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CASE REPORT

# Bacteremia caused by *Pantoea agglomerans* and *Enterococcus faecalis* in a patient with colon cancer

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## Summary

*A case of bacteremia caused by Pantoea agglomerans (P. agglomerans) and Enterococcus faecalis in a male patient with colon cancer is reported. He was successfully treated with vancomycin and meropenem. The clinical isolate was identified as P. agglomerans by 16S rRNA gene sequencing.*

**Key words:** colon cancer, DNA sequencing, meropenem, *Pantoea agglomerans*, polymicrobial bacteremia, 16S rRNA

## Introduction

*P. agglomerans* (formerly named *Enterobacter agglomerans*) is a Gram-negative organism of the *Enterobacteriaceae* family. It can be found in human and animal faeces, and in plants. Human infections caused by *P. agglomerans* more often involve bones and joints and occur mainly after plant thorn injuries [1,2]. In addition, clinical isolates have been recovered from blood and chronic ambulatory peritoneal dialysis fluid [3,4].

*P. agglomerans* is not as frequent a cause of endogenous nosocomial infection as *Enterobacter* species. This probably reflects the greater intrinsic susceptibility of *P. agglomerans* than other *Enterobacter* species to $\beta$-lactams antibiotics [5] and the opportunity for infection rather than the intrinsic virulence of the organism involved [6]. *P. agglomerans* has been implicated in a nosocomial outbreak of bacteremia associated with contaminated parenteral nutrition [7], whereas the organism is also associated with outbreaks of pseudobacteremia due to contaminated intravenous solutions, stored blood products and blood collection tubes [6,8]. Here, we describe a case of bacteremia caused by *P. agglomerans* and *Enterococcus faecalis* in a patient with colon cancer.

## Case presentation

A 72-year-old man suffering from colon cancer was admitted in October 2005 to our hospital because of mild hemorrhage from sigmoidostomy. In June 2004 he had undergone sigmoidectomy followed by adjuvant chemotherapy. Between the two hospital visits, prostate cancer had been diagnosed and treated with androgen blockage, while colon cancer had relapsed locally and had been re-operated twice. First- and second-line chemotherapy was given and continued until last presentation. On admission, blood pressure was 100/60 mmHg. Laboratory results showed hemoglobin 12.3 g/dl, leukocytes $10 \times 10^9$/l with 70% neutrophils and platelet count $360 \times 10^9$/l.

On day 5, while in an unstable condition, the patient developed rigors and a temperature of 40º C. No source of infection was clinically apparent. Laboratory data was notable for a leukocyte count of $13 \times 10^9$/l

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with 80% neutrophils, hemoglobin 9.0 g/dl, activated partial thromboplastin time (APTT) 46.1 sec (normal range, 26.0-38.0) and international normalized ratio (INR) 1.5 (normal range 0.8-1.2). Chest X-ray as well as CT scan of the brain, chest and abdomen showed no signs of infection. Urine, stool and sputum cultures were negative. Two sets of blood cultures, which had been taken from a peripheral vein on day 5 while no central venous catheter or other prosthetic material were in place, yielded a Gram-negative rod and a Gram-positive coccus later identified as *P. agglomerans* and *E. faecalis*, respectively. Because of the unstable condition the patient was treated with meropenem (6 g daily) and vancomycin (1.5 g daily) for 7 days, and recovered completely from his infection. There was no evidence of relapse until day 30 when the patient died because of heart failure.

Environmental samples, e.g., sinks, showerhead, taps, detergents, and disinfectants, from the patient's medical ward were collected and cultured as described previously [9]. All environmental samples failed to yield this organism. Finally, investigation revealed strict adherence to well-established guidelines for blood collection and blood culture processing [10].

## Microbiological investigation

Two sets of standard medium blood cultures in a total of 4 bottles were taken immediately after the onset of fever. Blood cultures were processed with the BacT/ALERT System (bioMerieux). The 4 bottles (2 aerobic and 2 anaerobic) were positive within 12 h of incubation for a yellow-pigmented, oxidase-negative, fermentative Gram-negative bacterium identified as *Pantoea* spp. (biotype 1007173; probability 95.1%) by the API 20E system (bioMerieux), and a Gram-positive coccus in pairs and chains identified as *E. faecalis* by the same commercial system.

