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Prevalence of CTX-M Gene as an ESBL Marker in Clinical Klebsiella pneumoniae Isolates

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ABSTRACT

Klebsiella pneumonia is a bacteria that causes many negative impacts on health, primarily due to its tendency to develop antibiotic resistance. This research aims to identify the prevalence of the CTX-M gene as an ESBL marker of the Clinical Isolate of K. pneumoniae of Wahidin Sudirohusodo General Hospital. The samples consisted of 50 Clinic isolates of K. pneumoniae which fulfilled the inclusion and exclusion criteria. The research procedure consisted of DNA extraction, amplification, and agarose gel electrophoresis. The research results indicate that 38 samples (76%) are positive for the CTX-M gene, while 12 samples (24%) are negative or not found CTX-M gene. Thus, the prevalence of the CTX-M gene in K. pneumoniae resistant to beta-lactam antibiotics in Wahidin Sudirohusodo General Hospital is relatively high.

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Introduction

Klebsiella pneumoniae is a gram-negative bacterium that causes many negative impacts in the health sector, mainly due to the tendency of this bacterium to develop antibiotic resistance. The global surveillance report on antibiotic resistance conducted by the World

Health Organization, shows that Klebsiella pneumoniae is one of nine bacteria of concern in antibiotic resistance. Antibiotic resistance of each bacteria has a different mechanism.

β-lactamase synthesis is considered to be a major mechanism that facilitates the development of clinically important resistance to gram-negative bacteria to β-lactam antibiotics [1]. Genetic mutations cause the replacement of several amino acids in the protein sequence which affects the structure of the enzyme in producing an expanded spectrum of hydrolyzed antibiotics. Mutations can appear rapidly, because the microorganism becomes resistant to antibiotics during the course of treatment. Resistance can be acquired from birth or acquired. Natural resistance can be characterized by the absence of the presence of target microorganisms that can produce chromosomes encoding β-lactamases, such as for example Klebsiella pneumoniae which produces SHV-1 and Enterobacter cloacae, Enterobacter aerogenes, Citrobacter freundii, Serrata spp., and Pseudomonas aeruginosa which produces β-class C lactamases. The ability of individual bacterial strains to maintain viability at concentrations of antibiotics that inhibit most microbial populations, is considered as acquired or secondary resistance [2].

The formation of acquired resistance is often due to the acquisition of new genetic information or changes in the expression level of wild-type genes. The determinants of secondary resistance are mobile genetic elements acquired simultaneously. Resistance in the Enterobacteriaceae family to β-lactam antibiotics is acquired resistance. ESBL genes are transferred via different mobile genetic elements such as plasmids, transposons, insertion sequence (IS) elements, class 1 integrons, and ISCR1 elements containing integron. Variations of genetic transfer mechanisms contribute to the rapid spread of genes [3].

Identification of genes that cause resistance is still rarely done in Indonesia. In fact, by identifying these genes, it can be seen the percentage of spread of resistant genes and the potential for a bacterium to develop resistance to certain classes of antibiotics, so that health workers become more vigilant in selecting the modalities of infection therapy. Based on this, identification of the prevalence of the CTX-M gene group is important to determine the percentage of these genes in causing resistant strains of Klebsiella pneumoniae.

Material And Methods

This research uses a cross-sectional study. The research was carried out from March to July 2018 at the Microbiology Laboratory at Hasanuddin University RSP, Makassar. The isolates came from the Clinical Pathology Laboratory of RSUP Wahidin Sudirohusodo Makassar. The population in the study were all K. pneumoniae isolates from March to May 2018. Sample collection was carried out by total sampling, where all isolates that met the

inclusion and exclusion criteria would be used as research samples. The inclusion criteria of this study were isolates from RSWS PK Lab, isolates from various samples (pus, blood, feces, throat swabs), K. pneumoniae isolates with identification test results using an automated tool (Vitek® 2 Compact), isolates entered during March to May 2018. The exclusion criteria from this study were damaged isolates (contaminated/dry isolates).

The tools used in this study include: PCR machine (Bio Rad), DOC Gel, Electrophoresis Machine, Centrifuge, Waterbath, Laminary air Flow, Type II BSC, Micropipette (1000 ul, 100ul, 20ul, 10ul), Agarose molds, Tips (1000 ul,100 ul, 20 ul,10 ul), Eppendorf tube, PCR tube, Erlenmeyer, Measuring cup, ose, Bunsen. The materials used in this study were: Sample, Primer, PCR Enzyme (Kappa Hot Star Taq DNA polymerase), RNAse Free water, Agarose, Ethidium Bromide, TBE 0.5, Loading Dye DNA Ladder/Marker (100 bp), PBS. PCR examination.

