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IDENTIFICATION OF THE CTX-M GENE IN KLEBSIELLA PNEUMONIAE PRODUCING ESBLS IN UTI PATIENTS AT A PRIVATE HOSPITAL IN PURWOKERTO, BANYUMAS REGENCY, CENTRAL JAVA - CROSS SECTIONAL STUDY

Research Report
Penelitian

IDENTIFIKASI GEN CTX-M PADA PENGHASIL ESBLS PADA PASIEN ISK DI RUMAH SAKIT SWASTA PURWOKERTO KABUPATEN BANYUMAS JAWA TENGAH - STUDI CROSS SECTIONAL

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A B S T R A C T

Background: *The CTX-M gene in ESBLs-producing Klebsiella pneumoniae is associated with virulence factors and encodes for antibiotic resistance. There are currently 128 different varieties of CTX-M, which gene can hydrolyze beta-lactam medicines like cefotaxime.* ***Purpose:*** *Identify the CTX-M gene in Klebsiella pneumoniae from patients with Urinary Tract Infections (UTI) at a private hospital in Banyumas Region of Central Java, Indonesia.* ***Method:*** *Cross-sectional survey method was employed in this study. The subjects of the study comprised 40 patients with UTI admitted to a private hospital in Banyumas Region, Central Java, Indonesia. Using a direct molecular approach called Polymerase Chain Reaction (PCR) directed against the CTX-M gene, DNA was isolated from isolates cultured in CHROMagar™ ESBLs obtained from catheter urine samples.* ***Result:*** *A total of 4 out of 40 urine samples (10%) tested positive for ESBL-producing Klebsiella pneumoniae. The PCR was performed to detect the CTX-M gene in 100% of the strains.* ***Conclusion:*** *These findings suggest the presence of CTX-M gene found in ESBLs-producing Klebsiella pneumoniae in individuals with urinary tract infections in a private hospital in Purwokerto, Banyumas regency, Central Java, Indonesia. This information can be used to assess antibiotic administration practices.*

A R T I C L E I N F O

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A B S T R A K

Latar belakang: Gen CTX-M telah dilaporkan terkait dengan faktor virulensi dan kode untuk resistensi antibiotik pada *Klebsiella pneumoniae* yang memproduksi ESBLs. Gen ini dapat menghidrolisis antibiotik beta-laktam, termasuk sefotaksim, dan saat ini ada 128 jenis CTX-M yang berbeda. **Tujuan:** Mengetahui gen CTX-M pada *Klebsiella pneumoniae* pada individu dengan infeksi saluran kemih di Rumah Sakit Swasta wilayah Banyumas, Jawa Tengah, Indonesia. **Metode:** Penelitian ini merupakan penelitian *cross-sectional*. Subyek penelitian terdiri dari 40 pasien yang terdaftar dengan infeksi saluran kemih di sebuah rumah sakit swasta di Wilayah Banyumas, Jawa Tengah, Indonesia. DNA diekstraksi dari isolat yang dibiakan menggunakan agar CHROM ESBLs yang diperoleh dari sampel urin kateter kemudian diamplifikasi menggunakan metode molekuler langsung *Polymerase Chain Reaction* (PCR) terhadap gen CTX-M. **Hasil:** Sebanyak 4 dari 40 sampel urin dikonfirmasi positif untuk *Klebsiella pneumonia* penghasil ESBLs (10%). Setelah itu, semua galur (100%) positif untuk gen CTX-M menggunakan metode PCR. **Kesimpulan:** Penemuan ini menunjukkan adanya gen CTX-M yang ditemukan pada *Klebsiella pneumoniae* penghasil ESBL pada individu dengan infeksi saluran kemih di rumah sakit swasta di Purwokerto, Kabupaten Banyumas, Jawa Tengah, Indonesia. Informasi ini dapat digunakan untuk mengevaluasi cara pemberian antibiotik.

Kata kunci:
Resistensi antibiotik, CTX-M, ESBLs, Infeksi saluran kemih, *Klebsiella pneumoniae*

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INTRODUCTION

Antibiotic resistance, often referred to as the alarming bacteria, is a life-threatening problem in many regions of the world (Afifah *et al*., 2017). Antibiotic resistance is predicted to kill 10 million population worldwide by 2050, with 4.7 million dying in Asia if the problem is not addressed. In the United States, antibiotic resistance kills roughly 23.000 individuals each year (Ahmed *et al*., 2013). Furthermore, antibiotic resistance is expected to cause 135.000 fatalities annually in Indonesia, incurring a cost of 56 trillion dollars for the country (AL-Subol and Youssef, 2015). In Indonesia, the incidence of sepsis remains high at 30.29% with a mortality rate ranging from 11.56 to 49%. The mortality rate of severe sepsis falls within the range of 15% to 40%, and the mortality rates due to septic shock range between 20% - 72% (Fernando *et al*., 2017).

