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. hareii* (Y07839). *Peptoniphilus* is a rare but important cause of BSI.

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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 choramnionitis [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 (*i*FA) and anaerobic (*i*FN) 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

TABLE 1. **Clinical characteristics and blood culture results of *Peptoniphilus* BSI cases**

Study ID#	Age & Sex	Significant past medical history	Discharge diagnoses	Blood culture –ID by Gram stain and either MALDI-TOF or Vitek2 ANC card[a]	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 2nd CMV	PROM (25 weeks), chorioamnionitis	MALDI: anaerobic Gram positive cocci	*Peptoniphilus sp.*DQ986463 (KF963587)	505	1	Discharged home, infant survived
03	78F	COPD on home oxygen	Acute exacerbation of COPD	Vitek2: *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, colon 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	Gastroenteritis	Vitek2: polymicrobial (*Peptoniphilus* sp., *E. coli*)	*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 urostomy and ilieal conduit, smoker	Complicated urinary tract infection, bacteraemia	MALDI: *Peptoniphilus* sp.	*Peptoniphilus* sp. DQ986463 (KF963582)	477	10	Discharged home
10	34F	End-stage renal disease on haemodialysis via fistula 2nd Goodpasture's disease, cardiac arrest 2004	Recurrent pericarditis	Vitek2: *Peptoniphilus* spp.	*Peptoniphilus duerdenii* (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* spp., *Eggerthella lenta*)	*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* spp.	*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. B. fragilis, S. viridans*)	*Peptoniphilus* sp. DQ986463 (KF963589)	500	3	Discharged to community hospital
15	96M	COPD, pacemaker	Acute exacerbation COPD, aspiration pneumonia, stroke	MALDI: polymicrobial (anaerobic Gram-positive cocci, *P. mirabilis, S. aureus*)	*Peptoniphilus* sp. DQ986463 (KF963586)	492	2	Deceased
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.
[a]For polymicrobial cultures identification was performed on all isolates individually.

confirmed the similarity of CLS sequence KF963596 with *P. duerdenii* 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. duerdenii* 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 choramnionitis ($n = 3$), acute exacerbation of COPD and/or pneumonia ($n = 3$), skin and soft tissue infection ($n = 2$) 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 streptococcus 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.

Only one of the three women with *Peptoniphilus* BSI secondary to septic abortion/choramnionitis 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

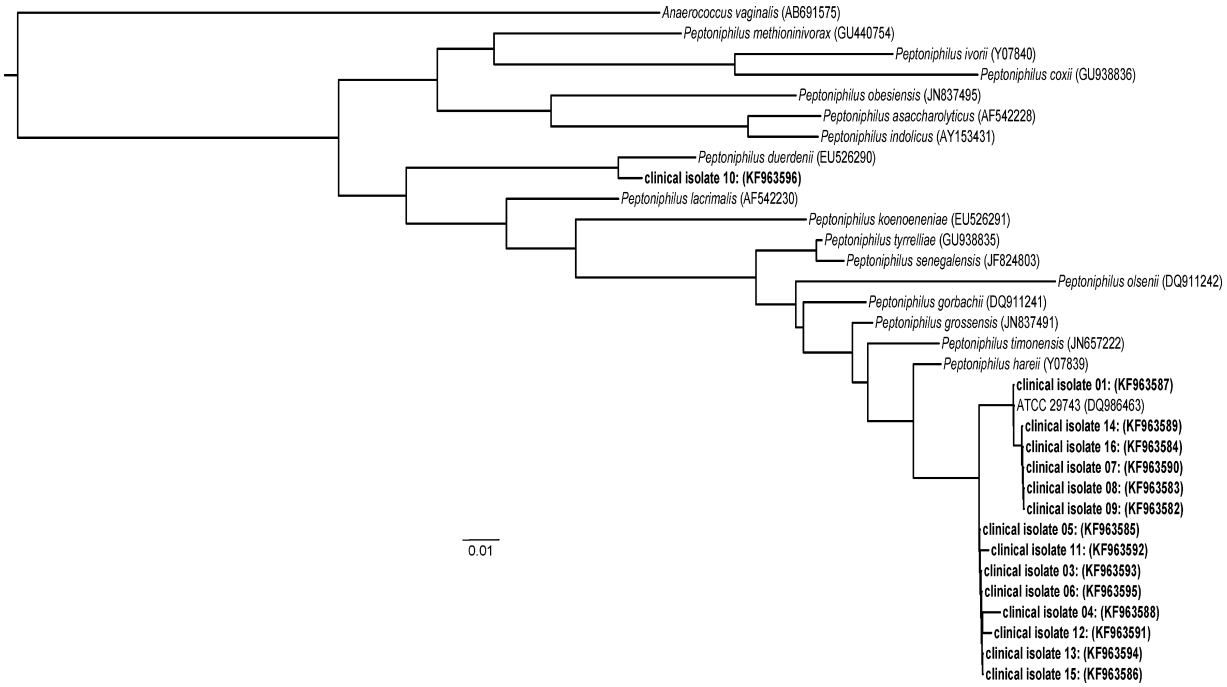

FIG. 1. Maximum likelihood phylogeny of partial 16S rDNA (variable regions V1–V3) sequences from the *Peptoniphilus* BSI isolates (GenBank accessions KF963582–KF96396) in relation to publicly available *Peptoniphilus* reference strains (Genbank accession in parentheses). Fasttree 2 software was used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

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. asaccharolyticus* 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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