Bloodstream infections due to *Peptoniphilus* spp.: report of 15 cases

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**Abstract**

*Peptoniphilus* spp. are Gram-positive anaerobic cocci (GPAC) that were formerly classified in the genus *Peptostreptococcus*. This study describes 15 cases of *Peptoniphilus* spp. bloodstream infection (BSI) diagnosed from 2007 to 2011 using 16S rDNA sequencing in patients with pneumonia, pre-term delivery, soft tissue infection or colon or bladder disease. Seven out of 15 (47%) of these cases had polymicrobial BSIs. One of the isolates was closely related to *P. duerdenii* (EU526290), while the other 14 isolates were most closely related to a *Peptoniphilus* sp. reference strain (ATCC 29743) and *P. harei* (Y07839). *Peptoniphilus* is a rare but important cause of BSI.

**Keywords:** Anaerobic bacteria, bacteraemia, Gram-positive cocci, *Peptoniphilus*, 16S ribosomal DNA sequencing

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Gram-positive anaerobic cocci (GPAC) are not routinely recovered or identified from many types of clinical specimens because they may be difficult to grow in culture and are frequently isolated as part of mixed polymicrobial infections [1]. *Peptoniphilus* are commensals of the human vagina and gut that were formerly classified in the genus *Peptostreptococcus* [2]. Recent implementation of non-biochemical methods including MALDI-TOF and 16S rDNA sequencing has allowed their accurate identification from a variety of clinical specimens. There are now more than 15 *Peptoniphilus* species within the genus, seven of which were discovered in 2012 [3–10]. Several new *Peptoniphilus* spp. were also recently discovered as part of a study of the human gut microbiome in which microbial culturomics was used to complement genetic analyses [11]. To date, *Peptoniphilus* spp. have most commonly been associated with diabetic skin and soft tissue infections, bone and joint infections and surgical site infections [12–15]. A recent study of pre-term labour and early neonatal sepsis also isolated *Peptoniphilus* spp. from amniotic fluid causing chorioamnionitis [16]. To our knowledge, our study is the first case series of *Peptoniphilus* spp. causing bloodstream infection (BSI) either alone or as part of a polymicrobial infection.

A retrospective review of all adult patients (>17 years) with at least one blood culture positive for *Peptoniphilus* spp. was carried out. All cases were diagnosed by the regional centralized clinical microbiology laboratory, Calgary Laboratory Services (CLS), between 1 July 2007 and 31 December 2012. The study protocol was approved by the Conjoint Health Research Ethics Board at the University of Calgary.

All isolates were recovered from blood cultures. The regional protocol for adult blood culture draws routinely collects two sets, each consisting of both an aerobic (iFA) and anaerobic (iFN) bottle with incubation, culture and growth monitoring being performed by the BacT/Alert 3D System (bioMérieux, Laval, QC, Canada). Positive blood cultures were immediately pelleted, Gram stained and plated to standard aerobic and anaerobic culture media. *Peptoniphilus* spp. isolates demonstrated typical GPAC morphology on Gram stain from anaerobic Brucella blood agar. Prior to 2011, anaerobic GPAC isolates were preliminarily identified using antibiotic (KVC) disc testing and biochemical analysis with the Vitek 2 ANC card (bioMérieux), but subsequently, MALDI-TOF (Vitek MS; bioMérieux) has been used. Antibiotic susceptibility testing was performed using E-test strips (bioMérieux) according to CLSI guidelines and the manufacturer’s instructions [17]. All isolates were susceptible to penicillin and metronidazole, and all but one of the isolates (case #7) was also susceptible to clindamycin.

