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Brief Report

Outbreak of *Ralstonia* spp. and *Burkholderia* spp. Catheter-Related Bloodstream Infection in Hemodialysis Unit

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Abstract: The *Ralstonia species* (*RB*) and *Burkholderia species* (*BB*) are bacteria responsible for nosocomial infections in frail patients such as hemodialyzed (HD) patients. Here, we report how we managed an outbreak caused by *RB* and *BB* that occurred in a dialysis unit. From the 7th to the 16th of April 2021, an infection due to *RB* and *BB* occurred in 7 out of 39 (17.9%) HD patients with central venous catheter (CVC). Disinfectants, CVC-lock therapy solutions, water by reverse osmosis unit (ROW) and dialysis concentrates were cultured, including the biofilm from the loading plastic tubes (LPTs) that connect the hemodialysis consoles (HCs) to the ROW delivery line. The antibiotic treatment was successful for all patients. *RB* and *BB* were isolated in the biofilm of 11/37 LPTs. Three out of 11 positive LPTs were associated with the infected patients. The ROW delivery line was modified to provide a whole disinfection with the HCs connected, avoiding the risk of new contamination of the LPTs. A filtration module of 0.01 mm was added prior to the ROW delivery line. Our experience suggests that outbreaks sustained by unusual bacteria such as *RB* and *BB* should be promptly investigated to treat the infected patients with the appropriate therapy and to identify the possible source of infection, making the needful changes to achieve a safer dialysis unit.

Keywords: bloodstream infection; *Burkholderia* spp.; central venous catheter; hemodialysis; *Ralstonia* spp.

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1. Introduction

The *Ralstonia* (*RB*) and *Burkholderia* (*BB*) *species* are environmentally aerobic, non-fermentative, Gram-negative bacilli, reclassified from the *Pseudomonas* group [1], which are commonly found in soil, wastewater and plants [2]. Although *RB* and *BB* have a low virulence [3], they could be responsible for sepsis, meningitis, pneumonia, peritonitis, and central venous catheters associate bacteremia more frequently in immunocompromised patients such as transplanted and hemodialyzed patients [2,4]. In addition, several nosocomial infections due to medical devices and solutions such as saline and sterile water

contaminated by *RB* and *BB* have been reported [3,5]. The proliferation of the *Ralstonia species* implies the formation of a biofilm that enhances their survival in the colonized environment or in medical devices [6–8]. The *Ralstonia species* can pass through 0.2 micrometer filters used for the sterilization of different medical products, such as saline solution, and they can potentially go through reverse-osmosis dialysis membranes [3]. *RB* can also survive for long periods in oligotrophic environments because of its low nutrient requirements and ability to derive energy from a large variety of substrates [9]. The *Ralstonia species* are resistant to disinfectants such as chlorhexidine [10], which is commonly used in clinical settings, including disinfection procedures used for the antiseptic handling of the vascular access of hemodialyzed patients. In addition, *RBs* are frequently resistant to antibiotics such as beta-lactam and carbapenems [3,11].

The most common species of *RB* reported in the literature are *Ralstonia pickettii* (formerly *Pseudomonas pickettii* and *Burkholderia pickettii*), *Ralstonia insidiosa* and *Ralstonia mannitolilytica* [3].

Here, we report an outbreak of bacteraemia caused by *RB* and *BB*, which occurred in our dialysis unit located in Ancona, in the central part of Italy.

How we managed the infected patients, what we have accomplished to identify the source of contamination and what we have implemented to make the dialysis unit safer against environmentally aerobic, non-fermentative, Gram-negative bacilli are reported.

