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An *Elizabethkingia meningoseptica* (*Chryseobacterium meningosepticum*) Outbreak in Intensive Care Units and Infection Control Measures

Yoğun Bakım Ünitelerinde *Elizabethkingia meningoseptica* (*Chryseobacterium meningosepticum*) Salgını

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ABSTRACT

Introduction: *Elizabethkingia meningoseptica is an emerging gram-negative opportunistic nosocomial pathogen reported in immunocompromised patients. We aimed to report an outbreak of E. meningoseptica acquisition in the intensive care units (ICUs) of a training and research hospital in Turkey and highlight the infection control measures.*

Materials and Methods: *Bacterial strains were determined using MALDI Biotyper (Bruker Daltonics, Bremen, Germany) minimum inhibitor concentrations were examined using MicroScan automatized system. Results were determined according to the CLSI guidelines. Environmental cultures were obtained and investigated for the presence of E. meningoseptica. Clonal relationships among E. meningoseptica strains were investigated using PFGE.*

Results: *Isolates were obtained from nine critically ill patients' clinical samples. All strains were resistant to tested antibiotics (amikacin, Amox/Clav, cefepime, cefotaxime, ceftzidime, cefuroxime, ertapenem, gentamycin, meropenem, trimethoprim/sulfamethoxazole (TMP–SMX), imipenem) except for levofloxacin (8/9), ciprofloxacin (5/9), and piperacillin/tazobactam. PFGE indicated that the strains involved in the outbreak were closely related.*

Conclusion: *Intrinsically multiple drug-resistant Elizabethkingia spp. isolates can be a common life-threatening pathogen in ICUs in our country, and prevention is possible through early notification of small-scale outbreaks and necessary infection control measures.*

Key Words: *Elizabethkingia meningoseptica; MDR; Outbreak; PFGE, MALDI-TOF.*

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ÖZ

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Giriş: *Elizabethkingia meningoseptica bağışıklığı baskılanmış hastalarda artan oranda bildirilen Gram negatif fırsatçı hastane infeksiyonu etkenidir. Yoğun bakım ünitelerinden (YBÜ) izole edilen E. meningoseptica suşları arasındaki klonal ilişkiler ve bunların salgınla ilişkisi araştırıldı.*

Materyal ve Metod: *İzolatlar MALDI-TOF kullanılarak tanımlandı (Bruker Dal-tonik GmbH, Bremen, Almanya). Antibiyotik Minimum inhibitör konsantrasyonları, MicroScan otomatize sistem kullanılarak belirlendi. Sonuçlar, Klinik ve Laboratuvar Standartları Enstitüsü kılavuzlarına göre raporlandı. Çevre kültürleri alındı ve E. meningoseptica varlığı açısından araştırıldı. E. meningoseptica suşları arasındaki klonal ilişkiler PFGE kullanılarak araştırıldı.*

Bulgular: *İzolatlar anestezi yoğun bakım ünitesinden iki kadın (62 ve 82 yaşında), bir erkek (58 yaşında) ve koroner yoğun bakım ünitesinden iki kadın (73 ve 60 yaşında), iki erkek (57 ve 75 yaşında) ve dahiliye yoğun bakım ünitesinden iki kadın (65 ve 67 yaşında) hastanın klinik örneklerinden izole edildi. Suşlar levofloksasin (8/9), siprofloksasin (5/9) ve piperasilin/tazobaktam dışında test edilen antibiyotiklere dirençli bulundu. PFGE ile salgına dahil olan suşların yakından ilişkili olduğu gösterildi.*

Sonuç: *Sonuç olarak, çoklu ilaç dirençli Elizabethkingia spp. izolatları ülkemizde yoğun bakım ünitelerinde tesbit edilen ve yaşamı tehdit eden bir patojendir. Salgından korunma küçük çaplı salgınların erken bildirilmesi ve gerekli infeksiyon kontrol önlemlerinin alınmasıyla mümkündür.*

Anahtar Kelimeler: *Elizabethkingia meningoseptica; Çok ilaca dirençli bakteri; Salgın; PFGE; MALDI-TOF.*

INTRODUCTION

Elizabethkingia meningoseptica is an opportunistic gram-negative pathogen that has been recognized as a causative agent of hospital-acquired infections[1,2]. It was first defined by King in 1959 as *Flavobacterium meningosepticum*[3]. In 1994, it was named as *Chryseobacterium meningosepticum* by Vandamme et al[4]. Finally, in 2005, *C. meningosepticum* was transferred to a new genus, *Elizabethkingia* gen. nov., with the name *E. meningoseptica* comb. nov. based on 16S rRNA gene sequencing[5]. It is a gram-negative rod shaped, nonfermenting, nonmotile, oxidase-positive bacterium widely distributed in nature (such as fresh water, salt water, or soil). Sink basins and taps, saline solutions, disinfectants, and medical devices, such as feeding tubes, arterial catheters, respirators, intubation tubes, and incubators are potential reservoirs of infection in hospital settings[6].

