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Major Article

Ralstonia mannitolilytica infection in a tertiary care center: An outbreak report

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Key Words:
Nosocomial infection
Ralstonia
Sodium chloride

Background: This paper describes an outbreak of *Ralstonia mannitolilytica* infection declared at our facility between January-2021 and January-2022.
Methods: To identify the source of the outbreak, we applied widespread epidemiological investigations and infection control measures, including device isolation, environmental sampling, and pulsed-field gel electrophoresis typing.
Results: Thirty-six cases of *R mannitolilytica* infection were identified, mostly adults (78%) and males (75%). Initially, neurological procedures were a common risk factor among cases, leading to sampling of related environmental settings. Cases with other medical procedures started to be reported. The pulsed-field gel electrophoresis results showed most *R mannitolilytica* isolates were indistinguishable, which expanded our investigation to all hospital areas. The outbreak source was traced to a specific lot of contaminated sodium chloride solution, which was recalled from all hospital units. The findings were reported to the Saudi Food and Drug Authority to communicate with the manufacturer and other health care organizations involved. No new cases of *R mannitolilytica* were identified thereafter.
Conclusions: It is essential to regularly monitor the compliance of manufacturers and suppliers with regulations related to the safety of solutions administered in medical practice. An extended incubation period might be considered to enhance the identification of *R. mannitolilytica* in future outbreaks.
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BACKGROUND

Ralstonia mannitolilytica (R mannitolilytica) is a nonfermenting gram-negative bacteria that can survive in extreme environmental conditions.[1,2] Until recently, the only *Ralstonia* species thought to be responsible for nosocomial infections was *Ralstonia pickettii*. However, other novel species of clinical importance, including *R mannitolilytica,* have been isolated over the past decades.[3,4] Due to the relatively low prevalence and virulence of *Ralstonia* spp, they have not formed a part of routine microbiological investigations in hospital settings. This has resulted in marked underdetection and undertreatment of *Ralstonia* species infections, which might have been managed as infections caused by other pathogens or as infections/fever/sepsis of unknown origin.[4-6]

R mannitolilytica has been responsible for several hospital outbreaks.[2] In earlier studies, most *R mannitolilytica* isolates were linked to contamination of a distilled-water source, leading to clinical infections such as bacteremia, bacteriuria, and wound infections.[7-10]

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Other outbreak reports followed; however, not all investigations were successful. Some were challenging and were concluded without definitive identification of the source due to microbiological challenges or untraceable sources used in practice.[11-16] This study was conducted as part of a clinical and epidemiologic investigation of a challenging outbreak of *R mannitolilytica* infections at a tertiary care center, which involved various hospital units. The outbreak investigation is reported in accordance with the Outbreak Reports and Intervention studies Of Nosocomial infection (ORION) statement.

METHODS

King Faisal Specialist Hospital and Research Centre is a tertiary care hospital in Riyadh, Saudi Arabia that offers a wide variety of health care services, including oncology, organ transplants, and cardiac surgeries, with approximately 960 beds. The Infection Control and Hospital Epidemiology Department at our institution monitors all hospital infections through an active surveillance system. Any positive organism detected by the local laboratory is flagged in the system for further investigation by infection control practitioners. The outbreak started when the first case of *R mannitolilytica* was detected on January 2021 and continued until the source was identified in January 2022. A case is confirmed if *R mannitolilytica* is detected microbiologically from sterile or non-sterile samples via accepted means of culturing specimens. Ethics approval was obtained from the Research Ethics Committee at King Faisal Specialist Hospital and Research Center.

Infection control measures

The infection control team initiated an epidemiologic investigation of the *R mannitolilytica* outbreak and carried out 4 phases of infection control interventions after identifying the first case. Data on cases were collected by a trained epidemiologist and analyzed to identify any overlap between cases that might reveal the source of infection. Infection-related outcomes were reported, including the number of new cases for each month, antimicrobial susceptibility, and 30-day all-cause mortality.

Microbiological methods

Clinical samples

Clinical samples were processed in the microbiology laboratory based on the infection site and specimen source. Growth from suspected *R mannitolilytica* colonies was identified using VITEK MS MALDI-TOF (Matrix Assisted Laser Desorption Ionization Time-of-Flight, bioMérieux, Inc).

