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Original Article

Isolation and Detection of *Erysipelothrix rhusiopathiae* and Its Distribution in Humans and Animals by Phenotypical and Molecular Methods in Ahvaz-Iran in 2015

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Abstract

Background: *Erysipelothrix rhusiopathiae* (*E. rhusiopathiae*) is generally transmitted into the gastrointestinal tract of animals by the intake of contaminated food or water and causes great economic loss in agriculture worldwide. Some of the *Erysipelothrix* spp. are the causative agents of erysipeloid, which is an occupational infection in humans. The aim of the present study was to isolate *E. rhusiopathiae* from animals as well as the hands of the butchers working in Ahvaz, Iran, and to determine their susceptibility to antibiotics.

Methods: Totally, 150 samples were taken from slaughterhouse workers, fishermen, and livers and hearts of sheep and calves by the swabbing method. Phenotypical methods and polymerase chain reaction (PCR) were used for the isolation and identification of *E. rhusiopathiae*. The isolates were tested for their susceptibility to commonly used antimicrobial agents using the disk diffusion protocol described by the Clinical and Laboratory Standards Institute.

Results: Out of the 150 samples examined via phenotypical and biochemical tests, 16 samples were positive as putative *Erysipelothrix spp*. twelve cases out of the 16 putative *Erysipelothrix spp*. were confirmed by PCR. The tested isolates were highly sensitive to the antibiotics used. The results of the sensitivity and specificity of PCR revealed that the sensitivity and specificity of indirect PCR were higher than those of direct PCR.

Conclusion: *E. rhusiopathiae* is widely distributed on seafood and presents as a commensal pathogen in nature and animals. Infection with this microorganism should be emphasized because it is a rare organism causing severe infections such as infectious endocarditis and polyarthritis following localized infections.

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Keywords ● Erysipelothrix rhusiopathiae ● Erysipeloid ● Occupational diseases ● Polymerase chain reaction

What's Known

- Swine or pig is the most common source for *Erysipelothrix rhusiopathiae*, which has a worldwide distribution with isolates detected in culture.
- Transmission of *E. rhusiopathiae* infection in Iran can be usually caused by contact with other animals such as fish, sheep, turkeys, and calves.

What's New

- In this study, from 150 samples taken from slaughterhouse workers, fishermen, fish handlers, fish, and the liver and heart of sheep and calves, 20 cases were positive for *E. rhusiopathiae* by PCR and 16 cases were positive by the phenotypical method.

Introduction

Erysipelothrix is a long and thin, facultative, anaerobic, Gram-positive, non-sporulating, intracellular, rod-shaped bacterium,

and is widely distributed in nature.[1] Some of the *Erysipelothrix* spp. are the causative agents of erysipeloid (a skin disease in humans) as well as swine erysipelas (a disease that can cause acute symptoms such as septicemia, lead to chronic syndromes like polyarthritis and endocarditis in pigs, and give rise to a wide spectrum of diseases in other animals such as birds, some fish, sheep, and other mammals).[2] *E. rhusiopathiae* is generally transmitted into the gastrointestinal track of animals by the intake of contaminated food or water and causes great economic loss in agriculture the world over.[3] The genus of *Erysipelothrix* comprises 4 species and 28 associated serotypes: *E. rhusiopathiae* (17 serotypes), *E. tonsillarum* (9 serotypes), *Erysipelothrix* sp. strain 1 (1 serotype), and *Erysipelothrix* sp. strain 2 (1 serotype).[1,4] Among the genus *Erysipelothrix*, *E. rhusiopathiae* is the most important pathogen in humans. Contact with infected animals, their products, or their waste is usually the major cause of *Erysipelothrix* infections in humans. Thus, it is often found among slaughterhouse workers, fishermen, farmers, fish handlers, and veterinarians.[5] In humans afflicted with *E. rhusiopathiae* infection, usually 3 well-defined clinical syndromes are seen. The most common symptom in erysipeloid is characterized by the redness and swelling of the infected parts of the body, fingers, and hands and frequently presents as acute cellulitis at the portal of entry. The cutaneous infection form, albeit intense, is rare. Bacteremia is the most common form of *E. rhusiopathiae* infection, to which endocarditis has always been linked as a systemic infection. Although endocarditis and bacteremia are relatively rare, these types of diseases appear to exhibit an increasing incidence.[5,6]

E. rhusiopathiae and infections caused by this organism occur world-wide. Infections of humans and animals have been reported from Africa, Australia, several countries in the Americas, Japan, China, and throughout Europe. Man disease can originate from animals or environmental sources.[7]

Eriksson et al.[8] studied the suitableness of different subordinate methods for genetic and phenotypical similarities among the Swedish isolates of the organism such as: 45 isolates from poultry (n=23), pigs (n=17), emus (n=2), and the poultry red mite Dermanyssus gallinae (n=3), checked by serotyping and pulsed-field gel electrophoresis (PFGE).[8]

The aim of the current study was to isolate and detect *E. rhusiopathiae* and its distribution in humans and animals by phenotypical and molecular methods and determine their susceptibility to antibiotics in Ahvaz, Iran, in 2015.

