Journal of Health Sciences and Medical Development
Vol. 1, No. 02, pp. 76-82
journal.iistr.org/index.php/HESMED
DOI: 10.56741/hesmed.v1i02.155

Tem Gene Detection as ESBL Marker in Clinical Isolates *Klebsiella pneumoniae*

A Case Study

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ARTICLE INFO

Article history
Received 19 September 2022
Revised 19 October 2022
Accepted 8 December 2022

Keywords
ESBL
Klebsiella pneumoniae
PCR
Tem Gene

ABSTRACT

Klebsiella pneumoniae is one of the most common ESBL-producing microorganisms. The research aims to detect the *Tem* gene in clinical isolates of *K. pneumonaie* from the Laboratory of clinical pathology of Wahidin Sudirohusodo General Hospital. Methods: This research is a descriptive cross-sectional design. Fifty samples of *K. pneumonieae* were obtained from the Laboratory of clinical pathology from March to May 2018. *Tem* gene was detected using primary *Tem* 164.SE and *Tem* 165.AS (445 bp) of Polymerase Chain Reaction (PCR). The research results indicate that 48 isolates are positive, containing the *Tem* gene with the highest number found in the sputum specimen. Genotype detection of bacteria-producing ESBL is essential so that antibiotic therapy given to the patients is more effective and efficient.

Introduction

Antibiotics are biochemical substances produced by microorganisms and used to inhibit or kill other microorganisms [1]. Using antibiotics that are not on target can lead to antibiotic resistance. Antibiotic resistance is an important problem in the health sector. Antibiotic resistance can occur through many mechanisms, for example: chromosomal mutations, expression of latent chromosomal resistance genes, bacteriophages,

extrachromosomal DNA plasmids, or acquired resistance through transformation mechanisms [2].

Ref. [3] said that the development of antibiotic resistance had spread worldwide; one example of MDRO is Extended-Spectrum β-Lactamases (ESBL) which are commonly found in gram-negative bacteria. Infection by ESBL-producing bacteria is a serious problem in hospitals. Bacteria with antibiotic-resistance genes can transfer these genes to other bacteria. If this resistant organism spreads, it will threaten society and cause the presence of new types of infections that are more difficult to treat, extend the hospitalization period, and cause higher medical costs [4-7]. ESBL enzyme-producing bacteria can cause many health problems, including urinary tract infections, peritonitis, cholangitis and intra-abdominal abscess [8, 9].

Based on the results of several studies in America and Europe, it was shown that 60% of clinical isolates were ESBL bacteria. *K. pneumonaie* is one of the bacteria recorded as having the highest ESBL activity compared to other bacteria [10, 11]. According to research by Ref. [12], at Arifin Achmad Pekanbaru Hospital, throughout 2015, out of 1121 bacterial isolates, 228 *K. pneumonaie* isolates were found, 151 of which were detected positive for ESBL (66.2%).

ESBL is a β-lactamase that causes resistance to penicillin, first, second and third-generation cephalosporins, and aztreonam by hydrolyzing these compounds [13]. In 1963, The β -lactamase enzyme was first discovered in E. coli and named *TEM* [14]. *TEM*, *SHV, OXA* and several other mutated genes are the genes responsible for the ability of ESBL strains to hydrolyze β-lactam class antibiotics extensively. The *TEM* gene is one of the ESBL-encoding genes that cause resistance to ampicillin, penicillin and first-generation cephalosporins such as cephalothin. This gene is widely used in detecting ESBL [14]. According to Ref. [16], in 2009 and 2010, 78 strains of *K. pneumonaie* were obtained from patients at Imam Reza Hospital, and Ghaem University Hospital, where as many as 56.01% of isolates were detected to produce ESBL and 20.6% of isolates were shown to have the *Tem* gene.

Early detection of ESBL-producing Enterobacteriaceae can assist in selecting appropriate antimicrobials and infection control [17]. ESBL detection can be done phenotypic and genotypic. The ESBL encoding gene can be detected through DNA amplification using a standard molecular method, namely the polymerase chain reaction or PCR. Based on this, the purpose of this study was to detect the presence of the *Tem* gene in clinical isolates of *K. pneumonaie* from the Clinical Pathology Laboratory, Tropical Infection Subdivision, Wahidin Sudirohudoso General Hospital for the period March-May 2018.

