the spread of the organism (Wood 1975). On a lobster fishing vessel, it may be impractical to carry this out, particularly for lobster pots. However, it is recommended that protective clothing and gloves be cleaned regularly with an appropriate disinfectant. In this unique working environment, injury prevention by education would be a more realistic way to reduce potential infection.

The results of this part of the investigation suggest that *E. rhusiopathiae* does have a role in the aetiology of "crayfish poisoning", however, further study into the natural history of this infection is still required.

It is likely that infection with *E.rhusiopathiae* is under-diagnosed because of the resemblance of erysipeloid to other infections, and the problems that may be encountered in isolation and identification of the organism (Brooke and Riley 1999)). One aim of this study therefore was to evaluate various cultural and molecular methods for the detection of *Erysipelothrix* spp. Much of the literature on the recovery of *Erysipelothrix* spp. from human, veterinary and environmental specimens is old; however, most investigations having employed a two stage process of enrichment in broth followed by plating onto solid media. A variety of selective agents, both antibiotic and chemical, have been used (Brooke and Riley 1999)). Three protocols were evaluated with subculture after both 24 and 48 h enrichment: non-selective enrichment in BHIB, followed by plating on BHIA made selective by the addition of kanamycin, neomycin and vancomycin (BHIA/S), selective enrichment in TSB with sodium azide and crystal violet as selective agents (TSB/S), followed by plating on BA, and enrichment in selective BHIB followed by plating on BA. Because of the unknown prevalence of *Erysipelothrix* spp. in routine human clinical specimens, a variety of seafood samples was chosen for the evaluation (Wood 1975). *Erysipelothrix* spp. were recovered from 21 of the 52 seafood parts cultured, 19 of which were *E. rhusiopathiae* and two, possibly, *E. tonsillarum.* Of the three protocols evaluated, the BHIB incubated

for 48 h, followed by subculture to BHIA/S was significantly better than the other two, and 19 isolates from the 52 seafood parts were recovered with this combination. Erysipelothrix selective broth has been regarded as the best selective medium, despite the problem relating to kanamycin susceptibility of some strains (Bratberg 1981). The slight modification of ESB performed quite poorly and the reasons for this are unclear.

In contrast, PCR detection of *Erysipelothrix* spp. was better following 48 h selective enrichment. Thirty-seven of the 52 samples of seafood were PCR positive following enrichment in TSB/S. There were an additional five PCR positives from other enrichment broths (Table 8). Why TSB/S was so superior for PCR detection is unclear. Many enrichment media are known to inhibit PCR (Lantz *et al*. 1998). This may be less so with TSB/S or the broth may have stabilised the template DNA. All samples that were culture positive were also PCR positive, however, PCR detected an extra 21 positives (Table 8). While it might be argued that these were false positives, it is well recognised that PCR techniques are significantly more sensitive than culture (Tompkins 1998). In several cases, the initial correlation between culture and PCR was not good. Four percent of enrichment cultures were PCR positive after 24 h incubation and negative after 48 h. This is likely to have been caused by too much DNA template being present, a factor known to reduce the efficiency of the PCR, or again inhibitors may have been present within the sample (Ausubel 1992) or the enrichment media (Lantz *et al*. 1998). In addition, 29% of the samples that were culture positive were PCR negative initially. Diluting these broths resulted in positive PCRs, suggesting again too much DNA or PCR inhibitors present in the initial sample. For optimal results, PCR at both 24 and 48 h incubation is recommended.

E. rhusiopathiae was widely distributed on the various seafoods tested and all fish were positive. It is well recognised that fish are a common source of *E. rhusiopathiae* (Pestana de Castro 1967; Shewan 1971) and infection can be most severe when contracted from a fish (Klauder 1938). *E. rhusiopathiae* was isolated also from WRLs. Early reports suggested that anglers may have been infected from crustaceans through puncture wounds made by spiny lobsters and crabs (Gilchrist 1904). *E. rhusiopathiae* was isolated also from both octopus and squid from the north of the state. Even though these seafoods are unlikely to cause puncture wounds, they may contaminate existing wounds leading to infection.

About one third of the seafoods were only PCR positive for *Erysipelothrix* spp. There was no particular pattern of distribution of these seafoods which came from through out Western Australia and New Zealand. Since these seafoods were PCR positive and culture negative, this suggests that *Erysipelothrix* spp. DNA was present and the organism was not viable. One possibility which may have to be considered is that *Erysipelothrix* spp. can exist in a viable but not culturable state as many other aquatic organisms have been shown to do (Desmonts *et al*. 1990). Another possibility is that there may have been some cross contamination between the different seafood types, occurring after they were caught. This needs to be investigated further.

One surprising finding was the isolation of two organisms that could not be distinguished from *E. tonsillarum.* *E. tonsillarum* has not been associated previously with seafood as it was in this investigation, with isolates from black bream and the shell of an Australian oyster. This organism is thought to be avirulent for swine, mice and

chickens, however, pathogenicity for humans has not been investigated (Brooke and Riley. 1999).

Overall, *Erysipelothrix* spp. were widely distributed on Australasian seafoods, although there was no correlation between the origin of the seafoods tested and the distribution of *Erysipelothrix* spp. *E. rhusiopathiae* was associated with fish, cephalopods and crustaceans, all of which may either cause injury or infect an existing wound during capture.

Contemporary antimicrobial susceptibility data for *E. rhusiopathiae* isolated from humans are still limited, and monitoring is required to determine the emergence of resistance to antimicrobials. In Japan, resistance to erythromycin and oxytetracycline in *E. rhusiopathiae* isolated from cases of swine erysipelas was first reported over 15 years ago (Takahashi *et al*. 1984b). This was most likely a result of the practice in Japan of feeding pigs food containing macrolides and tetracyclines as growth promotants.

One aim of this study was to investigate the susceptibility of *E. rhusiopathiae* isolates from diverse sources, both clinical and environmental, so that healthcare professionals could have access to contemporary data. Recent investigations have concentrated almost exclusively on isolates from warm-blooded animals, particularly pigs. Therefore isolates from humans, various other mammals and birds, aquatic creatures and the environment were tested. The results were in general agreement with previous reports. In Australia, *E. rhusiopathiae* remains susceptible to penicillins and macrolides, with MICs for susceptible strains similar to previous reports (Yamamoto *et al*. 1999; Yamamoto *et al*. 2000; Takahashi *et al*. 1984b; Takahashi *et al*. 1987). For cefotaxime, an MIC_{90} of 0.06 mg/l has been reported (Venditti *et al*. 1990), again similar to what we obtained for ceftriaxone (MIC_{90} 0.125 mg/l). The earlier first generation cephalosporin, cephalothin, was also active. Erysipeloid can be treated with oral penicillin (Reboli and Farrar 1992) and, for more serious *E. rhusiopathiae* infection, intravenous penicillin is recommended (Grieco and Sheldon 1970). Tetracycline MICs were slightly raised, with an MIC_{90} of 2 mg/l and some strains having MICs of 4 mg/l, and thus tetracycline could not be recommended, however, minocycline was still active. Interestingly, all isolates tested were resistant to rifampin, a fact not previously reported, although Soriano *et al*. (1998) tested six isolates of *E. rhusiopathiae* against the closely related rifapentine and found them all resistant.

As previously documented, *E. rhusiopathiae* was resistant to vancomycin with an MIC_{90} of 64 mg/l. In a patient with endocarditis and a history of skin lesions suggestive of erysipeloid, or with occupational risk factors such as lobster fishing, *E. rhusiopathiae* should always be considered and intravenous penicillin should be part of the empiric regime. Because of its low MICs against *E. rhusiopathiae*, ceftriaxone might still be suitable for patients who are mildly allergic to penicillin, as clindamycin and erythromycin are only bacteriostatic (Reboli and Farrar 1992), however, if serious penicillin allergy is a problem then ciprofloxacin should be considered. Overall, there did not appear to be emergence of antibiotic resistance amongst the isolates tested.

E. rhusiopathiae is a remarkably resilient organism, surviving in harsh environmental conditions and tolerant to numerous chemicals (Conklin and Steele 1970). The commercially available home disinfectants tested were quite active against *E. rhusiopathiae*. Pinocleen containing 1.5% w/v benzalkonium chloride was extremely

effective, with an MIC of only 0.001%. However, benzalkonium chloride is easily inactivated by organic matter and hard water (Fraise 1999), and *E. rhusiopathiae* is known to be able to survive in organic matter. For this disinfectant to be useful, prior cleaning is required to remove the excess organic matter from surfaces, followed by disinfection (Mutalib *et al*. 1993; Spencer 1959). Domestos, Linely and the Wheelie Bin Phenyl Cleanser were also active with an MIC/MBC of 0.03%. Some strains were killed by 0.016% Domestos reflecting, possibly, the greater amount of available chlorine in this disinfectant. The Wheelie Bin Phenyl Cleanser contained 2.9% mixed phenols, made up mainly of *m*- and *p*-cresols and with a small amount of *o*-cresols, in a soap base made from natural fat (the exact composition was unknown); however, all three of these cresols are used as disinfectants. It has been reported that *E. rhusiopathiae* can grow in 0.2% phenols (Wood 1965), but this was not the case in our investigation. Phenolic disinfectants have an advantage in not being inactivated by organic matter (Rutala 1987),however, they can be toxic and unpleasant to work with (Fraise 1999) and for this reason they are not recommended when there are alternatives. The biodegradable, environmental friendly toilet cleaner, containing citric acid and vinegar was not effective against *E. rhusiopathiae*.

Benefits

The proven involvement of a certain group of bacteria, and in particular *E. rhusiopathiae*, in "crayfish poisoning" has enabled contemporary information to be disseminated on the diagnosis and treatment of such infections. Information for lobster fisherman has been included in the WAFIC Code of Practice (Stevens 2003) and an information sheet and video is being prepared. The findings from this project should benefit all industry personnel involved in catching and processing WRL and, additionally, recreational fishermen may achieve some flow-on benefit because of this greater awareness. These benefits are exactly as outlined in the original application.

Further Development

Although anecdotal evidence suggests that similar infections occur in processing plants, this has not been investigated properly. Even if the microbiology is the same, and it is likely to be, some examination of risk factors is warranted.

Planned Outcomes

Since this project began there has been a greater awareness of these occupationally related infections in the fishing industry and by healthcare professionals. Anecdotally, the incidence of infection appears to have declined over the last few years. Information on the aetiology of these infections has been published in peer-reviewed medical literature and, more importantly, data on the types of therapy most likely to be successful provided. Knowledge of the extent of contamination of the work environment will stimulate research into ways of providing a safer working environment. In the interim, recommendations on ways to prevent infection, through the use of protective clothing and equipment, should contribute to the decline in infection.

Conclusions

This investigation was stimulated by the finding that *E. rhusiopathiae* may play a role in "crayfish poisoning", an occupational infection of lobster fishermen in Western Australia. It showed that *E. rhusiopathiae* is often isolated from lesions and many lesions are infected secondarily by *S. aureus* and possibly *Strep. pyogenes*. Improved methods for the detection and culture of *E. rhusiopathiae* were developed. The vast majority of fish, crustaceans and cephalopods carry *E. rhusiopathiae* as part of their normal microbial flora, and exposure to any of these marine animals is a risk factor for acquiring infection. The study also showed that lobster fishing boats are extensively contaminated with *E. rhusiopathiae*. Cleaning and disinfecting the work environment may be a way of reducing the opportunity for exposure and thus infection. Most surfaces on fishing boats are metal and therefore should be amenable to disinfection providing all organic matter is first removed by cleaning. However, professional lobster fishermen use either rectangular wooden pots or the traditional cane "beehive" pots. These porous surfaces are likely to harbour large numbers of organisms of all types and represent a hazard if workers are cut or grazed by them. Disinfection is not likely to be achievable unless pots are soaked in disinfectant solution which is not an option for the industry. There are several commercially available home disinfectants that may be suitable depending on the working environment or equipment needing cleaning and

disinfection. *E. rhusiopathiae* is susceptible the antibiotics commonly used to treat staphylococcal and streptococcal infections. Early detection and treatment is desirable to prevent serious sequelae. *E. rhusiopathiae* is intrinsically resistant to vancomycin and this antibiotic should never be used. Emphasis on the wearing of protective clothing is an important preventative measure, and making sure this protective clothing is regularly cleaned and keeping a clean work environment, will all contribute to a reduction in incidence of infection.

References

Ausubel FM. The polymerase chain reaction. 1992; p. 15.1.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), *Current protocols in molecular biology*, vol. 2. John Wiley and Sons Inc., Canada.

Blackmore DK, Gallagher GL. An outbreak of erysipelas in captive wild birds and mammals. *Vet Rec* 1964; 76: 1161-1164.

Bond MP. Polyarthritis of pigs in Western Australia: the role of *Erysipelothrix rhusiopathiae*. *Aust Vet Jl* 1976; 52: 462-467.

Bratberg AM. Observations on the utilisation of a selective medium for the isolation of *Erysipelothrix rhusiopathiae*. *Acta Vet Scand* 1981; 22: 55-59.

Brooke CJ, Riley TV**.** *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *J Med Microbiol* 1999; 48**:** 789-799.

Brooke CJ, McLaughlin V, Mee BJ, Riley TV. An investigation into "crayfish poisoning" in Western Australia (Letter). *Med J Aust* 1999; 170: 288.

Brooke CJ. Thesis. The University of Western Australia, 1996.

Chen S, Evans GA. Use of polymerase chain reaction for screening transgenic mice. In: White B.A. (ed). *Methods in molecular biology*. Ottowa: Human Press, 1993.

Conklin RH, Steele JH. *Erysipelothrix* infections. In: Steele JH editor. CRC Handbook. Series in Zoonoses Vol 1. Florida: CRC Press, 1979; 327-337.

Cottral GE. *Erysipelothrix*. In *Manual of standardized methods for veterinary microbiology* 1978; pp. 429-436, 671, 672, 679, 687. Ithaca, New York: Cornell University Press.

Cross GM, Edwards MJ. The detection of arthritis in pigs in an abattoir and its public health significance. *Aust Vet J* 1981; 57**:** 153-158.

Czachor JS. Unusual aspects of bacterial water-borne illnesses. *Am Fam Phys* 1992; 46: 797-804.

Dean AG. *et. Al.*, Epi Info, Version 5: a word processing, database, and statistics program for epidemiology on microcomputers. UDS, Incorporated, Stone Mountain, Georgia.

Desmonts CJ, Minet R, Colwell, Cormier M. Fluorescent-antibody method useful for detecting viable but nonculturable *Salmonella* spp. in chlorinated wastewater. *Appl Env Microbiol* 1990; 56: 1448-1452.

Dunbar SA, Clarridge JE III. Potential errors in recognition of *Erysipelothrix rhusiopathiae*. *J Clin Microbiol* 2000; 38: 1302-1304.

Ewald FW. The genus *Erysipelothrix*. 1981; p. 1688-1700. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), *The Prokaryotes: A handbook on habitats, isolation and identification of bacteria*. Springer-Verlag, New York.

Fidalgo SG, Wang Q, Riley TV. Comparison of methods for detection of *Erysipelothrix* spp. and their distribution in some Australasian seafoods. *Appl Environ Microbiol* 2000; 66: 2066-2070.

Fraise AP. Choosing disinfectants. *J Hosp Infect* 1999; 43: 255-64.

Gilchrist TC**.** Erysipeloid, with a record of 329 cases, of which 323 were caused by crab bites, or lesions produced by crabs. *J Cutan Dis* 1904; 22: 507-519.

Gorby GL, Peacock J. E, Jr**.** *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Rev Infec Dis* 1988; 10: 317-325.

Grieco MH, Sheldon C. *Erysipelothrix rhusiopathiae*. *Ann N Y Acad Sci* 1970; 174: 523-32.

Harlow E, Lane D. Immunoblotting. 1988, pp471-504. In *Antibodies: A laboratory manual*. Cold Spring Harbor: New York.

Harrington R Jr, Hulse DC. Comparison of two plating media for the isolation of *Erysipelothrix rhusiopathiae* from enrichment broth culture. *Appl Microbiol* 1971; 22: 141-142.

Heller HM, Tortora G, Burger H. *Pseudomonas putrefaciens* bacteremia associated with shellfish contact. *Am J Med* 1990; 88: 85-86.

Hillenbrand FKM**.** Whale finger and seal finger; their relation to erysipeloid. *Lancet* 1953; i: 680 - 681.

Hugo WB, Russell AD. In *Pharmaceutical microbiology*, 1992; 5th ed., Blackwell Scientific Publications: Oxford.

Hunter D**.** *The diseases of occupations*, 1974; p. 709-712, 5th ed. The English University Press Ltd, London.

Jones D, Morgan G. *A field guide to crustaceans of Australian waters*. Perth: Reed, 1994.

Jones D. Genus *Erysipelothrix* Rosenbach1909[367AL]. In *Bergey's Manual of Systematic Bacteriology* 9th ed. Sneath, PHA, Mair, NS, Sharpe, ME, Holt, JG. 1986; pp. 1245-1249: Baltimore, Williams and Wilkins.

Klauder JV**.** Erysipeloid as an occupational disease. *J Am Med Assoc* 1938; 111: 1345-1348.

Klauder JV. Erysipelopid and swine erysipelas in man. *JAMA* 1926; 86: 536-541.

Lantz PGR, Knutsson Y, Blixt WA, Soud Al, Borch E, Radstrom P**.** Detection of pathogenic *Yersina enterocolitica* in enrichment media and pork by multiplex PCR: a study of sample preparation and PCR-inhibitory components. *Int J Food Microbiol* 1998; 45: 93-105.

Makino S, Yamamoto K, Murakami S, Shirahata T, Uemura K, Sawada T, *et al*. Properties of repeat domain found in a novel protective antigen, SpaA, of *Erysipelothrix rhusiopathiae*. *Microbial Pathogenesis* 1998; 25: 101-109.

Makino SY, Okada T, Maruyama K, Ishikawa T, Takahashi M, Nakamura T, Ezaki, Morita H**.** Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J Clin Microbiol* 1994; 32: 1526-1531.

McClain JB. *Erysipelothrix rhusiopathiae*. Pp. 1599-1600. In: Mandell GL, Douglas RG, Bennet JE (eds.) *Principles and practice of infectious disease*. 3rd Ed. New York: Churchill Livingstone, 1990.

Molin G, Soderlind O, Ursing J, Norrung V, Ternstrom A, Lowenhielm C. Occurrence of *Erysipelothrix rhusiopathiae* on pork and in pig slurry, and the distribution of specific antibodies in a battoir workers. *J Appl Bacteriol* 1989; 67: 347-352.

Morgan MJ, Britt JO, Cockrill JM, Eiten ML. *Erysipelothrix rhusiopathiae* infection in an emu (*Dromaius novaehollandiae*). *J Vet Diag Invest* 1994; 6: 378-379.

Murase N, Suzuki K, Isayama Y, Murata M. Studies on the typing of *Erysipelothrix rhusiopathiae*. III Serological behaviour of the strains isolated from the body surface of marine fishes and their epizootiological significance in wine erysipelas. *Jap J Vet Sci* 1959; 21: 215-219.

Murray P, Baron EJ, Pfaller M, Tenover F, Yolken R. *Manual of clinical microbiology*, 1995; 6th ed., ASM Press: Washington.

Mutalib AA, King JM, McDonough PL. Erysipelas in caged laying chickens and suspected erysipeloid in animal caretakers. *J Vet Diagn Invest* 1993; 5: 198-201.

Nakazawa H, Hayashidani H, Higashi J, Kaneko K, Takahashi T, Ogawa M. Occurrence of Erysipelothrix spp. in broiler chickens at an abattoir. *J Food Protect* 1998a; 61: 907-909.

Nakazawa H, Hayashidani H, Higashi J, Kaneko K, Takahashi T, Ogawa, M. Occurrence of *Erysipelothrix* spp. in chicken meat parts from a processing plant. *J Food Prot* 1998b; 61: 1207-1209.

National Committee for Clinical Laboratory Standards. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 4th ed. Approved standard. NCCLS document M7-A4, 1997. National Committee for Clinical Laboratory Standards, Villanova, Pa.

Norrung V, Munch B, Larsen HE. Occurrence, isolation and serotyping of *Erysipelothrix rhusiopathiae* in cattle and pig slurry. *Acta Vet Scand* 1987; 28: 9-14.

Packer RA**.** The use of sodium azide and crystal violet in a selective medium for streptococci and *Erysipelothrix rhusiopathiae. J Bacteriol* 1943; 46: 343-349.

Patterson TF, Bell SR, Bia FJ. *Vibrio alginolyticus* cellulites following coral injury. *Yale J Biol Med* 1988; 61: 507-512.

Pestana de Castro AFO, Campedelli Filho, Troise C**.** Isolation of *Erysipelothrix rhusiopathiae* from marine fish. *Rev Inst Med Trop Sao Paulo* 1967; 9: 169-171.

Pezzlo MA, Valter PJ, Burns MJ. Wound infection associated with *Vibrio alginolyticus*. *Am J Clin Pathol* 1979; 71: 476-478.

Prociv P. *Vibrio alginolyticus* in Western Australia. *Med J Aust* 1978; 2: 296.

Proctor DM, Richardson IM. A report on 235 cases of erysipeloid in Aberdeen. *Br J Ind Med* 1954; 11: 175-179.

Reboli AC, Farrar WE. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clin Microbiol Rev* 1989; 2: 354-359.

Reboli AC, Farrar WE. The genus *Erysipelothrix*. In *The prokaryotes: a handbook on the biology of bacteria: ecophysiology, isolation, identification, application* ed. Balows, A, Truper, HG, Dworkin, M, Harder, W, Schleifer, K. 1992; pp. 1629-1642. New York: Springer-Verlag.

Robson JM, McDougall R, van der Valk S, Waite SD, Sullivan JJ. *Erysipelothrix rhusiopathiae*: an uncommon but ever present zoonosis. *Pathology* 1998; 30: 391-4.

Rutuala WA. *Prevention and control of nosocomial infections*. Baltimore: Williams and Wilkins, 1987; Chapter 18, Disinfection, Sterilization, and Waste Disposal.

Sato H, Hirose K, and Saito H. Protective activity and antigenic analysis of fractions of culture filtrates of Erysipelothrix rhusiopathiae. *Vet Microbiol* 1995; 43: 173-82.

Sheard K,Dicks HG. Skin lesions among fishermen at Houtman's Abrolhos, Western Australia, with an account of erysipeloid of Rosenbach. *Med J Aust* 1949; ii: 352-354.

Shewan JM**.** The microbiology of fish and fishery products - a progress report. *J Appl Bacteriol* 1971; 34: 299-315.

ShimojiY, Mori Y. and Fischetti VA. Immunological characterization of a protective antigen of *Erysipelothrix rhusiopathiae*: identification of the region responsible for protective immunity. *Infection & Immunity* 1999; 67**:** 1646-1651.

Shimoji Y, Mori Y, Hyakutake K, Sekizaki T, Yokomizo Y**.** Use of an enrichment broth

cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. *J Clin Microbiol* 1998; 36: 86-89.

Shimoji Y, Mori Y, Sekizaki T, Shibahara T, Yokomizo Y. Construction and vaccine potential of a capsular mutants of *Erysipelothrix rhusiopathiae*: use of excision of Tn916 to inactivate a target gene. *Infect Immun* 1998b; 66: 3250-3254.

Shuman RD. Erysipelothrix. In *Infectious and parasitic diseases of wild birds* ed. Davis, JW, Anderson, RC, Karstad, LH, Trainer, DO. 1971; pp. 141-145. Ames, IA: Iowa State University Press.

Sneath PHA, Abbott JD, Cunliffe AC. The bacteriology of erysipeloid. *Br Med J* 1951; 2: 1063-1066.

Soriano F, Fernandez-Roblas R, Calvo R, Garcia-Calvo G. In vitro susceptibilities of aerobic and facultative non-spore-forming gram-positive bacilli to HMR 3647 (RU 66647) and 14 other antimicrobials. *Antimicrob Agents Chemother*. 1998; 42: 1028-33.

Spencer R. The sanitation of fish boxes. I. The quantitative and qualitative bacteriology of commercial wooden fish boxes. *J Appl Bacteriol* 1959; 22: 73-84.

Stephenson EH, Berman DT. Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *Am J V Res* 1978; 39: 187-188.

Stevens, R.N. Best practice in the Western Australian Australian Rock Lobster industry. 2003; (in press)

Takahashi T, Fujisawa T, Benno Y, Tamura Y, Sawada T, Suzuki S, *et al*. *Erysipelothrix tonsillarum* sp. Nov. isolated from tonsils of apparently healthy pigs. *Int J Syst Bacteriol* 1987a; 37: 166-168.

Takahashi T, Fujisawa T, Tamura Y, Suzuki S, Muramatsu M, Sawada T, Benno, and Mitsuoka T**.** DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. *Int J Syst. Bacteriol* 1992; 42: 469-473.

Takahashi T, Sawada T, Muramatsu M, Tamura Y, Fujisawa T, Benno Y, *et al*. Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. *J Clin Microbiol* 1987b; 25: 536-539.

Takahashi T, Sawada T, Ohmae K, Terakado N, Muramatsu M, Seto K, *et al*. Antibiotic resistance of *Erysipelothrix rhusiopathiae* isolated from pigs with chronic swine erysipelas. *Antimicrob Agents Chemother* 1984b; 25: 385-386.

Takahashi T, Tamura Y, Yoshimura H, Nagamine N, Kijima M., Nakamura M, *et al*. *Erysipelothrix tonsillarum* isolated from dogs with endocarditis in Belgium. *Res Vet Sci* 1993; 54: 264-265.

Takahasi T, Fujisawa T, Yamamoto K, Kijima M, Takahashi T. Taxonomic evidence that

serovar 7 of *Erysipelothrix* strains isolated from dogs with endocarditis are *Erysipelothrix tonsillarum*. *Zentralblatt fur Veterinarmedizin - Reihe* 2000; *B* 47**,** 311-313.

Taylor DJ. *Pig diseases*. 1990; Cambridge: Burlington Press.

Timoney JF, Groschup MM. Properties of a protective protein antigen of *Erysipelothrix rhusiopathiae*. *Vet Microbiol* 1993; 37: 381-387.

Tompkins LS**.** Molecular epidemiology in infectious diseases, 1998; pp. 33-34. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), *Infectious diseases*, 2nd ed. WB Saunders Co., Philadelphia, Penn.

Venditti M, Gelfusa V, Tarasi A, Brandimarte C, Serra P. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae*. *Antimicrob Agents Chemother* 1990; 34: 2038-2440.

White TG, Shuman RD**.** Fermentation reactions of *Erysipelothrix rhusiopathiae J Bacteriol* 1961; 82: 595-599

Wood RL, Booth GD, Cutlip RC. Susceptibility of vaccinated swine and mice to generalized infection with specific serotypes of *Erysipelothrix rhusiopathiae*. *Am J Vet Res* 1981; 42: 608-614.

Wood RL**.** A selective liquid medium utilising antibiotics for isolation of *Erysipelothrix insidiosa*. *Am J Vet Res* 1965; 26: 1303-1308.

Wood RL. Erysipelas. In *Diseases of swine*, ed. Leman, AD. 1992; pp. 475-486. Ames, Iowa: Iowa State University Press.

Wood RL. *Erysipelothrix* infection. In *Diseases transmitted from animals to man*, 6th ed. Hubbert, WT, McCulloch, WF, Schnurrenberger, PR. 1975; pp. 271-281. Springfield, IL: Thomas.

Yamamoto K, Kijima M, Yoshimura H, Takahashi T. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae* isolated from pigs with swine erysipelas in Japan. *Zentralbl Veterinarmed [B]* 2000; 48: 115-26.

Yamamoto M, Kijima M, Takahashi T, Yoshimura H, Tani O, Kojyou T, *et al*. Serovar, pathogenicity and antimicrobial susceptibility of *Erysipelothrix rhusiopathiae* isolates from farmed wild boars (*Sus scrofa*) affected with septicemic erysipelas in Japan. *Res Vet Sci* 1999; 67: 301-303.

Appendix 1

Intellectual property issues: None.