Materials and Methods

Bacterial Isolation

Totally, 150 samples were taken from slaughterhouse workers, fishermen, fish handlers, fish, and livers and hearts of sheep and calves by the swabbing method. The samples were collected from March to September (2015) from different parts of the Iranian city of Ahvaz. Based on the manufacture's recommendations, a brain heart infusion (BHI) broth (Merck, Germany) was prepared and sterilized by autoclaving at 121 ºC for 15 minutes. All the samples were inoculated in the BHI broth and placed into candle jars and incubated for 48 hours at 37 ºC. Subculturing was performed from the BHI onto selective blood agar (Merck, Germany), supplemented with 5% sheep blood and kanamycin (40 µg/mL), neomycin (50 µg/mL), and vancomycin (70 µg/mL). All the antibiotic supplements were taken from Sigma Company. After 24 to 48 hours of incubation at 37 ºC, suspected small colonies (approximately 0.1 mm) were stained by the Gram method. Slender, straight, or slightly rod Gram-positive bacteria were selected and biochemically confirmed using standard laboratory methods (catalase and oxidase activities, H_2S production, motility, and carbohydrates fermentation on triple sugar iron agar [TSI] [Merck, Germany]) and H_2S, Indole, and Motility (SIM medium) (Merck, Germany) were used to confirm *Erysipelothrix* spp. The putative Gram-positive bacilli confirmed as *Erysipelothrix* spp. were kept for final confirmation by polymerase chain reaction (PCR). All the isolate bacteria were inoculated in skim milk plus 15% glycerol and stored at -80ºC for future works.[9,10]

Detection of E. rhusiopathiae by PCR

Genomic DNA was extracted using the High Pure PCR Template Preparation Kit (Roche, Germany). Furthermore, PCR was done with the DNA extracts first by using universal primers. The specific primers that were used for this study consisted of DNA sequence coding for 16S rRNA, EMB Laccessionno, and M23728. The primers, MO101 (5'AGATGCCAT-AGAAACTGGTA3'), and M0102 (5'CTGTATCCGCCATAACTA3') amplified a 407-bp DNA fragment in the *Erysipelothrix* spp. The amplification reactions were performed in a final volume of 25 µL, containing 0.2 µg of genomic DNA, 20 p mol of each primer, 1.25 U Taq DNA polymerase, and 100 Mm of dNTP. Initial denaturation at 95 °C

for 5 minutes, 30 cycles of denaturation at 95 °C for 1 minute, annealing at 54 °C for 2 minutes, extension at 72 °C for 2 minutes, and final extension at 72 °C for 7 minutes were carried out using a DNA thermal cycler, Eppendorf. Electrophoresis was applied for 60 minutes at 100 mV in 2% agarose gel and stained with ethidium bromide after electrophoresis in 0.5×TBE for the detection of amplified products. The specimens of this study with consistent PCR results were sequenced by Bioneer Company (Korea) and used as positive controls, while distilled water was used as a negative control.[9,10]

Gene Sequencing

The primers used in this study were specific for *Erysipelothrix* spp., and they did not differentiate between *E. rhusiopathiae* and *E. tonsillarum*. Subsequently, the PCR products were collected and sent for sequencing analysis and identification of different species at Bioneer Company, Korea.

