Identification of \textit{bla}_{imp} gene carbapenem-resistant \textit{Enterobacteriaceae} (CRE) isolated from patient infection

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Abstract. There have been increasing cases of carbapenem-resistant \textit{Enterobacteriaceae} (CRE) through the production of enzymes carbapenemase in the last decade. \textit{Bla}_{imp} gene acts as a gene encoding the enzyme production carbapenemase that hydrolyzes beta-lactam ring in carbapenem antibiotics. The mechanism of bacterial plasmid transfer among \textit{Enterobacteriaceae} facilitates the spread of carbapenem antibiotic resistance to other bacterial species. This study aims to identify the genes \textit{bla}_{imp} in carbapenem-resistant \textit{Enterobacteriaceae} at Dr. Mohammad Hoesin Hospital. This research is an observational research laboratory. The population of \textit{Enterobacteriaceae} bacteria is accumulated in clinical microbiology laboratories RSMH, period September-November 2017, then performed a test to get a sample of bacterial phenotype CRE through Vitex 2® compact bioMerieux tool. CRE phenotype sample obtained bacteria, later identified to determine genotype \textit{bla}_{imp} CRE bacteria samples by PCR and visualized on a 232 bp band. A total of 35 from 730 \textit{Enterobacteriaceae} bacteria population (4.8%) was identified as bacterial phenotype CRE. A total of three from 35 samples of the CRE phenotype (8.6%) was identified containing the gene genotype \textit{bla}_{imp}. The third came from sputum samples containing \textit{Klebsiella pneumoniae}, pus containing \textit{Enterobacter cloacae} and blood containing \textit{Serratia marcescens}. In this study identified three samples (8.6%) bacterial genes phenotype \textit{bla}_{imp} CRE.

1. Introduction

\textit{Enterobacteriaceae} is the largest family in the family of bacteria, which consists of 53 genus. A total of 80% of strains of bacteria in the family is an important gram-negative bacteria in medicine. A total of 50% all bacteria in the lab comes from the family \textit{Enterobacteriaceae}. Some examples of species that important in medicine are \textit{E.coli}, \textit{Salmonella spp}, \textit{Shigella spp}, \textit{Yersinia enterocolitica}. Bacteria in this family can cause opportunistic infections such as sepsis, pneumonia, meningitis and urinary bacterial infections. Bacterial species of this family are often the cause of infections acquired in the ICU (Intensive Care Unit), particularly respiratory tract infections and urinary tract infections [1,2,3].

Carbapenem is a broad-spectrum antibiotic beta-lactam class that used for serious infections in gram-negative and gram-positive. This drug is the first choice for treating Multi-Drug Resistance (MDR) against beta-lactam antibiotics [4]. The main factor for the occurrence of carbapenem-resistant \textit{Enterobacteriaceae} (CRE) cases is a carbapenem antibiotic used (doripenem, ertapenem, imipenem, and meropenem) as primary therapy without using other antibiotics to support in overcoming MDR \textit{Enterobacteriaceae} bacterial infection that bacteria adapt to antibiotics.
CRE mechanism consists of two causes, with the production of carbapenemase enzymes and nonenzymic carbapenemase, but the most common is Enterobacteriaceae bacteria produce carbapenemase enzymes on plasmid through bla\textsubscript{IMP} gene initiation the hydrolysis of beta-lactam ring, causing damage to carbapenem antibiotics. The resistance cases caused by plasmid as the gene bla\textsubscript{IMP} have the high prevalence for plasmid gene transfer process easier by conjugation in Enterobacteriaceae bacteria. The case of carbapenem resistance through the production of carbapenemase is a variant of the most frequent and dangerous than the other beta-lactamase [5].

Bla\textsubscript{IMP} gene is the common gene variants on the class of Metallo-beta-lactamase which synthesized firstly by P. aeruginosa in 1991 [6]. Bla\textsubscript{IMP} genes have strong hydrolysis activity against carbapenem, cephalosporin, and penicillin antibiotics but negative on aztreonam [7]. Other research in RSCM Jakarta in 2011 discovered the gene encoding carbapenemase were bla\textsubscript{IMP}-1 and bla\textsubscript{NDM}-1 of 27.6% [8]. CRE patient’s mortality rate was very high, reached 40-65%.

