Detection of $bla_{\text{TEM}}$ Gene of Klebsiella pneumoniae Isolated from Swab of Food-Producing Animals in East Java

M. H. Effendi*, I. G. Bintarib, E. B. Aksonoc, & I. P. Hermawanb

*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Airlangga University
bStudent, Faculty of Veterinary Medicine, Airlangga University

cDepartment of Basic Veterinary Medicine, Faculty of Veterinary Medicine, Airlangga University
Jalan Mulyorejo, Kampus C Mulyorejo Surabaya 60115, Indonesia

*Corresponding author: mheffendi@yahoo.com

(Received 22-01-2018; Reviewed 06-04-2018; Accepted 03-08-2018)

ABSTRACT

*Klebsiella pneumoniae* is one of 9 bacteria resistance to antibiotics in concern. This research aimed to detect any gene of $bla_{\text{TEM}}$ in bacteria of the *K. pneumoniae* isolated from swab of food-producing animals. In this study, 195 swab samples were taken from 17 sampling locations. Samples obtained were cultivated on selective medium and had several tests including identification, antibiotic sensitivity test using Kirby-Bauer method against antibiotics of ampicillin, cefotaxime, amoxicillin, meropenem, and trimetoprilm-sulfamethoxazole, and followed by PCR test for detecting the gene that was responsible for the antibiotic resistances. The results showed that 10 out of 195 samples were found to be *K. pneumoniae*, those were 4 samples originated from dairy cows (SP-S1, SP-S3, SP-B2, SP-G4), 2 samples originated from beef (SPT-K1, SPT-K2), 1 sample originated from chickens (A-W5), and 3 samples originated from fish (IN-P2, IN-P3, IN-S3). Most of isolates (9/10) were found to be resistant toward amoxicillin. These isolates were SP-S3, SP-B2, SP-G4, SPT-K1, SPT-K2, A-W5, IN-P2, IN-P3, and the IN-S3 and all of them also showed to be positive of $bla_{\text{TEM}}$ gene. It could be concluded that most of *K. pneumoniae* isolates from food animals harbour had *Extended Spectrum Beta-Lactamase* (ESBL) encoding gene.

Keywords: antibiotic resistance, ESBL, food-producing animals, Klebsiella pneumoniae, amoxicillin

INTRODUCTION

*Klebsiella pneumoniae* is a bacterium belonging to the genus of *Klebsiella* a member of the family of Enterobacteriaceae. The bacterium is a normal organism living in traktus digestivus so that it can be isolated from animal or human feces (Susilo et al., 2004). *K. pneumoniae* is a Gram-negative, basil, nonmotile, and one of the important pathogenic bacteria. This species is the agents of various diseases, such as pneumonia, urinary tract infection, bakteremia, infection in wounds, and abscesses of the liver (Rahamathulla et al., 2016). *K. pneumoniae* pose a great impact on the health sector. In a report on global surveillance on antimicrobial resistance carried out by the World Health Organization, *K. pneumoniae* is one of nine bacteria concerned in resistance to antibiotics (WHO, 2014). *K. pneumoniae* was found to be capable of being resistant towards many of the third-generation of cephalosporins and aztreonam which also belong to the group of beta-lactam antibiotics. In fact, this drug can not kill lactamase producing bacteria as a results of it spread of its resistance spectrum, to penicillin, cephalosporins, and aztreonam so that it called as the *Extended Spectrum Beta-Lactamase* (ESBL) bacteria. The ability of ESBL strains to hydrolyze beta-lactam antibiotics is generally due to a number of mutations in the gene, and one of which is $bla_{\text{TEM}}$ gene. These mutations are generally found to be at the active site of the enzyme that leads to a higher enzymatic activity (Yuwono, 2011). The existance of ESBL strain in food-producing animals is reported by Overdevest et al. (2011).

Animals can carry harmful bacteria in their intestines. When antibiotics are given to animals, antibiotics kill most of the bacteria. However, resistant bacteria are survive and multiply. Food-producing animals have been known as reservoirs for ESBL-producing bacteria. Food-producing animals are capable of spreading bacteria that are resistant to antibiotics through feces. Through feces, resistant bacteria contained in
animal waste and they can migrate around the farms, slaughterhouses or poultry slaughterhouses, and during meat processing. The surroundings of farms and slaughterhouses or chicken slaughterhouses will also be contaminated even though it is far from the source of contamination (Doosti et al., 2014).

Attempts to detect the existence of resistant genes in bacteria from food-producing animals in Indonesia, to our knowledge, remains limited. In fact, the gene detection should allow us to understand the pattern of genes spreading and possibility of the bacteria to gain further antibiotic resistance from certain groups. Accordingly, detection of the \( \text{bla}_{\text{TEM}} \) gene group in \( K. \) pneumonia strain is important to do.

