

Occurrence of antimicrobial resistance genes *sul* and *dfrA12* in hospital environmental isolates of *Elizabethkingia meningoseptica*

Xiaobing Jiang · Dapeng Wang · Yuxiao Wang ·
He Yan · Lei Shi · Lijun Zhou

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Abstract The aim of this study was to investigate the prevalence, antimicrobial susceptibility and resistant determinants of *Elizabethkingia meningoseptica* in a Beijing hospital. Four hundred and eighty-seven samples from medical devices, hospital surfaces and medical staff hands were collected. In total, 26 *E. meningoseptica* isolates were obtained. The sinks, faucets, and drains accounted for more than half of the total number of isolates recovered. Antimicrobial susceptibility testing revealed that 24 isolates were resistant to one or more antibiotics. All strains were susceptible to piperacillin/tazobactam and vancomycin. Although the trimethoprim/sulfamethoxazole has previously been shown to exhibit good activity against *E. meningoseptica*, in our study 15 strains were resistant to it. We detected trimethoprim/sulfamethoxazole resistance determinants using PCR; six isolates possessed the *sulII* gene and four possessed the *sulIII* gene, whilst the *dfrA12* gene was detected in only one of them. Pulsed-field gel electrophoresis (PFGE) analysis showed 9 distinct types and one dominant pattern with 12 strains was found. Our data indicate that antimicrobial resistant *E. meningoseptica* strains exist in the hospital environment and susceptibility testing revealed that vancomycin and piperacillin/tazobactam was the most effective antibiotics. These results have practical significance for treatment of *E. meningoseptica* infection.

Keywords *Elizabethkingia meningoseptica* · Hospital environment · Antimicrobial susceptibility · Trimethoprim/sulfamethoxazole

Introduction

Elizabethkingia meningoseptica (previously *Flavobacterium meningosepticum*, *Chryseobacterium meningosepticum*; Kim et al. 2005) is a Gram-negative, oxidase-positive, and non-glucose-fermenting bacterium which is widely distributed in soil, plants and water, but is not normally present in human microflora (Bernardet et al. 2005). *E. meningoseptica* is recognized as an occasional but serious opportunistic bacterial pathogen to humans, giving rise to meningitis, pneumonia, septic arthritis, endocarditis and conjunctivitis (Xie et al. 2009).

Environmental studies have revealed that this species can survive in water supplies, and often colonizes sinks, basins and taps, thus creating potential reservoirs of infection within hospitals (Hoque et al. 2001). *E. meningoseptica* can colonize patients via fluid contaminated medical devices (e.g., respirators, intubation tubes, mist tents, humidifiers, incubators for newborns and ice chests; Du Moulin 1979; Hoque et al. 2001). It can also be spread by wet and dry materials and surfaces, including hospital staff hands (Ceyhan et al. 2008). Nosocomial transmission of *E. meningoseptica* among immunocompromised adults in intensive care units (ICUs) has also been reported (Pokrywka et al. 1993).

Antimicrobial susceptibility data for *E. meningoseptica* is limited, since this pathogen has rarely been isolated from clinical specimens. Treatment of *E. meningoseptica* infections is challenging because of its increasing resistance to several classes of antimicrobial agents, for

X. Jiang · H. Yan · L. Shi
College of Light Industry and Food Sciences, South China
University of Technology, Guangzhou 510640, China

X. Jiang · D. Wang · Y. Wang · L. Zhou (✉)
Central Laboratory of Navy General Hospital,
Beijing 100048, China
e-mail: zhlj63@yahoo.com.cn

example, aminoglycosides, tetracyclines, chloramphenicol and erythromycin (Kirby et al. 2004; Lin et al. 2004). However, some fluoroquinolones have shown favourable results (Fraser and Jorgensen 1997; Kirby et al. 2004). Vancomycin has been successfully used for treating meningitis in infants in the past, but its efficacy against *E. meningoseptica* has been questioned (Fraser and Jorgensen 1997). Consequently, the lack of a broad spectrum agent against *E. meningoseptica* means that antimicrobial susceptibility testing is required to ensure effective treatment outcomes.