Appendix 2

Staff: Professor Thomas V Riley

Dr Brian J Mee

Ms Tanya Adams

Ms Silvana Fidalgo

Ms Chelsea Longbottom

Appendix 3 – Questionnaire and patient consent form

THE MICROBIOLOGY OF CRAYFISH POISONING

PATIENT INFORMATION

"Crayfish poisoning" is an occupationally related infectious disease which affects individuals in the fishing industry who handle crayfish (Western Rock Lobster) (*Panulirus cygnus*). Typically, infection follows punctures from lobster or fish spines but may occur after any cut or abrasion becomes contaminated. After injury to the skin, there is little reaction for several days, during which the injury apparently heals. Redness then develops around the wound, followed by pain and swelling, with or without fever. Healing usually takes place over 3 weeks, or secondary infection may occur. The causative organism for the disease is thought to be a bacterium called *Erysipelothrix* however, the only investigation of this disease in Western Australia occurred in the late 1940s. In that investigation conclusive identification of the pathogen was not possible. Despite an awareness of the problem in the crayfish industry, 26 cases of crayfish poisoning were recorded at the Leeman nursing post during 1993. The total number of cases reported to all nursing posts and medical practitioners will be much higher. These infections have a major impact on the multimillion-dollar crayfish industry.

The purpose of this study is to determine the cause of crayfish poisoning. If a convenient time can be arranged for a visit to see either Dr Clay Golledge or Dr David Speers, a swab or biopsy from your infection and a blood sample will be taken. You will also receive antibiotic treatment if required.

Any questions about the investigation may be directed to Dr Thomas Riley at the Department of Microbiology at the University of Western Australia. Telephone: (08) 9346 3690, Facsimile: (08) 9346 2912.

PATIENT CONSENT

I have read the above information and the details of the investigation have been explained to me to my satisfaction. I agree to allow specimens from my infection to be collected and processed at the Department of Microbiology at the University of Western Australia. I understand that the results of this investigation may be used for medical or scientific publications, however, my identity will not be disclosed. Any publication will not include any identifying information. All investigation details will be stored in a locked area within the Department of Microbiology. I understand that I have the right to withdraw from the study at any time without prejudice to my continuing treatment.

Patient's signature Date

..../...../.....

Attending nurse / physician's signature Date

..../...../.....

Case Code No:.....

CRAYFISH POISONING QUESTIONNAIRE

Please fill this form in for each injury (no matter how small) relating to crayfish poisoning that you may receive during the crayfish season. All the information will be treated as confidential.

SECTION A – To be filled in by person with the injury *(Please fill in the appropriate box or space).*

Name:_____ Date:_____/_____/_____

Sex: Male ☐ Female ☐ Age:_____ years

Occupation: _____

Contact phone number: ( )_____ or Processing plant:_____

1. Injury details –

Lesion 1	**Lesion 2**
Date of injury _____/_____/_____	Date of injury _____/_____/_____
Finger ☐ Hand ☐	Finger ☐ Hand ☐
Arm ☐ Body ☐	Arm ☐ Body ☐
Face ☐ Leg ☐	Face ☐ Leg ☐
Foot ☐	Foot ☐

2. What part of the crayfish caused the injury?

Carapace ☐ Legs ☐ Antennae ☐

Abdominal Spines ☐ Other:_____

3. Was any protective garment worn ie gloves, boots at the time of the injury? YES ☐ NO ☐

If YES, please specify the type of garment:

_____

4. Please describe how you obtained the injury(s):_____

_____

5. What did you do immediately after the injury?

Wash with fresh water ☐

Wash with soap and water ☐

Wash with antiseptic ☐

Other:_____

6. Did you treat the injury later? YES ☐ NO ☐

If YES, what was the treatment?

_____

7. Did you have any other symptoms? YES ☐ NO ☐

If YES, please list them and when they started below

(for example, headache 23 / 10 / 98 lasted 2 days)

SYMPTOM	DATE STARTED	HOW LONG DID IT LAST?

8. At the time of the injury were you taking any antibiotics? YES ☐ NO ☐

If YES, please state the name(s):_____

_____

9. Did you start taking antibiotics after the injury? YES ☐ NO ☐

If YES, please state the name(s):_____

_____

10. How many standard alcoholic drinks on average would you have per day?

1 bottle of full strength beer (750mL)	= 3 standard drinks	
1 can of full strength beer	= 11/2 standard drinks.	
1 can of light beer	= 1 standard drink.	Average number = ☐
A nip of spirits	= 1 standard drink.	
A small glass of wine	=1 standard drink.	

11. Did you take any time off work due to this injury? YES ☐ NO ☐

If YES, how many days:_____

SECTION B: To be filled in by Medical Staff *(Please fill in the appropriate box or space).*

Name of attending Doctor / Nurse: _____

Contact address: _____

Contact phone: ( )_____

Date: _____/_____/_____

1. Lesion details –

Lesion 1				Lesion 2			
Date of injury _____/_____/_____				Date of injury _____/_____/_____			
Abscess	☐	Furuncles	☐	Abscess	☐	Furuncles	☐
Blister	☐	Paronychia	☐	Blister	☐	Paronychia	☐
Cellulitis	☐	Superficial Abrasion	☐	Cellulitis	☐	Superficial Abrasion	☐
Erythema	☐	Dry ulcer	☐	Erythema	☐	Dry ulcer	☐
Suppurating ulcer	☐	Other:_____		Suppurating ulcer	☐	Other:_____	

2. Site of lesion #1_____

   Site of lesion #2_____

3. Does the patient have any systemic symptoms? YES ☐ NO ☐

   If YES, please state the symptom(s):_____

   _____

4. Was the patient prescribed antibiotics? YES ☐ NO ☐

   If YES, please state the name(s): _____

   _____

Please swab & / or take a biopsy and collect 5ml of clotted blood

(Please turn over)

Appendix 4 – Publications (attached)

Book Chapters:

Riley, T.V., Brooke, C.J. and Wang, Q. 2001. *Erysipelothrix rhusiopathiae*. In *Molecular Medical Microbiology* (Ed. M Sussman) Academic Press: London.

Fidalgo, S. and Riley, T.V. 2003. Detection of *Erysipelothrix rhusiopathiae* in clinical and environmental samples. In *Methods in Molecular Biology* (Ed. F Spencer) Humana Press: (in press)

Scientific Journals:

Brooke, C.J. and Riley, T.V. 1999. *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. *J Med Microbiol* 48: 789-799.

Fidalgo, S.G., Wang, Q. and Riley, T.V. 2000. Comparison of methods for the detection of *Erysipelothrix* spp. and their distribution in Australasian seafood. *Appl Environ Microbiol* 66: 2066-2070.

Wang, Q., Fidalgo, S., Chang, B.J., Mee, B.J. and Riley, T.V. 2002. The detection and recovery of *Erysipelothrix* spp. in meat and abattoir samples in Western Australia. *J Appl Microbiol* 92: 844-850.

Fidalgo, S.G., Longbottom, C.J. and Riley, T.V. 2002. Susceptibility of *Erysipelothrix rhusiopathiae* to antimicrobial agents and home disinfectants. *Pathology* 34: 462-465.

50

Erysipelothrix rhusiopathiae

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Erysipelothrix rhusiopathiae is a small Gram-positive bacillus classified in the genus *Erysipelothrix* together with *E. tonsillarum* and other two recently classified unnamed species (Takahashi *et al.*, 1992). *E. rhusiopathiae* was first established as a human pathogen late in the nineteenth century. Since then three forms of human disease have been recognised. These include a localised cutaneous lesion form, erysipeloid, a generalised cutaneous form and a septic form often associated with endocarditis (Grieco and Sheldon, 1970). The organism is ubiquitous and able to persist for a long period of time in the environment, including marine locations. It is a pathogen or a commensal in a wide variety of wild and domestic animals, birds and fish (Conklin and Steele, 1979). Swine erysipelas caused by *E. rhusiopathiae* is of great prevalence and economic importance (Wood, 1992). Diseases in other animals include erysipelas of farmed turkeys, chickens, ducks and emus, and polyarthritis in sheep and lambs. The organism causes no known disease in fish but can survive for long periods on the mucoid exterior slime of fish (Wood, 1975).

Infection due to *Erysipelothrix* spp. in humans is occupationally related, principally occurring as a result of contact with contaminated animals, their products or wastes, or soil. Erysipeloid is the most common form of infections in humans. Some other names have been used to describe this infection, including whale finger, seal finger, speck finger, blubber finger, fish poisoning, fish handler's disease, and pork finger (Hillenbrand, 1953; Hunter, 1975; Wood, 1975; Reboli and Farrar, 1992). It has been suggested that the incidence of human infection may be declining because of technological advances in animal industries, but infection still occurs in specific environments. Additionally, infection by *Erysipelothrix* spp. is possibly underdiagnosed because of the resemblance it bears to other infections, and problems encountered in its isolation and identification. Diagnosis of erysipeloid can be difficult if not recognised clinically, as culture is lengthy and the organism resides deep in the skin. Recent advances in molecular diagnostic techniques may help to establish the true prevalence of infections in humans.

Classification

Until recently, the genus *Erysipelothrix* consisted of only the type species, *E. rhusiopathiae*, which was

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known to demonstrate great serological, biochemical and antigenic variation (Reboli and Farrar, 1992). Genetic analysis has revealed a new species, *E. tonsillarum* (Takahashi *et al.*, 1987a) and it is likely that others exist. *Erysipelothrix* was originally considered a close relative of the *Listeria* spp., but numerous molecular taxonomic studies have concluded that the genus is a distinct cluster of organisms, most similar to the streptococci (Jones, 1986; Reboli and Farrar, 1992).

E. rhusiopathiae was the first described as a cause of human disease in 1870; further cases were documented in 1873 as erythema serpens (Barber, 1948). It was, however, not until 1884, when Rosenbach isolated an organism from a patient with localised cutaneous lesions, that *Erysipelothrix* was established as a human pathogen. He used the term 'erysipeloid' to differentiate between the human streptococcal disease erysipelas and the condition he had observed (Wood, 1975). Rosenbach distinguished three separate species of the organism, *E. muriseptica*, *E. porci* and *E. erysiploides*, based on their isolation, respectively, from mice, pigs and humans. It was later realised these three organisms were nearly identical strains of the same species, and they were named *E. insidiosa*, as originally proposed by Trevisan in 1885. This and all 36 other documented names for the organism were rejected in 1966 in favour of *E. rhusiopathiae*, a combination that originated in 1918 (Ewald, 1981).

Takahashi *et al.* (1987c) isolated a cluster of avirulent strains, later found to be genetically distinct from *E. rhusiopathiae* by DNA base composition and DNA–DNA homology studies. These strains formed the basis of a new species, *E. tonsillarum* (Takahashi *et al.*, 1987a). Originally, *E. tonsillarum* was considered morphologically and biochemically identical to *E. rhusiopathiae*, but it was shown later that *E. tonsillarum* could ferment sucrose, while *E. rhusiopathiae* could not (Takahashi *et al.*, 1992).

Isolation

Traditionally, culture methods for the isolation of *E. rhusiopathiae* have involved the use of selective and enrichment media. A number of media have been described. A commonly used medium is Erysipelothrix selective broth (ESB), a nutrient broth containing horse serum, kanamycin, neomycin, and vancomycin (Wood, 1965). Modified blood azide medium (MBA) is a selective agar that contains sodium azide and horse blood or serum (Harrington and Hulse, 1971). Packer's medium is a selective agar suitable for grossly contaminated specimens that contains sodium azide and crystal violet (Packer, 1943). Bohm's medium utilises sodium azide, kanamycin, phenol and water blue (Ewald, 1981). More recently, Japanese workers have described a selective enrichment broth containing tryptic soy broth, Tween 80, Tris-aminomethane, crystal violet and sodium azide (Shimoji *et al.*, 1998). All these media make use of the organism's resistance to antibiotics and tolerance of chemicals; each has good aspects, but none is ideal. ESB is regarded as the best selective medium. MBA requires less incubation time than Packer's medium, but is not as selective. Shimoji's selective broth combined with PCR has been used for the rapid diagnosis of erysipelas in swine (Shimoji *et al.*, 1998).

In a recent survey of the distribution of *Erysipelothrix* spp. in 19 Australasian seafoods, methodologies for detection of *Erysipelothrix* spp. were evaluated. Primary isolation of *Erysipelothrix* spp. was most efficiently achieved with brain-heart infusion broth enrichment, followed by subculture onto a selective brain–heart infusion agar containing kanamycin, neomycin and vancomycin, after 48 hours incubation. Selective tryptic soy broth with 48 hours incubation, followed by PCR, was the best culture method for detection of *Erysipelothrix* spp. The PCR detection method was 50% more sensitive than culture (Fidalgo *et al.*, 2000).

Identification

Identification of *Erysipelothrix* spp. is based on Gram's stain, cultural morphology, motility, haemolytic characteristics and biochemical properties, particularly the production of hydrogen sulphide (Reboli and Farrar, 1992). *E. rhusiopathiae* is a non-motile, non-spore-forming, and non-acid-fast bacillus. It is Gram-positive but easily decolourised (Jones, 1986). A capsule is present and may play a role in virulence (Shimoji *et al.*, 1994). Colonies on solid agar are described as clear, circular and very small with a diameter of 0.1–0.5 mm after 24 hours or 0.5–1.5 mm after 48 hours. Both smooth (S) and rough (R) forms are present. The role played in disease by different colonial forms has not been determined. Most strains show a narrow zone of α-haemolysis on blood agar, but β-haemolysis is never seen (Conklin and Steele, 1979; Jones, 1986).

The genus *Erysipelothrix* is relatively inactive in biochemical properties. It is negative for catalase, oxidase, methyl red, indole and Voges–Proskauer (Ewald, 1981). Sucrose is fermented by most strains of *E. tonsillarum* but not by *E. rhusiopathiae* (Takahashi *et al.*, 1992). Hydrogen sulphide is produced by 95% of strains of *Erysipelothrix* spp. and is well demonstrated

on triple sugar iron agar (Vickers and Bierer, 1958). Rapid identification of *E. rhusiopathiae* can be achieved with an API Coryne System strip (Soto *et al.*, 1994), and the mouse protection test is regarded as the best confirmatory test (Jones, 1986).

Three PCR methods have been developed to detect *Erysipelothrix* spp. Makino *et al.* (1994) described a PCR method that uses primers based on a region of the 16S rRNA gene and is genus-specific. A PCR that is *E. rhusiopathiae*-specific and designed from sequences associated with virulence of *E. rhusiopathiae* was described by Shimoji *et al.* (1998). The sensitivity of the Makino PCR is less than 20 bacteria, but the Shimoji PCR detects a minimum of 1000 bacteria per reaction mixture. Makino's method is specific for *Erysipelothrix* spp. but cannot differentiate between *E. rhusiopathiae* and *E. tonsillarum*. An improved PCR method that can distinguish four species of the genus *Erysipelothrix* has been reported (Takeshi *et al.*, 1999). This species-specific PCR method may provide a new approach for the rapid diagnosis of *Erysipelothrix* infections in animals and humans.

Serology

Watts (1940) showed that most strains of *Erysipelothrix* have two kinds of antigen, a species-specific heat-labile protein antigen and a heat- and acid-stable polysaccharide antigen, and these now form the basis for serotyping strains. Two major serotypes, A and B, were recognised and strains which did not react with these specific antisera were classified in group N (Jones, 1986). The Arabic numeral serotyping system of Kucsera (1973) superseded the alphbetical scheme because of variations in previous methods of antigenic extraction. The current standard serotyping method is a double agar-gel precipitation test with type-specific rabbit antisera and antigen recovered by hot aqueous extraction (Kalf and White, 1963; Kucsera, 1973). Strains of *Erysipelothrix* were determined as belonging to serotypes 1–26 and group N (Norrung and Molin, 1991). In swine, 75–80% of isolates are classified into serotype 1 or 2. A relationship between serotype and virulence was noted early. Serotype 1a is most commonly isolated from acute swine erysipelas, and serotype 2a is more prevalent in chronic forms of the disease. Some studies also gave contradictory results, however, and all clinical conditions can be induced experimentally in susceptible swine with a variety of serotypes (Wood and Harrington, 1978; Kucsera, 1979). It is therefore debatable whether serotyping provides any practical value in the classification of strains of *Erysipelothrix*. Sneath *et al.* (1951) reported that strains of human or pig origin are antigenically similar, but this has not been verified. The epidemiological significance of serotyping in human disease is uncertain.

Molecular and other typing methods have been applied in the classification of isolates of *Erysipelothrix*. These include DNA–DNA hybridization (Takahashi *et al.*, 1992), polyacrylamide gel electrophoresis (Tamura *et al.*, 1993), multilocus enzyme electrophoresis studies (Chooromoney *et al.*, 1994) and restriction-fragment length polymorphism (Ahrne *et al.*, 1995). By these methods serotypes 1a, 1b, 2, 4–9, 11, 12, 15–17, 19, 21, and 25 were all *E. rhusiopathiae* whereas serotypes 3, 7, 10, 14, 20, 22–24 were *E. tonsillarum*. Serotypes 13 and 18 were the two unnamed species. These studies clearly show that there is no direct relationship between serotype and virulence, which supports earlier investigators who noted that there are strains of high, low and non-virulence in each serotype. Factors other than serotype are important in the induction of disease in animal models (Takahashi *et al.*, 1985; Eamens, 1988).

Virulence Factors

Various *Erysipelothrix* spp. virulence factors have been suggested. The presence of a neuraminidase and a hyaluronidase was recognised early, and a correlation between the amount of neuraminidase produced and the virulence of strains was noted (Krasemann and Muller, 1975). Neuraminidase may serve as a key in the pathogenesis of arteritis and thrombocytopenia in rats experimentally infected with the organism. The adhesion of bacteria to a cultured cell line from the rat aorta was closely related to the release of sialic acid from endothelial cells. It is significant that more bacteria adhered to neuraminidase-treated endothelial cells and inhibition of bacterial adhesion by *N*-acetylneuraminlactose, a substrate of bacterial neuraminidase, was dose dependent. Bacterial invasion *in vivo* is always associated with desialated sites of arterial regions (Nakato *et al.*, 1986, 1987). Thus, it was suggested that *E. rhusiopathiae* neuraminidase plays a significant role in bacterial attachment. Hyaluronidase is a spreading factor that facilitates the dissemination of pathogens into tissues. It was detected in strains of *Erysipelothrix* spp. both virulent and avirulent for pigs (Norrung, 1970), and the role of hyaluronidase in the pathogenesis of the *Erysipelothrix* infection remains controversial.

The heat-labile capsule of *E. rhusiopathiae* is considered an important factor in the pathogenesis of infection. It is a major non-protein antigen with

molecular mass of 14–22 kDa. Tn916-generated mutants have been constructed to study the potential role of the capsule in virulence (Shimoji *et al.*, 1994). Capsule-deficient mutants are avirulent for mice. The virulent parent strain resisted phagocytosis by murine polymorphonuclear leucocytes, whereas all the capsule-deficient mutants were susceptible to phagocytosis. Intracellular survival mediated by the capsule was examined. Although the virulent Fujisawa-SmR strain of *E. rhusiopathiae* and its acapsular mutants were both ingested in the presence of normal serum, the number of ingested bacteria was 3–4-fold greater in the case of the acapsular mutants than in the case of the parent strain. These results are consistent with other studies that used macrophages for phagocytosis (Bohm and Suphasundha 1980; Bohm *et al.*, 1982). Thus, the virulence of *E. rhusiopathiae* is associated with a capsule, resistance to phagocytosis and intracellular survival.

Adhesion assays *in vitro* showed that strains of *E. rhusiopathiae* virulent for swine and mice adhere better to the porcine kidney cell lines PK-15 and ESK than do avirulent strains (Takahashi *et al.*, 1987b). Bratberg (1981) reported that *E. rhusiopathiae* isolated from swine with endocarditis or septicaemia showed a higher degree of adherence to fresh heart valves of swine in organ culture than did strains isolated from the various other sources. Adherence may therefore be an important determinant of virulence for *E. rhusiopathiae* in pigs. The role of this factor in human disease has not been further investigated.

Several surface proteins have been defined in *E. rhusiopathiae*. A 66–64 kDa antigen in Triton X-100 extracts of the organism is located on the cell surface (Galan and Timoney, 1990). This surface protein appears to play a role in virulence, because it is expressed less on strains of low or moderate virulence than on highly virulent strains, but its function has yet to be defined. Another surface protein is the SpaA antigen (Makino *et al.*, 1998). This is very similar in the structure and amino acid sequence of its C-terminal region to the choline-binding proteins of *Streptococcus pneumoniae*. This implies that SpaA, like the *Strep. pneumoniae* proteins, may play a role in the virulence of *E. rhusiopathiae*, but this role remains to be clarified. A gene that encodes the haemolysin of *E. rhusiopathiae* has been identified (Makino *et al.*, 1999). The gene encodes a surface protein with a molecular mass of 16 kDa that is commonly distributed amongst *Erysipelothrix* spp. It has been suggested that this surface protein is involved in haemolysis in all *Erysipelothrix* spp., but this has yet to be verified.

Epidemiology of *Erysipelothrix* Infections

E. rhusiopathiae and infections caused by it are worldwide in distribution, and affect a variety of animals, birds, marine and freshwater fish (Grieco and Sheldon, 1970; Wood, 1975). Human disease can originate from animal or environmental sources.

Swine erysipelas is seen in three different forms: an acute form characterised by sudden onset with death, a chronic form of infection with local arthritis or endocarditis, and a milder, subacute urticarial form characterised by purple diamond-shaped lesions on the skin (Grieco and Sheldon, 1970). Infection is economically detrimental to the pig industries of North America, Europe, Asia and Australia (Wood, 1992). Polyarthritis of sheep and lambs, and erysipelas in calves, ducks and domestic turkeys, are also economically significant diseases caused by *E. rhusiopathiae* (Wood, 1975; Conklin and Steele, 1979). In Australia, the organism is an emerging problem in farmed emus (Griffiths and Buller, 1991).

The domestic pig is the most important reservoir of *E. rhusiopathiae*. It has been estimated that 30–50% of healthy swine harbour the organism in their tonsils and other lymphoid tissues (Stephenson and Berman, 1978), but many of these strains may be *E. tonsillarum*. Carriers can discharge the organism in their faeces, urine, saliva and nasal secretions, to create an important source of infection. Soil, bedding, food and water can be contaminated by infected pigs leading to the indirect transmission of the organism. The more than 30 species of wild birds and at least 50 species of wild mammals (Shuman, 1971; Wood and Shuman, 1981) known to harbour *E. rhusiopathiae* provide a further extensive reservoir. The organism can survive for long periods in marine environments. It inhabits and grows on the exterior mucoid slime of fish, without causing disease in fish themselves (Wood, 1975). The slime on fish appears to be an important source of infection for humans. Although the organism has been isolated from the environment, this appears to be secondary in importance to animal reservoirs as a source of *E. rhusiopathiae*. It was long believed that the organism could live in soil indefinitely, but Wood (1973) found that with various changes in temperature, pH, moisture content, and organic content it survives for a maximum of only 35 days in soil.

E. rhusiopathiae infection in humans is related to occupation. It occurs mostly in those whose jobs are closely associated with contaminated animals, their product or wastes, or soil. Those with the highest risk of exposure include butchers, abattoir workers,

veterinarians, farmers, fishermen, fish handlers and housewives (Reboli and Farrar, 1989). The common names for human infection, including 'whale finger', 'seal finger', 'speck finger', 'blubber finger', 'fish poisoning', 'fish handler's disease', and 'pork finger' reflect this occupational mode of acquisition. Infection is initiated either by an injury to the skin with infective material or when a previous injury is contaminated (Wood, 1975).

The clinical manifestations of human infection closely parallel those seen in swine, with erysipeloid the most common form of human infection. This is an acute localised cutaneous infection that usually occurs on the hand or fingers and is described as a local cellulitis. The lesion consists of a well-defined, slightly elevated, violaceous zone the peripheral edge of which spreads as the centre fades. Pain is often severe and has been described as a burning, throbbing, or itching sensation. Systemic symptoms can occur in some cases, including fever, joint aches, lymphadenitis and lymphadenopathy. The disease is self-limiting and usually resolves in 3–4 weeks without therapy (Reboli and Farrar, 1992), but recent reports of fatal or near-fatal infections emphasise the importance of accurate diagnosis (Robson *et al.*, 1998; Dunbar and Clarridge, 2000).

The generalised cutaneous form of the disease caused by *E. rhusiopathiae* involves lesions that progress from the initial site to other locations on the body or appear at remote areas (Klauder, 1944). The lesions are similar to those of the localised form, but bullous lesions can occur also, and systemic symptoms are more frequent than in the localised form. The clinical course is more protracted and recurrences are common (Klauder, 1938).

Septicaemia is a more serious manifestation of *E. rhusiopathiae* infection and is always linked to endocarditis. In 15 years 50 cases with systemic infection have been reported, with an extremely high (90%) incidence of endocarditis (Reboli and Farrar, 1992). Gorby and Peacock (1988) found a high male-to-female ratio among patients with *E. rhusiopathiae* endocarditis, which may reflect occupational exposure, and the mortality was 38%, which is almost double the rate for endocarditis caused by other bacteria. Nearly 60% of patients had normal heart valves before they were affected. Congestive heart failure was the most common complication of endocarditis and was present in 80% of patients. Alcohol abuse and lose of immunocompetence were believed to be significant risk factors.

Other infections associated with *E. rhusiopathiae* include chronic arthritis (Ehrlich, 1946), cerebral infection (Silberstein, 1965) and necrosis of bone (Klauder, 1944). Recently, some new manifestations of infection have been reported. These include erysipeloid with coexisting orf (contagious pustular dermatitis of sheep) (Connor and Green, 1995), persistent bacteraemia in a hospitalised patient (Schuster *et al.*, 1993), bacteraemia in an HIV-positive patient (Hollick and Edinger, 1995), endocarditis with acute renal failure (Gimenez *et al.*, 1996), septicaemia and lupus nephritis (Totemchokchyakarn *et al.*, 1996), and septicaemia in a neonate (Jones and Khoosal, 1997). *E. rhusiopathiae* has also been implicated in an occupational infection known as 'crayfish poisoning' which affects lobster fishermen in Western Australia and bears a clinical resemblance to erysipeloid (Brooke *et al.*, 1999). A direct causal relationship between *E. rhusiopathiae* and 'crayfish poisoning' has not been proved, because of the presence of other potential pathogens, but the results were suggestive.

Treatment and Prevention

Antibiotic susceptibility data for *Erysipelothrix* are still limited (Reboli and Farrar, 1992), in spite of recent reports on the subject (Takahashi *et al.*, 1984, 1987c; Venditti *et al.*, 1990). *Erysipelothrix* is highly susceptible to penicillin, cephalosporins and clindamycin (Takahashi *et al.*, 1984; Gorby and Peacock, 1988). Most strains are resistant to aminoglycosides, trimethoprim–sulphamethoxazole, polymyxins, sulphonamides, streptomycin, novobiocin and vancomycin. The organism is variably susceptible to chloramphenicol, tetracyclines and erythromycin (Reboli and Farrar, 1992).

Erysipeloid can be effectively treated with oral penicillin (Reboli and Farrar, 1992). Although infection is usually self-limiting, relapses and progression to more serious forms are possible. Oral penicillin will resolve a case of erysipeloid in around 48 hours, but intravenous penicillin is recommended for more serious *E. rhusiopathiae* infections (Grieco and Sheldon, 1970). Although the mortality rate for endocarditis has been reduced from the 100% seen in the pre-antibiotic era, it is still 38% in spite of available treatment (Gorby and Peacock, 1988). This rate may partly be explained by the use of vancomycin (to which the organism is resistant) in the empirical therapy of endocarditis (Venditti *et al.*, 1990). Early diagnosis of all forms of *E. rhusiopathiae* infection is therefore essential (Grieco and Sheldon, 1970). In those allergic to penicillin, cephalosporins have been described as the most appropriate alternative, since clindamycin and erythromycin are only bacteriostatic for *E. rhusiopathiae* (Reboli and Farrar, 1992).

Before the advent of penicillin therapy, some alleviation of symptoms could be achieved with hyperimmune serum, but the resulting serum sickness was often more severe than an episode of erysipeloid, and this treatment was shown to be of little value for cutaneous infections (Grieco and Sheldon, 1970). Commercial vaccines in the form of bacterins, lysates or live attenuated strains of *E. rhusiopathiae* serotype 2 offer protection to pigs and turkeys (Groscup and Timoney, 1990). Vaccination of humans is not a viable option because clinical erysipeloid appears to convey little or no immunity (Proctor and Richardson, 1954), as is shown by relapse of infection.

