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# *Achromobacter spp.* bacteremia outbreak related to contaminated furosemide ampoules

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## SUMMARY

Nosocomial outbreaks related to medication contamination are reported world-wide. A sudden increase in cases of *Achromobacter* spp. bacteremia led to an outbreak investigation in our setting. Line listing and environmental sampling led to identification of contaminated furosemide ampoules as the source. Molecular identification helped in species identification and in this outbreak more than one species was identified. Prompt withdrawal of the contaminated batch of ampoules curtailed the outbreak.

*Key words: Achromobacter,* nosocomial infections, bacteremia, medication contamination, outbreak.

## INTRODUCTION

*Achromobacter xylosoxidans* is an aerobic, motile, oxidase-positive, non-fermenting Gram-negative rod widely distributed in the environment. In 1971 Yabuuchi and Ohyama first described this pathogen from purulent ear discharge in patients with chronic otitis media. They later proposed the name *A. xylosoxidans* which encompasses 2 different subspecies *A. xylosoxidans subsp. xylosoxidans* and *A. xylosoxidans subsp. denitrificans* [1]. *Achromobacter* is not a usual component of human endogenous flora and is commonly found in water sources, such as well water, hospital faucets, swimming pools, and moist soil. They can contaminate intravenous (IV) fluids, disinfectants and water in humidifiers [2]. Apart from primary bacteraemia, other clinical presentations include meningitis, urinary tract infections, abscesses, osteomyelitis, corneal ulcers, prosthetic valve endocarditis, peritonitis, pneumonia and wound infection. Majority of cases reported in literature have been associated with healthcare-associated infections and in patients with haematological malignancy and those who are immunosuppressed [3, 4]. Bacteremia outbreak in an ICU setting is a concern warranting urgent investigation, since nosocomial bacteremias are associated with increased morbidity, mortality, length of hospital stay and cost. The aim of the present paper is to describe an outbreak of *Achromobacter* bacteremia observed in our hospital. Here we describe the efforts and procedures adopted to identify the source of the infection. We have also reviewed the literature regarding the epidemiology and previous outbreaks due to *Achromobacter* spp. causing invasive nosocomial infections.

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## OUTBREAK DESCRIPTION

In April 2020, increasing number of bacteremia due to *Achromobacter* spp was noted, particularly

in our ICUs. A trend of *Achromobacter* bacteremia over the past 2 years was analysed. There were total three cases of *Achromobacter* bacteremia in 2019. Between March and mid- April 2020, there were six cases *Achromobacter* bacteremia in cardiac and medical ICUs, which was above the usual occurrence, suggesting an outbreak. Soon an outbreak investigation team was formed which included hospital infection control team, infectious diseases specialists, microbiologist, lead in clinical services and nurse lead, to help identify all cases of *Achromobacter* bacteremia and collect the clinical details both retrospectively and prospectively till the outbreak ended. Case was defined as one in whom one or more blood cultures grew *Achromobacter* spp. In the latter half of April and in May, six more cases were identified taking the total number of Achromobacter bacteremia to twelve. Line listing of the cases was done.

*Patient characteristics*

There were 12 patients with *Achromobacter* bacteremia between March and May 2020; the details are shown in Table 1. Eleven of them were in cardiac or medical ICUs while one was in the ward. Majority were males (10 out of 12) and age ranged from 30 to 81. All patients had primary bacteraemia. Duration from admission till blood culture positivity ranged from 1 to 16 days; 7 out of 12 had bacteraemia within 3 days of admission. Only 6 underwent some form of interventional procedure, either vascular or surgical and were not uniform; 5 had hemodialysis. In those who had hemodialysis, blood cultures which grew *Achromobacter* spp. were drawn before the initiation of dialysis. Six patients had central venous catheter and arterial line; the rest had peripheral vascular catheter only. All of them received at least two or three IV medications like inotropes, diuretics, proton pump inhibitors, heparin, antibiotics, fluids and saline flush. Intravenous diuretic, furosemide was used in all patients for pulmonary edema, either as bolus or infusion.

Blood cultures were done by inoculating in the BacT/ALERT® FA aerobic and FN anaerobic bottles and incubated in an automated system (BacT/ALERT 3D, bioMerieux, France) for five days. Bacterial identification and antimicrobial susceptibility testing were done using Vitek2, according to the Clinical Laboratory Standards Institute (CLSI) guidelines. Selected samples were further analysed to confirm species by molecular techniques by VITEK MS MALDITOF (bioMérieux) and whole genome sequencing (WGS) of DNA done on Illumina HiSeqX by Next Generation Sequencing technique. Blood culture from 10 patients grew either one of the species- *A. denitrificans* and *A. xylosoxidans*; both the species were identified in 2 patients. All isolates were resistant to quinolones, aminoglycosides and cotrimoxazole but were susceptible to ceftazidime, piperacillin tazobactam and carbapenem. All were treated with appropriate antibiotics according to the susceptibility results, and majority received piperacillin tazobactam. Four patients expired; all four had underlying heart failure.

