Carbapenem Resistance of OXA-48 Gene Coding in Klebsiella Pneumoniae and Escherichia Coli

by Nita Parisa

Submission date: 08-Feb-2022 07:39AM (UTC+0700)
Submission ID: 1757279339
File name: 228386-Article_Text-519799-1-10-20210402.pdf (245.69K)
Word count: 2922
Character count: 14562

CARBAPENEM RESISTANCE OF OXA-48 GENE CODING IN KLEBSIELLA PNEUMONIAE AND ESCHERICHIA COLI

Nita Parisa[1], Muhammad Fitrizal[2], Ella Amalia[3]

[1]Pharmacology, Faculty of Medical Science, Universitas Sriwijaya
[2]Student of Medical Science, Faculty of Medical Science, Universitas Sriwijaya
[3]Medical Microbiology, Faculty of Medical Science, Universitas Sriwijaya

Introduction

Infection is one of the diseases which occurred because of the bacteria, virus, fungi or protozoa. *Enterobacteriaceae* is a gram-negative bacteria shaped in trunk/rod and located in human intensive (1). One of the members of *Enterobacteriaceae* is *Escherichia coli* or a bacteria with pathogen characteristic that cause morbidity and mortality (2). *E.coli* has ability to show enhancement in resistance. During the first year *ciprofloxacin* resistance stay in 4.1% and increase to 31.8% after 10 years. *Amino glycoside* also showed slower enhancement in resistance, but still consistence, from 1999 to 2007 (3). *E.coli* appeared for the first time in 2004 with 0.4% resistance and up to 5.1% during five years period. Among *Klebsiella spp*. *Carbepenem* resistance isolate is rarely observed before 2003, but after the rapid enhancement of resistance level in 2007 and degradation in 2008 related with intervention control of local infection, the *Carbepenem* resistance started to analyze.

The use of anti-bacteria that incompatible can cause the occurrence of bacteria resistance if it is used repeatedly in a long term duration. For example *Carbepenem* resistance. This resistance had enhancement and become a phenomenon that should be aware of. According to European Centre for Disease Prevention and Control (ECDC) in 2009 until 2012, there was an increase in blood infection case, in five different countries, caused by *Klebsiella pneumonia* with *Carbepenem* resistance in 5% (3). OXA-48 gene is β-lactamase of D class which not inhibited by clavulanate acid, tazobactam, and sulbactam because its activity is probably inhibited by NaCl in in vitro (4). Some β-lactamase enzymes is hydrolyzed the *Carbapenem* and thus it defined as *Carbepenem* hydrolyzing class D of β-lactamase (CHDLs). OXA-48 gene is found in plasmid which produced isolate with ability to resist against many medicine and showed higher resistance to all β-lactamase, including wide spectrum of *cephalosporins*, *cephamycins*, *monobacton*, and *carbepenem*. BlaOXa-48 gene had relationship toward sequence insertion ISI999 on *K.pneumoniae* and expressed β-lactamase (5). It is proved that blaOXA-48 gene is a part of transposon composite named Tn1999 and made of two copies of ISI999. Bacteria has ability to adapt and mutate as its defend mechanism to survive (6). This study is a prevalence study that focused on identifying the presence of OXA-48 gene as *Carbepenem* coding in *K.pneumoniae* and *E.coli* using PCR method. It aimed to confirm the resistance case in genotype in Dr. Mohammad Hosein Hospital.

Methodology

This study is a descriptive observational methodology with laboratory based research to identify the availability of OXA-48 gene in *K.pneumoniae* and *E.coli Carbapenem* resistance using PCR. The place of the study is held in Molecular Biology Laboratory in Faculty of Medical Science, Sriwijaya University and Microbiology Laboratory of Dr. Mohammad Hosein Hospital.

The population was the isolate bacteria of *K.pneumoniae* and *E.coli* from the entire specimen types in *carbapenem* resistant infection patient in September-November 2017 period in Microbiology Laboratory. The sample was taken using purposive sampling, which consisted of 24 patients with infection in Dr. Mohammad Hosein Hospital in Palembang. The isolate bacteria from infection patient were collected in Microbiology Department of Dr. Mohammad Hosein Hospital and identified using Vitek 2 Compact. The inclusion criteria in this study were; (a) isolate bacteria of *K.pneumoniae* and *E.coli* which derived from infection patient, and (b) isolate bacteria is proven as *K.pneumoniae* and *E.coli* through the culture examination, gram coloring, and Vitek 2 Compact. The collected data were processed using *Microsoft Excel 2010* which then analyzed using simple statistic measurement in the form of percentage. The result then explained in narration and served in table.