Resistance of *E. rhusiopathiae* to penicillin has not been recorded. One experiment involving serial passage of 75 strains for 8 months did not produce resistance (Grieco and Sheldon, 1970). Though plasmids were not found in *E. rhusiopathiae* strains in early investigations (Takahashi *et al.*, 1984; Venditti *et al.*, 1990), they were found in later studies (Noguchi *et al.*, 1993). Plasmids do not appear to play a critical role in *E. rhusiopathiae* antibiotic resistance. Antibiotics contained in animal feed may have a role in the resistance of some strains of *E. rhusiopathiae*, but the mechanism remains uncertain (Takahashi *et al.*, 1987c).

Containment and control of *E. rhusiopathiae* are far more effective in preventing the spread of infection in humans and animals. For individuals working in at-risk occupations, an awareness of the infection is essential. Suggested preventative measures include the wearing of gloves or other protective handwear, good hygiene, especially frequent hand-washing with disinfectant soap and the prompt treatment of any small injuries (Wood, 1975). Good health is considered to be an important factor in prevention, since poor health, including alcoholism, may predispose to more serious forms of infection (McCarty and Bornstein, 1960; Gorby and Peacock, 1988). Control of animal disease by sound husbandry, herd management, good sanitation and immunisation procedures is recommended (Wood, 1992).

The removal or regular disinfecting of contaminated sources is an important method of limiting the spread of the organism throughout a work environment (Wood, 1975). *E. rhusiopathiae* can be killed by commonly available disinfectants (Conklin and Steele, 1979). Many investigators have noted, however, that structurally complex equipment is difficult to clean and, because the organism is able to survive in organic matter, disinfecting without cleaning is useless (Spencer, 1959; Mutalib *et al.*, 1993). If disinfection is impractical, other control measures become more important.

Control of reservoir populations of *E. rhusiopathiae* is impractical or impossible, because of the widespread distribution of the organism, the large variety of animal hosts, and its ability to persist in the environment. The possibility of human infection can, however, be lessened with awareness, safe work practices and sensible precautions.

References

Ahrne S, Tenstrom IM, Jensen NE *et al.* (1995) Classification of *Erysipelothrix* strains on the basis of restriction fragment length polymorphisms. *Int. J. Syst. Bacteriol.* 45: 382–385.

Barber M (1948) Discussion on swine erysipelas infection (*Erysipelothrix rhusiopathiae*) in man and animals. *Proc. R. Soc. Med.* 41: 328–330.

Bohm KH, Suphasindhu V (1980) Investigations on phagocytosis of erysipelas bacteria by neutrophil leukocytes with special reference to virulence. In: Nielsen NC, Hogh P, Bille N (eds) *Proceedings of the International Pig Veterinary Society Congress, Copenhagen*, p. 197.

Bohm KH, Soliman R, Leibold W (1982) Interactions between erysipelas bacteria and swine macrophages with special reference to virulence. In: Necoechaea RR, Pijioan C, Cesarin A, Guzman M (eds) *Proceedings of the International Pig Veterinary Society Congress, Mexico City*, p. 146.

Bratberg AM (1981) Selective adherence of *Erysipelothrix rhusiopathiae* to heart valves of swine investigated in an *in vitro* test. *Acta. Vet. Scand.* 22: 39–45.

Brooke CJ, Mclaughlin V, Mee BJ, Riley TV (1999) An investigation of 'crayfish poisoning' in Western Australia. *Med. J. Aust.* 170: 288.

Chooromoney KN, Hampson DJ, Eamens GJ, Turner MJ (1994) Analysis of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* by multilocus enzyme electrophoresis. *J. Clin. Microbiol.* 32: 371–376.

Conklin RH, Steele JH (1979) *Erysipelothrix* infections. In: Steele JH (ed.) *CRC Handbook. Series in Zoonoses*, Vol. 1, (section A). Boca Raton, FL: CRC Press, pp. 327–337.

Connor MP, Green AD (1995) Erysipeloid infection in a sheep farmer with coexisting orf. *J. Infect.* 30: 161–163.

Dunbar SA, Clarridge JE (2000) Potential errors in recognition of *Erysipelothrix rhusiopathiae*. *J. Clin. Microbiol.* 38: 1302–1304.

Eamens GJ (1988) Pathogenicity of field isolates of *Erysipelothrix rhusiopathiae* in mice, rats and pigs. *Aust. Vet. J.* 65: 280–284.

Ehrlich JC (1946) *Erysipelothrix rhusiopathiae* infection in man. *Arch. Intern. Med.* 78: 565–577.

Ewald FW (1981) The genus *Erysipelothrix*. In: Starr MP, Stolp H, Truper HG, Balows A, Schlegel HG (eds) *The Prokaryotes*. New York: Springer Verlag, pp. 1688–1700.

Fidalgo SG, Wang Q, Riley TV (2000) Comparison of methods for detection of *Erysipelothrix* spp. and their

distribution in some Australasian seafoods. *Appl. Environ. Microbiol.* 66: 2066–2070.

Galan JE, Timoney JF (1990) Cloning and expression in *Escherichia coli* of a protective antigen of *Erysipelothrix rhusiopathiae*. *Infect. Immun.* 58: 3116–3121.

Giménez M, Fernández P, Padilla E, Matas L, Ausina V (1996) Endocarditis and acute renal failure due to *Erysipelothrix rhusiopathiae* (letter). *Eur. J. Clin. Microbiol. Infect. Dis.* 15: 347–348.

Gorby GL, Peacock JE (1988) *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Rev. Infect. Dis.* 10: 317–325.

Grieco MH, Sheldon C (1970) *Erysipelothrix rhusiopathiae*. *Ann. N. Y. Acad. Sci.* 174: 523–532.

Griffiths GL, Buller N (1991) *Erysipelothrix rhusiopathiae* infection in semi-intensively farmed emus. *Aust. Vet. J.* 68: 121–122.

Groscup MH, Timoney JF (1990) Modified Feist broth as a serum-free alternative for enhanced production of protective antigen of *Erysipelothrix rhusiopathiae*. *J. Clin. Microbiol.* 28: 2573–2575.

Harrington R Jr, Hulse DC (1971) Comparison of two plating media for the isolation of *Erysipelothrix rhusiopathiae* from enrichment broth culture. *Appl. Microbiol.* 22: 141–142.

Hillenbrand FKM (1953) Whale finger and seal finger: their relation to erysipeloid. *Lancet* i: 680–681.

Hollick GE, Edinger R (1995) *Erysipelothrix* bacteraemia. *Clin. Microbiol. Newsletter* 17: 9–16.

Hunter D (1975) *The Diseases of Occupations*, 5th edn., pp. 709–712.

Jones D (1986) Genus *Erysipelothrix Rosenbach* 367[al]. In: Sneath PH, Mair NS, Sharpe ME (eds) *Bergey's Manual of Systematic Bacteriology*, Vol. 2. Baltimore, MD: Williams & Wilkins, pp. 1245–1249.

Jones N, Khoosal M (1997) *Erysipelothrix rhusiopathiae* septicemia in a neonate. *Clin. Infect. Dis.* 24: 511.

Kalf GF, White TG (1963) The antigenic components of *Erysipelothrix rhusiopathiae*. II. Purification and chemical characterization of a type-specific antigen. *Arch. Biochem. Biophys.* 102: 39–47.

Klauder JV (1938) Erysipeloid as an occupational disease. *JAMA* 111: 1345–1348.

Klauder JV (1944) *Erysipelothrix rhusiopathiae* infection in swine and in human beings: comparative study of the cutaneous lesions. *Arch. Dermatol. Syphilol.* 50: 151–159.

Krasemann C, Muller HE (1975) The virulence of *Erysipelothrix rhusiopathiae* strains and their neuraminidase production (author's transl). *Zbl. Bakteriol. Parasitenkunde. Infektionskr Hygiene Erste Abt Orig Reihe A: Med. Mikrobiol. Parasitol.* 231: 206–213.

Kucsera G (1973) Proposal for standardisation of the designations used for serotypes of *Erysipelothrix rhusiopathiae* (Migula) Buchanan. *Int. J. Syst. Bacteriol.* 23: 184–188.

Kucsera G (1979) Studies on the nature of the type specific antigen of *Erysipelothrix rhusiopathiae*. *Acta. Vet. Acad. Sci. Hung.* 27: 375–380.

Makino S, Okada Y, Maruyama T *et al.* (1994) Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J. Clin. Microbiol.* 32: 1526–1531.

Makino S, Yamamoto K, Murakami S *et al.* (1998) Properties of repeat domain found in a novel protective antigen, SpaA, of *Erysipelothrix rhusiopathiae*. *Microb. Pathog.* 25: 101–109.

Makino S, Katsuta K, Shirahata T (1999) A novel protein of *Erysipelothrix rhusiopathiae* that confers haemolytic activity on *Escherichia coli*. *Microbiology* 145: 1369–1374.

McCarty D, Bornstein S (1960) *Erysipelothrix* endocarditis. *Am. J. Clin. Pathol.* 33: 820–824.

Muller HE, Krasemann C (1976) Immunity against *Erysipelothrix rhusiopathiae* infection by means of active immunization using homologous neuraminidase (author's transl). *Z. Immunitatsforschung. Exp. Klin. Immunol.* 151: 237–241.

Mutalib AA, King JM, McDonough PL (1993) Erysipelas in caged laying chickens and suspected erysipeloid in animal caretakers. *J. Vet. Diagn. Invest.* 5: 198–201.

Nakato H, Shinomiya K, Mikawa H (1986) Possible role of neuraminidase in the pathogenesis of arteritis and thrombocytopenia induced in rats by *Erysipelothrix rhusiopathiae*. *Pathol. Res. Pract.* 181: 311–319.

Nakato H, Shinomiya K, Mikawa H (1987) Adhesion of *Erysipelothrix rhusiopathiae* to cultured rat aortic endothelial cells. Role of bacterial neuraminidase in the induction of arteritis. *Pathology Res. Pract.* 182: 255–260.

Noguchi N, Sasatsu M, Takahashi T, Ohmae K, Terakado N (1993) Detection of plasmid DNA in *Erysipelothrix rhusiopathiae* isolated from pigs with chronic swine erysipelas. *J. Vet. Med. Sci.* 55: 349–350.

Norrung V (1970) Studies on *Erysipelothrix insidiosa S. rhusiopathiae*. 1. Morphology, cultural features, biochemical reactions and virulence. *Acta. Vet. Scand.* 11: 577–585.

Norrung V, Molin G (1991) A new serotype of *Erysipelothrix rhusiopathiae* isolated from pig slurry. *Acta. Vet. Hung.* 39: 137–138.

Packer RA (1943) The use of sodium azide and crystal violet in a selective medium for *streptococci* and *Erysipelothrix rhusiopathiae*. *J. Bacteriol.* 46: 343–349.

Proctor DM, Richardson IM (1954) A report on 235 cases of erysipeloid in Aberdeen. *Br. J. Ind. Med.* 11: 175–179.

Reboli AC, Farrar WE (1989) Erysipelothrix rhusiopathiae: an occupational pathogen. *Clin. Microbiol. Rev.* 2: 354–359.

Reboli AC, Farrar WE (1992) The genus *Erysipelothrix*. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer K (eds) *The Prokaryotes*. New York: Springer-Verlag, pp. 1629–1642.

Robson JM, McDougall R, Valk S van der, Waite SD, Sullivan JJ (1998) *Erysipelothrix rhusiopathiae*: an uncommon but ever present zoonosis. *Pathology* 30: 391–394.

Schuster MG, Brennan PJ, Edelstein P (1993) Persistent bacteremia with *Erysipelothrix rhusiopathiae* in a hospitalized patient. *Clin. Infect. Dis.* 17: 783–784.

Shimoji Y, Yokomizo Y, Sekizaki T, Mori Y, Kubo M (1994) Presence of a capsule in *Erysipelothrix rhusiopathiae* and its relationship to virulence for mice. *Infect. Immun.* 62: 2806–2810.

Shimoji Y, Mori Y, Hyakutake K, Sekizaki T, Yokomizo Y (1998) Use of an enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. *J. Clin. Microbiol.* 36: 86–89.

Shuman RD (1971) *Erysipelothrix.* In: Davis JW, Anderson RC, Karstad LH, Trainer DO (eds) *Infectious and Parasitic Diseases of Wild Birds.* Ames: University of Iowa Press , pp. 141–145.

Silberstein EB (1965) *Erysipelothrix* endocarditis: report of a case with cerebral manifestations. *JAMA* 191: 862–864.

Sneath PHA, Abbott JD, Cunliffe AC (1951) The bacteriology of erysipeloid. *BMJ* 2: 1063–1066.

Soto A, Zapardiel J, Soriano F (1994) Evaluation of API Coryne system for identifying coryneform bacteria. *J. Clin. Pathol.* 47: 756–759.

Spencer R (1959) The sanitation of fish boxes. I. The quantitative and qualitative bacteriology of commercial wooden fish boxes. *J. Appl. Bacteriol.* 22: 73–84.

Stephenson EH, Berman DT (1978) Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *Am. J. Vet. Res.* 39: 187–188.

Takahashi T, Sawada T, Ohmae K *et al.* (1984) Antibiotic resistance of *Erysipelothrix rhusiopathiae* isolated from pigs with chronic swine erysipelas. *Antimicrob. Agents. Chemother.* 25: 385–386.

Takahashi T, Sawada T, Seto K *et al.* (1985) Pathogenicity of *Erysipelothrix rhusiopathiae* strains of serovars 1a, 3, 5, 6, 8, 11, 21, and type N isolated from slaughter pigs affected with chronic erysipelas. *Jap. J. Vet. Sci.* 47: 1–8.

Takahashi T, Fujisawa T, Benno Y *et al.* (1987a) *Erysipelothrix tonsillarum* sp. nov. isolated from tonsils of apparently healthy pigs. *Int. J. Syst. Bacteriol.* 37: 166–168.

Takahashi T, Hirayama N, Sawada T, Tamura Y, Muramatsu M (1987b) Correlation between adherence of *Erysipelothrix rhusiopathiae* strains of serovar 1a to tissue culture cells originated from porcine kidney and their pathogenicity in mice and swine. *Vet. Microbiol.* 13: 57–64.

Takahashi T, Sawada T, Muramatsu M *et al.* (1987c) Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from the tonsils of apparently healthy slaughter pigs. *J. Clin. Microbiol.* 25: 536–539.

Takahashi T, Fujisawa T, Tamura Y *et al.* (1992) DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum. Int. J. Syst. Bacteriol.* 42: 469–473.

Takeshi K, Makino S, Ikeda T *et al.* (1999) Direct and rapid detection of *Erysipelothrix* sp. DNAs prepared from bacterial strains and animal tissues. *J. Clin. Microbiol.* 37: 4093–4098.

Tamura Y, Takahashi T, Zarkasie K, Nakamura M, Yoshimura H (1993) Differentiation of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* by sodium dodecyl sulphate-polyacrylamide gel electrophoresis of cell proteins. *Int. J. Syst. Bacteriol.* 43: 111–114.

Totemchokchyakarn K, Janwityanujit S, Sathapatayavongs B, Puavilai S (1996) *Erysipelothrix rhusiopathiae* septicemia in systemic lupus erythematosus. *Int. J. Dermatol.* 35: 818–820.

Venditti M, Gelfusa V, Tarasi A, Brandimarte C, Serra P (1990) Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae. Antimicrob. Agents. Chemother.* 34: 2038–2040.

Vickers CL, Bierer BW (1958) Triple sugar iron agar as an aid in the diagnosis of erysipelas. *J. Am. Vet. Med. Assoc.* 12: 543–544.

Watts PS (1940) Studies on *Erysipelothrix rhusiopathiae. J. Pathol. Bacteriol.* 50: 355–369.

Wood RL (1965) A selective liquid medium utilizing antibiotics for isolation of *Erysipelothrix insidiosa. Am. J. Vet. Res.* 26: 1303–1308.

Wood RL (1973) Survival of *Erysipelothrix rhusiopathiae* in soil under various environmental conditions. *Cornell Vet.* 63: 390–410.

Wood RL (1975) Erysipelothrix infection. In: Hubbert WT, McCullough WF, Schnurrenberger PR (eds) *Diseases Transmitted from Animals to Man,* 6th edn. Springfield, IL: Thomas, pp. 271–281.

Wood RL (1992) Erysipelas. In: Leman AD, Straw BE, Mengeling WL, D'Allaire S, Taylor DJ (eds) *Diseases of Swine,* 7th edn. Ames: Iowa State University Press, pp. 475–486.

Wood RL, Harrington R Jr (1978) Serotypes of *Erysipelothrix rhusiopathiae* isolated from swine and from soil and manure of swine pens in the United States. *Am. J. Vet. Res.* 39: 1833–1840.

Wood RL, Shuman RD (1981) *Erysipelothrix* infection. In: Davis JW, Karstad LH, Trainer DO (eds) *Infectious Diseases of Wild Mammals,* 2nd edn. Ames: Iowa State University Press.

Detection of *Erysipelothrix rhusiopathiae* in clinical and environmental samples

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Running head: Detection of *Erysipelothrix* spp.

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1. Introduction

Erysipelothrix rhusiopathiae is pathogenic for both animals and humans, causing erysipelas in swine and erysipeloid in humans ***(1)***. In swine, disease may be either acute or chronic, and this results in the development of arthritis and endocarditis ***(2)***. In Japan, erysipelas remains an animal hygiene problem which results in great economic loss as infected swine are disused ***(3)***. Human infection closely resembles that seen in swine, with both acute and chronic forms also. The most common presentation is erysipeloid, a localised cutaneous infection ***(4)***. In Western Australia, an erysipeloid-like infection, referred to as "crayfish poisoning", occurs in lobster fishermen and handlers ***(5)***. A second type of presentation is a generalised cutaneous form, that involves lesions that progress from the initial site of infection or appear at remote areas ***(6)***. The third and most serious form of disease is a septicaemia which is almost always linked to endocarditis ***(4)***. The mortality rate in *Erysipelothrix* endocarditis is still high (38%) ***(7)*** and can be explained by the use of vancomycin, to which *Erysipelothrix* spp. are inherently resistant, as empirical therapy. Therefore, it is critical to have an early diagnosis of *E. rhusiopathiae* infection ***(8)***.

Unfortunately, several problems exist with the diagnosis of *E. rhusiopathiae* infections by conventional cultural procedures and these infections are often incorrectly diagnosed. Firstly, due to their very small colony size and slow growth rates, it is difficult to isolate *E. rhusiopathiae* from heavily contaminated specimens ***(4)***. Various selective media have been described to improve the isolation of *E. rhusiopathiae* from contaminated specimens, however, not all contaminants are inhibited. The development of two PCR methods has created an opportunity to greatly improve the efficiency with which these organisms are detected and identified. Makino *et al.* ***(9)*** designed a PCR method that amplifies a 407 bp

DNA fragment derived from the 16S rRNA coding sequence. The primers in this method are specific for the genus *Erysipelothrix*, and do not differentiate between the species ***(9).*** A second set of primers designed by Shimoji *et al.* ***(10)*** amplifies a 937 bp DNA fragment which is derived from a sequence associated with virulence of *E. rhusiopathiae*. These primers are specific for *E. rhusiopathiae* only. Shimoji *et al.* ***(10)*** also utilised a selective enrichment medium based on tryptic soy broth containing ethidium bromide and sodium azide. More recently, a third PCR method, which differentiates between four different species of *Erysipelothrix*, has been publisheded ***(11)***.

We recently undertook a survey of seafood where various methodologies for detection of *Erysipelothrix* spp. were evaluated ***(12).*** The tryptic soy broth used by Shimoji *et al.* ***(10),*** with 48 h incubation followed by a double round PCR, was the best method for the detection of *Erysipelothrix* spp. With some modifications to the two PCR methods designed by Makino *et al.* ***(9)*** and Shimoji *et al.* ***(10),*** and with the use of selective tryptic soy broth for enrichment, this chapter presents a method for the rapid detection of *E. rhusiopathiae* in both clinical and environmental samples.

2. Materials

2.1. Preparation of sample

1. Trypticase soy broth (TSB) (pH 7.6) (Becton Dickinson & Co., Cockeysville, Md.) supplemented and made selective with: 0.3% Tris, 0.1% Tween 80, 0.03% sodium azide and 5 μg/ml crystal violet (TSB/S).
2. M^{c}Cartney bottles.
3. 1.5 ml microcentrifuge tubes.

2.2. Preparation of PCR master mixes and amplification

1. 0.2 ml micro-tubes (Certified DNase/RNase free).

2. Openers for 0.2 ml micro-tubes.

3. Micro-tube racks.

4. Sterile diethylpyrocarbonate (DEPC) treated water: add 400 µl of diethylpyrocarbonate to 400 ml deionised water that has been filtered through a MilliQ filter system. Shake thoroughly, incubate at 37°C overnight, then autoclave at 15ibs/sq for 60 min in electric steriliser.

5. Thermal cycler (Perkin Elmer 9600 suggested).

6. 10 x PCR buffer II (100 mM Tris-HCl, pH 8.3 and 500 mM KCl) (Applied Biosystems, New Jersey, USA).

7. 25 mM $MgCl_2$ (Appled Biosystems).

8. 5 U/µl Ampli*Taq* Gold™ DNA polymerase (Appled Biosystems).

9. Deoxyribonucleoside triphosphate (dNTP) mix (25 mM of each dATP, dCTP, dGTP, dTTP) (Fisher Biotec).

10. Primer sets: see Table for sequences (Gibco BRL, Paisley, UK); for use make up 10 µM stocks in DEPC water and store at -20°C.

2.3. Analysis of PCR products

1. Electrophoresis gel tank, tray and comb.

2. Electrophoresis power supply capable of 200 V.

3. DNA Grade agarose (DNase and RNase free).

4. Ethidium bromide (10 mg/ml): add 1g ethidium bromide to 100ml of distilled water. Stir on magnetic stirrer for several hours, wrap the container in aluminium foil and store at 2 – 8°C.

5. 50 x TAE buffer: add 121g of Tris base, 23.6 ml glacial ascetic acid, 50 ml 0.5M EDTA pH 8.0. Dissolve and make up to 500 ml with deionised water.

6. Electrophoresis buffer: add 40 ml 50 x TAE buffer and 100 µl ethidium bromide stock (10 mg/ml) to 1960 ml deionised water. Store at 2 – 8°C.

7. 6 x loading dye: add 1g bromophenol blue and 160g sucrose to 400 ml deionised water. Mix and store at 2 – 8°C.

8. TE buffer pH 8.0: 10mM Tris buffer pH 8.0, 1 mM EDTA pH 8.0.

9. DNA Molecular Weight V III marker (Boehringer GmbH, Mannheim, Germany): for use, dilute 1:10 with TE buffer.

10. UV transilluminator.

11. Polaroid camera.

Table - Primers used for PCR detection of *Erysipelothrix* spp. and *E. rhusiopathiae.*

Primer	**Nucleotide sequence 5' →3'**	**Length of product (bp)**	**Detection**
MOIO1 [b]	AGATGCCATAGAAACTGGTA	407	*Erysipelothrix* sp
MOIO2 [b]	CTGTATCCGCCATAACTA		
ER1 [c]	CGATTATATTCTTAGCACGCACGCAACG	937	*E. rhusiopathiae*
ER2 [c]	TGCTTGTGTTGTGATTTCTTGACG		

[a] Primer sequences for MOIO1 & 2 and ER1 & 2 from references 6 and 8, respectively.

[b]GenBank/EMBL accession no. M23728

[c] GenBank/EMBL accession no. D64177

3. Methods (*see* Notes 1 and 2)

3.1. Preparation of PCR Master Mixes (*see* Note 3)

Volumes given are based on 16 µl and 20 µl per reaction mixture for first and second round mix tubes respectively. Thaw out the following reagents and keep them on ice at all times: 10 x PCR buffer II, 25 mM $MgCl_2$, 5 U/µl Ampli*Taq* Gold™ DNA polymerase, 100mM dNTP mix and 10 µM stocks of primers (MOIO1 & 2 and ER1 & 2).

3.2. Erysipelothrix *spp. Master Mixes*

1. Using aseptic techniques, add the following volumes of each reaction component to a sterile M°Cartney bottle to make 100 first round mix tubes: 1134 µl of DEPC water, 200 µl of 10 x PCR buffer II, 160 µl of 25 mM $MgCl_2$, 16 µl of dNTP pool, 10 µl of 5U/µl Ampli*Taq* Gold™ DNA polymerase, 40 µl of 10 µM MOIO1 & 2 primers.

2. To make 100 second round mix tubes, add the following volumes: 1527 µl of DEPC water, 204 µl of 10 x PCR buffer II, 163.2 µl of 25 mM $MgCl_2$, 16.3 µl of dNTP pool, 10 µl of 5U/µl Ampli*Taq* Gold™ DNA polymerase, 40.8 µl of 10 µM MOIO1 & 2 primers.

3. Use the vortex mixer and gently but thoroughly mix the contents.

4. Place rows of sterile 0.2 ml micro-tubes into a micro-tube rack with the lids open.

5. Dispense 16 µl and 20 µl of the reaction mixtures into the micro-tubes for the first and second round mix tubes, respectively.

6. Colour code the tips of the micro-tubes for first and second round, and make sure the lids are placed on firmly.

7. Place the rack with the micro-tubes in the -70°C freezer for 1 hour.

8. Once the reaction mixtures are frozen they can be removed from the rack and stored in boxes in the -70°C freezer till later required.

3.3. E. rhusiopathiae *Master Mixes*

1. Add the following volumes to a McCartney bottle for 100 first round mix tubes: 1174 µl of DEPC water, 200 µl of 10 x PCR buffer II, 120 µl of 25 mM $MgCl_2$, 16 µl of dNTP pool, 10 µl of 5U/µl Ampli*Taq* Gold™ DNA polymerase, 40 µl of 10 µM MOIO1 & 2 primers.

2. For 100 second round mix tubes, add the following volumes to another McCartney bottle: 1565.7 µl of DEPC water, 204 µl of 10 x PCR buffer II, 122.4 µl of 25 mM $MgCl_2$, 16.3 µl of dNTP pool, 10 µl of 5U/µl Ampli*Taq* Gold™ DNA polymerase, 40.8 µl of 10 µM MOIO1 & 2 primers.

3. Follow the same procedure as for *Erysipelothrix* master mixes from step 3.

3.4. *Preparation of samples* (*see* **Note 4**)

1.Dispense 10 ml sterile TSB/S into a McCartney bottle and inoculate organism to be tested.

2. Use the vortex mixer to thoroughly mix the contents.

3. Incubate the broth for 48 h at 37°C.

4. Following the 48 h incubation, vortex the broth culture vigorously.

5. With a sterile plastic transfer pipette remove 1.5 ml of the culture and transfer it to a sterile micro-centrifuge tube.

6. Centrifuge the culture at 10 000 x g for 3 min to pellet the suspension.

7. Remove the supernatant and add 1.5 ml DEPC water to the pellet.

8. Wash the pellet by vortexing it into suspension.

9. Centrifuge the suspension, once again at 10 000 x g for 3 min.

10. Remove the supernatant and resuspend the pellet in 100 µl DEPC water.

3.5. Preparation of DNA Template

1. With the 100 µl suspension boil them at 100°C for 15 min.

2. Pellet the debris at 10 000 x g for 3 min.

3. Transfer the supernatant containing the DNA to a sterile microcentrifuge tube.

4. Store the microcentrifuge tube containing the DNA extract in the -70°C freezer till later required.

3.6. PCR Amplification (*see* **Note 5**)

Inoculation of the mix tubes should be conducted in a laminar flow cabinet if possible, if not in a separate area or laboratory from where the mix tubes were prepared. Plastic sleeves should be worn whilst working in the cabinet. Whilst inoculating the second round mix tubes from the first, do not open them by hand, use the openers at all times.

3.6.1. First Round Amplification

1. Thaw out the DNA extract, first round *Erysipelothrix* and *E. rhusiopathiae* mix tubes, and then place them on ice.

2. Label mix tubes according to samples tested.

3. In a laminar flow cabinet, vortex the DNA extract and add 4 µl to the corresponding *Erysipelothrix* and *E. rhusiopathiae* mix tubes.

4. Place the lid firmly on the micro-tubes and gently mix the contents (*see* **Note 6**).

5. Place the *Erysipelothrix* mix tubes in the thermal cycler and the *E. rhusiopathiae* mix tubes on ice.

6. For the *Erysipelothrix* mix tubes amplify the reaction by using the following cycle parameters: 94°C for 15 min and then 45 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 72°C for 45 s, followed by an additional extension step at 72°C for 7 min and cooling to 4°C.