The initial step of PCR examination is by extracting DNA. DNA extraction was carried out according to the Geneaid PrestoTM DNA/RNA Extraction Kit protocol. Followed by DNA amplification using CTX-M-U1 primer : (5'- ATG TGC AGC ACC AGT AAA GTG ATG GC -3') and CTX-M-U2 : (5'- TGG GTA AAG TAA GTG ACC AGA ATC AGC GG -3'). The PCR mix consists of: 12.5 µl PCR Enzymes (Go Taq Master Mix); 0.5 µl CTX-M-U1 Primer; 0.5 µl CTX-M-U2 Primer; 5.0 µl of product DNA; 6.5 µl Nuclease Free water. The PCR conditions used were as follows: pre-denaturation (95°C for 15 seconds), denaturation at 94°C for 30 seconds with 35 cycles, annealing (61°C for 40 seconds) and extension (72°C for 2 minutes), then ended with final extension at 72°C for 2 minutes. Next, electrophoresis of the PCR product was visualized in 2% agarose. GelDoc is used to read electrophoresis results. Positive results are marked with. band formation at 593 bp. The data obtained were then analyzed descriptively.

Results and Discussions

Isolate *Klebsiella pneumonia*e obtained from the Clinical Pathology Laboratory, Microbiology and Tropical Infection subdivision, RSUP Dr. Wahidin Sudirohusodo Makassar from March-May 2018 and found 50 samples that met the inclusion and exclusion criteria. The samples came from various types of specimens, namely: 20 sputum (40%), 15 urine (30%), 6 feces (12%), 4 pus (8%), 3 blood (6%), 1 gastric rinse (2 %), and 1 bronchial washing (2%).

Based on the PCR results, it was found that 38 samples (76%) were positive for the CTX-M gene and as many as 12 samples (24%) were negative for not having the CTX-M gene. The positive and negative results were based on whether or not a band was formed at 593bp when carrying out agarose gel electrophoresis of PCR products. PCR product visualization results in 2% agarose gel.

Distribution of the presence of the CTX-M gene in various isolates*K. pneumonia* can be seen in Table 1 which shows that of the 38 samples that were positive as many as 12 samples came from sputum (24%), 13 samples came from urine (26%), 6 samples from feces (12%), 3 samples came from pus (6 %), 2 samples from blood (4%), 1 sample from gastric lavage (2%) and 1 sample from bronchial washing (2%). Most antibiotic resistance marker genes were found in urine samples.

Table 1. Distribution of the presence of the CTX-M gene in K. pneumoniae isolates

No	Specimen type	Number of isolates (%)	Gen CTX-M
1	Sputum	20 (40%)	12 (24%)
2	Urine	15 (30%)	13 (26%)
3	Stool	6 (12%)	6 (12%)
4	Put	4 (8%)	3 (6%)
5	Blood	3 (6%)	2 (4%)
6	Gastric lavage	1 (2%)	1 (2%)
7	Bronchial washings	1 (2%)	1 (2%)

Fig. 1 shows the measurement model visualization of PCR products on gel agarose 2%. A positive result is characterized by the appearance of the band at 445 bp. M is Marker; K- is a negative control; K+ is positive control; A1-A17 is isolates 1 through 17.

