Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Short Communication

Multiresistant *Elizabethkingia meningoseptica* infections in tertiary care

Maj Inam Danish Khan^{a,*}, Lt Col Mahima Lall^b, Col Sourav Sen^c,
Maj S.M. Ninawe^d, Surg Lt Cdr P. Chandola^d

^a Resident (Microbiology and Molecular Medicine), Army Hospital (R&R), New Delhi 110010, India

^b Classified Specialist (Microbiology and Molecular Medicine), Army Hospital (R&R), New Delhi 110010, India

^c Professor and Head, (Microbiology and Molecular Medicine), Army Hospital (R&R), New Delhi 110010, India

^d Resident (Microbiology & Molecular Medicine), Army Hospital (R&R), New Delhi 110010, India

ARTICLE INFO

Article history:

Received 20 September 2013

Accepted 6 February 2014

Available online 3 April 2014

Keywords:

Elizabethkingia meningoseptica

Intrinsic multiresistance

Paradoxical antimicrobial

susceptibility

characterization in routine diagnostic settings. *Elizabethkingia* is an eponym from its discoverer Elizabeth O. King. Earlier classified under CDC group IIa, *Flavobacterium* (1959) and *Chryseobacterium* (1994), it was assigned the genus *Elizabethkingia* under Flavobacteriaceae family in 2005 based on 16s rRNA phylogenetic studies.³ Few infections of *E. meningoseptica* have been reported from India. We report four cases of *Elizabethkingia meningoseptica* from an apex tertiary care setup.

Case 1

A 47-year-old male patient with Contrast induced nephropathy – Chronic Kidney Disease (CKD) – End stage renal disease on maintenance hemodialysis since 2007 was transplanted an unrelated cousin donated renal graft in Nov 2010. Induction with basiliximab was followed by maintenance immunosuppression by methylprednisolone, tacrolimus and mycophenolate mofetil. Sulfamethoxazole-Trimethoprim (SXT) prophylaxis was given till six months post transplant. The patient developed left moderate multiloculated pleural effusion along with precarinal, preaortic and paraaortic lymphadenopathy in Oct 2012. On clinical suspicion, non-rifampin based anti-tubercular (HZE) treatment was administered for 8 months on out-patient basis. In Jul 2013, the patient developed left pleural empyema. Laboratory investigations revealed hemoglobin 10.5 g/dl; leukocyte count 13,500/dl, 78% polymorphs, 20% lymphocytes, 2% eosinophils; and ESR 30 mm at 1 h. Chest

Introduction

Elizabethkingia meningoseptica is an emerging pathogen known to cause meningitis, pneumonia, endocarditis, bacteremia, sepsis, wound, soft tissue, abdominal, respiratory and ocular infections, dialysis associated peritonitis and prosthesis associated septic arthritis; especially in immunodeficient hosts.^{1,2} It is ubiquitous Gram-negative nonfermenter found in soil, plants, fresh and marine water, food products, hospital environments, chlorinated water and may colonize sinks and taps causing both community and nosocomial infections, though does not constitute human microflora.¹ It has many attributes of Gram-positive bacteria which confuse its

* Corresponding author. Tel.: +91 8860179989.

E-mail address: titan_afmc@yahoo.com (I.D. Khan).
<http://dx.doi.org/10.1016/j.mjafi.2014.02.002>

0377-1237/© 2014, Armed Forces Medical Services (AFMS). All rights reserved.

