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Infections caused by *Elizabethkingia meningoseptica* in a tertiary care centre – A retrospective study

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Abstract *Elizabethkingia meningoseptica* (EM) has emerged as an important opportunistic pathogen that can cause sporadic nosocomial outbreaks as well as infections in neonates. They are known for their multidrug resistance and their unique susceptibility pattern. The present study is a retrospective single-center study in which the sensitivity pattern and comorbidities associated with infection by this organism are systematically noted. All samples received in the microbiology laboratory and yielding EMas identified by an automated method (Vitek2, Biomerieux) from October 2020 to September 2021 were included in the study. The strains were reidentified to the species level by polymerase chain reaction amplification of the 16S r RNA gene.All samples where clinical significance could not be proven and repeat samples from the same patient were excluded from the analysis. The case sheets of the patients were collected from the record section and analyzed for the presence of various risk factors.In this study, 57 patients were included with a mean age of 47.12, and most of the patients belonged to the 51-60 age group (22.81%). The most common sample of isolation was blood (57.89%), followed by respiratory samples (26.32%). The majority (89.28%) of patients were in the ICU, and most of the infections were hospital acquired (92.9%). The mean duration of ICU stay before isolation was 13.8±8.07. Other major associated factors were diabetes mellitus (42.86%), high-dose steroid use (57.14%) and post-COVID pneumonia (35.71%). The isolates were resistant to beta-lactams and combination agents; minocycline (98.2%), followed by cotrimoxazole (47.2%) and levofloxacin (45.6%), and ciprofloxacin (35.1%) was the most useful antibiotic. EM is an emerging nosocomial pathogen in the ICU setting. Clinicians and microbiologists need to be aware of its unique sensitivity pattern. In contrast to previous studies, we found a high degree of resistance to tigecycline and *Piperacillin tazobactam*. Thus, in planning a combination regimen, this should be kept in mind.

Keywords: *E. meningoseptica*, Vitek 2, Nosocomial pathogen, Carbenicillin resistance

1. Introduction

Elizabethkingia meningoseptica (previously known as *Chryseobacterium meningosepticum and Flavobacterium meningosepticum*) is a ubiquitous gram-negative bacillus that is widely distributed in nature, particularly in soil and water (Issack and Neetoo 2011).Their isolation from other known pathogens and their constant presence in the hospital environment has often prompted microbiologists to label them as contaminants. In recent years, this organism has been reported to cause various invasive infections, such as meningitis, pneumonia, endocarditis, and bacteremia, in adults and neonates in association with severe underlying illness (Han et al 2017).They have emerged as important opportunistic pathogens that can cause sporadic nosocomial outbreaks and infections in immunocompromised or at-risk individuals (Moore et al 2016; Yung et al 2018; Nicholson et al 2016; Perrin et al 2017; Chew et al 2018; Jean et al 2014). However, the majority of the literature on this organism in India is from isolated case reports and case series only. Treatment of this organism remains difficult due to multiple drug resistance, lack of data on clinical response to different treatment regimens and its unique susceptibility pattern, thus necessitating the need to note the sensitivity pattern of these isolates. There is a lack of large-scale studies describing the prevalence, patient demographics associated with infection by this organism and sensitivity pattern of this organism in our area. The present study is a retrospective single-center study that aims to bridge this gap.

1.1. Objective

This study aims to determine the risk factors associated with EM infection and the commonly susceptible and resistant antibiotic patterns for its treatment.

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2. Material and Methods

This is a retrospective study conducted over a period of 1 year, from October 2020 to September 2021, undertaken in the Department of Microbiology of our hospital, a premier tertiary care teaching hospital in the eastern part of India. Owing to the rarity of isolation of this bacterium, a consecutive sampling strategy was undertaken.

Inclusion criteria - All clinically significant, nonrepetitive isolates that grew this organism during the study period were included.

Exclusion criteria - Duplicate samples, samples where clinical significance could not be ascertained and surveillance cultures were excluded from analysis.