*Possible sources of Achromobacter infection*

Further epidemiological details were collected based on the literature search of common sources of Achromobacter outbreak. Literature search was done with regard to the epidemiology and previous outbreaks due to *Achromobacter* spp. using PubMed Central and key words Achromobacter, outbreak and bacteremia. Subsequent cross references of important papers were done. *Achromobacter* spp. has been implicated in outbreaks of nosocomial infections associated with contaminated solutions including chlorhexidine atomizer, deionized water, intravenous preparations and contamination during diversion of morphine infusion [5-8]. With this information possible sources that were considered for the current bacteremia outbreak included contaminated chlorhexidine solution, water used in hemodialysis system, common procedures, personnel involved, instruments and medical supplies used and parenteral medications administered. Locations in the hospital where the outbreak occurred was also looked at and included in the line listing. During this period, visits were made to observe procedures, specifically regarding preparation and administration of intravenous medications and interview the area staff about the level of knowledge and training in the procedures they did.

Clinical variables looked at and noted were age, sex, hospitalization dates, location of admission, underlying diagnosis, dates when blood culture grew *Achromobacter* spp, intravenous medications received, presence and type of central venous catheter (CVC), symptoms, types of invasive procedures, and sample from which *Achromobacter* spp. was isolated.

*Investigation for environmental contamination*
Sample for culture was collected from chlorhexidine-alcohol skin disinfectant, de-ionised water used in humidifier and from work surfaces. Environmental samples were inoculated into MacConkey purple broth, and incubated at 37°C for up to 48 hrs. Turbidity/colour change to yellow were indicative of bacterial growth. Such samples were sub-cultured to blood agar and MacConkey agar, and the growth obtained was identified using Vitek 2 identification system. Samples from commonly used IV preparations were noted from the line listing and sent for cultures. Samples of IV medication solution were inoculated in thioglycolate broth, incubated for 18-24 hours at 37°C, and observed for turbidity. Samples showing turbidity were sub-cultured to blood agar and MacConkey agar, and the growth obtained was identified using Vitek 2 identification system.

## RESULTS

Chlorhexidine-alcohol skin disinfectant used for hand disinfection, as well as the one used for pre-procedure skin disinfection from the affected unit were found to be sterile. The de-ionised water used in humidifier and dialysis water source for the affected area yielded no growth. Neither did the environmental samples collected from work surfaces.
All IV medications that the patients received prior to taking blood culture were listed out. Majority of the patients had IV drugs like inotropes, pantoprazole, heparin, saline infusion, saline flush, but the one medicine that all patients uniformly received was furosemide (Table 1). Furthermore, data was collected from pharmacy regarding details of batches and the brands of furosemide which were despatched to these patients during this period. All of them had received one particular brand and batch of furosemide.
Samples of available 2 brands of furosemide (Brand 1 and 2), pantoprazole, heparin, single use saline flush, and saline bottles were given for culture. Brand 2 of furosemide, pantoprazole, heparin, single use saline flush and saline did not yield growth. Culture of furosemide Brand 1, belonging to the same batch of furosemide which were administered to these patients yielded growth of *Achromobacter* spp. Four more ampoules of the

**Table 1** - Details of those who had Achromobacter bacteremia during the outbreak.

| *Case* | *Procedure done* | *Day(s) from admission to positive blood culture sampling* | *IV Medications administered prior to blood culture draw* | *Achromobacter spp. isolated* |
|---|---|---|---|---|
| 1 | Nil | 2 | Saline, **furosemide**, enoxaparin, pantoprazole | *A. denitrificans* |
| 2 | Nil | 1 | **Furosemide**, pantoprazole, calcium gluconate | *A. denitrificans* |
| 3 | CAG and DVR | 9 | **Furosemide** | *A. denitrificans*<br>*A. xylosoxidans* |
| 4 | CAG | 3 | Pantoprazole, **furosemide** | *A. denitrificans* |
| 5 | PTCA | 7 | Pantoprazole, heparin, **furosemide** | *A. denitrificans* |
| 6 | Nil | 5 | **Furosemide**, pantoprazole, 25% dextrose | *A. xylosoxidans* |
| 7 | Nil | <1 | **Furosemide** | *A. denitrificans* |
| 8 | Nil | <1 | **Furosemide** | *A. denitrificans* |
| 9 | CAG | 4 | **Furosemide** | *A. denitrificans* |
| 10 | Nil | <1 | **Furosemide** | *A. xylosoxidans* |
| 11 | Nil | <1 | **Furosemide** | *A. denitrificans*<br>*A. xylosoxidans* |
| 12 | Sphenoidal mass excision, ICD insertion | 16 | **Furosemide** | *A. xylosoxidans* |

*Abbreviations:* IV- intravenous, CAG- coronary angiogram, PTCA- percutaneous transcatheter coronary angioplasty, DVR, double valve replacement, ICD- intercostal drainage.