**MATERIALS AND METHODS**

**Sample Collection and Preparation**

In this study 195 swab samples were obtained from 17 locations in East Java. Sampling was done by using swab aseptically from dairy cows, beef cattle, broiler, and tilapia in 17 sampling locations. The samples were directly analysed within 30-40 min or then transferred to the lab for further analysis in a cool box.

As many as 195 samples of dairy cows, beef cattle, broilers, and tilapia were cultivated by taking 1 ose and then scrawled by streak plate technique method on selective media of Mac Conkey and EMBA then incubated at 37°C for 24 h. In Mac Conkey media, \( K. \) pneumoniae looks pink with colonies culture looks very mucoid and in EMBA media \( K. \) pneumoniae looks red to brick (Masruroh et al., 2016).

**Characterization of Isolates**

Pure bacterial isolates were identified based on morphological characters that included colony morphology, cell morphology, and gram staining tests. Observation of colony morphology was based on the shape, color, and the edge of bacterial colonies. The morphological observations of bacterial cells include the shape and structure of bacterial cells. Furthermore, each isolate was biochemically characterized including carbohydrate fermentation test (glucose, lactose, mannitol, maltose, and sucrose), indole, motility, and citrate (Lestari et al., 2016).

**Antibiotic Test**

The antibiotic sensitivity test was performed using Kirby-Bauer agar diffusion method (Ningrum et al., 2016). The resulting clear zone was then grouped into sensitive groups (S), intermediates (I) or resistant (R) (Sagita et al., 2015; Kusumaningrum et al., 2016). Selection of antibiotic discs (disks) used previous research and based on some journals for reference. The types of antibiotics used were ampicillin, amoxicillin, cefotaxim, meropenem, and sulfamethazol-trimetoprim.

Pure cultures were prepared in suspensions with an equivalent of 0.5 McFarland (1-2 x 10^8 CFU/mL) turbidity. The cultures were taken using sterile swab cotton and distributed by means of a diole on the surface of the Mueller Hinton agar (MHA), and allowed to stand for ± 5 min. The antibiotic-containing discs were placed on the top of the MHA, which had been dispersed with pure cultures, at a distance of 25-30 mm. Furthermore, the culture was incubated at 35°C for 24 h (Masruroh et al., 2016).

**Genomic DNA Extraction**

DNA extraction by adding \( K. \) pneumoniae bacteria was performed using QIAamp DNA mini kit 50 (Qiagen, USA) according to manufacturer protocol. Briefly, samples were added with 5 μL lysozyme (5 mg/mL) enzyme and incubated for 30 min at 56°C. Furthermore, the extracted DNA was diluted to 100 μL with a buffer kit. DNA solution used for PCR amplification was as much as 1 μL.

**Amplification of \( \text{bla}_{\text{TEM}} \) gene: Polymerase Chain Reaction**

For the amplification was performed using Qiagen HotStarTag Master Mix (Qiagen, USA) according to manufacturer protocol with pure genomic DNA of \( K. \) pneumonia was used as a template. The primers used in this study were shown in Table 1. The amplification steps involved a denaturation process at 95°C for 15 min, 30 cycles denaturation at 94°C for 30 s, annealing at 50°C for 30 s, extension at 72°C for 2 min followed by the final extension on temperature of 72°C for 10 min (Moensstein et al., 2007).

Amplification product was then separated on 2% gel agarose, stained with gel-red, and visualized using UV light. \( \text{bla}_{\text{TEM}} \) gene detection was considered to be positive when bands at 445 bp of apparent size was observed in the gel.

**RESULTS**

The result showed that 10 out of 195 samples isolated from swab dairy cows, beef cattle, broiler chickens, and fish tilapia were positive \( K. \) pneumoniae. The presence of these bacteria on swab samples consisted of dairy cows by 4.8% (4/83), beef cattle by 40% (2/5), chicken broiler by 10% (1/10), and fish by 3% (3/97). All isolates also showed 90% resistance to the amoxicillin

<table>
<thead>
<tr>
<th>Gene target</th>
<th>Primary sequences</th>
<th>Amplicon (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{bla}_{\text{TEM}} )</td>
<td>F-5'-TCGCCCGCATACACTATTCCTCAGAATGA-3'</td>
<td>445</td>
<td>Monstein et al., 2007</td>
</tr>
<tr>
<td></td>
<td>R-5'-ACGCTCACCGGCTCCAGATTAT-3'</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 1. The primers used in this study.
(9/10) and sensitive to other types of antibiotics (Table 2, Figure 1).