7. Once the *Erysipelothrix* mix tubes amplification is completed place them in the fridge.

8. Gently mix the contents of the *E. rhusiopathiae* mix tubes and place them in the same thermal cycler.

9. For the *E. rhusiopathiae* mix tubes amplify the reaction by using the following cycle parameters: heating at 94°C for 5 min and then 30 cycles of denaturation at 94°C for 1 min, annealing at 63°C for 30 s and extension at 72°C for 1 min, followed by an additional extension step at 72°C for 7 min and cooling to 4°C.

10. Following amplification also place the *E. rhusiopathiae* mix tubes in the fridge.

3.6.2. Double (Second Round) Amplification

1. Place the second round mix tubes on a rack in line with the first round mix tubes.

2. Place all the equipment that is required in the cabinet and then put on plastic sleeves.

3. Use a micro-tube opener to open the first round mix tube.

4. Remove 0.4 µl from the first round mix tube.

5. Use a clean opener to open the second round mix tube.

6. Inoculate the 0.4 µl from the first round into the second round mix tube.

7. Remove the second round mix tubes from the block, gently mix the contents and place them in the thermal cycler.

8. Then amplify the second round mix tubes using the same cycle parameters as for first round for both *Erysipelothrix* and *E. rhusiopathiae* mix tubes.

9. Remove the samples from the thermal cycler and store them at 4°C.

3.7. Analysis of PCR Products

1. Prepare a 2% agarose gel (w/v) by adding 1.75 g of agarose to 70 ml deionised water, plus 1.4 ml 50 x TAE buffer and 3.5 µl ethidium bromide (10 mg/ml).

2. Once the gel is set, place it in the tank with the electrophoresis buffer just covering the surface of the gel.

3. Add 4 µl of the loading dye into each sample, gently resuspend and load 12 µl of the samples into the well. Also load the molecular weight markers.

4. When loading is complete, place the lid on the tank and plug into the power pack.

5. Run the gel at a constant voltage of 100 - 120 V till the dye front approaches the edge of the gel.

6. Switch off the power pack and remove the gel tray.

7. Place on the UV eye shield.

8. Place the gel on the UV transilluminator and switch on.

9. Visualise and photograph the products (*see* **Note 7**).

4. Notes

1. Each section of the methods should be performed either in separate laboratories or in different areas to avoid contamination. Preparation of the master mixes should be carried out in a specimen free laboratory. Inoculation of the first and second round mix tubes should be performed in separate laminar flow cabinets to prevent cross contamination. The analysis of the PCR products should also be performed in a separate area or laboratory.
2. Gloves should be worn at all times to avoid contamination with DNAses and RNAses.
3. When preparing the master mixes, overestimating the final required volume by one or two reactions to allow for volume losses and pipetting errors is recommended. Also, make a minimum batch of 25 reaction mixtures for first and second round. Large batch numbers are recommended as very small volumes of the primers are used.
4. It is recommended that the following be used as positive and negative controls. *E. rhusiopathiae* ATCC 19414 can be used as the positive control for both PCR methods,

and *E. tonsillarum* ATCC 43339 can be used as the negative control for the *E. rhusiopathiae* specific PCR.

5. A DEPC water negative control should be used for both PCR methods.
6. Make sure the lids on the centrifuge and microcentrifuge tubes are placed on firmly to prevent evaporation of the suspension during boiling or PCR amplification.
7. Samples should be tested twice on separate days to check for reproducibility. If discrepant results are obtained, then the analysis should be repeated. If the third PCR is positive, then the overall results are considered positive. If the third PCR was negative, then the overall result is considered negative.

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References

1. Wood, R. L., and Shuman, R. D. (1975) Swine erysipelas, in: Diseases of swine, (Scholl. E, and Leman. A. D.) Iowa State University Press, Ames, pp565-620.
2. Takahashi, T., Sawada, T., Takagi, M., Seto, K., Kanzaki, M., and Maruyama, T. (1984) Serotypes of *Erysipelothrix rhusiopathiae* strains isolated from slaughter pigs affected with chronic erysipelas. *Jpn. J. Vet. Sci.* **46**, 149-153.
3. Takahashi, T. (1996) Swine erysipelas and *Erysipelothrix. Jpn. J. Bacteriol.* **51**, 707-715.
4. Reboli, A. C., and Farrar, W. E. (1992) The genus *Erysipelothrix*, in: The Prokaryotes, A Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification and

Applications, (Balows, A., Truper, H. G., Dworkin, M., Harder, W., Schleifer, K.) New York, pp1629-1642.

5. Brooke, C. J., McLaughlin, V., Mee, B. J., and Riley, T. V. (1999) An investigation into "crayfish poisoning" in Western Australia. *Med. J. Aust.* **170**, 288.
6. Klauder, J. V. (1944) *Erysipelothrix rhusiopathiae* infection in swine and in human beings: comparative study of the cutaneous lesions. *Arch. Dermatol. Syphilol.* **50**, 151-159.
7. Gorby, G. L., and Peacock, J. E. Jr. (1988) *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Rev. Infect. Dis.* **10**, 317-325.
8. Grieco, M. H., and Sheldon, C. (1970) *Erysipelothrix rhusiopathiae. Ann. N. Y. Acad. Sci.* **174**, 523-532.
9. Makino, S., Okada, Y., Maruyama, T., Ishikawa, K., Takahashi, T., Nakamura, M., Ezaki, T., and Morita, H. (1994) Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J. Clin. Microbiol.* **32**, 1526-1531.
10. Shimoji, Y., Mori, Y., Hyakutake, K., Sekizaki, T., and Yokomizo, Y. (1998) Use of enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. *J. Clin. Microbiol.* **36**, 86-89.
11. Takeshi, K., Makino, S., Ikeda, T., Takada, N., Nakashiro, A., Nakanishi, K., Oguma, K., Katoh, Y., Sunagawa, H., and Ohyama, T. (1999) Direct and rapid detection of *Erysipelothrix* spp. DNAs prepared from bacterial strains and animal tissues. *J. Clin. Microbiol.* **37**, 4093-4098.
12. Fidalgo, S. G., Wang, Q., and Riley, T. V. (2000) Comparison of methods for detection of *Erysipelothrix* spp. and their distribution in some Australasian seafoods. *Appl. Environ. Microbiol.* **66**, 2066-2070.

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REVIEW ARTICLE

Erysipelothrix rhusiopathiae: bacteriology, epidemiology and clinical manifestations of an occupational pathogen

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Erysipelothrix rhusiopathiae **has been recognised as a cause of infection in animals and man since the late 1880s. It is the aetiological agent of swine erysipelas, and also causes economically important diseases in turkeys, chickens, ducks and emus, and other farmed animals such as sheep. The organism has the ability to persist for long periods in the environment and survive in marine locations. Infection in man is occupationally related, occurring principally as a result of contact with animals, their products or wastes. Human infection can take one of three forms: a mild cutaneous infection known as erysipeloid, a diffuse cutaneous form and a serious although rare systemic complication with septicaemia and endocarditis. While it has been suggested that the incidence of human infection could be declining because of technological advances in animal industries, infection still occurs in specific environments. Furthermore, infection by the organism may be under-diagnosed because of the resemblance it bears to other infections and the problems that may be encountered in isolation and identification. Diagnosis of erysipeloid can be difficult if not recognised clinically, as culture is lengthy and the organism resides deep in the skin. There have been recent advances in molecular approaches to diagnosis and in understanding of *Erysipelothrix* taxonomy and pathogenesis. Two PCR assays have been described for the diagnosis of swine erysipelas, one of which has been applied successfully to human samples. Treatment by oral and intramuscular penicillin is effective. However, containment and control procedures are far more effective ways to reduce infection in both man and animals.**

Introduction

The genus *Erysipelothrix* consists of two named species, *E. rhusiopathiae* and *E. tonsillarum*, and an as yet unnamed third species. All are gram-positive, non-sporing rods. *E. rhusiopathiae* is a pathogen or a commensal or saprophyte of a wide variety of wild and domestic animals, birds and fish. Diseases of economic importance in animals include swine erysipelas, erysipelas of farmed turkeys, chickens and emus, and polyarthritis in sheep and lambs. Man can be infected; the organism is an occupational pathogen, prevalent in those working in association with animals and animal products. Three forms of human disease are recognised, the mildest and most common of these is the skin infection known as erysipeloid.

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A review of *E. rhusiopathiae* and human infection has not been published for 10 years [1]. While few additional cases have been reported in this time, much has been achieved in the area of *Erysipelothrix* taxonomy and in molecular approaches to the diagnosis and pathogenesis of infection. It has been suggested that the frequency of human infection is declining, due to changes in technology by industry which have reduced the use of animal products and occupational exposure to the organism [1]. However, in specific environments, exposure to *E. rhusiopathiae* continues, and the threat of complications such as endocarditis is real. This review presents the recent developments against a background of older literature to ensure that awareness of the organism is maintained.

Bacteriology

History and nomenclature

Bergey's Manual [2] classifies *E. rhusiopathiae* as a regular non-sporing gram-positive rod. Until recently,

the genus consisted of only the type species, *E. rhusiopathiae*, which was known to demonstrate considerable serological, biochemical and antigenic variation [3]. Genetic analyses have revealed a new species, *E. tonsillarum* [4]. *Erysipelothrix* was originally considered a close relative of the genus *Listeria*, although numerous molecular taxonomic studies have concluded now that the genus is a distinct cluster of organisms, most similar to the streptococci [2, 3].

E. rhusiopathiae (from the Greek 'erysipelas' – a disease, 'thrix' – a hair or thread, 'rhusius' – reddish and 'pathus' – disease [5]), literally 'erysipelas thread of red disease' [1], has a long history and is the result of many name changes. Koch [6] first isolated a strain of the genus *Erysipelothrix* in 1876 from a mouse he inoculated with putrefying blood. He described the organism as the 'bacillus of mouse septicaemia' and named it *E. muriseptica*. In 1882, Loeffler isolated a similar organism from cutaneous blood vessels of a pig which had died from swine erysipelas, and was the first to describe fully the infectious agent and the disease it caused in swine [6].

The first description of human disease, later attributed to *Erysipelothrix*, was reported in 1870 in the *British Medical Journal*; further cases were documented in 1873 as erythema serpens [7]. However, it was not until 1884, when Rosenbach isolated an organism similar to Koch's from a patient with localised cutaneous lesions, that *Erysipelothrix* was established as a human pathogen. He coined the term 'erysipeloid' to differentiate between the human streptococcal disease erysipelas and the condition he had observed [6].

Rosenbach [6] distinguished three separate species of the organism, *E. muriseptica, E. porci* and *E. erysiploides*, based on their isolation from mouse, pig and man, respectively. It was later realised that these were three nearly identical strains of the same species [3], and they were named *E. insidiosa*, as originally proposed by Trevisan in 1885. This name, and all 36 other documented names for the organism, were rejected in favour of *E. rhusiopathiae* in 1966, a combination which originated in 1918 [8].

Takahashi *et al.* [9] isolated a cluster of avirulent serotype 7 strains which were later found to be genetically distinct from *E. rhusiopathiae* by DNA base composition and DNA–DNA homology studies [4]. These strains formed the basis of a new species, *E. tonsillarum* (from the Latin 'of the tonsils'). Originally, *E. tonsillarum* was considered morphologically and biochemically identical to *E. rhusiopathiae*, but it was shown later that *E. tonsillarum* could ferment sucrose, while *E. rhusiopathiae* could not [10, 11]. *E. tonsillarum* was described as avirulent for pigs, mice and chickens, but pathogenic for dogs [12, 13]. There have been no studies so far into the pathogenicity of this species for man.

Morphology and growth characteristics

E. rhusiopathiae is a non-motile, non-sporulating, non-acid-fast, slender gram-positive rod, which is easily decolourised [2]. Gram-negative forms are often seen [14], particularly if the culture is old [15]; thus, the organism has been cited occasionally as a gram-negative bacillus [16]. *E. rhusiopathiae* was long described as non-capsulate [2], until recent studies showed the presence of a capsule and suggested a role for it in virulence [17].

Based on the colonial appearance of the organism, *Erysipelothrix* morphology is described as smooth (S) or rough (R) [2, 8, 18]. S-form colonies are convex, with a smooth surface and entire edge [2, 15, 19]. R-form colonies are slightly larger with an irregular edge and a flattened, rough surface [14]. All colonies are clear, circular and very small (0.1–0.5 mm diameter after 24 h; 0.5–1.5 mm after 48 h [2, 19]), increasing in size and tending towards a pale blue opacity with further incubation or age [2, 19]. Most strains exhibit a narrow zone of α-haemolysis on blood agar, which can even show slight clearing after 48 h [2]. R-form colonies do not cause haemolysis [18].

Growth in broth was best described by Smith (cited by Jones [2]), who noted that the suspension had a 'faint opalescence ... which on shaking was resolved for a moment into delicate rolling clouds'. S forms cause slight turbidity and a powdery deposit, whereas R forms have a tangled hair-like appearance [3].

Cell morphology is closely linked to the colonial characteristics of each form [19]. S forms are slender, straight or slightly curved rods with rounded ends, 0.8–2.5 μm in length and 0.2–0.4 μm in diameter. The rods exist in various formations, often as small chains [2]. The R form exhibits a predominantly filamentous morphology, frequently likened to the mycelial formations of fungi, although branching does not occur. The filaments can be 4 μm to >60 μm in length and can have a beaded appearance with Gram's staining [2]. Long chains of distinct rods can also exist in this form [14, 18].

The origin of R and S forms and their role in disease have received much emphasis in the *Erysipelothrix* literature, possibly because different forms for other pathogens, such as *Streptococcus pneumoniae*, have distinct roles in virulence. For *E. rhusiopathiae* these roles are not definite, and there are conflicting observations on the role of each form. Furthermore, the distinction between S and R forms is not always clear. An intermediate (RS) form, which is the most common conformation [15], has been used to describe colonies sharing the characteristics of both types [8]. Some investigators have suggested that S forms are commonly isolated from acute pig diseases, such as septicaemia, and R forms from more chronic syn-

dromes such as arthritis and endocarditis [8]. However, there are conflicting reports relating to the virulence of R forms. Gorby [20] reported that, in pig disease, R forms are the more virulent type, while Taylor [21] stated that the R form was generally considered less virulent. Similar possible relationships for S and R forms in human disease have not been documented.

Media and conditions of incubation (see below) play a major role in morphology formation; R forms are favoured by incubation at 37°C in acidic pH, while S forms predominate in alkaline conditions (pH 7.6–8.2) with incubation at 33°C [14]. Changing growth conditions has allowed S forms to give rise to R and RS forms, and S forms to originate from R forms [3]. The S form often dissociates to the R form with age [8, 15]. These changes in morphology and cultural characteristics are reported to lead to changes in virulence and antigenic properties [8].

Growth conditions and requirements

E. rhusiopathiae is a facultative anaerobe [3]. Newly isolated strains are micro-aerophilic, but laboratory-adapted cultures grow both aerobically and anaerobically, with some strains being favoured by incubation in CO_2 5% or 10% [2]. The organism can grow at temperatures between 5° and 44°C [2], optimally between 30° and 37°C [18]. Best growth is favoured by an alkaline pH. The optimum pH range has been documented as 7.2–7.6 [2, 5] or 7.4–7.8 [15, 18, 19 ,22] and the limits of growth as 6.7–9.2 [23].

Growth is enhanced by the inclusion of serum 5–10%, blood, glucose 0.1–0.5%, protein hydrolysates, or surfactants such as Tween 80 in media [8, 18]. The exact nutritional requirements of the organism are not known [18], but riboflavin, small amounts of oleic acid and several amino acids [24] – particularly tryptophan and arginine [8, 25] – are needed for growth. Higher concentrations of glucose and oleic acid are inhibitory [5].

Biochemistry

The genus *Erysipelothrix* is relatively inactive and gives negative results for catalase, oxidase, methyl red, indole and Voges-Proskauer reactions [15]. Carbohydrate fermentations produce acid without gas, but reaction patterns are variable and depend on the basal medium and indicator used [14, 26]. Andrade's agar with horse serum 10% is the recommended medium for biochemical tests. The majority of strains produce H_2S gas, but again the extent of this production varies with the culture medium. The best reaction is demonstrated on triple sugar iron agar [27]. A more detailed description of the biochemical characteristics of *Erysipelothrix* can be found in the reviews of Ewald [8], Jones [2] or Reboli and Farrar [3]. Traditionally, biochemical reactions were used to differentiate between *Erysipelothrix* and morphologically similar bacteria, such as *Listeria* and *Corynebacterium* spp. Characteristics used for this purpose included α-haemolysis, lack of motility, lack of catalase production and resistance to neomycin [14]. While traditional biochemical testing may still be of value, particularly in discriminating between *E. rhusiopathiae* and *E. tonsillarum*, rapid identification can be achieved with an API Coryne System strip (bioMérieux) [28].

Chemical tolerances

E. rhusiopathiae is a remarkably resistant organism for one that does not form spores [18]. The survival of the organism in the environment is an important factor in the epidemiology of disease. *E. rhusiopathiae* is also tolerant to numerous chemicals. It can grow in the presence of phenol 0.2% and crystal violet 0.001%, and is said to be one of the organisms most resistant to sodium azide, tolerating 0.1% [23, 29]. Some of these chemical tolerances have been utilised in the development of selective media.

Antigenic structure

Watts [30] noted that most strains of *Erysipelothrix* had two kinds of antigen, a species-specific heat-labile protein antigen and a heat- and acid-stable polysaccharide antigen, which now form the basis for serotyping strains. Dedié (cited by Reboli and Farrar [3]) recognised two major serotypes, A and B. Strains that did not react with these specific antisera were named group N. The Arabic numeral serotyping system of Kucsera [31] superseded the alphabetical scheme, due to variations in previous methods of antigenic extraction. The current standard serotyping method is a double agar-gel precipitation test with type-specific rabbit antisera and antigen prepared by hot aqueous extraction [31, 32].

At present, strains of *Erysipelothrix* are classified as serotypes 1–26 [33]. A group N still exists for strains that have no type-specific antigen. In swine, 75–80% of isolates are of serotype 1 or 2 (previously group A or B) and the less common serotypes make up the remaining 20% [18]. Some investigators have noted a relationship between serotype and clinical condition in pigs, with serotype 1a most commonly isolated from acute swine illness, and serotype 2a more prevalent in chronic forms of disease [34]. However, other surveys have provided contradictory results, and all clinical conditions can be induced experimentally in susceptible swine with a variety of serotypes [18, 35]. Sneath *et al.* [23] reported that strains from human or pig origin were antigenically similar, but there has been no verification of this since the numerical typing system was established. There is a deficiency in the literature regarding serotypes in human infection, and the epidemiological significance of serotyping in human disease is questionable.

Molecular studies have cast further doubt on the value of serotyping as a reliable taxonomic and epidemiological method of classification. DNA–DNA hybridisation [10], polyacrylamide gel electrophoresis [36] and multilocus enzyme electrophoresis studies [11] classified serotypes 1a, 1b, 2, 4–9, 11, 12, 15–17, 19, 21 and 25 in the species *E. rhusiopathiae*, and serotypes 3, 7, 10, 14, 20, 22–24 in *E. tonsillarum*, with differing results for serotypes 13 and 18 [11]. When *Erysipelothrix* strains were analysed by restriction fragment length polymorphisms (RFLP), both *E. tonsillarum* and *E. rhusiopathiae* contained serotype 2 (virulent) and 7 (avirulent) strains. The creation of a third species of *Erysipelothrix* was suggested to account for a distinct cluster of strains [37].

These findings suggest that there is no direct relationship between serotype and virulence, supporting earlier investigators who noted that, within each serotype, strains of high, low and no virulence existed, and that factors other than serotype were important in the induction of disease in animal models [38, 39]. Further investigation is required to reach a consensus on typing schemes and their role, if any, in studies of pathogenicity.

Mechanisms of pathogenicity

Relatively little is known about the pathogenesis of *E. rhusiopathiae* infections. Strains of *E. rhusiopathiae* are known to vary considerably in virulence. Despite much investigation, there has been no conclusive evidence of a relationship between virulence and morphology, chemical structure or antigenic structure [18]. Various virulence factors have been suggested, although their relative importance is not yet clear. The presence of a hyaluronidase and a neuraminidase has been recognised [40, 41], but hyaluronidase was detected in strains both virulent and avirulent for pigs. A correlation between the amount of neuraminidase produced and the virulence of strains was noted [41], although later studies demonstrated that avirulent acapsular mutant strains also produced the enzyme [17].

Adhesion to porcine kidney cells *in vitro* was greater for virulent strains [42]; however, the role of this factor in disease has not been investigated further. Further work on adhesion has been carried out [43], but this was in relation to arthritis in swine as a model for rheumatoid arthritis in man. Recently, the presence of a labile capsule was reported and acapsular mutants were constructed by transposon mutagenesis. In contrast to the parental strain, the mutants failed to resist phagocytosis by murine polymorphonuclear leucocytes, and could not survive within murine macrophages, suggesting that the capsule was an important virulence factor [17, 44]. The mutant has been used in the development of a diagnostic PCR [45] (see below) and vaccine studies [46]. Further investigation of the pathogenesis of *E. rhusiopathiae* infection is required.

Epidemiology

E. rhusiopathiae and infections caused by this organism occur world-wide [6, 14]. Infections of man and animals have been documented from Africa, Australia, several countries in the Americas, Japan, China and throughout Europe [8]. Human disease can originate from an animal or environmental source.

Animal disease

Swine erysipelas caused by *E. rhusiopathiae* is the disease of greatest prevalence and economic importance [18]. There are three clinical forms: a severe acute septicaemic form of sudden mortality; a milder, subacute urticarial form characterised by purple diamond-shaped lesions on the skin and a chronic form with endocarditis or arthritis [14]. Swine erysipelas is economically detrimental to the pig industries of North America, Europe, Asia and Australia [18].

As well as affecting swine, *E. rhusiopathiae* causes infections in a wide variety of domestic and wild mammals (including marine mammals), domestic, game and wild birds, and man [3, 8, 19, 22]. Polyarthritis of sheep and lambs, and erysipelas in calves, ducks and domestic turkeys are also economically significant diseases caused by *E. rhusiopathiae* [6, 19]. In Australia, the organism is an emerging problem in farmed emus [47].

Domestic swine are believed to be the most important animal reservoir of *E. rhusiopathiae*. The organism is shed by diseased animals in faeces, urine, saliva and nasal secretions, which can contaminate food, water, soil and bedding, leading to indirect transmission of the organism [18]. Furthermore, an average of 20–40% of healthy swine, and in some herds up to 98%, harbour *Erysipelothrix* in the lymphoid tissue of the alimentary tract, particularly in the tonsils [18, 48, 49]. One study demonstrated that both virulent (serotypes 2, 6, 11, 12 and 16) and avirulent (serotype 7) serotypes were found on the tonsils [9]. Another showed that the faeces of apparently healthy animals contained virulent organisms [50]. The maintenance of *E. rhusiopathiae* in nature appears to result from asymptomatic carriage in animals and subsequent dissemination of the organism to the environment [6].

Mice are susceptible to infection [6], but other rodents seem to be affected only occasionally [51]. These animals can harbour the organism and are important reservoirs in some environments, such as meat packing plants [6]. Insects have been reported to carry *E. rhusiopathiae*, and are occasional vectors [6, 19, 52];

however, this is not a known route of infection for man.

Environmental reservoirs

The environment appears to be secondary in importance to animal reservoirs as a source of *E. rhusiopathiae*. However, in some circumstances, such as in marine environments, the organism may survive long enough to create a significant hazard to man [6].

E. rhusiopathiae is a saprophyte associated with some groups of animals, particularly marine fish, molluscs and crustaceans [6, 53, 54]. Freshwater fish and some species of bird are also hosts [14]. The organism survives and grows on the exterior mucoid slime of fish, without causing disease in the fish themselves [6]. It is likely that *E. rhusiopathiae* survives by a similar mechanism on the exterior slime layer of other marine creatures. The slime on fish appears to be an important source of infection for man. In early reports, fish caught under 'aseptic conditions' did not harbour *Erysipelothrix* [55], so investigators concluded that the organism was transmitted *via* the slime from other fish [54, 55]. Boxes used for transport of fish seemed to play a vital role in the transmission of *E. rhusiopathiae*, and many human cases resulted from contact with these objects [5, 54, 55].

Once in the environment, *E. rhusiopathiae* can survive for long periods although it does not form spores [18]. The organism is ubiquitous, and can be found wherever nitrogenous matter decomposes, retaining virulence and viability for months in putrid material [56]. Survival in swine faeces for 1–5 months, depending on seasonal conditions [19], and in soil for up to 5 years from the time of the last diseased pig [57], has been demonstrated. However, this latter report did not consider the possibility of asymptomatic shedding. *E. rhusiopathiae* has been recovered from sewage effluent from abattoirs, streams, drains and fertilizer [3, 15], surviving in drinking water for 4–5 days and sewage for 10–14 days [5]. It was long thought that the organism could live in soil indefinitely, and early reports suggested that the source of infection was soil [5]. However, studies by Wood [58] did not support this widely held belief; *E. rhusiopathiae* survived for a maximum of only 35 days, depending on temperature and soil condition. Despite this limited endurance, the organism does survive long enough in soil to be a potential source of infection to animals and man.

E. rhusiopathiae persists in animal tissues for long periods, despite chilling, freezing, or curing [6]. The organism is resistant to pickling, smoking and salting [14]. *Erysipelothrix* can also survive in decaying tissue, and will remain viable in a carcass for 12 days in direct sunlight, for 4 months in putrefied flesh, for 9 months in a buried carcass and at least 10 months in refrigerated tissue [19]. *E. rhusiopathiae* has been isolated from fresh fish, pork and chicken for human consumption [59, 60]. The widespread distribution of *E. rhusiopathiae* can be attributed to the ability of the organism to survive for long periods in the environment, and the fact that the organism can colonise or infect a wide variety of animals [6].

Human infection

Risk of humans infection is based on the opportunity for exposure, and factors such as age, sex, race and socio-economic status relate only to this opportunity [3, 61]. Individuals involved in occupations or recreations with contact with animals, animal products or animal wastes are at greatest risk. Thus, *E. rhusiopathiae* infection is said to be occupationally related [56]. It follows that those in occupations with most frequent animal contact, such as butchers, abattoir workers, veterinarians, farmers, fishermen, fish-handlers and housewives are the most commonly infected [5, 6, 19, 62]. However, cases have been documented from a very wide variety of occupations (see [1] for a complete list). The common names for human infection reflect this occupational mode of acquisition. These include seal finger, whale finger, blubber finger, fish hand, fish poisoning, fish handler's disease and pork finger [3, 6, 62, 63].

Human infection can occur from contact with infected animals, their secretions, wastes or products, or organic matter contaminated by any of these [6]. Infection is initiated either by an injury to the skin with infective material or when a previous injury is contaminated. There have been a few documented instances of penetration of the skin by the bacteria [61], and of infection by ingestion of contaminated food products [62]. There have been no reports of person-to-person transmission [3]. Modes of infection tend to be very occupation-specific, and transmission is generally by vehicles. These include contaminated objects causing wounds, such as knives, needles, dissecting instruments, fish teeth and spines, fish hooks, bone splinters, and crab, lobster and crayfish claws [6]. If a wound is already present, infection can result from contact with any of a very wide variety of contaminated objects [6, 62].

Infections in both man and animals appear to have a seasonal incidence, with most cases occurring in the summer months [6]. While it has been suggested that the biological activity of *E. rhusiopathiae* is related to temperature [55], others think that it is likely to be due to increased contact between people and sources of infection during these months [6].

Clinical manifestations in man

E. rhusiopathiae can cause three forms of human disease which closely resemble disease in swine. These

are erysipeloid (a localised cutaneous form), a generalised cutaneous form and a septic form often associated with endocarditis [14]. It is possible that the incidence of human infection is declining. However, infection is possibly under-diagnosed, because of the resemblance it bears to other infections, difficulties in isolation and identification of the causative organism, and the rapid response to empiric antimicrobial therapy [64].

Erysipeloid

Erysipeloid is the most common form of human infection [14]. It is an acute localised cutaneous infection, described as a local cellulitis [3]. Erysipeloid usually occurs on the hand or fingers, reflecting the occupational nature of acquisition of the disease [62]; however, lesions have been described on many areas of the body [6].

The incubation period is usually <4 days, but can be up to 7 days after exposure [65]. The infection consists of a distinctive, well-demarcated, slightly elevated violaceous lesion [19]. The peripheral edge spreads slowly as the centre fades [66], and while vesicles are occasionally present, there is no suppuration or pitting [14, 56]. There is associated local swelling, and an intense itching or a severe burning or throbbing pain [6], which is inconsistent with the mild look of the lesion [1]. Systemic symptoms can occur; 10% of cases experience fever and joint ache, and lymphadenitis and lymphadenopathy appear in 33% of patients [67]. Arthritis can manifest in an adjacent joint. The disease is self-limiting and usually resolves in 3–4 weeks without therapy [3], although relapses may occur if untreated [6].

