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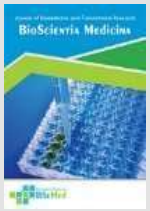

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Elizabethkingia meningoseptica Carbapenem Resistance in Cerebrospinal Fluid Cultures of Patients Suspected of Bacterial Meningitis: A Case Report

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A B S T R A C T

Background: *Elizabethkingia meningoseptica* is a Gram-negative bacterium that rarely causes meningitis in children. These infections are often resistant to carbapenem antibiotics, making diagnosis and treatment more complicated. **Case presentation:** A 1-month-old male patient was treated to the PICU of Dr. M. Djamil General Hospital Padang with symptoms of decreased consciousness, seizures, and fever. The patient was diagnosed with bacterial meningitis and was referred from Sungai Dareh Regional General Hospital with previous therapy for 4 days. A cerebrospinal fluid examination showed increased protein and decreased glucose, and a CT scan showed hydrocephalus. The cerebrospinal fluid culture showed growth of *Elizabethkingia meningoseptica* which was resistant to imipenem and meropenem. The patient was treated with a combination of non-carbapenem antibiotics and recovered from meningitis. **Conclusion:** Carbapenem-resistant *Elizabethkingia meningoseptica* is a rare causative agent of meningitis in children. Early diagnosis and treatment with appropriate non-carbapenem antibiotics are essential to increase the chance of cure.

1. Introduction

Elizabethkingia meningoseptica (EM), a Gram-negative bacterium belonging to the Flavobacteriaceae family, is usually found in aquatic and soil environments. Although rare, EM can be an opportunistic pathogen that attacks humans, especially children and causes serious infections such as meningitis. Meningitis, inflammation of the meninges that surround the brain and spinal cord, is a life-threatening medical condition. When EM infects the meninges, the disease is known as EM meningitis and can have severe neurological consequences in children. EM has several characteristics that make it different from other meningitis pathogens. EM is found in aquatic and soil environments, including freshwater, seawater, and contaminated soil. Children who play in these environments are at greater risk of exposure to EM. EM shows high adaptability to antibiotics, including carbapenems, which are the main choice for the treatment of bacterial meningitis. This resistance complicates diagnosis and effective therapy. Symptoms of EM meningitis are often non-specific, such as fever, headache, vomiting, and stiff neck. This can cause delays in diagnosis and treatment. EM meningitis can cause severe long-term neurological complications in children, including hydrocephalus, seizures, and intellectual disability.[1-3]

Several factors increase the risk of EM meningitis in children. Infants and young children are more susceptible to EM infections because of their immature immune systems. Children with chronic illnesses such as diabetes, immunodeficiency, or

kidney disease are more at risk of developing EM infections. Exposure to EM-contaminated water or soil may increase the risk of infection. Previous use of carbapenem antibiotics may increase the risk of EM resistance. Invasive medical procedures such as the placement of mechanical ventilation aids or catheters can provide an entry point for EM to infect the meninges. The diagnosis of EM meningitis is based on a combination of clinical factors, laboratory tests, and cerebrospinal fluid (CSF) culture. Clinical symptoms such as fever, headache, vomiting, and stiff neck may suggest meningitis. Laboratory tests such as CSF analysis show an increase in protein and white blood cells, as well as a decrease in glucose. CSF culture is an important step in identifying EM and determining antibiotic sensitivity. Treatment of EM meningitis involves a combination of non-carbapenem antibiotics that are effective against EM. The choice of antibiotic depends on the bacterial sensitivity pattern and the patient's clinical condition. Frequently used antibiotics include cefotaxime, amikacin, and tigecycline. Corticosteroids may also be given to reduce brain inflammation.[4-6]