Trimethoprim and sulfamethoxazole share both a wide antibacterial spectrum including common urinary tract pathogens, respiratory tract pathogens, skin pathogens, as well as certain enteric pathogens (Huovinen et al. 1995). Because of the wide range of clinical indications, the combination of trimethoprim and sulfamethoxazole has been used extensively everywhere in the world. In previous studies trimethoprim/sulfamethoxazole has shown good activity against *E. meningoseptica* in vitro and has been considered as the potential therapy for *E. meningoseptica* infections (Lin et al. 2004, 2009). The survey of SENTRY Antimicrobial Surveillance Program indicated that the rate of resistance to trimethoprim/sulfamethoxazole was 20.8 % in *E. meningoseptica* isolates (Kirby et al. 2004). The lower resistance rate to trimethoprim/sulfamethoxazole (3.1 %) was observed in clinical isolates of *E. meningoseptica* from Taiwan (Lin et al. 2009). However, susceptibility to trimethoprim/sulfamethoxazole appears variable. Weaver et al. (2010) reported that among the 18 patient *E. meningoseptica* isolates 12 were resistant to trimethoprim/sulfamethoxazole. In addition, studies demonstrated the rate of trimethoprim/sulfamethoxazole resistance in *E. meningoseptica* isolates was high in some provinces of China, e.g., 80 % in Hebei, 43.9 % in Liaoning, 83.8 % in Zhejiang, and 49.1 % in Tianjin (Jia and Qin 2002; Wang et al. 2005; Yang et al. 2008; Zhang 2008). The usefulness of trimethoprim/sulfamethoxazole against *E. meningoseptica* infections has been questioned. To counter bacterial resistance it is essential to understand the molecular background of resistance mechanisms. The acquisition of trimethoprim/sulfamethoxazole resistance determinants by horizontal transfer, mediated by mobile elements, is thought to play an important role in the spread of resistance. However, very little is known about the capacity of *E. meningoseptica* to carry trimethoprim/sulfamethoxazole resistance determinants.

In this study, we collected *E. meningoseptica* isolates from the hospital environment and investigated their susceptibility to various antimicrobial agents. We detected the presence of class 1 and 2 integrons and the genetic determinants responsible for trimethoprim/sulfamethoxazole resistance in this pathogen.

Materials and methods

Sampling

Sampling was performed over a 5-month period (March through July 2011) at a Beijing hospital. In this study, ICUs, neonatal department, obstetric department, operating rooms, catheterization rooms, hemodialysis rooms, and wards were selected for testing. Specimens were collected from various hospital surfaces, and inanimate objects, such as bed frames, bed sheets, night tables, door handles, lockers, light switches, tables, chairs, sinks, faucets, drains, stands for infusion apparatus, infant incubators, medicine cabinets, instruments, respirators and mobile instrument tables. The samples were taken with sterile cotton swabs moistened with sterile brain heart infusion broth (BHI; Huankai Ltd., Guangzhou, Guangdong, China). The samples were transported to the research laboratory immediately and cultures were then spread on BHI agar plates and incubated at 37 °C for 24–48 h.

Species identification

After incubation, colonies were streaked for purity and subcultured on BHI plates. All oxidase-positive Gram-negative isolates were selected for identification. Initially the isolates were typed by PCR amplification of 16S rRNA gene. The primers of 16S rRNA gene and PCR program were performed as described previously (Moreno et al. 2002). The isolates were PCR typed for identity and then confirmed using the VITEK 2 bacterial identification system (BioMérieux, Marcy l'Etoile, France).

Antimicrobial susceptibility testing

E. meningoseptica susceptibility to antimicrobials was determined using the broth microdilution method (Srinivasan et al. 2008). The Clinical and Laboratory Standards Institute (CLSI) MIC breakpoint for *Staphylococcus* spp. was applied to vancomycin (Lin et al. 2009). The CLSI MIC breakpoints for non-*Enterobacteriaceae* were applied to the other antimicrobials tested here.

PCR and gene sequence analysis

The isolates were screened for presence of the *suII*, *suIII* and *dfrA1–dfrA12* genes which are responsible for trimethoprim/sulfamethoxazole resistance, using the PCR primers and conditions as described previously (Chen et al. 2004). In addition, isolates were screened for the presence of class 1 and class 2 integrons using previously described PCR methods (Yaqoob et al. 2011). The PCR products were sequenced at Invitrogen Biotechnology Co., Ltd. (Beijing, China). The

Table 1 Distribution of *E. meningoseptica* strains isolated from the hospital environment

Sampling site	No. of isolates
Sink	7
Faucet	5
Drain	3
Table at ICUs	2
Door handle	2
Night table	2
Hand specimen	1
Mobile instrument table	1
Bed frame	1
Bed sheet	1
Locker	1

resulting DNA sequence data were compared to GenBank database sequences using the BLAST algorithm available at the National Center for Biotechnology Information web site (www.ncbi.nlm.nih.gov).

