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Continuous Medical Education Forum (CME from EB)

Continuous medical education activities; Answers to Case No. 3

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The considerations were *Listeria, Corynebactrium, Erysipelothrix* and *Arcanobacterium* species. The isolate was catalase-negative, which rules out *Corynebacteria* and *Listeria* species. The Gram-positive card of the Vitek 2 system can identify *Erysipelothrix* species, which eliminated that genus. At this point, a reverse CAMP test was performed to determine if the microorganism might be *Arcanobacterium hemolyticum (A. hemolyticum). Arcanobacterium hemolyticum* typically inhibits the beta hemolysis of *Staphylococcus aureus* on blood agar, hence resulting in a positive reverse CAMP test. The patient's isolate neither inhibited nor enhanced the β-hemolysis of *S. aureus* and was not *A. hemolyticum.* Additional biochemical tests were performed. The mystery microorganism is a tiny, Gram positive rod that is strongly β-hemolytic, catalase negative, ferments xylose and demonstrates no reaction on the reverse CAMP test. What is your diagnosis? (To be continued…).

The considerations were *Listeria, Corynebactrium, Erysipelothrix* and *Arcanobacterium* species. The isolate was catalase-negative, which rules out *Corynebacteria* and *Listeria* species. The Gram-positive card of the Vitek 2 system can identify *Erysipelothrix* species, which eliminated that genus. At this point, a reverse CAMP test was performed to determine if the microorganism might be *Arcanobacterium hemolyticum (A. hemolyticum). Arcanobacterium hemolyticum* typically inhibits the beta hemolysis of *Staphylococcus aureus* on blood agar, hence resulting in a positive reverse CAMP test **(Figure 1)**. The patient's isolate neither inhibited nor enhanced the β-hemolysis of *S. aureus* and was not *A. hemolyticum.* Additional biochemical tests were performed and are summarized in **table (1)**. The mystery microorganism is a tiny, Gram positive rod that is strongly β-hemolytic, catalase negative, ferments xylose and demonstrates no reaction on the reverse CAMP test [1]. What is your diagnosis?

Background

Arcanobacterium pyogenes is a well-known pathogen in animals, causing a number of pyogenic infections in cattle and swine. Very few cases of infection in humans due to *A. pyogenes* are reported in English literature, and they are almost always exclusive to rural settings. *Arcanobacterium pyogenes* was initially known as *Corynebacterium pyogenes* and later as *Actinomyces pyogenes.* Based on 16S rRNA gene sequences, it was assigned to the genus *Arcanobacterium* in the year 1997. It is classified as a member of the class, *Actinobacteria;* order, *Actinomycetales;* family, *Actinomycetaceae* and genus *Arcanobacterium* [2].

It belongs to the lineage of Gram-positive bacteria with high guanine + cytosine content. It is generally discussed under coryneform Gram-positive bacilli, but it does not exhibit club shaped morphology which is restricted to *Corynebacterium* spp. In *Arcanobacteria* spp, lysine is the amino acid in the cell wall, but in the phylogenetically related *Actinomyces*

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spp, lysine or ornithine can be found. Palmitic, oleic and stearic acid are the main cellular fatty acids. The genus *Arcanobacterium* currently contains 6 species, 3 of which have been recovered from human clinical specimens: *A. hemolyticum, A. bernadiae, A. pyogenes.* Unlike *A. hemolyticum,* which is a relatively well-known pathogen that causes pharyngitis in humans, the role of *A. pyogenes* in human infections is not clearly established and based on the limited number of case reports. This may partly be due to the fact that it can be misidentified as *A. hemolyticum*. Some authors believe that *A. pyogenes* infection in humans is underreported, as it may be regarded as a coryneform contaminant. *A. pyogenes,* however, has unique biochemical characteristics that can assist in its laboratory identification [3].

Reservoir

Arcanobacterium pyogenes is a commensal in the upper respiratory and genital tracts of domestic animals. It is a well-known opportunistic pathogen in these animals, causing a number of pyogenic infections such as liver abscesses, arthritis, and pneumonia. It has not been described as part of the normal human flora [2].

Clinical significance

Arcanobacterium pyogenes is a rare cause of infections in humans. Unlike *A. hemolyticum,* which produces a well-defined clinical infection in the upper respiratory tract, confirmed cases of human *A. pyogenes* infections are not site specific. Reported cases include soft tissue abscesses, otic infections, intraabdominal infections, cystitis, pneumonia, endocarditis and bloodstream infections, foot ulcers, and others. A common factor in majority of cases is the presence of underlying illnesses, such as cancer and diabetes mellitus, as seen in our index case. Infections are most commonly reported in people living in rural areas, who have close contact with animals [2].

