Journal of Microbiology, Immunology and Infection 55 (2022) 80—85

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Original Article

An outbreak of *Ralstonia pickettii* bloodstream infection among pediatric leukemia patients

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Received 2 December 2019; received in revised form 12 November 2020; accepted 15 December 2020
Available online 6 January 2021

KEYWORDS
Ralstonia picketti;
Outbreak;
Malignancy;
Nosocomial infection

Abstract *Background: Ralstonia pickettii* is an opportunistic waterborne microbe which can survive in many kinds of solutions. Contamination of these solutions may result as outbreaks, which can be mortal for immuncompromised patients. Herein we report an outbreak of *R. pickettii* related to contaminated saline infusion in our center.
Methods: This study was conducted in Ankara Pediatric City Hospital. An outbreak occured in Pediatric Hematology and Oncology Unit between August 28, 2019 and September 13, 2019. When the outbreak occured, infection control team began an investigation. Environmental samples were collected in order to find the source of the outbreak.
Results: A total of 11 patients with catheter related blood stream infection caused by *R. pickettii* who were diagnosed with leukemia were affected. None of the patients infected with *R. pickettii* died during the outbreak. A total of seventy environmental samples were cultured with the purpose of finding the source of outbreak. *R. pickettii* grew in normal saline solution culture and all isolates had the same clone of *R. pickettii*. The outbreak lasted two weeks and was controlled by stopping the usage and sending back the saline solutions belonging to the same manufacturing batch.
Conclusions: We reported an outbreak of *R. pickettii* BSIs in highly immunocompromised patients due to contaminated intravascular solution, which was rapidly controlled by infection

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https://doi.org/10.1016/j.jmii.2020.12.004

control measures. Vigilant surveillance by hospital infection control teams and prompt investigation to identify the source of nosocomial infections are crucial to stop an outbreak.

Introduction

Ralstonia pickettii is a nonfermentative gram-negative bacillus and an opportunistic pathogen in both the hospital setting and in the environment.[1] It is a waterborne microorganism that can survive in any kind of water source and can contaminate intravenous drugs, blood culture bottles, distilled water, saline solutions, and other solutions used for patient care in hospitals.[2—5] Moreover, *R. pickettii* tends to form and maintain biofilms in plastic industrial water piping.[6] The pathogenicity of the microorganism is not high; however, it is more virulent in immunosuppressed patients.[7]

The contamination of solutions may result in outbreaks of bloodstream infections (BSIs).[8,9] Such contamination is generally introduced during manufacture.[8,9] Solutions contaminated during manufacture have been associated with outbreaks of BSIs or catheter-related infections.[2]

Here we report an outbreak of catheter-related BSI due to *R. pickettii* that was related to contaminated saline infusion at our center.

Materials and methods

Clinical setting

The present study was conducted at Ankara Pediatric City Hospital. An outbreak occurred in the Pediatric Hematology-Oncology Unit and Hematopoietic Stem Cell Transplantation (HSCT) between August 28, 2019 and September 13, 2019. All patients who were culture positive for *R. pickettii* between these dates were eligible for the study. A total of 11 patients who were diagnosed with leukemia and were culture positive for *R. pickettii* were included in the study. Inclusion criteria was based on BSI definitions in guidelines of Infectious Diseases Society of America (IDSA).[10] A definitive diagnosis of catheter related BSI required that the same organism grew from at least 1 percutaneous blood culture and from a culture of the catheter tip.[10] Patients whose blood culture through central venous catheters was positive for *R. pickettii* but peripheral blood culture was not were excluded from the study. In addition, patients who had a positive blood culture but no clinical findings were excluded from the study. All the patients had a central venous catheter (Hickman or Port-a-Cath). The patients were hospitalized in the hematology ward during the study period. They had minimum one blood culture through the central venous catheter and one blood culture through the peripheral vein concurrently positive for *R. pickettii*. All the patients had neutropenia for at least five days.