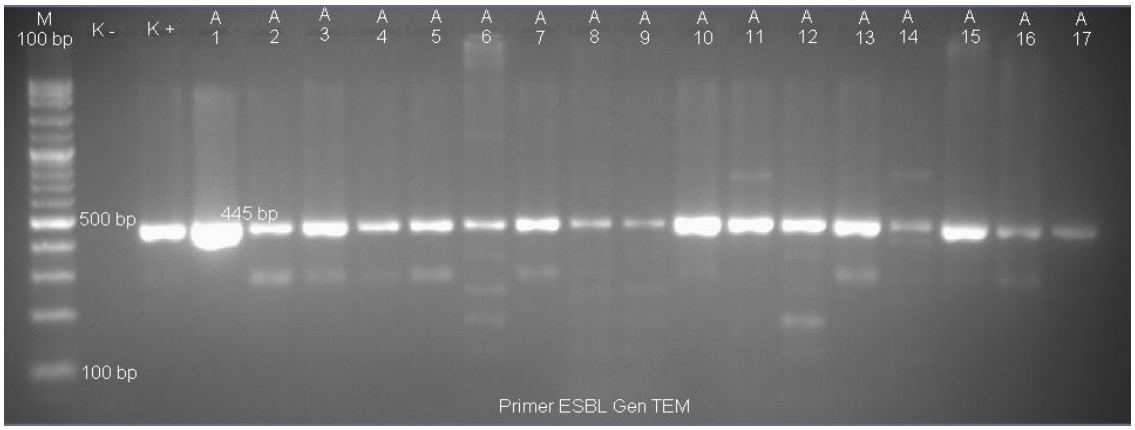


Fig. 1. Measurement Model Visualization of PCR products on gel agarose 2%

This study showed a fairly high prevalence where the percentage of the CTX-M gene in ESBL isolate K. pneumoniae from the Clinical Pathology Laboratory Tropical Infection Subdivision Wahidin Sudirohusodo Hospital for the period March-May 2018 was 76%. A high prevalence was also found in a study conducted at Sanglah General Hospital in Denpasar on 97 samples of K. pneumoniae and found a prevalence of the CTX-M-1 gene of 77.3% [4]. In this study the most CTX-M gene was found in urine samples, this is in line with research conducted at Al-Zahra Hospital Isfahan Iran in patients with urinary tract infections (ISP), the prevalence

of the CTX-M gene and the TEM K. pneumoniae gene in 25 strains ESBL-producing found 23 (92%) to contain CTX-M and 19 (76%) to contain TEM [5].

In the last decade, CTX-M type ESBLs have increased clinically in Enterobacteriaceae isolates. This was confirmed by a study in Lebanon where 86.7% of 68 ESBL isolates of K. pneumoniae contained blaCTX-M [6]. Research in the United States in 1990 showed that the prevalence of blaCTX-M was still rare but increased by 25% in 2000 and 90% in 2005 [7]. A study in New York also showed agreement with the increase in the prevalence of blaCTX-M K. pneumoniae, namely a significant increase from 2005 to 2009 of 1.7% and from 2010 to 2012 it increased to 26.4% ($p<0.0001$) [8]. Nosocomial infections caused by CTX-M-15 which are resistant to more than one drug (multidrug resistant) produce K. pneumoniae (CTX-M-15KP), which is known to have increased dramatically in recent years [9] [10] [11].

Conjugative plasmids are considered as one of the main factors causing the successful spread of CTX-M type ESBLs in K. pneumoniae [12]. The blaCTX-M-15 gene is often associated with specific insertion sequences (ISs) (eg ISEcp1) and plasmids of the F incompatibility group [13]. In previous studies it was explained that blaCTX-M-3 was thought to be the ancestor of blaCTX-M-15 which was obtained from the Kluyvera spp chromosome. via ISEcp1. Other mobile elements are then involved in the transfer of ISEcp1 blaCTX-M-15 from among plasmids to the chromosomes of other members of Enterobacteriaceae [12].

The CTX-M enzyme has almost replaced other ESBL enzymes in Enterobacteriaceae, including TEM and SHV variants [14][15]. This substitution occurred not only as a result of the spread of the blaCTX-M gene in its extraordinary mobilization of genetic material transfer including plasmids and transposons, but also because of its success in cloning [16]. The increased resistance phenomenon that occurs in CTX-M-producing organisms to aminoglycosides and fluoroquinolones also facilitates the selection process for resistance [17].

Conclusion

Based on the research that has been carried out, it can be concluded that the prevalence of the CTX-M gene group in *Klebsiella pneumoniae* in Wahidin Sudirohusodo General Hospital Makassar for the period March - May 2018, was quite high at 76%. CTX-M-15, a multidrug-resistant K. pneumoniae, has dramatically increased in recent years. Conjugative plasmids are considered the main factor in the spread of CTX-M type ESBLs in K. pneumoniae, with the blaCTX-M-15 gene often associated with ISs and F incompatibility group plasmids. CTX-M enzyme has replaced other ESBL enzymes due to its successful mobilization of genetic material transfer, including plasmids and transposons, as well as its cloning success. The increased

resistance of CTX-M-producing organisms to aminoglycosides and fluoroquinolones also contributes to its selection.

Conflict of Interest

The authors should declare that there is no conflict of interest.

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