The samples received in the central laboratory of the hospital were processed in the laboratory as per standard protocol. Blood and bile culture was processed by a Bac T Alert automated system, Biomerieux, and then plated on blood and Mac Conkey agar plates when flagged positive by the system, while all other samples were processed directly on appropriate solid culture media. The 1-2 mm, smooth, circular, moist, grayish white nonhemolytic colonies on blood agar (Figure 1) with poor growth on MacConkey agar were processed further. Catalase, oxidase-positive, nonmotile, nonfermenting gram-negative bacilli on TSI, which are indole and urease positive and citrate negative, were preliminarily identified as EM, and further confirmation was performed using Vitek2, Biomerieux, Version 8.01, North Carolina, USA, automated method using GN cards. The sensitivity patterns of the isolates were determined by the Vitek2 automated system, and breakpoints of CLSI for non-Enterobactarales were used for interpretation.

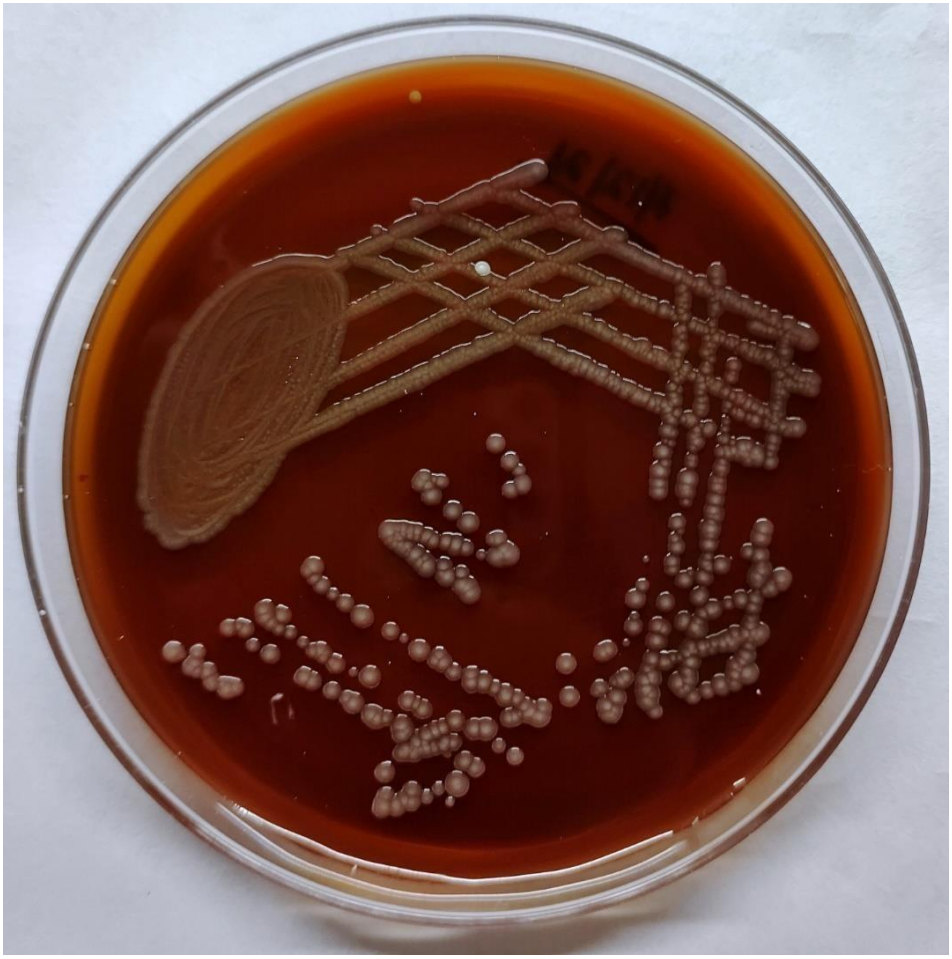

Figure 1 Grayish white nonhaemolytic colonies of E. meningoseptica on blood agar.