Antimicrobial susceptibility testing was performed using an automated system, VITEK II (bioMérieux, Inc), and/or manual ETEST strips. Susceptibility testing was interpreted with reference to the American Clinical and Laboratory Standards Institute (CLSI M100, 31st edition). [17]

Environmental samples

Initially, water samples were received in the laboratory and screened for the presence of *R mannitolilytica.* Upon receipt, each sample was inoculated onto blood and MacConky agars and incubated aerobically at 37 °C for 48 hours. Identification was performed by VITEK MS MALDI-TOF (bioMérieux, Inc).

In some cases, *R mannitolilytica* may be present in water samples below culturable levels, which might lead to the organism's inability to grow on regular culture plates. For that reason, the environmental culture procedure was slightly modified. Instead of delivering the sample to the laboratory in sterile containers, the water samples were delivered in blood culture bottles which were incubated in the BACT/ALERT VIRTUO system for 5 days until no growth or any microorganism was detected by the special indicators in the system. Subsequently, when the system flagged the blood culture bottle as positive, it was retrieved from the BACT/ALERT system for further processing. A few drops of the positive blood culture were aseptically placed on a slide to perform a gram stain. The sample was inoculated onto blood agar, MacConkey agar, and chocolate agar plates. Each plate was streaked with 1 drop from the positive blood culture bottle. After an aerobic overnight incubation at 37 °C, colonies were validated for purity and similarity to *Ralstonia* spp, and bacterial identification was performed from the bacterial colonies using VITEK MS MALDI-TOF (bioMérieux, Inc). The identification result was validated and verified before being reported to the laboratory integration system.

Pulsed-field gel electrophoresis

Pulsed-field gel electrophoresis (PFGE) methods are explained in detail in the Supplementary material. The dendrogram was generated using the unweighted pair-group method with arithmetic average, with a 4% dice coefficient and 2% tolerance. The criteria for interpreting PFGE patterns recommended by Tenover et al[18] were used as a reference.

RESULTS

Table 1 provides a summary of cases reported in the *R mannitolilytica* outbreak. Thirty-six *R mannitolilytica* isolates were identified between January 2021 and January 2022. The mean age of the affected patients was 41 years. The majority of cases were male (75%) and adult (78%). The outbreak was not exclusive to 1 U; however, most cases were associated with clinical procedures, mostly craniotomy, ureterostomy, and bronchoscopy. *R mannitolilytica* was primarily isolated from cerebrospinal fluid (n = 9) and bronchoalveolar lavage (n = 6). Most of the affected patients (n = 22) were symptomatic, supporting the notion of a true outbreak, with fever being the most reported symptom. The identified infectious disease clinical diagnoses included meningitis (n = 9), bacteremia (n = 6), intra-abdominal infection (n = 4), and others. Of the 36 cases identified, 7 patients died within 30 days despite receiving multiple courses of therapy. The 30-day all-cause mortality rate reported in this outbreak was 19 per 100 patients.

Table 1
Summary of cases identified in *Ralstonia mannitolilytica* outbreak in Saudi Arabia (n = 36)

Characteristic	n (%)
Age (mean ± SD)	41 ± 24
Pediatrics	8 (22)
Adults	28 (78)
Sex (male)	27 (75)
Clinical procedure	
Craniotomy	11 (31)
Exploratory laparotomy	6 (17)
Ureteroscopy	3 (8)
Bronchoscopy	7 (19)
Insertion of central venous catheter	3 (8)
Other	7 (19)
Multidrug resistant infection	23 (64)
30-d mortality	7 (19)

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Table 2
Antimicrobial resistance pattern in 36 *Ralstonia mannitolilytica* isolates

Antibiotics	Susceptible	Intermediate	Resistant
Piperacillin/Tazobactam	-	3/33 (9)	30/33 (91)
Ceftazidime	1/32 (3)	-	31/32 (97)
Gentamicin	14/36 (39)	-	22/36 (61)
Tobramycin	13/25 (52)	-	12/25 (48)
Amikacin	1/22 (5)	-	21/22 (95)
Cefepime	19/33 (58)	7/33 (21)	7/33 (21)
Imipenem	1/35 (3)	9/32 (28)	25/35 (71)
Meropenem	-	-	36/36 (100)
Ciprofloxacin	30/30 (100)		-
Minocycline	9/10 (90)	1/10 (10)	-
Trimethoprim/ Sulfamethoxazole	23/24 (96)		1/24 (4)
Ampicillin/Sulbactam		4/4 (100)	
Cefotaxime		4/5 (80)	1/5 (10)
Ceftazidime-Avibactam			4/4 (100)
Ceftolozane/Tazobactam			4/4 (100)
Colisitn			1/1 (100)
Tetracycline	1/1 (100)		
Doxycycline	1/1 (100)		
Chloramphenicol			1/1 (100)
Levofloxacin	1/1 (100)		

NOTE. The denominator refers to the number tested.