2. Case Presentation

A 1-month-old male patient was treated in the PICU of the children's health department of Dr. M. Djamil General Hospital Padang on September 15th, 2022 with clinical information from Susp. Bacterial meningitis. The patient had a main complaint of decreased consciousness 2 days before admission to the hospital. Decreased consciousness since 2 days before entering the hospital. Seizure 5 days before entering the hospital, the first seizure occurred at home, during the seizure the patient appeared stiff in the legs and arms, accompanied by abnormal movements in the eyes. Seizure frequency 4-5x/day. The child was initially conscious and in contact again after the seizure. After having a seizure for a day at home, the patient was taken for treatment to Sungai Dareh Regional General Hospital and was treated for 4 days. The child experienced seizures once with the same seizure characteristics while being treated at the Regional General Hospital. While in the ER (Emergency Department) of Dr. M. Djamil General Hospital Padang, the child had seizures twice with both eyes turning upwards, and hands and feet stiff, the seizures stopped when he was given Sibital once. Fever since 5 days before entering the hospital. The fever is not too high. There are no complaints about urinating and defecating. Referred child to Sungai Dareh Regional General Hospital with diagnosis of Susp. meningitis and hydrocephalus. The child has received therapy for 4 days. The child was born vaginally with a history of LBW 2400 grams. The child was hospitalized for approximately 2 weeks due to complaints of LBW and a history of jaundice after birth. Physical examination revealed a general condition that looked seriously ill. Consciousness appears somnolence (GCS 9). Blood pressure 116/64 mmHg; Pulse 130x/min; Respirations 30x/min; Temperature 38.5°C; O_2 saturation 99%; Poor nutritional status. The crown is prominent and tense, the head circumference is 39cm, there is a stiff neck, and there is Kernig's sign. Hematological examination was within normal limits. Clinical chemistry examination showed decreased total protein. Cerebrospinal fluid analysis showed increased CSF protein and decreased CSF glucose. CT scan showed hydrocephalus.

A 1-month-old male patient's cerebrospinal fluid sample was sent in a screw tube from the pediatric intensive care unit (PICU) room to the microbiology laboratory at Dr. M. Djamil General Hospital Padang on September 20th, 2022 for culture examination and antibiotic sensitivity testing with clinical information Susp. Bacterial meningitis. Cerebrospinal fluid samples were placed in thioglycollate enriched media and then incubated for 24 hours at 37°C. Cloudy enrichment media indicates bacteria are growing. The samples were then planted on blood agar and MacConkey agar media. Colonies that grow on MacConkey agar appear round, small to medium in size, smooth, pale in color, and transparent, and there is no visible hemolytic reaction on blood agar. Colonies that grow on agar culture are subjected to Gram

staining. The results of Gram staining found Gram-negative bacilli. The examination was continued using the automated microbiology system utilizing the growth-based Technology (VITEK 2) tool and *Elizabethkingia meningoseptica* results.

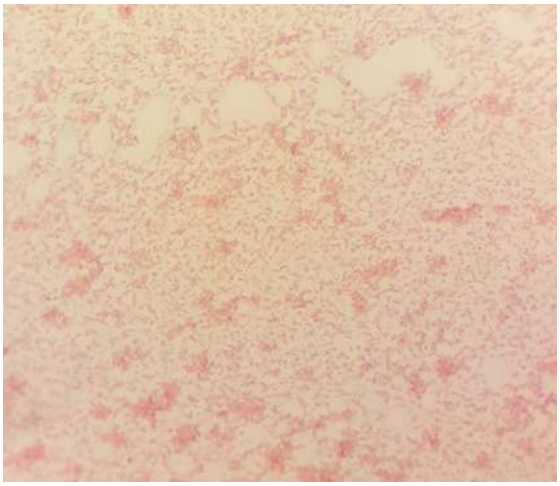
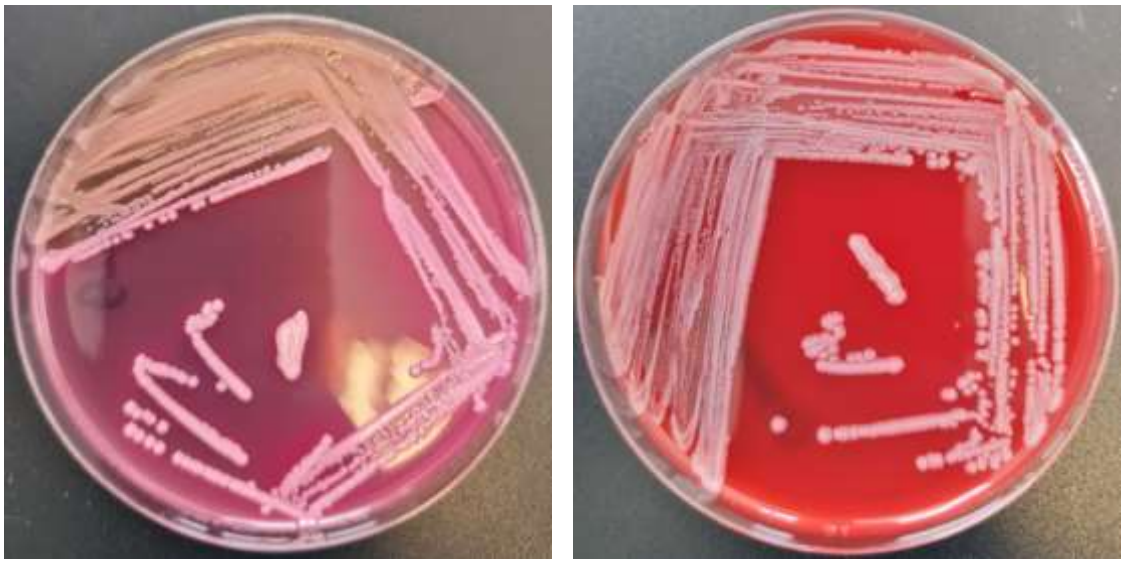