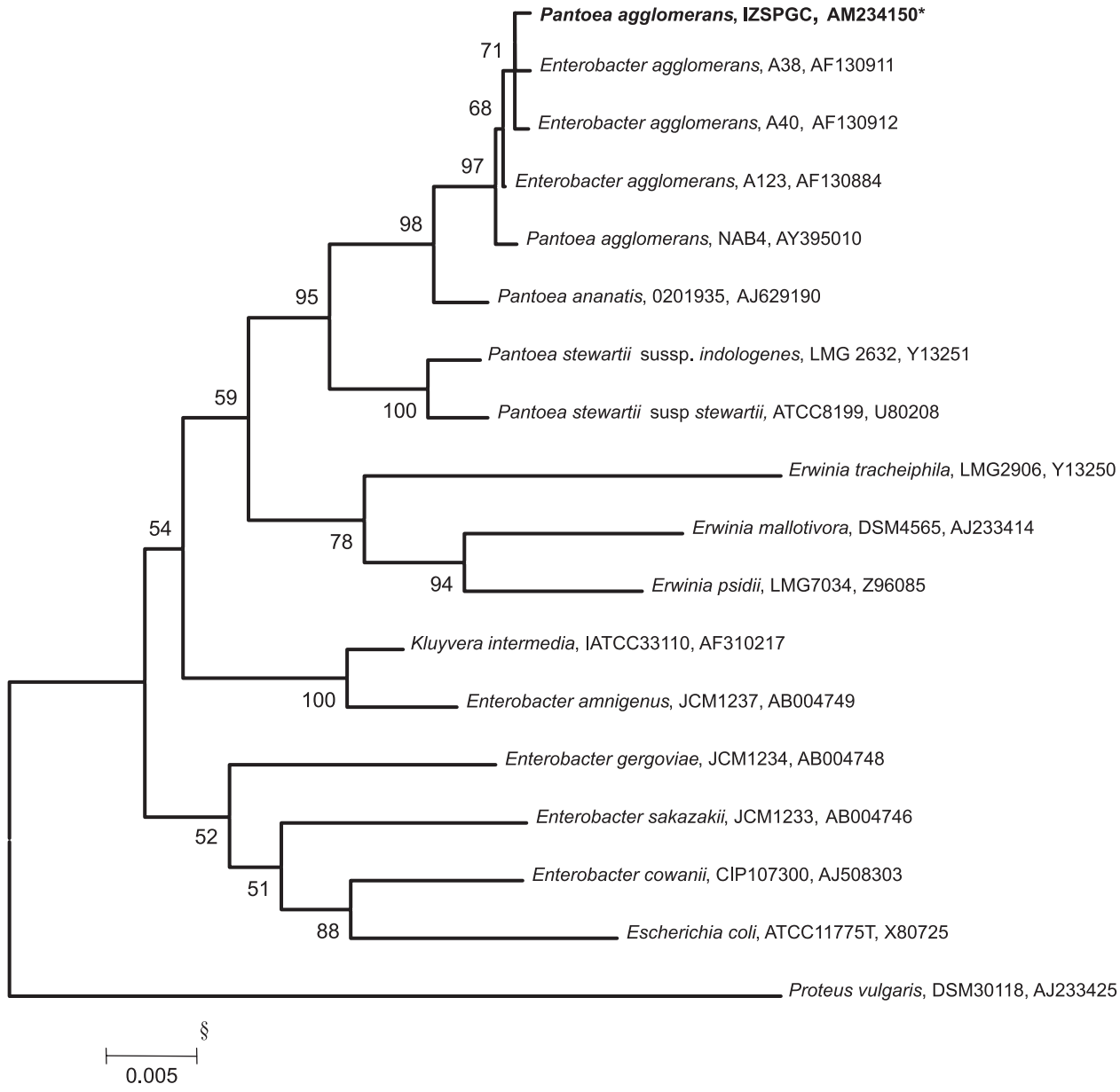
In order to identify the primary structure of 16S rRNA gene, chromosomal DNA extraction, PCR amplification and sequencing of the 16S rRNA gene were performed as described previously [11]. Amplification of the 16S rRNA gene was carried out using the primers PANTF1: 5′-GCTCAGATTGAACGC TGGC G-3′ and PANTR1: 5′-CCCTACGGTTACCTTGT-TACG-3′. The PCR product, 1,486 bp in size, was subcloned in a pGEM-T plasmid vector (Promega) and sequenced by universal primers at least twice for both strands. The nucleotide sequence of the PCR product, which deposited in the EMBL bank (GenBank accession number AM234150), was compared with known 16S rRNA gene sequences, using the BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/). Genetic analysis confirmed that the isolate belonged to the genus *Pantoea,* as it shared 95-99.5% identity in the 16S rRNA sequences with *P. agglomerans* and 86-95% identity with other *Enterobacter* strains. Regarding the species level, the isolate showed the highest identity score with the strains: *E. agglomerans* strain A40 (GenBank AF130912) and *E. agglomerans* A38 (GenBank AF130911), exhibiting 10 and 21 bases difference, respectively. Multiple alignment of 16S rRNA gene sequences was performed with the CLUSTAL W program [12] and phylogenetic trees were constructed with the neighbor-joining, minimum evolution and parsimony analyses using the MEGA version 3 software [13]. The isolate clustered with *P. agglomerans* species is shown in Figure 1.