Pulsed-field gel electrophoresis (PFGE)

PFGE of all isolates was performed as described previously (Weaver et al. 2010). *E. meningoseptica* DNA plugs were lysed, washed and digested with 50 U of the restriction enzyme *ApaI* (CHIMERx, Madison, Wisconsin, USA) at 30 °C overnight. The PFGE was performed for 22 h at 14 °C with the CHEF MAPPER apparatus (Bio-Rad, Hercules, CA, USA) at 6 V/cm with switch time ranging from 5 to 35 s. The gel was stained in ethidium bromide solution and photographed using a Bio-Rad Gel Doc. PFGE patterns were compared using Quantity One software (Bio-Rad).

Results

Bacterial isolation

A total of 32 nursing staff hand samples and 455 samples from hospital surfaces and medical devices were screened for *E. meningoseptica*. Twenty-six isolates were obtained from the hospital environment and hand specimens. The overall isolation rate of *E. meningoseptica* was 5.3 %. The distribution of the isolates from various samples is presented in Table 1.

Antimicrobial susceptibility

The results of the sensitivity tests for the isolates to 17 different antibiotics are shown in Table 2 and Fig. 1. Out

of 26 isolates tested, 24 (92.3 %) were resistant to one or more antibiotics, while only two (7.7 %) were fully susceptible. All strains were susceptible to piperacillin/tazobactam and vancomycin. Resistance to trimethoprim/sulfamethoxazole (57.7 % of the isolates were resistant), resistance to aztreonam (53.8 %), and resistance to amikacin (53.8 %) were observed most often, whereas resistance to piperacillin, levofloxacin, and ciprofloxacin were observed less frequently.

Detection of integrons and trimethoprim/sulfamethoxazole resistance determinants

Class 1 and class 2 integrons bearing antibiotic resistance determinants were not detected in the *E. meningoseptica* strains. Of the 15 trimethoprim/sulfamethoxazole resistant isolates, six isolates possessed the *suI* gene and four possessed the *suII* gene. None of the susceptible isolates yielded positive *suI* PCR products. Among the four *suII* positive isolates, one harboured the *dfrA12* gene which was responsible for trimethoprim resistance.

PFGE

PFGE analysis revealed 9 different pulsotypes among the 26 *E. meningoseptica* tested (Fig. 1). The dominant pattern I included 12 isolates, while the majority of remaining types identified (n = 7) had 2 or 1 strains. There was no complete correlation between resistance pattern and PFGE.

Discussion

Hospitals contain a reservoir of microorganisms, many of which are multiply resistant to antimicrobials (Dancer 1999). Hospital staff and patients are considered the most important sources of nosocomial microorganisms, however there is growing evidence that the hospital environment itself is also of substantial importance (Dancer 1999). The aim of this short-term study, therefore, was to provide insight into the prevalence of *E. meningoseptica* in a hospital environment in China.

In present study, 487 samples were collected for environmental surveillance, and twenty-six *E. meningoseptica* isolates were obtained. Especially the sinks, faucets, and drains, accounted for 57.7 % of the total number of isolates recovered, which was consistent with the previous report that *E. meningoseptica* often colonizes in humid environments (Du Moulin 1979; Hoque et al. 2001). Three isolates were recovered from the surfaces of tables in ICUs and one from hand specimens of a nurse. Although there were no reported cases of infection associated with *E. meningoseptica* in the hospital during our sampling periods, the

Table 2 Antimicrobial resistance of *E. meningoseptica* isolates from hospital environment

Antimicrobial agent	Breakpoint concn ($\mu\text{g/mL}$)	Susceptible (%)	Intermediate susceptibility (%)	Resistant (%)
Piperacillin	128	22 (84.6)	2 (7.7)	2 (7.7)
Piperacillin/tazobactam	128/4	26 (100)	0	0
Ceftazidime	32	13 (50.0)	0	13 (50.0)
Ceftriaxone	64	16 (61.5)	0	10 (38.5)
Cefepime	32	15 (57.7)	0	11 (42.3)
Aztreonam	32	12 (46.2)	0	14 (53.8)
Imipenem	16	15 (57.7)	0	11 (42.3)
Meropenem	16	13 (50.0)	0	13 (50.0)
Amikacin	64	12 (46.2)	0	14 (53.8)
Gentamycin	16	14 (53.8)	0	12 (46.2)
Tobramycin	16	13 (50.0)	0	13 (50.0)
Ciprofloxacin	4	19 (73.1)	1 (3.8)	6 (23.1)
Levofloxacin	8	24 (92.3)	0	2 (7.7)
Trimethoprim/sulfamethoxazole	4/76	11 (42.3)	0	15 (57.7)
Vancomycin	16	26 (100)	0	0
Tetracycline	16	16 (61.5)	4 (15.4)	6 (23.1)
Chloramphenicol	32	16 (61.5)	2 (7.7)	8 (30.8)

