Phenotypic and Molecular Detection of OXA-48 Gene Carbapenem-resistant Klebsiella Pneumoniae and Escherichia Coli Isolates in Haji Adam Malik Hospital Medan, Indonesia

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Abstract: Carbapenem-resistant to *Klebsiella pneumoniae* and *Escherichia coli*, increasingly reported as a major cause of infection in hospitals and healthcare facilities. Carbapenemase is an enzyme produced by gram-negative bacteria that causes failure of antibiotic therapy, especially the carbapenem. The study aimed to characterize phenotypically using Vitek 2 Compact and detect OXA-48 clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* belonging to Carbapenem-Resistant using PCR. The results showed that all were beta-lactamase producers, which of 12 (14.11%) were resistant to carbapenem. The phenotype distribution of carbapenem is 10/12 (11.75%) *Klebsiella pneumoniae* and 2/12 (2.35%) *Escherichia coli*. From 12 isolates Carbapenemase phenotypically, in which 10 (11.77%) of both bacteria bearing OXA-48 gene with the distribution 9 (10.6%) *Klebsiella pneumoniae* and 1 (1.17%) *Escherichia coli*, respectively. The study shows that prevalence of OXA-48 genes in North Sumatra, Indonesia in *Klebsiella pneumoniae* and *Escherichia coli* which cause failure of therapeutic types of antibiotics Carbapenem. The only antibiotic that is still sensitive to carbapenem-resistant bacteria based on antimicrobial susceptibility is amikacin, which can be recommended as carbapenemases therapy.

1 INTRODUCTION

The spread of resistance to carbapenem by Enterobacteriaceae, especially Klebsiella pneumoniae and *Escherichia coli* (Maryam et.al, 2017). The emergence of bacterial resistance to the carbapenem is a global problem that is growing rapidly and requires urgent action for the international scientific community (CDC, 2013). Tropical countries such as Indonesia, infections by *Klebsiella pneumoniae* and *Escherichia coli* are strongly associated with health care such as the use of health facilities that cause urinary tract infections, as well as postoperative wound care (Raka et.al, 2006).

Carbapenem-resistant over the past decade, healthcare settings have emerged due to the bacterial infection of Enterobacteriaceae and are strongly related to the ability of bacteria to produce β-lactamase, which is capable of hydrolyzing carbapenems (Nordmann et.al, 2012). Most of these phenomena are related to the spread of various types of β-lactamases. Carbapenem-hydrolysing β-lactamase major in Enterobacteriaceae is class A *Klebsiella pneumoniae carbapenemases* and class B acquires Metallo beta-lactamases (MBLs) which is dominated by Klebsiella pneumoniae which has shown rapid international spread. In the D-class β-lactamase OXA-48 despite its weak activity in resistance but is increasingly reported to be significant in Enterobacteriaceae. General epidemiological observations and recent research shows that OXA-48 producers are increasingly being identified in various developing countries (Nordmann et.al, 2014).

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The increasing prevalence of carbapenem-resistant almost all over the world provides the basis for the importance of phenotypic characterization and molecular detection of the Carbapenemase gene in the Laboratory of Clinical Microbiology. Molecular detection of the OXA-48 gene in bacteria that produce carbapenemase enzymes is needed to control antibiotic resistance, avoid bacterial transmission and improve therapy management. The aim of the study was to characterize phenotypically and detect OXA-48 genes in isolates of *Klebsiella pneumoniae* and in *Escherichia coli* resistant to carbapenem in Haji Adam Malik Hospital Medan, Indonesia.

2 METHOD

2.1 Phenotypic Characterization

Phenotypic characterization was carried out by identification of bacterial types and sensitivity testing of antibiotics as well as carbapenemase phenotype using Vitek 2 Compact. The sample was taken purely sampling based on germ pattern in the first semester of 2015. Bacterial isolates were obtained from routine examination of the Laboratory of Clinical Microbiology, Installation of Diagnostic Laboratory Haji Adam Malik Hospital Medan.

2.2 Isolation of DNA

Isolation of DNA was isolated by the freeze-thaw cycling method before the isolate was subcultured on Mac-Conkey Agar medium with a 24-hour incubation period at 37°C. One colony was put into a tube containing 20µl of aquabidest and homogenized, then frozen for 10 minutes at 20°C, followed by heating for 10 minutes at 90°C, hot and frozen cycles were carried out 6 cycles. The isolation of DNA was centrifuged for 5 minutes at a speed of 13.000 rpm. The supernatant part is separated, DNA purity is measured using a nanophotometer.