Infections due to *Elizabethkingia* are mostly reported from immunocompromised patients, such as meningitis, sepsis, bacteremia, pneumonia, endocarditis, skin and soft tissue infections, wound infection, abdominal abscess, ocular infections, sinusitis, bronchitis, epididymitis, dialysis-associated peritonitis, and prosthesis-associated septic arthritis[7-11]. However, prolonged hospital stay, antimicrobial use, and central venous catheter use are important risk factors for *E. meningoseptica* infection[12]. Although the laboratory diagnosis of this organism is complicated, matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS), has good performance in identification of nonfermenting gram-negative organisms (NFGNB)[6].

Treatment of *Elizabethkingia* is challenging because these organisms are intrinsically resistant to antimicrobials, including extended-spectrum beta-lactams, tetracycline, aminoglycosides, and

chloramphenicol due to multiple β-lactams. Three MBL genes have been identified in *E. meningoseptica*: first bla_{CME}, coding for the class D serine-β-lactamase (SBL) CME and bla_{BlaB} and bla_{GOB}, coding for two other MBLs, BlaB (subclass B1) and GOB (subclass B3)[13-15]. *E. meningoseptica* infections are empirically treated with antibiotics such as fluoroquinolones, rifampin, trimethoprim/sulfamethoxazole (TMP–SMX)[16].

Outbreaks with *Elizabethkingia* species have been increasingly reported from different countries, including Turkey[17], United States[18], and England[6]. We aimed to report an outbreak of *E. meningoseptica* acquisition in the intensive care units (ICUs) of a training and research hospital in Turkey and highlight the infection control measures.

MATERIALS and METHODS

Strain Collection

This outbreak occurred in critical care units of 1500-bed hospital (in two weeks). The critical care units have single suite rooms (each with a room sink). Taps on the sinks are all infrared. All doors are equipped with sensors. By contrast, the hospital has a multidisciplinary infection control team who routinely screens hospital-acquired infections and immediately implements appropriate infection control measures. Isolates were obtained from seven tracheal aspirates, one catheter culture, and one blood culture (Table 1).

Strain Identification and Antimicrobial Susceptibility Tests

Bacterial strains were identified using the MALDI Biotyper (Bruker Daltonics, Bremen, Germany)[19]. Minimum inhibitor concentrations (MIC) were determined using the MicroScan automatized system. Antimicrobial added plates were also investigated for minimum inhibitor consantrations by eye at the out of the machine. Results were determined according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (with CLSI criteria for other non-*Enterobacteriaceae*)[20].

Environmental Cultures

Multiple environmental samples were obtained from intubation devices, ventilator machines, humidifier boxes, hospital trolley, nebulizer, bed curtains, bed railings, saline bottles, dialysis fluids, tap water, ICU sinks. For liquid samples, membrane filtration method was used because low-count specimens were expected, and Min 100 mL was used for each sampling. The samples were filtered through the membrane, and the filter was applied directly face up onto the surface of the agar plate and incubated. Swabs were used to collect enviromental cultures and they were inoculated by swab at the time of the collection[21].

Pulse-Field Gel Electrophoresis

Clonal relationships among *E. meningoseptica* strains were investigated using pulse-field gel electrophoresis (PFGE). Genomic DNA was prepared