Preliminary GPAC identifications were confirmed by performing partial sequencing of the 16S rDNA gene using standard methods [2,10,18] (ABI Prism 3130 sequencer; Applied Biosystems, Foster City, CA, USA). The SmartGene Integrated Database Network System (IDNS) indicated the most closely related species (per cent identity >99%) as shown in Table 1. A maximum likelihood phylogeny was produced illustrating the relation of the 15 clinical isolates with publicly available sequences [19,20]. Genus-level identity (99–100%) was achieved for all 16S rDNA sequences in this study. Species level identification by the IDNS Smartgene database often resulted in multiple species with >99% sequence similarity. Independent phylogenetic analysis of the partial 16S rDNA sequences
| Study ID# | Age & Sex | Significant past medical history | Discharge diagnoses | Blood culture - ID by Gram stain and either MALDI-TOF or Vitek2 ANC card<sup>a</sup> | ID by 16S sequence using Smartgene IDNS database (Genbank accession) | Sequence length (bp) | Days antibiotics (IV & PO) from day of positive blood draw | Outcome |
|----------|-----------|----------------------------------|---------------------|----------------------------------|----------------------------------------------------------------|----------------|---------------------------------------------------------------|---------|
| 01       | 20F       | Remote Guillan + Barre Syndrome 2<sup>nd</sup> CMV on home oxygen | PROM (25 weeks), chorioamnionitis, Acute exacerbation of COPD | MALDI: anaerobic Gram positive cocci | Peptoniphilus sp. DQ986463 (KF963587) | 505            | 1                                                             | Discharged home, infant survived |
| 03       | 78F       | COPD on home oxygen | | Peptoniphilus sp. | Peptoniphilus sp. DQ986463 (KF963593) | 518            | 11                                                            | Discharged to rehabilitation facility |
| 04       | 84F       | Remote stroke with deficits requiring total care, nutrition via gastrostomy tube | Pneumonia, sepsis | MALDI: anaerobic Gram-positive cocci | Peptoniphilus sp. DQ986463 (KF963588) | 488            | 17                                                            | Deceased |
| 05       | 96M       | Colon cancer diagnosed on admission with probably metastases | Urosepsis, coloan cancer invading bladder | MALDI: polymicrobial (Peptoniphilus sp. Aerococcus urinae) | Peptoniphilus sp. DQ986463 (KF963585) | 488            | 18                                                            | Discharged back to long-term care facility |
| 06       | 42F       | Remote tubal ligation | | Vitek2: polymicrobial | Peptoniphilus sp. DQ986463 (KF963595) | 508            | 4                                                             | Discharged home |
| 07       | 54F       | Type 2 diabetes mellitus | Diabetes mellitus extremity infection requiring above-knee amputation | MALDI: polymicrobial (Peptoniphilus sp., Group B Streptococcus) | Peptoniphilus sp. DQ986463 (KF963590) | 499            | 12                                                            | Discharged to rehabilitation facility |
| 08       | 33F       | None | Septic abortion (8 weeks) | MALDI: Peptoniphilus sp. | Peptoniphilus sp. DQ986463 (KF963583) | 496            | 18                                                            | Discharged home |
| 09       | 66F       | Remote bladder cancer with urostenosis and ileal conduit, smoker | Complicated urinary tract infection, bacteremia | MALDI: Peptoniphilus sp. | Peptoniphilus sp. DQ986463 (KF963582) | 477            | 10                                                            | Discharged home |
| 10       | 34F       | End-stage renal disease on haemodialysis via fistula 2<sup>nd</sup> Goodpasture’s disease, cardiac arrest 2004 | Recurrent periendritis | Vitek2: Peptoniphilus sp. | Peptoniphilus duodenii (KF963596) | 380            | 7                                                             | Discharged home |
| 11       | 82M       | Remote prostate cancer, Crohn’s disease on prednisone | Ischaemic colitis with pelvic abscess, B-cell lymphoma of colon | Vitek2: polymicrobial | Peptoniphilus sp. DQ986463 (KF963592) | 518            | 21                                                            | Deceased |
| 12       | 42M       | Renal transplant, on sirolimus, tacrolimus and prednisone | Sepsis, deep vein thrombosis | MALDI: Peptoniphilus sp. | Peptoniphilus sp. DQ986463 (KF963591) | 494            | 13                                                            | Discharged home |
| 13       | 32F       | Unknown (paper chart unavailable) | Stillbirth (18 weeks), chorioamnionitis | Vitek2: Peptoniphilus sp. | Peptoniphilus sp. DQ986463 (KF963594) | 438            | 15                                                            | Discharged home, foetal death |
| 14       | 80M       | Metastatic prostate cancer with recent venous thromboembolism | Bladder tumour eroding into rectum | MALDI: polymicrobial | Peptoniphilus sp. DQ986463 (KF963589) | 500            | 3                                                             | Discharged to community hospital |
| 15       | 96M       | COPD, pacemaker | Acute exacerbation COPD, aspiration pneumonia, stroke | MALDI: polymicrobial (Peptoniphilus sp. B. fragilis, S. viridans) | Peptoniphilus sp. DQ986463 (KF963587) | 492            | 2                                                             | Discharged |
| 16       | 87M       | Type 2 diabetes mellitus | Acute renal failure secondary to obstruction, urinary tract infection | MALDI: polymicrobial | Peptoniphilus sp. (E. faecalis) | Peptoniphilus sp. DQ986463 (KF963584) | 499            | 13                                                            | Discharged home |

CMV, cytomegalovirus; PROM, premature rupture of membranes; COPD, chronic obstructive pulmonary disease.

<sup>a</sup> For polymicrobial cultures identification was performed on all isolates individually.
confirmed the similarity of CLS sequence KF963596 with *P. duerenii* while the remaining 14 CLS sequences shared highest similarity with a *Peptoniphilus* spp. (ATCC 29743). These sequences were also highly similar (99.6%) to *P. harei* (Fig. 1).