Surveillance data

When the outbreak occurred, the infection control team began an investigation. For the identification of source and transmission process of this infection outbreak field visits were organized. Environmental samples, including samples of tap water, saline, hand soap, total parenteral nutrition fluids, intravenous fluids, sterile distilled water used for respiratory therapy, antiseptic and antibiotic solutions, and sodium heparin solutions, and samples from water tanks were collected to determine the source of the outbreak. These samples were incubated for 24 h in enriched media and cultured in 5% sheep blood agar and eosin methylene blue lactose sucrose agar. After 72 h of incubation, bacterial growth was assessed.

Bacterial identification

Each blood culture bottle was placed in the BacT/Alert 3D automated blood culture system (bioMerieux, France) and incubated for five days or until bacterial growth was observed.[11] Blood samples were cultured in 5% sheep blood agar plates (RTA, Turkey) and incubated at 37 °C under 5% CO_2 for 24 h (maximum, 72 h), according to the national laboratory guidelines. The microorganisms were identified using the VITEK MS system (bioMerieux, France). Antibiotic susceptibility tests [for assessing the minimum inhibitory concentration (MIC) and detecting the presence of extended-spectrum beta-lactamases (ESBLs) and carbapenem resistance] were performed for each isolate using the VITEK 2 system (bioMerieux, France), according to the manufacturer's instructions and the criteria of the European Committee of Antimicrobial Susceptibility Testing.[12]

All the bacterial isolates were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker MALDI Biotyper; Bruker Daltonics, Bremen, Germany).

Genomic DNA typing by pulsed-field gel electrophoresis (PFGE)

PFGE was performed as described previously.[13] In brief, bacterial cells embedded in 1% low-melting-point agarose (Bio-Rad Lab, Hercules, CA, USA) plugs were lysed with lysozyme and proteinase K. Following this, chromosomal DNA was digested with SpeI (Thermo Scientific-Fermantas Corporation, Vilnius, Lithuania). Fragmented DNA samples were electrophoresed in 1% pulsed field certified agarose (Bio-Rad Lab, Hercules, CA, USA) using a CHEF-DR III system (Bio-Rad Lab, Nazareth, Belgium) with a pulse time of 5—70 s for 18 h at 14 °C at 6 V cm^{-2}. The gel was stained

with ethidium bromide (5 μg mL^{-1}), visualized under UV light, and photographed using the ChemiDoc MP Imaging System (Bio-Rad Company, United Kingdom). PFGE patterns were analyzed using BioNumerics software, version 7.5 (AppliedMaths, Saint-Matins-Latem, Belgium) and compared using the Dice coefficient with a tolerance of 1.5% and an optimization of 1%.[14] Isolates with identical patterns were considered genotypically indistinguishable, those that differed by one to three bands were considered closely related, those that differed by four to six bands were considered possibly related, and those that differed by more than seven bands were considered unrelated or different.[15]

Results

Fifty patients were hospitalized in our hospital's Pediatric Hematology-Oncology-HSCT Unit during the period of the outbreak. The infection rate was 22%. Moreover, the catheter-related infection rate in the hospital was 2.7%. Eleven patients who had positive cultures for *R. pickettii* were included in the study. Of these, seven patients were male and four were female. The patients' median age was 6.5 years (range, 2–14 years). Five of these patients were diagnosed with pre-B-cell acute lymphoblastic leukemia, one was diagnosed with acute myeloid leukemia, one was diagnosed with T-cell leukemia, and four were diagnosed with B-cell leukemia. All the patients were hospitalized with febrile neutropenia and treated with at least one antibiotic. The patients had at least one of the following findings on the day of culture, in addition to fever (>38 °C): chills, hypotension, tachycardia, and altered consciousness. These signs and symptoms and positive laboratory results were not related to an infection at another site. The primary diagnosis and main characteristics of the patients are shown in Table 1. None of the patients infected with *R. pickettii* died during the outbreak. *R. pickettii* was the sole microorganism isolated from all the positive clinical specimens. Positive blood cultures were obtained through Port-a-Cath in 81.8% of the patients and Hickman catheters in 18.2% of the patients. The central venous catheter had to be removed because of clinical deterioration and sepsis and repetitive positive cultures in four patients.

The median absolute neutrophil count of the patients was 100/μL (range, 10–690/μL). The C-reactive protein level was elevated in 90.9% of the patients, with a median of 64 mg/L (range, 6–192 mg/L).