Strain confirmation was performed with PCR. A Gene JET PCR Purification Kit was used to extract genomic DNA. Species-specific primers (Forward 5′-GATTCGGCATCGGATTATATTG-3′ and Reverse 5′-CCACTTCAACCTTACTCAAGACTAAC-3′) were designed according to the conserved 16S rRNA region in EM accession No. AY468482, AF207074 to perform the polymerase chain reaction (PCR). The PCR mixture (50 µL) consisted of 25 pM primers (each), 1 µL genomic DNA, and 10 µL of 2× PCR Master Mix (DreamTaq Green PCR Master Mix). The cycle was performed as follows: denaturation at 95°C for 5 minutes; 30 cycles of denaturation at 94°C for 15 seconds, annealing at 50.5°C for 35 seconds, and extension at 72°C for 30 seconds; and a final extension at 72°C for 7 minutes. The 475-bp product was analyzed by 1.5% agarose gel electrophoresis and visualized with ethidium bromide staining (Figure 2).

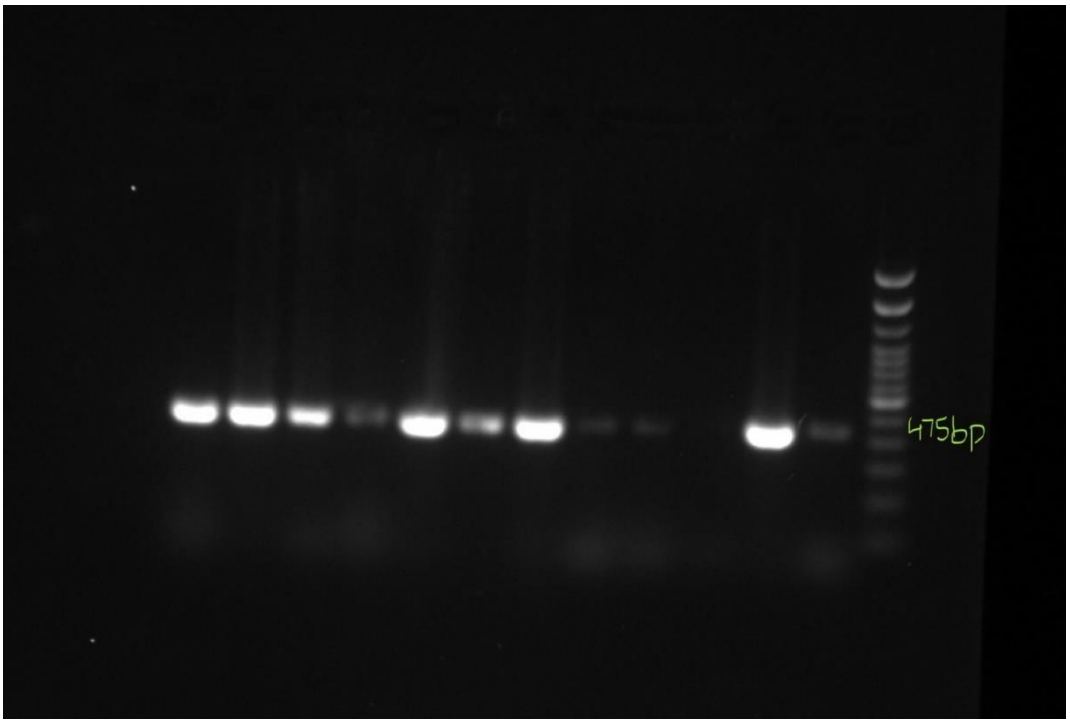

Figure 2 16 s rRNA amplification of *E. meningoseptica.*

Clinical significance was ascertained for blood culture-positive samples when the same organism was isolated from two simultaneous peripheral blood cultures with similar antibiotic sensitivity patterns. For respiratory samples, the culture was reported when there was significant colony counts in tracheal aspirate (colony count > 10^5 CFU/ml), bronchoalveolar lavage fluid (colony count >10^4 CFU/ml), evidence of infection on Gram stain and radiology. Any infection developing in a patient other than the presenting complaint after 48 hours of admission was termed hospital acquired. When patients had fever with leukocytosis occurring after 48 hours of endotracheal intubation or mechanical ventilation with radiological evidence of new infiltrates, worsening of oxygen status and mucoid or purulent sputum, they were considered to have ventilator-associated pneumonia.