Figure 1. Gram staining where Gram-negative bacilli appear at 1000x magnification.



A **B**

Figure 2. *Elizabethkingia meningoseptica* colony: (A) Agar MacConkey. Colonies appear round, smooth, small-medium size, pale, non-fermenter (B) Blood Agar. The colonies appear round, small-medium size, anhemolysis.

3. Discussion

The *Elizabethkingia* genus is an opportunistic pathogen that includes deadly infections with resistance to various drugs. The mortality rate associated with this pathogen is approximately 30-54%. The *Elizabethkingia* genus consists of saprophytic bacteria that are able to survive in chlorinated water, hospital equipment, and child care. *Elizabethkingia* species are not part of the normal microbial flora of humans, although they are

considered opportunistic pathogens that commonly infect immunocompromised subjects. Bacteria belonging to this genus are known to have virulence properties such as protease, catalase, acetyltransferase, peroxidase, capsular polysaccharides, and lipooligosaccharides. The adhesion ability of *E. meningoseptica* to abiotic surfaces such as intravascular devices through biofilm formation is well known.[7,8]

Elizabethkingia meningoseptica is a rare cause of meningitis in infants and children, most cases in neonates are associated with premature birth. Meningitis is the most common infection associated with *Elizabethkingia meningoseptica*. Cases of endocarditis, pneumonia, cellulitis, wound infection, bacteremia after burns, abdominal abscess, dialysis-associated peritonitis, or endophthalmitis, especially in immunocompromised patients, have also been reported. The impact of *Elizabethkingia meningoseptica* on susceptible hosts, either by simple colonization or invasive infection, demonstrates the influence of the immune response on multiple pathogenic mechanisms. The source of infection cannot be accurately identified in the majority of reported symptomatic cases, although nosocomial transmission is usually considered. *Elizabethkingia meningoseptica* shows in vitro resistance to many antibiotics, where the antibiotic profile of *Elizabethkingia meningoseptica* is different from other Gram-negative rods. No CLSI breakpoints are set. This bacterium is characterized by the inherent resistance to aminoglycosides, β-lactam agents, chloramphenicol, and carbapenems, but also by susceptibility to Rifampicin, Ciprofloxacin, Vancomycin, and Trimethoprim-Sulfamethoxazole. The significance of *Elizabethkingia meningoseptica* antibiotic susceptibility is limited, as there are no validated susceptibility testing methods or antimicrobial treatment guidelines.[9-11]