Fig. 1 – Kirby–Bauer disk diffusion showing inhibition zones and inducible clindamycin resistance (Left/clockwise: E – Erythromycin, CD – Clindamycin, CIP – Ciprofloxacin, LZ – Linezolid, VA – Vancomycin, TEI – Teicoplanin, LE – Levofloxacin; Right/clockwise: IPM – Imipenem, COT – Cotrimoxazole, LZ – Linezolid, CD – Clindamycin, LE – Levofloxacin, VA – Vancomycin, CL – Colistin; Figures represent drug concentrations in $\mu\text{g/ml}$).

radiograph revealed left pleural effusion with subjacent consolidation. Approximately 520 ml effusion fluid drained through pigtail catheter on the first day revealed long slender Gram-negative bacilli 5–20/OIF with 5–10 polymorphs/HPF on direct uncentrifuged smears and grew *E. meningoseptica* which was isolated on two more subsequent OPD visits. Empyema fluid revealed total cells 26,500/dl (polymorphs 14,700/dl), protein 4.3 g/dl, albumin 2 g/dl, LDH 3474 U/l and ADA 61.8 U/l.

E. meningoseptica was isolated on sheep blood and chocolate agars (both bioMérieux, France) while there was no growth on MacConkey agar. Culture of pigtail catheter revealed scanty shiny raised colonies while blood cultures were negative. Non-motile nonsporing noncapsulated Gram-negative bacilli, positive for catalase, oxidase, indole and ONPG; oxidative in O–F glucose and negative for urease and nitrate were seen. Gram-negative identification card of VITEK 2 compact automated system (bioMérieux, France)

provided 99% identification probability. Gram-positive card tested positive for β galactosidase, α glucosidase, arginine dihydrolase, phosphatidylinositol phospholipase C, L-pyrrolidonyl arylamidase (PYR) and other amino acid arylamidases, although it could not identify any organism. Inhibition zones by Kirby–Bauer disk diffusion exhibited variability in zone sizes on repeat test and could not be conclusively interpreted. However, flattening of clindamycin inhibition zone proximal to erythromycin indicated inducible clindamycin resistance (Fig. 1). Phenotypic combined disk tests (PCDT) for Extended Spectrum β -Lactamase (ESBL) + Amp C (Rosco Diagnostica, Denmark, Ref 98008; Lot 1203-1); and *Klebsiella pneumoniae* carbapenemase (KPC) + Metallo- β -Lactamase (MBL) (Rosco Diagnostica, Ref 98006; Lot 1202-1) interpretations as per CLSI and/or European Committee on Antimicrobial susceptibility testing (EUCAST) guidelines revealed coexistent ESBL, Amp C and MBL (Fig. 2).⁴ Susceptibility only to SXT with minimal

Fig. 2 – Detection of coexistent Extended spectrum, Amp C and Metallo β -Lactamases (Rosco Diagnostica, Denmark) (CTX30–Cefotaxime 30 $\mu\text{g/ml}$, CTX + C–Cefotaxime + Clavulanic acid, CTXCC–Cefotaxime + Clavulanic acid + Cloxacillin, MRP10–Meropenem 10 $\mu\text{g/ml}$, MRPDP–Meropenem + Dipicolinic acid, MRPCX–Meropenem + Cloxacillin, MRBPB– Meropenem + Boronic acid) (Interpretation explained in second paragraph of discussion).

inhibitory concentration (MIC) 40 µg/ml was confirmed by Vitek 2 compact AST-N090 card (Fig. 3). Prompt treatment with SXT lead to improvement.

Case 2

An 8-year young male patient was diagnosed with CKD on continuous ambulatory peritoneal dialysis (CAPD) since 18 months, was diagnosed a case of peritonitis two days after admission for conservative management. Immediate and repeat CAPD fluid cultures revealed *E. meningoseptica* susceptible to cefoperazone-sulbactam and nalidixic acid with MIC 16 µg/ml each by Vitek AST-N090 card. Coexistent ESBL, Amp C and MBL were seen by PCDT (Rosco Diagnostica, Denmark). Prompt treatment with cefoperazone-sulbactam led to improvement.

