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Microbiology of *Elizabethkingia* spp. isolates in hospitalized patients

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SUMMARY

An increasing number of infections due to *Elisabethkingia* spp. have been observed and reported in recent years. Here we report the microbiological aspects of 13 cases with positive cultures for *Elizabethkingia* spp. from 1998 to 2017. *Elizabethkingia* isolates were identified using the Vitek 2 Compact 60 (AES software) Gram-Negative Identification test (GNI) card (bioMérieux. Marcy-l'Etoile, France). The MICs were determined using Vitek 2 and interpreted according to CLSI guidelines based on the interpretive MIC breakpoints for *Acinetobacter spp*. Vitek 2 susceptibility results were confirmed using the manual E-test and the colistin result was confirmed using the broth dilution method. Twelve cases were observed between 2010 and 2017 with four cases in 2017. Of the 2017 samples, three out of four isolates were obtained outside the intensive care units compared to one out of eight samples between 2010 and 2016. There was variable susceptibility to trimethoprim/sulfamethoxazole (58.3%), ciprofloxacin (41.7%), piperacillin/tazobactam and gentamicin (16.6% each), and all were resistant to colistin. There is a high rate of drug resistance and further studies to identify the source of the infection are needed to build up a profile of *Elizabethkingia* spp. to inform public health policy in this context.

Keywords: *Elizabethkingia*; Saudi Arabia; nosocomial; antimicrobial resistance.

INTRODUCTION

Elizabethkingia meningoseptica (previously termed *Flavobacterium meningosepticum* and *Chryseobacterium meningosepticum*) is a non-fastidious, oxidase-positive, Gram negative bacillus of the genus *Elizabethkingia* [1, 2]. It is widely found in soil, plants and water, including adequately chlorinated municipal water supplies, but is not part of normal human microflora [2, 3]. In recent years, an increase in healthcare associated cases and outbreaks of *E. meningoseptica* infection has been documented in several countries including the United States, the United Kingdom, Taiwan, India and Brazil [4-10]. *E. meningoseptica* has been described as responsible for numerous meningitis outbreaks in hospital neonatal wards affecting mainly premature infants [11-13]. Outbreaks and clusters of cases have also occurred among immunocompromised adults in intensive care units (ICUs) and in long-term acute care hospitals [5, 8, 14-16]. Risk factors for infection include a history of antibiotic exposure and comorbidities associated with immune impairment (such as organ transplantations and malignancies), as well as long-term hospital stays and use of invasive medical devices (central venous catheters and mechanical ventilation) [1, 5, 15, 17, 18]. Hospital cases and outbreaks have frequently been associated with hospital water supplies and contamination of taps and sinks, as well as equipment and solutions, often exacerbated by shortcomings in infection prevention and control measures [7, 14, 16].

Apart from meningitis, *E. meningoseptica* is associated with bacteraemia, sepsis, respiratory tract

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infections, endocarditis and eye infections and colonization of contact lenses [5, 8, 19-22]. Mortality rates is high, ranging from 23 to 52%, partially due to multidrug resistance and the ability of the organism to form biofilms [5, 17, 23, 24]. *E. meningoseptica* is extensively resistant to β-lactam antibiotics, including carbapenems and cephalosporins, due to carriage of *blaB* and bla_{GOB} resistance determinants, extended spectrum β-lactamases (ESBLs), and aminoglycosides and polymixin resistance [1, 25]. Tigecycline has been reported to be efficacious in studies from Taiwan, while reports on susceptibility levels to trimethoprim-sulfamethazole and ciprofloxacin are more variable [5, 17, 23, 25, 26]. Levofloxacin is a possible therapeutic option; however, some studies have indicated relatively high levels of resistance and a significantly increased risk of bacteraemia-associated mortality with levofloxacin-resistant strains [27]. Newer quinolone antibiotics such as levofloxacin may be promising, based on *in vitro* data, while there are some conflicting reports on effectiveness of vancomycin or rifampicin against *E. meningoseptica* [23, 28-30]. The Clinical and Laboratory Standards Institute (CLSI) have not defined interpretive breakpoints establishing the minimum inhibitory concentrations (MICs) for *E. meningoseptica*. Therefore, interpretive breakpoints are based on MIC breakpoints for other bacterial species [1]. The multidrug resistance and the lack of defined breakpoint guidelines complicates effective treatment of *E. meningoseptica* infection.

