Original Research Article

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Epidemiological analysis of *Elizabethkingia meningoseptica* infection cluster among mechanically ventilated pediatric intensive care patients

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ABSTRACT

Background: *Elizabethkingia meningoseptica* is frequently found in hospital environments and usually associated with healthcare-associated infections (HAIs), particularly in patients in the intensive care units (ICU). The current study report an outbreak of *E. meningoseptica* infection/colonization in the pediatric intensive care unit, highlighted the infection control methods used to stem the spread.

Methods: During a period of 7 months, May-November 2015, 4 patients were infected/ colonized by *E. meningoseptica*. Infection control measures were re-emphasized after each case and environmental swabs were cultured to detect possible source. Follow up for 25 months to ensure eradication of the pathogen.

Results: Four patients were colonized/ infected with *E. meningoseptica*, their mean age 22 months. The average time patients spent in ICU between admission and isolation of E. *meningoseptica* was 27.5±19.2 days. All patients were mechanically ventilated. 25% *E. meningoseptica* isolated from blood causing healthcare associated Central Line Associated Blood Stream Infection (CLABSI) while it was isolated from endotracheal tube (ETT) secretion in 75% as healthcare associated colonization. The 4 isolates confirmed as identical using pulsed field gel electrophoresis (PFGE).

Conclusions: Intensive infection control measures including healthcare workers education, emphasizing hand hygiene, comprehensive cleaning and disinfection of equipment and the environment are important to eradicate the bacterium.

Keywords: Elizabethkingia meningoseptica, Healthcare associated infections, Mechanical ventilation, Pediatric intensive care unit

INTRODUCTION

Elizabethkingia meningoseptica, formerly known as Flavobacterium meningosepticum and Chryseobacterium meningosepticum or CDC II-a, is a non-motile, non-fastidious, catalase-and oxidase-positive, aerobic glucose

non-fermenting Gram-negative bacillus first described by King in 1959. Let is commonly found in the environment worldwide and has been detected in soil, river water and reservoirs. However, it rarely causes human infections although it has been reported as causative agent of meningitis in newborn babies and meningitis or

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bloodstream and respiratory infections in people with weakened immune systems.³

E. meningoseptica is usually isolated from any aquatic environment in the hospital; sinks, taps, fluids for preparing disinfectants, flushing saline used for medical devices, including feeding tubes, arterial catheters, and respirators. It has been shown that this organism can survive in chlorine-treated water deliveries, often colonizing sink basins and taps, intubation tubes, humidifiers and newborns 'incubators, and has become a probable reservoir for hospital infections. 4-6

E. meningoseptica causes potentially risky infections in patients on admission to critical care areas because of its multidrug-resistant (MDR) character and its ability to get used to different environments. It is organisms of low virulence as only a small percentage of colonized patients develop sepsis while others remain asymptomatic. However, it is associated with high mortality rates (23-52%), partly due to being an MDR organism. The prevalence of nosocomial infection by *E. meningoseptica* has increased, predominantly in patients with severe underlying diseases, prolonged hospitalization, treatment with invasive procedures, prior use of broad-spectrum antimicrobials and concomitant infections. These factors have impacted survival rates. 11

Several cases of *E. meningoseptica* infections have been recognized in outbreaks related to contamination of hospital tap-water, saline, disinfectants antibiotic solutions, lipid solution, sink drains and respiratory equipment, evolving it to be potentially important cause of hospital infections.^{6,12,13} The objectives of this study are to, report unusual clustering of *E. meningoseptica* infections/colonization over a short period and highlight the infection control measures taken to control the infection.

METHODS

Study design

A prospective cohort study with ongoing daily epidemiological and microbiological surveillance for a cluster of *Elizabethkingia meningoseptica* that occurred in the Pediatric Intensive Care Unit (PICU) was carried out.

Setting

The PICU is a 7-bed unit (6-bed bay with 2 hand washing stations in addition to 1 cubicle isolation room with clinical sink). Infrared taps are used in all sinks. Infection control team provides support with daily rounds. Nurse-to-patient ratio is one-to-one. The PICU is located in the Chest Diseases Hospital, which is a 358-bed tertiary care hospital that is specialized in management of cardiac and cardiothoracic cases in Kuwait.

Study population

All pediatric patients admitted to the PICU in the study hospital with different cardiac and cardiothoracic diseases and stayed to receive medical management or postoperative care after open heart/thoracic surgeries.

Sample size

Investigation of the cluster of *Elizabethkingia* meningoseptica cases among all patients residing in PICU from May 2015 till December 2017.

