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Research Paper

A Retrospective Study on *Elizabethkingia Meningoseptica* - An Emerging Pathogen

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ABSTRACT: *Elizabethkingia meningoseptica is a ubiquitous Gram negative bacilli and is an emerging pathogen for hospital acquired infection. The mortality rate is high and thus timely diagnosis is essential. Even more so because it is inherently resistant to a lot of antibiotics.*
In this retrospective study carried out over a period of 3 months at our tertiary care hospital, 12 patients were detected with Elizabethkingia meningoseptica predominantly from Endo-Tracheal tube aspirate.In 83.33% instances they caused nosocomial infection in COVID 19 patients.Mechanical ventilation, ECMO and prolonged exposure to carbapenems were significant risk factors associated with the infection. Cotrimoxazole was most sensitive among the antibiotics tested, followed by vancomycin and levofloxacin. Most of the other antibiotics active against Gram negative bacilli showed very high resistance including recent antibiotics like meropenem + EDTA, ceftazidime+avibactam and ELORES (ceftriaxone+ sulbactum + EDTA). The outcome was not favourable in most cases

KEYWORDS: *Elizabethkingia meningoseptica , Carbapenem resistant,ECMO,Ceftazidime-avibactum, vancomycin, cotrimoxazole, ELORES*

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I. INTRODUCTION

Elizabethkingia meningoseptica is an environmental inhabitant which can befound in various niches, most commonly in soil and water reservoirs. In case of the hospital patients the source of infection includes hospital water supplies, incubators, sinks, faucets, tap water, hemodialysis systems and saline solutions [1], [2], [3].Though they are not a part of normal human micro-biota, they can colonize in respiratory tract during hospitalization. This colonization often results from the exposure to contaminated water or medical devices (e.g., respiratory equipment) [4],[5]. Beside this, *E. meningoseptica* occasionally be transmitted from the birth canal to the neonate [6].

E. meningoseptica generally grows on routine laboratory media like MacConkey agar (less growth), blood agar and chocolate agar media [7]. The key biochemical and physiologic characteristics of this non-motile bacteria are oxidase positive, non-lactose fermenting, capable of oxidizing mannitol, indole positive, gelatin liquefaction positive, urease negative, nitrate reduction negative and esculin hydrolysis positive [8]. Still now the specific virulence factors of *E. meningoseptica*are less known. As it can survive in chlorinated water, there may be some possibilities that *E. meningoseptica*(the species most commonly associatedwith human infections) is capsulated or it produces proteases and gelatinases, by which host cells and tissues are destroyed [9].

In adultsthe life threatening diseases caused by *E. meningoseptica* includes pneumonia, endocarditis, wound infections, postoperative bacteremia, and meningitis. It also attributes to the serious risk factor for immunocompromised patients or those with serious underlying diseases [10]. In premature infants acquiring of this infection leads to neonatal meningitis with a mortality rate as high as 55% [11]. *E. meningoseptica*has increasingly been reported as a cause of healthcare-associated infections particularly those who are on devices such as ventilator, and dialysis machines [12] etc.

The appropriate choice of effective antimicrobial agents for treatment of *Elizabethkingia* infections is difficult as they are inherently resistant to many antimicrobial agents commonly used to treat infections caused by Gram-negative bacteria (like aminoglycosides, β-lactam antibiotics, tetracyclines, chloramphenicol), but are

surprisingly susceptible to agents generally used for treating infections caused by Gram-positive bacteria (like rifampin, clindamycin, erythromycin, sparfloxacin, trimethoprim-sulfamethoxazole, and vancomycin)[13],[14], [15].