potential risk of *E. meningoseptica* infection in hospital patients still existed. Since *E. meningoseptica* was isolated from medical personnel hand samples, it is possible that transmission of the bacterium could occur between nursing staff and patients. Environmental surfaces and shared medical devices may also place patients at risk of *E. meningoseptica* infection. Our study indicates that surveillance of potential reservoirs of *E. meningoseptica* is important, as is maintenance of strict infection control measures in the operating rooms and ICUs (Lin et al. 2009).

According to previous studies, quinolones, rifampin, and trimethoprim/sulfamethoxazole were most active against *E. meningoseptica* in vitro, whilst the β -lactams, aminoglycosides and carbapenems exhibited poor activity (Kirby et al. 2004; Lin et al. 2004; Hung et al. 2008). Most *E. meningoseptica* clinical isolates have been reported as resistant or even multiresistant to antibiotics (Xie et al. 2009). Since more than 90 % of the *E. meningoseptica* strains tested here were resistant to antimicrobials, it is clear that the isolates originating within this hospital displayed a similarly high level of resistance as known clinical isolates of this bacterium (Lin et al. 2004, 2009). We found that among the β -lactams, the most active agents overall were the piperacillin/tazobactam combination, whilst cephalosporins and carbapenems exhibited poor activity against these isolates. Moreover, the strains showed substantially high rates of resistance to aminoglycosides. The quinolones were once considered the treatment of choice

for *E. meningoseptica* infections (Hung et al. 2008). In our study, ciprofloxacin and levofloxacin also showed the higher potency of activity against the overall collection of *E. meningoseptica*. In addition, some reports have documented the efficacy of vancomycin for treating *E. meningoseptica* infections, hence this antimicrobial agent was recommended as the therapeutic choice (Di Pentima et al. 1998). Our study confirmed the effectiveness of vancomycin against all *E. meningoseptica* strains, which is consistent with previous results (Di Pentima et al. 1998; Xie et al. 2009). We noted that the MICs for the *E. meningoseptica* strains tested were slightly different from other recent studies (Kirby et al. 2004; Lin et al. 2009); this is most likely due to our small sample size. What is clear is that there is no absolutely optimal regimen to treat *E. meningoseptica* infections, hence antimicrobial therapy should be based on the MIC data from standardized susceptibility tests.

PFGE analysis revealed 9 distinct types among *E. meningoseptica* strains. The dominant pattern I accounted for 46.2 % of all isolates. Such clear predominance of one clone indicates that *E. meningoseptica* may be capable of permanent existence within the hospital environment. One the whole, there was no complete correlation between resistance pattern and PFGE. Some isolates belonging to the same PFGE patterns had variable resistance profiles. On the other hand, some isolates with the different PFGE types shared the common resistance profiles.