Table 1. Antibiotic MIC values for *Elizabethkingia meningoseptica* strains

Isolate no	CTX	XM	CAZ	CIP	LEV	CEP	PTc	AK	GEN	IMP	MEM	VA
1	>32	>16	>16	1	≤1	>16	≤8	>16	>8	>8	>8	16
2 HD	>32	>16	>16	1	≤1	>16	≤8	>16	>8	>8	>8	16
3 AT	>32	>16	>16	1	≤1	>16	>64	>16	>8	>8	>8	16
4 GE	>32	>16	>16	1	≤1	>16		>16	>8	>8	>16	16
5 İG	>32	>16	>16	1	≤1	>16	≤8	>16	>8	>8	>16	>16
6 HM	>32	>16	>16	1	1	>16	>16	>16	>8	>8	>16	16
7 ST	>32	>16	>16	1	≤1	>16	64	>16	>8	>8	>16	16
8EK	>32	>16	>16	1	≤5	>16	≤8	>16	>8	>8	>16	16
9 EA	>32	>8	>16	>1	1	>16	>16	>16	>8	>4	>16	16

CTX: Cefotaxime, XM: Sefixime, CAZ: Ceftazidime, CIP: Ciprofloxcacin, LEV: Levofloxcacin, PTc: Piperacyline tazobactam, AK: Amikacin, IMP: Imipenem, MEM: Meropenem, VA: Vancomycin, MIC: Minimum inhibitor consantrations.

in agarose blocks and digested with the restriction enzyme XbaI. The DNA fragments were separated for 20 h at 6 V/cm and 14°C with initial and final pulse times of 0.5 and 30 s, respectively. The cluster analysis was performed using the GelCompar II software (Applied Maths, Sint-Martens-Latem, Belgium). Percentages of similarity were determined using the Dice correlation coefficient, and a dendrogram was produced via the unweighted pair group method with arithmetic mean clustering[22].

RESULTS

Clinical Characteristics of Patients

The mean age of the patients was 66.55 ± 8.53 years. The patients had the following comorbidities: coronary heart disease (n= 5), diabetes mellitus (n= 2), chronic obstructive pulmonary disease, solid tumor (n= 1), cerebrovascular disease and Alzheimer disease (n= 1). Table 1 shows the demographic and clinical characteristics of the patients. All patients were mechanically ventilated and had pneumonia symptoms during receipt of culture results (in addition to pneumonia symptoms, temperature, white cell count, C-reactive protein level, and procalcitonin levels were elevated). Four of the patients died.

Identification of Strains and Antimicrobial Susceptibility Testing

Identification of isolates using MALDI-TOF MS showed *E. meningoseptica* with a relative intensity of matched peak scores of >2.0 for all isolates. All strains were resistant to tested antibiotics, except for levofloxacin (8/9), ciprofloxacin (5/9), and piperacillin/tazobactam (Table 2).

Table 2. Clinical characteristics and outcomes of patients with *Elizabethkingia meningoseptica* infections

Case	Age (years)	Sex	Location	Underlying condition	Antibiotic susceptibility patterns of the strains	Treatment	Death
1	62	F	AICU 1, Catheter culture	Acute renal failure, COPD	Sensitive to LEV/TZP, resistant to CIP, CAZ, VA, GEN	TZP/CIP	No
2	82	F	AICU 2, Tracheal aspirate	Cerebrovascular disease, Alzheimer disease	Sensitive to TZP, LEV resistant to, CIP, CEP, GEN, VA	LEV	Yes
3	58	M	CICU, Catheter culture	Diabetes, COPD	Sensitive to CIP, LEV, resistant to PIP, TZP, VA, GEN	MOX	No
4	60	F	CICU, Tracheal aspirate	Coronary heart disease	Sensitive to CIPLEV-resistant to CAZ, TZP, VA CEF, PIP, GEN, AZT	LEV	Yes
5	57	M	ICU, Tracheal aspirate	Chronic renal failure	Sensitive to TZP, CIP, LEV resistant to PIP, CEF, CAZ, VA, GEN	LEV	No
6	73	F	CICU, Tracheal aspirate	Coronary heart disease	Sensitive to LEV, CIP, resistant to PIP, TZP, VA, GEN, CEF, CAZ	LEV	Yes
7	65	F	ICU, Tracheal aspirate	Coronary heart disease	Sensitive to LEV, GEN resistant to CIP, CEF, TZP, PIP	LEV	Yes
8	67	F	ICU, Tracheal aspirate	Acute renal failure	Sensitive to CIP, LEV, TZP, resistant to CEF, CAZ, GEN,	MOX, PIP	No
9	75	M	ICU, Blood culture	Diabetes mellitus, chronic obstructive pulmonary disease, Ca	Sensitive to LEV, resistant to CIP, CEF, CAZ, TZP, GEN, VA	LEV, TZP	No