Diffuse cutaneous form

This form is more generalised than erysipeloid, and includes the rare cases in which lesions progress from the initial site to other locations on the body, or in which there is development of lesions remote from the site of inoculation [68]. The lesions are similar to those of the localised form, but bullous lesions can also occur [69]. Systemic symptoms are more frequent and include fever, malaise, joint and muscle pain and severe headaches [6], and polyarthritis in rare instances. The clinical course is more protracted and recurrences are more frequent than with erysipeloid [56]. Very few cases have been documented; only 1 of 100 cases reported by Klauder [68] was generalised and none of the 500 cases reported by Nelson [67] was of this form.

Septicaemia and endocarditis

A more serious manifestation of *E. rhusiopathiae* infection is septicaemia, to which endocarditis has almost always been linked. In 49 cases of systemic infection in 15 years, 90% were associated with endocarditis [20]. Although septicaemia and endocarditis are relatively uncommon, there does appear to be an increase in incidence [14, 20], which could either reflect increased exposure or improved diagnosis.

E. rhusiopathiae endocarditis has a mortality of 38% and presents as an acute or subacute form, the latter being more frequent. In their summary of cases of endocarditis, Gorby and Peacock [20] discussed predisposing factors, and comparisons with other forms of endocarditis were made. *E. rhusiopathiae* endocarditis had an increased male to female ratio, possibly the result of occupational exposure, and mortality was almost double the rate of endocarditis of other aetiologies. The majority of patients had normal native heart valves and were immunocompetent. A history of alcohol abuse, believed to be a risk factor for the development of this complication, was noted in 33% of patients. Only 36% reported a previous erysipeloid lesion, and 89% of patients were in occupations involving contact with animals.

Recent reports [16, 70] have demonstrated that *Erysipelothrix* bacteraemia without endocarditis is more common than was thought previously, occurring mainly in immunocompromised patients. This increased rate was linked to a more thorough identification of blood culture isolates from these patients.

Miscellaneous infections

There are reports of other infections associated with *Erysipelothrix*. These have included chronic arthritis [69], cerebral infection, [71, 72] and osseous necrosis [68]. Recent case reports have focused on novel presentations and complications of *Erysipelothrix* infection. These have included erysipeloid with co-existing orf [64], persistent bacteraemia in a hospitalised patient [73], bacteraemia in an HIV-positive patient [74], endocarditis with acute renal failure [75], septicaemia and lupus nephritis [76], and septicaemia in a neonate [77]. In reports of systemic infection, the typical predisposing factors have been involved: either immunocompromised patients with atypical infection without cardiac involvement or immunocompetent patients with endocarditis. Renal involvement and alcoholism were factors noted in this second group.

E. rhusiopathiae has been implicated recently in a syndrome known as 'crayfish poisoning', which affects lobster fishermen in Western Australia and bears a clinical resemblance to erysipeloid [78]. A possible association between the organism and infections in fishermen was noted in 1947 by Sheard and Dicks [79], but proper identification was hampered by field conditions. It was not until 1996 that this infection was investigated further. The presence of other potential pathogens did not allow a direct causal relationship between *E. rhusiopathiae* and 'crayfish

poisoning' to be established; however, the results were suggestive of this [78].

Treatment and prevention

Susceptibility data are still limited [3], despite recent reports on the subject [9, 80, 81]. *Erysipelothrix* is highly susceptible to penicillin, cephalosporins and clindamycin [20, 23, 80]. Most strains are resistant to aminoglycosides, trimethoprim-sulphamethoxazole, polymyxins, sulphonamides, streptomycin, novobiocin and vancomycin. The organism is variably susceptible to chloramphenicol, tetracyclines and erythromycin [3].

Erysipeloid can be treated effectively with oral penicillin [3]. Although infection is usually self-limiting, relapses and progression to more serious forms are possible. Oral penicillin will resolve a case of erysipeloid in around 48 h, while intravenous penicillin is recommended for more serious *E. rhusiopathiae* infections [14]. While the mortality rate for endocarditis has been reduced from 100% in the pre-antibiotic era, there is still a 38% fatality rate despite available treatment [20]. This rate could be partly explained by the use of vancomycin, to which the organism is resistant, in empiric therapy for endocarditis [81]. Therefore, early diagnosis of all forms of *E. rhusiopathiae* infection is essential [14]. In those individuals allergic to penicillin, cephalosporins have been described as the most appropriate alternative, as clindamycin and erythromycin are only bacteriostatic towards *E. rhusiopathiae* [3].

Before the advent of penicillin therapy, some alleviation of symptoms could be achieved with hyperimmune serum. However, the resulting serum sickness was often more severe than an episode of erysipeloid, and this treatment was confirmed to be of little value for cutaneous infections [14, 82]. Commercial vaccines in the form of bacterins, lysates or live attenuated strains of *E. rhusiopathiae* serotype 2 offer protection to pigs and turkeys [25]. Vaccination is not a viable option in man, because clinical erysipeloid appears to convey little or no immunity [55, 66], as evidenced by relapse and/or re-infection.

Since Heilman and Herrel [52] first reported the success of penicillin therapy for *Erysipelothrix* infections, there has been no recorded resistance of the organism to this antibiotic. One experiment involving serial passage of 75 strains for 8 months did not produce resistance [14]. Plasmids were not found in *E. rhusiopathiae* in early investigations [80, 81], but later studies were able to detect them [83]. Plasmids appear to play no critical role in *E. rhusiopathiae* resistance. Antibiotics contained in animal feed have been reported to influence the resistance of some strains of *E. rhusiopathiae*, although the mechanism remains uncertain [9].

Containment and control of *E. rhusiopathiae* are the most effective means of preventing the spread of infection in man and animals. An awareness of the infection is essential for individuals in occupations which put them at risk. Suggested preventive measures include: the wearing of gloves or other protective hand wear, good hygiene – especially frequent hand washing with disinfectant soap – and the prompt treatment of any small injuries [6]. Good health is considered an important factor in prevention, as a poor state of health, including alcoholism, may predispose to the serious forms of infection [20, 84]. Control of animal disease by sound husbandry, herd management, good sanitation and immunisation is recommended [18].

The removal or regular disinfection of contaminated sources has been shown to be an important method of limiting the spread of the organism throughout a work environment [6]. *E. rhusiopathiae* can be killed by commonly available disinfectants [19]. However, many investigators have noted that structurally complex equipment is difficult to clean, and because the organism is able to survive in organic matter, disinfecting without cleaning is useless [85, 86]. If disinfection is impractical, other control measures become even more significant.

Control of reservoir populations of *E. rhusiopathiae* is impractical or impossible, because of the widespread distribution of the organism, the large variety of animal hosts and its ability to persist in the environment. However, the possibility of human infection can be reduced by awareness, safe work practices and sensible precautions.

Isolation and laboratory identification

It has been reported that medical practitioners who see cases of human erysipeloid regularly find the lesion and other symptoms are 'so typical that a biopsy and subsequent isolation is neither necessary nor justifiable' [87]. As a result, the majority of identification protocols have been developed with swine erysipelas in mind. However, if human cases are declining, doctors could be less likely to recognise the infection, and, therefore, isolation techniques and methods for identification of *E. rhusiopathiae* may gain new importance.

Cultural methods

Traditional cultural methods for *E. rhusiopathiae* isolation involve the use of selective and enrichment media. Identification is based on Gram's stain, cultural morphology, motility, haemolytic characteristics and biochemical properties, particularly H_2S production [3]. For bacteraemia or endocarditis, a blood sample cultured in standard blood culture media is sufficient for isolation [19], as *E. rhusiopathiae* is not particu-

larly fastidious [3]. The organism is more difficult to isolate from cases of erysipeloid, because it is said to live deep in the skin [2]. A biopsy at the advancing edge of the lesion and extending the entire thickness of the dermis is required. Aspirates of the lesion or associated bullae and vesicles are usually less rewarding [19]. Swabbing does not usually detect the pathogen [65].

Biopsies and aspirates are incubated in an infusion broth of glucose 1%, in air or CO_2 5–10% and subcultured to blood agar every 24 h. If a sample is likely to be heavily contaminated, such as from soil, faeces, or animal tissue, selective measures are required [2]. A number of selective and enrichment broths have been described. The most commonly used is *Erysipelothrix* selective broth (ESB), a liquid medium containing serum, tryptose, neomycin, vancomycin and kanamycin [88]. Packer's medium (SACV) makes use of the organism's tolerance of sodium azide and crystal violet [29], and is frequently used for subculture after growth in ESB [18]. Modified blood azide (MBA) is similar to SACV, but does not include crystal violet [89]. Bohm's medium contains azide, kanamycin, phenol and water blue [8].

Incubation of liquid media at 37°C for 18–24 h is usually sufficient for growth. Colonies are visible after 24 h on most solid media, although incubation for 48 h is recommended for ESB and 72 h for SACV. An alternative for biopsy specimens is to refrigerate them at 4–5°C for 4–5 weeks in a liquid enrichment media and then subculture to SACV [15].

Each medium has advantages, but none is ideal. ESB is still regarded as the best selective medium, despite a report showing that some strains grow poorly due to kanamycin susceptibility [90]. MBA requires less incubation time than SACV, but is not as selective and is not suitable for heavily contaminated samples [89]. Bohm's medium does not seem to have been widely used, although the reasons for this are unclear.

Mouse protection test

This test is traditionally regarded as the best confirmatory test of *E. rhusiopathiae* identity, because most strains of the organism are highly virulent for laboratory mice. One group of animals is inoculated subcutaneously with a 24-h broth culture and equine hyperimmune *E. rhusiopathiae* antiserum, and the second group receives broth culture but not antiserum. If the organism is *E. rhusiopathiae* the second group, but not the first, will die within 5–6 days [2]. However, strains must be pathogenic for mice for this method to be useful. While this test is useful in studies of swine erysipelas, the reliability of this method for human pathogenic strains remains unknown.

Fluorescent antibody test

Direct and indirect assays have been used to confirm the identity of *E. rhusiopathiae* in tissues [91, 92], in broth [93] and in human infection [94]. However, Harrington [93] noted that this method was not as sensitive as cultural methods, and as a result it has not been used widely.

API Coryne system

The API Coryne system (bioMérieux) is a commercial strip system for the identification of coryneform bacteria. Soto [28] compared conventional biochemical reactions with the commercial system for corynebacteria and related genera, including *Erysipelothrix*. The system had few misidentifications and all four strains of *E. rhusiopathiae* tested were correctly identified by use of the strip. The investigators concluded that the commercial strip was a good alternative to traditional biochemical methods, permitting reliable and rapid identification of coryneform bacteria.

PCR

Two PCR methods are available for the detection of *Erysipelothrix* species [45, 95]. While they were both developed for swine erysipelas, a PCR method was employed for detection of organisms in human specimens in a recent study in Australia [78]. Makino *et al.* [95] based their primers on a region of the 16S rRNA gene, specific to *Erysipelothrix* but shared by both *E. rhusiopathiae* and *E. tonsillarum*. Shimoji *et al.* [45] made use of the avirulent transposon mutant created during capsule studies to develop an *E. rhusiopathiae*-specific PCR. The primers were designed from sequences presumed to be associated with the virulence of *E. rhusiopathiae*. This assay would be particularly useful for monitoring of swine disease. However, the value of this test in a human clinical situation is uncertain, due to the lack of information regarding the pathogenicity of *E. tonsillarum* for humans. The time saved by using the PCR is the greatest advantage it has over all other methods for detecting *Erysipelothrix*.

Erysipelothrix PCR is very sensitive; Makino *et al.* [95] detected <20 bacteria in a mouse spleen, although the limit detected by Shimoji [45] was only 1000 per reaction mixture, despite a broth enrichment procedure and the use of a DNA extraction kit. Other advantages include the ability to detect an organism in a contaminated sample, as the primers recognise only the specific sequences. With the Makino PCR, the organism does not have to be alive for detection. However, a major drawback is that organism viability cannot be assessed and other procedures would be required for this task. The use of broth enrichment as described by Shimoji [45] could be used to overcome this problem; a modification of this technique was also used in the Australian study [78].

PCR is a powerful tool for the detection of *Erysipelothrix* in all kinds of samples and is able to overcome many problems inherent in other diagnostic methods. However, it is likely that a combination of culture and molecular techniques will be used for accurate diagnosis in the future.

Conclusions

Although uncommon, it is likely that human infections with *E. rhusiopathiae* are under-diagnosed. The organism's slow growth and small colony size mean that it may be overlooked in the routine diagnostic laboratory or overgrown with secondary pathogens such as *Staphylococcus aureus* and *Streptococcus pyogenes*. A high index of suspicion and the application of modern molecular techniques will no doubt improve this situation. As a pathogen of animals, particularly swine, *Erysipelothrix* is of great economic importance and good animal husbandry practice is essential to reduce impact. However, the organism's resilience and ability to survive are important in both human and veterinary medicine. Most human infections result from occupational exposure and this possibility can be reduced through awareness and safe work practices.

References

1. Reboli AC, Farrar WE. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clin Microbiol Rev* 1989; **2**: 354–359.
2. Jones D. Genus *Erysipelothrix* Rosenbach1909^{367AL}. In: Sneath PHA, Mair NS, Sharpe ME, Holt JG (eds) Bergey's Manual of systematic bacteriology, 9th edn, vol 2. Baltimore, Williams and Wilkins. 1986: 1245–1249.
3. Reboli AC, Farrar WE. The genus *Erysipelothrix*. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer K (eds) The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification, applications. New York, Springer-Verlag. 1992: 1629–1642.
4. Takahashi T, Fujisawa T, Benno Y *et al. Erysipelothrix tonsillarum* sp. nov. isolated from tonsils of apparently healthy pigs. *Int J Syst Bacteriol* 1987; **37**: 166–168.
5. Woodbine M. *Erysipelothrix rhusiopathae*: bacteriology and chemotherapy. *Bacteriol Rev* 1950; **14**: 161–178.
6. Wood RL. *Erysipelothrix* infection. In: Hubbert WT, McCulloch WF, Schnurrenberger PR (eds) Diseases transmitted from animals to man, 6th edn. Springfield, IL, Thomas. 1975: 271–281.
7. Barber M. Discussion on swine erysipelas infection (*Erysipelothrix rhusiopathiae*) in man and animals. *Proc R Soc Med* 1948; **41**: 328–330.
8. Ewald FW. The genus *Erysipelothrix*. In: Starr MP, Stolp H, Trüper HG, Balows A, Schlegel HG (eds) The prokaryotes: a handbook on habitats, isolation, and identification of bacteria, Vol 2. New York, Springer-Verlag. 1981: 1688–1700.
9. Takahashi T, Sawada T, Muramatsu M *et al.* Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. *J Clin Microbiol* 1987; **25**: 536–539.
10. Takahashi T, Fujisawa T, Tamura Y *et al.* DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. *Int J Syst Bacteriol* 1992; **42**: 469–473.
11. Chooromoney KN, Hampson DJ, Eamens GJ, Turner MJ. Analysis of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* by multilocus enzyme electrophoresis. *J Clin Microbiol* 1994; **32**: 371–376.
12. Takahashi T, Tamura Y, Yoshimura H *et al. Erysipelothrix tonsillarum* isolated from dogs with endocarditis in Belgium. *Res Vet Sci* 1993; **54**: 264–265.
13. Takahashi T, Tamura Y, Endoh YS, Hara N. Cellular fatty acid composition of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum*. *J Vet Med Sci* 1994; **56**: 385–387.
14. Grieco MH, Sheldon C. *Erysipelothrix rhusiopathiae*. *Ann NY Acad Sci* 1970; **174**: 523–532.
15. Cottral GE. Erysipelothrix In: Manual of standardized methods for veterinary microbiology. Ithica, NY, Cornell University Press. 1978: 429–436, 671, 672, 679, 687.
16. García-Restoy E, Espejo E, Bella F, Llebot J. Bacteremia due to *Erysipelothrix rhusiopathiae* in immunocompromised hosts without endocarditis. *Rev Infect Dis* 1991; **13**: 1252–1253.
17. Shimoji Y, Yokomizo Y, Sekizaki T, Mori Y, Kubo M. Presence of a capsule in *Erysipelothrix rhusiopathiae* and its relationship to virulence for mice. *Infect Immun* 1994; **62**: 2806–2810.
18. Wood RL. Erysipelas. In: Leman AD, Straw BE, Mengeling WL, D'Allaire S, Taylor DJ (eds) Diseases of swine, 7th edn. Ames, IA, Iowa State University Press. 1992: 475–486.
19. Conklin RH, Steele JH. *Erysipelothrix* infections. In: Steele JH (ed) CRC handbook. Series in zoonoses, vol 1 (section A). Boca Raton, FL, CRC Press. 1979: 327–337.
20. Gorby GL, Peacock JE. *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Rev Infect Dis* 1988; **10**: 317–325.
21. Taylor DJ. Pig diseases, 5th edn Cambridge, Burlington Press. 1990.
22. Shuman RD. Erysipelothrix. In: Davis JW, Anderson RC, Karstad LH, Trainer DO (eds) Infectious and parasitic diseases of wild birds. Ames, IA, Iowa State University Press. 1971: 141–145.
23. Sneath PHA, Abbott JD, Cunliffe AC. The bacteriology of erysipeloid. *BMJ* 1951; **2**: 1063–1066.
24. Hunter SH. Some growth requirements of *Erysipelothrix* and *Listeria*. *J Bacteriol* 1942; **43**: 629–640.
25. Groschup MH, Timoney JF. Modified Feist broth as a serum-free alternative for enhanced production of protective antigen of *Erysipelothrix rhusiopathiae*. *J Clin Microbiol* 1990; **28**: 2573–2575.
26. White TG, Shuman RD. Fermentation reactions of *Erysipelothrix rhusiopathiae*. *J Bacteriol* 1961; **82**: 595–599.
27. Vickers CL, Bierer BW. Triple sugar iron agar as an aid in the diagnosis of erysipelas. *J Am Med Vet Assoc* 1958; **133**: 543–544.
28. Soto A, Zapardiel J, Soriano F. Evaluation of API Coryne system for identifying coryneform bacteria. *J Clin Pathol* 1994; **47**: 756–759.
29. Packer RA. The use of sodium azide (NaN_3) and crystal violet in a selective medium for streptococci and *Erysipelothrix rhusiopathiae*. *J Bacteriol* 1943; **46**: 343–349.
30. Watts PS. Studies on *Erysipelothrix rhusiopathiae*. *J Pathol Bacteriol* 1940; **50**: 355–369.
31. Kucsera G. Proposal for standardization of the designations used for serotypes of *Erysipelothrix rhusiopathiae* (Migula) Buchanan. *Int J Syst Bacteriol* 1973; **23**: 184–188.
32. Kalf GF, White TG. The antigenic components of *Erysipelothrix rhusiopathiae*. II. Purification and chemical characterization of a type-specific antigen. *Arch Biochem Biophys* 1963; **102**: 39–47.
33. Norrung V, Molin G. A new serotype of *Erysipelothrix rhusiopathiae* isolated from pig slurry. *Acta Vet Hung* 1991; **39**: 137–138.
34. Kucsera G. Serological typing of *Erysipelothrix rhusiopathiae* strains and the epizootiological significance of the typing. *Acta Vet Acad Sci Hung* 1979; **27**: 19–28.
35. Wood RL, Harrington R. Serotypes of *Erysipelothrix rhusiopathiae* isolated from swine and from soil and manure of swine pens in the United States. *Am J Vet Res* 1978; **39**: 1833–1840.
36. Tamura Y, Takahashi T, Zarkasie K, Nakamura M, Yoshimura H. Differentiation of *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* by sodium dodecyl sulfate-polyacrylamide gel electrophoresis of cell proteins. *Int J Syst Bacteriol* 1993; **43**: 111–114.
37. Ahrne S, Sternstrom I, Jensen NE, Pettersson B, Uhlen M, Molin G. Classification of *Erysipelothrix* strains on the basis of restriction fragment length polymorphisms. *Int J Syst Bacteriol* 1995; **45**: 382–385.

38. Eamens GJ, Turner MJ, Catt RE. Serotypes of *Erysipelothrix rhusiopathiae* in Australian pigs, small ruminants, poultry, and captive wild birds and animals. *Aust Vet J* 1988; **65**: 249–252.
39. Takahashi T, Sawada T, Seto K, Muramatsu M, Maruyama T, Kanzaki M. Pathogenicity of *Erysipelothrix rhusiopathiae* strains of serovars 1a, 3, 5, 6, 8, 11, 21, and type N, isolated from slaughter pigs affected with chronic erysipelas. *Nippon Juigaku Zasshi* 1985; **47**: 1–8.
40. Norrung V. Studies on *Erysipelothrix insidiosa – S. rhusiopathiae*. I. Morphology, cultural features, biochemical reactions and virulence. *Acta Vet Scand* 1970; **11**: 577–585.
41. Krasemann C, Muller HE. [The virulence of *Erysipelothrix rhusiopathiae* strains and their neuraminidase production.] *Zentralbl Bakteriol Orig A* 1975; **231**: 206–213.
42. Takahashi T, Hirayama N, Sawada T, Tamura Y, Muramatsu M. Correlation between adherence of *Erysipelothrix rhusiopathiae* strains of serovar 1a to tissue culture cells originated from porcine kidney and their pathogenicity in mice and swine. *Vet Microbiol* 1987; **13**: 57–64.
43. Franz B, Davies ME, Horner A. Localization of viable bacteria and bacterial antigens in arthritic joints of *Erysipelothrix rhusiopathiae*-infected pigs. *FEMS Immunol Med Microbiol* 1995; **12**: 137–142.
44. Shimoji Y, Yokomizo Y, Mori Y. Intracellular survival and replication of *Erysipelothrix rhusiopathiae* within murine macrophages: failure of induction of the oxidative burst of macrophages. *Infect Immun* 1996; **64**: 1789–1793.
45. Shimoji Y, Mori Y, Hyakutake K, Sekizaki T, Yokomizo Y. Use of an enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. *J Clin Microbiol* 1998; **36**: 86–89.
46. Shimoji Y, Mori Y, Sekizaki T, Shibahara T, Yokomizo Y. Construction and vaccine potential of acapsular mutants of *Erysipelothrix rhusiopathiae*: use of excision of Tn*916* to inactivate a target gene. *Infect Immun* 1998; **66**: 3250–3254.
47. Griffiths GL, Buller N. *Erysipelothrix rhusiopathiae* infection in semi-intensively farmed emus. *Aust Vet J* 1991; **68**: 121–122.
48. Rowsell HC. The effects of stomach contents and the soil on the viability of *Erysipelothrix rhusiopathiae*. *J Am Vet Med Assoc* 1958; **132**: 357–361.
49. Stephenson EH, Berman DT. Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *Am J Vet Res* 1978; **39**: 187–188.
50. Wood RL. Isolation of pathogenic *Erysipelothrix rhusiopathiae* from feces of apparently healthy swine. *Am J Vet Res* 1974; **35**: 41–43.
51. Shuman RD. Erysipelas. In: Davis JW, Karstad LH, Trainer DO (eds) Infectious diseases of wild mammals. Ames, IA, Iowa State University Press. 1970: 267–272.
52. Heilman FR, Herrell WE. Penicillin in the treatment of experimental infections due to *Erysipelothrix rhusiopathiae*. *Proc Staff Meet Mayo Clin* 1944; **19**: 340–345.
53. Klauder JV. Erysipeloid and swine erysipelas in man. A clinical and bacteriologic review; swine erysipelas in the United States. *JAMA* 1926; **86**: 536–541.
54. Murase N, Suzuki K, Isayama Y, Murata M. Studies on the typing of *Erysipelothrix rhusiopathiae*. III. Serological behaviour of the strains isolated from the body surface of marine fishes and their epizootiological significance in swine erysipelas. *Jpn J Vet Sci* 1959; **21**: 215–219.
55. Proctor DM, Richardson IM. A report on 235 cases of erysipeloid in Aberdeen. *Br J Ind Med* 1954; **11**: 175–179.
56. Klauder JV. Erysipeloid as an occupational disease. *JAMA* 1938; **111**: 1345–1348.
57. Weaver RE. Erysipelothrix. In: Lennette A, Balows A, Hausler WJ, Shadomy HJ (eds) Manual of clinical microbiology, 4th edn. Washington, DC, American Society for Microbiology. 1985: 209–210.
58. Wood RL. Survival of *Erysipelothrix rhusiopathiae* in soil under various environmental conditions. *Cornell Vet* 1973; **63**: 390–410.
59. Ternstrom A, Molin G. Incidence of potential pathogens on raw pork, beef and chicken in Sweden, with special reference to *Erysipelothrix rhusiopathiae*. *J Food Protection* 1987; **50**: 141–146.
60. Stenstrom I-M, Norrung V, Ternstrom A, Molin G. Occurrence of different serotypes of *Erysipelothrix rhusiopathiae* in retail pork and fish. *Acta Vet Scand* 1992; **33**: 169–173.
61. McGinnes GF, Spindle F. Erysipeloid condition among workers in a bone button factory due to the bacillus of swine erysipelas. *Am J Public Health* 1934; **24**: 32–35.
62. Hunter D. The diseases of occupations, 5th edn. London, English University Press. 1975: 709–712.
63. Hillenbrand FKM. Whale finger and seal finger: their relation to erysipeloid. *Lancet* 1953; **1**: 680–681.
64. Connor MP, Green AD. Erysipeloid in a sheep farmer with coexisting orf. *J Infect* 1995; **30**: 161–163.
65. McClain JB. *Erysipelothrix rhusiopathiae*. In: Mandell GL, Douglas RG, Bennett JE (eds) Principles and practice of infectious diseases, 3rd edn. New York, Churchill Livingstone. 1990: 1599–1600.
66. King PF. Erysipeloid. Survey of 115 cases. *Lancet* 1946; **2**: 196–198.
67. Nelson E. 500 cases of erysipeloid. *Rocky Mountain Med J* 1955; **52**: 40–42.
68. Klauder JV. *Erysipelothrix rhusiopathiae* infection in swine and in human beings: comparative study of the cutaneous lesions. *Arch Dematol Syphil* 1944; **50**: 151–159.
69. Ehrlich JC. *Erysipelothrix rhusiopathiae* infection in man. Report of a case with cutaneous bullae, in which cure was achieved with penicillin. *Arch Intern Med* 1946; **78**: 565–577.
70. Fakoya A, Bendall RP, Churchill DR, Doherty JF, Ridgway GL. *Erysipelothrix rhusiopathiae* bacteraemia in a patient without endocarditis. *J Infect* 1995; **30**: 180–181.
71. Torkildsen A. Intracranial erysipeloid (swine erysipelas) abscess. Variety of abscess not hitherto observed. *Bull Hyg* 1943: 1013.
72. Silberstein EB. *Erysipelothrix* endocarditis: report of a case with cerebral manifestations. *JAMA* 1965; **191**: 862–864.
73. Schuster MG, Brennan PJ, Edelstein P. Persistent bacteremia with *Erysipelothrix rhusiopathiae* in hospitalized patient. *Clin Infect Dis* 1993; **17**: 783–784.
74. Hollick GE, Edinger RE. *Erysipelothrix* bacteremia. *Clin Microbiol Newsl* 1995; **17**: 9–16.
75. Giménez M, Fernández P, Padilla E, Matas L, Ausina V. Endocarditis and acute renal failure due to *Erysipelothrix rhusiopathiae*. *Eur J Clin Microbiol Infect Dis* 1996; **15**: 347–348.
76. Totemchokchyakarn K, Janwityanujit S, Sathapatayavongs B, Puavilai S. *Erysipelothrix rhusiopathiae* septicemia in systemic lupus erythematosus. *Int J Dermatol* 1996; **35**: 818–820.
77. Jones N, Khoosal M. *Erysipelothrix rhusiopathiae* septicemia in a neonate. *Clin Infect Dis* 1997; **24**: 511.
78. Brooke CJ, McLaughlin V, Mee BJ, Riley TV. An investigation of 'crayfish poisoning' in Western Australia. *Med J Aust* 1999; **170**: 288.
79. Sheard K, Dicks HG. Skin lesions among fishermen at Houtman's Abrolhos, Western Australia, with an account of erysipeloid of Rosenbach. *Med J Aust* 1949; **2**: 352–354.
80. Takahashi T, Sawada T, Ohmae K *et al*. Antibiotic resistance of *Erysipelothrix rhusiopathiae* isolated from pigs with chronic swine erysipelas. *Antimicrobial Agents Chemother* 1984; **25**: 385–386.
81. Venditti M, Gelfusa V, Tarasi A, Brandimarte C, Serra P. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae*. *Antimicrob Agents Chemother* 1990; **34**: 2038–2040.
82. Gledhill AW. Discussion on swine erysipelas infection (*Erysipelothrix rhusiopathiae*) in man and animals. *Proc R Soc Med* 1948; **41**: 330–332.
83. Noguchi N, Sasatsu M, Takahashi T, Ohmae K, Terakado N, Kono M. Detection of plasmid DNA in *Erysipelothrix rhusiopathiae* isolated from pigs with chronic swine erysipelas. *J Vet Med Sci* 1993; **55**: 349–350.
84. McCarty D, Bornstein S. *Erysipelothrix* endocarditis. Report on a septicemic form of the erysipeloid of Rosenbach. *Am J Clin Pathol* 1960; **33**: 39–42.
85. Spencer R. The sanitation of fish boxes. I. The quantitative and qualitative bacteriology of commercial wooden fish boxes. *J Appl Bacteriol* 1959; **22**: 73–84.
86. Mutalib AA, King JM, McDonough PL. Erysipelas in caged laying chickens and suspected erysipeloid in animal caretakers. *J Vet Diagn Invest* 1993; **5**: 198–201.
87. Price JEL, Bennett WEJ. The erysipeloid of Rosenbach. *B MJ* 1951; **2**: 1060–1062.
88. Wood RL. A selective liquid medium utilizing antibiotics for isolation of *Erysipelothrix insidiosa*. *Am J Vet Res* 1965; **26**: 1303–1308.

