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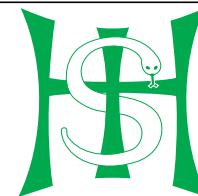
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Short report

Nosocomial outbreak of *Pantoea agglomerans* bacteraemia associated with contaminated anticoagulant citrate dextrose solution: new name, old bug?

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SUMMARY

We describe an outbreak investigation of *Pantoea agglomerans* bacteraemia associated with anticoagulant citrate–dextrose 46% (ACD) solution prepared in-house. A healthy man presented with septic shock during plasmapheresis for granulocyte donation. The solution used for priming and blood samples were sent for culture. Identification of the isolate to species level was performed by *gyrB* sequencing. Typing was performed by pulsed-field gel electrophoresis (PFGE). In total, eight cases were identified during a three-week period. *P. agglomerans* was also cultured from six ACD solution bags. Isolates from patients and ACD bags were identical by PFGE. All isolates were susceptible to ampicillin, cephazolin, gentamicin, ciprofloxacin, cefepime and imipenem.

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Introduction

Pantoea spp. constitute a genus formerly classified as *Erwinia herbicola*–*Enterobacter agglomerans* complex.¹ *Pantoea ananatis* and *Pantoea dispersa* are causes of human infections.^{2,3} Biochemical heterogeneity of the genus causes difficulty with identification.

Pantoea spp. have been isolated from environmental niches and from humans and animals as a commensal.¹ *P. agglomerans* is the most frequent species associated with human infections.¹ Nosocomial outbreaks due to contamination of total parenteral nutrition, propofol, blood products and transfusion tubes used for intravenous hydration have previously been described.^{4–7}

This study describes an outbreak of *P. agglomerans* in a tertiary care centre related to in-house-produced anticoagulant solution used for priming of the plasmapheresis machine and for haemodialysis in acute care.

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Methods

Setting

Hospital das Clínicas is a tertiary care teaching hospital affiliated to the University of São Paulo, Brazil. It has about 2500 beds divided among six institutes. The hospital has a haemodialysis unit and a blood bank service that are located in the central institute and serve the whole hospital.

The central pharmacy produces the anticoagulant solution ACD (anticoagulant citrate–dextrose 46%) and distributes it to the whole hospital including to the division of haematology and to the haemodialysis unit. In the haematology department, ACD is added to plasmin (pentamide–hydroxyethylamide) and it is used to prime the plasmapheresis system before each procedure. The haemodialysis unit uses ACD in acute care as an anticoagulant for cases in which heparin is contraindicated.

The outbreak

On 4 January 2010, a previously healthy 44-year-old man presented with rigors, chills and fever (temperature $>38^{\circ}\text{C}$) while donating granulocytes at the blood bank. The attending physician interrupted the procedure and collected a sample of the ACD/plasmin solution used to prime the plasmapheresis system. Additional blood samples were obtained from the patient and all samples were sent for culture. Culture of the ACD/plasmin solution yielded *Pantoea* sp.

Control measures

The infection control team implemented the following measures:

- The infection control teams of the five other institutes were notified.
- Weekly meetings took place involving infection control, central pharmacy, microbiology laboratory, haematology and nephrology and the hospital administration.
- Visits to the pharmacy and the hospital units. Points evaluated in the pharmacy were packaging, sterilization, traceability of batches and storage. Evaluation of patient units included the storage of ACD, handling, infusion and practices related to infusion therapy.
- The pharmacy traced the whole ACD batch related to the index case. A recall was implemented. Surveillance of blood cultures to identify new cases was maintained for six months.

Investigation

Although the blood culture obtained from the granulocyte donor was negative, we considered him as the index case.

Case definitions were as follows:

1. Confirmed case: isolation of *Pantoea* sp. in blood cultures, on 28 November 2009 (date of production of ACD batch used on index patient) or later, from patients who had undergone plasmapheresis or haemodialysis for acute care at our hospital.

2. Possible case: fever, chills or shivering during or after dialysis or plasmapheresis with a negative blood culture, on 28 November 2009 or later.
3. Suspected case: septic shock or sepsis in patients undergoing dialysis or plasmapheresis, on 28 November 2009 or later, in which *Enterobacter* sp. or *Citrobacter* sp. were identified from blood cultures. These micro-organisms were included based on the possibility of misidentification of *Pantoea* spp. due to potential limitations of our automated system (Vitek 1, bioMérieux Vitek, Hazelwood, MO, USA) used during the whole second semester of 2009.

Data from cases were collected from patients' records and the computer laboratory.