ESBLs are enzymes that confer resistance to most beta-lactam antibiotics, including penicilins, chepalosporins, and the monobactam aztreonam (Dia *et al*., 2019). The prevalance of ESBLs and molecular detection of blaTEM, blaSHV, and blaCTX-M genotypes among Enterobacter isolates from patients in Sudan, according to the Ambler clasification, indicate that ESBLs belong to class A. There is a predominance of pasmid-borne EBSLs, such as CTX-M lactanase, widely distributed in *Escherichia coli* and GES, which are prevalent in *Enterobacterales*, *Pseudomonas aeruginosa*, and *A. Baumannii*.

A previous study conducted at the Government Hospital in the Banyumas Regency, Central Java Indonesia, reported that 2 out of 40 *Klebsiella pneumoniae* analyzed were resistant to antibiotics (Afifah *et al*., 2017). These bacteria contribute to healthcare-associated illnesses and are the most frequent cause of urinary tract infections in hospital (Musdalipah, 2018). *Klebsiella pneumoniae* can produce biofilms, making it more challenging to treat and increasing antibiotic resistance in UTI patients (CDC, 2019). Genes encoding resistance are related to one of the virulence factors against a bacterium. They are encoded by bla-CTX-M, bla-SVH, bla-OXA, and bla-TEM in ESBLs-producing *Klebsiella pneumoniae*. In cases of microorganism-induced resistance, the CTX-M gene is the most commonly detected in bacteria. CTX-M is a gene that can hydrolyze beta-lactam antibiotics, such as cefotaxime. There are currently 128 different varieties of CTX-M (Ghafourian *et al*., 2015).

In 2005 to 2009, the prevalence of the CTX-M gene in *Klebsiella pneumoniae* increased by 1.7% in New York. Subsequently, from 2010 to 2012, it increased to 26.4% (Dirar *et al*., 2020). According to research conducted at Sanglah Hospital in Denpasar Bali that the CTX-M gene was detected in 77.3% of 97 *Klebsiella pneumoniae* samples (Endriani *et al*., 2016). This study aims to detect the CTX-M gene, which is the most common ESBL-encoding gene associated with antibiotic resistance. The CTX-M gene is highly effective at hydrolyzing cefotaxime and ceftazidime, causing failure in therapy and increasing morbidity.

MATERIAL AND METHOD

Collecting urine samples

The ethical approval for this study was obtained from the ethics committee of the Faculty of Medicine, Jenderal Soedirman University, number 054.KEPK.III. 2021. Cross-sectional survey method was employed in this study. The participants consisted of 40 patients who were enrolled with urinary tract infections in a private hospital in Banyumas Regency, Central Java, Indonesia. Prior to participation, the patients were given informed consent forms and received detailed explanations, thus their participation was entirely voluntary, and no was involved. Urine specimens were collected using the following method. The catheter tube was clamped (no more than 30 minutes). Then the sampling location on the catheter was wiped with 70% alcohol. An injection syringe (21 G needle) was inserted at the sampling site, then 15 ml of urine was suctioned. The urine was, then, put in a sterile pot aseptically, and the clamp was re-opened. The urine samples were taken to the research laboratory of the Faculty of Medicine of Jenderal Soedirman University, Purwokerto, Indonesia. Any urine samples that could not be analyzed within 30 minutes of collection were refrigerated. Urine culture was performed after 24 hours after the received sample was cooled (Hermawan HA and Guntur, 2008).

Identification of ESBLs-producing *Klebsiella pneumoniae*

ESBL-producing *E.coli* bacteria were cultured by streaking the urine sample on HiCrome™ ESBL agar medium^{+} HiCrome™ ESBL agar supplement. The media were, then, incubated for 24 hours at 37^{0}C. Chromogenic mixture was used to distinguish the pigment of ESBL-producing organisms. HiCrome™ ESBL selective medium was used in order to suppress the growth of contaminating microorganisms. ESBL *Klebsiella pneumoniae* in the selective medium appeared bluish green, *E.coli* showed purple and *Pseudomonas aeruginosa* exhibited light green.

