Monoclonal outbreak of *Ralstonia solanacearum* catheter-related bloodstream infection associated with contaminated package of normal saline solution in a tertiary care hospital

Abstract

**Background/Aim.** *Ralstonia solanacearum* is a very rare cause of infection in humans. There is no described nosocomial outbreak due to *R. solanacearum* until yet. We determined *R. solanacearum* as the source of catheter-related bloodstream infection (CRBSI) outbreak.

**Methods:** This outbreak analysis was carried out in a 1000-bed tertiary care university hospital in Turkey. The outbreak analysis included haematology, oncology, nephrology, gastroenterology wards, emergency department and intensive care units. The first case with *R. solanacearum* CRBSI was detected on May 20, 2019 and *R. solanacearum* was isolated in catheter blood cultures in 34 patients until October 3, 2019

**Results:** Standart outbreak analysis procedures were applied. Culture samples were taken from the fluids administered via catheters. Cultures did not yield any bacteria. As a result of the investigation in storage area, it was found that there were leaks, air bubbles and water drops inside the packaging of saline solutions. *R. solanacearum* was yielded in the cultures obtained from the surface of saline bags and inner side of plastic packings. To validate our hypothesis, a clonal analysis was performed by Arbitrarily Primed - PCR method and the 16S rRNA gene for identification among isolates. All *R. solanacearum* isolates were monoclonal and identical.

**Conclusion:** This is the first outbreak of *R. solanacearum* CRBSI described in hospital settings. The source of the outbreak was a contamination in the surface of saline bag and inner side of plastic packing. Efficacy of an active surveillance system, accurate and rapid conduction of microbiological identification are essential for outbreak management.

**Key words:** *Ralstonia solanacearum*, outbreak, nosocomial, bloodstream infection, contaminated saline
1. Introduction

*Ralstonia* spp are emerging opportunistic pathogens within the nonfermenting gram-negative bacillus group that is present in both hospital and environmental settings and can be found in soil and water resources [1]. It is reported that *Ralstonia* spp infection are increasing in parallel to the increase in the patient population at risk (older populations, neonates, immunosuppressed and critically ill patients) and the implementation of more invasive procedures. *Ralstonia* spp. have been shown to be the causative agent of severe invasive infections, including bacteraemia (especially central venous catheter related), pneumonia, meningitis, osteomyelitis, etc. [1]. It can cause nosocomial outbreaks due to its resistance to disinfection procedures, its ability to live in water sources and low-nutrient conditions [2-4]. *Ralstonia pickettii* has caused contamination of pharmaceutical solutions in various countries, resulting in healthcare infections. *Ralstonia solanacearum* is a soil-borne bacterium causing the widespread disease known as bacterial wilt and it is also the causal agent of Moko disease of banana and brown rot of potato. Although there are a few cases of infection, no described outbreak due to *R. solanacearum* in humans [5].

In this article, we described a monoclonal outbreak of *R. solanacearum* catheter related bloodstream infection (CRBSI). The source of the outbreak was a contamination in the surface of saline bags and inner side of plastic packings. This is the first study which proved *R. solanacearum* as the source of a nosocomial outbreak.

2. Material and Method

2.1a Hospital Settings and outbreak

This outbreak analysis was carried out in a 1000-bed tertiary care university hospital in Turkey. The outbreak analysis included pediatric and adult hematology, oncology, nephrology,
gastroenterology wards, emergency department and intensive care units. These units, which include epidemic analysis, are located in different blocks and floors of the hospital and there is no staff mobility between them. The first case of *Ralstonia solanacearum* catheter related bloodstream infection (CRBSI) was detected on May 20, 2019 and *Ralstonia solanacearum* was isolated in catheter or/and peripheral blood cultures in 34 patients until October 3, 2019.

2.1b Microbiological sampling

After the literature review, case definition and hypothesis were conducted. All adults and pediatric patients with positive blood cultures were included in the study. Microbiological samples were obtained from environmental sources (distilled waters, saline, dextrose solutions, batticons, bedside oxygen jars, humidifiers, heparin solutions, tap water and sinks) beside the blood cultures of patients.

2.2 Microbial Identification

Blood samples were evaluated by an automated blood culture system (Bact/ALERT® 3D, bioMerieux, Durham, NC). Blood culture bottles signaling positive were removed from the Bact/ALERT® 3D system, aliquots were taken for subculture on the sheep blood agar and EMB agar media and then incubated at 37 °C for 24 hours to 48 hours. After the incubation period, identification and susceptibility testing were performed. Isolates grown on solid media were identified to the species level using the MALDI-TOF MS system (Bruker Biotyper; Bruker Daltonics, Bremen, Germany). VITEK-2 antimicrobial susceptibility testing system (bioMerieux, Mercy L’Etoil, France) was used to determine the antibiotic susceptibilities of the isolates.