ACD samples from the same batch received by the case patients were cultured.

Microbiology

The micro-organisms were initially identified using Gram-negative cards of Vitek 1 (bioMérieux, Hazelwood, MO, USA). Because of doubts with the identification, conventional biochemical tests were performed. During January 2010, the microbiology laboratory gradually migrated over a few weeks from the automated system Vitek 1 to Vitek 2, and the three initial isolates were identified as *Enterobacter intermedius*/*Rahnella aquatilis*. These were re-identified later as *Pantoea* sp. Antimicrobial susceptibility testing was performed using broth microdilution according to the Clinical and Laboratory Standards Institute recommendations. Isolates were re-identified by API20E (bioMérieux, Marcy l'Etoile, France) and species confirmed by *gyrB* sequencing. Nucleotide sequencing to confirm the species was performed by MegaBACE 1000 (GE Healthcare). The sequences were analysed using the software Sequence Analyzer with the Base Caller Cimarron 3.12. The genetic sequence was compared with the database BLAST (<http://www.ncbi.nlm.nih.gov/blast/>).

Molecular typing

Pulsed-field gel electrophoresis (PFGE) was performed for isolates obtained from ACD bags and patients, using CHEF-DRII (Bio-Rad Laboratories Inc., Berkeley, CA, USA), after digestion by *Xba*-I restriction enzyme. The interpretive criteria were those by Tenover *et al.*⁸ Controls used were *Enterobacter cloacae* ATCC 13047 and *Pseudomonas aeruginosa* ATCC 27853.

Endotoxin assay

Bacterial endotoxin assay was performed using the gel formation method as described elsewhere (Pharmacopeia Americana).⁹

Results

From 31 December 2009 through 19 January 2010 there were six confirmed cases (Figure 1) of *Pantoea* sp. infection plus the index case. Later an eighth individual who did not fulfil criteria, because he had not undergone plasmapheresis or haemodialysis, was included as a case because he presented with a positive blood culture with the same clone of *Pantoea* sp. The patients' characteristics are presented in Table I.

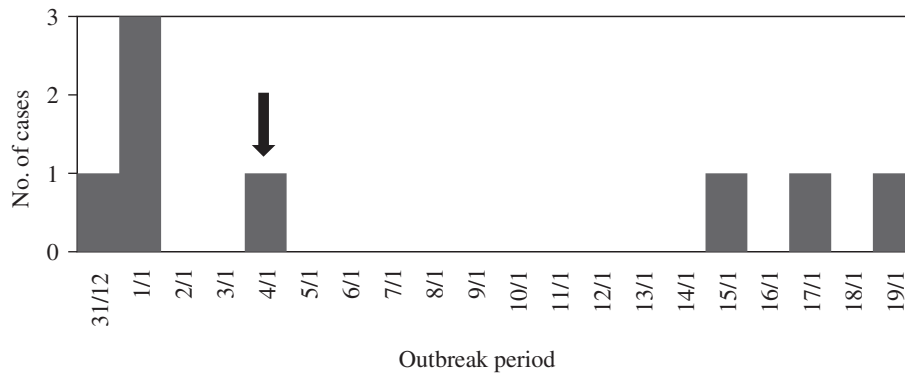


Figure 1. Distribution of *Pantoea agglomerans* bacteraemia during the outbreak period. The arrow indicates the day of the index case (donor of granulocytes) which initiated the investigation.

No possible cases were identified. One unrelated case of *Pantoea agglomerans* bacteraemia was identified in the neonatology ward.

Although seven patients fulfilled the criteria for suspected cases they were not confirmed as *Pantoea* sp.

The pharmacy traced 500 ACD bags belonging to the same batch as the one used by the index patient: 50 ACD bags in the central institute (22 were withdrawn and 28 had been already used) where four cases occurred; and 405 in the heart institute (292 were withdrawn and 113 had been used) where the four other cases occurred. In addition, 45 bags were traced to the children’s institute, with no cases (43 were withdrawn and two had been used).

Evaluation of the hospital’s compound pharmacy did not indicate technical problems during production although the final step, bottling, was totally manual. During visits to the hospital units it was noticed that ACD bags were stored in the wards, which was not recommended. We promoted training sessions about hand hygiene and revision of good practices on intravenous drug manipulation. We recommended implementation of traceability of delivered drugs to the patient level. Finally, we discussed with the hospital’s chief executive officer the cost-benefit of in-house preparation of intravenous solution and advocated changes in order to acquire industry-produced solutions. After the control measures had been implemented and the implicated ACD batch had been withdrawn, no new cases occurred during follow-up.

