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RESEARCH ARTICLE

Ralstonia pickettii bacteremia in a cardiac surgery patient in Belgrade, Serbia

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ABSTRACT

Ralstonia pickettii is an opportunistic bacterium found in the water environment with an increasing incidence as a nosocomial pathogen. The objectives of this study were to describe *R. pickettii* bacteremia in a cardiac surgery patient and to evaluate its ability to grow in a saline solution and to form biofilm. The patient in this study underwent mitral and aortic valve replacement surgery with two aortocoronary bypasses. She developed signs of respiratory and renal failure, therefore hemodialysis was started. After 25 days in an intensive care unit, the patient had recurrent episodes of fever with signs of bacteremia. *R. pickettii* was identified from blood cultures by MALDI-TOF MS. Antimicrobial susceptibility testing was performed using disc diffusion and broth microdilution methods in accordance with EUCAST methodology and results were interpreted following clinical breakpoints for *Pseudomonas* spp. The isolate was susceptible to all tested antimicrobial agents except aminoglycosides and colistin. Survival of *R. pickettii* was analyzed in saline solution with four different starting concentrations at 25 °C and 37 °C for six days. Biofilm capacity was tested using the microtiter plate method. *R. pickettii* showed substantial growth in saline solution, with starting concentration of 2 CFU ml^{-1} reaching 10^7 CFU ml^{-1} after six days. There was no significant difference between growth at 25 °C and 37 °C. This indicates that storage of contaminated solutions at room temperature can enhance the count of *R. pickettii*. Our strain did not show the capacity to form biofilm. The patient responded well to adequate treatment with ceftazidime, and after 48 days in ICU she was discharged to convalesce.

KEYWORDS

Ralstonia pickettii, cardiac surgery patient, hemodialysis, saline solution, biofilm

INTRODUCTION

Non-fermenting Gram-negative bacilli (NFGNB) are a rising heterogeneous group of pathogens that cause infections in humans, especially in intensive care units (ICUs). The most frequently isolated species of this group are *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, particularly multi-drug resistant strains, which Centers for Disease Control and Prevention reported as microorganisms with a serious threat level [1]. In recent years, there has been an increase in the incidence of other bacteria in this group, and an example of this is *Ralstonia pickettii* [2]. *R. pickettii* was first described by Ralston et al. in 1973, and it was classified as *Pseudomonas pickettii* [3]. After reviewing genomic and phenotypic characteristics of seven *Pseudomonas* strains, in 1992 *P. pickettii* was reclassified in *Burkholderia* spp. [4].

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In 1995, the same group of authors revised *B. pickettii* and transferred it to the new genus *Ralstonia*, based on its phenotypic characteristics, cellular lipid and fatty acid analysis, rRNA–DNA hybridization, and analysis of 16s rDNA nucleotide sequences [5].

R. pickettii can be found in soil and water, still the main concern is that it can be habitually found in the hospital environment [6, 7]. Although it is of low virulence, this bacterium has been identified as causative agent of many potentially harmful and fatal infections [7]. It is described as a pathogen that can have a major impact on immunocompromised patients such as neonates, patients with blood malignancies, patients on hemodialysis [8–10], and major conditions associated with *R. pickettii* infection are bacteremia and septicemia [7].

R. pickettii is a slow-growing waterborne bacterium requiring a low intake to survive and it can even grow in ultra-pure water [11]. Many cases have discovered that the source of *R. pickettii* is different contaminated solutions used in hospital settings [8, 12]. Additionally, a few studies described outbreaks of *R. pickettii* in hemodialysis units [13]. One of the reasons for infecting patients during hemodialysis is its ability to form biofilm on PVC plastic pipes [14].

The aims of our study were to present a case of *R. pickettii* bacteremia in a cardiac surgery patient who underwent hemodialysis, to evaluate the ability of the isolate to survive and grow in saline solution, and to form biofilm.