Case sheets of these patients were collected retrospectively from records departments, and the data were retrieved by the investigators from them in a specified format. As per the local regulations, the case sheets of the government-funded ICUs and medico-legal cases were not made available for the investigators and were thus excluded from the analysis of the patient demographics factors.The present study is a retrospective study without any involvement with patient care activities. All the samples collected for testing were received by the laboratory after receiving implied consent from the patient for diagnosis or treatment purposes in this hospital. During the tenure of the study, no individual history was disclosed in any form. This work was reviewed and approved by the institutional ethical committee (IEC registration No- ECR/627/Inst/OR/2014/RR-20) via letter no IEC/IMS.SH/SOA/2022/342 dated 11th April 2022.

3. Results

In the period of study, 57 isolates of EM were obtained from various areas of the hospital. The mean age of patients in the study was 47.12 with a standard deviation of 21.4 with a range from 0- 88 years with a median age of 67. The most common age group of the patients was 51-60 years, followed by 61-70 years, with rates of 22.81% and 17.54%, respectively. Males outnumbered females in the affected patients, with a male:females ratio of 1.85.There were 4 patients younger than 10 years of age and 6 patients older than 70 years, accounting for 17.5% of patients at extreme ages.

The most common sample that revealed EM on culture was blood (57.89%), followed by respiratory samples (26.32%). We had 10.53% cases revealed from bile. CSF and urine constituted the least common samples (Figure 3).

Of the 57 cases, we retrieved case sheets of 28 patients from the records department. The data were retrieved from these case sheets, and the preexisting illnesses are tabulated in Table 1. Apart from 2 patients (7.1%), the rest of the cases were hospital-acquired infections. Most of the cases were in the ICU (89.28%). The mean duration of acquiring infection by this organism post admission was 13.8±8.07. All the patients (100%) had a history of carbapenem antibiotic administration, and 57.14% of the patients were on colistin. Common comorbidities associated with the cases were diabetes mellitus (42.86%), high-dose steroid use (57.14%), and post-COVID pneumonia (35.71%).

Table 1 Coexisting factors associated with the isolation of *Elizabethkingia meningoseptica* isolates (N=28).

Coexisting illness	Number (%)

ICU admission	25 (89.28%)
Post covid pneumonia	10 (35.71%)
Coexisting malignancy	08 (28.57%)
Previous Surgery within 1 month	06 (21.42%)
History of carbapenem use within 15 days	28 (100%)
Colistin use nebulized/Intravenous	16 (57.14%)
Hospital acquired Infection	26 (92.86%)
Diabetes mellitus	12 (42.86%)
Steroid	16 (57.14%)
Chronic Kidney Disease	06 (21.43%)
Asthma/COPD	06 (21.43%)
Congestive cardiac failure	06 (21.43%)

Note- Previous surgery included caesarian section in three patients, hernia repair with mesh in1 patient, and GI malignancy for colectomy in 2 patients.