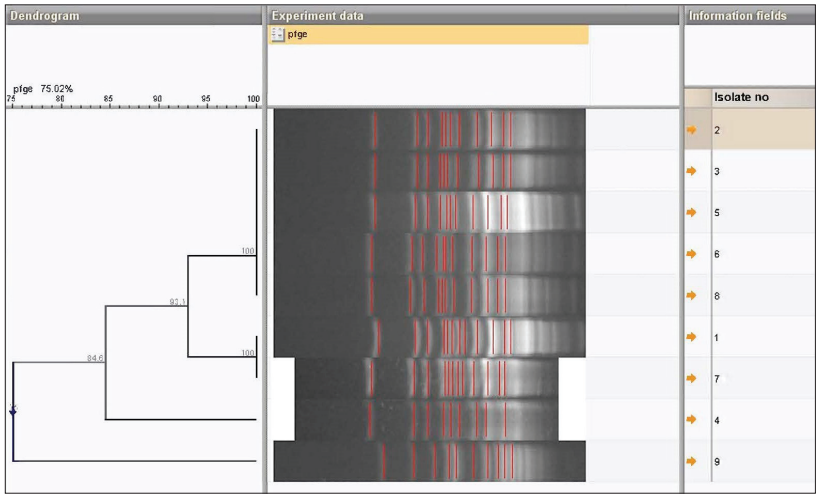

Figure 1. Clonal relationships among *E. meningoseptica* strains isolated from intensive care units (ICUs).

Environmental Cultures

No growth was documented for *E. meningoseptica* from multiple environmental samples listed previously. *Tsukamurella*, *Staphylococcus haemolyticus*, *Staphylococcus epidermidis*, *Staphylococcus cohnii*, *Staphylococcus hominis*, *Delftia acidovorans*, *Pseudomonas aeruginosa*, *Pseudomonas pseudoalcaligenes*, *Pseudomonas oleovorans*, *Streptococcus anginosus*, *Stenotrophomonas maltophilia*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Rothia muciloginosa* were the other strains that was isolated in the ICU's environment during the study period.

PFGE Results

PFGE indicated that strains involved in the outbreak were closely related (similarity> 85%). Only one strain (isolate 9) isolated from blood culture showed an unrelated pattern (Figure 1).

DISCUSSION

E. meningoseptica is an opportunistic pathogen that is well adapted to live for a long period in the ICU environment due to their ability to form biofilms[23]. Health care workers' hands are the most important mode of transmission for this bacteria. Numerous outbreaks related to tap or sink drain contamination by *E. meningoseptica* and other NFGNB were reported[24-26]. Thus, environmental sampling is necessary to identify the possible source. In this study, no growth was documented for *E. meningoseptica* from the multiple environmental samples listed previously. Saline water or dialysis fluids that were used and discarded could not be sampled, and these are thought to be potential sources of this outbreak. The outbreak occurred in different ICUs; thus, it can be due to these commonly used solutions or cleaning staff exchange between different parts of the hospital. By contrast, the most common strains isolated in the ICU environment during the study period were *Pseudomonas* spp. *Acinetobacter* spp. and *S. maltophilia* and *Staphylococcus* species (Table 2). Unfortunately, no direct relationship was detected between contamination of ICU environment and colonization or NFGB infection of patients in this study period.

The most widely used microbial identification systems include API/ID32 Phenotyping Kits (BioMérieux, Marcy l'Etoile, France), Phoenix 100 ID/AST Automated Microbiology System (Becton Dickinson Co., Sparks, MD, USA), Vitek 2 Automated Identification System (BioMérieux) are the most widely used identification systems for identifying *Elizabethkingia* species. MALDI-TOF MS systems with expanded spectrum databases could identify *E. anophelis* and *E. meningoseptica*, while these systems cannot distinguish between the other species of the genus *Elizabethkingia*[11,13,27,28].

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ID/AST Automated Microbiology System (Becton Dickinson Co., Sparks, MD, USA), Vitek 2 Automated Identification System (bioMйrieux) are the most widely used identification systems for identifying *Elizabethkingia* species. MALDI–TOF MS systems with expanded spectrum databases could identify *E. anophelis* and *E. meningoseptica*, while these systems cannot distinguish between the other species of the genus *Elizabethkingia*[11,13,27,28]. This outbreak occurred after MALDI-TOF MS started to be used, suggesting that outbreaks with microorganisms that are difficult to define, such as *Elizabethkingia*, may be overlooked in many hospitals. We accepted the highest scores (2.1) for these patients as *E. meningoseptica*. Otherwise, 16S rRNA gene sequencing is gold standard method for identifying *Elizabethkingia* species[29]. Unfortunately, as a limitation of our study, 16S RNA sequencing could not be performed for the clinical isolates because of economic burdens.