Antimicrobial Susceptibility Test

All the isolated *E. rhusiopathiae* were inoculated in the BHI (Merck, Germany) broth overnight at 37°C. Then, antibacterial susceptibility patterns were performed using the disk diffusion method (Kirby Bauer's technique). The suspension of each isolated bacterium was prepared and confirmed by the turbidity of a 0.5 McFarland tube. Then each strain of the *E. rhusiopathiae* was inoculated on Müller-Hinton agar (Merck, Germany). Seven antimicrobial disks, comprising penicillin G (PC-G), erythromycin (EM), ciprofloxacin (CIP), imipenem (IMP), ampicillin (AMP), cefazolin (CEZ), and cefotaxime (CTX), (PadtanTeb, Iran), were placed on the inoculated agar plates. The growth inhibition zone was measured around the disks after incubation for 24 hours at 37 °C, according to the guidelines published by the Clinical and Laboratory Standards Institute (CLSI).[11]

Results

Isolation of the Bacteria by Culture

All the 150 samples were examined using phenotypical and biochemical tests, after 24 hours and 48 hours of incubation. Sixteen (10.6%) samples were positive as putative *Erysipelothrix* spp. The colonies of these bacteria on the blood agar were smooth, transparent, and small and with α hemolysis. No samples suspected to contain *Erysipelothrix spp*. were isolated from the hand wounds of the butchers.

All the results concerning the putative *Erysipelothrix spp*. based on phenotypical and biochemical tests are depicted in table 1.

Detection of Erysipelothrix spp. by PCR

Based on the phonotypical method (culture and biochemical tests), 16 (10.7%) cases were recovered from the 150 samples with similar properties related to *E. rhusiopathiae*. Twelve isolates out of the 16 culture-positive isolates were confirmed by PCR. Also, 134 samples that were culture-negative were subjected directly to PCR. Out of the 134 samples, another 8 cases were detected by PCR (table 2). Accordingly, 20 (13.3) cases were detected

Table 1: Phenotypical and biochemical tests for the identification of *Erysipelothrix rhusiopathiae*

Isolate	Specimen	Catalase	Oxidase	H2s production	Citrate	Motility	Indole	Fructose	Sucrose	Mannitol	Lactose
71	Fish	-	-	+	-	-	-	weak	weak	weak	weak
82	Fish	-	-	+	-	-	-	weak	weak	weak	weak
95	Fish	-	-	+	-	-	-	weak	weak	weak	weak
74	Fish	-	-	+	-	-	-	weak	weak	weak	weak
86	Fish	-	-	+	-	-	-	weak	weak	weak	weak
94	Fish	-	-	+	-	-	-	weak	weak	weak	weak
108	Sheep	-	-	+	-	-	-	weak	weak	weak	weak
112	Cow	-	-	+	-	-	-	weak	weak	weak	weak
114	Cow	-	-	+	-	-	-	weak	weak	weak	weak
137	Fish	-	-	+	-	-	-	weak	weak	weak	weak
148	Slaughter glove	-	-	+	-	-	-	weak	weak	weak	weak
125	Calf	-	-	+	-	-	-	weak	weak	weak	weak
144	Fish	-	-	+	-	-	-	weak	weak	weak	weak
104	Goat	-	-	+	-	-	-	weak	weak	weak	weak
135	Sheep	-	-	+	-	-	-	weak	weak	weak	weak
113	Cow	-	-	+	-	-	-	weak	weak	weak	weak

Table 2: distribution of *Erysipelothrix rhusiopathiae* in different specimens, using both culture and PCR results

Specimen type	No. of samples	Culture-Positive		Culture-Negative	
		PCR positive (%)	PCR negative (%)	PCR positive (%)	PCR negative (%)
Fish	39	7 (4.7)	1 (0.7)	2 (1.3)	29 (19.33)
Cow and calf	41	4 (2.7)	0 (0.0)	1 (0.7)	36 (24)
Sheep	38	1 (0.7)	3 (2)	4 (2)	31 (20.7)
Butcher's hand wound	24	0 (0.0)	0 (0.0)	0 (0.0)	24 (16)
Turkey and hen	8	0 (0.0)	0 (0.0)	1 (0.7)	7 (4.7)
Total positive samples	20	12 (8%)		8 (5.33%)	

as *E. rhusiopathiae* by the molecular method. Among the 20 positive cases, 4 (3.33%) cases were determined as *E. rhusiopathiae* by both culture and direct PCR methods (figure 1).

DNA Sequencing Analysis

All the PCR products (20 cases) were sequenced at Bioneer Company (Korea), and all the sequences were compared with GenBank. All the cases were recognized as *E. rhusiopathiae*.

Antimicrobial Susceptibility Test

The diameters of the inhibition zones of 7 commercial antibiotics against the 12 PCR-confirmed isolates were measured with a ruler (table 3). These 12 strains were highly sensitive to PC-G, IMP, EM, AMP, CEZ, CTX, and CIP.