Initially vancomycin was used for treating serious infection with *E. meningoseptica*,[16], [17] but recent studies have shown greater in *vitro* activity of minocycline, rifampin, trimethoprim–sulfamethoxazole, and quinolones[14], [18], [19]. The choice of appropriate antimicrobial therapy is further a complicated process due to the fact that minimum inhibitory concentration (MIC) breakpoints for resistance and susceptibility of this group have not been established by the Clinical Laboratory Standards Institute and the results of disk diffusion testing have been shown to be unreliable in predicting antimicrobial susceptibility to Elizabethkingia species.[20], [21], [22], [14] The E test has been shown to be a possible alternative to the standard agar dilution method for testing cefotaxime, ceftazidime, amikacin, minocycline, ofloxacin, and ciprofloxacin, but not piperacillin.[23]

During this study period COVID 19 was sweeping over the country. A good number of such COVID patients were admitted to our tertiary care hospitalrequiring critical care support. Most of them required support of invasive ventilation and extra corporeal membrane oxygenation (ECMO). These patients also suffered from a lot of secondary bacterial infections. *E. meningoseptica*was isolated in a greater number than usual which drove us to look for possible risk factors contributing to this infection in COVID 19 patients.

In this study we also tried to find out possible treatment options for *E. meningoseptica*. We have investigated some newer antibiotic combinations like ceftazidine+avibactum, meropenem+EDTA and ELORES (ceftriaxone+ sulbactam+ EDTA) to find out whether they could be a treatment option to treat this bacterium.

II. MATERIALS AND METHODS

This retrospective study was done based on laboratory data and patient electronic record from March 2021 to May 2021 in a tertiary care hospital of Eastern India.

In our study*Elizabethkingia meningoseptica* were isolated from12 patients . The samples [blood and endotracheal tube aspirate (ETA)] were collected from indoor patients and sent to the laboratory for culture& sensitivity. Blood samples were cultured in automated blood culture system Bactec Fx 200 (Becton, Dickinon, USA).The respiratory samples were cultured on Blood agar and Mac Conkey agar media [HiMedia, Mumbai, India]. Samples from positive blood culture bottles were also inoculated on the same. The plates were incubated for 24 hours at 37° C. Gram stain and Oxidase test were done from the positive growths. Identification and antibiogram was obtained with help of Microscan walkaway 40 (Beckman Coulter, USA) instrument.Tofurther verify the identification of the organism, the results were compared with two different laboratories, using the Vitek 2 compact and BD Phoenix machine.

When the isolate was identified as *Elizabethkingia meningoseptica*, it was again inoculated on plates of Muller Hinton agar to obtain a lawn culture. E strips of vancomycin, meropenem+EDTA, ceftazidime+avibactam and ELORES (ceftriaxone + sulbactam + EDTA) discs were used to know the susceptibility to the same.The clinical breakpoints to interpret the MIC as sensitive or resistant has been adopted from CLSI criteria for other Non-Enterobacteriaceae; for interpreting vancomycin, brakpoints for Staphylococcus spp other than S. aureus from CLSI has been used [24].

Retrospectively, information were collected about those patients to find out possible risk factors apart from their demographic information. We monitored their stay in hospital before the organism was isolated, whether they required support of any invasive devices like ECMO machine, ventilator or dialysis machine. Their total leucocyte counts (TLC/ WBC count) as well as inflammatory markers like C-reactive protein (CRP) and procalcitonin were noted on the day the organism was isolated.Drug exposure during the current stay particularly broad spectrum antibiotics and steroids, along with the final outcome of the patients were also recorded.All the above data were correlated and analyzed.

III. RESULTS

The samples examined were ETA or blood. In 7 patients the sample was ETA (58.33%) and in 5 patients the same organism was isolated from blood (41.67%).In two among these 12 patients the organism was isolated from ETAfollowed by blood. [Table 1]. The colony appeared as small, round, 1-2 mm in diameter, translucent, pinkish in Mac Conkey agar. On blood agar the colonies were larger 3-4 mm in diameter, round, moist and pale in colour, with a blackish hue surrounding the colonies.[Figure 1]. The isolated organism was Gram negative short bacilli and Oxidase positive. Microscan Walkaway system identified the organism as *Elizabethkingia meningoseptica.*Same results were obtained from both Vitek 2 compact and BD Phoenix.

