Case Description: A 20-year-old male with a history of spina bifida in the lumbar region, type II diabetes mellitus, solitary left kidney, neurogenic bladder and bowel, and chronic sacral decubiti presented to the emergency department at Cincinnati Children’s Hospital Medical Center with fever, nausea, and vomiting. On examination, there were multiple sacral ulcers, with the largest stage 4 ulcer present on the left buttock and measuring 5cm in diameter by 5cm deep with purulent drainage. A swab specimen of the wound from the left buttock ulcer was initially sent for culture. Following processing and incubation in the laboratory, this culture grew seven different colony-types with no predominant type. A more representative specimen was requested. The patient was subsequently taken to the operating room for debridement. Tissue obtained during this procedure from the ulcer bed was sent for culture. The specimen was cultured on 5% sheep blood agar (SBA) and chocolate (CHOC) agar. The media were incubated at 35°C in 5% CO₂ for 24-48 hours. Following incubation, there was a predominant growth of many tiny grey colonies on CHOC agar. These colonies were small and β-hemolytic on SBA. (Fig.1).
At this point, the major consideration was a possible *Streptococcus* spp. A Gram stain, catalase test, and latex *streptococcus* grouping test to identify Lancefield *streptococcal* groups A, B, C, D, F and G were performed. The microorganism, however, was a pleomorphic small, v-shaped, branching Gram-positive bacillus (Fig. 2), catalase-negative, and unreactive with the Lancefield *streptococcal* grouping antibodies. The Vitek 2 bioMerieux system reported the microorganism as an UNIDENTIFIED organism. In the meantime, the patient was treated with piperacillin-tazobactam and daily wound dressing. He promptly improved after 6 days of antibacterial therapy, remained afebrile with improvement of nausea and vomiting, and continued antibiotics for an additional week after discharge. Which bacteria should be considered at this time and what should be done with the isolate at this point?
Case Twenty Three: Not Just in Animals

Oluyomi Asojo, Ashley Bowman, and Joel Mortensen

The mystery microorganism is a tiny, Gram positive rod that is strongly \( \beta \)-hemolytic, catalase negative, ferments xylose and demonstrates no reaction on the reverse CAMP test. What is your diagnosis?

**Background**

*Arcanobacterium pyogenes* is a well known pathogen in animals, causing a number of pyogenic infections in cattle and swine.\(^6\) 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.\(^1-10\) *A. pyogenes* was initially known as *Corynebacterium pyogenes*\(^4\) and later as *Actinomyces pyogenes*. Based on 16S rRNA gene sequences, it was assigned to the genus *Arcanobacterium* in the year 1997.\(^7\) It is classified as a member of the class, *Actinobacteria*; order, *Actinomycetales*; family, *Actinomycetaceae* and genus *Arcanobacterium*. It belongs to the lineage of Gram-positive bacteria with high guanine + cytosine content.\(^12\) It is generally discussed under coryneform Gram-positive bacilli but it does not exhibit club shaped morphology which is restricted to *Corynebacterium* spp. In *Arcanobacterium* spp, lysine is the amino acid in the cell wall, but in the phylogenetically related *Actinomyces* spp, lysine or ornithine can be found. Palmitic, oleic and stearic acid are the main cellular fatty acids.\(^12\) The genus *Arcanobacterium* currently contains 6 species, 3 of which have been recovered from human clinical specimens: *A. hemolyticum*, *A. bernardiae*, *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.\(^3\)\(^4\) *A. pyogenes*, however, has unique biochemical characteristics that can assist in its laboratory identification.

**Reservoir**

*A. pyogenes* is a commensal in the upper respiratory and genital tracts of domestic animals.\(^3\)\(^6\) It is a well known opportunistic pathogen in these animals, causing a number of pyogenic infections such as liver abscesses, arthritis, and pneumonia.\(^1\)\(^3\)\(^5\) It has not been described as part of the normal human flora.\(^2\)

**Clinical Significance**

*A. pyogenes* is a rare cause of infections in humans. Unlike *A. hemolyticum*, which produces a well defined clinical infection in the upper respiratory tract\(^7\), 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 [Table 2]. 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. Table 2 summarizes cases of *A. pyogenes* infections in humans reported in English literature.

**Laboratory Identification**

*A. 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 CO\(_2\) enriched atmosphere when incubated at 35-37°C for 24-48hrs. *A. 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 \(\beta\)-hemolytic. Of all *Arcanobacterium* spp., *A. pyogenes* shows the sharpest zone of hemolysis. Polymysin is the protein responsible for the \(\beta\)-hemolysis, and is an important virulence factor in-vivo.\(^12\)

**Biochemical Characteristics**

*A. pyogenes* is catalase-negative and metabolizes sugars by fermentation. It ferments glucose with succinic and lactic acid as the main end products. *A. pyogenes* is the only *Arcanobacterium* spp. of medical relevance that expresses \(\beta\)-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*.\(^2\) The ability to produce \(\beta\)-glucuronidase, hydrolyze gelatin, ferment xylose and produce a negative reverse CAMP test differentiates *A. pyogenes* from *A. hemolyticum*. The 3 medically rele-
vant 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.

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

*A. 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 antiserum. Human infections may be more common than reported. Its characteristic biochemical properties can assist in identification and differentiate it from *A. hemolyticum*.

References


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# Questions for STEP Participants

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| 1.       | *Arcanobacterium pyogenes* is primarily pathogenic in which of the following? | A. humans  
B. plants  
C. animals  
D. fish |
| 2.       | Colonies of *Arcanobacterium pyogenes* on sheep blood agar are most commonly exhibit which of the following? | A. a hemolytic  
B. β hemolytic  
C. γ hemolytic  
D. None of the above |
| 3.       | The following have been associated with *Arcanobacterium pyogenes* infection except which of the following? | A. Exposure to pigs and cattle  
B. Residing in a rural environment  
C. Tending hen houses  
D. Intravenous drug abuse |
| 4.       | All of the following *Arcanobacterium spp.* have been recovered from human clinical specimens except which of the following? | A. *A. pyogenes*  
B. *A. pluranimalium*  
C. *A. bernadiae*  
D. *A. hemolyticum* |
| 5.       | Based on Gram-stain morphology, which of the following organisms appear similar and can be confused with *Arcanobacterium pyogenes*? | A. *Listeria* spp.  
B. *Erysipelothrix* spp.  
C. *Streptococcus* spp.  
D. A and B |
| 6.       | Which of the following has a positive reverse CAMP test? | A. *A. pyogenes*  
B. *A. pluranimalium*  
C. *A. hemolyticum*  
D. *A. bernadiae* |
| 7.       | All of the following are true concerning *A. pyogenes* except which of the following? | A. It is part of the normal human flora.  
B. It may be disregarded as a coryneform contaminant.  
C. Most infections are associated with underlying illness.  
D. It has unique biochemical characteristics that can assist in laboratory identification. |
| 8.       | Biochemical properties of *A. pyogenes* include which of the following? | A. Xylose fermentation  
B. Hydrolyzes gelatin  
C. β-glucuronidase activity  
D. All of the above |
| 9.       | *A. pyogenes*  
A. Reacts with antisera against Lancefield group G streptococci  
B. Colonies on sheep blood agar range from <0.5 to 1mm, even though they are the largest of all Arcanobacterium colonies  
C. A and B  
D. None of the above |
| 10.      | *A. pyogenes* infections in humans: | A. Have a well defined clinical pattern  
B. Have been reported in patients with no known exposure to animals  
C. Occur mainly in the upper respiratory tract  
D. Are commonly encountered in clinical and laboratory practice |