Case 3

A 23 year old female patient underwent a Medical Termination of Pregnancy (MTP) by suction and evacuation for suspected blighted ovum or missed abortion in primary care settings. Post procedure fever, bleeding and an ultrasonographically suspected perforation was followed up by a diagnostic laparoscopy revealing adherent gut and omentum over uterine perforation and empirical antimicrobials were administered. An exploratory laparotomy 14 days after MTP revealing chronic left tubal ectopic pregnancy was followed by left

salpingectomy at our center. Two sets of blood culture bottles sent on the day of surgery revealed pure growth of *E. meningoseptica* four days after incubation, susceptible to SXT (MIC 80 µg/ml) by Vitek AST-N090 card which was confirmed from repeat culture. Coexistent ESBL, Amp C and MBL were seen by PCDT (Rosco Diagnostica, Denmark). She was managed by SXT along with an aggressive post-operative cover by Piperacillin-tazobactam, amikacin, teicoplanin and metronidazole.

Case 4

A 19 year old female patient, known case of severe Mitral Regurgitation underwent Mitral Valve Replacement. *Elizabethkingia meningoseptica* susceptible to SXT (MIC 160 µg/ml) by Vitek AST-N090 card along with coexistent ESBL, Amp C and MBL by PCDT, was isolated from blood on third post-operative day. Unfortunately, the patient had a fatal outcome because of post-operative complications.

Discussion

E. meningoseptica may present diagnostic and therapeutic challenges and even surpass routine laboratory identification by standard techniques. It may be confused with oxidase positive multiresistant nonfermenters such as *Pseudomonas*, *Burkholderia*, *Sphingomonas*, *Sphingobacterium* and *Empedobacter*, which may not be identified to genus/species level by

Location: OPD		Physician:	
Lab ID: 201307/3184		Isolate Number: 1	
Selected Organism : Elizabethkingia meningoseptica			
Source: BMP YMA		Collected:	
Comments:			

Identification Information	Analysis Time: 4.00 hours	Status: Final
Selected Organism	99 % Probability	Elizabethkingia meningoseptica
Organism Quantity:	Bionumber:	5462101100271020
ID Analysis Messages		

Susceptibility Information	Analysis Time: 10.50 hours		Status: Final		
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>= 32	R	Meropenem	>= 16	R
Amoxicillin/Clavulanic Acid	>= 32	R	Amikacin	>= 64	R
Piperacillin/Tazobactam	>= 128	R	Gentamicin	>= 16	R
Cefuroxime	>= 64	R	Nalidixic Acid	>= 32	R
Cefuroxime Axetil	>= 64	R	Ciprofloxacin	>= 4	R
Ceftriaxone	>= 64	R	Tigecycline	>= 8	R
Cefoperazone/Sulbactam	>= 64	R	Nitrofurantoin	>= 512	R
Cefepime	>= 64	R	Colistin	>= 16	R
Ertapenem			Trimethoprim/Sulfamethoxazole	40	S
Imipenem	>= 16	R			

+ Deduced drug * = AES modified ** = User modified

AES Findings	
Confidence:	Analysis not performed

Fig. 3 – Vitek 2 automated report on identification and susceptibility of *Elizabethkingia meningoseptica*.

many laboratories.^{5,6} Non-diffusible yellow pigment and a surrounding greyish discoloration due to proteases and gelatinases may be seen.⁵ It is esculin and DNase positive. Identification by manual techniques is demanding in time and effort and employs many uncommon tests and reagents. Molecular methods such as DNA hybridization, 16s rDNA amplification with species specific primers and pulsed-field gel electrophoresis are highly specific for identification, resistance characterization, strain typing and outbreak investigation, but are limited to advanced laboratories with higher acquisition, maintenance and output capacities.¹ Comprehensive diagnosis by phenotypic automated systems is not only advantageous over both standard manual and molecular techniques, but may also form the only available modality for identification of *Elizabethkingia* especially for medium sized laboratories. These systems facilitate rapidity, compaction, storage, execution, standardization, quality control and reproducibility of results. Limitations of misidentification of *Elizabethkingia* for *Sphingobacterium* and *Aeromonas salmonicida* have been reported for Vitek 2 and ID32 GN respectively.⁷