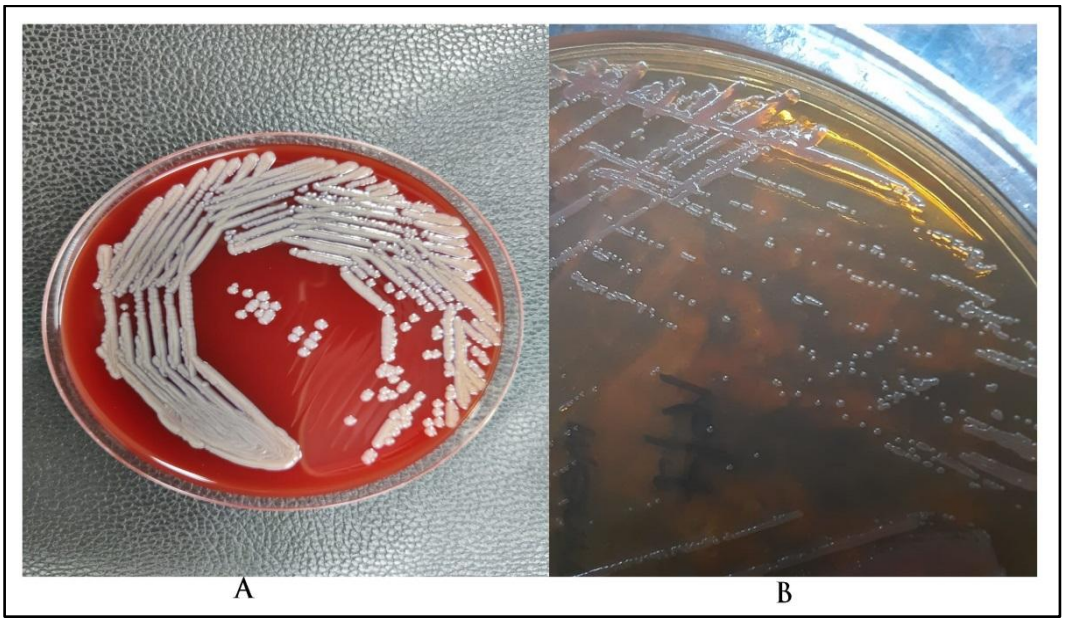

Figure 1: Colony morphology of *Elizabethkingiameningoseptica.* (A) Growth on blood agar media, (B) Growth on Mac Conkey agar media

All the patients were male except one. There was no age predilection. 4 patients were over 60 years (33.3%), 4 were below 40(33.3%) and 4 were between 40 and 60(33.3%) [Table 1].

WBC count was highly raised in most of the patientswhen the organism was isolated. 10 patients had TLC of >20000/ cmm. Mean TLC being 24074/ cmm with a range from 8350 to 31240/ cmm [Table 1]. Inflammatory markers like CRP and Procalcitonin were not recorded in all patients. CRP was raised above 10 mg/dl in all 9 patientswho were tested for(100 %, n=9), with a mean of 144 mg/dl and ranging from 26.16 to 348.37 mg/dl. Procalcitonin was recorded in 8 patients, among which 3 patients had procalcitonin >2 ng/ mL (37.50%, n = 8) [Table 1]. All of the patients stayed long in Critical care ward. The interval between admission in critical care area and isolation of the organism ranging from14 to 95 days (median 32.5 days)[Table 1].All-cause mortality was very high in these patients. All the patients had expired except 2, giving a very high mortality rate of 83.33% [Table 1].

