Case Report

Two cases of severe sepsis caused by *Bacillus pumilus* in neonatal infants

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Bacillus pumilus is an environmental contaminant that has been rarely associated with clinical infections. Here, two cases of severe sepsis caused by *B. pumilus* are described in two full-term neonates; one in a female infant with no factors predisposing her to infection and the other in a male infant requiring mechanical ventilation and an intravenous catheter. In both cases, the micro-organism was recovered from repeated blood cultures and was identified using biochemical assays and 16S rRNA gene sequencing. Both infants were successfully treated with vancomycin. This report reveals the potential role of *B. pumilus* as a bloodstream pathogen during infancy.

Introduction

Other than *Bacillus anthracis*, species of the genus *Bacillus* are rarely associated with infection, despite their widespread distribution in the environment, and are more frequently isolated as culture contaminants (Logan *et al.*, 2009). Bacteraemia caused by *Bacillus* species is mainly attributed to *Bacillus cereus* and has been reported mostly in immunocompromised patients with haematological malignancies (Farrar, 1963; Banerjee *et al.*, 1988; Drobniewski, 1993). The clinical significance of the isolation of these micro-organisms requires careful evaluation, thus the initial report of a blood culture growing a *Bacillus* species often creates a therapeutic dilemma, even in high-risk patients. Here, we report, to the best of our knowledge, the first two cases in literature of severe sepsis caused by *Bacillus pumilus* in neonatal infants, one of which had no predisposing factors.

Case reports

The first case concerns a female infant, weighing 3.39 kg, who was born to a healthy 21-year-old primigravida woman at term (38-week gestation). Routine ultrasonography at 32 gestational weeks speculated gastric or duodenal atresia. Soon after birth, mild abdominal distension and gastric residue were noticed in the neonate. Radiological studies were normal, without anatomical evidence of gastrointestinal tract anomaly. Oral feeds were started on day 2 of her life,

which were well tolerated. On postnatal day 8, however, the infant appeared to be septicaemic.

Laboratory testing showed a white blood cell count of $20\,180\ \mu\text{l}^{-1}$ and a high C-reactive protein level ($120\ \text{mg l}^{-1}$). Urine and cerebrospinal fluid (CSF) cultures were sterile. Blood culture demonstrated growth of Gram-positive rods after overnight incubation at 35 °C in broth (BacT/ALERT, PF Paediatric FAN; bioMérieux). Subsequently, the micro-organism was identified as *B. pumilus* with 99.9 % confidence based on the results of tests performed in API 50 CHB (*Bacillus*) test strips (bioMérieux) and the first 12 tests of the API 20 E gallery (bioMérieux; Table 1). It should be noted that the combination of API 50 CHB and API 20 E tests represents the most accepted method for *B. pumilus* identification in the clinical laboratory (Logan & Berkeley, 1984; Logan *et al.*, 2009).

Antimicrobial susceptibility testing of the micro-organism was determined by the agar-gradient diffusion technique (Etest; bioMérieux). Susceptibility breakpoints were interpreted using the CLSI MIC breakpoint criteria for *Bacillus* species (other than *B. anthracis*), which were adapted from those used for *Staphylococcus* species (CLSI, 2010). The isolate was found to be susceptible to penicillin, ampicillin, imipenem, vancomycin, erythromycin, levofloxacin, clindamycin and trimethoprim–sulfamethoxazole (Table 2).

This micro-organism was, at first, identified as a contaminant, until a blood culture collected on postnatal day 10, once again, yielded *B. pumilus* growth. Antibiotic treatment was initiated with vancomycin ($10\ \text{mg kg}^{-1}$ body weight,

Abbreviation: CSF, cerebrospinal fluid.

Table 1. Biochemical characteristics identifying the *B. pumilus* isolates recovered from bloodstream infections using API 50 CHB and API 20 E tests.

In both cases the isolates gave identical results. –, Negative; +, positive.

Characteristic	<i>B. pumilus</i>
API 50 CHB tests for utilization of:	
L-Arabinose, ribose, D-glucose, D-fructose, D-mannose, arbutin, aesculin, salicin, cellobiose, sucrose, trehalose, β -gentiobiose, D-tagatose	+
Glycerol, erythritol, D-arabinose, D-xylose, L-xylose, adonitol, β -methylxyloside, galactose, L-sorbose, rhamnose, dulcitol, inositol, mannitol, sorbitol, α -methyl-D-mannoside, α -methyl-D-glucoside, N-acetylglucosamine, amygdalin, maltose, lactose, melibiose, inulin, melezitose, raffinose, starch, glycogen, xylitol, turanose, D-lyxose, D-fucose, L-fucose, D-arabitol, L-arabitol, gluconate, 2-ketogluconate, 5-ketogluconate	–
API 20 E tests	
β -Galactosidase (ONPG) activity, citrate utilization (Simmons test), Voges–Proskauer test, gelatinase activity	+
Hydrolysis of L-arginine, L-lysine, L-ornithine and L-tryptophan, H ₂ S production, urease activity, indole production, nitrate reduction	–

every 8 h). Biochemical identification confirmed the isolates as *B. pumilus* as did sequencing of the 16S rRNA gene, using the universal primers 16S-27f (5'-AGAGTT-TGATCMTGGCTCAG-3') and 16S-907r (5'-CCGTCAT-TCMTTTRAGTTT-3') for the PCR, and 16S-27f and 16S-519r (GWATTACCGCGGCKGCTG) for the sequencing chemistry mixtures (Mellmann *et al.*, 2008). The infant promptly responded to the treatment and was discharged on day 20 of admission in good health.