MATERIALS AND METHODS

Case report

A 77-year-old female patient was admitted to the Cardiovascular Institute Dedinje, Belgrade, Serbia, with a history of fatigue, exhaustion, and leg swelling. Echocardiogram showed an enlarged left atrium (48 mm), mitral regurgitation 3+, EF 40%, hypokinesis of the septum, inferior wall, and posterior wall, aortic stenosis AVA 0.4 cm^2, pressure gradient 96/62 mmHg, tricuspid regurgitation 2+, and SPDK 57 mmHg. After evaluating the patient's condition, cardiac surgery was performed on April 1st, 2021, and it included: mitral valve replacement (Cabomedics), aortic valve replacement (SJM Regent), and two aortocoronary bypasses. Immediately after surgery, empirical treatment with vancomycin began due to the presence of collections on the mitral valve. On day 2 after surgery, the patient developed a fever of up to 38.5 °C with neutrophilic leukocytosis and increased inflammatory markers (Le = 15×10^9/L, CRP = 237 mg L^{-1}, Pct = 2.288 ng ml^{-1}). Vancomycin was substituted with meropenem and metronidazole, and corticosteroids were introduced due to a further increase of CRP (309.6 mg L^{-1}) and procalcitonin (6.12 ng ml^{-1}). Two blood cultures, a central venous catheter tip, a mitral valve biopsy sample, and a urine sample were sent for a microbiology examination and were all negative. Signs of respiratory insufficiency were observed and the patient was reintubated on day 3 after surgery. Additionally, the patient developed signs of renal failure, and continuous venovenous hemodiafiltration (CVVHDF) was started. Moreover, on day 5 after cardiac surgery, the patient underwent cholecystectomy due to the pain in the right hypochondrium, increased liver enzymes, and signs of inflammation and calculi in the gallbladder visualized by ultrasound. After cholecystectomy, the patient was afebrile, and leucocytes and inflammatory markers were at normal levels. The patient stayed in ICU and her condition was stable. On day 15, the patient started having recurrent episodes of fever and multiple blood cultures were negative again. During that time, the patient developed *Candida albicans* urinary tract infection, and fluconazole was administered. Recurrent episodes of fever and signs of bacteremia continued and on day 25 after surgery, two blood cultures were positive and *R. pickettii* was isolated. Ceftazidime was introduced in accordance with antimicrobial susceptibility testing and was administered for 13 days. The patient had a good clinical condition and was discharged from ICU after 48 days, in all, without sequelae.

Culture and antimicrobial susceptibility of the strain

Multiple blood cultures were incubated in a BacT/ALERT system (bioMérieux, France), and two blood culture sets obtained on day 25 after surgery became positive after 32 and 45 h of incubation, respectively. Gram stain showed Gram-negative rods (Fig. 1). Positive blood cultures were subculture on blood agar and MacConkey agar (Promedia,

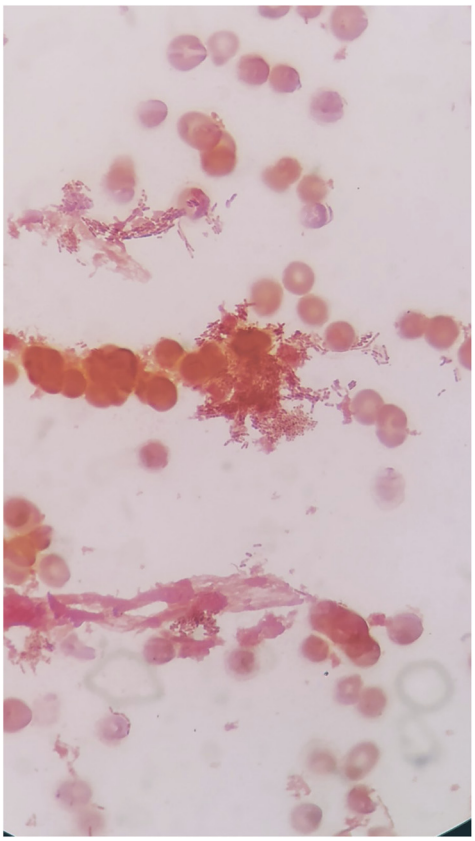

Fig. 1. Gram stain of blood culture showing Gram-negative bacilli

Serbia) at 37 °C. After 48 h, tiny catalase-positive and delayed oxidase-positive colonies were observed. Rapid and accurate identification was achieved using MALDI-TOF MS, Vitek MS (bioMérieux, France) and *R. pickettii* was identified.

Antimicrobial susceptibility testing (AST) was performed by disk diffusion method and broth microdilution for colistin (ComASP Colistin, Liofilchem, Italy). As there are no EUCAST susceptibility breakpoints available for *Ralstonia* spp., the AST results were interpreted following clinical breakpoints used for *Pseudomonas* spp. and *Acinetobacter* spp. for trimethoprim-sulfamethoxazole.

Growth in saline solution and biofilm formation

To determine its ability to grow in 0.9% saline solution, we used a modified method previously described by Andersen et al. [15]. In short, the overnight culture of *R. pickettii* strain was suspended in saline solution and adjusted to 0.5 McFarland standard. Following that, the working solution was diluted with saline solution, and two inocula were made: low inoculum 10^3 CFU ml^{-1} and high inoculum 10^5 CFU ml^{-1}. Both concentrations were inoculated in two volumes of sterile saline solution: 5 and 50 ml, to get four starting concentrations of *R. pickettii* in saline solutions: 2 CFU ml^{-1}; 20 CFU ml^{-1}; 200 CFU ml^{-1}, and 2.000 CFU ml^{-1}. All concentrations were processed in duplicates. Bacterial growth was evaluated five times: after 0, 24, 48, 72, and 144 h of incubation at 25 °C and 37 °C. Further, the 10^{-1}, 10^{-3}, and 10^{-5} dilutions were made from starting concentrations and inoculated on Mueller-Hinton agar with a sterile glass stick. After 24 h of incubation at 37 °C, colonies were counted.

