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Case report

Bacteroides pyogenes causing serious human wound infection from animal bites

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ABSTRACT

Bacteroides pyogenes is part of the normal oral flora of domestic animals. There is one previous report of human infection, with *B. pyogenes* bacteremia following a cat bite (Madsen 2011). We report seven severe human infections where *B. pyogenes* was identified by Bruker matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDTI-TOF MS), but not by VITEK MS and was misidentified by VITEK ANC card.

1. Introduction

Bacteroides pyogenes, *B. tectus*, *B. suis* are anaerobic Gram negative bacilli that colonise the mouth of mammals, and share similar phenotypic and biochemical characteristics [1]. There has been one reported case of human infection with *B. pyogenes* isolated in blood following a cat bite [2]. Advances in microbiological diagnostic techniques, including the increasing use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS), has brought with it increasing isolation of "new" or previously rarely described organisms [3].

In the two years after the introduction of MALDI-TOF MS into our clinical microbiology laboratory, *B. pyogenes* was isolated from tissue specimens and/or wound swabs in seven cases, all of which were serious human wound infections. A search of our laboratory information system showed that in the 12 months prior to the introduction of MALDI-TOF no isolates of *B. pyogenes* had been identified.

2. Clinical characteristics

The clinical characteristics of seven cases are summarised in Table 1. Patient age ranged from 10 to 69 years, with a median age of 54.4 years (4 male, 3 female) and three patients had diabetes mellitus. All patients had infection affecting a distal limb. Six of the seven patients had been bitten by a cat or a dog, and the time from trauma to presentation ranged from 1 day to 6 weeks. Four were treated with oral and/or intramuscular antibiotics prior to hospitalization. All patients required hospital admission, intravenous antibiotic therapy and surgical management. Two patients required skin grafts, and one required amputation of the forefoot. The median length of stay was 7 days. One patient was treated with a prolonged intravenous course of antibiotics for osteomyelitis. All patients made a full recovery.

3. Microbiological characteristics

The microbiological findings of the seven cases are outlined in Table 2. In three cases only *B. pyogenes* was isolated; the other four had polymicrobial growth including *Pasteurella* spp. (3 cases) and *Staphylococcus* spp. (2 cases). The *B. pyogenes* isolates were obligately anaerobic, non-sporeforming, non-motile, Gram-negative

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Table 1
Bacteroides pyogenes clinical case details.

Case no.	Age/ sex	Site	Cause of injury	Time from injury to hospital admission	Pre-hospital management	Antimicrobial therapy	Surgical procedures performed (in order of procedures)	Length of hospital stay
1	46/F	Left index finger	Dog bite	1 week	IM ceftriaxone	IV piperacillin-tazobactam, oral amoxicillin-clavulanate	1. Debridement, washout 2. Washout, full thickness skin graft	5 days
2	64/ M	Right lower leg	Cat bite	1 week	Oral dicloxacillin, IM ceftriaxone	IV piperacillin-tazobactam, oral amoxicillin-clavulanate	1. Debridement, washout 2. Debridement, washout 3. Debridement, split skin graft	14 days
3	69/F	Right forearm abscess	Cat bite	6 weeks	Oral cephalexin, flucloxacillin, amoxicillin-clavulanate IM ceftriaxone	IV piperacillin-tazobactam, oral amoxicillin-clavulanate	1. Incision and drainage 2. Debridement	2 days
4	47/F	Left index finger	Dog bite	1 day	Nil	IV piperacillin-tazobactam, oral amoxicillin-clavulanate	1. Debridement, washout	3 days
5	76/ M	Left forefoot	Trauma to toe, has two dogs	2 weeks	Nil	IV piperacillin-tazobactam, IV flucloxacillin and iv ciprofloxacin, oral amoxicillin-clavulanate and ciprofloxacin	1. Left forefoot amputation 2. Revision of stump, washout	19 days
6	10/ M	Right hand 1st webspace	Dog bite	1 week	Nil	IV piperacillin-tazobactam, oral amoxicillin-clavulanate	1. Incision and drainage, washout 2. Incision and drainage, washout	5 days
7	67/ M	Left hand 2nd metacarpophalangeal joint	Cat bite	4 weeks	Oral dicloxacillin, amoxicillin	IV piperacillin-tazobactam (6 weeks)	1. Debridement, washout 2. Debridement, washout 3. Debridement, washout 4. Flap closure 5. Debridement, washout	9 days

Note: F = female; IM = intramuscular; IV = intravenous; M = male.

rods. On blood agar plates, colonies after 48 h incubation were 1 mm in diameter, circular with entire edge, low convex, light gray, shiny and smooth and no hemolysis or pigment was detected.