In Saudi Arabia, there is a lack of epidemiological information on incidence of *E. meningoseptica* infection levels in hospitals and healthcare facilities. Case reports have been presented on a liver transplant patient and a case of meningitis in a premature neonate [18, 31]. There is a need to document cases of *E. meningoseptica* infection in Saudi hospitals and determine the antibiotic resistance profiles of cases in order to improve diagnosis and treatment of this infection and help recognition of potential outbreaks. In this study, we report the epidemiologic and microbiologic characteristics of *Elizabethkingia* spp. isolates in our hospital in the recent years.

METHODS

Patients and bacterial isolates

The study was conducted at the microbiology laboratory of the John Hopkins Aramco Healthcare, a 450-bed hospital in Dhahran City in Saudi Arabia. *Elizabethkingia* spp isolates were collected from thirteen patients between December 1998 and July 2017. Information were collected on date of admission, the location within the hospital where the patient was treated, and days to positive culture. Bacterial identification was performed using the Vitek 2 Compact 60 (AES software) Gram-negative Identification test (GNI) card (bioMérieux, Marcy l'Etoile, France).

Antimicrobial sensitivity testing

The MICs of the isolates to eleven antimicrobial agents (amikacin, ampicillin/sulbactam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, colistin, gentamicin, imipenem, piperacillin/tazobactam and trimethoprim/sulfamethoxazole) were determined using Vitek 2. N291 and N292 sensitivity cards (bioMérieux, Marcy l'Etoile, France) were inoculated with suspension vials at the Smart Carrier Station™ and loaded into the Vitek 2 automated reader-incubator, and interpreted according to the manufacturer's instructions and CLSI guidelines [32]. Interpretive MIC breakpoints for *Acinetobacter* spp. were used for all antimicrobial agents. Table 1 shows the concentration ranges of the agents used [32]. In addition, Vitek 2 susceptibility results were confirmed using manual E-test and colistin result was confirmed using broth dilution method.

RESULTS

During the study period, thirteen strains of *Elizabethkingia* spp. were isolated from different samples collected from patients hospitalized in different wards. Specimens included sputum (n=8), bile (n=2), blood (n=1), tracheal aspirate (n=1) and wound (n=1). Demographic data and dates of admission are shown in Table 2. There was only one case identified in 1998, one in 2010, one case in 2012, two in 2013, one in 2014, two in 2015, and one in 2016. Up to July, there were four cases identified in patients admitted in 2017 (Table 1). There were four cases in 2017 and no identified epidemiologic link between these cases was identified. The annual rate of *Elizabethkingia spp.* infection per 1000 patient-days ranged between 0.04 and 0.15 per 1000 patient-days.

No information was available on the 1998 patient about the specimen (Table 2). Of the remain-

Table 1 - Antimicrobial *in vitro* susceptibilities.

Antimicrobial agent	*Concentration range (mcg/mL)*	*Number (%) susceptible isolates*	*Number (%) intermediate isolates*	*Number (%) resistant isolates*
Amikacin	2-64	1 (8.3%)	0	11 (91.7%)
Ampicillin/sulbactam	2-32	1 (8.3%)	0	11 (91.7%)
Cefepime	2-64	1 (8.3%)	0	11 (91.7%)
Ceftazidime	2–64	1 (8.3%)	0	11 (91.7%)
Ceftriaxone	2–64	1 (8.3%)	0	11 (91.7%)
Ciprofloxacin	0.25–4	5 (41.7%)	2 (16.6%)	5 (41.7%)
Colistin	2-64	0	0	12 (100%)
Gentamicin	1–16	2 (16.6%)	2 (16.6%)	8 (66.7%)
Imipenem	0.25–16	1 (8.3%)	0	11 (91.7%)
Piperacillin/tazobactam	4/4–128/4	2 (16.6%)	0	10 (83.4%)
Trimethoprim/ sulfamethoxazole	1/19–16/304	7 (58.3%)	0	5 (41.7%)

ing twelve isolates identified since 2010, seven (58.3%) isolates were from male patients and five (41.7%) from female patients. Average age of the patients was 65.6±16.0 years (range 28-87 years). All except one had known underlying disease or clinical condition, most commonly respiratory failure/arrest (n=6; 50%) or diabetes mellitus (n=5; 41.7%) (Table 1). Eight of the twelve isolates

Table 2 - Demographic and outcome data.