Methods

The research design is cross-sectional. The research was conducted in March - July 2018 at The Cell and Molecular Biology Laboratory, HUM-RC, Makassar. The isolates came

from the Clinical Pathology Laboratory of RSUP Wahidin Sudirohusodo Makassar. The population in the study were all *K. pneumonaie* isolates from March-May 2018. Sample collection was carried out by total sampling, where all isolates that met the inclusion and exclusion requirements would be used as research samples. The inclusion criteria in this study were isolated from RSWS PK Lab, isolates from various samples (pus, blood, faeces, throat swabs), and K. pneumonia isolates with identification test results using an automated tool (Vitek® 2 Compact). It isolates entered during March–May 2018. The exclusion criteria in this study were damaged isolates (contaminated/dry isolates). The equipment used in this study included: PCR machine (Biorad), DOC Gel, Electrophoresis Machine, Centrifuge, Water bath, Laminary air flow, BSC Type II, Micropipette (1000 ul, 100 ul, 20 ul, 10 ul), Agarose Molds, Tips (1000 ul,100 ul,20 ul,10 ul), Efendorf 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.

DNA extraction was carried out according to the DNA extraction protocol Genead Presto TM DNA/RNA Extraction Kit. DNA amplification was performed using primers 164.SE (5'- TCG CCG CAT ACA CTA TTC TCA GAA TGA -3') and 165.AS (5'- ACG CTC ACC GGC TCC AGA TTT AT -3'). The PCR mix consists of: 12.5 µl PCR Enzymes (Go Taq Master Mix); 0.5 µl Primer *Tem* 164.SE; 0.5 µl Primer *Tem* 165.AS; 5.0 µl of product DNA; 6.5 µl Nuclease Free water. The PCR conditions used were as follows: predenaturation (95°C for 15 seconds), denaturation at 94°C for 30 seconds for 35 cycles, annealing (61°C for 40 seconds) and extension (72°C for 2 minutes), then ended with a final extension at 72°C for 2 minutes. The PCR product was visualized in 2% agarose. GelDoc is used to read electrophoresis results. Positive results are marked with band formation at 445 bp. PCR results from data were recorded and processed using Microsoft Excel and then displayed in tabular form.

Results

The research was conducted from March to July 2018 at the Wahidin Sudirohusodo Central General Hospital and Hasanudin University Teaching Hospital. During the sample collection process, 50 samples were obtained that matched the inclusion and exclusion criteria of the study. The samples came from various types of specimens, namely: 20 sputum (40%), 15 urine (30%), six faeces (12%), one gastric rinse (2%), one bronchial rinse (2%), three blood (6%) and four pus (8%). Visualization results in 2% agarose showed the formation of a band at 445 bp (if the isolate has the *Tem* gene). Of the 50 isolates, 48 (96%) isolates were detected to have the *Tem* gene. The distribution of the presence of the *Tem* gene in *K. pneumonaie*

isolates at Wahidin Sudirohusodo Hospital from March to May 2018 can be seen in Table 1, while the results of visualization of PCR products in 2% agarose gel can be seen in Figure 1.

Table 1. Distribution of the presence of the *Tem* gene in *K. pneumonaie*

No	Specimen Type	Number of isolates (%)	*Tem* gene (%)
1	Sputum	20 (40%)	20 (41.7%)
2	Urine	15 (30%)	13 (27.1%)
3	Stool	6 (12%)	6 (12.5%)
4	gastric lavage	1 (2%)	1 (2.1%)
5	Bronchus rinsing	1 (2%)	1 (2.1%)
6	Pussy	4 (8%)	4 (8.3%)
7	Blood	3 (6%)	3 (6.2%)
	Total	50	48