89. Harrington R, Hulse DC. Comparison of two plating media for the isolation of *Erysipelothrix rhusiopathiae* from enrichment broth culture. *Appl Microbiol* 1971; **22**: 141–142.
90. Bratberg AM. Observations on the utilization of a selective medium for the isolation of *Erysipelothrix rhusiopathiae*. *Acta Vet Scand* 1981; **22**: 55–59.
91. Dacres WG, Groth AH. Identification of *Erysipelothrix insidiosa* with fluorescent antibody. *J Bacteriol* 1959; **78**: 298–299.
92. Avilag C, Unzucta BB, Olguin RF. Indirect immunofluorescence test for the diagnosis of porcine erysipelas. *Veterinaria (Mexico)* 1972; **3**: 33–39.
93. Harrington R, Wood RL, Hulse DC. Comparison of a fluorescent antibody technique and cultural method for the detection of *Erysipelothrix rhusiopathiae* in primary broth cultures. *Am J Vet Res* 1974; **35**: 461–462.
94. Heggers JP, Buddington RS, McAllister HA. *Erysipelothrix* endocarditis diagnosis by fluorescent microscopy: report of a case. *Am J Clin Pathol* 1974; **62**: 803–806.
95. Makino S, Okada Y, Maruyama T *et al.* Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *J Clin Microbiol* 1994; **32**: 1526–1531.

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Comparison of Methods for Detection of *Erysipelothrix* spp. and Their Distribution in Some Australasian Seafoods

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For many years, *Erysipelothrix rhusiopathiae* has been known to be the causative agent of the occupationally related infection erysipeloid. A survey of the distribution of *Erysipelothrix* spp. in 19 Australasian seafoods was conducted, and methodologies for the detection of *Erysipelothrix* spp. were evaluated. Twenty-one *Erysipelothrix* spp. were isolated from 52 seafood parts. Primary isolation of *Erysipelothrix* spp. was most efficiently achieved with brain heart infusion broth enrichment followed by subculture onto a selective brain heart infusion agar containing kanamycin, neomycin, and vancomycin after 48 h of incubation. Selective tryptic soy broth, with 48 h of incubation, was the best culture method for the detection of *Erysipelothrix* spp. with PCR. PCR detection was 50% more sensitive than culture. *E. rhusiopathiae* was isolated from a variety of different fish, cephalopods, and crustaceans, including a Western rock lobster (*Panulirus cygnus*). There was no significant correlation between the origin of the seafoods tested and the distribution of *E. rhusiopathiae*. An organism indistinguishable from *Erysipelothrix tonsillarum* was isolated for the first time from an Australian oyster and a silver bream. Overall, *Erysipelothrix* spp. were widely distributed in Australasian seafoods, illustrating the potential for erysipeloid-like infections in fishermen.

Erysipelothrix spp. are widely distributed throughout nature, in soil as a saprophyte or wherever nitrogenous substances are decomposing. Currently, the genus *Erysipelothrix* is comprised of two species, *Erysipelothrix rhusiopathiae* and *Erysipelothrix tonsillarum* (23). Much of the literature on *Erysipelothrix* is old, and it is likely that organisms described as *E. rhusiopathiae* may actually be a mixture of *E. rhusiopathiae*, *E. tonsillarum*, and, potentially, other species. Commensal and pathogenic *E. rhusiopathiae* have been found in a variety of animals, including swine, sheep, and cattle, as well as in crustaceans and fresh and saltwater fish. Although not a pathogen of fish, *E. rhusiopathiae* can survive for long periods of time on the mucoid exterior slime of fish (18). Of the mammals, swine are the most profoundly affected by *E. rhusiopathiae* infection, and this has great economic importance (9, 18). Human infection, or erysipeloid, is mostly related to the opportunity for exposure to the organism. It is common among those who have direct contact with animals or with organic matter in which the organism is usually found, and thus infection is mostly occupationally related. Historically, erysipeloid has been referred to as fish poisoning, seal finger, whale finger, and pork finger (11, 12, 26). Those at highest risk of infection are fishermen, fish handlers, butchers, abattoir workers, and housewives; however, infection has also been associated with other occupations (18). In the marine environment, most infections occur following a scratch or puncture of the skin by the scales, teeth, bones, or spines of fish or lobster (12, 18). In Western Australia, an erysipeloid-like infection referred to as crayfish poisoning is known to occur in lobster fishermen and handlers (4).

One problem in the diagnosis of erysipeloid is that *E. rhusiopathiae* is difficult to recover from lesions, possibly because of inadequate specimens, but also because of its cultural characteristics. *E. rhusiopathiae* grows to form very small colonies after 24 to 48 h of incubation on most media (19), and it may be difficult to isolate in heavily contaminated samples. Thus, various selective media have been described, including sodium azide-crystal violet (SACV) or Packers medium containing tryptose broth or agar, sodium azide, crystal violet, and horse serum (16). While SACV medium is satisfactory for selecting *E. rhusiopathiae*, some difficulties in differentiating the very small *E. rhusiopathiae* colonies from *Streptococcus* spp. may occur (7). A modified blood-azide medium containing heart infusion agar, sodium azide, defibrinated bovine blood, and horse serum allowed for more rapid isolation of *E. rhusiopathiae* compared to SACV (24 h versus 48 h for growth); however, modified blood-azide medium was not as selective (10). A liquid medium, *Erysipelothrix* selective broth (ESB), containing tryptose broth base, 5% horse serum, and antibiotics (kanamycin [400 μg/ml], neomycin [50 μg/ml], and vancomycin [25 μg/ml]), was successful in culturing *E. rhusiopathiae*, although not all contaminants were inhibited (27). ESB was further investigated by Bratberg (3), who showed that not all *E. rhusiopathiae* isolates grew in this broth. This failure was thought to be due to the moderate susceptibility of some isolates to kanamycin and could be overcome by using lower concentrations of kanamycin (3).

In addition to culture methods, detection of *E. rhusiopathiae* has been achieved by using two PCR methods. One detects a 407-bp DNA fragment derived from the 16S rRNA coding sequence. While the primers used in this method are specific for *Erysipelothrix* spp., they do not differentiate between *E. rhusiopathiae* and *E. tonsillarum* (15). Shimoji et al. (22) developed a method which utilized a broth similar to SACV for the enrichment of *Erysipelothrix* spp. followed by PCR. In this case, detection of a 937-bp DNA fragment allowed for specific identification of *E. rhusiopathiae* (22).

The objective of our study was to evaluate several combinations of media, both selective and nonselective, for primary

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TABLE 1. Types of seafoods investigated in this study and their origins

Seafood type	Common name	Species	Origin
Fish	Blue mackerel	*Scomber australasicus*	Fremantle, WA[a]
	Herring	*Arripis georgianus*	Fremantle, WA
	Pink snapper	*Chrysophrys unicolor*	North of WA
	Sardine	*Sardinops sagnax*	Fremantle, WA
	Scaly mackerel	*Amblygaster postera*	Fremantle, WA
	Silver bream	*Acanthopagrus butcheri*	Fremantle, WA
	Yellow tail	*Seriola lalandi*	Fremantle, WA
Other	Australian oyster	*Saccostrea commercialis*	Tasmania and South Australia
	Banana prawn	*Penaeus merguiensis*	North of WA
	Blue manna crab	*Portunus pelagicus*	Farm in Fremantle, WA
	Coral prawn	*Metapenaeopsis* spp.	Carnarvon, WA
	Cuttlefish	*Sepia officinalis*	Carnarvon, WA
	King prawn	*Penaeus latisulcatus*	North of WA
	Local mussel	*Mytilus edulis*	South of WA
	New Zealand mussel	*Perna canaliculus*	New Zealand
	Octopus	*Octopus australis*	North of WA
	Pacific oyster	*Crassostrea gigas*	New Zealand
	Squid	*Teuthoidea* spp.	North of WA
	Western rock lobster	*Panulirus cygnus*	Fremantle, WA

[a] WA, Western Australia.

isolation of *Erysipelothrix* spp. and to assess a PCR detection method by using primers described previously (15). In order to achieve this, a survey of the distribution of *Erysipelothrix* spp. in different seafoods was undertaken.

MATERIALS AND METHODS

Bacterial strain. *E. rhusiopathiae* ATCC 19414 was used as a positive control and *E. tonsillarum* ATCC 43339 was used as a negative control for both cultural and molecular identification procedures.

Seafood. Seafood samples were purchased from three seafood markets and directly from lobster fishing boats in the port of Fremantle, Western Australia. Over one-third of the samples (37%) had been caught in local waters and 37% had been caught in waters 1,000 km north of Fremantle. The remaining samples were from southern Western Australia, New Zealand, South Australia, Tasmania, and an aquaculture establishment at Fremantle. None of the seafood had been washed, and lobsters were purchased alive: two directly from a lobster fishing boat and one from a live storage facility. The seafoods and their origins are given in Table 1.

Media. Two types of selective enrichment broths and one nonselective broth, as well as selective and nonselective agar plates, were assessed. Brain heart infusion broth (BHIB) and agar (BHIA) (Oxoid, Ltd., Basingstoke, Hampshire, United Kingdom) were prepared according to the manufacturer's recommendations and were sterilized by autoclaving at 121°C for 15 min. Once cooled to room temperature, nonselective BHIB and selective BHIB (BHIB/S) were supplemented with 5% horse serum. For BHIB/S, the following antibiotics were added: kanamycin (40 μg/ml), neomycin (50 μg/ml), and vancomycin (25 μg/ml). Selective BHIA (BHIA/S) consisted of molten agar cooled to 50°C supplemented with serum and antibiotics as for BHIB/S. Selective Trypticase soy broth (TSB/S) (Becton Dickinson and Co., Cockeysville, Md.) was prepared as described previously (22) and contained tryptic soy broth (pH 7.6) supplemented with 0.3% Tris, 0.1% Tween 80, 0.03% sodium azide, and crystal violet (5 μg/ml).

Isolation of *Erysipelothrix* spp. from seafood. While handling seafood, gloves were worn at all times. After collection, seafood was placed into separate clean plastic bags, and care was taken to ensure no cross contamination. The seafood was cut aseptically into small portions which were immersed in 10-ml portions of each of the three broths and was vortexed for 30 s. All broths were incubated at 37°C. After 24 and 48 h, BHIB was subcultured onto BHIA/S, and TSB/S and BHIB/S was subcultured onto horse blood agar (HBA) (Oxoid). The agar plates were incubated at 37°C and, after 24 and 48 h, were examined for growth of suspected *Erysipelothrix* colonies which were small (approximately 0.1 mm), convex, circular, and transparent (19). Suspect colonies were Gram stained, and gram-positive rods were kept for confirmation by PCR. Isolates were stored as heavy suspensions in BHIB plus 10% glycerol at −70°C.

PCR detection of *Erysipelothrix* spp. DNA extraction from pure and enrichment cultures. Frozen isolates were thawed, streaked onto HBA, and incubated at 37°C for 24 h. After 24 h, a few colonies were suspended in 100 μl of sterile diethylpyrocarbonate (DEPC) water (2) in sterile Eppendorf tubes and then heated to 100°C for 15 min. Heated samples were centrifuged in a microcentrifuge at 10,000 × *g* for 2 min, and the supernatant was transferred to sterile Eppendorf tubes and was frozen at −70°C for later use in the PCR.

PCR was performed on all enrichment cultures at both 24 and 48 h. Broths were vortexed vigorously, 1.5 ml of the suspension was transferred to sterile Eppendorf tubes, and organisms were pelleted by centrifugation at 10,000 × *g* for 3 min. The pellet was then washed by resuspension in 1.5 ml of DEPC water. After further centrifugation, the supernatant was removed, and the pellet was resuspended in 100 μl of sterile DEPC water. The samples were then treated in the same way as the pure cultures.

PCR primers. The primers used were MOIO1 and MOIO2 (Gibco BRL, Paisley, United Kingdom), derived from the DNA sequence coding for 16S rRNA of *Erysipelothrix* spp. (GenBank/EMBL accession no. M23728) (15).

DNA amplification. The PCR method was modified from that previously described (15). PCR was carried out in a DNA thermocycler (GeneAmp 9700; Perkin-Elmer, Foster City, Calif.) in 20 μl of a reaction mixture containing 1× Perkin Elmer buffer II (100 mM Tris-HCl, pH 8.3, and 500 mM KCl) 2.0 mM $MgCl_2$, 0.2 mM each deoxyribonucleotide triphosphate (dNTP) (Boehringer GmbH, Mannheim, Germany), 0.2 μM each primer (Gibco BRL), 0.5 U of *Taq* DNA polymerase (Perkin-Elmer), and 4.0 μl of extracted DNA sample.

The amplification consisted of initially heating at 94°C for 15 min and then 45 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 45 s, followed by an additional extension step at 72°C for 7 min and cooling to 4°C.

In preliminary experiments, attempts were made to enhance the sensitivity of the PCR by including a second reaction with 0.4 μl of the first-round reaction product as template. In a sample of 23 seafoods cultured in various enrichment broths, the proportion of positive PCRs increased from 31 to 38% with a second PCR. Only one of the 115 PCRs changed from positive to negative with a second amplification and therefore, double amplification PCR was used throughout the study.

All broth culture samples were tested twice on separate days. If discrepant results were obtained, then the analysis was repeated. If the third PCR was positive, then the overall result was considered positive. If the third PCR was negative, then the overall result was considered negative. The pure culture samples were tested once to confirm for *Erysipelothrix* spp.

Agarose gel electrophoresis. Aliquots of the amplification reactions were electrophoresed on 2% (wt/vol) agarose gels (Seakem LE agarose; FMC Bioproducts, Rockland, Maine) containing ethidium bromide (0.5 μg/ml). Bands were visualized on a UV transilluminator and were compared to the positive control, *E. rhusiopathiae* ATCC 19414, and a DNA Molecular Weight VIII (Boehringer) standard. A band of 407 bp was considered diagnostic for *Erysipelothrix* spp.

Differentiation of *Erysipelothrix* spp. Carbohydrate fermentation. To differentiate *E. rhusiopathiae* from *E. tonsillarum*, fermentation of glucose and sucrose was used. The method described by White and Shuman (25) was modified by replacing Andrade's fermentation broth with agar.

PCR detection of *E. rhusiopathiae*. The PCR method described by Shimoji et al. (22) was used with slight modifications to differentiate *E. rhusiopathiae* from *E. tonsillarum*. The basic protocol was the same as for PCR detection of *Erysipelothrix* spp. from the pure and enriched cultures with alterations to the reaction mixtures and amplification cycles.

PCR primers. The primers used were ER1 and ER2 (Gibco BRL), derived from a region possibly coding for *E. rhusiopathiae* virulence (GenBank/EMBL accession no. D64177) (22).

DNA amplification. PCR was carried out in 20-μl samples of reaction mixture as before; however, the $MgCl_2$ concentration was decreased to 1.5 mM. Amplification consisted of initially heating at 94°C for 5 min and then 30 cycles of denaturation at 94°C for 1 min, annealing at 63°C for 30 s, and extension at 72°C for 1 min, followed by an additional extension step at 72°C for 7 min and cooling to 4°C. A band of 937 bp was considered diagnostic of *E. rhusiopathiae*.

In preliminary experiments, the specificity of *Erysipelothrix*- and *E. rhusiopathiae*-specific PCR methods was tested with several marine bacteria (*Vibrio*, *Pseudomonas*, *Acinetobacter*, *Proteus*, and *Aeromonas*). The results indicated that the primers were specific for *Erysipelothrix* spp. with MOIO1 and -2 and *E. rhusiopathiae* with ER1 and -2.

Statistical analysis. The chi-square test was used to detect statistically significant differences between proportions.

TABLE 2. Distribution of *Erysipelothrix* spp. from different seafoods according to culture and PCR results[a]

Seafood type	No. of parts tested	Culture positive		Culture negative	
		PCR positive	PCR negative	PCR positive	PCR negative
Bivalves	10	1 (2)	0 (0)	2 (4)	7 (13)
Cephalopods	7	2 (4)	0 (0)	3 (6)	2 (4)
Crustaceans	18	3 (6)	0 (0)	14 (27)	1 (2)
Fish	17	15 (29)	0 (0)	2 (4)	0 (0)

[a] Numbers in parentheses indicate percent positive out of the total of 52 seafood parts.

RESULTS

Evaluation of culture methods for the isolation of *Erysipelothrix* spp. A total of 52 different seafood parts from 19 different aquatic species were examined. Twenty-one suspected *Erysipelothrix* spp. were isolated, some by more than one of the six culturing methods. The best recovery of *Erysipelothrix* spp. was achieved by using BHIB enrichment followed by subculture to BHIA/S. After 24 and 48 h of incubation, 15 (29%) and 19 (37%) of the 52 different seafood parts, respectively, were culture positive. TSB/S enrichment followed by subculture to HBA resulted in the recovery of *Erysipelothrix* spp. from 8 (15%) and 11 (21%) of the 52 seafood parts after 24 and 48 h of incubation, respectively. The poorest recovery of only two isolates (4%), at both 24 and 48 h, was achieved by enrichment in BHIB/S followed by subculture to HBA. The number of *Erysipelothrix* spp. isolated by using the 48-h BHIB to BHIA/S protocol was significantly higher than those obtained by using the BHIB/S to HBA protocol and the 24-h TSB/S to HBA protocol ($P < 0.05$).

Evaluation of PCR detection of *Erysipelothrix* spp. from different broths. The best PCR detection of *Erysipelothrix* spp. was achieved with the TSB/S 48-h enrichment culture, where 35 (67%) of the 52 seafood parts were positive. From BHIB/S 48-h and TSB/S 24-h enrichment cultures, 29 (56%) and 26 (50%), respectively, of the 52 seafood parts were positive. The BHIB 48-h enrichment cultures produced 25 positives (48%), while the lowest detection rate of nine positives (31%) came from BHIB/S 24-h enrichment cultures. TSB/S at 48 h was significantly better than all of the other combinations of enrichment broths and incubation times ($P < 0.05$), except 24-h TSB/S and 48-h BHIB/S followed by subculture to HBA.

Four percent of enrichment cultures were PCR positive after 24 h and PCR negative after 48 h of incubation, and 23% were PCR negative after 24 h and PCR positive after 48 h of incubation.

Of 56 culture-positive enrichment broths, 16 (29%) were initially PCR negative. Dilutions of these samples achieved PCR-positive results in all cases.

Differentiation of *Erysipelothrix* spp. Of the 21 isolates which were positive by *Erysipelothrix*-specific PCR, 19 fermented glucose but not sucrose, consistent with *E. rhusiopathiae*. The other two fermented both glucose and sucrose, possibly suggesting *E. tonsillarum*. The 19 glucose-fermenting isolates were also positive by *E. rhusiopathiae*-specific PCR.

Distribution of *Erysipelothrix* spp. in different seafoods by culture and PCR. The distribution of *Erysipelothrix* spp. in different seafood groups, using both culture and PCR results, is shown in Table 2. All isolates that were cultured were also detected by PCR; however, PCR gave additional positives. The highest isolation (culture positive) and detection (PCR positive) of *Erysipelothrix* spp. was 15 positives (29%) from all the different fish parts. Fourteen of these isolates were *E. rhusiopathiae*. In addition to the silver bream being culture and PCR positive for *E. rhusiopathiae*, *E. tonsillarum* was cultured. *E. tonsillarum* was also cultured from the Australian oyster shell. The antennae, leg, and carapace of the Western rock lobster obtained directly from the boat and surfaces of one octopus and the squid were all culture and PCR positive for *E. rhusiopathiae*. The highest PCR detection of *Erysipelothrix* spp. was achieved from crustaceans, with 14 positives (27%). All the prawns, various parts of the Blue manna crab, and the other two Western rock lobsters were culture negative and PCR positive, as were the two Pacific oyster shells and the cuttlefish. The lowest PCR detection of two positives (4%) was from the bivalves. *Erysipelothrix* spp. were not isolated or detected from the flesh of either the Australian or Pacific oysters or from the local and New Zealand mussel shells.

DISCUSSION

Infection with *Erysipelothrix* has been recognized for over 100 years. It has been suggested that the frequency of human infection is declining (18). While this may be true, it is also likely that infection is underdiagnosed because of the resemblance of erysipeloid to other infections and the problems that may be encountered in isolation and identification of the organism (5). One aim of our study, therefore, was to evaluate various cultural and molecular methods for the detection of *Erysipelothrix* spp. Much of the literature on the recovery of *Erysipelothrix* spp. from human, veterinary, and environmental specimens is old; however, most investigations have employed a two-stage process of enrichment in broth followed by plating onto solid media. A variety of selective agents, both antibiotic and chemical, have been used (5). We chose to evaluate three protocols with subculture after both 24 and 48 h of enrichment: nonselective enrichment in BHIB followed by plating on BHIA made selective by the addition of kanamycin, neomycin, and vancomycin (BHIA/S); selective enrichment in Trypticase soy broth with sodium azide and crystal violet as selective agents (TSB/S) followed by plating on HBA; and enrichment in selective BHIB followed by plating on HBA. Because of the unknown prevalence of *Erysipelothrix* spp. in routine human clinical specimens, a variety of seafood samples was chosen for the evaluation (26). *Erysipelothrix* spp. were recovered from 21 of the 52 seafood parts cultured, 19 of which were *E. rhusiopathiae* and two of which were possibly *E. tonsillarum*. Of the three protocols evaluated, the BHIB incubated for 48 h followed by subculture to BHIA/S was significantly more selective than the other two, and 19 isolates from the 52 seafood parts were recovered with this combination. ESB has been regarded as the best selective medium, despite the problem relating to

the kanamycin susceptibility of some strains (3). Our slight modification of ESB performed quite poorly, and the reasons for this are unclear.

In contrast, PCR detection of *Erysipelothrix* spp. was better following 48 h of selective enrichment. Thirty-seven of the 52 samples of seafood were PCR positive following enrichment in TSB/S. There were an additional five PCR positives from other enrichment broths (Table 2). Why TSB/S was so superior for PCR detection is unclear. Many enrichment media are known to inhibit PCR (14). This may be less so with TSB/S, or the broth may have stabilized the template DNA. All samples that were culture positive were also PCR positive; however, PCR detected an extra 21 positives (Table 2). While it might be argued that these were false positives, it is well recognized that PCR techniques are significantly more sensitive than culture (24). In several cases, the initial correlation between culture and PCR was not good. Four percent of enrichment cultures were PCR positive after 24 h of incubation and were PCR negative after 48 h of incubation. This is likely to have been caused by too much DNA template being present, a factor known to reduce the efficiency of the PCR, or inhibitors may have been present within the sample (1) or the enrichment media (14). In addition, 29% of the samples that were culture positive were initially PCR negative. Diluting these broths resulted in positive PCRs, again suggesting that too much DNA or PCR inhibitor was present in the initial sample. For optimal results, PCR at both 24 and 48 h of incubation is recommended.

E. rhusiopathiae was widely distributed on the various seafoods tested, and all fish were positive. It is well recognized that fish are a common source of *E. rhusiopathiae* (17, 21), and infection can be most severe when contracted from a fish (13). *E. rhusiopathiae* was also isolated from Western rock lobster. Early reports suggested that anglers may have been infected from crustaceans through puncture wounds made by the claws of spiny lobsters and crabs (8). In Western Australia, infections associated with *E. rhusiopathiae* have been recently recognized in lobster fishermen (4). *E. rhusiopathiae* was also isolated from both octopus and squid from the north of the state. Even though these seafoods are unlikely to cause puncture wounds, they may contaminate existing wounds, leading to infection.

About one-third of the seafoods were only PCR positive for *Erysipelothrix* spp. These seafoods had no particular pattern of distribution, and they had originated from throughout Western Australia and New Zealand. The fact that these seafoods were PCR positive and culture negative suggests that *Erysipelothrix* spp. DNA was present and the organism was not viable. One possibility which may have to be considered is that *Erysipelothrix* spp. can exist in a viable but not culturable state, as many other aquatic organisms have been shown to do (6). Another possibility is that there may have been some cross contamination between the different seafood types after they were caught; this needs to be investigated further.

One surprising result of this study was the isolation of two organisms that could not be distinguished from *E. tonsillarum*. *E. tonsillarum* has not been previously associated with seafood as it was in this investigation, with isolates from silver bream and the shell of an Australian oyster. This organism is thought to be avirulent for swine, mice, and chickens; however, pathogenicity for humans has not been investigated (5).

Overall, *Erysipelothrix* spp. were widely distributed on Australasian seafoods, although there was no correlation between the origins of the seafoods tested and the distribution of *Erysipelothrix* spp. *E. rhusiopathiae* was associated with fish, cephalopods, and crustaceans, all of which may either cause injury or contaminate an existing wound during capture, resulting in infection.