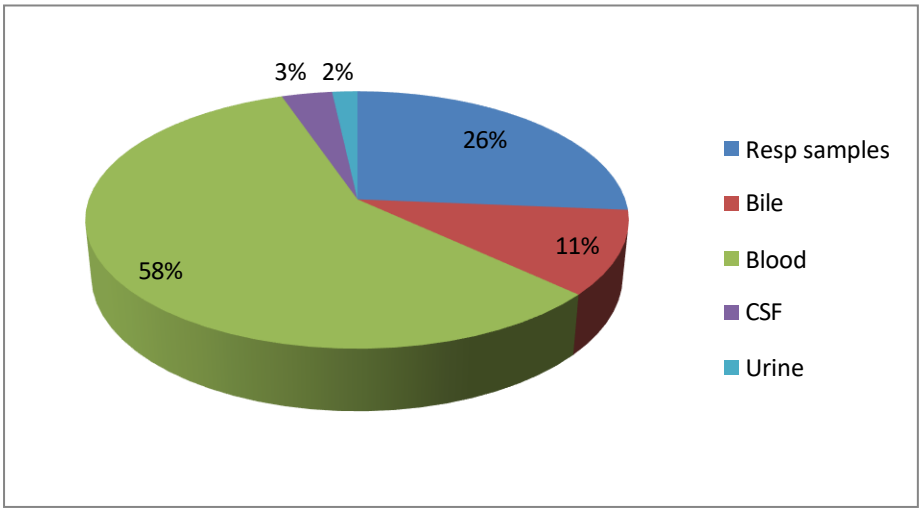

Figure 3 Different samples of *E.meningoseptica.*

The sensitivity pattern of the various isolates is shown in Figure 4. All the isolates were resistant to commonly used antipseudomonal antibiotics, such as ceftazidime, cefepime, aztreonam, piperacillin-tazobactam, ticarcillin-clavulanic acid, meropenem, amikacin and colistin. The most sensitive antibiotic was minocycline (98.2%), followed by cotrimoxazole (47.2%). Quninolones are the other useful choice of antibiotics, with sensitivity percentages of 45.6% and 35.1% for levofloxacin and ciprofloxacin, respectively. The MIC values of various antibiotics are presented in Table 2.

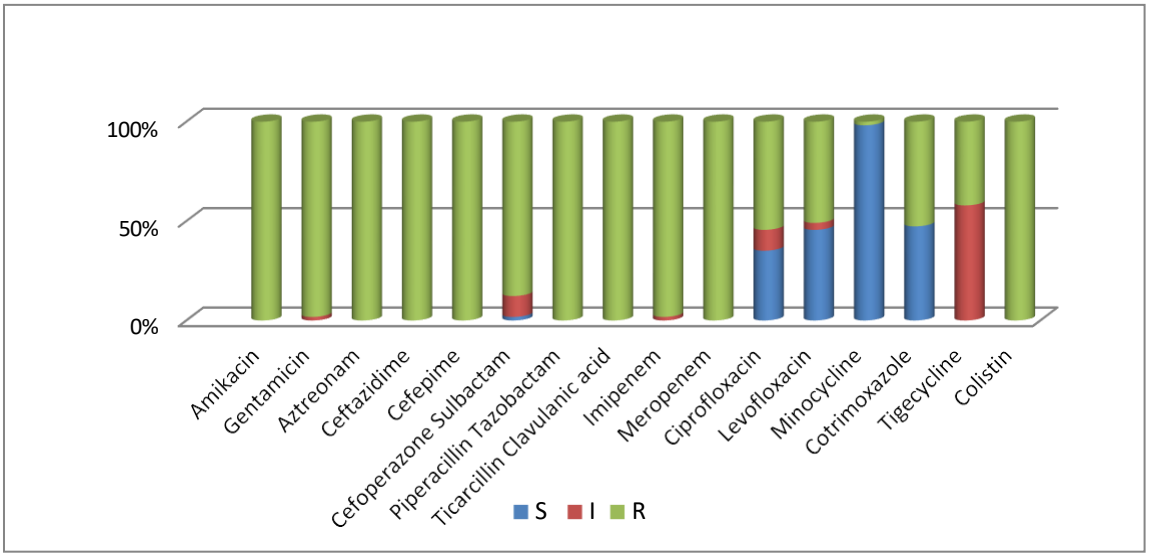

Figure 4 Sensitivity pattern of various isolates.

Table 2 MIC of the isolates (N=57) for various antibiotics by the Vitek2 method.

	Sensitive MIC (n)	Intermediate MIC (n)	Resistant MIC (n)
Ceftazidime	0	0	≥64 (57)
Aztreonam	0	0	≥64 (57)
Ciprofloxacin	1 (20)	2 (6)	≥4 (31)
Levofloxacin	0.5 (15)	4 (2)	≥8 (29)
	1 (5)		
	2 (6)		
Minocycline	≤1 (54)	0	≥16(1)
	2 (2)		
Cefoperazone Sulbactam	≤8 (1)	32 (6)	≥64 (50)
Piperacillin-Tazobactam	0	0	≥128 (57)
Cotrimoxazole	40 (27)	0	80 (8)
			160 (5)
			≥320 (17)
Tigecycline	0	4 (33)	≥8 (24)
Imipenem	0	8 (1)	≥16 (56)
Gentamicin	0	8 (1)	≥16 (56)
Amikacin	0	0	≥64 (57)