Laboratory identification

Arcanobacterium pyogenes is an aerobic, asporogenous, pleomorphic Gram-positive, non-motile bacillus. Due to its morphology, it may be mistakenly regarded as a coryneform contaminant in specimens. *A. pyogenes* grows well on SBA and can be isolated aerobically in CO2 enriched atmosphere when incubated at 35-37°C for 24-48 hrs. *Arcanobacterium pyogenes* colonies are the largest of all *Arcanobacterium* colonies, with diameters up to 1mm after 48hrs of incubation. The colonies are convex, white to grey and β-hemolytic. Of all *Arcanobacterium* spp, *A. pyogenes* shows the sharpest zone of hemolysis. Polymysin is the protein responsible for the hemolysis and is an important virulence factor in-vivo [3].

Biochemical characteristics

Arcanobacterium pyogenes is **catalase-negative** and metabolizes sugars by **fermentation.** It ferments glucose with succinic and lactic acid as the main end products.

Arcanobacterium pyogenes is the only *Arcanobacterium* spp. of medical relevance that expresses β-**glucuronidase** and ferments **xylose.** *A. pyogenes* has the ability to **hydrolyze gelatin** and reacts with antisera against Lancefield group G streptococci resulting in possible misidentification as *Streptococcus*. The ability to produce β-glucuronidase, hydrolyze gelatin, ferment xylose and produce a negative reverse CAMP test differentiates *A. pyogenes* from *A. hemolyticum.* The 3 medically relevant Arcanobacteria are correctly identified by the API Coryne database system (API Coryne 1 sensitivity 56-85%, API Coryne 2 sensitivity close to 100%). This commercial test system uses a combination of standard biochemical tests and fermentation tests. Definitive identification is best achieved by molecular methods using 16SrRNA-targeted PCR amplification, sequencing and editing. Amplified products are then compared to sequences available in the NCBI Genbank bacterial DNA database [3].

Antimicrobial susceptibilities

Isolates from infected animals are susceptible to β-lactams, gentamicin, macrolides, vancomycin, linezolid and rifampin and are resistant to TMP/SMX, streptomycin and tetracycline1. Susceptibility standards in human infections are not available; however, cases reported show a similar susceptibility pattern as seen in animal infections.

Conclusion

Arcanobacterium pyogenes is a common zoonotic pathogen that can cause infections in humans. Due to morphology on Gram stain, it may be mistaken for a coryneform contaminant. It can also be confused with *Streptococcus* due to reaction with Lancefield group G antisera. Human infections may be more common than reported. Its characteristic biochemical properties can assist in identification and differentiate it from *A. hemolyticum.*

Figure 1. Reverse CAMP test: zone of hemolysis around *S. aureus* not inhibited.

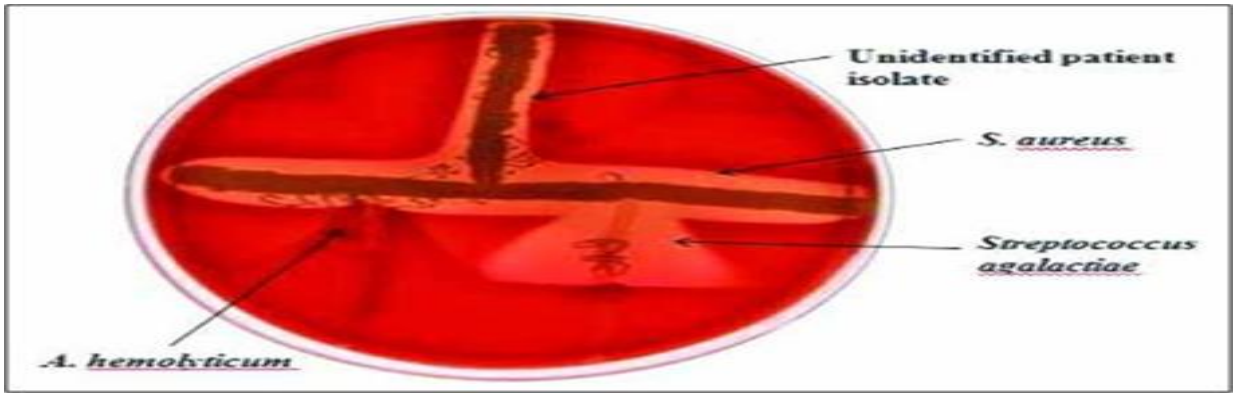

Table 1. Biochemical reactions of the unidentified isolate

BR	Catalase	Glucose	Maltose	Xylose	Lactose	Nitrate reduction	Voges Proskauer	Esculin hydrolysis	PYR
Result	-	+	+	+	+	-	+	-	-

BR: Biochemical reaction, PYR: L - pyrrolidonyl-β- naphthylamide test, -: Negative result, +: Positive result

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