Challenge M171-3

May 2017

Wound: *Erysipelothrix rhusiopathiae*

HISTORY

A simulated wound sample collected from a 38 year old pig farmer with hand wound was sent to category A and B laboratories.

Participants were expected to isolate and identify *Erysipelothrix rhusiopathiae*/species. No susceptibility testing was required.

CMPT QA/QC/STATISTICS

All simulated wound samples are produced at CMPT according to CMPT internal protocols. The sample contained a pure culture of *Erysipelothrix rhusiopathiae*.

The samples are assessed for homogeneity and stability using in-house quality control methods and random selection of samples before and during production, and post sample delivery. The number of random samples selected is 15% of the total production batch.

The challenge sample lot was confirmed to be homogeneous and stable for at least **15** days.

Organism identification was confirmed by a reference laboratory.

All challenge components have in-house assigned values based on the most clinically appropriate result; the most clinically appropriate result is determined by expert committee evaluation. No further statistical analysis is performed on the results beyond that described under "Suitability for grading."

SURVEY RESULTS

Reference laboratories: 13/13 (100%) laboratories reported *Erysipelothix rhusiopathiae*

Participants: 54/61 (89%) processing laboratories reported *E. rhusiopathiae* or *Erysipelothrix* species

Suitability for Grading

A challenge is considered suitable for grading if agreement is reached by 80 percent of selected reference group and at least 50 percent of the participants.

MAIN EDUCATIONAL POINTS from M171-3

1. Gram positive rods isolated from superficial sites can be significant causes of infection.
2. Details of the clinical history can provide indications for increased risk for infection with certain species.
3. *Erysipelothrix rhusiopathiae* is an unusual but significant cause of superficial and deep infection.

Organism identification was correctly performed by at least 80 percent of reference laboratories and greater than 50 percent of all laboratories and was thus, determined to be suitable for grading.

COMMENTS ON RESULTS

Of the 61 laboratories that identified the organism, 59 gave excellent results. An identification of *Erysipelothrix rhusiopathiae* or species was given a grade of 4; as new species become recognized in the genus, speciation, or referral, may become necessary but at this time the other species are unusual and of veterinary interest only, although they might conceivably occur as contaminants.

Five laboratories were graded 4 because, even though the isolate was not identified, it was recognized as potentially significant and referred for further testing. *Erysipelothrix* species are not part of the normal human flora. The

Grading

Maximum grade: 4

Reporting *Erysielothrix rhusioathiae* or species was graded 4.

Reporting gram positive bacilli and referring the isolate for identification was graded 4.

Reporting gram negative bacilli or normal skin flora was graded 0.

Reporting *P. bivia* was graded 0.

Table 1. Identification results

Reported	Total	Grade
Erysipelothrix rhusiopathiae ± refer ± presumptive/possible	50	4
Erysipelothrix species, refer	3	4
Erysipelothrix species	1	4
gram positive bacilli, refer	4	4
gram negative bacilli - unidentified, refer	1	0
4+ normal skin flora	1	0
Prevotella bivia	1	0
snnp	3	ungraded
Total	**64**	

laboratory that identified the isolate as *P. bivia* should confirm that the isolate grew aerobically and was not over decolourized.

ISOLATION and IDENTIFICATION

E. rhusiopathiae is a facultatively anaerobic non-motile, non-sporulating, gram positive bacillus.[3] It occurs as short rods, singly or in short chains, but may form long non branching filaments. Organisms may be easily over-decolourized, especially if the culture is old, or in clinical specimens. [11]

The optimal sample for the isolation of *E. rhusiopathiae* is a biopsy covering the entire thickness of the skin. Aspirates of vesicles or bullae are less sensitive while superficial swabs do not usually pick-up the pathogen. *E. rhusiopathiae* can be recovered in standard blood culture media in cases of bacteremia or endocarditis. [1]

Erysipelothrix grows well on blood agar. Aspirates and biopsies should be inoculated into broth, as well, and subcultured every 24 hours. 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. [2]

Selective media is available and often used in veterinary laboratories to take advantage of the remarkable resistance this organism shows towards some chemicals and antimicrobial agents. It can grow in the presence of phenol 0.2%, crystal violet 0.001% and is tolerant to 0.1% sodium azide. Examples of selective media include *Erysipelothrix* selective broth (ESB – a liquid medium containing serum, tryptose, neomycin, vancomycin, and kanamycin), Packer's medium (containing sodium azide and crystal violet, frequently used for subculture after growth in ESB), modified blood azide (MBA – similar to SACV, but without crystal violet), and Bohm's medium (containing azide, kanamycin, phenol, and water blue). [1]

Two types of colony have been described: smooth (S) and rough (R) colonies. These morphology characteristics change with alterations in pH and incubation temperature. Microscopically, smooth colonies appear as small, slightly curved, slender rods with rounded ends, while rough colonies present as long filaments up to 60 µm or more, and may appear beaded or even gram negative.[11]

Members of the genus *Erysipelothrix* are relatively inactive biochemically. *Erysipelothrix* species can be differentiated from morphologically similar bacteria such as *Listeria* and *Corynebacterium* species as *Erysipelothrix spp* are alpha-hemolytic, non-motile, lack catalase production, and are resistance to neomycin. [4] It is oxidase, methyl red, indole, esculin, and Voges-Proskauer negative. It does not reduce nitrate nor does it liquefy gelatin. [5] The production of H_2S in triple sugar iron is the major discriminatory characteristic as very few clinically significant gram positive bacteria produce H_2S. [6]

Some species of *Lactobacillus* are resistant to vancomycin which in addition to the Gram stain, colony morphology, and negative catalase results, make *Lactobacillus* species difficult to differentiate from *Erysipelothrix* species. Dunbar et al. [4] recommends confirming the identity of catalase-negative gram positive rods isolated in the presence of PMNs from a normally sterile site by performing an H_2S test using a TSI slant.