2.3 Molecular Detection of OXA-48

Molecular detection using specific primers: GCG-TGG-TTA-AGG-ATG-AAC-AC forward and CAT-CAA-GTT-CAA-CCC-AAC-CG reverse. PCR preparation began with DNA amplification at 25µl, consisting of 12.5µl mastermix green go-Taq, 8.5µl nuclease-free water, 1µl of forward and reverse primer and 2µl bacterial DNA. Amplification is carried out on Thermocycling reactions. PCR results were electrophoresed and documented in UV Reader.

3 RESULTS AND DISCUSSIONS

3.1 Phenotype

Profile of the Vitek 2 Compact antimicrobial susceptibility from 85 clinical isolates showed that all isolates are ESBLs, consisting of 52% of Klebsiella pneumoniae and 48% of Escherichia coli, respectively. The percentage profile of antimicrobial sensitivity of the samples is presented in Table 1.

Table 1: Percentage of Antibiotic Sensitivity Tests.

Group	Antibiotics Agents	S (%)	R (%)
Penicillin	Amoxycillin	0	100
	Ampicillin	0	100
Cephalosporin	Cefotaxime	0	100
	Ceftriaxone	0	100
	Ceftazidime	0	100
	Cefepime	0	100
Monobactam	Aztereonam	0	100
Beta-lactamase Inhibitor	Amoxicillin/ Clavulanic Acid	27	73.0
	Piperacillin/ Tazobactam	43.5	56.4
	Cefoperazone/ Sulbactam	63.5	36.4
Aminoglicosides	Gentamycin	35.3	64.7
	Amikacin	100	0
Carbapenem	Ertapenem	85.9	14.1
	Meropenem	85.9	14.1
	Imipenem	85.9	14.1
Fluoroquinolone	Ciprofloxacin	0	100
	Levofloxacin	25.8	74.2
Fosfomycin	Fosfomycin	86.5	13.5
Folate Pathway Inhibitor	Cotrimoxazole	0	100
Tigecil	Tigecycline	84.5	15.5

All isolates showed resistance (R) to beta-lactam group antibiotics (penicillins, monobactams, cephalosporins), which indicates that all isolates carrying Extended-Spectrum Beta-Lactamases (ESBLs). Resistance was also shown in the beta-lactamase inhibitor group of 73% amoxicillin/ clavulanic acid, 56% piperacillin/tazobactam and

36.4% cefoperazone/sulbactam, respectively. Resistance is also shown in other classes of antibiotics such as ciprofloxacin and cotrimoxazole. From 85 isolate were found to be 12 (14.2%) resistant to carbapenem (ertapenem, meropenem imipenem), of which 2 (2.35%) *Escherichia coli* and 10 (11.77%) *Klebsiella pneumoniae*. which indicated that the isolates are carrying ESBls and *Carbapenemases*. The only antibiotic that is still sensitive (S) to all isolates based on antimicrobial susceptibility is amikacin.

Resistance occurs because of the genes that encode resistant and expressed phenotypically. Resistance occurs because of the genes that encode resistant and expressed phenotypically. This is very important because enzymes produced by bacteria will express genes for resistance (Thenmozhi et.al, 2014). The reliability of the Vitek 2 Compact System as an ESBLs and Carbapenemases detection system was verified in comparison with another method such as double disc synergy which is recommended by Clinical Laboratory Standards Institute. The characterization of the Carbapenemase phenotype with a combination of Modified Hodge Test (MHT) using discs and ertapenem and meropenem is preferred. In addition, the MBL strip E-test was also used to evaluate Metallo-β-lactamase production (CLSI, 2013).

From the results of the Carbapenemase phenotyping using Vitek 2 Compact, it is necessary to proceed at the molecular level to detect ESBLs encoding genes as well as the accuracy of both methods. Then amplification and molecular detection of OXA-48 gene against these 12 isolates by using PCR.

3.2 Molecular Detection of OXA-48 Gene

Molecular detection of the OXA-48 gene showed DNA amplification 389 bp in *Klebsiella pneumoniae* and *Escherichia coli* (figure 1.). Based in this study found 10/12 OXA-48 gene with the percentage of 11.77%.

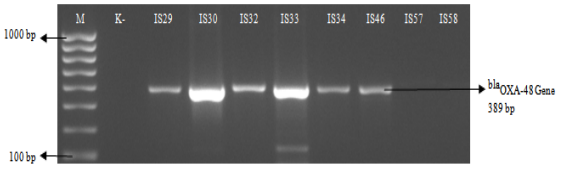

Figure 1: profiles of OXA-48 gene

K- (*Escherichia coli* Non-*Carbapenemase*),
IS29 (*Klebsiella pneumoniae* 11474),
IS30 (*Klebsiella pneumoniae* 11527),
IS32 (*Klebsiella pneumoniae* 11583),
IS33 (*Klebsiella pneumoniae* 11665),
IS34 (*Klebsiella pneumoniae* 11417),
IS46 (*Klebsiella pneumoniae* 11311),
IS57 (*Escherichia coli* 11926),
IS58 (*Klebsiella pneumoniae* 11688).