Intrinsic multiresistance of *Elizabethkingia* including resistance to polymyxins and tigecycline is known owing to production of both ESBL and chromosomal MBL.^{1,2} *Elizabethkingia* species have been proposed as potential reservoirs of novel β -lactamases.^{1,8} Two unrelated wide spectrum heterogenous MBLs; BlaB (subclass B1) and GOB (subclass B3) have been described.¹ Nosocomial *Elizabethkingia* infections are known to occur under intensive Gram-negative antimicrobial cover. Paradoxical susceptibility to vancomycin paved way for treatment of *E. meningoseptica* infections, eventually leading to emerging vancomycin resistance (MIC ≥ 16 μ g/ml). Only 4% isolates are vancomycin susceptible according to SENTRY database.⁹ *Burkholderia cepacia* is a confusing multiresistant nonfermenter, which does not grow on MacConkey agar and is susceptible to vancomycin, although it is motile and negative for indole and PYR. Alternative treatment with SXT, quinolones, rifampin, piperacillin, piperacillin-tazobactam, minocycline, macrolides, clindamycin and novobiocin is being considered for establishing guidelines, albeit being classic drugs for Gram-positive bacteria, they are not routinely tested on Gram-negative bacteria. Susceptibility testing by disk diffusion may be unreliable and there are no CLSI guidelines for performance and interpretation of antimicrobial susceptibility. However, inducible clindamycin resistance is easily detectable. Phenotypic combined disk tests from Rosco Diagnostica, Denmark for ESBL and Amp C; and KPC and MBL rapidly detect complex β -lactamase resistance mechanisms.⁴ Vitek 2 may rapidly test broth microdilutions, however, the available susceptibility cards such as AST-N025, AST-N090 and AST-N280 customized for Gram-negative organisms does not cater to the unique susceptibility pattern of *Elizabethkingia*. Eradication is difficult and may mandate therapeutic drug monitoring which is conducted in very few laboratories. Prolonged treatment with higher than usual dosages may be required to achieve pharmacokinetic/pharmacodynamic targets.¹⁰ Prolonged combination of rifampin with vancomycin, SXT, minocycline or fluoroquinolones may have better clinical outcome.²

E. meningoseptica does not constitute human microflora, though may colonize tissues through wash basins, medical devices such as intubation tubes, humidifiers, neonatal incubators, respirators and even antiseptic and saline solutions.^{2,9} A batch contamination of povidone iodine pads led to the product being recalled from US markets.¹¹ *E. meningoseptica* infections in these cases represent its expanding pathogenicity and host range. Person to person transmission is unusual. A high index of suspicion for a possible outbreak was considered. *E. meningoseptica* could not be isolated from surveillance air, water and surface cultures; antiseptic solutions or medical devices from nephrology, respiratory medicine and obstetrics-gynecology and open heart Intensive Care Unit; which are located on different sections in the hospital. No possibility of transfer of infection between these centers was evident. The four isolates were temporally separated and the antibiograms obtained by Vitek 2 compact were different. Strain characterization by nucleic acid sequencing is ideal in such a case but could not be performed. The cases had extensive community exposure prior to admission. In the present scenario, the distinction of nosocomial or community acquired infection couldn't be arrived at. Repeated isolation from pleural cavity in an immunodeficient patient, CAPD fluid in a patient of CKD, bacteremia in a patient undergoing extensive tissue trauma; with improvement by susceptibility tested antimicrobials, establishes its pathogenicity. The rising populace of immunodeficient hosts warrants a high index of suspicion to diagnose emerging *Elizabethkingia* infections. A difficult identification being a nonfermenter along with paradoxical antimicrobial susceptibility makes it a challenging infection to deal with. *Elizabethkingia* is the dominant gut bacteria in *Anopheles stephensi* and *A. gambiae*, making further research on vector based transmission, an imperative.¹² Dedicated efforts by clinicians and microbiologists targeted at early diagnosis, surveillance and further research are needed to optimize management and control of *E. meningoseptica* infections.