Detection of the ESBLs-producing *Klebsiella pneumoniae* CTX-M gene by PCR

Klebsiella pneumoniae bacteria from the culture results were, then, extracted using the Geneaid Presto™ Mini Plasmid Kit to obtain bacterial DNA. The extracted DNA was, then, amplified using *Polymerase Chain Reaction* (PCR). In cycle 1 there was a pre-denaturation 1x with a temperature of 95^{0}C for 15 seconds. Then the denaturation process, was carried out for 35 cycles. The next process was the annealing process, which took

place in two stages. In stage 1, the temperature was set at 94^{0}C for 30 seconds, and in stage 2, the temperature was set at 54^{0}C for 40 second. The primers used were the forward primer (5'- ATG TGC AGY ACC AGT AAR GT 3') and the reverse primer ('5 - TGG GTR AAR TAR GTS ACC AGA 3'). The size of the CTX-M gene amplification was 560 bp[1]. The amplification process included an initial step at a temperature of 95^{0}C for 15 seconds, followed by the cycle 2 that was carried out for 35 cycles. In stage 1, the denaturation process took place at a temperature of 94^{0}C for 30 seconds, while in stage 2, the annealing process occurred at a temperature of 54^{0}C for 40 seconds (aneling process). The primers used were the forward primer (5'- ATG TGC AGY ACC AGT AAR GT 3') and the reverse primer ('5 - TGG GTR AAR TAR GTS ACC AGA 3'). The size of the CTX-M gene amplification was 560 bp[1].

RESULT

Identification of ESBLs-producing *Klebsiella pneumoniae*

According to the identification of ESBLs producing *Klebsiella pneumoniae* based on the analysis of 40 urine samples, the study revealed four isolates of *Klebsiella pneumoniae*, all of which produced ESBLs as shown in Figure 1.

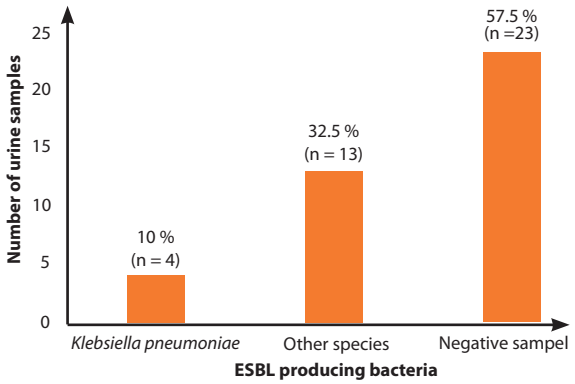

Figure 1. Percentage of ESBLs-producing *Klebsiella pneumoniae*

Based on Figure 1, it is evident that out of 40 UTI patients' urine samples, 17 tested positive ESBL isolates, consisting of 4 *Klebsiella pneumoniae* isolates (24%), 13 isolates from other species (32.5%) and 23 (57.5%) negative ESBL-producing bacteria samples.

Detection of the ESBLs-producing *Klebsiella pneumoniae* CTX-M gene

The results showed that all 4 isolates of ESBLs-producing *Klebsiella pneumoniae* were positive for the CTX-M gene as a code for antibiotic resistance as seen in Figure 1. The results of the electrophoresis of PCR products on agarose gel were shown in Figure 2. The CTX-M gene was detected in the positive samples, as indicated by the bands in lanes 1, 2, 3, and 4 with a size of 560 bp in the PCR.

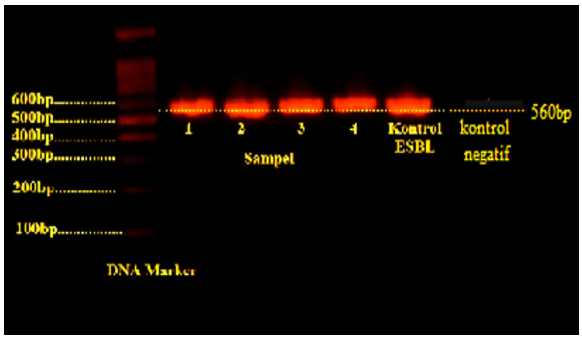

Figure 2. PCR results of CTX-M gene with 560 bp amplicon on *Klebsiella pneumoniae* producing ESBLs bacteria

DISCUSSION

According to the data analysis in Figure 1. *Klebsiella pneumoniae* ESBLs-producing bacteria is a common cause of urinary tract infections in a private hospital in Banyumas Regency, Central Java, Indonesia. *Klebsiella pneumoniae* was the second most common bacteria, accounting for 20% of all cases (Hamam *et al*., 2019). *Klebsiella pneumoniae* was the second most common ESBLs-producing bacteria in Sri Lanka with a prevalence (13.1%) and *E.coli* bacteria (86.8%) (Higgins, 2008).