2. Materials and Methods

From the 7th to the 16th of April 2021, we identified six patients who were regularly hemodialyzed in our dialysis unit and who experienced symptoms related to infection, such as fever and malaise soon after dialysis treatment. The identification of more cases of infection developing over a few days in our population of hemodialyzed patients led us to define it as an outbreak [12]. All affected patients had undergone hemodialysis with a long-term indwelling central venous catheter (CVC). Cultures of blood drained from the CVC and the peripheral vein of the six symptomatic patients revealed the growth of *Ralstonia insidiosa* in four patients and *Ralstonia pickettii* and *Burkholderia cepacia* in the remaining two patients. We reasoned that the presence of the CVC might be a cause of *RB* and *BB* infection, allowing the proliferation of bacteria in the biofilm. Thus, all patients carrying a CVC (39 patients) underwent cultures of blood drained from the CVC and the peripheral vein. A prompt antibiotic therapy was started based on the reported antibiogram checked for each isolated bacterium. Specifically, the 3 patients (#1, #4 and #5) infected by *R. insidiosa* were treated with the combination of intravenous ciprofloxacin at the dose of 200 mg bid and meropenem 500 mg bid for a mean of 11 days; the remaining 4 infected patients were treated only with intravenous ciprofloxacin at the dose of 200 mg bid for a mean of 11 days. Although, antibiotic treatment was successful in all patients without any problem of CVC patency, we decided to remove the colonized CVC in all the patients in accordance with our infectologist and microbiologist consultants.

Concomitantly, in order to identify the source of the infection, we performed cultures on the disinfectants used in the dialysis unit for antiseptic purposes in handling vascular access, including drugs such as heparin and sodium citrate used as lock therapy for CVCs. In addition, we cultured samples of the water produced by the reverse osmosis unit (ROW) and used for diluting the dialysis concentrates using a system for the production of an acidic dialysis concentrate, namely Granumix (Fresenius Medical Care). Cultures of the dialysis fluids at different points, starting from the output of the reverse osmosis unit through the ROW delivery line and including the dialysis fluid wasted from the hemodialyzer console (HC), were also performed.

Due to the absence of *RB* and *BB* in all the disinfectants, as well as the drugs and water from the ROW delivery line, we speculated in accordance with D'Amico et al. [13] that the bacteria might have colonized the biofilm of the loading pipes (LPs) and the loading plastic tubes (LPTs) that connect the HC to the LPs of the ROW delivery line.

All the LPTs connecting the 25 HC to the ROW delivery line used in each dialysis session, including those connecting the ROW delivery line to the stored ones (14 HC), were dissected, and the fragments obtained were placed in dedicated boxes and sent to the laboratory for microbiological analysis. Briefly, the boxes containing the LPT fragments were opened under a laminar flow hood and covered with a saline solution [14–16]. Sonication of the biofilm attached to the LPT fragments was performed using BactoSonic-Bandelin at 30–40 KHz 0.22 + 0.04 W/cm^2 for 5 min [16–18]. Sonication products, 0.1 mL, were inoculated into Columbia Blood Agar, Chocolate Agar and Mac Conkey Agar plates. The incubation times were different according to the culture growth mediums as reported below: Columbia Blood Agar at 37 °C in anaerobiosis for 14 days; Chocolate Agar plates in 5% CO2 atmosphere at 37 °C for 4 days; and Mac Conkey Agar plates at 37 °C for 48 h [16–18].

The bacteria strains were identified using MALDI-TOF VITEK® MS (Biomerieux, Grassina, Italy), an automated mass-spectrometry microbial identification system based on the Matrix Assisted Laser Desorption Ionization Time-of-Flight technology.

The blood cultures from the CVC and peripheral vein were incubated in VIRTUO BIOMERIEUX®, an automated and continuous-monitoring blood culture microbial detection system. The bacteria positivity of the blood samples was checked using the BACT/ALERT® VIRTUO® (Biomerieux, Italy). Subsequently, the positive blood samples were inoculated into Chocolate Agar and Columbia Blood Agar plates.

After the identification of *RB* and *BB* in the LPTs, the ROW delivery line was modified to provide a scheduled chemical and physical disinfection with the hemodialysis consoles connected to avoid the risk of new contamination of the LPTs. Briefly, on alternate months, the whole system, which includes the ROW delivery line (PE-Xa Medical Device®, Fresenius Medical Care), loading pipes, LPTs and hemodialysis consoles, are disinfected by hot water (90 °C) produced by a water heater (AquaB plus HF®, Fresenius Medical Care) and by a chemical disinfectant of 5% peracetic acid, according to the protocols made by the console firm. We planned also the replacement of LPTs every 6 months.

