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Identification and control of a *Pseudomonas spp* (*P. fulva* and *P. putida*) bloodstream infection outbreak in a teaching hospital in Beijing, China

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SUMMARY

Objectives: An outbreak of bacteremia caused by *Pseudomonas spp* (*P. fulva* and *P. putida*) was first identified in our hospital in the summer of 2010 and reoccurred in the following year. Based on the epidemiological data collected in these 2 years, we initiated an investigation on the source of the outbreak. The aim of this study was to report the results of the investigation, as well as the intervention strategies that resulted in successful control of the outbreak.

Methods: An infection control team was set up consisting of infectious disease specialists, microbiologists, infection control practitioners, and head nurses. The microbiology and medical records of case-patients with *P. fulva* or *P. putida* bloodstream infections were reviewed. Environmental samples and intravenous (IV) solutions from the wards and the pharmacy center were collected for culturing. The molecular characteristics of the bacterial isolates were studied by pulsed-field gel electrophoresis (PFGE). Strict infection control strategies were implemented.

Results: A total of 20 case-patients from five inpatient wards were identified during three summer seasons from 2010 to 2012. Nineteen of them recovered with proper antibiotics. Unfortunately one died from complications of heart failure. A total of 19 isolates of *P. fulva* and four of *P. putida* were identified, of which 20 were from blood, two from environmental surface samples from the hospital pharmacy, and one from an in-use compounded solution from a case-patient in the cardiology ward. Molecular analysis revealed that the *P. fulva* isolated from the in-use compounded solution (5% glucose solution containing insulin, isosorbide dinitrate, and potassium magnesium aspartate) and the environmental samples had the same PFGE type as the clinical isolates.

Conclusions: The investigation identified that contaminated IV solution was the source of the *P. fulva* bacteremia, which prompted us to implement intensified control measures that resulted in successful control of the outbreak.

1. Introduction

Pseudomonas fulva is isolated mainly from environmental sources.[1] One report of *P. fulva* isolation from the sputum of a patient with cystic fibrosis has been documented in the literature, however the authors did not report the clinical significance of this finding.[2] The first report of a bloodstream infection caused by *P. fulva* was reported in South Korea in 2010.[3] In addition, *P. fulva* was identified from cerebrospinal fluid culture by Almuzara et al.[4] More importantly, this isolate carried the blaVIM-2 gene cassette.

Pseudomonas putida, a low-virulence opportunistic pathogen, primarily causes nosocomial infection in immunocompromised patients and in patients with medical devices or catheters.[5–7] *P. putida* has also been implicated in outbreaks of bacteremia due to transfusion of contaminated blood or fluid.[7,8] Due to their ability to metabolize a wide range of compounds, members of this species are able to colonize moist and inanimate environmental surfaces.[9] This can serve as a reservoir and cause nosocomial infections.

P. fulva and *P. putida* have rarely been detected in clinical specimens at our hospital in the past. However, a number of

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bacteremia cases caused by *P. fulva* or *P. putida* have been reported recently, mostly in the cardiology ward. We describe herein an outbreak of 16 cases of *P. fulva* and four cases of *P. putida* bacteremia that occurred in our hospital from August 2010 to September 2012, with emphasis on the molecular epidemiology of the outbreak, as well as the intervention strategies that resulted in successful control of the outbreak.

2. Methods

2.1. Background of the outbreak

The Beijing Chao-Yang Hospital is a 1500-bed, tertiary-care, academic hospital in Beijing, China. The hospital pharmacy center routinely prepares various reconstituted solutions for patients with special needs, which are then distributed to different wards every day. On September 5, 2012, two patients in the cardiology ward experienced a sudden onset of fever following intravenous fluid infusion. Peripheral blood samples that were drawn on site were sent for microbiological analysis. The next day, *Pseudomonas spp* (*P. fulva*) was recovered from the blood samples of the patients. Since similar cases had been reported during the summer season over the past 2 years in the cardiology ward in our hospital, the Chao-Yang Hospital Department of Infection Control was notified on September 7, 2012. An infection control team was set up on the same day, consisting of infectious diseases physicians, microbiologists, infection control practitioners, and head nurses from the hospital. The team members discussed the problems and made decisions on control measures with the agreement of the hospital authority, and initiated an investigation within 24 h. A third patient in the same ward was identified as having a *Pseudomonas spp* (*P. fulva*) bloodstream infection in the following 5 days.