The second case was detected 14 months later in a male term infant of 38 weeks of gestational age, who was born to a 30-year-old gravida two mother with a family history of myotonic dystrophy. The clinical manifestations were consistent with a diagnosis of Prader–Willie syndrome, without excluding the possibility of congenital myotonic dystrophy. Soon after birth, the infant developed respiratory distress syndrome and required mechanical ventilation for 6 days. An umbilical vein catheter was inserted, which was replaced in 5 days by a peripheral intravenous central catheter. Oral feeds were started on day 7 of his life, which were not tolerated due to hypotonia, so parenteral nutrition was continued. On postnatal day 19,

the infant became febrile and lethargic, with signs of septicaemia. The leukocyte count was 17 080 μl^{-1} and a high C-reactive protein level (105 mg l^{-1}) was detected. CSF examination was normal. Blood culture after overnight incubation yielded *B. pumilus*, which was identified using biochemical tests (Table 1) and 16S rRNA gene sequencing, as described earlier. This isolate was found to be resistant to clindamycin and susceptible to penicillin, ampicillin, imipenem, vancomycin, erythromycin, levofloxacin and trimethoprim–sulfamethoxazole (Table 2). The same micro-organism was isolated from blood cultures performed over four consecutive days. Once these blood culture results were known, vancomycin was administered (10 mg kg^{-1} body weight, every 8 h for 10 days) and the neonate promptly responded to the treatment. Approximately 2 months after birth, the infant was discharged in good health.

Discussion

Bacillus species, apart from *B. anthracis* and *B. cereus*, have little or no pathogenic potential and are rarely associated

Table 2. Antibiotic susceptibilities of the *Bacillus pumilus* isolates

S, Susceptible; R, resistant.

Antibiotic	Isolates recovered from the first case		Isolates recovered from the second case	
	MIC ($\mu\text{g ml}^{-1}$)	Susceptibility	MIC ($\mu\text{g ml}^{-1}$)	Susceptibility
Penicillin	0.016	S	0.016	S
Ampicillin	0.016	S	0.016	S
Imipenem	1	S	1	S
Vancomycin	0.38	S	0.5	S
Erythromycin	0.032	S	0.38	S
Levofloxacin	0.094	S	0.047	S
Clindamycin	0.064	S	24	R
Trimethoprim /sulfamethoxazole	0.032	S	0.008	S

with clinical infections (Drobniewski, 1993). A computerized search in the National Library of Medicine database provided a total of 18 cases of clinically significant infections caused by *B. pumilus*. These infections fall into three broad groups: (i) 10 cases of bloodstream infections (nine cases in adults and one case in an 8-year-old child) (Farrar, 1963; Cotton *et al.*, 1987; Banerjee *et al.*, 1988; Galanos *et al.*, 2003; Ozkocaman *et al.*, 2006; Bentur *et al.*, 2007; Farhat *et al.*, 2008); (ii) three cases of cutaneous infections (Tena *et al.*, 2007); and (iii) five cases of food poisoning, characterized by toxin-induced emetic and diarrhoeagenic syndromes (Suominen *et al.*, 2001; From *et al.*, 2007).

The present study describes, to the best of our knowledge, the first cases of septicaemia caused by *B. pumilus* in neonates. Documented cases of clinically significant bloodstream infections in neonates or older infants due to non-anthraxis *Bacillus* species are very limited and include only cases caused by *B. cereus* (Farrar, 1963; Patrick *et al.*, 1989; Hilliard *et al.*, 2003; Adler *et al.*, 2005). This is despite the fact that the neonatal population is particularly susceptible to disseminated disease caused by environmental organisms due to molecular, cellular and functional deficiency of both cellular and humoral immunity (Schelonka & Infante, 1998). The low number of cases could be partially attributed to the fact that clinical laboratories may not attempt to identify *Bacillus* organisms at the species level, arbitrarily designating them as contaminants, without adequate consultation with clinicians. Previous studies have shown that *Bacillus* species should be recognized as true pathogens, especially in neonates and other immunosuppressed hosts and when isolated from blood cultures collected at the same time or from at least two samples collected at different time (Cotton *et al.*, 1987; Weber *et al.*, 1989). In our cases, *B. pumilus* grew in two and four consecutive blood cultures collected from different days, which, in association with clinical picture, fulfils the appropriate criteria for distinguishing true bloodstream infection from sample contamination.

Risk factors for *B. pumilus* bacteraemia in adults have included use of a central venous catheter (Bentur *et al.*, 2007), cancer (Banerjee *et al.*, 1988; Farhat *et al.*, 2008), particularly haematological malignancies (Cotton *et al.*, 1987; Ozkocaman *et al.*, 2006), and spinal anaesthesia (Farrar, 1963). Predisposing factors were not identified in our first neonate, while the second neonate required prolonged total parenteral nutrition, mechanical ventilation, umbilical vein catheterization and use of long-term, intravascular catheters.

B. pumilus isolates from both infants were susceptible to penicillin, as well as to the vast majority of the remaining antibiotics tested. However, isolates from the second case exhibited resistance to clindamycin (Table 2). The different susceptibility profiles makes it unlikely that there is an epidemiological association between the two cases, beside the fact that the two neonates were hospitalized in the same

neonatal ICU within a time interval of 14 months. Both neonates promptly responded to vancomycin treatment, which is considered the drug of choice for *Bacillus* infections (Weber *et al.*, 1989). In conclusion, our study highlights the fact that *B. pumilus* is a potential bloodstream pathogen in neonates, and should be recognized as such, especially if this micro-organism is isolated from subsequent blood cultures.

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