Initially, patients were primarily treated with meropenem, but antibiotics were later tailored based on sensitivity results. Multidrug resistant *R mannitolilytica* was detected in 64% of the cases (Supplementary Table S1 provides a detailed line list of identified cases). The majority of isolates were resistant to meropenem (100%), ceftazidime (97%), amikacin (95%), piperacillin/tazobactam (91%), imipenem (71%), and gentamicin (61%) and were susceptible to ciprofloxacin (100) and trimethoprim/sulfamethoxazole (96). Table 2 provides more details on antimicrobial susceptibility.

Outbreak investigation

Our outbreak investigation and interventions could be summarized in 4 phases (Supplementary Table S2).

Phase 1: Detection of the outbreak

This phase involved identifying the first 4 cases of *R mannitolilytica* infections over 1 week in January 2021. In response to the detected cases, which occurred within a short period (< 1 week between the first and fourth cases), the problem was declared an outbreak and was immediately announced facility-wide. Three of the detected cases were in patients who had undergone neurosurgical procedures and had been receiving postoperative care; therefore, a link between the outbreak and neurosurgeries was considered. Actions at this stage included close monitoring of all incident cases through the infection control surveillance system, regular communication with the microbiology and neurosurgery departments, maintaining a log of all cases, raising awareness among all team members, conducting a detailed review of all cases, and saving all bacterial isolates for PFGE. The initial case definition was any admitted patient with a positive culture for *R mannitolilytica* from sterile or nonsterile specimens. Evidence from previous outbreaks suggests that contaminated solutions, such as water for injection, saline solutions, disinfectants, and antiseptics, can be a source.[7-10] Therefore, our investigation focused on culturing any solutions or equipment that contained solutions (Supplementary Table S3). Unfortunately, all cultures of saline solutions, antiseptics, and devices used in operating rooms were negative. The PFGE results showed that the 4 initial bacterial isolates were indistinguishable, supporting the hypothesis of a point source for the outbreak (Fig. 1).

Phase 2: Confirmation of the outbreak

To exclude the possibility of this outbreak being part of a national or international outbreak, the Saudi Ministry of Health and the USA Centers for Disease Control and Prevention were contacted. Both organizations confirmed that no similar occurrences had been reported or were under investigation.

The laboratory was instructed to store all samples positive for *R mannitolilytica* by freezing them in 30% glycerol tryptic soya broth at −80 °C to enable further testing for typing and surveillance. During this phase, a case-control study was performed. Control cases were selected from patients admitted during the same period and matched for age, sex, and ward. Various variables were explored, including age, sex, operating room suites, surgeons, anesthetists, assistant surgeons, circulating nurses, surgical procedures, material and devices (Cavitronic Ultrasonic Surgical Aspirator) used, and solutions used. The study aimed to identify common risk factors for *R mannitolilytica* infection among the initially confirmed cases. This revealed that a procedure, primarily a neurological procedure, was the only shared risk factor. During this time, additional positive cases of *R mannitolilytica* infection mainly occurred among patients who had undergone neurosurgery, and this further affirmed a potential source within this setting. The case-control study also identified that most patients who tested positive for *R mannitolilytica* infection had ventriculoperitoneal shunts. An internal review of these cases revealed that the shunts were immersed in saline prior to insertion. Based on this information, a decision was made to postpone ventriculoperitoneal shunt procedures except in emergencies. Despite this action, the number of cases continued to rise, reaching a total of 19 by the end of August 2021 (Fig. 2). Some of those cases were outside the neurosurgery department and included patients with a history of urological procedures, bronchoscopy, and endoscopy. This shifted the thoughts of dealing with a localized problem to a hospital-wide outbreak. Communication with the logistics department was initiated to determine if any products, devices, or consumables, including irrigation solutions, had been introduced or replaced prior to the outbreak. No such changes were noted or reported.