Table 1. Distribution and clinical parameters of the patients

Sl. No	Age/ Sex	Sample	Location	Interval (Days)	TLC / cmm	CRP (mg/L)	Procalcitonin(ng/mL)	Outcome
1	62/M	BLOOD	ICU	14	31240	75	28.8	EX
2	53/M	BLOOD	ICU	65	22560	226.59	-	DIS
3	77/M	BLOOD	ICU	17	23330	64.93	0.38	EX
4	53/M	BLOOD	ICU	14	30850	-	-	EX
5	58/M	ETA	ICU	35	29640	-	-	EX
6	33/M	ETA	ICU	25	9540	348.37	1.18	EX
7	34/M	ETA	ICU	42	29940	45.37	1.33	EX
8	54/M	ETA+BLOOD	ICU	95	8350	-	6.83	EX
9	33/M	ETA	ICU	35	30200	296.96	10.6	EX
10	32/M	ETA	ICU	48	21800	146.11	-	EX
11	74/F	ETA+BLOOD	HDU	30	23820	26.16	0.35	LAMA
12	81/M	BLOOD	ICU	18	27620	72.07	0.08	EX

Notes: M-male; F-female; ETA- endotracheal tube aspirate; ICU- intensive care unit; HDU- High Dependency Unit; EX- expired; DIS- discharged; LAMA- Left against medical advice; TLC- Total Leucocyte count; CRP- C reactive protein

Except for two patients all had COVID 19 positive pneumonia and received steroids[Table 2]. Only one patient was on BIPAP, 11 out of 12 patients requiredmechanical ventilator support (91.67%, n = 12) among which 6

were on ECMO (50%, n = 12) as well.1 patient was on BIPAP andonly 1 patient had received heamo dialysis [Table 2].

Table 2: Possible risk factors associated with the patients

	DEVICES				PRIOR ANTIBIOTICS USED (DAYS)			
Sl No.	**ECMO**	**VENT**	**DIALYSIS**	**COVID/STEROID**	**POLY**	**CARB**	**TIGE**	**TEICO**
1	N	Y	N	Y	4	11	-	12
2	N	Y	N	N	-	20	-	-
3	N	Y	N	Y	-	8	-	8
4	Y	Y	N	Y	11	11	11	-
5	Y	Y	N	Y	22	22	22	-
6	Y	Y	N	Y	8	8	7	-
7	Y	Y	N	Y	14	18	9	-
8	N	Y	Y	N	20	37	20	-
9	Y	Y	N	Y	27	7	27	-
10	Y	Y	N	Y	25	13	25	-
11	N	Y	N	Y	-	14	8	14
12	N	BI PAP	N	Y	-	5	-	-

Notes: ECMO- extra corporal membrane oxygenation; VENT- ventilator; POLY- Polymixins; TEICO- Teicoplanin; CARB- Carbapenems; TIGE- Tigecycline ; n- number of days of antibiotics, Y-Present; N- Not present

All of the patients received broad spectrum antibiotics before isolation of the organism. All 12 patients received carbapenems, polymixins were given to 8 patients and tigecycline also was given to 8 patients. 7 patients received three drugs together at a time, 1 patient was on carbapenem + polymixin and 1 patient received carbapenem + tigecycline. Duration of these antibiotics was, for carbapenems 5 - 37 days (median 12 days), for polymixins 4 - 27 days (median 17 days) and for tigecycline 7 - 27 days (median 15.5 days) [Table 2].

The susceptibility testing revealed *in vitro* resistance to most of the antibiotics used to treat Gram-negative bacteria as well as recent antibiotics like meropenem+EDTA, ceftazidime+avibactam and ELORES (ceftriaxone+ sulbactam + EDTA) The susceptibilities of the *E. meningoseptica* isolates to, co-trimoxazole, levofloxacin and vancomycin were found to be 66.67%, 33.3%, 50 % respectively [Table 3].

Figure 2: MIC of vancomycin against *Elizabethkingia* determined using vancomycin E strip.

Sensitivity was found to be different in ETA and blood samples. Sensitivity of levofloxacin, co-trimoxazole and vancomycin in blood isolates were 20%, 60% and 60% respectively. Sensitivity of levofloxacin, co-trimoxazole and vancomycin in ETA isolates were 28.57%, 71.42% and 42.85% respectively [Figure 3].