In addition, we added to the reverse osmosis system a filtration module of 0.01 mm (AquaUF®, Fresenius Medical Care) prior to the ROW delivery line to reduce the microbiological risk of contamination by *RB* and *BB* (Figure 1).

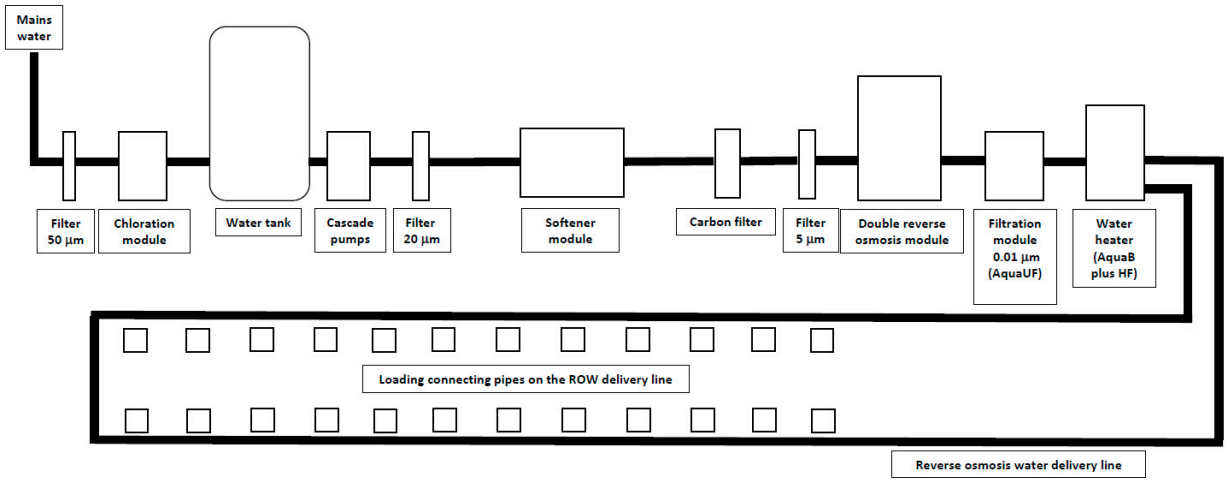

Figure 1. Hemodialysis Water Treatment System. ROW: reverse osmosis treated water.

3. Results

At the time of the study the total number of patients who underwent regular hemodialysis treatment was 94. 39 out of 94 patients (41.5%) underwent HD with CVC. 7 patients out of 39 (17.9%) developed infection associated with bacteremia. 6 patients were symptomatic and one asymptomatic. None of the patients undergoing HD treatment with arterio-venous fistula developed symptoms related to vascular access infection nor bac-

teremia. Demographic, clinical and microbiological data of the patients with bacteremia are shown in Table 1.

Table 1. Demographic clinical and laboratory data of the hemodialyzed patients with bacteremia.

Patient (#)	Age (Years)	Gender (M: Male; F: Female)	Dialysis Duration (Months)	Comorbidities	Strain of Isolated Bacterium	CRP (mg/dL)	PCT (ng/mL)	WBC (Cells/mmc)
#1	76	M	36	Previously kidney transplant; HTN; ST	*R. insidiosa*	3.3	14.9	7.680
#2	51	F	7	HTN; O	*R. insidiosa*	3.9	5.35	7.100
#3	70	F	33	HTN; DM; O	*R. pickettii*	5.6	9.95	7.430
#4	83	M	36	HTN; DM	*R. insidiosa*	4.5	27.5	6.530
#5	78	M	43	History of cancer; previously kidney transplant; HTN; ST	*R. insidiosa*	3.7	6.43	7.600
#6	57	F	44	HTN; DM	*B. cepacia*	5.9	15.5	7.420
#7	63	F	6	DM	*R. pickettii*	0.8	1.02	4.350

CRP: C-reactive protein; PCT: procalcitonin; WBC: white blood cell count; HTN: hypertension; DM: diabetes; O: obesity (BMI $\geq$ 30 kg/m^2); and ST: steroid therapy.