Research tools

Surveillance form

Included; socio-demographic data of patients, hospital file number, location, and bed number. Date of admission, date of surgery, type and date of insertion and removal of invasive devices. Diagnosis on admission, history in details, underlying condition, clinical, laboratory and radiological evidence. Type of laboratory samples, collection date and report result. Medications name and dosage, daily progress of the condition and patient outcome.

Outbreak notification form

Included the following; type of outbreak, Incubation period, etiological agent, mode of transmission, date of outbreak was detected /reported to infection control, outbreak location. Healthcare facility source, Index case identified or not, if healthcare facility source was the index case; if it was a HCW or not. Date of onset of the first ill person, date of outbreak commenced, total number of affected cases; number of laboratory confirmed cases, number of patients still hospitalized, number of deceased patients. Outbreak status; ongoing or controlled, date of onset of last till person resolved, the date of outbreak completed.

Outbreak case list

This form is the summary information of the previous two forms.

Data collection

All cases' information was collected by infection control team from medical files, laboratory reports, nursing notes and environmental screening reports using the previous forms through their daily visits to the study location.

Data for the current research was retrieved from the surveillance form, outbreak notification form and outbreak case list for the diagnosed nosocomial *Elizabethkingia meningoseptica* infected and colonized cases.

Laboratory work

Bacterial identification and antibiotic susceptibility testing

The isolates had been identified at the microbiology laboratory of the hospital using the API 20 NE system (bioMerieux, Marcy-l Étoile, France) and VITEK 2 ID System (bioMerieux). Antibiotic susceptibility testing against amikacin, ceftazidime, ciprofloxacin, colistin, gentamycin. imipenem, meropenem, piperacillin, piperacillin-tazobactam, trimethoprim-sulfamethoxazole, rifampicin and vancomycin was performed using the Etest (bioMérieux, Marcy-l Étoile, France) method according to the manufacturer's protocol. E. coli ATCC 25922 was included in each run for quality control. were interpreted according Results recommendations of the Clinical Laboratory Standard Institute.14

Genomic fingerprinting

Four E. meningoseptica isolates obtained from blood and endotracheal tube (ETT) secretions were sent to the anaerobic and hospital infection reference laboratory, to be fingerprinted by pulsed-field gel electrophoresis (PFGE) typing of the extracted whole-cell genomic DNA embedded in 1% agarose plugs and digested with Xba1. The Xba1-digested genomic DNA was electrophoresed in a 1% certified agarose gel with a voltage gradient of 6 V/h at 14°C at an angle of 120° using the CHEF-MAPPER XI System (Bio-Rad Laboratories, Hercules, CA, USA). Banding patterns were analyzed using FP Quest TM software (Bio-Rad Laboratories) and strains defined as having PFGE profiles of >94% similarity. A lambda ladder (New England Biolabs, Beverly, MA, USA) was included in each gel run. DNA relatedness was estimated using the criteria of Tenover and his colleagues in 1995.15

Environment screening

Water from all clinical taps in the critical care unit was sampled for bacterial colonization. A total of 100 mL of water was collected from each tap, filtered by using a 0.45-µ filter membrane, and incubated on MacConkey agar in air at 37°C for 48 hours. Oxidase-positive non-lactose-fermenting colonies were sub-cultured onto nutrient agar and a 10-µg meropenem disk placed on the inoculum. Organisms displaying meropenem resistance were further identified by the API 20 NE system (bioMérieux) and VITEK 2 ID System (bioMérieux).

RESULTS

Epidemiological analysis of the cluster of cases

Healthcare associated colonization/infections with E. meningoseptica were identified in 4 female patients. Their mean age was 22 months (range, 1 day - 7 years).

They were admitted on the PICU during a period of 7 months (May – November, 2015 The average time spent in the intensive care unit between admission and isolation of *E. meningoseptica* was 27.5±19.2 days (range 5-44 days).

Table 1: Frequency of possible risk factors in the study cluster.

Intervention	Percentage (%)
PICU admission	100
Mechanical ventilation	100
Colistin use	25
Cefazolin	75
Central line	100
Arterial line	75
Parenteral nutrition	25
Bronchoscopy	25
Urinary catheter	100
Nasogastric tube	25
Tracheostomy care	25

All patients were mechanically ventilated for a period ranged 14-65 days, and had other invasive devices (central lines and urinary catheters). Three patients had open heart surgery. Cefazolin was the prophylactic antibiotic used for all cases before surgery and continued for 2-5 days post-operative. In 1 patient, E meningoseptica was isolated from blood associated with healthcare-associated central line associated blood stream infection (CLABSI) while it was isolated from endotracheal tube (ETT) secretion in 3 patients classified as healthcare-associated colonization. Three of the cases had other clinically diagnosed healthcare-associated infections, such as catheter-associated urinary tract infection (CAUTI), ventilator-associated pneumonia (VAP), peritonitis, and CLABSI with Stenotrophomonas maltophilia, Pseudomonas aeruginosa, Klebseilla pneumoniae, Staphylococcus epidermis Staphylococcus hominies (Table 1 and 2). Three of the 4 patients expired (75% mortality rate) and the remaining 1 patient was boarded to United Kingdom to facilitate extubation and possible rehabilitations.