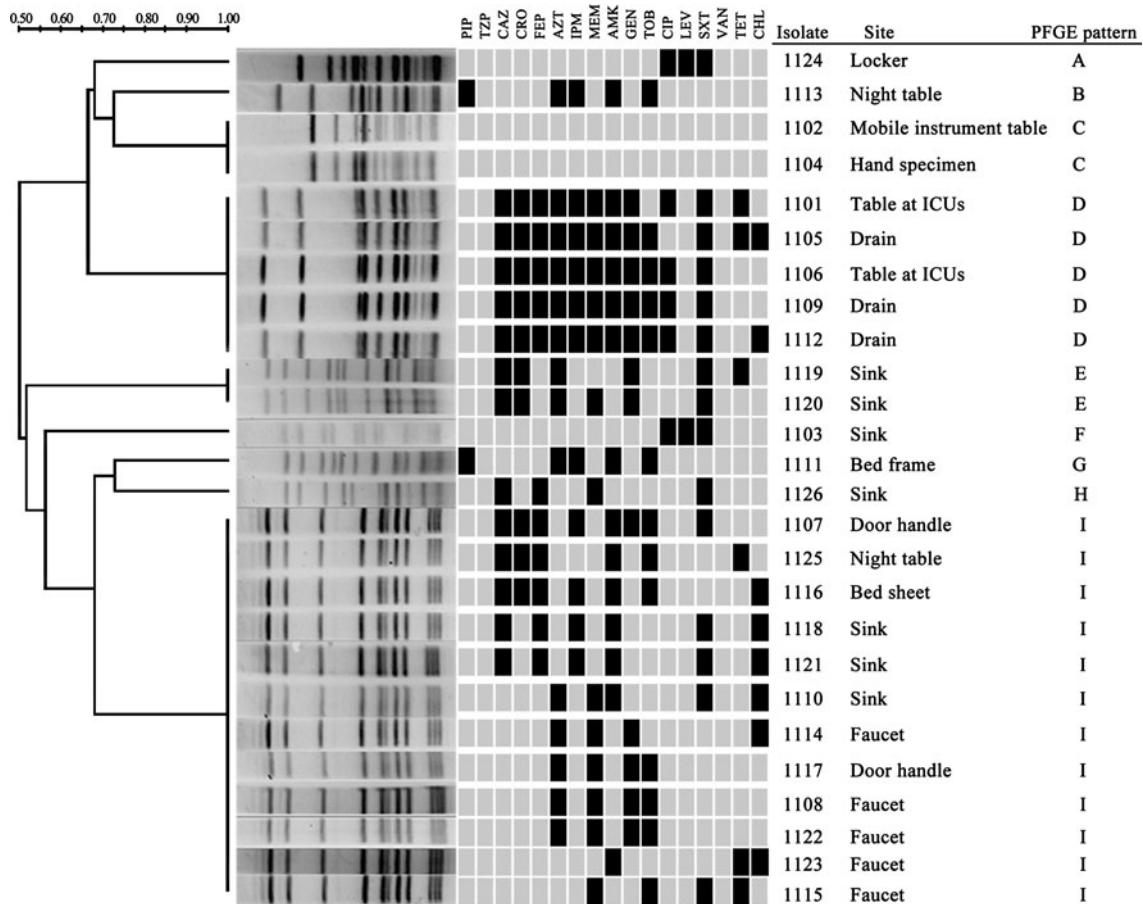


Fig. 1 PFGE patterns and antimicrobial resistance profiles of *E. meningoseptica* isolates. Black boxes indicate resistance to a particular antimicrobial. PIP piperacillin; TZP piperacillin/tazobactam; CAZ ceftazidime; CRO ceftriaxone; FEP cefepime; AZT

aztreonam; IPM imipenem; MEM meropenem; AMK amikacin; GEN gentamycin; TOB tobramycin; CIP ciprofloxacin; LEV levofloxacin; SXT trimethoprim/sulfamethoxazole; VAN vancomycin; TET tetracycline; CHL chloramphenicol

Interestingly, studies have shown that trimethoprim/sulfamethoxazole exhibited good activity against *E. meningoseptica* (Kirby et al. 2004); however, more than 50.0 % of the isolates were resistant to this agent in our study. Of the 26 isolates, six isolates possessed the *suII* gene and four possessed the *suIII* gene. Among the four *suIII* positive isolates, one harboured the *dfrA12* gene. None of the corresponding resistance genes evaluated were detected in other resistant isolates, suggesting that the possibility of other resistance mechanisms are responsible for trimethoprim/sulfamethoxazole resistance in these isolates. Since the *suII* gene was commonly found to be associated with integron, we investigated whether the *E. meningoseptica* isolates carried integrons. Unfortunately no class 1 or class 2 integron bearing antibiotic resistance determinants were detected in all of the isolates. Given that the sulfamethoxazole resistance gene *suIII* is predominantly located on plasmids (Enne et al. 2001), we speculate that these resistant genes might be located on resistance plasmids in the resistant

E. meningoseptica strains. The acquisition of drug resistance genes by horizontal transfer, mediated by plasmids, is currently thought to play an important role in the development of multi-drug resistance. However, additional longer-term studies conducted in more locations of these resistance genes are needed to validate this hypothesis.

In summary, we collected twenty-six *E. meningoseptica* isolates from various hospital environmental samples and hand specimens, thus indicating a need for greater infection control surveillance. The PFGE typing results presented indicate that some *E. meningoseptica* strains may be capable of adaptation to hospital environment conditions and continuous existence in this environment. Our results show that vancomycin and piperacillin/tazobactam are most active against *E. meningoseptica*, however, trimethoprim/sulfamethoxazole combination may not represent effective option. Nevertheless, the use of antimicrobial agents to treat infections caused by this bacterium needs further clinical evaluation.

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