The results of antimicrobial susceptibility tests revealed that all *R. pickettii* isolates were susceptible to ceftazidime, ciprofloxacin, imipenem, and meropenem and resistant to aztreonam, cefepime, gentamicin, amikacin, and piperacillin–tazobactam.

The antibiotic chosen for therapy was meropenem in four patients, piperacillin–tazobactam in three patients, cefepime in two patients, and ciprofloxacin in two patients. Fever resolved in median of 1.5 days. No complications were observed in patients who were empirically treated with piperacillin–tazobactam and cefepime.

Ten samples were characterized by PFGE. The results revealed that all isolates belonged to the same clone of *R. pickettii* (Fig. 1). One sample could not be characterized.

Table 1 Patient demographics and clinical characteristics

	Number of patients	Percentage
Sex		
Male	4	36.3
Female	7	63.7
Age (years)		
Mean ± standart deviation	6.36 (±3.5)	
Median (range)	6.5 (2–14)	
Main diagnosis		
Pre B cell ALL	5	45.4
AML M2	1	9.15
B cell ALL	4	36.3
T cell ALL	1	9.15
Department		
Pediatric Hematology Department	10	90.9
HSCT Unit	1	9.1
Intravascular device		
Hickman catheter	2	18.2
Port-a-Cath	9	81.8
Treatment		
Meropenem	4	36.4
Piperacillin-tazobactam	3	27.2
Cefepime	2	18.2
Ciprofloxacin	2	18.2
Fever	11	100
Shaking chill	11	100
C-reactive protein (mg/L)		
Mean ± standart deviation	69.72 ± 59.11	
Median (range)	64.0 (6–192.00)	
Absolute neutrophil count (/microL)		
Mean ± standart deviation	224.5 ± 267.6	
Median (range)	100 (10–690)	

ALL: Acute lymphoblastic leukemia, AML M2: Acute myeloblastic leukemia, HSCT: Hematopoietic stem cell transplantation.

The time course of infection and characteristics of *R. pickettii* isolates are shown in Table 2. A total of 60 environmental samples, including those obtained from unopened bottles containing medications (n = 9), aqueous and alcoholic solutions in use for skin hygiene before venous punctures (n = 9), bottles of water for injection (n = 8), glucose solution (n = 10), tap water (n = 8), water tanks (n = 8), and pipelines (n = 8), were cultured to identify the source of the outbreak. *R. pickettii* was found to grow in normal saline solution. Ten bottles of normal saline were sampled; *R. pickettii* grew in one of these samples. The product with the same lot number was collected by the company due to contamination warning. PFGE showed that the *R. pickettii* clones isolated from the saline solution and blood culture were the same. The same saline solution was used in other pediatric units. However, no other infectious complication occurred in these units.