Infection control measures and interventions

Urgent infection control committee meeting discussed the situation and agreed up on the following measures:

Healthcare workers education

Training sessions for doctors, nurses, physiotherapist as well as respiratory therapist were done to emphasis on hand-hygiene (HH) and aseptic techniques. Training sessions for cleaners and their supervisors to stress upon the appropriate methods for cleaning, disinfection and preparation of cleaning solution with appropriate concentration of sodium hypochlorite were conducted.

Table 2: Description of Elizabethkingia meningoseptica cluster of cases in pediatric intensive care unit.

Cases	Age	Gender	Underlying condition	E. meningoseptica Detected	Mechanical ventilation	Duration of ventilation	Isolated from	Other HAIs	Outcome
1	1 day	F	Congenital heart	HAIs	Yes	21 days	Blood	CLABSI	Expired
2	2 month	F	Congenital heart Trisomy 21	colonization	Yes	65 days	ETT	CLABSI Peritonitis	Expired
3	2 month	F	Congenital heart	colonization	Yes	14 days	ETT	VAP CAUTI	Expired
4	7 year	F	Post cardiac arrest VF and VT Chronic Myocarditis	colonization	Yes	30 days	ETT	Did not meet criteria for any HAIs	Boarded to UK

vf = ventricular fibrillation; vt = ventricular tachycardia; hais = healthcare-associated infections; ett = endotracheal tube; clabsi = central line-associated blood stream infection; vap = ventilator-associated pneumonia; cauti= catheter-associated urinary tract infection.

Table 3: The antimicrobial susceptibility of the isolates.

Antimicrobial substance			Susceptibility %	
	S	I	R	
Amikacin	≤16	32	≥64	100
Piperacillin-Tazobactam	≤16	32-64	≥128	100
Trimethoprim/Sulfa	≤2	-	≥4	100
Ciprofloxacin	≤1	2	≥4	100
Levofloxacin	≤2	4	≥8	100
Gentamicin	≤4	8	≥16	100
Rifampicin	≤10	-	≥25	75
Amoxicillin-clavulanic acid	≤8	16	≥32	0
Ceftazidime	≤8	16	≥32	0
Ceftriaxone	≤8	16-32	≥64	0
Cefuroxime	≤8	16	≥32	0
Imipenem	≤2	4	≥8	0
Meropenem	≤2	4	≥8	0
Colistin	≤2	4	≥8	0

s: sensitive, i: intermediate, r: resistance; mic: minimum inhibitory concentration µg/ml.

HH compliance was measured to evaluate the impact of training for PICU team; doctors' HH compliance was 51.6% but improved to 67.9% after the training. While nurses' HH compliance were 85.6% and increased to 92.9% after education.

Screening of environmental samples

Environmental samples were collected from (ventilators, humidifiers, incubators, bed rails, sinks, treatment trolley, bedside tables, cleaning solutions open one and new one as well as water sample. Two samples (sink swab and a water sample) of the 24 (8.3%) environmental specimens obtained were identified positive for *E. meningoseptica*.

Patients and healthcare workers measures

Cohorting of patients as well as healthcare workers were done. Evacuation of the patients from the PICU was carried out to allow extensive cleaning and disinfection.

Environmental cleaning and disinfection

These were achieved by cleaning and disinfecting all equipment (e.g. ventilators) as per manufacturer instructions. All open containers of cleaning and disinfection solutions were discarded. Direct observation was carried out to ensure meticulous cleaning and disinfection of all environmental surfaces in PICU including beds, floors, and cleaning equipment (e.g. mops and buckets). In addition, removal and laundry of all curtains, cleaning and disinfection of the ceiling and air condition ducts, filters, outlets and inlets and hyperchlorination of water supply to the unit, were all carried out.

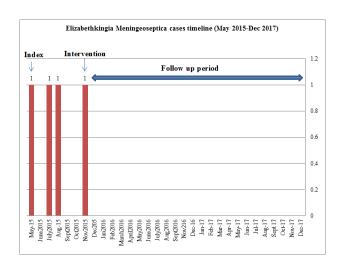


Figure 1: Timeline of *Elizabethkingia meningoseptica* cluster of cases with 25 months of follow-up.