E. rhusiopathiae is usually correctly identified by the API Coryne® identification strips as well as the Vitek automated systems. [6] Maldi –tof identification to the genus level has been shown to be reliable for the Bruker system.[12]

ANTIMICROBIAL SUSCEPTIBILITY

As stated previously, the CLSI document, M45-A2 "Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria" [7] provides interpretive criteria for susceptibility testing of *E. rhusiopathiae* by means of broth microdilution.

Because *Erysipelothrix* is highly susceptible to penicillin, cephalosporins, and fluoroquinolones, antimicrobial susceptibility testing is not required, however, for patients with penicillin allergy, testing of erythromycin and clindamycin may be warranted. [7]

Erysipelothrix is considered intrinsically resistant to vancomycin and thus, proper and prompt identification of this organism is very important, since vancomycin is often used empirically to treat gram positive bacterial infections.

Most strains are also resistant to aminoglycosides, trimethoprim-sulfamethoxazole, polymyxins, sulfonamides, streptomycin, and novobiocin. [1, 5]

CLINICAL RELEVANCE

Infection due to *E. rhusiopathiae* in humans is occupationally related, principally occurring as a result of contact with contaminated animals, their products or wastes, or soil. [2] Most cases in humans and other animals may occur via scratches or puncture wounds of the skin.

Domestic swine are believed to be the most important animal reservoir of *E. rhusiopathiae.*[2] Molluscs, crustaceans, marine and freshwater fish are also sources of infection as the organism survives and grows on the exterior slime of fish. [8] The organism is able to survive in the environment for extended periods of time, and can survive in sunlight for 12 days, or in soil for months. It is also resistant to smoking, pickling, and salting.[2]

Occupations that are at particular risk of infection include veterinarians, abattoir workers, butchers, and workers who handle fish and other seafood, such as fishermen, fishmongers, and cooks. Veraldi et. al. reported a case in a woman who had contact with a scorpion fish. [9]

There are three clinical manifestations of infection in humans: erysipeloid, which may be localized or a generalized, cutaneous form, and a septicemic form which is often associated with endocarditis. [3, 5]

Erysipeloid is the most common form of human infection. It is an acute localized cellulitis that usually occurs on the hand or fingers. [3] It is characterized by local pain with swelling. The affected area is bright red/purplish and forms a localized plaque, with well defined raised borders, occasionally forming bullae or vesicles. Seal finger and whale finger are varieties of this infection.

The generalized cutaneous form involves lesions that progress from the initial site to other locations on the body and is characterized by fever, joint pain and myalgia. It is even less common than the localized form.

Systemic spread is very rare, but sepsis can occur or the development of endocarditis, often in patients with a history of alcohol abuse. The aortic valve is most commonly affected, especially if previously damaged. The disease can be acute or subacute and is complicated by heart failure in 80% and death in 38%.[2] Other systemic infection is rare but bone and joint infection has been reported and Feasi et al reported a perihepatic abscess.[10]

Treatment with penicillin hastens healing and is used in high doses for systemic infection. Empiric use of vancomycin or trimethoprim sulfamethoxazole for cellulitis is ineffective for this species as the isolates are resistant: thus the need for prompt identification to ensure appropriate antimicrobial therapy. [5]

REFERENCES

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

2. Reboli AC, Farrar WE. *Erysipelothrix rhusiopathiae*: an occupational pathogen. *Clinical Microbiology Reviews*. 1989;2:354-359.

3. Veraldi S, Girgenti V, Dassoni F, Gianotti R. Erysipeloid: a review. *Clin Exp Dermatol*. 2009;34:859-862.

4. Dunbar SA, Clarridge JE. Potential Errors in Recognition of *Erysipelothrix rhusiopathiae*. *Journal of Clinical Microbiology*. 2000;38:1302-1304.

5. Wang Q, Chang BJ, Riley TV. *Erysipelothrix rhusiopathiae*. *Vet Microbiol*. 2010;140:405-417.

6. Wellinghausen N. *Listeria* and *Erysipelothrix*. In: Versalovic ea, ed. *Manual of Clinical Microbiology.* Vol 1. 10th ed. ed. Washington, DC.: ASM; 2011:403.

7. Clinical Laboratory Standards Institute. Methods for Antimicrobial Dilution and Disk susceptibility Testing of Infrequently Isolated or Fastidious Bacteria; Approved Guideline- Second Edition. Wayne, PA.: CLSI; 2010;30:M45-A2 CLSI Wayne, PA.

8. Lehane L, Rawlin GT. Topically acquired bacterial zoonoses from fish: a review. *Med J Aust*. 2000;173:256-259.

9. Veraldi S, V Girgenti, R Gianotti. Erysipeloid. Clinical and Experimental Dermatology2009. 34: e605-e607.

10. Feasi M, L Bacigalupo, S Cappato, E Pontali, D Usiglio, G Rollandi, M Filauro, M Mori, G Cassola. *Erysipelothrix rhusiopathiae* intra abdominal abscess. International Journal of Infectious Diseases 2010. 14:e81-e83.

11 Wellinghausen N, *Listeria* and *Erysipelothrix* in Manual of Clinical Microbiology 11 Ed. (Editor in Cheif J Versalovic) 2011. ASM Press, American Society for Microbiology, Washington DC US, 408-9.

12. Eriksson H, E Bagge, V Båverud, C Fellström, D Jansson. *Erysipelothrix rhusiopathiae* contamination in the poultry house environment during erysipelas outbreaks in organic laying hen flocks. Avian Pathology 2014, 43:3 231-237.