Sample of cerebrospinal fluid (CSS) from a 1-month-old male patient with clinical information of suspected bacterial meningitis. sent to the Microbiology Laboratory of Dr. M. Djamil General Hospital Padang for culture examination and antibiotic sensitivity testing. The results of bacterial identification showed that *Elizabethkingia meningoseptica* with antibiotic sensitivity test results was resistant to carbapenems. Liquid samples were received in screw tubes, then placed in thioglycollate enriched media and incubated for 24 hours at 37°C. Cloudy enrichment media indicates bacteria are growing. The samples were then planted on blood agar and MacConkey agar media. Colonies that grow on Mac Conkey agar appear round, small to medium in size, smooth, pale in color, and translucent, and there is no visible hemolytic reaction on blood agar. Colonies that grow on agar culture are subjected to Gram staining. The results of Gram staining found Gram-negative bacilli. CSF culture is the gold standard in diagnosing bacterial meningitis. Positive test results can be found in 70-85% of patients who have not received antibiotic therapy and can decrease to less than 50% if the patient has received previous antibiotic therapy. Apart from being influenced by antibiotic therapy, the pre-analytic process plays a major role in CSF culture. The CSF specimen in this patient was not sent using transport media, therefore the specimen must be sent to the microbiology laboratory as soon as possible. Specimens for culture should not be refrigerated or exposed to extreme cold, excessive heat, or sunlight and transported at temperatures between 20°-35°C. CSF specimens must be cultured within 1 hour for proper culture results.[12,13]

This patient's CSF sample was immediately inoculated with thioglycollate broth. Inoculation with thioglycollate during processing of cerebrospinal fluid specimens for bacterial and fungal culture is a widely practiced procedure for the recovery of microbial pathogens. Broth cultures are usually used as an adjunct to support cultures on solid media. The use of this broth is aimed at recovering fastidious organisms or organisms present in the sample in small numbers, such as may occur early in the disease process or in the setting of previous antibiotic therapy. Patient CSF samples that were positive on thioglycollate media

were then planted on blood agar and MacConkey agar media. Colonies that grew on the media were made into smear preparations for Gram staining and Gram-negative bacilli were found. The next examination was carried out using the VITEK 2 tool, which is an automatic system for bacterial identification and antibiotic sensitivity testing based on fluorescent technology. The bacterial identification results obtained were *Elizabethkingia meningoseptica*. The results of the antibiotic sensitivity test in this patient were found to be sensitive to tigecycline and trimethoprim/sulfamethoxazole. The *Elizabethkingia* that infected this patient included carbapenem-resistant E. Meningoseptica.[14,15]

Elizabethkingia meningoseptica is a rare cause of meningitis in infants and children, but meningitis is the most common infection associated with *E. Meningoseptica*. Cases of endocarditis, pneumonia, cellulitis, wound infection, bacteremia after burns, abdominal abscess, dialysis-associated peritonitis or endophthalmitis, especially in immunocompromised patients, have also been reported in *E. meningoseptica* infections. Based on several studies, *E.* meningoseptica only accounts for approximately 1–21% of all *Elizabethkingia* pathogens isolated from clinical specimens. In contrast, *E. anophelis* is the most common pathogen in this genus, accounting for 59–99% of all isolates. Some automated tools, including Vitek 2, are only able to detect *E. meningoseptica*, so bias in the epidemiology of *Elizabethkingia* infections is very likely. The impact of *E. meningoseptica* on susceptible hosts, either by simple colonization or invasive infection, demonstrates the influence of the immune response on various pathogenic mechanisms. The source of infection cannot be accurately identified in the majority of reported symptomatic cases, although nosocomial transmission is usually considered primarily in newborns. The possible source of transmission in this patient occurred through nosocomial infection because the patient was treated for 2 weeks when he was just born and the patient's current condition occurred less than 30 days after being discharged from the hospital.[15,16]

Elizabethkingia exhibits a unique sensitivity pattern. Resistance occurs to many antibiotics commonly used to treat infections caused by Gram-negative bacteria. Resistance commonly occurs to aminoglycosides and β-lactam agents, including carbapenems, due to the production of extended-spectrum β-lactamases (ESBLs) and metallo-b-lactamases. In contrast, *E. meningoseptica* is often susceptible to agents used for Gram-positive bacteria. An increasing number of studies show that *Elizabethkingia* species (even the same species) isolated from different geographic regions have different antibiotic susceptibilities, indicating that there is a complex spectrum of antimicrobial resistance in *Elizabethkingia*. The mechanism of resistance to most β-lactam antibiotics is through the production of extended-spectrum β-lactamases and carbapenems that hydrolyze metallo-β-lactamases (MBL). *Elizabethkingia meningoseptica* is the only known microorganism with two chromosomally inherited intrinsic MBL genes. Metallo-β-lactamases (also known as class B β-lactamases) have been classified into three subclasses according to sequence homology: B1 (which covers most of the broad-spectrum, clinically relevant MBLs), B2 (the smallest subclass, consisting exclusively of carbapenemases), and B3 (a more distant group in terms of phylogeny). MBL-producing clinical pathogens, in general, escape the action of carbapenems by expressing a single MBL whose catalytic mechanism is efficient.[16,17]