Follow up

Active daily surveillance of infections and follow-up for any possible colonization in patients who were in PICU

risk was performed by infection control team. There were no further cases of infection/colonization with *E. meningoseptica* from November 2015 till December 2017 (Figure 1).

Antimicrobial susceptibility of the isolates

All the 4 isolates were susceptible to the aminoglycosides, fluoroquinolones, Piperacillin-Tazobactam and trimethoprim/sulfamethoxazole (Table 3).

Genomic typing of isolates

All the 4 isolates were identical (clonally related) on the pulsed-field gel electrophoresis (PFGE) as shown in Figure 2. The isolate from the index case was a blood isolate shown in lane 1 while the subsequent isolates from the ETT secretions of the 3 other patients are demonstrated in lanes 2, 3 and 4. Unfortunately, the 2 environmental isolates were lost in transit to the Reference Laboratory that performed the genomic typing.

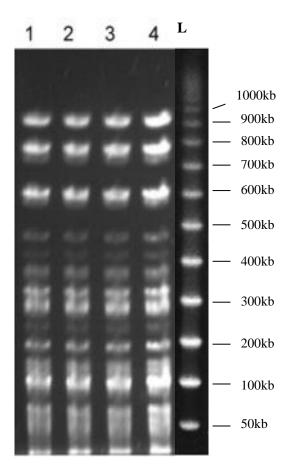


Figure 2: Pulsed-field gel electrophoretic pattern of the four isolates of *Elizabethkingia meningoseptica* from blood stream infection and endotracheal tube (ETT) secretions of patients on the pediatric intensive care unit.

Note: lanes 1 is the blood isolate, lanes 2, 3 and 4 are isolates from the ett secretions, and lane l is the ladder.

DISCUSSION

E. meningoseptica has a strong predilection for extremes of age with attendant high mortality rates. In this study, our patients were aged between 1 day and 7 years with a mortality rate of 75%. The organism is also well known to cause hospital-acquired infections in premature newborns and infants and associated with high mortality rate of approximately 43% in some studies although lower mortality rate of 9.1% has been reported for community-acquired infections.¹⁶

The predisposing factors for infection/colonization, in our study, were presence of comorbidity, congenital heart disease, open heart surgery and mechanical ventilation. These findings are supported by previous reports elsewhere which showed that admission to the ICU, the underlying severe illness, shock at presentation, tachycardia, use of life support devices, and prolonged antibiotic treatment were the main predisposing factors. Place Also being immuno-compromized and elderly may represent important host factors that predict susceptibility to *E meningoseptica* infection. Host susceptibility factors are critical determinants of risk of *E. meningoseptica* infection.

It is also apparent from our study that the time interval between the admission of the patients to the hospital and development of infection/colonization with E. meningoseptica vary. In this series, this interval appeared to have occurred relatively earlier, 5-44 days (mean 27.5 \pm 19.2 days), compared to other study conducted in 2011; where late infection was the common experience as it developed 50-70 days after hospital admission. 11

clinical spectrum of disease due meningoseptica may range from simple colonization to symptomatic acute infection and furthermore to infectionrelated sequelae. 19 Some hosts may be colonized by E. meningoseptica organism in the absence of signs and symptoms of active infection and thus may act as a source of an outbreak.²⁰ It is reasonable to assume that contact to exogenous sources can lead to colonization with E. meningoseptica at mucous membranes as the respiratory tract and non-intact skin sites and that colonized patients may act as sources of infection for susceptible individuals. Therefore, identification of patients colonized or infected with E. meningoseptica is of paramount importance in preventing further dissemination of the bacterium during an outbreak. 7

Meningoseptica infection is very challenging to both clinicians and microbiologists, as the organism is intrinsically resistant to multiple antibiotics, such as the β -lactams, aminoglycosides, tetracycline, tigecycline, colistin, chloramphenicol and carbapenems.⁴ However, it is susceptible to the agents used to treat Gram-positive bacterial infections such as rifampicin, ciprofloxacin, vancomycin and trimethoprim— sulfamethoxazole. Yet

adequate treatment for this organism has not been properly outlined. Although, vancomycin alone or when it is combined with rifampicin had been used efficiently, recent studies raised many inquiries about its efficacy.²⁰

In our study, the 4 isolates were resistant to all β -lactam antibiotics, tigecycline, and colistin but susceptible to the other classes of antibiotics. Some studies have shown that susceptibility of E. meningoseptica was relatively high (>50%) to piperacillin, piperacillin-tazobactam, cotrimoxazole, ciprofloxacin, moxifloxacin, levofloxacin, tigecycline, vancomycin and showed multidrug resistance ampicillin-sulbactam, ticarcillin. ceftazidime. ceftriaxone, cefepime, cefoperazone-sulbactam, cefepime-tazobactam, tetracycline, chloramphenicol, imipenem, amikacin, gentamicin, meropenem, tobramycin, and colistin. 10, 21 Our study showed that the E. meningoseptica isolates were fully susceptible to amikacin, piperacillin-tazobactam, trimethoprim/sulfa, ciprofloxacin, levofloxacin, and gentamicin.