Phase 3: Recognition of the outbreak source

Due to the inability to identify the source of the *R mannitolilytica* outbreak, several hypotheses were considered, including air conditioning and water sources as potential origins of the outbreak. Specimens from air conditioning units in the operating rooms and intensive care units were collected and cultured, but the results were negative. At this stage, the culture method was modified for fastidious culture by culturing fluid specimens in blood culture bottles. A more extensive collection of samples was undertaken from the operating rooms, including fluids, machines, devices, and other equipments used during neurosurgery. Furthermore, samples from sterile solutions used throughout the hospital were collected and cultured, including specimens from sterile saline used to flush the endoscope. The samples from the endoscopy unit (from company X, a local product) tested positive for *R mannitolilytica*, and these isolates were identical to those isolated from patients. Samples from new bottles of the saline solution with the same lot number were cultured and tested positive with the same *R mannitolilytica* (Fig. 1). Random samples taken from the same fluid type but different lot numbers returned negative results, confirming that this specific

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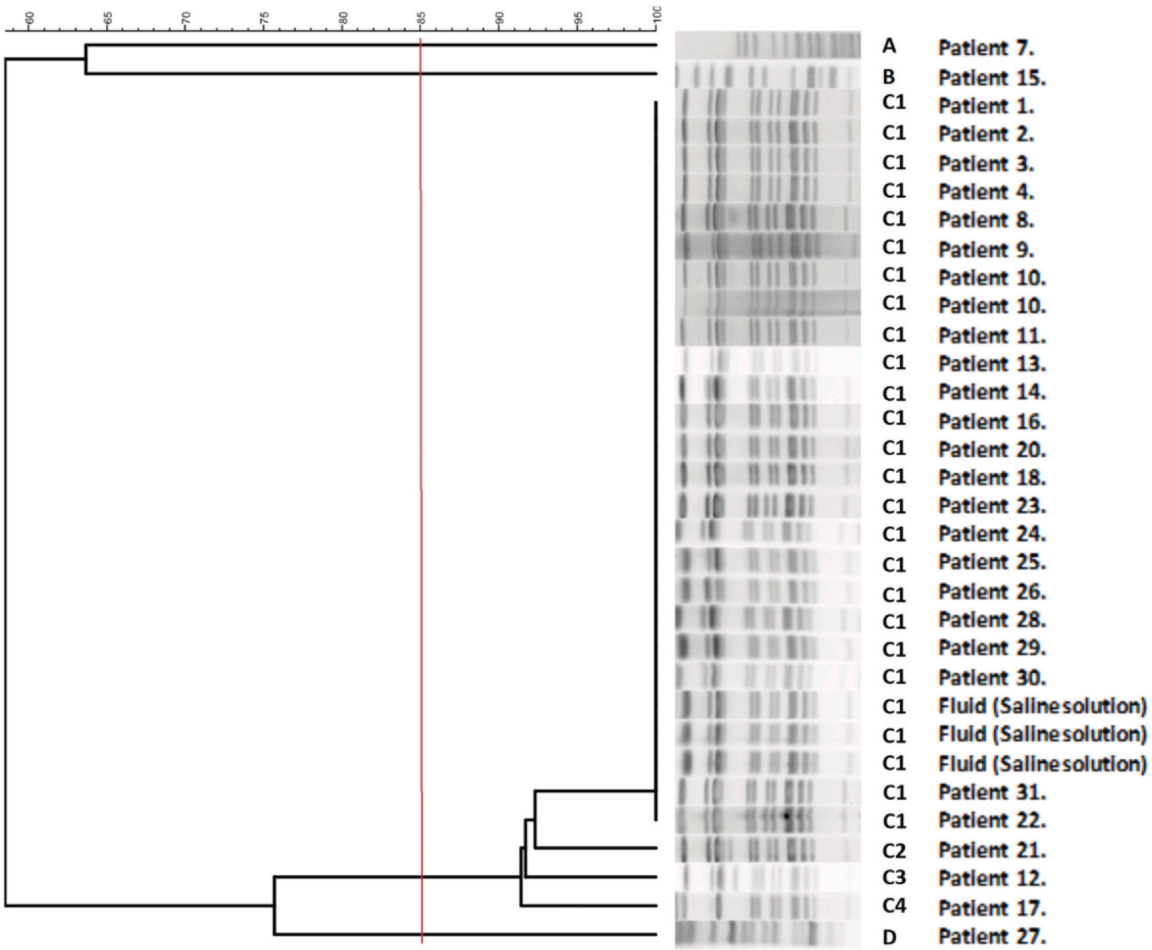