2.2. Case definition

Microbiology and medical records were re-examined to identify patients with *Pseudomonas spp* (*P. fulva* or *P. putida*) bloodstream infections in the whole hospital. A case patient was defined as any patient with symptoms and signs consistent with sepsis and a blood culture positive for *Pseudomonas spp* (*P. fulva* or *P. putida*) during the period January 1, 2010 to September 30, 2012.

2.3. Infection control strategies implemented

The first intervention was the prompt collection and quarantine of all the intravenous (IV) solutions used by patients in the cardiology ward, followed by culturing of solution samples in the clinical microbiology laboratory. At the same time, unopened stock solutions from the hospital pharmacy center including 0.9% NaCl, 5% glucose, 10% glucose, etc., in their original packaging, were also cultured. Environmental cultures from the cardiology ward and hospital pharmacy center were obtained, including samples from work surfaces, baskets for carrying compounded solutions, equipment, and door handles. Meanwhile, all health care workers were informed of the *P. fulva* bacteremia outbreak through the hospital's local internet. Heightened infection control measures, especially hand hygiene, were enforced. Environmental surfaces in the cardiology ward were rigorously cleaned twice a day with 5% sodium hypochlorite throughout the outbreak.

2.4. Identification of the isolates

Originally, the organisms were identified as *P. putida* (99% probability) in all of the samples (n = 23) using a phenotypic identification systems, Vitek 2 Compact (bioMérieux, France). Then DNA sequencing analysis was performed using four pairs of primers, including 16S rRNA, *gyrB*, *rpoB*, and *rpoD*, as described previously,[3] to further confirm the identity of the isolates.

2.5. Pulsed-field gel electrophoresis (PFGE)

PFGE was conducted as described previously, with a slight modification.[10] Briefly, whole-cell genomic DNA of culture lysed cells representing each isolate embedded in 1% agarose plugs (Bio-Rad, Richmond, CA, USA) were digested with the restriction enzyme *SpeI* (TaKaRa Biotechnology, Dalian, China) and separated by electrophoresis through 1% pulsed-field-certified agarose (Bio-Rad) using a CHEF-Mapper instrument (Bio-Rad). Electrophoretic switch times of 4–40 s were used with a 6 V/cm current and a switch angle of 120° under a constant temperature of 14 °C. PFGE patterns were interpreted using the criteria proposed by Tenover et al.[11]

2.6. Ethics statement

Permission to use the information in the medical records of the patients and the *P. fulva and P. putida* isolates for research purposes was given by the ethics committee of Beijing Chao-Yang Hospital.

3. Results

3.1. Epidemiological findings

A list of 20 case-patients from five wards was identified using the microbiology records for the period August 8, 2010 to September 30, 2012. The clinical features and outcomes of the 20 patients with *Pseudomonas spp* (*P. fulva* or *P. putida*) bacteremia are summarized in Table 1. Case-patients had been admitted for various underlying medical conditions, including myocardial infarction, hypertension, hyperlipidemia, pneumonia, cancer, diabetes mellitus, anemia, etc., and had been admitted to different wards: cardiology ward (n = 12), neurosurgery ward (n = 3), surgery intensive care unit (SICU) (n = 2), gastrointestinal disease ward (n = 2), and cardiac care unit (CCU) (n = 1). Fifteen (75%) patients were male. The case-patients had all been administered medication and/or nutrients and electrolytes via the central or peripheral vein within 48 h of developing *Pseudomonas spp* (*P. fulva* or *P. putida*) bacteremia during their hospital stay. Most of the patients in the cardiology ward had received continuous intravenous infusions for more than 24 h. All the patients received antimicrobial drugs for the *Pseudomonas spp* (*P. fulva* or *P. putida*) bloodstream infection. Nineteen out of the 20 (95%) patients became free of *Pseudomonas spp* (*P. fulva* or *P. putida*) bloodstream infection and were subsequently discharged from the hospital without complications from the bacteremia. Unfortunately one patient (patient 9) died of heart failure.