All 4 clinical isolates were clonally related. Moreover, 2 samples from the environment revealed the growth of E. meningosepticas howling the same sensitivity pattern suggesting a point-source spread either from the index case or the environment. It is conceivable that the environment must have played a part in maintaining the organism within the PICU for the 7-month period. Although not proven, the hands of the healthcare workers in the unit would have been responsible for the spread. Outbreaks of meningitis due to E. meningoseptica have been reported on neonatal wards and ICUs in the past. 5,17,22,23 Some of these outbreaks were investigated in order to determine whether transmission of E. meningoseptica from the environment to patients might have occurred. Despite examination of large numbers of screening cultures no firm evidence was obtained. 17,23 However, another investigation considered that the hands of healthcare workers were the likely route of transmission of E. meningoseptica between pediatric patients.5

Screening of the environment and patients for E. meningoseptica and other infection control measures by contrast with outbreaks of other bacteria such as vancomycin-resistant enterococci, has been debatable for a while. So far, there is no concrete agreement about who to screen and when to initiate active screening during a suspected outbreak of E. meningoseptica infection.²⁴ However, we believe that implementation of screening strategy to detect asymptomatic carriers should be considered in an outbreak setting (or where E. meningoseptica is endemic). Previous investigations have included mechanical ventilation equipment, catheters, infant incubators, parenteral and antiseptic solutions, feeding bottles and bags, components of the hospital water system (sinks, basins, and faucets), environmental surfaces and equipment, such as doors and door handles, electrical buttons, telephones, computer keyboards, covers of medical charts as part of screening program. of these, infusion containers and sinks have been identified most often as likely sources of contamination, although in our study, sink and tap water were the contaminated sources; a finding which is partly supported by the study of Amer et al in which the organism was found as colonizer in tap water, tubing of ventilators and in sink basins of the hospital wards. Several cases of *E. meningoseptica* infections have been reported as part of outbreaks and source was traced to contaminated hospital water supply, saline, disinfectants, antibiotic solutions, water sinks, and respirators. Several cases

Studies have also shown that the organism can survive in chlorine-treated water supplies as sink basins and taps, and becoming a possible reservoir for hospital infections.4,27 Thus, outbreaks may be controlled with strong emphasis on infection control measures. Given the importance of environmental sources of the bacterium in the epidemiology of nosocomial outbreaks of E. meningoseptica infection, it is not surprising that some investigators have reported success in controlling outbreaks using enhanced cleaning protocols, cite control of a cluster of infection involving 13 pediatric patients.⁵ Although an environmental source was not identified, the outbreak was terminated following introduction of two disinfectants (hypochlorite solution, and isopropanol spray) daily cleaning of the unit with particular emphasis on objects containing, or in contact with water.

Other measures that have successfully controlled *E. meningoseptica* outbreaks in hospital settings include modification of empirical antimicrobial protocols, restriction of staff exchange and stoppage of new admissions, supplementation of hand hygiene regimens with chlorhexidine gluconate 4%, as well as actions relating to hospital water such as hyper chlorination, isolation of tanks from the common hospital feeder tanks and toileting of babies with sterile rather than tap water. It is satisfying to note that the same measures that have been used successfully by to eradicate *E. meningoseptica* outbreaks in pediatric units including, among others, restriction of further admissions and thorough disinfection of the unit were effective to eradicate the cluster of *E. meningoseptica* cases in our study. ^{6,12,28}

CONCLUSION

Early recognition of patients colonized or infected with *E. meningoseptica* assisted in preventing spread of the bacterium. Efficient investigations to identify and control the source of the microorganism and comprehensive cleaning of all equipment and environmental surfaces are necessary. Furthermore, the strengthening of standard infection control measures, included proactive contact isolation precautions, restricted patient movement and transfer, in addition to enhanced staff HH compliance and good antibiotic stewardship together were the actions that have eradicated *E. meningoseptica* cluster.

Recommendations

Development of robust interventions to contain outbreaks of this pathogen among critically ill patient is urgently needed to reduce the morbidity and mortality associated with this infection. Environmental sampling is required to identify the possible source.

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