All isolates were susceptible to ampicillin [minimum inhibitory concentration (MIC₅₀ and MIC₉₀: 1 µg/mL), cephazolin (MIC₅₀ and MIC₉₀: 1 µg/mL), gentamicin (MIC₅₀ and MIC₉₀ <0.25 µg/mL), ciprofloxacin (MIC₅₀ and MIC₉₀ <0.25 µg/mL), cefepime (MIC₅₀ and MIC₉₀ <0.25 µg/mL) and imipenem (MIC₅₀ and MIC₉₀ <0.5 µg/mL).

GyrB sequencing and BLAST analysis demonstrated 100% correspondence with *P. agglomerans* ATCC 27990 (formerly known as *Enterobacter* sp. ATCC 27990). PFGE demonstrated that all isolates were identical. No bacterial endotoxin was detected in ACD samples.

Discussion

This study reports an outbreak of *P. agglomerans* bacteraemia, caused by the contamination of ACD solution produced by the hospital’s compound pharmacy. The outbreak occurred in patients receiving haemodialysis and plasmapheresis. Poor adherence to hand hygiene, low quality of hand hygiene, environmental contamination (not investigated), may have contributed to the contamination. *Pantoea* spp. have been involved in outbreaks of healthcare-associated infections (HAIs) probably due to its ability to grow in glucose-enriched solutions widely used in hospitals.^{2–4} Its importance as a cause of HAI is probably underestimated because, until recently, *Pantoea* belonged to the genus *Enterobacter*, a much better known hospital pathogen.

Table I

Demographic data, exposure factors and outcomes of patients with *Pantoea agglomerans* bloodstream infection during an outbreak caused by contaminated anticoagulant citrate–dextrose (ACD), Hospital das Clínicas, December 2009–January 2010

Patient	Institute	Sex	Age (years)	Diagnosis	Dialysis	Anticoagulant used in dialysis	Plasmapheresis	ACD priming	Outcome
Index case	Central	M	44	Granulocyte donor	No	–	Yes	Yes	Cure
2	Central	F	22	TTP	No	–	Yes	Yes	Cure
3	Central	M	68	GBS	Yes	Heparin	Yes	Yes	Cure
4	Central	M	52	TTP	No	–	Yes	Yes	Death
5	Heart	F	83	CCF	Yes	ACD	No	NA	Death
6	Heart	F	55	Lung Tx	Yes	ACD	No	NA	Death
7	Heart	F	72	CCF	Yes	ACD	No	NA	Death
8	Heart	M	67	Lung Tx	No	NA	No	NA	Death

M, male; F, female; TTP, thrombotic thrombocytopenic purpura; GBS, Guillain–Barré syndrome; CCF, cardiac congestive failure; Tx, transplant; NA, not applicable.

In this study difficulties with identification delayed the recognition of the outbreak and introduction of early preventive measures. The occurrence of an entirely new genus causing bacteraemia (identified by Vitek 2) brought this to our attention, but there was the possibility that other cases had been undetected because they had been identified differently (identified by Vitek 1) such as *Enterobacter* sp. and *Citrobacter* sp. This led us to create a definition for suspected cases but no such cases were identified.

Species of the genus *Pantoea* are closely related phenotypically, making rapid species identification difficult. In the present study *gyrB* gene sequencing provided the identification. With the problems in identification, the outbreak may have been overlooked and the control measures delayed. In our study susceptibility was universal to beta-lactams, aminoglycosides, and quinolones.

Contamination of medication or solutions can occur during the process of manufacturing or the manipulation of multidose vials. In our outbreak, the contamination probably occurred during manufacturing. Drugs and products produced in compound pharmacies have been reported to be the cause of outbreaks.¹⁰ Industry production of intravenous solutions is probably safer. In addition, sterility control performed before the release of the solutions failed to detect contamination. We hypothesize that a small inoculum contaminated the dextrose-rich solution which acted as a culture medium for the agent. Furthermore, the conditions of storage were frequently inadequate.

In conclusion, strict protocols of good practices should be assured in order to prevent contamination, when in-house manipulation of solutions cannot be avoided. This should include continuous education on good quality hand hygiene and rigorous observation of environment control in the handling area. Finally, we advocate that in-house manipulation should be abandoned and hospitals should only use industry-produced intravenous solutions. Efforts to trace all drugs used in patients should be employed if contamination occurs, to identify problems early and avoid new exposures.

Conflict of interest statement

None declared.

Funding sources

None.

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