To evaluate the capacity of *R. pickettii* strain to form biofilm on a plastic surface, we used the previously described method by Stepanovic et al. [16]. *P. aerugonosa* ATCC 27853 strain was used for comparison.

RESULTS

The *R. pickettii* strain was susceptible to meropenem and trimethoprim-sulfamethoxazole; susceptible, increased exposure to piperacillin, piperacillin-tazobactam, ticarcillin, ceftazidime, cefepime, imipenem, aztreonam, ciprofloxacin, and levofloxacin; and resistant to amikacin, tobramycin, and colistin (minimum inhibition concentration higher than 16 mg L^{-1}).

The growth of *R. pickettii* in 0.9% saline solution at 25 °C and 37 °C was shown in Fig. 2. Logarithmic growth was observed during the incubation period at both temperatures in all evaluated solutions with different starting bacterial concentrations and there were no significant differences in growth rate. There was also a similar number of bacteria in all analyzed solutions after six days of incubation and

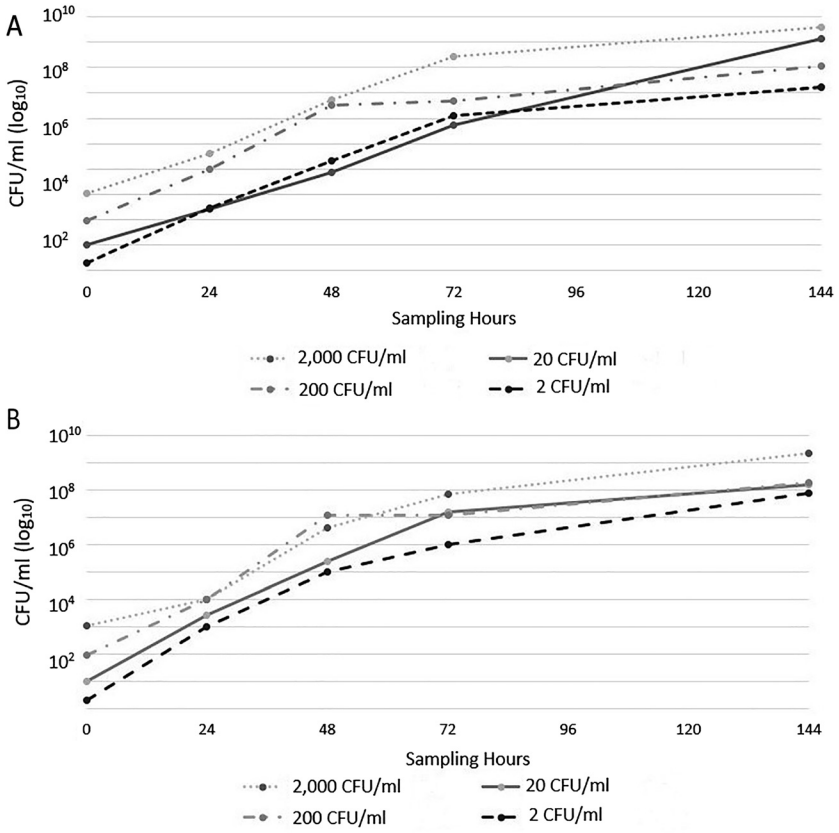

Fig. 2. Growth of *R. pickettii* in saline solution at 25 °C (A) and 37 °C (B). Four starting concentrations of *R. pickettii* in saline solutions were: 2 CFU ml^{-1}, 20 CFU ml^{-1}, 200 CFU ml^{-1}, 2.000 CFU ml^{-1}

reached concentrations were 10^7–10^9 CFU ml^{-1}. Even the saline solution with starting concentration of 2 CFU ml^{-1} reached 10^6 CFU ml^{-1} at 25 °C and 10^7 CFU ml^{-1} at 37 °C.

R. pickettii strain did not demonstrate the capacity to produce biofilm on the plastic surface after 24 h of incubation compared to *P. aeruginosa* ATCC 27853 strain, which showed moderate biofilm formation.

DISCUSSION

R. pickettii is an emerging infrequent causative agent of bloodstream infections [10], meningitis [17], endocarditis [18], and osteomyelitis [19], although it has also been identified in several nosocomial outbreaks [9, 12]. One of the most affected groups of patients is hemodialysis patients [10, 13]. Intravascular-administered commercially available solutions are usually been identified as a source of infection in hospital patients and cause nosocomial outbreaks [12]. Contamination of these solutions happens most often during the manufacturing stage, due to *R. pickettii* ability to pass through 0.2-µm filters which are often used for sterilization of medical solutions [20]. During the hospital stay, our patent had hemodialysis for 5 days and stayed in the ICU for more than 40 days. Considering findings in the literature [10] and the patient's medical history, we assumed that the source of the infection could have been a hemodialysis solution.