3.2. Laboratory findings

Originally, the organisms were identified as *P. putida* (99% probability) using a phenotypic identification system, the Vitek 2 Compact (bioMérieux, France). DNA sequencing showed 19 out of 23 of the samples to be most closely related to *P. fulva* (>98% homology to all four fragments). However, the remaining four samples showed 95% homology to *P. putida* (Table 1).

Among the 23 isolates, 20 were from positive clinical samples of patients in five wards, two were from environmental surface samples of the hospital pharmacy, and one was from the used compounded solution (5% glucose solution containing insulin, isosorbide dinitrate, and potassium magnesium aspartate) from patient 18 in the cardiology ward. The two positive environmental surface samples were isolated from the hospital pharmacy bench

Table 1
Demographic, clinical, and laboratory characteristics of 20 case-patients with *Pseudomonas fulva* and *Pseudomonas putida* bloodstream infections after receiving intravenous treatment during the period August 2010 to September 2012

Case No.	Age, gender	Ward	Medical treatment prior to culture	Date of isolation	Antibiotic treatment following culture	Outcome	Isolate ID	PFGE type
1	64, M	Cardiology	5% glucose solution, insulin, ID, PMA, KCl	Aug 8, 2010	TZP	Survived	*P. putida*	C
2	72, F	Cardiology	Normal saline	Aug 8, 2010	TZP, CIP	Survived	*P. putida*	C
3	76, M	Neurology	5% glucose solution, insulin, PMA, normal saline	Aug 25, 2010	AMC	Survived	*P. fulva*	A
4	60, M	Neurology	5% glucose solution, insulin, BH, PMA, alprostadil	Sep 16, 2010	AMC	Survived	*P. fulva*	A
5	91, M	Gastrointestinal disease	10% glucose solution, omeprazole, glutathione, PMA	Oct 10, 2010	CXM, cefmetazole	Survived	*P. fulva*	A
6	42, M	Cardiac care unit	5% glucose solution, insulin, ID, PMA, KCl	Apr 29, 2011	CSL	Survived	*P. fulva*	E
7	71, M	Cardiology	5% glucose solution, insulin, ID, PMA	Aug 14, 2011	CSL, AZI	Survived	*P. fulva*	B
8	52, F	Cardiology	5% glucose solution, insulin, ID, PMA	Aug 13, 2011	Cefminox	Survived	*P. fulva*	A
9	70, M	Cardiology	5% glucose solution, insulin, ID, PMA	Aug 21, 2011	CSL, LEV	Died	*P. fulva*	B
10	67, M	Surgical ICU	5% glucose solution, KCl	Aug 21, 2011	CSL	Survived	*P. putida*	D
11	55, M	Cardiology	5% glucose solution, insulin, ID, PMA	Aug 22, 2011	CSL, ofloxacin	Survived	*P. fulva*	B
12	58, M	Cardiology	5% glucose solution, insulin, ID, PMA	Sep 7, 2011	TZP, MOX	Survived	*P. fulva*	A
13	71, M	Neurology	5% glucose solution, insulin, KCl, alprostadil	Sep 10, 2011	FOX	Survived	*P. fulva*	A
14	48, F	Gastrointestinal disease	5% glucose solution, PMA	Sep 10,2011	FOX	Survived	*P. fulva*	A
15	68, F	Cardiology	5% glucose solution, insulin, ID, PMA	Sep 22, 2011	Cefmetazole	Survived	*P. fulva*	A
16	62, M	Surgical ICU	Lactated Ringer's, omeprazole	Sep 25, 2011	FOX	Survived	*P. putida*	D
17	81, M	Cardiology	5% glucose solution, insulin, ID, PMA	Dec 13, 2011	CSL	Survived	*P. fulva*	B
18	55, M	Cardiology	5% glucose solution, insulin, ID, PMA	Sep 5, 2012	CSL, MOX	Survived	*P. fulva*	F
19	33, M	Cardiology	5% glucose solution, insulin, ID, PMA	Sep 5, 2012	AMC	Survived	*P. fulva*	B
20	72, F	Cardiology	5% glucose solution, insulin, ID, PMA	Sep 9, 2012	CSL, MOX	Survived	*P. fulva*	F