Examination of DNA from samples using agarose electrophoresis gel (Figure 2) showed that the \( \text{bla}_{\text{TEM}} \) gene was successfully amplified with \( \text{bla}_{\text{TEM}} \) primers. The 10 samples tested by PCR in the study showed a positive result of 9 samples (90%) of the \( \text{bla}_{\text{TEM}} \) gene (Figure 2 and Figure 3).

**DISCUSSION**

The results of biochemical identification showed that *K. pneumoniae* bacteria did not contain indole (-) and Methyl Red (-), contained urea (+), Simmon’s Citrate (+), Voges Prokauer (+), and positive fermentation test of carbohydrate (+). The isolation and identification results were confirmed by *K. pneumoniae* character according to Holt et al. (2000).

*K. pneumoniae* bacteria is a bacterium with a size of 2.0-3.0 x 0.6 μm and this bacterium is a normal flora in the gastrointestinal and respiratory tracts. *K. pneumoniae* has a large capsule so that in its colonies culture looks very mucoid (Brooks et al., 2005).

Based on the morphology of colonies grown on Mac Conkey agar and biochemical test, *K. pneumoniae* isolates were found from a dairy cow feces, chicken broiler, and tilapia fish as much as 10 out of 45 stool samples. The presence of bacteria of the *K. pneumoniae* on swab samples were 4.8% (4/83) in dairy cows, 40% (2/5) in beef cattle, 10% (1/10) in the broiler chicken, and 3% (3/97) in the tilapia fish.

*K. pneumoniae* bacteria resistance test against antibiotics indicated as much as 90% (9/10) resistant to amoxicillin. The results are similar to the results found by Sagita et al. (2015) that the bacteria *K. pneumoniae* was resistant to the antibiotic amoxicillin. Resistance occurs due to the ability of the bacteria to produce penicillinase enzymes that are capable of breaking down the beta lactam ring. With this effect, penicillin is converted into penicilloid acid that is not so active. Resistance is produced by taking action against degraded penicillin by beta-lactamase. Beta lactamase enzymes protect Gram-positive and Gram-negative bacteria. In a Gram-positive bacteria, the enzyme is liberated in the medium and destroys antibiotics before it reached the cell and in gram negative it is located on the route where antibiotics must

### Table 2. Antibiotic inhibition zone interpretation

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Antibiotic discs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SAM</td>
</tr>
<tr>
<td></td>
<td>D (mm)</td>
</tr>
<tr>
<td>SP-S1</td>
<td>25</td>
</tr>
<tr>
<td>SP-S3</td>
<td>24</td>
</tr>
<tr>
<td>SP-B2</td>
<td>24</td>
</tr>
<tr>
<td>SP-G4</td>
<td>22</td>
</tr>
<tr>
<td>SPT-K1</td>
<td>25</td>
</tr>
<tr>
<td>SPT-K2</td>
<td>24.5</td>
</tr>
<tr>
<td>AW-5</td>
<td>22</td>
</tr>
<tr>
<td>IN-P2</td>
<td>23</td>
</tr>
<tr>
<td>IN-P3</td>
<td>21.1</td>
</tr>
<tr>
<td>IN-S3</td>
<td>24</td>
</tr>
</tbody>
</table>

Note: R (resistant), I (intermediates), S (sensitive), SP-S (dairy cow in Senduro), SP-B (dairy cow in Batu), SP-G (dairy cow in Grati), SPT-K (beef cattle), AW (broiler in Wonokromo), IN-P (custom tilapia), IN-S (tilapia in Sedila), SAM (ampicillin 10μg), SXT (sulfamethonazole-trimetrophrim 27.75 μg), CTX (cefotaxime 30 μg), MEM (meropenem 10 μg), AML (amoxicillin 15 μg).

**Figure 1. Inhibition zone diameter interpretation.** (1) SAM (ampicillin 10μg), (2) SXT (sulfamethonazole-trimetrophrim 27.75 μg), (3) CTX (cefotaxime 30 μg), (4) MEM (meropenem 10 μg), (5) AML (amoxicillin 15 μg).

**Figure 2. Agarose gel electrophoresis 2% product used primer for \( \text{bla}_{\text{TEM}} \) gene detection in *Klebsiella pneumoniae* bacteria.** Lane M: marker; lane 1-6 sample; lane K (-): negative control. Note: lane 1= SP-S1; lane 2= SP-S3; lane 3= SP-B2; lane 4= SP-G4; lane 5= IN-P2; lane 6= SPT-K1.
The Authors declare that there is no conflict of interest with any financial, personal, or other relationships with other people or organization related to the material discussed in the manuscript.

**ACKNOWLEDGEMENT**

Thanks to the Integrated Applied Research Flagship Universities of the Ministry of Research, Technology and Higher Education of the Republic of Indonesia the 2017 Budget that supported this research.

**REFERENCES**