ESBLs-producing *Klebsiella pneumoniae* was discovered in four out of the 40 urine samples (10%). This finding is higher than that reported the Government Hospital in a previous study. In a study involving 40 samples from inpatient and intensive care rooms, researchers discovered two cases of positive ESBLs-producing *Klebsiella pneumoniae* bacteria (Bahr *et al*., 2021). Many studies have demonstrated that ESBLs-producing bacteriahave a higher mortality rate than those that do not produce ESBLs (James, 2011). *Polymerase Chain Reaction* (PCR) examination (Figure 2) showed that all ESBLs-producing *Klebsiella pneumoniae* were positive for the CTX-M gene (Izadpanah and Khalili, 2015). A previous study reported a similar result, CTX-M-1 phylogenetic subgroup was positive in seven *E. coli* and three *Klebsiella pneumoniae* isolates. In an examination of 102 *E. coli* bacteria and 21 *Klebsiella pneumoniae* bacteria, four ESBL-producing *Klebsiella pneumoniae* isolates (19%) and 12 ESBL-producing *E. coli* isolates (11.7%) were identified. Among these samples, 3 samples of *Klebsiella pneumoniae* produced positive ESBL gene CTX-M (75%), and 7 samples of *E. coli* produced positive ESBL gene CTX-M (58.3%) (Martinez *et al*., 2012). Another study conducted in Lebanon found that 68 (86.7%) *Klebsiella pneumoniae* were positive for the CTX-M gene (Obeid Charrouf *et al*., 2014). This gene of *Klebsiella pneumoniae*, is the most prevalent or dominant (Ming *et al*., 2017). This gene can be transferred through conjugative transfer and is often located on bacterial plasmids.

Factors that influence this gene include the use of antibiotics, geographical location, and changes in the patient's condition (Fatmawati *et al*., 2015). ESBLs-producing bacteria can rapidly spread among Gram-negative bacteria due to this gene (Mohajeri *et al*., 2018). This gene originates from the *Kluyvera* genus, which is only found in medical laboratories on a very occasional basis (Dia *et al*., 2019). The selection procedure for administering antibiotics to bacteria *Kluyvera* resulted in a mutation in this gene (Fatmawati *et al*., 2015).

Presently, CTX-M-producing bacteria are increasingly prevalent in various illnesses, both in the community and in hospitals (O'Neill, 2016). However, the high global distribution of the CTX-M enzyme increases to two or more β-lactamases in the same strain, and is becoming a common bacterial strategy to increase antibiotic resistance (Charrouf *et al*., 2014). Since ESBLs-producing bacteria have more genes for antibiotic resistance, they frequently become resistant to antibiotic classes outside beta-lactams. This limits the treatment choices for hospital patients infected with CTX-M bacteria to carbapenems. Because of the current appearance of bacteria that produce carbapenemases (beta-lactamases enzyme can deactivate carbapenems), there are no safe antibiotics left to treat these infections; it could possibly inpact on the decline on health-care systems all over the world (Sahputri *et al*., 2018).There are several types of CTXM Genes which are divided into 5 sub-clusters, namely CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, CTX-M-25 and there are also several enzymes that have not been published (CTX-M-22, CTX-M-23 and CTX-M-28 (Schwaber and Carmeli, 2008). The research results, it was discovered that the percentage of the CTX-M gene in *Klebsiella pneumoniae* bacteria producing ESBL as a gene encoding antibiotic resistance was (100%). The CTX-M β-lactamase gene causes an epidemic of resistance to more than one type of drug (multidrug resistance).

CONCLUSION

CTX-M was identified in the ESBLs-producing, *Klebsiella pneumoniae* as the gene encoding antibiotic resistance at a percentage level, according to the results of this study (100%). This information can be used to evaluate how antibiotics are being administered.

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