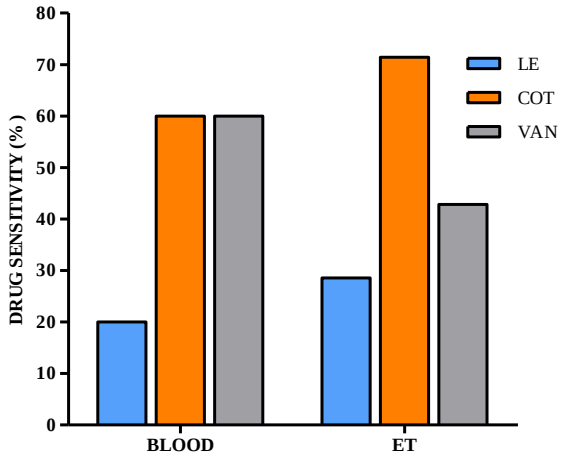

Figure 3: Sensitivity pattern of drugs in isolates from blood and ETA
Notes: LE- levofloxacin; COT- cotrimoxazole; VAN- vancomycin; ET- endotracheal tube aspirate ; n- number of sensitive samples

Table 3. Antibiotic sensitivity of *E. meningoseptica*

SL	Carbapenem	Cephalosporin	Vancomycin	Levofloxacin	Cotrimoxazole	Piperacillin Tazobactum	ELORES CEFT+SUL +EDTA)	CZA+ AV	MRP+ EDTA
1	R	R	S	S	S	R	R	R	R
2	R	R	S	R	R	R	R	R	R
3	R	R	R	R	S	R	R	R	R
4	R	R	R	R	R	R	R	R	R
5	R	R	S	I	R	R	R	R	R
6	R	R	R	R	R	I	R	R	R
7	R	R	S	R	S	R	R	R	R
8	R	R	R	R	S	R	R	R	R
9	R	R	R	R	S	I	R	R	R
10	R	R	R	S	S	I	R	R	R
11	R	R	S	S	S	R	R	R	R
12	R	R	S	S	S	R	R	R	R

Notes: CEFT- ceftriaxone; SUL- sulbactum; MRP - Meropenem; CZA+AV – Ceftazidime+avibactum; S – sensitive; R – resistant; I – intermediate.

IV. DISCUSSION

Elizabethkingia meningoseptica is an emerging cause of nosocomial infection and this study further corroborates this.

Our study showed that the organism shows no predilection to age but 91.6% were males. However a study conducted in Kasturba Medical College, Mangalore by Venkatesh et al showed a strong preference for extremes of the period, with 81.8% being male patients [25].In a study conducted in Central Taiwan, the mean age of the patient was 72.2 years (excluding one child patient), of which 79.9% were male [26].

The predominant source for the organism was from bloodin a number of studies, like 38.5% by Venkatesh *et al* [25] 48.7% by Chang *et al* [26] and 52.6% by Singh *et al* [27]. Few studies on the other hand showed respiratory samples as more common source; such asthestudy conducted in a Trauma Center in New Delhi showed that the primary source for culture was Boncho Alveolar Lavage (70%), followed by blood (22%) and CSF (4%) [28], Han *et al*also found *E. meningoseptica* in 76.7% from respiratory samples [24].In our study all the patients developed the infection as hospital acquired infection (HAI), 58.33% (7 samples) of the samples were ETA and 41.67% (5 samples) were blood.

All the patients except two in the studywere affected by severe COVID 19 and received steroids.Comparing with incidences of *E. meningoseptica*infection of pre COVID era, it is evidentthat*E. meningoseptica* infectionis strongly associated with COVID 19 diseases,but more data incorporating large number of COVID patients and statistical study is required.

All our patients were admitted for a long time in critical care wards. The median interval between admission and isolation of the organism was 32.5 days (14-95 days). The interval in non COVID patients being much more than COVID patients. Median and highest interval in COVID patients was 27.5 and 48 days, whereas in non COVID patients the intervals were 80 and 95 days respectively. This is comparable to the result of lin *et al*. where the mean duration of hospital stay was 32 days (range 13–99 days) [29] and Singh *et al* where the average duration was28 days (range 10-45 days) [27].