2.3 Molecular characterization of the isolates
We used arbitrarily primed-polymerase chain reaction (AP-PCR) technique in order to study clonal relationship among the *Ralstonia solanacearum* isolates. M13 universal primers were used for this purpose. AP-PCR was performed according to the method described by Prashanth [6]. The photographs of AP-PCR fingerprints were used for further analysis. To identify clonal strains, improved Sanger sequencing of the 16SrRNA gene technique were used as described by Chen [7]. GenBank database [http://www.ncbi.nlm.nih.gov/16S Ribosomal RNA Sequences [Bacteria and Archaea] were used for nucleotide BLAST analysis. The highest identity was selected as the identified species or genus [7]

3. Results

3.1 Epidemiological surveillance and investigation

*Ralstonia solanacearum* was first isolated from the blood culture [catheter] in a patient in the adult hematology unit on May 20, 2019. No new cases were detected in the same unit within next two weeks. However, blood cultures of seven patients yielded *R. solanacearum* in the 5th week. The infection control team investigated the epidemiological data, including predisposing risk factors of patients with *R. solanacearum* CRBSI. All of the patients (the range of age were between 1 month and 85 years old) were immunocompromised and had a long-term central venous or hickman catheter. Insertion and maintenance care check-lists for central venous catheters and compliance with these check-lists were reviewed. Treatment solutions (saline, dextrose or ringer lactate, etc.) and catheter care supplies were evaluated due to a previously reported possible relationship between *Ralstonia spp* associated bacteremia and contaminated solutions [2,3,8-10] Standart outbreak analysis procedures were applied. Culture samples were taken from the fluids administered via catheters. Cultures did not yield any bacteria. As a result of the investigation in storage area, it was found that there were leaks, air bubbles and water drops inside the packaging of saline solutions. *R. solanacearum* was yielded in the cultures obtained from the surface of saline bags and inner side of plastic packings.
Since the saline solutions were also used throughout the hospital, the surveillance of Ralstonia spp was extended to the hospitalwide. As a result of this surveillance, within the past 4 weeks, R. solanacearum catheter-related bacteremia was detected in Adult hematology unit (10 cases), pediatric hematology (8 cases), nephrology (6 cases), intensive care unit (4 case) and oncology unit (2 cases) and others (4 cases) (Figure 1. Distribution of cases by weeks).

It was thought that these contaminated packages caused to R. solanacearum catheter colonization or bacteremia especially in immunocompromised patients via hands of healthcare workers.

All the saline solutions were withdrawed from the units and sent to manufacturer. It is reported to the health authorities. To validate our hypothesis, a clonal analysis was performed by Arbitrarily Primed - PCR methods and Sanger Sequencing of the 16S rRNA Gene for Identification among R. solanacearum isolates. All R. solanacearum isolates were monoclonal and identical. (Figure 2. AP-PCR profiles of Ralstonia solanacearum)

Antibiotic susceptibility test results revealed that all patients and environmental strains were susceptible to quinolones, penicillins, 3rd generation cephalosporins, carbapenems, trimethoprim- sulfamethoxazole, but resistant to aminoglycosides.

The outbreak, which has been proven to be associated with contaminated saline solution packs, is controlled by collecting all possible contaminated saline solutions distributed in units. No new cases were detected in the next four months period since the last case occurred. All patients were fully recovered by catheter removal and antibiotic treatment.

4. Discussion

Ralstonia solanacearum infections are not common in clinical settings, although a few reported cases have been reported with R. pickettii. In this outbreak analysis, a total of 34 cases of R. solanacearum related bacteremia were reported. A monoclonal outbreak resulting from
contaminated saline solution packs was identified which affecting patients in different wards. This is the first described outbreak of *R. solanacearum* catheter-related bloodstream infection in hospital settings.