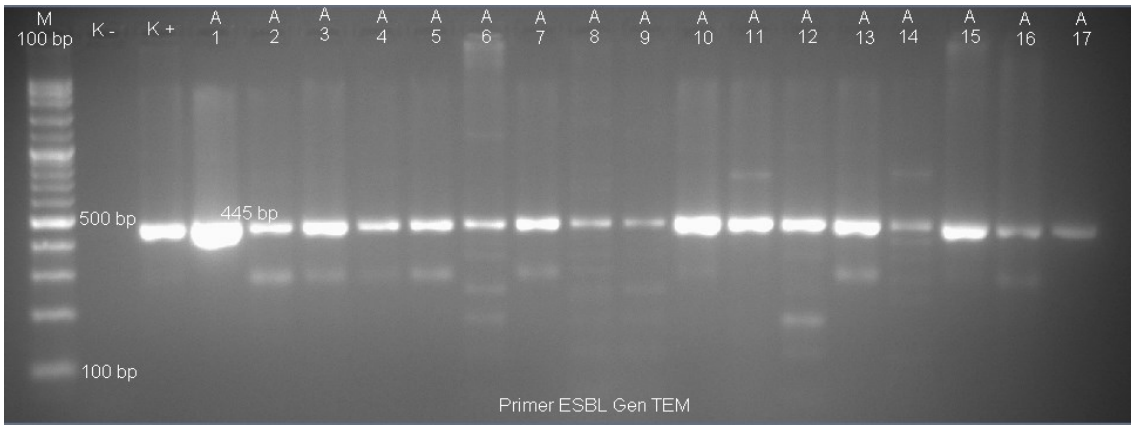

Fig. 1. Visualization of PCR products on 2% agarose gel. A positive result is indicated by the appearance of a band at 445 bp. M is Marker; K- is a negative control; K+ is a positive control; A1-A17 are isolated from 1 to 17

Discussion

In this study, ESBL examination was carried out by detecting the presence of the *Tem* gene in *K. pneumonaie* clinical isolates. This study showed that out of a total of 50 samples, 48 samples (96%) were detected to have the *Tem* gene. It is in line with a study conducted by Ref. [18] at Wahidin Sudirohusodo General Hospital, where the *Tem* gene was the most frequently detected gene in *K. pneumonaie* isolates (100%). K pneumoniae is the main pathogenic bacteria in hospitals, resulting in the increased incidence of ESBL (extended-spectrum β-lactamase) producing bacteria [19]. ESBL is a β-lactamase that causes resistance to penicillin, first, second and third-generation cephalosporins, and aztreonam by hydrolyzing these compounds [13]. The β-lactamase enzyme was first discovered in E. coli and was named *Tem* [14]. This gene is the most widely used gene detecting ESBL [15].

ESBL, as a beta-lactamase-mediated plasmid, can quickly spread its resistance genes to increase the incidence of ESBL. Increased resistance to ESBL-producing *K. pneumonaie* can have a negative impact because it can increase mortality and morbidity and cause negative economic impacts [20-22]. Most specimens in this study successively were: sputum, urine, faeces, pus, blood, gastric washings and bronchial washings. *K. pneumonaie* is a bacterium easily found in bodily fluids such as sputum, urine and blood [23].

Irrational use of antibiotics, disease severity, urinary catheterization, intubation and mechanical ventilation, and a long history of hospitalization are some risk factors that increase the percentage of ESBL spread. Therefore extra attention is needed so that the spread due to these factors can be minimized. Identification of ESBL-producing organisms is a significant challenge for clinical microbiology laboratories. Several factors influence this, namely the production of several different types of β-lactamases in single bacterial isolates and the production of ESBLs by organisms that also produce AmpC β-lactamases, carbapenemases and other antibiotic resistance enzymes [24]. Genotypic detection of ESBL-producing bacteria is essential so that antibiotic therapy given to patients is more effective and efficient.

Conclusion

This study concludes that there is the *Tem* gene in clinical isolates of *K. pneumonaie* from Wahidin Sudirohudoso General Hospital Makassar for March - May 2018, with most of the isolates coming from sputum. Suggestions for further research are to detect other ESBL-encoding genes such as SHV, CTX-M, PER, OXA, and VEB and their gene variants.The authors can write the limitation of the study and the suggestion for future researchers here.

Conflict of Interest

The authors declare that there is no conflict of interest.

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