The mean age of infected patients was 68 $\pm$ 16 years (Table 1). All symptomatic patients presented an increase in the C-reactive protein (CRP) levels (mean CRP: 4.48 $\pm$ 1.3 mg/dL, normal values < 0.5 mg/dL) and the procalcitonin (PCT) levels (mean PCT: 13.27 $\pm$ 11.07 ng/mL, normal values < 0.05 ng/mL). Fever (mean body temperature: 37.9 $\pm$ 1.4 °C) and malaise were present in the symptomatic patients. The asymptomatic patient presented normal levels of C-reactive protein and a little increase in the procalcitonin levels (PCT: 1.02 ng/mL). None of the patients had a significant increase in the white blood cells count (mean WBC 6.872 $\pm$ 1.177 cells/mmc, normal values 4.000–10.000 cells/mmc).

In consideration of the type of pathogens that primarily affect immunocompromised patients, we checked our infected patients for additional factors contributing to immunodeficiency beyond the ESKD with the need of dialysis. Briefly, the seven patients who developed infections in our dialysis unit were older (median age 70) with a moderate duration of dialysis (median 36 months), six patients had a history of longstanding hypertension with organ damage and four had a history of longstanding diabetes with related severe vasculopathy. Severe obesity (BMI $\geq$ 30 kg/m^2) was present in two patients, one patient had a history of cancer and two patients had a history of graft failure and were taking low doses of steroid, see Table 1.

The antibiotic sensitivity analysis of the isolated bacteria demonstrated an overlap of an antimicrobial sensitivity pattern both for the *RB* and *BB* strain. Specifically, there was a good sensitivity to quinolones and carbapenems (Table 2).

All the infected patients, irrespective of the presence of symptoms, were treated with intravenous antibiotic therapy according to their antibiotic sensitivity patterns for a mean duration of 11 days (see the Section 2). Specifically, 3 patients (#1, #4 and #5) infected by *R. insidiosa* were treated with a combination of ciprofloxacin and meropenem; the remaining 4 infected patients were treated only with ciprofloxacin. All symptomatic infected patients reached prompt and full recoveries with the disappearance of fever and malaise together with the normalization of inflammatory markers. The colonized CVCs were removed in all infected patients, irrespective of the presence of symptoms within 6 to 11 days (9.1 $\pm$ 2.5 days) from the diagnosis of bacteremia. Concomitantly, a new CVC was inserted in place of the colonized one. Blood cultures from both the new CVC and the peripheral vein after the end of antibiotic therapy had negative results.

The microbiological cultures of the solutions used for CVC disinfection purposes, including povidone iodine and alcoholic solutions and CVC-washing solutions such as

saline and CVC-lock solutions (heparin, sodium citrate and urokinase), did not show any bacterial growth.

Table 2. Strains of bacteria isolated in the hemodialyzed patients' blood cultures with the relative antibiotic sensitivity pattern analysis.

Type of Antibiotic	*Ralstonia insidiosa* (Patients: #1, #2, #4)	*Ralstonia insidiosa* (Patient: #5)	*Ralstonia pickettii* (Patient: #3)	*Burkholderia cepacia* (Patient: #6)	*Ralstonia pickettii* (Patient: #7)
Amikacin	R 32		S <= 4		S <= 4
Cefepime	S <= 2	S 2	S 4		S 4
Cefotaxime	I 2	S 1	I 2		R 4
Ceftazidime	R 16	R 16	R > 16	R > 256	R > 16
Ciprofloxacin	S <= 0.25	S 0.12	S <= 0.25		S <= 0.25
Ertapenem	R 4		R >4		R > 4
Gentamicin	R > 8	R >= 16	S <= 1		S <= 1
Imipenem	S <= 1		S 2		S 2
Levofloxacin	S <= 1		S <= 1	S 0.19	S <= 1
Meropenem	S <= 1	S 1	R > 8	S 1.05	R > 8
Piperacillin/ tazobactam	I <= 8	S <= 4	I <= 8		I <= 8
Tigecycline	I 0.5		S <= 0.25		S <= 0.25
Tobramycin	R > 8		S <= 1		S <= 1
Minocycline				S	
Temocillin				S 2	
Cotrimoxazole				S 0.19	

S = susceptibility, I = intermediate susceptibility, and R = resistance.