**Table 2** - Details of the clinical and contaminated furosemide sample isolates.

| *Sample* | *Method of species identification* | | | *Antibiotic susceptibility by Vitek* | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | *Vitek 2* | *MALDI-TOF* | *WGS* | *Ctz* | *Cpm* | *AG* | *Cipro* | *TMP-SMX* | *BL-BLI* | *Carba* |
| Patient sample 1 | *A. denitrificans* | *A. insolitus* | *A. insolitus* | S | R | R | R | R | S | S |
| Patient sample 2 | *A. xylosoxidans* | *A. insolitus* | *A. denitrificans* | S | R | R | I | R | S | S |
| Furosemide ampoule | *A. denitrificans* | *A. xylosoxidans* | *A. xylosoxidans* | ND | ND | ND | ND | ND | ND | ND |

*Abbreviations:* Ctz: ceftazidime, Cpm: cefepime, AG: aminoglycosides, Cipro: ciprofloxacin, TMP-SMX: cotrimoxazole, BL-BLI: betalactam- betalactamase inhibitor, Carba.: Carbapenem, S: sensitive, R: resistant, ND: not done.

same batch were cultured. All 4 ampoules grew non-lactose fermenting colonies in MacConkey agar and turbidity in the broth which were identified by Vitek2 as 2 different species, *A. denitrificans* and *A. xylosoxidans*. Thus, the source of the outbreak of *Achromobacter* spp. bacteraemia was identified as contaminated furosemide injection preparations.

Following identification, the pharmacy was instructed to withhold dispatch of the contaminated batch of furosemide and recall was done from all clinical areas. Following this, no new cases of *Achromobacter* bacteraemia were noted in the subsequent months.

Three random isolates (2 patient samples and one furosemide ampoule) were also tested by MALDITOF and WGS. The characteristic of the isolates that were obtained by WGS was interpreted using Kraken2 tool, the results of which are shown in Table 2. Multiple *Achromobacter* spp. as well as a discrepancy in identification of genus by Vitek 2 and molecular technique were noted in this outbreak.

## DISCUSSION

Between March and April 2020, we had 12 cases of *Achromobacter* bacteremia in our institution which warranted an outbreak investigation. Seven of them had bacteremia within three days after admission and four had blood cultures taken on day of admission growing *Achromobacter*. Previous studies on *Achromobacter* infections have pointed to its occurrence commonly in hospital setting [4, 9]. Though seven cases had a very short period of hospitalization before occurrence of bacteremia, nosocomial infection was strongly considered, as their initial presentation were as cardiac failure and not with fever. More over community onset *Achromobacter* endogenous infection is uncommon. Any contaminated agent directly coming in contact with blood can cause bacteremia in a short interval. Pseudo-bacteremia due to contamination of blood culture during venipuncture, in the preparation of culture media, or during laboratory processing of the culture was also considered, but many developed symptoms of infection later on.

In one of the earliest review articles on *A xylosoxidans* infections, Chandrasekar, et al. highlighted the pathogenic potential of this organism in immune-compromised host and therefore they should not be disregarded as contaminants [3]. *Achromobacter* nosocomial infections in newborn carry significant mortality. In one of the earliest reports in 1960, Linde et al., in his analysis of 205 cases of congenital heart surgery, described 2 *Achromobacter* endocarditis due to contaminated heart- lung machine [10]. Aerosolization of the organism resulting in pneumonia, meningitis and secondary bacteremia has also been described in new born units [11, 12].

Nosocomial bacteremias due to *Achromobacter* due to contaminated environmental sources, devices or IV preparations are summarized in Table 3. In initial reports contaminated water sources and equipment were noted as the source. Reverdy, et al., in his study describes the epidemiological investigation on *Achromobacter* spp. outbreak in ICU setting leading to identification of *A. xylosoxidans* in deionized water from the faucets of the hemodialysis system and the outbreak was controlled by disinfecting the deionized water system with sodium hypochlorite solution [13]. Gomez-Cerezo J, et al. describe 54 cases of *A xylosoxidans* bacteremia of which 52 episodes were nosocomial and

the source of infection in this series was identified as tap water and the contaminated hands of two healthcare workers. As several studies have implicated faucet aerators as reservoirs of nosocomial pathogens, by removing faucet aerators and instituting the use of gloves the outbreak was curtailed [5]. In another outbreak, Tena, et al. explain that catheter related bacteremia due to *A xylosoxidans* where the source was an atomizer with diluted chlorhexidine which was used for skin disinfection. The isolates from blood and atomizer were tested by pulse field gel electrophoresis (PFGE) to confirm atomizer as the source [6]. In another outbreak, *A xylosoxidans* contaminated transducers caused 15 cases of hospital infection. The problem arose from the re-use of disposable equipment after disinfection with benzalcone [14, 15].