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REFERENCES

1. **Ausubel, F. M.** 1992. The polymerase chain reaction, p. 15.1.5. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology, vol. 2. John Wiley and Sons, Inc., Toronto, Canada.
2. **Ausubel, F. M.** 1995. Preparation and analysis of RNA, p. 4.4.4. *In* F. M. Ausubel, R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith, and K. Struhl (ed.), Current protocols in molecular biology, vol. 1. John Wiley and Sons, Inc., Toronto, Canada.
3. **Bratberg, A. M.** 1981. Observations on the utilisation of a selective medium for the isolation of *Erysipelothrix rhusiopathiae*. Acta Vet. Scand. **22**:55–59.
4. **Brooke, C. J., V. McLaughlin, B. J. Mee, and T. V. Riley.** 1999. An investigation of 'crayfish poisoning' in Western Australia. Med. J. Aust. **170**:288.
5. **Brooke, C. J., and T. V. Riley.** 1999. *Erysipelothrix rhusiopathiae*: bacteriology, epidemiology and clinical manifestations of an occupational pathogen. J. Med. Microbiol. **48**:789–799.
6. **Desmonts, C., J. Minet, R. Colwell, and M. Cormier.** 1990. Fluorescent-antibody method useful for detecting viable but nonculturable *Salmonella* spp. in chlorinated wastewater. Appl. Environ. Microbiol. **56**:1448–1452.
7. **Ewald, F. W.** 1981. The genus *Erysipelothrix*, p. 1688–1700. *In* M. P. Starr, H. Stolp, H. G. Truper, A. Balows, and H. G. Schlegel (ed.), The prokaryotes: a handbook on habitats, isolation and identification of bacteria. Springer-Verlag, New York, N.Y.
8. **Gilchrist, T. C.** 1904. Erysipeloid, with a record of 329 cases, of which 323 were caused by crab bites, or lesions produced by crabs. J. Cutan. Dis. **22**:507–519.
9. **Gorby, G. L., and J. E. Peacock, Jr.** 1988. *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. Rev. Infect. Dis. **10**:317–325.
10. **Harrington, R., Jr., and D. C. Hulse.** 1971. Comparison of two plating media for the isolation of *Erysipelothrix rhusiopathiae* from enrichment broth culture. Appl. Microbiol. **22**:141–142.
11. **Hillenbrand, F. K. M.** 1953. Whale finger and seal finger; their relation to erysipeloid. Lancet **i**:680–681.
12. **Hunter, D.** 1974. The diseases of occupations, 5th ed., p. 709–712. The English University Press, Ltd., London, England.
13. **Klauder, J. V.** 1938. Erysipeloid as an occupational disease. JAMA **111**: 1345–1348.
14. **Lantz, P. G., R. Knutsson, Y. Blixt, W. A. Al Soud, E. Borch, and P. Radstrom.** 1998. Detection of pathogenic *Yersinia enterocolitica* in enrichment media and pork by multiplex PCR: a study of sample preparation and PCR-inhibitory components. Int. J. Food Microbiol. **45**:93–105.
15. **Makino, S., Y. Okada, T. Maruyama, K. Ishikawa, T. Takahashi, M. Nakamura, T. Ezaki, and H. Morita.** 1994. Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. J. Clin. Microbiol. **32**:1526–1531.
16. **Packer, R. A.** 1943. The use of sodium azide and crystal violet in a selective medium for *streptococci* and *Erysipelothrix rhusiopathiae*. J. Bacteriol. **46**:343–349.
17. **Pestana de Castro, A. F., O. Campedelli Filho, and C. Troise.** 1967. Isolation of *Erysipelothrix rhusiopathiae* from marine fish. Rev. Inst. Med. Trop. Sao Paulo **9**:169–171.
18. **Reboli, A. C., and W. E. Farrar.** 1989. *Erysipelothrix rhusiopathiae*: an occupational pathogen. Clin. Microbiol. Rev. **2**:354–359.
19. **Reboli, A. C., and W. E. Farrar.** 1992. The genus *Erysipelothrix*, p. 1629–1642. *In* A. Balows, H. G. Truper, M. Dworkin, W. Harder, and K. Schleifer (ed.), The prokaryotes. A handbook on the biology of bacteria: ecophysiology, isolation, identification and applications. Springer-Verlag, New York, N.Y.
20. **Sheard, K., and H. G. Dicks.** 1949. Skin lesions among fishermen at Houtman's Abrolhos, Western Australia, with an account of erysipeloid of Rosenbach. Med. J. Aust. **ii**:352–354.
21. **Shewan, J. M.** 1971. The microbiology of fish and fishery products—a progress report. J. Appl. Bacteriol. **34**:299–315.
22. **Shimoji, Y., Y. Mori, K. Hyakutake, T. Sekizaki, and Y. Yokomizo.** 1998. Use of an enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. J. Clin. Microbiol. **36**:86–89.
23. **Takahashi, T., T. Fujisawa, Y. Tamura, S. Suzuki, M. Muramatsu, T. Sawada, Y. Benno, and T. Mitsuoka.** 1992. DNA relatedness among *Erysip-*

elothrix rhusiopathiae strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. Int. J. Syst. Bacteriol. **42**:469–473.
24. **Tompkins, L. S.** 1998. Molecular epidemiology in infectious diseases, p. 33–34. *In* S. L. Gorbach, J. G. Bartlett, and N. R. Blacklow (ed.), Infectious diseases, 2nd ed. WB Saunders Co., Philadelphia, Pa.
25. **White, T. G., and R. D. Shuman.** 1961. Fermentation reactions of *Erysipelothrix rhusiopathiae*. J. Bacteriol. **82**:595–599.
26. **Wood, R. L.** 1975. *Erysipelothrix* infection, p. 271–281. *In* W. T. Hubbert, W. F. McCullough, and P. R. Schnurrenberger (ed.), Diseases transmitted from animals to man, 6th ed. Thomas, Springfield, Ill.
27. **Wood, R. L.** 1965. A selective liquid medium utilising antibiotics for isolation of *Erysipelothrix insidiosa*. Am. J. Vet. Res. **26**:1303–1308.

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The detection and recovery of *Erysipelothrix* spp. in meat and abattoir samples in Western Australia

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Aims: To investigate the occurrence of *Erysipelothrix rhusiopathiae* and other *Erysipelothrix* spp. in abattoir and meat samples in Western Australia.

Methods and Results: Samples were collected from various parts of pig and sheep carcasses, as well as different sections of slaughtering line, pen soil and effluent. Previously evaluated culture methods were applied for the isolation of *Erysipelothrix* spp., in conjunction with phenotypic and genotypic detection and identification procedures. Of 109 samples from the two abattoirs, 35 (32·1%) were *Erysipelothrix* genus-specific PCR-positive. These came from swabs of animal exterior surfaces and joints, slaughtering areas, pig pen soil and abattoir effluent. Four samples (3·7%) from sheep arthritic joints and pig abattoir effluent were also *E. rhusiopathiae* species-specific PCR-positive. Of 123 carcass washing samples, 12 (9·8%) were genus-specific PCR-positive, and these came from all five kinds of meat samples tested, including beef, lamb, mutton, pork and chicken. Four of them (3·3%) were also species-specific PCR-positive. A total of 25 isolates was recovered from the samples, of which seven were identified as *E. rhusiopathiae*, seven were consistent with *E. tonsillarum*, and the remaining 11 were other species of *Erysipelothrix*.

Conclusions: *Erysipelothrix* spp. can still be isolated and identified from specimens of animal origin with relative ease, provided that appropriate cultural and molecular procedures are used. Clinical microbiology laboratories may need to improve their diagnostic protocols.

Significance and Impact of the Study: This study confirms that *E. rhusiopathiae* and other species of *Erysipelothrix* continue to colonize and contaminate farmed animals and animal products. *Erysipelothrix* infection still poses a potential threat to the economy of the farmed animal industry, as well as being a potential human public health hazard.

INTRODUCTION

Erysipelothrix rhusiopathiae is the causative agent of erysipelas in animals and erysipeloid in humans. The organism is distributed worldwide and has been isolated from many species of wild and domestic mammals and birds, as well as from reptiles, amphibians and fish (Shuman 1971; Reboli and Farrar 1992). Diseases of economic importance in animals include: swine erysipelas, which presents as skin lesions, acute septicaemia and chronic arthritis; polyarthritis, commonly seen in sheep and lambs (Conklin and Steele 1979); and erysipelas of farmed turkeys, chickens and emus (Blackmore and Gallagher 1964; Morgan *et al.* 1994). Human infection is usually a consequence of occupational contact with infected animals, their products or waste. It is prevalent in abattoir workers, butchers, farmers and fishermen (Hillenbrand 1953; Hunter 1975; Wood 1975). Erysipeloid is the most common manifestation of human disease and is characterized by swelling and redness of the infected parts of the body, typically the fingers. Cases of bacteraemia with endocarditis have been reported also (Gorby and Peacock 1988). It has been suggested that *Erysipelothrix* infections in man and animals may be declining due to better animal handling practices (Reboli and Farrar 1989).

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The genus *Erysipelothrix* consists of at least two species, *Erysipelothrix rhusiopathiae* and *E. tonsillarum*; other unnamed species have been reported (Takahashi *et al.* 1992). Traditionally, identification of *E. rhusiopathiae* has been based on morphology, biochemical reactions such as sugar fermentation, haemolysis on blood agar and a mouse protection confirmatory test (Jones 1986). *Erysipelothrix tonsillarum* is morphologically and biochemically indistinguishable from *E. rhusiopathiae*, apart from the ability to ferment sucrose (Takahashi *et al.* 1992). A PCR method of identification based on a DNA sequence encoding 16 s rRNA was developed for the diagnosis of swine erysipelas (Makino *et al.* 1994). This method is *Erysipelothrix* genus-specific and produces a 407 bp band. In 1998, Shimoji *et al.* (1998a) developed a species-specific *E. rhusiopathiae* PCR which allows the separation of *E. rhusiopathiae* from other species of *Erysipelothrix* and makes the identification of *E. rhusiopathiae* faster and more specific.

In this study, these methods were used to investigate the occurrence of *E. rhusiopathiae* in Western Australia. Samples were collected from pig and sheep abattoirs near Perth. Previously evaluated culture methods (Fidalgo *et al.* 2000) were applied for the isolation of *Erysipelothrix* spp. in conjunction with molecular detection and identification.

MATERIALS AND METHODS

Bacterial strains

Two type strains, *E. rhusiopathiae* ATCC 19414 and *E. tonsillarum* ATCC 43339, were used as controls in both genotypic and phenotypic identification procedures.

Samples

From April to December 1999, surveys were conducted separately in a pig abattoir and a sheep abattoir located approximately 80 km east of Perth, Western Australia. Samples were collected from various parts of pig and sheep carcasses, as well as from different sections of the slaughtering line, pen soil and effluent. Samples from the carcass exterior, some inner organs, and the slaughtering line were collected with sterile cotton swabs. Samples of tonsil, lymph node, heart, lung and liver were collected by removing a small piece of the tissue with a sterile scalpel and placing it in a 50 ml sterile plastic container. Joint fluid samples were collected by aseptically opening the joints and collecting fluid in the joint chamber with a sterile swab. Abattoir pen soil samples from different locations were collected using moistened swabs. Effluent from drains was collected into 50 ml sterile plastic containers. All samples were taken to the microbiological laboratory on the day of collection for immediate processing. Carcass washing samples were obtained from Food Hygiene and Waters Examination Laboratories of the Western Australian Centre for Pathology and Medical Research. These samples were collected by environmental health workers as part of a monitoring programme conducted by the Health Department of Western Australia for food-borne pathogens. Meat washing samples screened for the presence of *Erysipelothrix* spp. included beef, lamb, mutton, pork and chicken.

Isolation and identification of *Erysipelothrix* spp.

Erysipelothrix spp. were isolated and identified as described previously (Fidalgo *et al.* 2000). Briefly, each swab sample or 1·5 ml of carcass washings was placed into 10 ml brain heart infusion broth containing 5% horse serum (BHIB/N). After incubation for 48 h at 37°C, a loopful of the enriched culture broth was streaked onto selective brain heart infusion agar plates containing kanamycin (40 mg l^{-1}), vancomycin (50 mg l^{-1}), neomycin (25 mg l^{-1}) and 5% horse serum (BHIA/S). After incubation for 48 h at 37°C, the BHIA/S agar plates were examined for growth of suspected *Erysipelothrix* colonies. The Gram-positive rods were kept for identification by PCR, biochemical testing and sugar fermentation. Colonies were subcultured for purity onto blood agar and, once purified, stored in BHIB plus 10% glycerol at − 70°C. Some primary culture plates gave two different colonial morphotypes (see Results).

Biochemical tests

API Coryne strips (bioMérieux SA, France) were used in the identification of the isolates. Additional glucose and sucrose fermentation tests were conducted using the method described by White and Shuman (1961). The production of H_2S was tested on triple sugar iron (TSI) agar supplemented with 10% horse serum.

DNA preparation

Cells from 1·5 ml enriched broth culture were recovered by centrifugation in a 1·5 ml Eppendorf tube for 3 min at 12 000 *g*. The supernatant fluid was discarded, then the pellet was washed three times with 1·5 ml sterile diethylpyrocarbonate (DEPC) water, resuspended in 100 μl DEPC water and boiled for 15 min. After centrifugation for 1 min at 12 000 *g*, the supernatant fluid was transferred to another tube and stored at − 70°C for PCR analysis. DNA was extracted from single colonies by picking 8–10 colonies following 24 h incubation on blood agar plates at 37°C. The cells were resuspended in 100 μl sterile DEPC water in

Eppendorf tubes which were heated to 100°C for 15 min. The samples were centrifuged at 12 000 *g* for 1 min and the supernatant fluid frozen at − 70°C for PCR analysis.

PCR primers

The oligonucleotide primers were obtained from Gibco BRL (Paisley, UK). They were genus-specific primers MO101 (5′-AGA TGC CAT AGA AAC TGG TA-3′) and MO102 (5′-CTG TAT CCG CCA TAA CTA-3′) based on the DNA sequence encoding 16S rRNA of *E. rhusiopathiae* (Makino *et al.* 1994), and species-specific primers ER1 (5′-CGA TTA TAT TCT TAG CAC GCA ACG-3′) and ER2 (5′-TGC TTG TGT TGT GAT TTC TTG ACG-3′) based on the virulence-coding region of the *E. rhusiopathiae* chromosome (Shimoji *et al.* 1998a).

DNA amplification

The genus-specific PCR method described by Makino *et al.* (1994) was used with some modification. PCR was carried out in a DNA thermocycler (GeneAmp 9700; Perkin-Elmer, Foster City, CA, USA) in 20 μl of a reaction mixture containing 1 × PCR buffer (100 mmol l^{-1} Tris–HCl, pH 8·3 and 500 mmol l^{-1} KCl), 2·0 mmol l^{-1} $MgCl_2$, 0·2 mmol l^{-1} each deoxyribonucleotide triphosphate (dNTP, Boehringer), 0·2 μmol l^{-1} each of primers (MO101 and MO102), 0·5 unit *Taq* DNA polymerase (Perkin-Elmer) and 4·0 μl DNA sample. The PCR amplification consisted of initially heating at 94°C for 15 min and then 45 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 45 s, followed by an additional extension at 72°C for 7 min and cooling to 4°C. The amplified products were electrophoresed at 100 V on 2% (w/v) agarose gel in Tris–borate buffer containing 0·5 mg l^{-1} ethidium bromide, and the bands were photographed under u.v. light. The type strain ATCC 19414 was used for control, producing a band of 407 bp. DNA Molecular Weight VIII (Boehringer) was used as size standard. The species-specific PCR method described by Shimoji *et al.* (1998a) was used with slight modifications for the detection of *E. rhusiopathiae.* PCR was conducted in 20 μl reaction mixture with 1·5 mmol l^{-1} $MgCl_2$ and the primers ER1 and ER2. The PCR amplification consisted of initially heating at 94°C for 5 min, and then 30 cycles of denaturation at 94°C for 1 min, annealing at 63°C for 30 s and extension at 72°C for 1 min, followed by an additional extension at 72°C for 7 min and cooling to 4°C. A band of 937 bp was specific for *E. rhusiopathiae.*

RESULTS

Detection of *Erysipelothrix* spp. in pig and sheep abattoirs

A total of 109 samples from two abattoirs was examined. Thirty-five samples (32·1%) were genus-specific PCR-positive, most coming from pig pen soil, animal joints, abattoir effluent, slaughtering areas and animal exterior surfaces. Four samples (3·7%) from sheep arthritic joints and pig abattoir effluent were species-specific PCR-positive. Culture yielded 15 putative *Erysipelothrix* isolates (13·8%), 12 of which were genus-specific PCR-positive but species-specific PCR-negative. Three isolates, two from sheep arthritic joints and one from the pig abattoir effluent, were positive for both PCRs. These results are summarized in Table 1.

Table 1 PCR results from pig and sheep abattoir samples and isolates

		Samples		Isolates	
Source	*n*	G-PCR*(+)	S-PCR†(+)	G-PCR(+)	S-PCR(+)
Joints	12	5	2		2
Effluent	10	5	2	2	1
Carcass surface	3	2		2	
Blood	7	4		1	
Soil	23	11		7	
Mouth	10	3			
Lungs, liver and intestine	4	1			
Slaughtering areas	21	4			
Tonsil, heart and lymph nodes	19	0			
Total	109	35(32·1%)	4(3·7%)	12(11·0%)	3(2·8%)

*Genus-specific PCR (Makino *et al.* 1994).
†Species-specific PCR (Shimoji *et al.* 1998a).

Occurrence of *Erysipelothrix* spp. in carcass washing samples

A total of 123 carcass washing samples was processed. The 12 samples (9·8%) that were genus-specific PCR-positive came from all five kinds of meat tested. Of the 12 positives, four (3·3%), from samples of lamb, pork and mutton, were species-specific PCR-positive. Five isolates resembling *Erysipelothrix* were cultured from washings; four from lamb, pork and mutton washings were both genus- and species-specific PCR-positive and one was genus-specific PCR-positive but species-specific PCR-negative. The sample sources and results are given in Table 2.

Identification of isolates

In total, 20 isolates resembling *Erysipelothrix* spp. were initially recovered. All stained as Gram-positive rods. Pure cultures were obtained by subculturing the suspected colonies, which were small (approximately 0·1 mm in diameter), convex, circular and transparent, from BHIA/S onto blood agar plates. Five of the isolates, D22, D23, D65, D67 and M4, demonstrated two forms of colonies on BHIA/S plates after 48 h incubation at 37°C: one form was rough, larger (approximately 0·5–1·0 mm in diameter) and light blue, and was termed -fbl, and the other was smooth, small (around 0·1–0·2 mm in diameter) and yellowish, and was termed -yws. Both forms had identical morphology by light microscopy.

All 25 isolates were genus-specific PCR-positive and fermented glucose (Table 3). Seven were species-specific PCR-positive, sucrose-negative and produced H_2S on TSI agar slants. They were further characterized with API Coryne strips and confirmed as *E. rhusiopathiae*. For the 18 species-specific PCR-negative isolates, seven produced H_2S and were sucrose-positive, suggesting *E. tonsillarum*. Of the rest, three were H_2S-positive but sucrose-negative, seven did not produce H_2S but were sucrose-positive, and one was both H_2S- and sucrose-negative. All isolates with two colonial forms were species-specific PCR-negative. Three of them, D23, D67 and M4, had the same biochemical features when comparing the two colony types (Table 3). For the other two isolates, D22 and D65, the different colony forms gave varying biochemical results (Table 3). Isolates that were H_2S- and sucrose-positive were classified as *E. tonsillarum*. Isolates that were species-specific PCR-negative and H_2S- and/or sucrose-negative are likely to be other species of *Erysipelothrix* (Table 4) and require further taxonomic investigation.

DISCUSSION

Erysipelothrix infection occurs widely in nature. Swine erysipelas in pigs is the most frequently encountered consequence of infection and this has significant economic impact around the world. *Erysipelothrix* infection in other farmed animals results in polyarthritis in sheep and erysipelas in poultry. Human infections, commonly seen as erysipeloid, usually occur following occupational contact with infected animals. Due to a resemblance to other human infections, such as erysipelas caused by *Streptococcus* or *Staphylococcus* (Reboli and Farrar 1989), and problems in isolation and identification of the organism, *Erysipelothrix* infections in humans may be clinically underdiagnosed. The PCR method described by Makino *et al.* (1994) was originally conceived as a method to diagnose *Erysipelothrix* infection in animals. It is genus-specific and highly sensitive, and was used in a survey to detect the presence of *Erysipelothrix* in a variety of samples. A species-specific PCR protocol (Shimoji *et al.* 1998a) was used to distinguish *E. rhusiopathiae* from other species of *Erysipelothrix*.

When surveying the 109 abattoir samples by PCR, 35 (32·1%) were genus-specific PCR-positive and 15 (13·8%) were culture-positive. The positives were distributed throughout nearly all the sources examined, including the surface of carcasses, slaughtering area, pen soil, effluent, arthritic joints and internal organs. Surprisingly, no

Table 2 PCR results from carcass wash samples and isolates

		Samples		Isolates	
Source	*n*	G-PCR*(+)	S-PCR†(+)	G-PCR(+)	S-PCR(+)
Beef	26	1			
Lamb	23	4	2		2
Pork	23	3	1		1
Mutton	11	2	1	1	1
Chicken	40	2			
Total	123	12(9·8%)	4(3·3%)	1(0·8%)	4(3·3%)

*Genus-specific PCR (Makino *et al.* 1994).
†Species-specific PCR (Shimoji *et al.* 1998a).

Table 3 Biological characteristics of isolates from meat and abattoir samples

No.	Isolate	G-PCR*	S-PCR†	TSI‡	GLU§	SUC¶	Source
1	M1	+	+	+	+	−	Lamb
2	M2	+	+	+	+	−	Lamb
3	M3	+	+	+	+	−	Mutton
4	M5	+	+	+	+	−	Pork
5	S16	+	+	+	+	−	Sheep arthritic joint
6	S19	+	+	+	+	−	Sheep arthritic joint
7	D14	+	+	+	+	−	Pig abattoir effluent
8	D23fbl	+	−	+	+	−	Pig skin
9	D23yws	+	−	+	+	−	Pig skin
10	D22fbl	+	−	+	+	−	Pig skin
11	D22yws	+	−	+	+	+	Pig skin
12	D11	+	−	+	+	+	Pig abattoir effluent
13	D25	+	−	+	+	+	Pig blood
14	D55	+	−	+	+	+	Pig pen soil
15	D66	+	−	+	+	+	Pig pen soil
16	D67fbl	+	−	+	+	+	Pig pen soil
17	D67yws	+	−	+	+	+	Pig pen soil
18	D13	+	−	−	+	+	Pig abattoir effluent
19	D56	+	−	−	+	+	Pig pen soil
20	D57	+	−	−	+	+	Pig pen soil
21	D63	+	−	−	+	+	Pig pen soil
22	M4fbl	+	−	−	+	+	Mutton
23	M4yws	+	−	−	+	+	Mutton
24	D65yws	+	−	−	+	+	Pig pen soil
25	D65fbl	+	−	−	+	−	Pig pen soil
	E. rhusiopathiae (ATCC 19414)	+	+	+	+	−	
	E. tonsillarum (ATCC 43339)	+	−	+	+	+	

*Genus-specific PCR (Makino *et al.* 1994). †Species-specific PCR (Shimoji *et al.* 1998a). ‡Triple sugar iron agar plus 10% horse serum. §Glucose fermentation. ¶Sucrose fermentation.

Table 4 Biological classification scheme of *Erysipelothrix* spp.

Species	Property					Isolates
	G-PCR*	S-PCR†	TSI‡	GLU§	SUC¶	
E. rhusiopathiae	+	+	+	+	−	M1, M2, M3, M5, S16, S19, D14
E. tonsillarum	+	−	+	+	+	D22yws, D11, D25, D55, D66, D67fbl, D67yws
Erysipelothrix sp.	+	−	+	+	−	D23fbl, D23yws, D22fbl
Erysipelothrix sp.	+	−	−	+	+	D13, D56, D57, D63, M4fbl, M4yws, D65yws
Erysipelothrix sp.	+	−	−	+	−	D65fbl

*Genus-specific PCR (Makino *et al.* 1994). †Species-specific PCR (Shimoji *et al.* 1998a). ‡Triple sugar iron agar plus 10% horse serum. §Glucose fermentation. ¶Sucrose fermentation.

positives were detected in tonsil tissue, which had previously been shown to be a site of *E. rhusiopathiae* and *E. tonsillarum* colonization (Stephenson and Berman 1978; Takahashi *et al.* 1987a, b).

The occurrence of *E. rhusiopathiae* in abattoirs was reported in the early literature. In Australia, *E. rhusiopathiae* was the major cause of arthritis in pigs. The prevalence of arthritis in carcasses from a state abattoir in New South

Wales was 1% (Cross and Edwards 1981). The organism was isolated from the joints of 63% of pigs condemned for polyarthritis in a survey carried out at three metropolitan abattoirs in Western Australia (Bond 1976). In a later survey in Denmark, *E. rhusiopathiae* was found in samples from 39 to 49% of cattle and pig herds investigated (Norrung *et al.* 1987). In Sweden, *E. rhusiopathiae* was found in pig slurry from two of 19 pig farms. One strain was isolated from one of the 16 hand infections of slaughterhouse workers, and 20 of 138 workers had antibodies against *E. rhusiopathiae* (Molin *et al.* 1989). Recently, in a chicken abattoir in Japan, 263 out of 2188 chicken samples (12%) contained *Erysipelothrix*, with 273 isolates of *E. rhusiopathiae* and 24 isolates of *E. tonsillarum* recovered from these positive samples (Nakazawa *et al.* 1998a). In a meat processing plant, 30% of chicken meat samples were contaminated with *Erysipelothrix* spp. (Nakazawa *et al.* 1998b). The present results confirm that *E. rhusiopathiae* can still frequently be found in abattoirs and animal products, suggesting that these products might be a potential source of human *Erysipelothrix* infections. Microbiological examination of carcass washings is usually carried out to look for food-borne pathogens such as *Salmonella*. The recovery of *Erysipelothrix* spp. from these samples indicates both the extent of contamination of carcasses during the slaughtering process and the resilience of the organism.

The identification of *Erysipelothrix* spp. was based on isolation on selective media combined with colonial morphology and Gram stain, followed by both genus- and species-specific PCR. Using this protocol in a previous study of Australian seafood, efficient isolation of *Erysipelothrix* spp. was achieved and 21 isolates of *Erysipelothrix* spp. were identified from 52 samples (Fidalgo *et al.* 2000). In the present study, 20 *Erysipelothrix* isolates, five of which exhibited two colonial forms on BHIA/S agar, were recovered from a total of 232 abattoir and meat wash samples. Seven were confirmed as *E. rhusiopathiae* and another seven were consistent with *E. tonsillarum*. Of the remaining species-specific PCR-negative strains, three produced H_2S and were sucrose-negative, seven were sucrose-positive but did not produce H_2S, and one was both sucrose- and H_2S-negative. Whether these represent further species of *Erysipelothrix*, and whether they include the unnamed species reported by Takahashi *et al.* (1992), remains to be determined.

As with other bacterial pathogens, *E. rhusiopathiae* may present with different colonial morphologies and appear as smooth (S), rough (R) or intermediate (RS) forms (Cottral 1978; Jones 1986). The role of these forms in virulence has not been clearly defined and conflicting results have been reported. The varying colonial forms observed in this study for some isolates were similar to S and R forms, but some of these variants were identified as non-*E. rhusiopathiae* by PCR and biochemical testing. These findings may explain the conflicting observations concerning virulence of each form mentioned in earlier literature (Gorby and Peacock 1988; Taylor 1990), and further detailed work on the taxonomy of these strains needs to be conducted. In addition, *Erysipelothrix* infections in both animals and humans were always thought to be due to *E. rhusiopathiae*. The pathogenicity of other *Erysipelothrix* spp. is not well understood. *Erysipelothrix tonsillarum* was reported to be avirulent for swine and mice (Takahashi *et al.* 1987a). However, some studies identified *E. tonsillarum* as a cause of endocarditis in dogs, indicating that some strains of *E. tonsillarum* are canine pathogens (Takahashi *et al.* 1993, 2000). Both the taxonomy and pathogenicity of other *Erysipelothrix* spp. requires further investigation.

Erysipelothrix infection can cause substantial economic losses in animal industries, and appropriate prophylactic measures need to be taken for the treatment and control of this disease. Antibiotic therapy is effective and penicillin is usually the drug of choice. Vaccination is considered as a proper procedure for controlling the problem in animal farms. Most current commercially-available vaccines are attenuated live *E. rhusiopathiae* strains or bacterins (Wood *et al.* 1981; Wood 1992). However, new potential vaccines have been suggested (Timoney and Groschup 1993; Makino *et al.* 1998; Shimoji *et al.* 1998b, 1999). Before these new vaccines can be assessed properly, it is imperative that the taxonomy of this group of organisms is adequately described and various host–pathogen relationships better defined.

From this survey, it is concluded that *Erysipelothrix* infection still exists in farmed animals in Western Australia. The animal-processing environment of abattoirs is highly contaminated with this pathogen. Also, animal products for human consumption are at risk of contamination, and there is an obvious risk that consumers will become infected with virulent *Erysipelothrix* strains from retail pork, lamb and mutton should they sustain a stabbing injury from bone, or should an existing wound be contaminated. *Erysipelothrix* infection still poses a potential threat to the economy of the farmed animal industry as well as being a potential public health hazard for the human population.

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REFERENCES

Blackmore, D.K. and Gallagher, G.L. (1964) An outbreak of erysipelas in captive wild birds and mammals. *Veterinary Record* 76, 1161–1164.

Bond, M.P. (1976) Polyarthritis of pigs in Western Australia: the role of *Erysipelothrix rhusiopathiae*. *Australian Veterinary Journal* 52, 462–467.

Conklin, R.H. and Steele, J.H. (1979) *Erysipelothrix* infections. In *CRC Handbook* ed. Steele, J.H. pp. 327–337. Boca Raton, FL: CRC Press.

Cottral, G.E. (1978) *Erysipelothrix*. In *Manual of Standardized Methods for Veterinary Microbiology*. pp. 429–436, 671, 672, 679, 687. Ithaca, New York: Cornell University Press.

Cross, G.M. and Edwards, M.J. (1981) The detection of arthritis in pigs in an abattoir and its public health significance. *Australian Veterinary Journal* 57, 153–158.

Fidalgo, S.G., Wang, Q. and Riley, T.V. (2000) Comparison of methods for detection of *Erysipelothrix* spp. and their distribution in some Australasian seafoods. *Applied and Environmental Microbiology* 66, 2066–2070.