Age (y)	*Sex*	*Source*	*Underlying disease*	*Year*	*Length of stay before the isolation of* E. meningoseptica	*Antibiotics used*	*Central line*	*Mechanical Ventilation*
69	m	Sputum	NA	1998	14	Imipenem	NA	NA
46	M	Sputum	Respiratory failure	2010	45	multiple	NA	NA
62	M	Sputum	HTN, respiratory arrest, tracheostomy	2012	132	NA	NA	NA
69	F	Sputum	DM, CVA, tracheostomy	2013	31	multiple	NA	NA
69	F	Sputum	DM, CRF, respiratory failure	2013	257	no	yes	yes
86	M	Sputum	RA, malignancy, respiratory failure	2014	107	no	yes	yes
81	M	Tracheal aspirate	Respiratory failure, DM, HTN	2015	15	multiple	yes	yes
87	F	Blood PICC line	DM, liver cirrhosis, CRF	2015	102	multiple	no	no
71	F	Sputum	Pancreatitis, MOF	2016	15	multiple	yes	yes
66	M	Bile	None	2017	0	imipenem	NA	NA
66	M	Wound	DM	2017	8	multiple	NA	NA
28	F	Bile	Hydatid disease	2017	14	multiple	NA	NA
56	M	Sputum	Respiratory failure	2017			yes	yes

CRF: chronic renal failure; CVA: cerebrovascular diseases; DM: diabetes mellitus; HTN: hypertension; RA: rheumatoid arthritis; MOF: multi-organ failure; NA: Data not available.

(66.7%) were obtained from patients hospitalized in the intensive care unit (ICU) (Table 2). Two (16%) patients had no antibiotic use in the proceeding 30-days, two (16%) had imipenem and the other patients had multiple antibiotics.

In 2017, one out of four samples were sputum samples, whereas between 2010 and 2016, six out of eight were sputum samples (Table 2).

Susceptibility of the isolates to eleven antimicrobial agents was determined (Table 1). There was variable susceptibility to trimethoprim/sulfamethoxazole (58.3%) (MIC 40 mcg/mL), ciprofloxacin (41.7%) (MIC 0.125-4 mcg/mL), piperacillin/tazobactam (MIC 8-128 mcg/mL) and gentamicin (MIC 2-64 mcg/mL) (each 16.6%) (Table 1). All isolates were resistant to colistin (MIC 256 mcg/mL). For colistin, essential agreement, and categorical agreement between VITEK 2 and microdilution method were 97% and 100%, respectively. The total essential agreement and categorical agreement between VITEK 2 and E-test method were 98% and 96.9%, respectively.

Comparing isolates obtained in 2017 to those obtained between 2010 and 2016, there was no significant difference in distribution of resistance for any tested antimicrobial agent, except for piperacillin/tazobactam for which all eight isolates between 2010 and 2016 were resistant, whereas two out of four isolates taken in 2017 were sensitive (Chi square statistic =4.800; p=0.029).

DISCUSSION

Of the 13 isolates of *Elizabethkingia* spp in our microbiology laboratory since 1998, four were isolated in 2017. This is consistent with the observed increase in healthcare associated cases and outbreaks of *E. meningoseptica* infection in other countries in recent years [4-10]. However, while seven out of the eight *Elizabethkingia* spp. -positive isolates were between 2010 and 2016 and were from patients in the ICU, those strains isolated in 2017 were from patients hospitalized in four different non-ICU wards. The average age of our patients admitted between 2010 and 2016 was 65.6±16.0 years, which is older than some previous reports but consistent with other reports, for example from Taiwan, which suggest that older adults may be at higher risk [2, 5, 17, 27, 33]. Most patients had prolonged hospitalization prior to *E. meningoseptica*-positive culture identification, had received antibiotic treatment and most also had underlying comorbid diseases, all of which are recognized risk factors for *E. meningoseptica* infection [1, 5, 15, 17, 18].