Fig. 1. cDendrogram based on pulsed-field gel electrophoresis of DNA macrorestriction fragments of 32 isolates of *Ralstonia mannitolilytica*. The dendrogram is shown on the left with a percent homology on the top, with a cut-off value of 85%. Patients number is equivalent to the number indicated in Supplementary Table S1. Generated pulsed-field gel electrophoresis patterns of the isolates displayed a genetic similarity coefficient that ranged between 58.47% and 100%. Banding pattern interpretation was conducted as previously recommended.[18] Indistinguishable patterns were given same name types, and related patterns were allotted same letters in their typed names. Eighty-five percent interlinkage homology between patterns was used to determine the clusters and define a close genetic relationship between strains. Based on the dendrogram generated for combined gel images, 4 major pulsotypes were exhibited from 32 isolates. Cluster C was the largest, comprised of 29 members, while the remaining were singletons (A, B, and D). Cluster C consisted of 4 *R mannitolilytica* strains (C1, C2, C3, and C4). Subcluster C1 had 26 isolates of clonally indistinguishable members, no fragment differences were observed among this group. C2, C3, and C4 subclusters (each with 1 isolate) were closely related to C1, with 2 to 3 band differences. Similarity values of 92.31% for C2 and 91.67% for both C3 and C4 subclusters compared with C1 were observed. For the unrelated isolates, less consonance was observed, with 60.87% for A and B and 72.00% for the D cluster compared to C1.

batch (lot number) of saline solution was the source of the outbreak. Further positive cases in non-neurosurgical departments supported the likelihood that the infected batch of saline was the origin due to its shared use across surgical departments.

Phase 4: Final corrective measures and cessation of outbreak

The identified contaminated saline batch product from company X, a local product, was immediately recalled from different hospital areas. Hospital management was updated about this progress, and various departments were advised not to use the product. The infected batch was communicated to the manufacturer, local public health authorities, the Saudi Food and Drug Authority, and the Saudi Ministry of Health to initiate a wider investigation and inform other health care organizations. As a result of our hospital investigations, definitive infection control measures were implemented in line with infection prevention guidelines.[19] Ongoing surveillance of *R mannitolilytica* infections was maintained. The reporting of and elimination of the contaminated saline batch from company X were closely monitored, and ongoing culturing of newly opened fluids in neurosurgery and other treatment units continued. Our department reiterated the quality control policy for endoscopes, which mandates monthly microbiologic surveillance testing to verify the cleaning process and the integrity of internal channels, as the source of the outbreak was identified in the endoscopy unit. No new cases of *R mannitolilytica* were identified thereafter. The outbreak concluded by the end of January 2022 with a total of 36 cases.

DISCUSSION

This hospital investigation into an outbreak of 36 cases of *R mannitolilytica* infections revealed that a single contaminated aqueous source was responsible for the incidents and subsequent cases. The contaminated source was a single batch of sodium chloride solution used in a wide range of surgical and pharmaceutical procedures. This conclusion was supported by PFGE results that