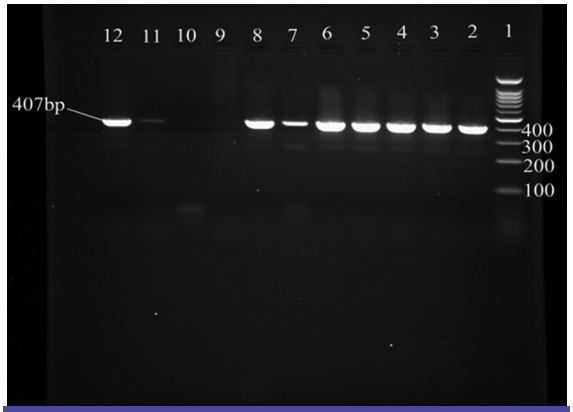

Figure 1: Agarose gel electrophoresis of PCR assay for the identification of *Erysipelothrix rhusiopathiae*. Lane 1: 100bp DNA marker; Lanes 2–8 and 11: positive samples; Lanes 10: negative control; Lane 12: *Erysipelothrix rhusiopathiae ATCC* 19414 as positive control.

Discussion

E. rhusiopathiae was first described in1909 by Rosenbach as a pathogenic microorganism and the infection agent in the cutaneous lesions of erysipeloid in humans.[12] This bacterium is also the causative agent of diseases in animals such as turkeys, pigs, sheep, chickens, shellfish, and ducks. Occupational diseases in humans are caused by contact with infected animals or their infected products. Most infections in humans may be caused through open wounds. The most common related disease in humans is a cutaneous form known as erysipeloid, which can be mild and localized; nonetheless, a severe diffuse form such as sepsis may also be found, which is rarely associated with diseases such as endocarditis, pneumonia, and arthritis in immunocompromised individuals.[13] Erysipeloid typically is an acute infection of the skin, and it improves by itself and resolves without any subsequences. Individuals with the systemic form of erysipeloid, in which organs other than the skin are involved, may have neurologic, cardiologic, or other impairments. Individuals with systemic infection may even die of sepsis if the proper diagnosis is not made and treatment is not initiated early on. Erysipeloid affects every racial type with no predilection. Males and females may be equally affected; however, males are more affected by erysipeloid due to occupational exposure. In addition, erysipeloid can affect any age group.[14,15] Erysipeloid appears in 3 clinical forms in humans: 1) erysipeloid of Rosenbach (localized cutaneous form), 2) spread cutaneous form, and 3) generalized or systemic infection. Local burning or pain at lesion sites is the symptom in the localized and spread forms of erysipeloid. Those afflicted may or may not have fever, malaise, and other constitutional symptoms. In the generalized form, patients present with fever, chills, weight loss, and a variety of other symptoms such as joint pain, cough, and headache, depending on the organ system involved. In the localized form of erysipeloid, lesions most commonly affect the hands (mainly the webs of the fingers); nevertheless, any exposed area of the body may be affected. Lesions consist of well-demarcated, bright red-to-purple plaques with a smooth, shiny surface. Lesions are warm and tender. They leave a brownish discoloration on the skin when resolving. Sometimes vesicles may be present.[7] In the diffuse cutaneous form of erysipeloid, multiple lesions appear on various parts of the body. Lesions are quite demarcated,

Table 3: Antimicrobial susceptibility of 12 isolated *Erysipelothrix rhusiopathiae* strains

Antimicrobial susceptibility disc (Diameter of bacteriostatic circle [mm])										
No. of isolates	**PC-G**	**AMP**	**CEZ**	**CEZ**	**CTX**	**EM**	**CIP**	**GM**	**N**	**IPM**
71	40/S	34/S	34/S	38/S	32/S	35/S	30/S	0/R	0/R	35/S
86	42/S	38/S	32/S	34/S	34/S	39/S	34/S	0/R	0/R	35/S
113	42/S	35/S	35/S	34/S	33/S	44/S	38/S	8.5/R	0/R	34/S
95	40/S	36/S	36/S	42/S	35/S	29/S	37/S	0/R	0/R	36/S
112	38/S	35/S	33/S	34/S	24/S	35/S	36/S	8/R	0/R	33/S
82	39/S	34/S	32/S	38/S	34/S	31/S	38/S	6/R	0/R	34/S
94	42/S	36/S	46/S	41/S	41/S	33/S	40/S	7.5/R	0/R	41/S
114	38/S	34/S	36/S	36/S	37/S	36/S	38/S	0/R	0/R	36/S
125	40/S	35/S	34/S	36/S	33/S	34/S	41/S	8/R	0/R	38/S
74	54/S	38/S	41/S	33/S	39/S	36/S	36/S	7/R	0/R	40/S
135	34/S	36/S	33/S	37/S	32/S	32/S	33/S	9/R	0/R	38/S
144	41/S	40/S	34/S	47/S	30/S	35/S	35/S	7.5/R	0/R	36/S