Antibiotic sensitivity was tested on Mueller–Hinton agar by the Kirby-Bauer disc diffusion method, and zone diameters were interpreted according to Clinical Laboratory Standard Institute guidelines [14]. Quality control was performed by testing American Type Culture Collection (ATCC) strains *Escherichia coli* 25922, *E. coli* 35218 and *Staphylococcus aureus* 25923. The *P. agglomerans* strain was sensitive to aminoglycosides, quinolones, chloramphenicol, trimethoprim-sulfamethoxazole and $\beta$-lactams except ampicillin, while the *E. faecalis* strain was susceptible to vancomycin, teicoplanin and linezolid.

## Discussion

The clinical experience with *P. agglomerans* infection is relatively limited because of the recent establishment of the genus [15] and the lack of confident identification in most clinical laboratories [16]. Members of the genus *Enterobacter* appear to confound commercial identification systems more often than do other genera in the *Enterobacteriaceae*, because of the heterogenicity of several of the species. For example, 6 commonly employed systems failed to identify one or more of several species (*E. cloacae*, *E. aerogene*s, *E. sakazakii*, and *P. agglomerans*) with $\geq$ 90% accuracy a total of 14 times [17]. An additional reason is the very low incidence of *P. agglomerans* infections. A total of 183 consecutive patients with *Enterobacter* bacteremia were analyzed during the period of January 1998 through December 2002 at Seoul National University Hospital. *P. agglomerans* strains were isolated only in 3 (1.6%) of the initial blood cultures [18].

We believe that *P. agglomerans* was one of the causative organisms in the case described here for several reasons. First, the patient's systemic symptoms




*Sequence obtained during this study; §Bar, 0.005 nucleotide changes per nucleotide position.

**Figure 1.** Dendrogram of the 16S rDNA sequences of the obtained clinical strain and related sequences. A rooted tree resulted from a neighbour-joining bootstrap analysis (100 replications) of 16S rRNA gene sequences from the clinical isolate and related species published in GenBank, with *Aeromonas hydrophilia* as an outgroup.

were at their height on the day that the positive blood cultures were drawn. Second, it grew from both aerobic and anaerobic bottles and bacterial growth took 12 h, which is consistent with rapid-growth properties of the organism [2]. In addition, positive blood cultures are significantly more likely to be true positives if obtained from patients with malignancies [19]. Third, our patient made a complete recovery with treatment, whereas *E. faecalis* was resistant to meropenem and *P. agglomerans* resistant (intrinsically) to vancomycin. Finally, polymicrobial bacteremia due to *Enterobacter* species is not an uncommon clinical finding [6] whereas no further instances of colonization or infection with *P. agglomerans* occurred in the months shortly before or after the patient in question. Although *P. agglomerans* was not isolated from stool we suggest that the damaged gastrointestinal tract was the portal of entry to the systemic circulation for both isolates. With the exception of the underlying illness, no other risk factor was found.

In conclusion, this report adds one additional patient to the few published cases of *P. agglomerans* infection since its new taxonomic status [15] and emphasizes the necessity of a sequence-based typing

approach for the definite identification of the organism. We also suggest that before beginning treatment of a bacteremia by *P. agglomerans*, it is very important to carry out a clinical evaluation of the patient to eliminate the possibility of a pseudobacteremia, and thereby avoid unnecessary treatment.