PFGE, pulsed-field gel electrophoresis; M, male; F, female; ICU, intensive care unit; ID, isosorbide dinitrate; PMA, potassium magnesium aspartate; TZP, piperacillin–tazobactam; CIP, ciprofloxacin; AMC, amoxicillin–clavulanic acid; BH, buflomedil hydrochloride; CXM, cefuroxime; CSL, cefoperazone–sulbactam; AZI, azithromycin; LEV, levofloxacin; MOX, moxifloxacin; FOX, cefoxitin.

surface and a basket for carrying the compounded solutions. Culturing of the unopened solutions in their original packaging produced no growth during the outbreak period in 2012. Intrinsic contamination was ruled out by negative culture of the unopened stock solutions used for preparing the compounded solutions. A total of 25 intravenous fluids used by patients in the cardiology ward were cultured; the compounded solution (5% glucose solution containing insulin, isosorbide dinitrate, and potassium magnesium aspartate) used by patient 18 tested positive for *P. fulva*, while the rest showed negative culture results.

PFGE analysis of the 23 strains revealed six PFGE types, as show in Figure 1 (the banding patterns are designated PFGE A to F). Isolates from eight patients in different wards over a 3-year period and an environmental surface isolate from the bench of the hospital pharmacy shared the same PFGE pattern (type A), suggesting a clonally related origin. Two isolates from patient 18 in the cardiology ward were identified from different sites (peripheral blood and used solution sample), and showed the same PFGE type (type F) to that of the environmental isolate from the basket in the hospital pharmacy center, also indicating clonal relatedness. Therefore, the compounded solution (5% glucose solution containing insulin, isosorbide dinitrate, and potassium magnesium aspartate) was strongly suspected as the source of the outbreak, having become contaminated when it was prepared or when administered to that patient.

4. Discussion

Outbreaks of bloodstream infection caused by various agents linked to contaminated infusions and medications are well

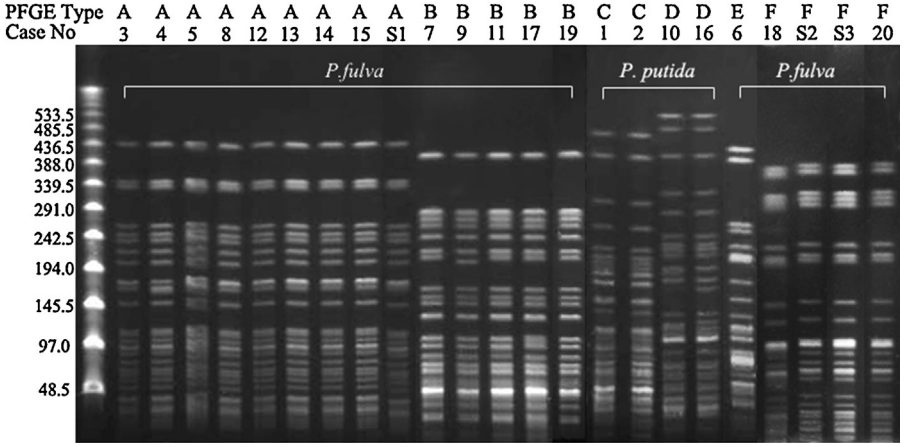

Figure 1. PFGE of *Pseudomonas fulva* and *Pseudomonas putida* isolates obtained during August 2010 to September 2012, Beijing, China. Numbers 1–20: bloodstream isolates from patients in different wards; S1: isolate from a pharmacy bench surface; S2: isolate from in-use solution; S3: isolate from a tray on which the prepared solution was placed in the pharmacy. First lane on the left hand side is the molecular weight marker.