The microbiological cultures of the samples of water produced by the ROW resulted negative at all points of the hemodialysis water treatment system, starting from the output of the reverse osmosis unit through the ROW delivery line, including the hemodialysis concentrate derived from Granumix.

RB and *BB* were isolated in the biofilm of 11 out of 37 (29.7%) LPTs connecting the loading pipes of the ROW delivery line to the HCs (Table 3). Specifically, 3 out of 11 of the LPTs connected to the HCs used to perform hemodialysis of the patients who developed *RB* and *BB* resulted positive for the same bacteria isolated in the patients.

Table 3. Results of the microbiological culture of the biofilm detached by using sonication from the loading plastic tubes connecting the hemodialysis consoles to the reverse osmotic water treatment-delivery line. The bacteria responsible for the infection are highlighted in bold.

Strains of Isolated Bacteria
Serratia marcescens (13 LPTs)
***Ralstonia insidiosa* (8 LPTs)**
Microbacterium flavescens (5 LPTs)
Ochrobactrum anthropi (4 LPTs)
***Burkholderia contaminans* (3 LPTs)**
Enterobacter cloacae/asburiae (3 LPTs)
Enterococcus faecalis (3 LPTs)
Pseudomonas aeruginosa (2 LPTs)
Sphingomonas paucimobilis (3 LPTs)
Alcaligenes xylosoxidans, *Brevundimonas diminuta*, *Brevibacterium casei*, *Citrobacter werkmanii*, *Clostridium* spp., *Corynebacterium jeikeium*, *Enterobacter* spp., *Microbacterium arborescens*, *Micrococcus* spp. (1 LPTs)

LPTs: loading plastic tubes.

4. Discussion

The outbreak of CVC-related bacteremia sustained by *RB* and *BB* in the hemodialyzed patients described in our study suggests the need for a strictly clinical and microbiological surveillance of the patients undergoing hemodialysis and of the system set to produce the reverse osmosis water, including the loading pipes of the ROW delivery line and the loading plastic tubes connecting the hemodialysis consoles.

Our experience can be divided in three main issues: (1) how we managed the infected patients, (2) what we implemented to detect the bacteria in the dialysis's ROW distribution system and (3) what we established to ameliorate the safety of the dialysis's ROW distribution system.

4.1. How We Managed the Infected Patients

The majority of the patients infected by *RB* and *BB* in our study were symptomatic except one who was asymptomatic. The presence of infection in the asymptomatic patient was identified by performing blood cultures on all hemodialyzed patients carrying CVCs. The percentage of the infected patients over the total of the hemodialyzed patients in our dialysis unit was significant, representing 7.4%. The antibiotic treatment was promptly started based on the antimicrobial sensitivity analysis, and it was efficacious in all patients. Nevertheless, considering the possibility of bacteria developing antibiotic resistance, we decided to remove the colonized CVC as a possible reservoir of bacteria, avoiding the risk of re-infection due to proliferation of the bacteria that remained alive because they were included in the CVC's biofilm.

The fact that only patients with CVCs developed infections by *RB* and *BB* led us to hypothesize that, due to *RB* and *BB*'s low virulence, they need to colonize the CVC biofilm, a protected niche that allows them to proliferate and thereby determine the infection. This hypothesis could explain the absence of infection in patients with autogenous or prosthetic arteriovenous fistulas. In fact, it is known that prosthetic arteriovenous graft underwent an epithelization of the inner site in contact with the blood, avoiding the possibility of *RB* and *BB* adhering and proliferating.