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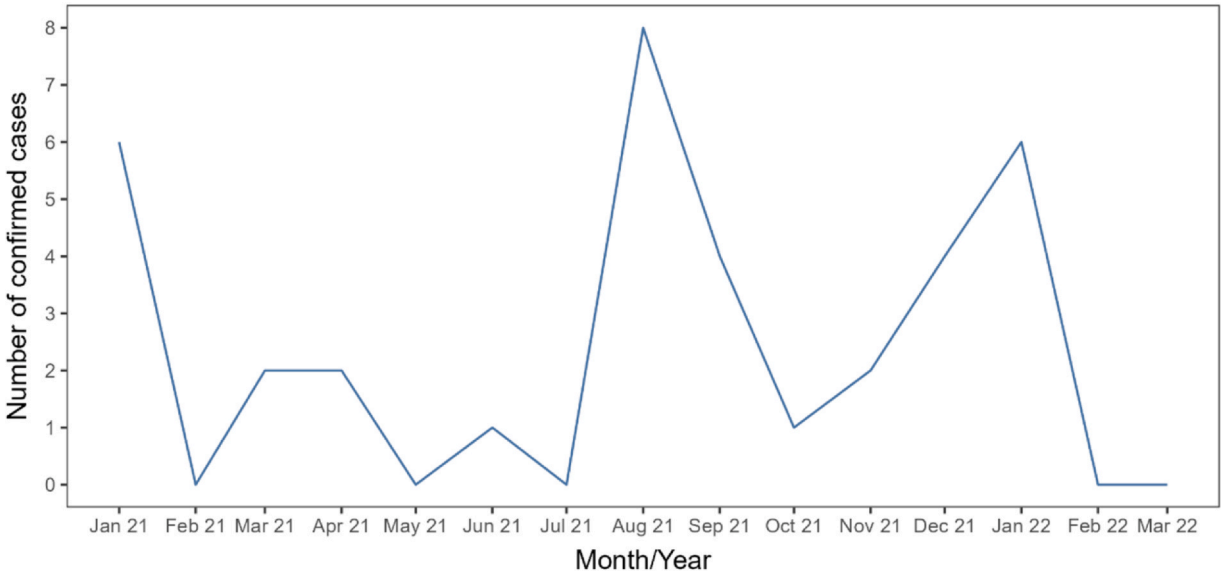

Fig. 2. Confirmed *Ralstonia mannitolilytica* cases in a local hospital in Saudi Arabia.

showed genetic similarities between *R mannitolilytica* identified in the contaminated saline and the clinical cases.

The findings of this investigation are consistent with previous outbreaks.[13,15,20] The increasing emergence of *R mannitolilytica* within the acute hospital environment is linked to its ability to resist and survive extreme environmental conditions.[2] A common observation across the literature on *R mannitolilytica* outbreaks is that a large proportion of affected patients have severe illnesses or immunosuppression status due to various diseases, medications, or stress related to surgery or chronic illness.[20-22] *R mannitolilytica* has been identified as an opportunistic pathogen that takes advantage of the host's underlying conditions. Patients with impaired defense mechanisms are at particular risk for infections. Our investigation found that a large group of affected patients had primary diagnoses of brain tumors, liver disease, and renal impairment/disease. Unfortunately, these patients were more susceptible to poor outcomes. Another potential risk factor for *R mannitolilytica* infection includes pediatric age status; notably, 22% of cases in this study occurred in children.[23]

Although the antimicrobial susceptibility profile of *R mannitolilytica* varies across the literature, most studies report resistance to aminoglycosides and carbapenems, which agrees with our results.[12,24,25] Multidrug resistance was also common among *R mannitolilytica* bacteria isolated in our outbreak (64%). Infections with these organisms complicate the hospital course for infected patients and can progress to sepsis and death. The choice of empirical therapy for sepsis caused by *R mannitolilytica* can be challenging due to its potential resistance to broad-spectrum antibiotics. Knowledge gained from cumulative antimicrobial susceptibility data is essential for improving the treatment plan for affected patients.

Overall, this appears to be the first published outbreak of *R mannitolilytica* in Saudi Arabia, as previous research only reported the organism's presence in indoor air spaces of schools.[26] The morbidity and mortality linked to this outbreak cannot be directly attributed to *R mannitolilytica* infection, as patients often had severe acute or chronic comorbid health problems that may have increased their risk of adverse outcomes. Yet, our evidence highlights the growing impact of atypical nosocomial infections in acute hospital settings, which contributes to poor outcomes and avoidable deaths both locally and worldwide. These findings emphasize the need for clinicians to become more aware of these opportunistic pathogens, which could aid in the proper management of their incidence.

CONCLUSIONS

Due to the growing number of outbreaks of *R mannitolilytica* linked to contaminated fluids, it is highly recommended to update our policies and regulations that pertain to the microbiological safety of aqueous solutions and devices used in clinical practice. It is also essential that sufficient equipment sterilization is maintained and microbiological techniques are enhanced to attain an accurate diagnosis of the underlying organism. Overall, these measures may help to prevent incidents or recurrent outbreaks in health care settings and reduce associated morbidity and mortality.

APPENDIX A. SUPPLEMENTARY DATA

Supplementary data related to this article can be found at doi:10.1016/j.ajic.2024.09.019.

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