PC-G: Penicillin G; EM: Erythromycin; CIP: Ciprofloxacin; IMP: Imipenem; N: Neomycin; AMP: Ampicillin; CEZ: Cefazolin; CTX: Cefotaxime; GM: Gentamycin; S: Susceptible; R: Resistant

with violet plaques. In the systemic form of erysipeloid, skin lesions may not be apparent. If present, skin lesions appear as localized areas of swelling surrounding a necrotic center. Skin lesions may also present as several follicular, erythematous papules. Endocarditis is rare, but it is recognized as the most common systemic form of erysipeloid.[16]

In the present study, we evaluated molecular and cultural methods for the isolation and identification of *E. rhusiopathiae* from humans working with animals and animal samples. Additionally, we assessed the antimicrobial susceptibility of some selected antibiotics on the isolated bacteria. Based on the phonotypical method (culture and biochemical tests), 16 (10.7%) cases were recovered from 150 samples with similar properties related to *E. rhusiopathiae*. Twelve isolates out of 16 culture-positive isolates were confirmed by PCR. This phenomenon is due to the similarity of the phenotypical properties of some bacteria with *E. rhusiopathiae*. There were 4 false-positive isolates according to the culture method. Also, 134 samples that were culture-negative were subjected directly to PCR. Out of the 134 samples, another 8 cases were detected by PCR (table 2). Therefore, 20 (13.3) cases were detected as *E. rhusiopathiae* by the molecular method.

In the current study, none of the collected cases of *E. rhusiopathiae* was isolated from human wounds or skin scrapes. In a similar investigation in Iran, 1 case of *E. rhusiopathiae* was isolated from an aborted lamb.[17] However, we isolated *E. rhusiopathiae* from 5 (13.15%) sheep. Addidle et al.[18] reported *E. rhusiopathiae* as the causative agent of reproductive problems in sows. Ersdal et al.[19] investigated the causative agent of infective polyarthritis in lambs and reported that 16 cases had chronic polyarthritis among 48 infected lambs according to PCR and 7 (16.7%) cases out of the 48 cases contained *E. rhusiopathiae* according to the culture method. The swine or pig is the most common source of *E. rhusiopathiae*, with a worldwide distribution with isolates detected in the culture from Africa, Japan, China, Australia, Americas, and Europe; in Iran, however, swine is rare.[7] Then transmission of *E. rhusiopathiae* infection in Iran can be usually caused by contact with other animal sources such as fish, sheep, turkeys, and calves. The distribution of *E. rhusiopathiae* in the different samples tested in the present study was varied. Based on our findings (table 2), fish (31%) was the most common source of *E. rhusiopathiae*. It is well documented that this kind of infection can be most severe when contracted from a fish.[7] Based on our study and different reports from other countries,[20] the isolated strains of *E. rhusiopathiae* exhibit susceptibility to most commercial antimicrobial agents. However, Xu et al.[21] have recently for the first time reported the macrolide resistance gene erm(T), harbored by a novel small plasmid from *E. rhusiopathiae*.

With respect to the importance of the present study, it should be noted that previous research in Iran focused, aside from 1 case of abortion in sheep, solely on diseases in boilers. Indeed, the current literature lacks studies on meat products and the possibility of the development of this disease on the hands of butchers and resultant health implications thereof in our country. The current investigation is the first of its kind to isolate *E. rhusiopathiae* from animals as well as the hands of the butchers working in the Iranian city of Ahvaz and to determine their susceptibility to antibiotics.

Conclusion

E. rhusiopathiae is widely distributed on seafood and presents as an opportunistic pathogen in nature and animals. Humans are liable to become infected through occupational exposure with infected animals, their products, or waste. Infection by eating incorrectly cooked meat or fish is rare. Sufficient attention should be paid to infection by *E. rhusiopathiae* in as much as it is a rare organism that can be the causative agent of severe infections such as infectious endocarditis and polyarthritis following localized infections. We employed molecular and culture methods and detected *E. rhusiopathiae* in 20 (13.3) cases out of 150 samples. All the isolated target bacteria were sensitive to the tested commercial antibiotics. In our study, *E. rhusiopathiae* was mostly isolated from fish samples.

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Conflict of Interest: None declared.

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