Line-listing helped in suggesting that intravenous furosemide, which was administered for pulmonary edema was common for all patients. The only IV medication that every patient had received prior to blood culture sampling was furosemide. Several reports on Gram negative bacteremia due to contaminated IV preparations have been reported previously and majority are non-lactose fermenters; two of them have been related to *Achromobacter* species [16-21]. Reina, et al. in their brief communication have reported a nosocomial outbreak of *A xylosoxidans* related to administration of intravenous contrast agent. The authors had suspected contrast solution contamination, but could not prove it [8]. Multidose vials of heparin or saline flushes were identified as a source and confirmed by PGFE in another outbreak of blood stream infections due to *Alcaligenes xylosoxidans*, in an out-patient oncology setting [7].

In some outbreaks, even after extensive search, source of the outbreak could not be identified [22]. In a study from India published in 2005 by Kumar, et al., during one-year period between 1996 and 1997, they found 20 strains of *A. xylosoxidans* from clinical specimens, and majority of the patients were neonates. Pulse field gel electrophoresis confirmed that 90% belonged to a single genotype. In this period environmental surveillance did not

**Table 3** - Prior reports of *Achromobacter* bacteremia in hospital settings due to contaminated environmental sources or IV preparations

| *Ref. No* | *Authors* | *Article* | *Nature of infection* | *No. of cases* | *Source of the outbreak* |
|---|---|---|---|---|---|
| 10 | Linde LM, et al., 1960 | Bacterial endocarditis following surgery for congenital heart disease. | Endocarditis | 2 | Heart lung machine |
| 12 | Hanson. L A, et al., 1966 | *Achromobacter* Septicemia in a Premature- Clinical and Bacteriological Aspects. | Septicaemia with meningitis | 1 | Aerosol from an infected humidifier |
| 13 | Reverdy ME, et al., 1984 | Nosocomial Colonization and Infection by *A. xylosoxidans*. | Peritonitis pneumonia with septicemia | 2 | Deionized water |
| 14 | Gahrn-Hansen B, et al., 1988 | Outbreak of infection with *A. xylosoxidans* from contaminated intravascular pressure transducers. | Bacteremia | 15 | Intravascular pressure transducer |
| 8 | Reina J, et al., 1998 | Nosocomial outbreak of *A. xylosoxidans* associated with a diagnostic contrast solution. | Bacteremia | 5 | Contamination of IV contrast |
| 7 | Kim M, et al., 2002 | Blood stream infections in out-patient oncology office. | Bacteremia | 12 | Probably related to contaminated multi dose vials of heparin and saline flushes |
| 6 | Tena D, et al., 2005 | Outbreak of long-term intravascular catheter-related bacteremia due to *A. xylosoxidans* in a hemodialysis unit. | Bacteremia | 4 | Atomizer containing diluted chlorhexidine |

point to any potential source and transmission probably occurred from person to person over a period of time [23]. In another outbreak study, 34 episodes of bacteremia occurred in 22 neonates during a 6-month period. The investigation was not able to single out the source of the outbreak. Despite intensive efforts to control the outbreak containment could be achieved only after the neonatal intensive care unit was relocated [24].

In the current outbreak two different species of *Achromobacter* were identified by Vitek 2 and in 2 patients, each one of their blood culture sample grew different species. This can be explained by contamination of the furosemide ampoules with multiple species of *Achromobacter* or due to difficulty in species identification by Vitek 2. Species level identification of non-lactose fermenting Gram negative rods by conventional method is still a challenge and non-molecular tests may be inadequate for reliable identification [25, 26]. Molecular methods to confirm the species identification showed variation as depicted in Table 2. It is noted that there may be species mismatch between conventional method and MALDITOF MS with non-fermenting Gram-negative rods and WGS is considered the reference standard for species identification.

In the current outbreak, recall of the contaminated batch of furosemide ampoules resulted in control of Achromobacter bacteremia. The manufacturer and distributor were alerted about details of contaminated batch of ampoules. In the following months no further cases of Achromobacter bacteremia were reported from this facility [26].

In conclusion, this evaluation highlights the importance of microbiology alerts in identifying early and rapid control of the nosocomial outbreaks. Contamination of IV preparations with non-lactose fermenters have been previously reported and should be considered in any outbreak of bacteremia. Species identification of Gram-negative non-lactose fermenter can be a challenge and may require molecular methods to confirm it.

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**Conflicts of interests**
None.

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