Detection of bla\textsubscript{IMP} genes in carbapenem-resistant Enterobacteriaceae (CRE) bacteria is an appropriate measure for the management of patients who can provide vital information for preventing infection, controlling and tracking of these organisms in the surveillance data. Detecting genes that cause resistance in Enterobacteriaceae performed by PCR (Polymerase Chain Reaction) which a molecular detection tool. Failing to detect harmful pathogens can cause improper administration of antibiotics that target the increasingly widespread these resistant bacteria.

2. Methods

This study was a descriptive observational laboratory to identify bla\textsubscript{IMP} genes in Enterobacteriaceae bacteria isolated from infected patients at Dr. Mohammad Hoesin Hospital (RSMH) Palembang period September-November 2017. The population in this study were all Enterobacteriaceae isolates containing the bacteria in clinical microbiology laboratories RSMH, while the sample was the population identified as carbapenem-resistant Enterobacteriaceae (CRE). The sampling technique in this study using purposive sampling. Enterobacteriaceae bacteria population obtain identified a sample of the bacteria phenotype CRE with Vitex 2®compact bioMerieux. This tool identifies the sensitivity of antibiotics to identified MIC (Minimum Inhibitory Concentration) was compared with a standard MIC for each antibiotic, taken from CLSI 2014. The process was carried out in the clinical microbiology laboratory at RSMH.

The samples which bacterial phenotype CRE performed isolation of DNA. The materials needed for the DNA isolation process is phosphate buffered saline (PBS) pH 7.4, 0.5% saponin in PBS and chelex-100 20% in ddH2O. The supernatant portion was taken and performed the PCR process in the process of DNA isolation.

Identification of the presence of genes bla\textsubscript{IMP} using two specific primers, the forward primer (5’ GGAATAAGAGGGCTTA ACTCTC3’) and reverse primer (5’ GTTTA ACAAAACAACCC ACC 3’). The PCR reactions were performed with 25 mL volume consisting of 9µl ddH2O, 10 mL taq Go Green, and each 0.5 mL for primer forward and reverse primer and 5 mL DNA template.

The process of gene amplification bla\textsubscript{IMP} regulated by initial temperature denaturation at 94°C for 10 minutes, followed by 36 cycles of amplification (denaturation 94°C for 30 seconds, annealing 52°C for 40 seconds and extension at 72°C for 50 seconds), and at the end were given a final extension for 5 minutes at 72°C [9]. The PCR process is carried out in the laboratory of biomolecular FK Unsri.

The results of bla\textsubscript{IMP} gene amplification by PCR, viewed by using agarose gel electrophoresis technique (concentration of 3%). Electrophoresis apparatus used Horizontal MiniSubDNA Biorad containing TAE and added substances 3 µl ethidium bromide. A total of 5 mL amplicon intercalator PCR results were entered in wells which located the gel. It was used DNA marker 100 bp as a size marker DNA bands in the gel for electrophoresis results. The gel was electrophoresed with an electric voltage of 110 Volt, 400 mA for 25 minutes. The electrophoresis results will be visualized with ultraviolet light. The samples were positive for the gene bla\textsubscript{IMP} will be formed along the 232 bp band.

Processing data was performed using Microsoft Word 2010. The data will be presented in the dummy table form. The presented data and charts can be further analyzed by simple statistical calculation in the percentage form. The results of the data analysis will be described in the narrative.
3. Results

A total of 730 Enterobacteriaceae bacterial derived from infected patients in clinical microbiology laboratories RSMH has identified 35 samples (4.8%) phenotype CRE bacteria that detected by vitex 2® compact bioMerieux. The distribution of CRE phenotype by means Vitex 2® compact bioMerieux can be seen in table 1.

| Phenotype    | n  | %  |
|--------------|----|----|
| positive CRE | 35 | 4.8|
| negative CRE | 695| 95.2|
| Total        | 730| 100|

CRE phenotype bacteria samples were from different patient specimens. The specimen type infection mostly found are 12 sputum samples (34.3%), 8 urinary samples (22.9%), 5 and pus samples (14.3%). Klebsiella pneumoniae is the most bacterial species which founded. It was reached 18 samples (48.6%).