described.[12,13] Contamination can be extrinsic or can occur during the manipulation of multi-dose vials.[14,15] The preparation of drug products can lead to an outbreak if stringent quality-control standards are not implemented.[16] In China, medications are reconstituted for the special needs of the patients in the hospital pharmacy center. In the present case, the major cause of the outbreak was probably *P. fulva* in the environment, which was introduced into the mixed fluids either during manipulation of the solution in the hospital pharmacy or during administration to multiple patients in the hospital wards. According to PFGE, type A found in the environment showed an identical pattern to that isolated from the patients (patients 3, 4, 5, 8, 12, 13, 14, and 15) in three different wards; type F found in the environment had an identical pattern to the patient 18 clinical isolate and the isolate from the used compounded solution. In most outbreaks, the source of the pathogens responsible is difficult to identify by environmental surveillance, even if the bacteria are isolated from patients or the environment.[17,18] Fortunately, the present investigation was able to establish the link between the outbreak and the environmental pathogen because the hospital infection control team traced the solution administered to each patient and cultured the unused solutions effectively. Therefore, we were able to identify patients who had received the contaminated solutions. The findings from this investigation indicate the risks of using intravenous formulations of medications.

Another finding in the current study was that the hospital pharmacy prepares compounded solutions for more than 30 wards within our hospital every day. However, the outbreak involved only five different wards over a 3-year period and most of the cases were detected in the cardiology ward. After reviewing common exposures for case-patients, we focused on intravenous infusions, medications, case-patient clinical signs, treatments, and outcomes. One possible explanation is the fact that in the cardiology ward, most patients required the use of venous catheters for more prolonged periods of time compared to patients in the other wards. It appears that a prolonged injection time may contribute to the development of bacteremia in these patients.

The outbreak seemed to be under control from September 2012 after an intensification of infection control practices. Up to the end of October 2013, no new *P. fulva* or *P. putida* isolates had been detected in our hospital. One possible explanation for the success may be the prompt recognition of the outbreak, the infection control intervention, and the early administration of antimicrobial therapy. Another possibility may be the low human pathogenicity of *P. fulva* and *P. putida*. However, the outbreak lasted for 3 years, suggesting the potential for long-lasting persistence of this microorganism and the possible role of *P. fulva* and *P. putida* as a long-lasting reservoir in the hospital environment. As these pathogens cannot be eradicated from the hospital environment, constant infection control measures are needed in order to prevent future nosocomial infections.

Regarding the hospital pharmacy, quality assurance/quality control of the pharmacy was implemented routinely before, during, and after the outbreak in accordance with hospital regulations. In order to avoid a similar incidence in the future, several new practices and control measures have been put in place, such as increasing staff awareness of hand washing, more frequent cleaning of environmental surfaces with disinfectants and sterilization of the working environment, modifying the preparation methods of the reconstituted solutions to ensure minimum handling of the solutions, and altering the IV regimen in order to shorten the infusion time. To facilitate the efficient identification of possible contaminated compounded solutions, sterility testing of the reconstituted solutions is performed regularly.

A limitation of this report is that we only identified the contaminated intravenous compounded solutions as the source of nosocomial *P. fulva* bacteremia during the year 2012. Although the outbreaks in 2010 and 2011 were only investigated retrospectively, they shared similarities (same pathogen, same season, and same wards) with that in 2012; we could therefore reasonably hypothesize that contaminated solutions might also have been responsible for the outbreaks in the past years.

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