Patient characteristics and blood culture results are summarized in Table 1. During the study period, 15 hospitalized patients were diagnosed with *Peptoniphilus* BSI; nine women and six men. The mean age was 62 years (range, 20–96 years; median, 66 years) and the mean length of stay was 18 days (range, 4–62 days; median, 11 days). BSI due to *P. duerenii* was confirmed in a 34-year-old female with end-stage renal disease secondary to Goodpasture’s disease and recurrent pericarditis. All of the other patients with *Peptoniphilus* spp. BSI were due to septic abortion with chorangionitis (*n* = 3), acute exacerbation of COPD and/or pneumonia (*n* = 2) or underlying bowel and/or bladder disease (*n* = 6) (Table 1). Seven (50%) patients had polymicrobial BSIs with other bacteria besides *Peptoniphilus* spp., including (i) *Aerococcus urinae*, (ii) *Escherichia coli*, (iii) Group B Streptococcus, (iv) *Eggerthella lenta*, (v) *Bacteroides fragilis* and viridians streptococci group, (vi) *Proteus mirabilis* and *Staphylococcus aureus*, and (vii) *Enterococcus faecalis*. Most polymicrobial BSIs originated from a bowel or bladder source, except for one patient with diabetes mellitus and severe necrotizing infection of the leg, and one elderly patient with aspiration pneumonia.

FIG. 1. Maximum likelihood phylogeny of partial 16S rDNA (variable regions V1–V3) sequences from the *Peptoniphilus* BSI isolates (GenBank accessions KF963582–KF96396) in relation to publicly available *Peptoniphilus* reference strains (Genbank accession in parentheses). FastTree 2 software was used to create the tree using the generalized time-reversible nucleotide substitution model [20].

Only one of the three women with *Peptoniphilus* BSI secondary to septic abortion/chorangionitis was discharged home with a live infant. Three of 15 (20%) of the patients died, and all of them were elderly with significant underlying co-morbidities. All of the other patients with *Peptoniphilus* BSI were successfully treated with antibiotics and discharged from hospital.

*Peptoniphilus* spp. would typically be recovered as a component of mixed bacterial flora in complex abscesses in the abdomen or pelvis. We would therefore expect to recover *Peptoniphilus* spp. as part of a polymicrobial BSI infection in patients with underlying bowel or bladder disease as occurred in our study. The recovery of *Peptoniphilus* alone indicates that this organism is a rare but important cause of BSI as a primary pathogen in certain clinical settings (i.e. septic abortion, soft tissue infection in immunocompromised patients and pneumonia).

Sequencing of the 16S rDNA gene is a valuable tool for definitive molecular identification of important clinical isolates that cannot be readily identified by phenotypic methods or MALDI-TOF [21]. *Peptoniphilus* spp. are often misidentified using biochemical methods [22], and MALDI-TOF databases currently do not include most species. Most of our isolates had highly similar sequences to a reference *Peptoniphilus* spp. strain that has been called *P. assacharolyticus* ATCC 29743 (GenBank DQ986463). However, it is clear from our phylogenetic
analysis (Fig. 1) that this reference strain is divergent from the other typed *P. assacharolyticus* ATCC 14963 (AF542228), and most closely related to *P. harei* (Y07839). Our results support a previous report of 89.5% 16S rDNA similarity between *P. assacharolyticus* strains ATCC 29743 and ATCC 14963 while the ATCC 29743 shares 99.4% similarity with *P. harei* (Y07839) [22]. We have therefore chosen for now to designate most of our isolates as *Peptoniphilus* spp.

In summary, clinical microbiology laboratories should be aware that *Peptoniphilus* are rare but important causes of BSI infection either as the primary pathogen or as part of a polymicrobial infection. Isolates meeting the preliminary phenotypic characteristics for GPAC should be referred for definitive identification using partial 16S rDNA sequencing. Delineation of the clinical and epidemiological significance and pathogenic potential of *Peptoniphilus* spp. in humans is dependent upon further isolates from clinical samples and full phenotypic and genotypic characterizations.

**Nucleotide Sequence Accession Numbers**

The sequences of the *Peptoniphilus* strains described here have been deposited in GenBank under accession numbers KF963582–KF963596.

**Author Contributions**

Kristen Brown: study design, patient chart reviews and manuscript preparation. Tarah Lynch: composed phylogenetic tree and manuscript preparation. Deirdre Church and Daniel Gregson: study design, 16S rDNA sequencing and manuscript preparation.

**Transparency Declaration**

The authors declare that they have no conflicts of interest.

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