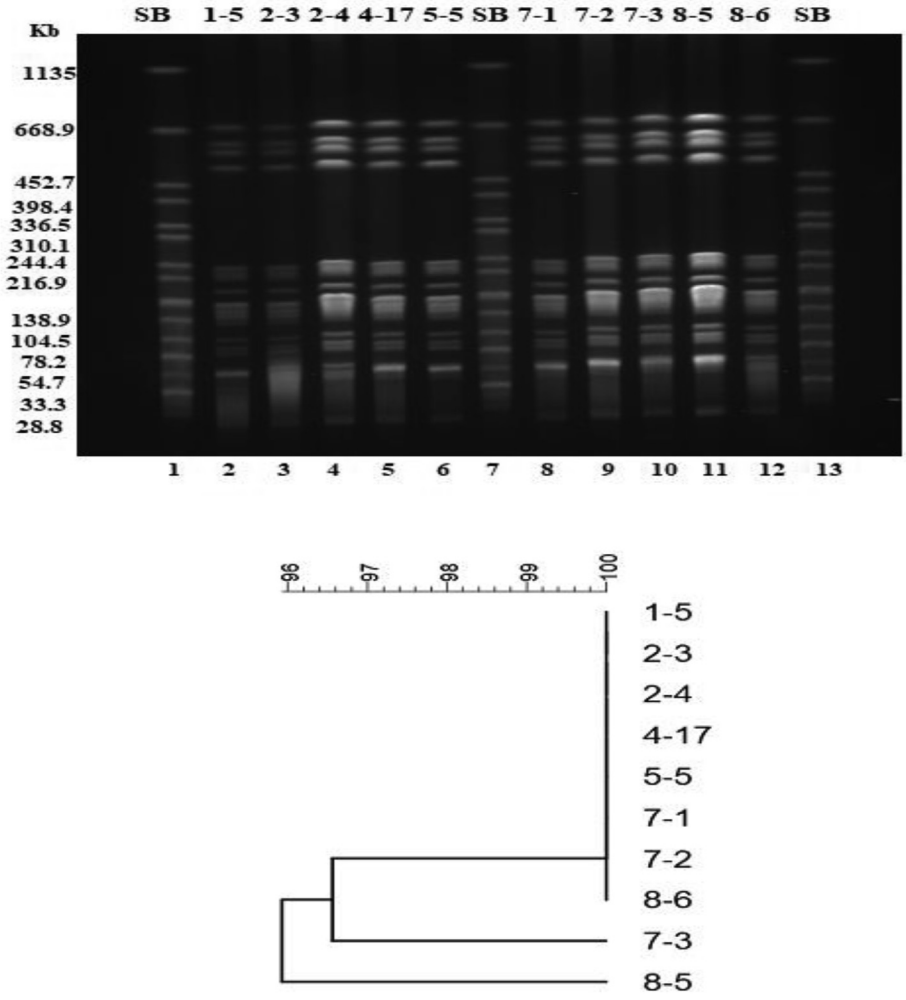

Figure 1. Pulsed-field Gel Electrophoresis Results of *Ralstonia pickettii* isolate. Pulse field gel electrophoresis (PFGE) profiles of SpeI-digested chromosomal DNA and dendrogram resulting from Bionumerics 7.5 software analysis of the profiles shown in a and b, *Ralstonia pickettii* isolates. PFGE lanes 1, 7, 13 molecular size markers (*Salmonella enterica subsp. enterica serovar Braenderup* (ATCC BAA664)).

Table 2 Characteristics of *Ralstonia pickettii* isolates related to the nosocomial outbreak of bloodstream infections.

Patient	Age (year)	Isolate identification	Isolate source	Date of isolation	Strain Code
Case 1	7	*R. pickettii*	Port-a-cath + peripheral blood	26.08.2019	1—5
Case 2	7	*R. pickettii*	Port-a-cath + peripheral blood	04.09.2019	2—3
Case 3	8	*R. pickettii*	Port-a-cath + peripheral blood	26.08.2019	2—4
Case 4	6.5	*R. pickettii*	Hickman catheter + peripheral blood	02.09.2019	4—17
Case 5	10.5	*R. pickettii*	Port-a-cath + peripheral blood	03.09.2019	5—5
Case 6	2.5	*R. pickettii*	Port-a-cath + peripheral blood	31.08.2019	7—1
Case 7	4.5	*R. pickettii*	Port-a-cath + peripheral blood	31.08.2019	7—2
Case 8	4	*R. pickettii*	Port-a-cath + peripheral blood	03.09.2019	non-typable
Case 9	4	*R. pickettii*	Hickman catheter + peripheral blood	29.08.2019	7—3
Case 10	14	*R. pickettii*	Port-a-cath + peripheral blood	04.09.2019	8—5
Case 11	2	*R. pickettii*	Port-a-cath + peripheral blood	03.09.2019	8—6

The outbreak lasted two weeks and was controlled by stopping the usage and sending back the saline solutions belonging to the same manufacturing batch of the pharmaceutical company with the same serial code.

Discussion

A highly unusual outbreak of *R. pickettii* BSIs occurred among 11 patients with leukemia at Ankara Pediatric City Hospital during August—September 2019. All the clinical isolates were suggested to have a common source due to their identical PFGE pattern. We believe that this is the first outbreak reported in pediatric patients with leukemia.