4. Discussion

EM has generally been reported as a causative agent of outbreaks of meningitis mainly in premature newborns and infants in neonatal intensive care units (ICUs). Apart from this, it is known to cause pneumonia, endocarditis, soft tissue infection, osteomyelitis, peritonitis, surgical site infections and sepsis (Lin et al 2004; Ghafur et al 2013). Most of our strains, as in previous studies, were isolated as causative agents of septicemia followed by pneumonia. Interestingly, we also isolated a number of organisms (6, 10.53%) from bile samples of the empyma gallbladder in choledocholithiasis cases. Peritonitis by EM following choledocholithisis has been described in the literature (Swain et al 2014). Although most cases in the literature have been seen in neonates, our study shows an expanding horizon of this organism, and it may be more common as a hospital-acquired pathogen in the ICU.

In a review of the literature, most of the nonneonatal infections are nosocomial, as in our case, where 92.9% were hospital-acquired infections. EM is capable of survival in hospital environments. Most likely sources are contaminated water supply (since it survives chlorine treatment), hospital equipment, saline solutions used for flushing procedures, disinfectants, hands of hospital staff, infant formulas, respiratory equipment, contaminated syringes in ice chests, vials, sink drains, sink taps, tube feedings, flush solutions for arterial catheters, etc. (Govindaswamy et al 2018). Analysis of our hospital water supply used for various procedures and washing was performed fortnightly in the hospital, which never yielded EM in the study period. However, other sites, such as sink, saline, and instrument surface sampling, could not be used because of the retrospective nature of the study, which may be regarded as a limitation of the present study. We noted 2 cases (7.1%) of community-acquired pneumonia that yielded EM on tracheal aspirate culture upon admission. Cases of community-acquired sepsis caused by EM have also been reported previously in various studies (Lin et al 2004; Swain et al 2014; Tuon et al 2007).

ICU admission has been noted as an important factor that has been noted in 72% and 60% of cases in Ghafur et al (Ghafur et al 2013) and Lin et al (Lin et al 2009), respectively. In our study, 89% of cases were admitted to the ICU. Multiple device use and prolonged use of antibiotics in the ICU may be contributing factors. The organism probably tends to form biofilms in various devices that become inaccessible to antibiotics (Lin et al 2009). Khan et al. (Khan et al 2015) showed that previous exposure to gram-negative antibiotic cover predisposes patients to hospital infections caused by EM. In our study, all the patients whose case history could be probed had multiple devices and carbapenem exposure, including 57.14% who were also on colistin. This may have contributed to the selection of organisms resistant to these antibiotics.

The mean duration of ICU stay before isolation of the organism was 13.8 ± 8.07 days. In another case series in nonneonatal patients in India, the authors also found that the mean duration of hospital stay was 12 days. Late infection occurring 50-70 days after hospital admission has been noted in some studies (Pereira et al 2013). A study (Ratnamani and Rao 2013) on hemodialysis patients showed an average duration of 5 days before retrieval of the organism, and the authors of that study proposed that tap water used for hand washing purposes may be the reason for such early affliction.

The risk factors for infection by this organism include diabetes mellitus, steroid use, malignancies, organ transplantation, neutropenia, prolonged hospitalization, prior exposure to multiple antibiotics, immunocompromised host and chronic hemodialysis (Ceyhan et al 2008). In another study (Govindaswamy et al 2018), prolonged hospital stay, especially in ICUs, the presence of a central venous catheter (CVC), exposure to carbapenem antibiotics, the presence of shock and recent surgery were the major risk factors. Common preexisting factors in the present study, apart from ICU stay and carbapenem exposure, were diabetes mellitus (42.86%), high-dose steroid use (57.14%) and post-COVID pneumonia (35.71%).