Although clinical infections with *Ralstonia* species are rare, disease progression to severity tends to be more serious once individuals are exposed. A large oncology hospital in Rome recently reported *R. mannitolilytica* infections among 12 oncology outpatients attending a day ward [11]. Liu et al., similarly reported three cases of bloodstream infections with *R. mannitolilytica* [12]. There is no report about the outbreak of *R. solanacearum* in humans until yet. Shi et al reported a case of hemophagocytic lymphohistiocytosis secondary to *Ralstonia solanacearum* infection [5]. *Ralstonia spp* was considered an unusual outbreak agent when compared with other outbreak agents. *Ralstonia spp* with low virulence is considered as a non-major nosocomial agent [3]. The development of an outbreak of bacteremia by this agent was made possible by direct access of the microorganism to the parenteral treatment area with contaminated saline packs.

The first nosocomial outbreak of *R. pickettii* previously called *Pseudomonas pickettii*, was an outbreak of contaminated chlorhexidine-induced bacteremia in 1983 [13]. Since then, nosocomial outbreaks associated with *R. pickettii* (*Pseudomonas pickettii* or *Burkholderia pickettii*) have been reported in different years and in different patient groups [2,3,8,9,14]. When these outbreaks are evaluated, it is seen that contaminated solutions, drugs and disinfectants play an important role in *R. pickettii* related outbreaks [3,10,15-17]. *R. pickettii* isolates can be found in hospitals and industrial production areas, especially in water resources and can form a biofilm [4,18-20]. These literature data suggest that saline contamination, which may cause hospital outbreaks, may have developed during industrial production. In our outbreak, contamination was detected in saline solution packs, whereas intrinsic contamination
of saline solution was not detected. This situation is thought to cause the number of cases to be limited.

In our outbreak, although the possible contaminated saline solution has been widely used throughout the hospital, the number of cases has been limited to certain units. This can be explained by the fact that bacteria with low virulence can only cause bacteremia in the susceptible host. Patients with hematologic malignancies, intensive care patients, patients with central venous catheters, transplant recipients and newborns are reported to be susceptible to *Ralstonia* spp. associated infections [1,2,21,22]. *R. pickettii*-related infections have also been reported in patients with diabetes, cystic fibrosis, chronic liver and kidney disease [1,4,23-25]. There was an underlying hematologic malignant in approximately half of our cases. In these patients, malignancy or chemotherapy-related immune suppression was thought to facilitate the occurrence of *R. pickettii* related bacteremia. Previously outbreaks of *Ralstonia* spp-related bacteremia in patients with hematologic malignancies have been described in the literature [2,21,22]. Also, almost all of the patients we evaluated in the outbreak analysis had central venous or port catheters. Therefore, in addition to immune suppression and comorbid diseases, CVC are also seen as an important risk factor for *Ralstonia* spp related bacteremia.

Ensuring outbreak control is possible with the continuation of effective surveillance, continuity of microbiological data analysis, and rapid and effective interventions. *R. solanacearum* related bacteremia cases were detected at certain intervals within approximately 5 months. The epidemic was finally diagnosed 10 weeks after the first case due to clustering of cases in the adult hematology department. After the outbreak definition, source identification, identification of new cases and planning of interventions to end the outbreak were carried out within approximately one month. However, the slowdown in elimination by collecting possible contaminated saline solutions spread throughout the hospital has led to the prolongation of the process between intervention and epidemic outcome.
It is stated that MALDI-TOF method improves infection control applications by increasing the bacterial identification rate and makes a positive contribution to public health [26]. This contribution is valid in the outbreak analysis and control process. In our outbreaks, the use of the MALDI-TOF MS method for microbiological identification of *R. solanacearum* isolates facilitated and accelerated the identification of new cases and detection of possible sources.

Strength side of our article is proving the identicality by molecular methods between clinical and environmental *Ralstonia* strains. In addition, using MALDI-TOF in identifying *Ralstonia* strains is another strength side. There are a few limitations in our study. Outbreak analysis is done only in wards which *Ralstonia* spp isolated from clinical samples. Due to retrospective aspects of the study, we could not make further analysis in other wards. Detected number of cases might be lower than actual number of cases.

In conclusion, the number of immunosuppressed patients and the increase in invasive procedures increase the clinical effect and importance of low virulence pathogens such as *R. solanacearum*. This is the first outbreak of *R. solanacearum* CRBSI described in hospital settings. The source of the outbreak was a contamination in the surface of saline bag and inner side of plastic packing. Efficacy of an active surveillance system, accurate and rapid conduction of microbiological identification are essential for outbreak management. Accurate identification of the outbreak agent facilitates the identification and control of possible outbreak sources with the evaluation of previous data.

**Conflict of Interest:** No conflict of interest was declared by the authors.

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Figure 1. Distribution of cases by weeks
Figure 2. AP-PCR profiles of *Ralstonia solanacearum*