From Figure 1 it can be seen that the appearance of the DNA band pattern indicates that the bacteria has the OXA-48 gene. Proving that the primary can be used OXA-48 produces 389 bp amplicon. In the previous study, detecting the OXA-48 gene using the same primary, where 27 of the 28 isolates of the Motahari Hospital, Tehran, Iran (Azimi et.al, 2014).

From 12 isolates carbapenemase phenotypically, it was found that 10 (11.77%) *Klebsiella pneumoniae* and 2 (2.35%) of *Escherichia coli* isolates showed carbapenemase, isolates containing OXA-48 gene were 10 (11.77%) which are presented in table 2. Another 2.35% possibility contains other genes that cause carbapenem-resistance.

Table 2: Percentage of ESBLs, Carbapenemases phenotype and OXA-48 gene.

Bacterial species	Phenotype		OXA-48 gene
	ESBLs	Carbapenemase	
K.pneumoniae	44 (52%)	10(11.77%)	9(10.6%)
E.coli	41 (48%)	2(2.35%)	1(1.17%)
total :	85(100%)	12 (14.12%)	10(11.77%)

The results of this study showed the spread of OXA-48 gene in Indonesia. In this study, the OXA-48 gene was not only found in *Klebsiella pneumoniae*, but was also found in the *Escherichia coli*. In 2 phenotypic Carbapenemase isolates, not found the blaOXA-48 gene. There may be other genes such as IMP-1, NDM-1 and other types of carbapenemase genes. Molecular detect of ESBLs genes by Karuniawati *et.al* (2013) in RSPN Cipto Mangunkusumo Jakarta, Indonesia. From the 61 Gram-negative bacteria-producing carbapenemases in phenotype, did not have the OXA-48 type gene. However, the gene encoded carbapenemases such as IMP-1 and NDM-1.

The presentation of carbapenem-resistant in Enterobacteriaceae poses a major problem for health services, for example the limited choice of antibiotic therapy. As a bacterium producing Carbapenemase, these bacteria are not only resistant to carbapenem but almost resistant to all beta-lactam groups, except monobactam (aztreonam) for MBL and other compounds such as OXA-48 (Jacoby et.al, 2004). In this study, it was found that all isolates were even resistant to aztreonam. In addition, the extended spectrum β-lactamase (ESBL) is strongly associated

with carbapenem resistance in Enterobacteriaceae, this is due to the female bacteria producing AmpC β-lactamase or loss of porin in the bacterial cell wall (Woodford, 2007).

This gene OXA-48 is also known as the carbapenemase coding gene in gram-negative bacteria. The first identified OXA-48 gene manufacturer was derived from the *Klebsiella pneumoniae* strain isolated in Turkey in 2003. Since then, manufacturers of ^{bla}OXA-48 have been widely reported in Turkey as a source of bacteria that cause nosocomial infections (Nordmann et.al, 2004). The worldwide distribution of OXA-48 manufacturers now covers countries in Europe, Africa, America, and even Asia. The spread of multi-resistant pathogens worldwide has been linked to a variety of epidemiological factors including international patient transfer originating from endemic areas (Girmenia et.al, 2016). The argument about the spread of *Klebsiella pneumoniae* as a type of bacteria that causes carbapenemase is considered a high-risk organism (Munoz-Price et.al, 2013).

The threat of antimicrobial resistance has been recognized by World Health Organization, where this threat involves and requires all actions related to the agency and society as a whole (WHO, 2013). Clone expansion is a major driver of the spread of carbapenemase in gram-negative bacteria, especially in Enterobacteriaceae. In addition, carbapenemase transmission as a clonal lineage of Enterobacteriaceae (CPE) is stable in the defense of the carbapenemase coding gene. Horizontal transfer of this type of gene is very likely to occur through the moving genetic element, the plasmid (Kitchel et.al, 2009).

Study on the detection of OXA-48 genes is still rare in Indonesia, especially in North Sumatera. This study shows the presence of the OXA-48 gene as a cause of Carbapenemase in *Klebsiella penumoniae* and *Escherichia coli*. This study also provides an overview of the prevalence and incidence of resistance to carbapenem, for this reason the awareness of all hospitals is the importance of controlling resistance in the present and avoiding the transmission of resistant bacteria in health facilities.

4 CONCLUSIONS

The prevalence of carbapenemase has been shown in this study, found of the phenotype 12 (14.12%) and molecularly detected 10 (11.77%) OXA-48 genes. However, there were 2 other isolates that were not found in the OXA gene, possibly another gene that causes carbapenemase.

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