R. pickettii is verified to be associated with nosocomial infections; however, it is thought to be less virulent and is not considered a major pathogen. Because of its low virulence, clinicians should suspect the contamination of intravascularly administered commercially distributed solutions in the event of an outbreak of *R. pickettii* bacteremia.[16]

Multiple nosocomial outbreaks have been caused by commercial solutions intrinsically contaminated with *R. pickettii*.[1,2,16] This microorganism can contaminate sterile solutions because of its ability to grow in a wide range of temperatures (15 °C—42 °C) and in saline solution.[17] In addition, it can pass through both 0.45-mm and 0.2-mm filters, which are used for the terminal sterilization of several medicinal products.[17] Chen et al. have reported a case series of BSIs due to catheter flushing with contaminated normal saline.[18] We have presented 11 cases of *R. pickettii* due to contaminated saline solutions. Our findings are consistent with previous findings. We think that *R. pickettii* may have colonized the normal saline solution used in our clinic because of its ability to pass through filters used for terminal sterilization.

It is well known that *R. pickettii* can form biofilms inside plastic catheters, making it more resistant to biocidal agents and more difficult to eradicate.[19] This makes *R. pickettii* a more important and serious agent responsible for catheter-related BSIs. In addition, *R. pickettii* may cause prolonged febrile reactions in patients with temporary dialysis catheters, possibly because of early biofilm development.[20] In the study by Chen et al., 18 Port-a-Caths had to be removed, and the catheter tips of 50% of these were culture positive.[18] In our study, catheter removal was needed in four patients (i.e., 36.3% of the affected patients). Only one patient had repetitive culture positivity for *R. pickettii* under treatment, which was accepted as colonization.

Because of its low virulence, *R. pickettii* infection is rarely fatal.[21,22] In accordance with this finding, although our patient group was immunocompromised and fragile, no death occurred during the outbreak. However, the chemotherapy of these patients could be delayed because of the infection. In addition, catheter removal is a distressing process. Further, the patients were exposed to anesthesia and surgery, and there was an overuse of antibiotics. Although the healing of these patients is a positive outcome, these disadvantages cannot be ignored.

We have reported the rapid control of an outbreak of *R. pickettii* BSIs in highly immunocompromised patients by initiating antibiotic therapy and by improving infection control practices in a short period of around two weeks. The limitation of our study is that although our findings suggest that the source of the outbreak was the saline solution, solid evidence was missing. Ongoing surveillance by hospital infection control teams and prompt and robust investigation to identify the source of nosocomial infections are crucial to stop an outbreak.[16] It is important for clinicians to be aware of the possibility of contamination of intravascular solutions with *R. pickettii*. Moreover, infection control practices should involve direct sampling of these fluids in order to prevent further contamination and new cases.

Funding

None.

Declaration of competing interest

None.

Acknowledgments

We want to thank the Infection Control Team of Ankara Children's City Hospital for their vigorous and hard work in controlling the outbreak.

References

1. Ryan MP, Pembroke JT, Adley CC. *Ralstonia pickettii*: a persistent gram negative nosocomial infectious organism. *J Hosp Infect* 2006;**62**(3):278—84.
2. Nosocomial Ralstonia pickettii Colonization Associated with Intrinsically Contaminated Saline Solution — Los Angeles, California, 1998. *MMWR* 1998;**47**(14):285—6. Available at External Link: https://www.cdc.gov/mmwr/preview/mmwrhtml/00052013.htm. [Accessed 10 January 2021].
3. Boutros M, Gonullu N, Casetta A, Guibert M, Ingrand D, Lebrun L. *Ralstonia pickettii* traced in blood culture bottles. *J Clin Microbiol* 2002;**40**:2666—7.
4. Choudhuri H, Jindal A, Pathengay A, Flynn Jr HW. An outbreak of *Ralstonia pickettii* endophthalmitis following intravitreal methotrexate injection. *Clin Ophthalmol* 2015;**9**:1117—20.
5. Kendirli T, Çiftçi E, İnce E, İncesoy S, Güriz H, Aysev AD, et al. *Ralstonia pickettii* outbreak associated with contaminated distilled water used for respiratory care in a paediatric intensive care unit. *J Hosp Infect* 2004;**56**(1):77—8.
6. Anderson RL, Holland BW, Carr JK, Bond WW, Favero MS. Effect of disinfectants on pseudomonas colonized on the interior surface of PVC pipes. *Am J Public Health* 1990;**80**:17—21.
7. Vincenti S, Quaranta G, De Meo C, Bruno S, Ficarra MG, Carovillano S, et al. Non-fermentative gram-negative bacteria in hospital tap water and water used for haemodialysis and bronchoscope flushing: prevalence and distribution of antibiotic resistant strains. *Sci Total Environ* 2014;**499**:47—54.
8. O'Grady NP, Alexander M, Burns LA, Dellinger EP, Garland J, Heard SO, et al. Guidelines for the prevention of intravascular catheter-related infections. *Am J Infect Contr* 2011;**39**:1—34.
9. Maki DG, Mermel LA. Infections due to infusion therapy. In: Jarvis WR, editor. *Bennett and Brachman's hospital infection*. 5th ed. Philadelphia: Lippincott Williams & Wilkins; 2007. p. 611—47.