Hospital-associated outbreaks are often due to shortcomings in infection prevention and control practices. For example, in an outbreak in Singapore where five patients were infected with *E. meningoseptica* in two intensive care units (ICUs), non-sanctioned practices such as use of hand-hygiene sinks for disposing of secretions from patients or rinsing re-usable patient care items led to colonization of taps and hence spread of infection to patients [14]. Another recent outbreak in a regional maternity hospital was linked to isolates recovered from storage boxes for pacifiers and pacifier covers [13]. Replacing the boxes with stainless steel boxes that were regularly sterilized by autoclaving resulted in no further infections with *E. meningoseptica*. The potential danger of *E. meningoseptica* is further compounded by its ability to form biofilms, which may contribute to antimicrobial resistance and to persistence, for example in tanks or in taps [34]. Persistent biofilm formation in taps was associated with a hospital outbreak of neonatal meningitis in the United Kingdom [35]. Consistent with data from other countries, *Elizabethkingia* spp. isolates from our hospital had a high level of resistance to multiple antibiotics including the β-lactams cefepime, ceftazidime, ceftriaxone and imipenem, the aminoglycoside amikacin and gentamicin, the polymixin colistin, as well as the combination penicillin-like antibiotic/β-lactamase inhibitor treatments piperacillin/tazobactam and ampicillin/sulbactam. There was a higher level of susceptibility to the fluoroquinolone ciprofloxacin (41.7%) and to trimethoprim/sulfamethoxazole (58.3%), consistent with previous reports showing susceptibility levels to trimethoprim-sulfamethoxazole and ciprofloxacin varying between approximately 33-92% and 43-87% respectively [5, 17, 23-26, 29]. We did not find that strains isolated between 2010-2016 were susceptible *in vitro* to piperacillin/ tazobactam, contrary to data from some *in vitro*, case series, and surveillance studies which suggested that this might be a potentially effective treatment [5, 17, 29]. However, two out of four isolates in 2017 were sensitive to piperacillin/tazobactam, suggesting that there may be a different profile of susceptibility to this antimicrobial agent among

isolates from that period of time. Given its extensive antibiotic resistance pattern, there is at the moment a lack of consensus on an appropriate treatment for infections caused by *E. meningoseptica* [1].

There is lack of epidemiological information on *E. meningoseptica* infections in healthcare facilities in Saudi Arabia, with published information being mainly limited to case reports [18, 31]. In view of the increasing recognition in other countries that healthcare associated *E. meningoseptica* infection incidence is increasing, there is a need to address this gap in knowledge in this part of the world. There is some debate as to whether *E. meningoseptica* is an emerging pathogen or a pseudo-emerging pathogen, which was previously present but under-identified or unidentified due to less advanced detection methods [7]. In any case, it has recently been identified in healthcare associated outbreaks in countries including the United States, the United Kingdom, Taiwan, India and Brazil [4-10].

Earlier CLSI guidelines did not differentiate species of *Elizabethiakingae*, however, the identification to the species level using Vitek system later was proved to be not enough. In addition to *E. meningoseptica*, additional novel species were proposed to be members of *Elizabethkingia* genus. Among these species, *E. anophelis* has emerged as a cause of life-threatening infection in humans, particularly in immunocompromised patients. *E. anophelis* isolates can be misidentified as *E. meningoseptica* even by MALDI-TOF MS, due to the absence of *E. anophelis* spectra in database [36, 37]. Thus, one of the limitations of this study is that we could not perform DNA sequencing. Thus, the designation of *Elizabethkingia* spp. seems to be more appropriate. In addition, the small sample size and the retrospective nature of the study were other limitations of the study.

In conclusion, we present the epidemiologic and microbiological data of *Elizabethkingia* spp isolates in Saudi Arabia. The study did not identify the source of the isolates and further studies to identify the environmental source of the infection are needed. In addition, it would be interesting to have similar studies in other hospitals and healthcare facilities in Saudi Arabia in order to build up a profile of *Elizabethkingia* spp to inform public health policy in this context.

Ethical statement

This study has been conducted after attaining formal approval from the Institutional Review Board at Johns Hopkins Aramco Healthcare.

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Conflict of interest

The author confirms that this article content has no conflict of interest.

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