A total of 35 samples of the CRE has identified three samples (8.6%) bacterial genes genotype blaIMP. The results of three samples positive visualization can be seen in figure 1.

These three positive samples were from a different specimen which sputum, pus, and blood. The three positive samples were from Klebsiella pneumoniae, Enterobacter cloacae, and Serratia marcescens.

The test results of antibiotic resistance to bacteria CRE on the positive and negative blaIMP genes shows different results. Antibiotic resistance rates in a sample CRE with positive blaIMP gene higher in terms of quantity and types of antibiotics. Figure 2 shows the percentage difference between the samples of the bacterial antibiotic resistance CRE with blaIMP gene positive than blaIMP gene negative.

4. Discussion

Carbapenem-resistant Enterobacteriaceae (CRE) is a serious problem that increases in the last decade. Carbapenem is broad-spectrum antibiotics and the most effective antibiotics to overcome bacterial infections of gram-negative and gram-positive bacteria than the other beta-lactam antibiotics [10].

CRE phenotype detection through vitex 2® compact bioMerieux has been conducted in various hospitals laboratories included in Dr. Mohammad Hoesin Hospital Palembang. PCR is a method for detecting genotype a blaIMP gene which has a very high sensitivity and specificity than the other method [11].

This study was conducted during the September-November 2017 of 730 bacterial populations Enterobacteriaceae obtained 35 samples (4.8%) bacterial phenotype CRE. This result was lower than...
the data surveillance CRE Indonesia was 5.8%. Indonesia is a country with the highest percentage of CRE in Southeast Asia, compared with Vietnam 3.0% and the Philippines 3.7% [12].

Most bacterial species found phenotype CRE sequence was 17 samples Klebsiella pneumonia (48.6%), 8 samples Escherichia coli (22.9%), 7 samples Enterobacter cloacae (20%), and 3 samples Serratia marcescens (8.7%). The data from CDC said two bacteria that cause most cases of CRE derived from carbapenem-resistant Klebsiella pneumoniae and Escherichia coli [13]. Klebsiella pneumonia role in Community-Acquired Pneumonia (CAP). Sputum is the most specimen found in this research that 12 samples (34.3%). It is related to Klebsiella pneumonia bacteria that the most common bacterial causes of respiratory infections and lung tissue consolidation so relevant as a sputum specimen found most [14].

A total of three from 35 samples CRE (8.6%) which sampled with genotype genes blaIMP were detected by PCR. A total of 32 other samples were not positive blaIMP gene does not mean there CRE but is likely to have a gene genotype other types CRE such as blaVIM, blaNDM, blaCTX, blakPC and others.

The prevalence of blaIMP genes was found higher than the study conducted by Anis Kurniawati in RSCM Jakarta in 2011. A total of 27 samples CRE derived from the ICU (Intensive Care Unit) that not found the samples contained a genotype blaIMP gene [8]. Study about Enterobacteriaceae bacterial resistance rates against carbapenem antibiotics was relatively little in Indonesia. That is not often found other data for comparison in addition to data on research conducted in RSCM Jakarta in 2011. Comparison of CRE research in various countries around the world can be seen in table 2.

A study in Singapore by Ling et al. (2015) obtained 15 samples (5.6%). The samples proved to have a genotype blaIMP gene of 203 samples CRE by the same method that PCR [15]. The high prevalence rates in this study can be caused by various factors such as variations CRE bacteria at Dr. Mohammad Hoesin Hospital higher than the other studies, although there may be other factors that differences influence in the results of research such as geographical location, individual, infectious disease prevention programs and policies of different antibiotic use in hospitals and in the country.

Based on the results of sensitivity tests on 35 samples of bacterial phenotype Carbapenem-resistant Enterobacteriaceae (CRE) to various antibiotics, mostly beta-lactam group found that the highest number of bacterial resistance to the antibiotic ampicillin, which is 35 samples (100%), followed by ceftazolin with 28 samples (80%), then 24 samples ceftriaxone (68.6%), and ceftriaxone 23 samples (65.7%). All of four the group with a carbapenem antibiotic which is a beta-lactam antibiotic and an antibiotic with the highest resistance to bacterial numbers CRE. The results of the same study also found in studies in China by Rong Zhang (2015). He found that the rates of bacterial resistance to antibiotics were the highest CRE cefotaxime (100%), ceftazidime (97.6%), amoxicillin-clavulanic (90.2% ). The third is a group of beta-lactam antibiotics [17].