EM has been said to have a strong predilection for extremes of age. However, the mean age of patients in our study was 47.12. A similar finding was also seen by Ratnamani et al (Ratnamani and Rao 2013) and Govindswami et al (Govindaswamy et al 2018) in their studies on dialysis and trauma patients, respectively.

Although this organism is a nonfermenter, in contrast to *P. aeruginosa* and *A. baumannii,* it has a unique resistance pattern. EM is resistant to most antibiotics implicated in gram-negative infections, and the use of inappropriate drugs as empirical therapy may contribute to the poor outcome in these infections (Steinberg and Burd 2010). Its susceptibility testing poses many limitations, such as unreliable results in commonly used disk diffusion methods, nonavailability of appropriate standards of interpretation in CLSI guidelines and sensitivity to drugs mostly used for gram-positive organisms (Forbes et al 2007).

Due to the production of two beta lactamases, one class A ESBL and one class B carbapenem-hydrolyzing metallolactamase, many EM strains are usually resistant to extended-spectrum beta-lactam agents, including carbapenems and aztreonam (Forbes et al 2007; Marchiaro et al 2008). Similarly, in our study, the isolates were resistant to all beta lactam antibiotics, while 98.2% of cases were resistant to imipenem. Piperacillin tazobactam, which has been reported in previous studies (Zong 2014) to be one of the useful choices, was resistant in all the isolates in the present study. Among the beta lactam-beta lactam inhibitor combination drugs, the most useful was cefoperazonesulbactam in 12.3% of cases. Similar to previously published literature, we also noted a high degree of resistance to aminoglycosides (amikacin-100%, gentamicin-98.2%). Fluoroquinolones have been reported to be active, while vancomycin, trimethoprim-sulfamethoxazole, doxycycline and congeners are other alternatives (Steinberg and Burd 2010; Forbes et al 2007; Marchiaro et al 2008; Fraser and Jorgensen 1997; Di Pentima et al 1998; Yoon 2007). A recent study showed variable susceptibilities of this organism to levofloxacin, ciprofloxacin, piperacillin-tazobactam and tigecycline (Lin et al 2009). Minocycline was detected in 98.2% of cases, followed by levofloxacin (45.6%) and ciprofloxacin (35.1%) in the present study. Although antibiotics such as minocycline are sensitive, their bacteriostatic nature proves to be a bottleneck. In contrast to prior studies (Lin et al 2009) where EM is highly susceptible (88.5%) to tigecycline, we did not find any isolate sensitive to it. (Interpreted according to the US Food and Drug Administration criteria of susceptible: ≤2 mg/mL; intermediate- 4; resistant: ≥8 mg/mL). For the treatment of these infections, combinations such as piperacillin-tazobactam plus rifampicin, vancomycin plus rifampicin, or a fluoroquinolone combined with vancomycin and rifampicin have been suggested (Bhat et al 2016; Issack and Neetoo 2011; Lin et al 2004). However, in our area, cefoperazone sulbactam and levofloxacin may be considered in drug formulations targeting this organism, omitting carbenicillins.

The present study has various limitations. First, it is a retrospective study. Second, we could also retrieve only 28 patient case sheets, which is a meager number for any statistical analysis. Thus, the associated factors could be confounders instead of being an actual risk factor. However, this study will help to formulate research questions for further prospective studies. This study will also help raise awareness regarding this organism as a pathogen in the ICU setting and guide decisions regarding antibiotic choices.

5. Conclusion

EM is an emerging pathogen that causes nosocomial infections commonly in the ICU setting. The most common associated factors for acquiring this infection are ICU admissions, prior multiple broad-spectrum antibiotics and colistin, diabetes mellitus, high-dose steroid use and post-COVID status. The organism is resistant to commonly used beta-lactams, including combination agents, carbapenems, and colistin. The quinolones minocycline and cotrimoxazole are the most effective antibiotics. There is a rising incidence of resistance to piperacillin tazobactam and tigecycline, which were previously used in combination therapy for this organism.

Acknowledgment

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Ethical considerations

Not applicable.

Conflict of Interest

The authors declare that they have no conflict of interest.

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