4.2. What We Implemented to Detect the Bacteria in the Dialysis's Reverse Osmotic Water Treatment-Distribution System

In the presence of an outbreak of an *RB* and *BB* infection in the dialysis unit, we performed an in-depth search to identify the source of the contamination, planned with the microbiologists and infectious disease specialists of our hospital. First, we checked the potential source of the contamination, including disinfectants, drugs and solutions that were usually administrated during the hemodialysis treatment. According to the national dialysis guidelines, we tested samples of the ROW at different points along the delivery line, including the hemodialyzer consoles. In addition, according to the present literature on the dialysis outbreak of *RB* and *BB* [13,19–23] that demonstrates the presence of *RB* and *BB* in the biofilm detached by sonication from the LPTs that connect the loading pipes of the ROW delivery line to the hemodialysis consoles, we performed a microbiological analysis of the biofilm derived from the LPTs. In fact, a swab of the LPTs or loading pipes is not able to remove a sufficient material from the biofilm to perform a reliable microbiological analysis. We found a significant percentage of LPTs that were positive for *RB* and *BB* (29.7%) and a strict correlation between the positive LPTs and the blood positivity of the patients (Table 3).

4.3. What We Implemented to Ameliorate the Safety of the Dialysis's Reverse Osmotic Water Treatment-Distribution System

We promptly replaced the contaminated LPTs and loading pipes with new ones. Nevertheless, the contamination of the LPTs displayed a locus minoris resistentiae of the ROW delivery system. Thus, we decided in accordance with the microbiologists to modify the ROW delivery line to provide a scheduled chemical and physical disinfection of the

ROW delivery line with the hemodialysis consoles connected, avoiding the risk of new contamination of the LPTs and loading pipes, as described in the Section 2. While waiting for the replacement of the new ROW delivery line, the patients with CVC were dialyzed by using dedicated hemodialyzer consoles connected to a portable water treatment unit.

At present, after more than 30 months from the modification of the whole system for the production and delivery of reverse osmotic water, including the new integrated disinfection protocol, none of the hemodialyzed patients developed infections by *RB* and *BB*.

In summary, our experience suggests that every effort should be made to reduce the number of hemodialyzed patients carrying a CVC irrespectively by age and prognosis due to other comorbidities beyond end-stage kidney disease. A strictly microbiological surveillance protocol approved by nephrologists, infectious disease specialists and microbiologists should be applied in the dialysis unit, and any infection outbreak sustained by unusual bacteria should be promptly investigated by a multidisciplinary team. Based on the site of contamination in the dialysis water treatment and/or the delivery system, the appropriate modifications should be applied, taking into account the characteristics of the pathogen.

In the future, because of the climate modifications, previously neglected bacteria from the *Pseudomonas* group, which belong to the telluric family, could rise in the environment, especially in the water [24,25] and consequently may negatively impact the safety of hemodialyzed patients. Thus, the nephrologists involved in a dialysis unit should be aware of the potential contamination of the water treatment system by unusual bacteria. In addition, nephrologists need to know the appropriate modality for identifying the bacteria species and what they can do to make the needful changes to achieve a safer dialysis unit.

Author Contributions: M.V. and A.R. conceived the report, interpreted the results, and wrote the manuscript. F.B., M.I.M. and R.M. contributed to the interpretation of the data. F.O. and M.M. (Marco Moretti). contributed to the microbiology analysis and the interpretation of the microbiological data. M.M.D., M.T., M.S.F.C., M.V., M.M. (Massimo Marchi), E.M. and A.R. contributed to the interpretation of the data and conceived the microbiological surveillance protocol and the modifications of the dialysis water treatment and delivery system. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was approved by the Research Ethics Committee of the Università Politecnica delle Marche (protocol number 0111661).

Informed Consent Statement: The aim of this study was to identify the possible source of infection in the dialysis unit, especially in the water treatment unit. Microbiological analysis was performed in the dialysis-infected patients according to the standard medical practice, including the choice of antibiotic therapy. The analysis performed to evaluate the correlation between the bacteria identified in the water treatment unit and those identified in the patients was made by anonymizing the patients' personal data. Accordingly, the Informed Consent Statement is not applicable in this study.

Data Availability Statement: The data underlying this article will be shared upon reasonable request to the corresponding author.

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