Based on specific groups of patients in whom *R. pickettii* infection is most often found, risk factors for this infection could be low immunological barriers with high IV fluids intake [7, 13]. Furthermore, the majority of these patients need prolonged hospital stay which also gives *R. pickettii* more opportunity to occur in a hospital environment. Despite the low mortality in patients with *R. pickettii* infection, long-term consequences are not observed. Carrell et al. suggest that this infection could lead to secondary infertility in men [21]. Our patient was discharged for rehabilitation without sequelae and after a one-month checkup, she was stable.

Identification and differentiation of *Ralstonia* spp. isolates with commercial biochemical identification systems are challenging [22]. Due to biochemical similarities, it is believed that many *R. pickettii* strains in clinical samples are overlooked and misidentified as *Burkholderia* spp. or *Pseudomonas* spp. [23]. Some authors described problems with the identification of *Ralstonia* spp. using automated systems (i.e. Vitek2) which identify these bacteria only at the genus level [24]. Confirmation of identification could be accomplished by 16S rDNA gene sequencing and Matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-TOF MS), which are more often used nowadays [24]. In our study, the use of the MALDI-TOF MS method for microbiological identification of *R. pickettii* strain accelerated the identification of this slow-growing pathogen. This provides fast reaction time and allows timely application of adequate antimicrobial therapy.

Most NFGNB are intrinsically resistant to many antibiotics, including benzylpenicillin, first- and second-generation cephalosporins, macrolides, and glycopeptides. Additionally, there is an increasing number of *Ralsotonia* spp. strains that are multi-drug resistant. *R. pickettii* is intrinsically resistant to colistin [25] and numerous studies described the increase in resistance to aminoglycosides [26], which was also detected in our strain. Moreover, Ryan et al. described treatment failure with meropenem and ceftriaxone in infections caused by *Ralstonia* spp. [7]. We also observed no response to meropenem treatment in our patient, even though that the *R. pickettii* strain was susceptible to this antibiotic *in vitro*. The resistance to ceftriaxone in *Ralstonia* spp. could be explained by its similarity to *P. aeruginosa,* which is intrinsically resistant to this antibiotic. In the presented case, the patient reacted well on ceftazidime and the patients' signs of bacteriemia were resolved.

R. pickettii is an oligotrophic bacterium that can live in low-nutrient solutions, including environmental water and a variety of hospital water sources [11, 27]. This enhances survival in saline solutions and other solutions used in hospitals, which are described as a source of nosocomial infections [8, 12]. Chetoui et al. found this organism in a "sterile" saline solution used in oncological patients, which led to systemic infection [28]. Additionally, the results of our study demonstrated the significant ability of *R. pickettii* to grow in low-nutrient medical solution. Our findings indicate that even when *R. pickettii* is present in saline solution in a small concentration it can survive and potentially cause an infection.

In our investigation, the growth of *R. pickettii* in saline solution was analyzed at 25 °C and 37 °C, and no difference in bacterial concentration was found after six days of incubation at both temperatures. This indicates that storage of contaminated solutions at room temperature can enhance the count of *R. pickettii* in it and increase the infectious inoculums, which could lead to more severe infections in immunocompromised patients.

A few studies investigated the ability of *R. pickettii* strains isolated in a hospital environment to produce biofilm [14, 24]. However, the result of our investigation showed that the bacterium had a significantly lower capacity in biofilm production in comparison with the *P. aeruginosa* ATCC strain. A possible explanation of this finding is the common presence of *R. pickettii* in polymicrobial biofilms (with *P. aeruginosa* and other NFGNB) [29] in which other organisms assume to have a more significant role in biofilm formation.

CONCLUSION

We reported an unusual case of *R. pickettii* bacteremia in our cardiac surgery patient. After adequate treatment with ceftazidime, the patient's infection resolved without sequelae. The *R. pickettii* strain was susceptible, increased exposure to many antibiotics and it did not show the capacity to form biofilm on a plastic surface after 24 h. On the other hand, the strain grew well in sterile saline solution, reaching concentrations of 10^9 CFU ml^{-1} after six days of

incubation. This indicates that the preparation and sterilization of solutions for IV application should be carefully managed. Fast identification of bacteria and predicting *R. pickettii* infection in hemodialysis patients could provide better outcomes. The limitation of our study is that although our findings suggest that the source of the infection was the hemodialysis solution, solid evidence was missing. Ongoing surveillance by hospital infection control teams and fast investigation in order to identify the source of healthcare-associated infections is crucial to stop outbreaks.

Conflicts of interest: The authors declared that they have no conflicts of interest in this work.

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