We put an effort to search for possible other risk factors associated with *E. meningoseptica* infection.Prolonged use of broad spectrum antibiotics has been mentioned as a significant risk factor in several studies like Rastogi *et al* where all the patients under study were on broad-spectrum antibiotics before procuring the infection [28] and Lin P Y *et al* [30]. We found in our study that carbapenems and polymixins are the two antibiotics our patients were most exposed to and that also with a median duration of >10 days. So continuous therapy with these drugs can increase chance of secondary infection with *E. meningoseptica*beyond 10 days. Judicious use of these drugs should be necessary to reduce the incidence.

Presence of invasive devices like mechanical ventilator, central venous line, haemodialysis has been identified as potential risk factors in different studies like done by Pereria *et al* [12] and Govindaswamy *et al* [10]. Most of our patients were on invasive devices. 91% of the isolates (11 patients) were associated with mechanical ventilation and 50% patients (6 patients) required ECMO.All of them had central veinous line in place but only 1 patient received dialysis. Unfortunately very little comparative data is available with respect to ECMO.

Infections with *E. meningoseptica* were associated with poor outcome, mortality varying from 23% to 52% has been reported [31].The 28-day mortality in a study conducted by Lin *et al*. was found to be 41% [32].In our study mortality rate was found to be as high as 83.3%.

The organism was said to be resistant to most of the β-lactam antibiotics including carbapenems and aztreonam, the aminoglycoside group of drugs and chloramphenicol, but was susceptible to drugs such as cotrimoxazole, fluoroquinolones, minocycline, tigecycline, and piperacillin [8] [12] [33,34].Variation in susceptibility profile has been found in different studies.In a study by Wang *et al*susceptibility rates of *Elizabethkingia* isolates to ciprofloxacin, levofloxacin and rifampin were 50.0%, 71.2% and 76.9%, respectively. Minocycline was more active rather than doxycycline and tigecycline (susceptible rates, 100% versus 96.2% and 78.8%, respectively). MIC50/MIC90 values of vancomycin and linezolid against *Elizabethkingia* isolates were 16/16 mg/L and 16/32 mg/L [35].In another study carried by Singh *et alElizabethkingia* showed almost 100% resistance to levofloxacin. Susceptibilities to cotrimoxazole was found to be 33.4%. Minocycline and piperacillin-tazobactam showed 100% and vancomycin showed 27.8 % sensitivity in *E. meningoseptica*[27].

In our study the susceptibility testing revealed in vitroresistance to most of the antibiotics used to treat Gram-negative bacilli (GNB).The susceptibilities of the *E. meningoseptica* isolates to, cotrimoxazole, levofloxacin and vancomycin were found to be 66.67%, 33.3% and 50 % respectively.Sensitivity also differed depending upon the sample type. Isolates from ETA were more sensitive to cotrimoxazole than vancomycin, whereas isolates from blood were equally sensitive to cotrimoxazole and vancomycin.

Some newer antibiotic combinations like meropenem+EDTA, ceftazidime+avibactam and ELORES (ceftriaxone+sulbactam + EDTA) are being increasingly used as a treatment option for carbapenem resistant Klebsiella and Acinetobacter infections. *E. meningoseptica*as found to play a significant role in nosocomial infection, it was speculated whether these antibiotics could be a treatment option for this.This study shows 100% in vitro resistance to all the three antibiotic combinations, thus should not be considered as a primary treatment option.

V. CONCLUSION

E. Meningoseptica is a rare but important causative agent of nosocomial infection. Particularly when a patient has developed HAI with oxidase positive Gram negative bacilliafter long stay in ICU, has invasive devices like ventilator and ECMO, exposed to broad spectrum antibiotics for prolonged time, possibility of *E. meningoseptica* should be kept in mind. For treatment cotrimoxazole is a better alternative than vancomycin, but that should be guided by institutional or local sensitivity data. The newer antibiotic combinations has little effect on *E. meningoseptica.* Mortality could be very high in *E. meningoseptica*infection particularly when associated with COVID.

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