Bacterial sensitivity test CRE gene genotype blaIMP show of 3 positive samples tested, there are five classes of antibiotics that have numbers 100% resistant to bacterial genes genotype blaIMP, the fifth such antibiotics as ampicillin, aztreonam, ceftazolin, cefazidime, and ceftriaxone. Based on the journals from the American Society for Microbiology in 2007, bacterial phenotype CRE with genotype genes blaIMP have activity profile hydrolysis was higher against the antibiotic penicillins (amoxicillin, ampicillin, penicillin G, and others), cephalexin early generation (cefoxitin, cefapirin, cefadroxil, etc.), and cephalexins broad spectrum especially cephalexins 2nd and 3rd generation
(cefotaxime, ceftriaxone, ceftazidime, ceftizoxime, etc.), bacteria carrying the gene hydrolysis class MBLs (including \textit{blaIMP}) can hydrolysed all beta-lactam antibiotics except aztreonam [7].

**Table 2.** Study results of \textit{blaIMP} genes sample carbapenem-resistant \textit{Enterobacteriaceae} (CRE) in various cities and countries around the world.

| Researcher (years) | Population | Number of Samples | The percentage of \textit{blaIMP} genes |
|-------------------|------------|-------------------|----------------------------------------|
| Marques et al., (2015) [11] | Santa Maria, Brazil | 32 | 12.5% |
| Moi Lin Ling (2015) [15] | Singapura | 268 | 5.6% |
| Deogratius Okoche (2014) [16] | Kampala, Uganda | 196 | 6.1% |
| Rong Zhang (2015) [17] | China | 1105 | 3.1% |
| Anis karuniawati (2011) [8] | Jakarta, Indonesia | 90 | 0% |
| This research (2017) | Palembang, Indonesia | 35 | 8.6% |

This statement is relevant to bacterial sensitivity test results that demonstrate antibiotic CRE with the highest resistance rates are ampicillin, cefazolin, ceftazidime, and ceftriaxone. All of that is a class of antibiotics penicillin and cephalosporin. The results obtained in antibiotic aztreonam inconsistent with the statement that the bacterial CRE gene genotype \textit{blaIMP} cannot hydrolyze the antibiotic aztreonam. This is a new finding that may indicate that the possibility of an increase in cases of aztreonam antibiotics resistance and spread of antibiotic resistance spreading.

CRE samples with genes \textit{blaIMP} positive has resistance rates higher than samples with genes \textit{blaIMP} negative, it indicates that the bacteria CRE with genotype genes \textit{blaIMP} be at high risk of increased treatment failure using a type of antibiotic that has been described previously as well as increase the morbidity and mortality of patients with infectious diseases as a result of CRE bacteria than bacteria non \textit{blaIMP} genes for antibiotic resistance has a number of higher and more.

Some choices proved effective management to overcome the problem CRE. Polymyxin, aminoglycosides, tigecycline can inhibit the in vitro activity of bacteria CRE and is an option that often used as well as the final choice of drug (drugs of last resort) to address the problem CRE [18].

WHO has made a guideline about preventive and control efforts particularly on health care facilities which can contribute to a reduction in the spread of cases of CRE in 2017. One of the protocols established by WHO is IPC (infection prevention and control) strategies include hand hygiene before and after touching the patient, the data surveillance complete regarding the data CRE, patient isolation, vigilance in contact with the patient (contact precautions) and cleaning the infected area CRE [3]. In addition to the application of the policy regarding prescribing and use of antibiotics through periodic reporting resistance rates is very important to reduce the number of carbapenem antibiotic resistance.

5. Conclusions
A total 730 Enterobacteriaceae bacteria population in clinical microbiology laboratories RSMH during the period September-November 2017 was identified 35 samples (4.8%) bacteria phenotype CRE. They were identified as three samples (8.6%) bacterial genes phenotype \textit{blaIMP}. The third sample \textit{blaIMP} genotype gene is derived from sputum containing \textit{Klebsiella pneumoniae}, pus-containing \textit{Enterobacter cloacae} and blood containing \textit{Serratia marcescens}.

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