10. Mermel LA, Allon M, Bouza E, Craven DE, Flynn P, O'Grady NP, et al. Clinical practice guidelines for the diagnosis and management of intravascular catheter-related infection:2009 update by the infectious diseases society of America. *Clin Infect Dis* 2009;**49**(1):1—45.
11. Mirret S, Reller LB, Petti CA, Woods CW, Varizani B, Sivadas R, et al. Controlled clinical comparison of BacT/ALERT standart aerobic medium with BACTEC Standard aerobic medium for culturing blood. *J Clin Microbiol* 2003;**41**(6):2391—4.
12. The Europian Committee on Antimicrobial Susceptibility Testing, EUCAST. *Breakpoint tables for interpretation of MICs and zone diameters*. 2018., Version 8.0. http://www.eucast.org.
13. Centers for Disease Control and Prevention. *Modified pulse-net procedure for pulsed-field gel electrophoresis of select gram negative bacilli purpose, document. No.: CEM.TE.C.0002*. 2014.
14. Morrison D, Woodford N, Barrett SP, Sisson P, Cookson BD. DNA banding pattern polymorphism in vancomycin-resistant *Enterococcus faecium* and criteria for defining strains. *J Clin Microbiol* 1999;**37**:1084—91.
15. Tenover FC, Arbeit RD, Goering RV. How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: a review for healthcare epidemiologists. Molecular Typing Working Group of the Society for Healthcare Epidemiology of America. *Infect Control Hosp Epidemiol* 1997;**18**:426—39.
16. Kimura AC, Calvet H, Higa JI, Pitt H, Frank C, Padilla G, et al. Outbreak of *Ralstonia pickettii* bacteremia in a neonatal intensive care unit. *Pediatr Infect Dis J* 2005;**24**:1099—103.
17. Anderson RL, Bland LA, Favero MS, McNeil MM, Davis BJ, Mackel DC, et al. Factors associated with *Pseudomonas pickettii* intrinsic contamination of commercial respiratory therapy solutions marketed as sterile. *Appl Environ Microbiol* 1985;**50**:1343—1348.
18. Chen YY, Huang WS, Chen CP, Sun SM, Kuo FM, Chan YJ, et al. An outbreak of *Ralstonia pickettii* bloodstream infection associated with an intrinsically contaminated normal saline solution. *Infect Contr Hosp Epidemiol* 2017;**38**(4):444—8.
19. Dombrowsky M, Kirschner A, Sommer R. PVC-piping promotes growth of *Ralstonia pickettii* in dialysis water treatment facilities. *Water Sci Technol* 2013;**68**:929—33.
20. Thet MK, Pelobello Ma LF, Das M, Alhaji MM, Chong VH, Khalil MAM, et al. Outbreak of nonfermentative Gram-negative bacteria (*Ralstonia pickettii* and *Stenotrophomonas maltophilia*) in a hemodialysis center. *Hemodial Int* 2019;**23**:83—9.
21. Roberts LA, Collignon PJ, Cramp VB, Mc Neil MM, Davis BJ, Mackel DC, et al. An Australia-wide epidemic of Pseudomonas pickettii bacteraemia due to contaminated "sterile" water for injection. *Med J Aust* 1990;**152**:652—5.
22. Pellegrino FL, Schirmer M, Velasco E, de Faria LM, Santos KR, Moreira BM. *Ralstonia pickettii* bloodstream infections at a Brazilian cancer institution. *Curr Microbiol* 2008;**56**:219—23.