All seven isolates were identified as *B. pyogenes* with confidence scores ≥2.000 by MALDI-TOF MS using the MALDI Biotyper system version 3.1 (Bruker Daltonik Bremen, Germany). *B. tectus/suis* are not included in the Bruker MALDI-TOF MS database. Five isolates were available for analysis using the VITEK MS database version 2 (bioMérieux, Marcy l'Etoile, France), and none were able to identified. *B. pyogenes* was not included on the list of species able to be identified using the VITEK MS database.

Prior to MALDI-TOF implementation, anaerobic organisms were identified in our laboratory using the VITEK-2 ANC card (bioMérieux, Marcy l'Etoile, France). Five isolates were available for analysis using the VITEK-2 ANC card, and all were misidentified with >90% confidence level as *Prevotella* spp., in particular *P. oralis* (4 isolates). Biochemical results from VITEK ANC card showed all isolates were positive for beta-galactopyranosidase, maltotriose, phosphatase, ala-phe,pro-arylamidase, D-glucose, 5-Bromo-4-chloro-3-indoxyl-beta- *N*-acetylglucosamide and D-mannose. All isolates were negative for tyrosine arylamidase, arbutin, L-arabinose, 5-bromo-4-chloro-3-indoxyl-alpha-galactoside, phenylalanine arylamidase, 5-bromo-4-chloro-3-indoxyl-beta-glucoside, phenylphosphonate, L-proline arylamidase, urease, arginine GP, 5-bromo-4-chloro-3-indoxyl-alpha-mannoside, alpha-l-arabinofuranoside, 5-bromo-4-chloro-3-indoxyl-beta-glucuronide, pyruvate and D-xylose. Variable results were obtained for other biochemical tests on the VITEK ANC card.

Bacterial DNA was extracted, amplified, and sequenced using a MicroSEQ 16S rRNA bacterial identification kit (PerkinElmer/Applied Biosystems Inc., Foster City, CA, USA). The resulting 16S rRNA gene sequences were then analyzed with MicroSeq 500 software (version 2.2.1). All isolates were identified by 16S rRNA sequencing as *B. pyogenes* with a sequence homology of >99% using 490 bp with organism CCM 3724 (Czech Collection of Microorganisms); this organism can be traced back to ATCC 35418 (GenBank Accession Number AB542766).

Antimicrobial susceptibility testing was performed for two available isolates using direct colony suspension of 1 McFarland inoculated onto Brucella Blood agar (Thermo Fisher Scientific, Australia). Etest gradient strips (bioMérieux, Marcy l'Etoile, France) were placed onto the media and incubated in anaerobic conditions at 35–37 °C for 42–48 h. The results were interpreted using the Clinical and Laboratory Standards Institutes (CLSI) criteria for Anaerobes [4]. Both isolates tested susceptible to amoxicillin-clavulanate, metronidazole, moxifloxacin, penicillin and piperacillin-tazobactam.

4. Discussion

Bacteroides pyogenes is an anaerobic, gram-negative, non-motile bacillus first isolated in pigs [1], and subsequently reported in the oral flora of dogs [5] and cats [6]. *B. tectus*, *B. suis* and *B. pyogenes* have very similar phenotypic and biochemical characteristics, and the latter two strains have been revealed to be heterotypic synonyms for *B. pyogenes* [7]. Madsen et al. found that only a few biochemical reactions separated *B. pyogenes* from *B. suis/tectus* and that the differentiation between the three species is difficult [4]. It is important to note that *B. tectus/suis* are not on the Bruker MALDI-TOF MS database and that the gene sequence similarity between *B.*