The appropriate choice of effective antimicrobial agents for the treatment of *E. meningoseptica* is difficult. The antimicrobial susceptibility profile of uncommon NFGNB was evaluated using the SENTRY antimicrobial surveillance program. Based on the results of the program, all *Chryseobacterium* spp. isolates were resistant to imipenem and meropenem, and 75% of strains were also resistant to amikacin. TMP-SMX was active against only 36.4% of *Chryseobacterium* spp. strains, and gatifloxacin and levofloxacin were the most active compounds[30]. In accordance with the program, in this study, strains were resistant to all tested antibiotics, except for fluoroquinolones. Therefore, levofloxacin was used to treat the infections. However, in a recent study, a resistance gene (*gyrB*) was discovered in an Elizabethkigia strain, so AST is necessary to treat the infection with fluoroquinolones[23].

The successful use of vancomycin to treat patients with infections caused by *E. meningoseptica* was reported in the literature but with high MICs. However, several clinical failures were reported for vancomycin treatment. In a study that investigated effect of vancomycin alone and with different regimens against *Elizabethkingia* meningoseptica bacteraemia, infections could not be treated with intravenous (i.v.) vancomycin-only therapy[31]. In this study, vancomycin MICs were between 16 and ≥64 μg/m[32].

By contrast, potential alternative treatment options for *Elizabethkingia* infections have been reported[33]. Chan et al. showed that combination therapy with piperacillin/tazobactam and TMP-SMX or fluroquinolone has high rates of microbiological cure for *E. meningoseptica* meningitis cases[34].

By PFGE, patterns that differ from the outbreak pattern by two or three fragment differences were considered to be subtypes of the outbreak pattern[35]. In this study, based on PFGE results, isolates had 85% similarity, except a strain was different from the others with 75% similarity. Although all strains were isolated form tracheal aspirates, this subtype was isolated form blood. In accordance with this study, isolates from bloodstream infections were found as clonally unrelated in a study conducted in northern Taiwan[36].

This outbreak was observed in adult ICUs. However, it did not expand to the neonatal ICUs due to early infection control measures. In Turkey, the first *E. meningoseptica* outbreak was reported in 2003 by Güngör et al. in an intensive neonatal care unit[37]. Subsequently in 2008, Ceyhan et al. described an outbreak in both neonatal and non-neonatal pediatric patients. They isolated *C. meningosepticum* strains from the hand culture of a senior resident, a powdered infant formula, an electrical button, and a computer keyboard in general use. Thus, in that study, findings suggest that health care worker hands are the most important source for the outbreaks[17]. As a limitation of this study, hand cultures could not be done since high staff changing circulation between the ICUSs and services.

Implementation of appropriate infection control measures is an essential part of the outbreak management. In this study, the infection control team was alarmed, and all infection control measures were implemented. All equipment were cleaned and disinfected. All open solutions were discarded. All environmental surfaces, including

beds, floors, door handles, and cleaning equipment (e.g., mops and buckets) were disinfected. In addition, health care workers and cleaners were examined by doctors and infection control nurses. After implementation of appropriate infection control measures, the outbreak was contained.

In conclusion, intrinsically multiple drug-resistant *Elizabethkingia* spp. isolates can be a common life-threatening pathogen in ICUs in our country, and prevention is possible by early notification of small-scale outbreaks and necessary infection control measures. Collaboration between microbiologists and clinicians is essential to control hospital outbreaks[5,6]. By contrast, further studies are needed to discover alternative treatment options for *Elizabethkingia* spp. isolates.

ETHICS COMMITTEE APPROVAL

This study was approved by Adana City Training and Research Hospital Clinical Research Ethics Committee (Date: 28.02.2018, Decision no: 170).

CONFLICT of INTEREST

None of the authors had conflict of interest.

AUTHORSHIP CONTRIBUTIONS

Concept and Design: FE, FK, TB

Data Collection or Processing: FE, TB, MMÖ, YK, NÜ

Analysis/Interpretation: FE, TB, NÜ

Literature Search: FE, TB

Writing: FE

Final Approval: All of authors

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