Quinolones, rifampicin, trimethoprim-sulfamethoxazole, and piperacillin-tazobactam are the most active agents against *E. meningoseptica*. In examining the anti-microbial sensitivity of this patient, it was found that the germs were sensitive to trimethoprim-sulfamethoxazole, so the patient was given this antibiotic as a replacement therapy for the empirical antibiotics that had been used previously. The patient's history revealed a decrease in consciousness since 2 days of SMRS. The patient also showed recurrent seizures since 5 days of SMRS. During the seizure, the patient appeared stiff in the

legs and arms, accompanied by abnormal movements in the eyes, and was last seen twice in the emergency room. The patient's history also revealed that the patient had a fever that was not too high. The patient showed somnolence (GCS 9) with an increase in temperature to 38.5°C on physical examination. Signs of meningeal stimulation were found in patients in the form of a stiff neck and Kernig's sign. The patient's fontanel also appears prominent and tense when palpated. Several pathological reflexes were found in patients in the form of Babinski, Gordon, and Schaefer reflexes. Neonates with bacterial meningitis often present with nonspecific symptoms, whereas in children other than neonates, the most common clinical characteristics of bacterial meningitis are fever, headache, stiff neck, and vomiting. The patient was found to have a fever. Fever is the most frequently reported symptom in childhood bacterial meningitis, with an incidence rate of 92–93%. The patient was also found to have seizures and decreased consciousness. Seizures have been reported on admission in 10-56% of children, while mental status changes have been reported in 13-56% of cases of childhood bacterial meningitis.[18,19]

The results of laboratory examinations in this patient were within normal limits, except for a decrease in total protein and globulin. The patient has symptoms of fever, but the leukocyte examination is normal and globulin is decreased. This can occur because the immune system of neonates and babies is still limited and they have a higher risk of infection. Studies have shown that white blood cell (WBC) screening is less predictive of infection in neonates. Apart from that, several studies also stated that total protein decreased in all babies, and this decrease was mainly caused by low globulin. Cerebrospinal fluid analysis is usually the first step to confirm the presence of bacterial meningitis and is performed on patients before culture. Typical CSF abnormalities associated with bacterial meningitis include turbidity, increased leukocytes >10 cells/mm^3 (especially PMN), decreased glucose concentration (<45 mg/dL), and increased protein concentration (>45mg/dL). On macroscopic examination of this patient's fluid, the fluid was yellowish with positive turbidity. Apart from that, the patient's CSF microscopic examination also complies with the CSS criteria for bacterial meningitis, in the form of an increase in the CSF leukocyte count to 137 cells/mm^3 with a PMN : MN ratio of 98 : 2, as well as a decrease in glucose to <5 mg/dL and an increase in protein to 450.5 mg/dL. Infections caused by *Elizabethkingia* spp. It is very difficult to treat and has a high mortality rate, especially in immunocompromised patients. One study reported a 28-day mortality rate of 41% for nosocomial infections and 9.1% for community-acquired infections. Normalization of CSF analysis was achieved in this case after six weeks, but complications of hydrocephalus developed, and other neuropsychological complications were possible over the next few years. Hydrocephalus can occur early in the process of bacterial meningitis or after treatment with antibiotics for days or weeks. In other studies, the interval from first symptoms to diagnosis of hydrocephalus was usually less than 4 weeks, with the shortest recorded time being 5 days.[19,20]

4. Conclusion

This case demonstrates the complexity of carbapenem-resistant EM meningitis in an infant. Typical clinical presentation, appropriate supporting examinations, cerebrospinal fluid culture, and antibiotic selection based on bacterial sensitivity are the keys to effective diagnosis and treatment. Early treatment with appropriate non-carbapenem antibiotics can increase the patient's chances of recovery.

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