Conflicts of interest

All authors have none to declare.

Acknowledgment

The authors acknowledge the technical contributions of Sub Pratap and Sep Subhash.

REFERENCES

1. Ceyhan M, Celik M. *Elizabethkingia meningosepticum* (*Chryseobacterium meningosepticum*) Infections in Children. *Int J Pediatrics*. 2011;2011:215–237.
2. Maraki S, Scoulica E, Manoura A, et al. A *Chryseobacterium meningosepticum* colonization outbreak in a neonatal intensive care unit. *Eur J Clin Microbiol Infect Dis*. 2009;28:1415–1419.

3. Kim KK, Kim MK, Lim JH, et al. Transfer of *Chryseobacterium meningosepticum* and *Chryseobacterium miricola* to *Elizabethkingia* gen. nov. as *Elizabethkingia meningoseptica* comb. nov. and *Elizabethkingia miricola* comb. nov. *Int J Syst Evol Microbiol*. 2005;55:1287–1293.
4. Rosco Diagnostica. User's guide. Neo-Sensitabs Susceptibility Testing, 19th ed. Rosco Diagnostica A/C, Taastrup, Denmark. 2007–2008. http://www.rosco.dk/pdf/Neo-Sensitabs_19th_ed_Users_Guide_09-2007-2008.pdf. Accessed 14.11.13.
5. Vanechoutte M, Dijkshoorn L, Nemec A, et al. *Acinetobacter*, *Chryseobacterium*, *Moraxella*, and other nonfermentative Gram-negative rods. In: Versalovic J, ed. *Manual of Clinical Microbiology*. 10th ed. Washington D.C.: ASM Press; 2011:714–738.
6. Khan ID, Sahni AK, Bharadwaj R, et al. Emerging Organisms in a Tertiary Healthcare Set Up. *MJAFI*; 2013. <http://dx.doi.org/10.1016/j.mjafi.2013.09.005>.
7. Chiu CH, Waddington M, Hsieh WH, et al. Atypical *Chryseobacterium meningosepticum* and meningitis and sepsis in newborns and the immunocompromised. *Taiwan. Emerg Infect Dis*. 2000;6(5):481–486.
8. Cartwright EJ, Prabhu RM, Zinderman CE, et al. Transmission of *Elizabethkingia meningoseptica* (formerly *Chryseobacterium meningosepticum*) to tissue-allograft recipients: a report of two cases. *J Bone Jt Surg Am*. 2010;92:1501–1506.
9. Kirby JT, Sader HS, Walsh TR, et al. Antimicrobial susceptibility and epidemiology of a worldwide collection of *Chryseobacterium* spp.: report from the SENTRY Antimicrobial Surveillance Program (1997–2001). *J Clin Microbiol*. 2004;42:445–448.
10. Neuner EA, Ahrens CL, Groszek JJ, et al. Use of therapeutic drug monitoring to treat *Elizabethkingia meningoseptica* meningitis and bacteraemia in an adult. *J Antimicrob Chemother*. 2012;67(6):1558–1560.
11. US Food and Drug Administration. H & P Industries Povidone Iodine Prep Pads: Recall – Potential Microbial Contamination. US Food and Drug Administration, US Department of Health and Human Services; 2011. <http://www.fda.gov/Safety/MedWatch/SafetyInformation/SafetyAlertsforHumanMedicalProducts/ucm247743.htm>. Accessed 14.09.13.
12. Ngwa CJ, Glöckner V, Abdelmohsen UR, et al. 16S rRNA gene-based identification of *Elizabethkingia meningoseptica* (Flavobacteriales: flavobacteriaceae) as a dominant midgut bacterium of the Asian malaria vector *Anopheles stephensi* (Diptera: Culicidae) with antimicrobial activities. *J Med Entomol*. 2013;50(2):404–414.