Gorby, G.L. and Peacock, J.E. Jr (1988) *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Reviews of Infectious Diseases* 10, 317–325.

Hillenbrand, F.K.M. (1953) Whale finger and seal finger: their relation to erysipeloid. *Lancet* 1, 680–681.

Hunter, D. (1975) The diseases of occupations. In *The Diseases of Occupations* 5th edn. pp. 709–712. London: English University Press.

Jones, D. (1986) Genus *Erysipelothrix* Rosenbach1909^{367AL}. In *Bergey's Manual of Systematic Bacteriology* 9th edn, ed. Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G. pp. 1245–1249. Baltimore: Williams & Wilkins.

Makino, S., Okada, Y., Maruyama, T. et al. (1994) Direct and rapid detection of *Erysipelothrix rhusiopathiae* DNA in animals by PCR. *Journal of Clinical Microbiology* 32, 1526–1531.

Makino, S., Yamamoto, K., Murakami, S. et al. (1998) Properties of repeat domain found in a novel protective antigen, SpaA, of *Erysipelothrix rhusiopathiae*. *Microbial Pathogenesis* 25, 101–109.

Molin, G., Soderlind, O., Ursing, J., Norrung, V., Ternstrom, A. and Lowenhielm, C. (1989) Occurrence of *Erysipelothrix rhusiopathiae* on pork and in pig slurry, and the distribution of specific antibodies in abattoir workers. *Journal of Applied Bacteriology* 67, 347–352.

Morgan, M.J., Britt, J.O., Cockrill, J.M. and Eiten, M.L. (1994) *Erysipelothrix rhusiopathiae* infection in an emu (*Dromaius novaehollandiae*). *Journal of Veterinary Diagnostic Investigation* 6, 378–379.

Nakazawa, H., Hayashidani, H., Higashi, J., Kaneko, K., Takahashi, T. and Ogawa, M. (1998a) Occurrence of *Erysipelothrix* spp. in broiler chickens at an abattoir. *Journal of Food Protection* 61, 907–909.

Nakazawa, H., Hayashidani, H., Higashi, J., Kaneko, K., Takahashi, T. and Ogawa, M. (1998b) Occurrence of *Erysipelothrix* spp. in chicken meat parts from a processing plant. *Journal of Food Protection* 61, 1207–1209.

Norrung, V., Munch, B. and Larsen, H.E. (1987) Occurrence, isolation and serotyping of *Erysipelothrix rhusiopathiae* in cattle and pig slurry. *Acta Veterinaria Scandinavica* 28, 9–14.

Reboli, A.C. and Farrar, W.E. (1989) *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clinical Microbiology Reviews* 2, 354–359.

Reboli, A.C. and Farrar, W.E. (1992) The genus *Erysipelothrix*. In *The Prokaryotes: a Handbook on the Biology of Bacteria: Ecophysiology, Isolation, Identification, Application* ed. Balows, A., Truper, H.G., Dworkin, M., Harder, W. and Schleifer, K. pp. 1629–1642. New York: Springer-Verlag.

Shimoji, Y., Mori, Y. and Fischetti, V.A. (1999) Immunological characterization of a protective antigen of *Erysipelothrix rhusiopathiae*: identification of the region responsible for protective immunity. *Infection and Immunity* 67, 1646–1651.

Shimoji, Y., Mori, Y., Hyakutake, K., Sekizaki, T. and Yokomizo, Y. (1998a) Use of an enrichment broth cultivation-PCR combination assay for rapid diagnosis of swine erysipelas. *Journal of Clinical Microbiology* 36, 86–89.

Shimoji, Y., Mori, Y., Sekizaki, T., Shibahara, T. and Yokomizo, Y. (1998b) Construction and vaccine potential of acapsular mutants of *Erysipelothrix rhusiopathiae*: use of excision of Tn916 to inactivate a target gene. *Infection and Immunity* 66, 3250–3254.

Shuman, R.D. (1971) Erysipelothrix. In *Infectious and Parasitic Diseases of Wild Birds* ed. Davis, J.W., Anderson, R.C., Karstad, L.H. and Trainer, D.O. pp. 141–145. Ames, IA: Iowa State University Press.

Stephenson, E.H. and Berman, D.T. (1978) Isolation of *Erysipelothrix rhusiopathiae* from tonsils of apparently normal swine by two methods. *American Journal of Veterinary Research* 39, 187–188.

Takahashi, T., Fujisawa, T., Benno, Y. et al. (1987a) *Erysipelothrix tonsillarum* sp. nov. isolated from tonsils of apparently healthy pigs. *International Journal of Systematic Bacteriology* 37, 166–168.

Takahashi, T., Fujisawa, T., Tamura, Y. et al. (1992) DNA relatedness among *Erysipelothrix rhusiopathiae* strains representing all twenty-three serovars and *Erysipelothrix tonsillarum*. *International Journal of Systematic Bacteriology* 42, 469–473.

Takahasi, T., Fujisawa, T., Yamamoto, K., Kijima, M. and Takahashi, T. (2000) Taxonomic evidence that serovar 7 of *Erysipelothrix* strains isolated from dogs with endocarditis are *Erysipelothrix tonsillarum*. *Zentralblatt fur Veterinarmedizin – Reihe B* 47, 311–313.

Takahashi, T., Sawada, T., Muramatsu, M. et al. (1987b) Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. *Journal of Clinical Microbiology* 25, 536–539.

Takahashi, T., Tamura, Y., Yoshimura, H. et al. (1993) *Erysipelothrix tonsillarum* isolated from dogs with endocarditis in Belgium. *Research in Veterinary Science* 54, 264–265.

Taylor, D.J. (1990) *Pig Diseases*. Cambridge: Burlington Press.

Timoney, J.F. and Groschup, M.M. (1993) Properties of a protective protein antigen of *Erysipelothrix rhusiopathiae*. *Veterinary Microbiology* 37, 381–387.

White, T.G. and Shuman, R.D. (1961) Fermentation reactions of *Erysipelothrix rhusiopathiae*. *Journal of Bacteriology* 82, 595–599.

Wood, R.L. (1975) *Erysipelothrix* infection. In *Diseases Transmitted from Animals to Man* 6th edn, ed. Hubbert, W.T., McCulloch, W.F. and Schnurrenberger, P.R. pp. 271–281. Springfield, Ill: Thomas.

Wood, R.L. (1992) Erysipelas. In *Diseases of Swine* ed. Leman, A.D. pp. 475–486. Ames, Iowa: Iowa State University Press.

Wood, R.L., Booth, G.D. and. Cutlip, R.C. (1981) Susceptibility of vaccinated swine and mice to generalized infection with specific serotypes of *Erysipelothrix rhusiopathiae*. *American Journal of Veterinary Research* 42, 608–614.

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MICROBIOLOGY

Susceptibility of *Erysipelothrix rhusiopathiae* to antimicrobial agents and home disinfectants

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Summary

Aim: *Erysipelothrix rhusiopathiae* causes the occupationally-related infection erysipeloid in humans, and may be responsible for infections in lobster fishermen in Western Australia. There are little recent data pertaining to antimicrobial susceptibility, or susceptibility to disinfectants that might be used in the environment. The aim of this study was to determine the susceptibility of *E. rhusiopathiae* from human, animal and environmental sources to various antimicrobial agents and disinfectants.

Methods: The susceptibility of 60 *E. rhusiopathiae* isolates was determined using a recommended agar dilution procedure. Susceptibility to disinfectants was achieved using a broth microdilution method.

Results: Penicillin and ceftriaxone, with low minimum inhibitory concentrations (MICs) (MIC_{90} 0.03 mg/l and 0.125 mg/l, respectively), remained active against *E. rhusiopathiae* and should continue to be recommended for treatment. Ciprofloxacin MICs were particularly low (MIC_{90} 0.06 mg/l), offering an alternative agent for the penicillin allergic patient. *Erysipelothrix rhusiopathiae* is still resistant to vancomycin (MIC_{90} 64 mg/l), highlighting the importance of early diagnosis of *E. rhusiopathiae* infection in cases of endocarditis. In addition, 31 *E. rhusiopathiae* isolates were tested against several commercially available home disinfectants. Most were effective in killing *E. rhusiopathiae* with minimum bactericidal concentrations of 0.001% for Pine O Cleen, and 0.03% for Domestos, Linely and the Wheelie Bin Phenyl Cleanser.

Conclusions: There appeared to be no new emergence of antibiotic resistance in *E. rhusiopathiae*. Various disinfectants could be used following mechanical cleaning of work environments, such as fishing boats, and equipment, to reduce the risk of infection with *E. rhusiopathiae*.

Key words: *Erysipelothrix rhusiopathiae*, antimicrobial agent, disinfectants.

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INTRODUCTION

Erysipelothrix rhusiopathiae is pathogenic to both humans and animals. There are three forms of human infection, which closely resemble those seen in swine. The most common form is erysipeloid, a localised cutaneous infection often described as a local cellulitis. It usually occurs on the hands and fingers and is self-limiting.[1] In Western Australia, an occupational infection known as 'crayfish poisoning', thought to be caused by *E. rhusiopathiae*, affects lobster fishermen after spiking injuries and closely resembles erysipeloid.[2] A second generalised cutaneous form involves lesions that progress from the initial site of infection. Systemic symptoms are more frequent and relapse may occur. The most serious *Erysipelothrix* infection is a septicaemic form which is almost always linked with endocarditis.[1] Even though the mortality rate of endocarditis due to *E. rhusiopathiae* has dramatically decreased, from 100% in the pre-antibiotic era to 38%,[3] mortality is still high. This can be partly explained by the use of vancomycin for empirical therapy of endocarditis. Therefore, early diagnosis of *E. rhusiopathiae* infection is essential.[4]

There have been only two recent reports on the susceptibility of *E. rhusiopathiae* to antimicrobial agents, and these contains data for animal isolates only.[5,6] Previous reports[7,8] suggested *Erysipelothrix* was highly susceptible to penicillins, cephalosporins and clindamycin. Most strains were resistant to aminoglycosides, trimethoprim-sulphamethoxazole, polymyxins, sulphonamides, streptomycin, novobiocin and vancomycin. Variable susceptibility was recorded for chloramphenicol, tetracyclines and erythromycin, with the suggestion of development of resistance to both macrolides and tetracyclines.

Environmental control of *E. rhusiopathiae* seems to be the most effective means of preventing the spread of infection in man and animals. *Erysipelothrix rhusiopathiae* is killed by commonly available disinfectants, such as phenols, chlorine-based solutions, alkalies, and quaternary ammonium compounds,[9] however, such reports are rare. Regular cleaning and disinfection of contaminated sources, such as fishing boats and their equipment, may assist in controlling this organism in the work environment. However, disinfectants that are not inhibited by organic matter are required, as *E. rhusiopathiae* can survive in organic matter. In addition, an awareness of infection for people at high risk, usage of preventative measures such as wearing protective gear, prompt treatment of any injury, and good hygiene including frequent washing of hands with soaps and disinfectants, reduces the spread of *E. rhusiopathiae*.[10]

The purpose of this investigation was to determine the susceptibility of a variety of *E. rhusiopathiae* isolates to a range of antimicrobial agents, particularly some that are currently suggested for treatment of infection, and to determine the *in vitro* efficacy of several commercially available home disinfectants that might be suitable for environmental decontamination.

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TABLE 1 MIC values for 60 isolates of *E. rhusiopathiae* against 13 antimicrobial agents

Antimicrobial agent	MIC (mg/l)		
	Range	50%	90%
Penicillin	0.03	0.03	0.03
Oxacillin	0.125–0.5	0.125	0.125
Tetracycline	2–4	2	2
Minocycline	0.125–0.25	0.25	0.25
Erythromycin	0.125	0.125	0.125
Clindamycin	0.5–2	1	2
Chloramphenicol	8–16	16	16
Cephalothin	0.125–1	0.125	0.25
Ceftriaxone	0.06–0.125	0.06	0.125
Fusidic acid	0.125–1	0.25	0.25
Rifampin	>8	>8	>8
Ciprofloxacin	0.06–0.125	0.06	0.06
Vancomycin	64–128	64	64

MATERIALS AND METHODS

Bacterial isolates

A total of 60 *E. rhusiopathiae* isolates was examined from a variety of sources. These included isolates from human infections (8), pigs (8), sheep (7), emus (2), crustaceans (10), fish (18), dolphins (2), cephalopods (2), and an abattoir (1) and fishing boat (2) environment. All the isolates were confirmed as *E. rhusiopathiae* with an *E. rhusiopathiae*-specific PCR method.[11] *Erysipelothrix rhusiopathiae* ATCC 19414 was used as a control for all susceptibility testing.

Antimicrobial agents

The antimicrobial agents tested were: penicillin (CSL, Australia), cephalothin (Eli Lilly, Australia), oxacillin, chloramphenicol, clindamycin, erythromycin, tetracycline and vancomycin (Sigma Chemical Co., Australia), ceftriaxone (Roche Products, Australia), minocycline (Lederle Laboratories, Australia), ciprofloxacin (Bayer Pharmaceuticals, Australia), fusidic acid (Leo Pharmaceuticals, Australia) and rifampin (Alphapharm, Australia). Stock solutions were prepared according to the instructions of the manufacturers and stored at −70°C for not more than 6 months.

Disinfectants

The following disinfectants were purchased from the manufacturers: two chlorine-based disinfectants, Linely (Ramprie Laboratories, Australia) containing sodium hypochlorite with 1% available chlorine (w/v), and Domestos (Lever Rexona, Australia) containing sodium hypochlorite with 5% available chlorine (w/v); one phenolic disinfectant, Wheelie Bin Phenyl Cleanser (Recochem Inc., New Zealand), containing 2.9% phenols; one quaternary ammonium disinfectant, Pine O Cleen (Reckitt and Colman, New Zealand), containing 1.5% (w/w) benzalkonium chloride; and one disinfectant containing citric acid and vinegar, Down to Earth Toilet Cleaner (Reckitt and Colman, Australia).

Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) were determined using the agar dilution technique and recommended breakpoints approved by the National Committee for Clinical Laboratory Standards (NCCLS),[12] with some modifications. Agar plates were prepared by adding 5% horse blood to Mueller Hinton agar (MHB) (Oxoid, UK), and then adding the antibiotics in doubling dilutions ranging from 512 to 0.008 mg/l, except for rifampin which was tested up to a maximum concentration of 8 mg/l. Control plates containing only 5% horse blood and Mueller Hinton agar were also included.

The isolates were streaked on horse blood agar (HBA) (Oxoid) and incubated at 37°C. After 24 h incubation, a few colonies were suspended in 0.85% saline to make a suspension with an opacity equivalent to that of a 0.5 McFarland turbidity standard. The suspension was then diluted in 0.85% saline to obtain approximately 10^7 CFU/ml. To confirm the inoculum size, viable counts were performed.

The plates were dried for 20 min and then inoculated with the 10^7 CFU/ml bacterial suspensions with a multipoint replicator (Mast Laboratories, UK). With the replicator, 1–2 µl spots containing approximately 10^4 CFU/ml of each organism were transferred. The inoculated plates were then incubated at 37°C for 48 h. The MICs were determined after 48 h incubation as the lowest concentration of antimicrobial that completely inhibited growth, disregarding one or two colonies or a faint haze due to the inoculum. The minimum concentrations of antimicrobial that inhibited at least 50 and 90% of the isolates were defined as the MIC_{50} and MIC_{90}, respectively. Testing was performed in triplicate on separate days, and the module MIC values were recorded.

Disinfectant susceptibility testing

MICs were determined using the broth dilution technique approved by the NCCLS, with some modifications. A range of dilutions of the disinfectants was prepared in BHIB (Oxoid), plus 0.05% Tween 80 (Sigma) in 96-well microtitre trays (Falcon; Becton Dickinson, USA).

Bacterial suspensions were prepared as for the agar dilution method, but diluted in 0.85% saline so that a final concentration of approximately 5.0×10^5 CFU/ml was obtained in each well following inoculation of the microtitre tray. The trays were then placed in plastic bags to prevent drying and incubated at 37°C. After 48 h incubation, 10 µl of the cultures was taken from each well and spotted on BHIA (Oxoid) plates that had been previously dried. The plates were then incubated at 37°C for 48 h. The MIC was defined as the lowest concentration of disinfectant that completely inhibited growth of the organism in the wells. The minimum bactericidal concentration (MBC) was defined as the lowest concentration of disinfectant resulting in death of 99.9% of the initial inoculum. The MIC_{50}, MIC_{90}, MBC_{50} and MBC_{90}, were also determined. Growth and sterility controls were included in every tray. Tests were performed in triplicate on separate days, and the module MIC and MBC values recorded.

RESULTS

The MIC_{50} and MIC_{90} values obtained for the 60 *E. rhusiopathiae* isolates are shown in Table 1. The most active antimicrobial against *E. rhusiopathiae* was penicillin with MIC_{50} and MIC_{90} values of 0.03 mg/l, followed by ciprofloxacin with MIC_{50} and MIC_{90} values of 0.06 mg/l, and ceftriaxone with MIC_{50} and MIC_{90} values of 0.06 mg/l and 0.125 mg/l, respectively. However, all antimicrobials tested apart from vancomycin, rifampin and tetracycline showed good activity, with no evidence of any resistance development. Vancomycin with a very high MIC_{50} and

TABLE 2 MIC and MBC values for 31 *E. rhusiopathiae* against five disinfectants

Disinfectant	MIC (%)			MBC (%)		
	Range	50%	90%	Range	50%	90%
Linely	0.03	0.03	0.03	0.03	0.03	0.03
Wheelie Bin Phenyl Cleanser	0.016–0.03	0.03	0.03	0.03–0.06	0.03	0.03
Pine O Cleen	0.001	0.001	0.001	0.001	0.001	0.001
Domestos	0.016–0.03	0.03	0.03	0.016–0.03	0.03	0.03
Toilet cleaner	2.0–4.0	2.0	4.0	2.0–4.0	2.0	4.0

MIC_{90} of 64 mg/l remained inactive against *E. rhusiopathiae*, as did rifampin (MIC_{50} and MIC_{90} >8 mg/l). The tetracycline MICs of 2–4 mg/l suggest borderline susceptibility.

The MIC and MBC values obtained for the 31 *E. rhusiopathiae* isolates tested against disinfectants are shown in Table 2. Pine O Cleen appeared to be extremely active against *E. rhusiopathiae*, and only 0.001% was required to inhibit (MIC) and kill (MBC), 50 and 90% of the isolates. The Wheelie Bin Phenyl Cleanser, Linely and Domestos disinfectants were also effective in inhibiting and killing *E. rhusiopathiae*, with MIC and MBC values of 0.03%. However, the biodegradable toilet cleaner with citric acid and vinegar had poor activity, with MIC_{90} and MBC_{90} values of 4.0%.

DISCUSSION

Human infections with *Erysipelothrix* appear to be rare today. While the older literature contains large series of patients with such infections,[13] most recent reports about *Erysipelothrix* spp. relate to swine erysipelas. However, occasional case reports do appear and emphasise the fact that isolation and identification of *Erysipelothrix* can be difficult.[14,15] Thus, contemporary antimicrobial susceptibility data for *E. rhusiopathiae* isolated from humans are still limited, and monitoring is required to determine the emergence of resistance to antimicrobials. In Japan, resistance to erythromycin and oxytetracycline in *E. rhusiopathiae* isolated from cases of swine erysipelas was first reported over 15 years ago.[7] This was most likely a result of the practice in Japan of feeding pigs food containing macrolides and tetracyclines as growth promoters.

One of our aims was to investigate the susceptibility of *E. rhusiopathiae* isolates from diverse sources, both clinical and environmental. Recent investigations have concentrated almost exclusively on isolates from animals, particularly pigs. Therefore, we tested isolates from humans, various animals and birds, aquatic creatures and the environment. Our results were in general agreement with previous reports. In Australia, *E. rhusiopathiae* remains susceptible to penicillins and macrolides, with MICs for susceptible strains similar to previous reports.[5–7,16] For cefotaxime, an MIC_{90} of 0.06 mg/l has been reported,[8] again similar to what we obtained for ceftriaxone (MIC_{90} 0.125 mg/l). The earlier first generation cephalosporin, cephalothin, was also active. Erysipeloid can be treated with oral penicillin[1] and, for more serious *E. rhusiopathiae* infection, intravenous penicillin is recommended.[4] Tetracycline MICs were slightly raised, with an MIC_{90} of 2 mg/l and some strains having MICs of 4 mg/l, and thus tetracycline could not be recommended; however, minocycline was still active. Interestingly, all isolates tested were resistant to rifampin, a fact not previously reported, although Soriano *et al.*[17] tested six isolates of *E. rhusiopathiae* against the closely related rifapentine and found them all resistant.

As previously documented, *E. rhusiopathiae* was resistant to vancomycin with an MIC_{90} of 64 mg/l. In a patient with endocarditis and a history of skin lesions suggestive of erysipeloid, or with occupational risk factors, *E. rhusiopathiae* should always be considered and intravenous penicillin should be part of the empirical regime. Because of its low MICs against *E. rhusiopathiae*, ceftriaxone might still be suitable for patients who are mildly allergic to penicillin, as clindamycin and erythromycin are only bacteriostatic;[1] however, if serious penicillin allergy is a problem then ciprofloxacin should be considered. Overall, there did not appear to be emergence of antibiotic resistance amongst the isolates tested.

Erysipelothrix rhusiopathiae is a remarkably resilient organism, surviving in harsh environmental conditions and tolerant to numerous chemicals.[10] The commercially available home disinfectants we tested were quite active against *E. rhusiopathiae*. Pine O Cleen containing 1.5% w/v benzalkonium chloride was extremely effective, with an MIC of only 0.001%. However, benzalkonium chloride is easily inactivated by organic matter and hard water,[18] and *E. rhusiopathiae* is known to be able to survive in organic matter. For this disinfectant to be useful, prior cleaning is required to remove the excess organic matter from surfaces, followed by disinfection.[19,20] Domestos, Linely and the Wheelie Bin Phenyl Cleanser were also active with an MIC/MBC of 0.03%. Some strains were killed by 0.016% Domestos reflecting, possibly, the greater amount of available chlorine in this disinfectant. The Wheelie Bin Phenyl Cleanser contained 2.9% mixed phenols, made up mainly of *m*- and *p*-cresols and with a small amount of *o*-cresols, in a soap base made from natural fat. The exact composition was unknown, however, all three of these cresols are used as disinfectants. It has been reported that *E. rhusiopathiae* can grow in 0.2% phenols,[21] however, this was not the case in our investigation. Phenolic disinfectants have an advantage in not being inactivated by organic matter,[22] however, they can be toxic and unpleasant to work with[18] and for this reason they are not recommended when there are other alternatives. The biodegradable, environmental friendly toilet cleaner, containing citric acid and vinegar was not effective against *E. rhusiopathiae*.

This investigation was stimulated by our finding that *E. rhusiopathiae* may play a role in 'crayfish poisoning', an occupational infection of lobster fishermen in Western Australia.[2] We also showed that the fishing boat

environment is extensively contaminated with *E. rhusiopathiae*.[23] Cleaning and disinfecting the work environment may be a way of reducing the opportunity for exposure and thus infection. Most surfaces on fishing boats are metal and therefore should be amenable to disinfection providing all organic matter is first removed by cleaning. However, professional lobster fishermen use either rectangular wooden pots or the traditional cane 'beehive' pots. These porous surfaces are likely to harbour large numbers of organisms of all types and represent a hazard if workers are cut or grazed by them. Disinfection is not likely to be achievable unless pots are soaked in disinfectant solution. However, depending on the working environment or equipment needing cleaning and disinfection, there are several commercially available home disinfectants that may be suitable.

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References

1. Reboli AC, Farrar WE. The genus *Erysipelothrix*. In: Balows A, Truper HG, Dworkin M, Harder W, Schleifer K, editors. *The prokaryotes. A handbook on the biology of bacteria: Ecophysiology, isolation, identification and applications*. New York: Springer-Verlag, 1992; 1629–42.
2. Brooke CJ, McLaughlin V, Mee BJ, Riley TV. An investigation into 'crayfish poisoning' in Western Australia. (Letter.) *Med J Aust* 1999; 170: 288.
3. Gorby GL, Peacock JE Jr. *Erysipelothrix rhusiopathiae* endocarditis: microbiologic, epidemiologic, and clinical features of an occupational disease. *Rev Infect Dis* 1988; 10: 317–25.
4. Grieco MH, Sheldon C. *Erysipelothrix rhusiopathiae. Ann NY Acad Sci* 1970; 174: 523–32.
5. Yamamoto M, Kijima M, Takahashi T, *et al.* Serovar, pathogenicity and antimicrobial susceptibility of *Erysipelothrix rhusiopathiae* isolates from farmed wild boars (*Sus scrofa*) affected with septicemic erysipelas in Japan. *Res Vet Sci* 1999; 67: 301–3.
6. Yamamoto K, Kijima M, Yoshimura H, Takahashi T. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae* isolated from pigs with swine erysipelas in Japan. *Zentralbl Veterinarmed [B]* 2000; 48: 115–26.
7. Takahashi T, Sawada T, Ohmae K, *et al.* Antibiotic resistance of *Erysipelothrix rhusiopathiae* isolated from pigs with chronic swine erysipelas. *Antimicrob Agents Chemother* 1984; 25: 385–6.
8. Venditti M, Gelfusa V, Tarasi A, Brandimarte C, Serra P. Antimicrobial susceptibilities of *Erysipelothrix rhusiopathiae. Antimicrob Agents Chemother* 1990; 34: 2038–40.
9. Wood RL. *Erysipelothrix* infection. In: Hubbert WT, McCullough WF, Schnurrenberger PR, editors. *Diseases transmitted from animals to man*. 6th ed. Illinois: Thomas, Springfield, 1975: 271–81.
10. Conklin RH, Steele JH. *Erysipelothrix* infections. In: Steele JH editor. *CRC handbook. Series in zoonoses*. Vol 1. Florida: CRC Press, 1979; 327–37.
11. Fidalgo SG, Wang Q, Riley TV. Comparison of methods for detection of *Erysipelothrix* spp. and their distribution in some Australasian seafoods. *Appl Environ Microbiol* 2000; 66: 2066–70.
12. National Committee for Clinical Laboratory Standards (NCCLS). Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 4th ed. Approved standard. NCCLS document M7-A4, 1997. Villanova, Pa: NCCLS, 1997.
13. Gilchrist TC. Erysipeloid, with a record of 329 cases, of which 322 were caused by crab bites or lesion produced by crabs. *J Cut Dis* 1904; 22: 507–19.
14. Dunbar SA, Clarridge JE III. Potential errors in recognition of *Erysipelothrix rhusiopathiae. J Clin Microbiol* 2000; 38: 1302–4.
15. Robson JM, McDougall R, van der Valk S, Waite SD, Sullivan JJ. *Erysipelothrix rhusiopathiae*: an uncommon but ever present zoonosis. *Pathology* 1998; 30: 391–4.
16. Takahashi T, Sawada T, Muramatsu M, *et al.* Serotype, antimicrobial susceptibility, and pathogenicity of *Erysipelothrix rhusiopathiae* isolates from tonsils of apparently healthy slaughter pigs. *J Clin Microbiol* 1987; 25: 536–9.
17. Soriano F, Fernandez-Roblas R, Calvo R, Garcia-Calvo G. In vitro susceptibilities of aerobic and facultative non-spore-forming gram-positive bacilli to HMR 3647 (RU 66647) and 14 other antimicrobials. *Antimicrob Agents Chemother* 1998; 42: 1028–33.
18. Fraise AP. Choosing disinfectants. *J Hosp Infect* 1999; 43: 255–64.
19. Mutalib AA, King JM, McDonough PL. Erysipelas in caged laying chickens and suspected erysipeloid in animal caretakers. *J Vet Diagn Invest* 1993; 5: 198–201.
20. Spencer R. The sanitation of fish boxes. I. The quantitative and qualitative bacteriology of commercial wooden fish boxes. *J Appl Bacteriol* 1959; 22: 73–84.
21. Wood RL. A selective liquid medium utilising antibiotics for isolation of *Erysipelothrix insidiosa. Am J Vet Res* 1965; 26: 1303–8.
22. Rutala WA. *Prevention and Control of Nosocomial Infections*. Baltimore: Williams and Wilkins, 1987; Chapter 18, Disinfection, sterilization, and waste disposal.
23. Brooke CJ. An investigation into *Erysipelothrix rhusiopathiae* as the causative organism of crayfish poisoning. Nedlands: The University of Western Australia, 1996. Thesis.