## References


1. Vincent K, Szabo RM. *Enterobacter agglomerans* osteomyelitis of the hand from a rose thorn: a case report. Orthopedics 1988; 11: 465-467.
2. De Champs C, Le Seaux S, Dubost JJ, Boisgard S, Sauvezie B, Sirot J. Isolation of *Pantoea agglomerans* in two cases of septic monoarthritis after plant thorn and wood sliver injuries. J Clin Microbiol 2000; 38: 460-461.
3. Cicchetti R, Iacobini M, Midulla F, Papoff P, Mancuso M, Moretti C. *Pantoea agglomerans* sepsis after rotavirus gastroenteritis. Pediatr Infect Dis J 2006; 25: 280-281 (Letter to the Editor).
4. Lim PS, Chen SL, Tsai CY, Pai MA. *Pantoea* peritonitis in a patient receiving chronic ambulatory peritoneal dialysis. Nephrology (Carlton) 2006; 11: 97-99.
5. Pitout JDD, Moland ES, Sanders CC, Thomson KS, Fitzsimmons SR. *β*-lactamases and detection of *β*-lactam resistance in *Enterobacter* spp. Antimicrob Agents Chemother 1997; 41: 35-39.
6. Sanders WE Jr, Sanders CC. *Enterobacter* spp.: Pathogens poised to flourish at the turn of the century. Clin Microbiol Rev 1997; 10: 220-241.
7. Habsah H, Zeehaida M, Van Rostenberghe H et al. An outbreak of *Pantoea* spp. in a neonatal intensive care unit secondary to contaminated parenteral nutrition. J Hosp Infect 2005; 61: 213-218.
8. Astagneau P, Gottot S, Gobin Y et al. Nosocomial outbreak of *Enterobacter agglomerans* pseudobacteraemia associated with non-sterile blood collection tubes. J Hosp Infect 1994; 27: 73-75.
9. Perola OT, Nousiainen S, Suomalainen S et al. Recurrent *Sphingomonas paucimobilis*-bacteremia associated with a multi-bacterial water-borne epidemic among neutropenic patients. J Hosp Infect 2002; 50: 196-201.
10. Dunne WM Jr, Nolte FS, Wilson ML. Cumitech 1B. Blood cultures III. Coordinating ed. J. A Hindler. Washington, DC: American Society for Microbiology, 1997: 2-3.
11. Woo PC, Leung PK, Leung KW, Yuen KY. Identification by 16S ribosomal RNA gene sequencing of an *Enterobacteriaceae* species from a bone marrow transplant recipient. Mol Pathol 2000; 53: 211-215.
12. Thomson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions-specific gap penalties and weight matrix choice. Nucleic Acids Res 1994; 22: 4673-4680.
13. Kumar S, Tamura K, Nei M. MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 2004; 5: 150-163.
14. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. Sixteenth information supplement. CLSI document, M100-S16. 2006; 26: 32-37.
15. Gavini F, Mergaert J, Beji A et al. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb. nov. and description of *Pantoea dispersa* sp. nov. Int J Syst Bacteriol 1989; 39: 337-345.
16. Janda JM, Abbott SL. Bacterial identification for publication: when is enough enough? J Clin Microbiol 2002; 40: 1887-1891.
17. Abbott SL. *Klebsiella*, *Enterobacter*, *Citrobacter*, *Serratia*, *Plesiomonas*, and other *Enterobacteriaceae*. In: Murray PR, Baron EJO, Jorgensen JH, Pfaller MA, Yolken RH (Eds): Manual of Clinical Microbiology. (8th edn). Washington, DC: American Society for Microbiology, 2003, pp 690-693.
18. Kang CI, Kim SH, Park WB et al. Bloodstream infections caused by *Enterobacter* species: Predictors of 30-day mortality rate and impact of broad-spectrum cephalosporin resistance on outcome. Clin Infect Dis 2004; 39: 812-818.
19. Little JR, Trovillion E, Fraser V. High frequency of pseudobacteremia at a university hospital. Infect Control Hosp Epidemiol 1997; 18: 200-202.