Procedure

Identification of Carbapenem Resistent of K.pneumoniae and E.coli

The identification of *K.pneumoniae and E.coli Carbapenem* resistance was done using Vitek 2 Compact (bioMerieux, USA). The phase in identify *K.pneumoniae and E.coli Carbapenem* resistance (7), were;

- Defining the isolate bacteria with gram coloring.
- Putting 3ml of saline sterile 0.45-0.5%, pH 4.5-7.0 into a tube size 12x75mm
- Pouring the isolate bacteria to the tube that has been filled with saline solution with swab to make the suspension of bacteria. The suspension then homogenized

DOI: 10.5281/zenodo.4657761

with turbidity 0.50-0.63 (gram-negative bacteria) using VITEK 2 DENSICHEK.

- Putting the tube of bacteria suspension and VITEK 2 DENSICHEK to special rack (cassette).
- The cassette then put into the vacuum chamber station.

Isolate DNA

Tools and Materials:

- Eppendorf tube 1.5ml
- Pipet with various size (10-100µl and 100-1000µl)
- Pipet tip volume 100µl
- Vortex
- ddH_2O
- Phosphate Buffered Saline (PBS)
- Chelex 20% in ddH_2O
- Micro centrifugal
- Water bath
- Saponins in 0.5% PBS
- Bacteria colony of *K.pneumoniae* and *E.coli*

Procedure:

- Take out the sample from freezer and sit in room temperature.
- Prepare the Eppendorf tube and put the bacteria colony to isolate its DNA.
- Wash the bacteria with 1ml PBS pH 7.4 then incubate for 10 minutes
- Centrifuge the bacteria with 5000 rpm speed during 5 minutes and throw the supernatant with pipet and repeat this step in three times.
- Add 500µl (0.5%) saponin to PBS and put into vortex to make it blend.
- The mixture then incubated in freezer in -20°C.
- The next day, the mixture is centrifuged with 12.000 rpm in 10 minutes and throw the supernatant.
- Add 50µl of chelex (20%) to ddH_2O with pH 10.5 and add 100µl ddH_2O.
- Incubate the mixture in boiled water for 10 minutes.
- Centrifuged the mixture in 12.000 rpm for 10 minutes and the DNA is moved to the sterilize Eppendorf tube and stored in -20°C temperature.

Primary Design

There are two primers used in multiplex PCR study, those are; forward sequence 5′-GCGTGGTTAAGGATGAACAC-3′ and reverse sequence 5′-CATCAAGTTCAACCCAACCG-3′.

OXA-48 Gene PCR

The result from DNA isolation then processed in PCR to identify the gene within.

Table 1. The PCR material composition

Material	Volume (µl)
ddH_2O	12
Taq polymerase	8
Primer *forward*	1
Primer *reverse*	1
DNA	3
Total	25

Table 2. The sequence of base, primary length, and PCR product

Primary	**Base**	**Primary Length (nt)**	**PCR Product (bp)**
Forward	5′-GCGTGGTTAAGGATGAACAC-3′	20	477
Reverse	5′-CATCAAGTTCAACCCAACCG-3′	20	

Source: (5)

The phase in implementing PCR method can be seen as below;

- Tag polymerase, ddH_2O, and DNA put into Eppendorf tube, in each volume and multiplied by the number of samples, using pipet in 100-1000µl and pipet tip 1000µl.
- Move the mixture to Eppendorf tube 0.2ml as much as the sample number with volume 25µl.
- Put the Eppendorf tube to PCR machine and adjust the thermal temperature during the process.

Agarose Gel Electrophoresis

The PCR result then visualized with agarose gel electrophoresis, with procedures as below;

DOI: 10.5281/zenodo.4657761

- Dissolve 0.4gr of agarose powder to 50ml buffer TAE by boiling it, after that let it sit in ±50°C temperature.
- Assemble the gel comb to electrophoresis tank and pour the agarose solution into it and freeze until it harden.
- After the gel harden, the gel comb can be taken and ready to be used.
- Agarose gel soaked in buffer TAE and 1µl of loading dye mixed with 3µl of DNA product in parafilm with the help of micropipette.
- The electrophoresis tank then covered and connected to the power supply (400mA, 90v) for 30 minutes.

Result

From the analysis above, it showed that *K.pneumoniae* bacteria was often found for 16 (66.3%) than *E.coli* bacteria for 8 (33.7%).

Table 3. Distribution of OXA-48 toward *Carbapenem* based on Specimen Types

No	Specimen	Total	Gen blaOXA-48			
			Positive	(%)	Negative	(%)
1	Pus	4	0	0	4	100
2	Sputum	9	0	0	8	100
3	Urine	7	1	14.3	6	85.7
4	Tissue	1	0	0	1	100
5	Pleura Fluid	1	0	0	1	100
6	Wound Swap	1	0	0	1	100
7	Feces	1	0	0	1	100
Total		24				

Table 3 above showed there were 7 specimens which tested to identify blaOXA-48 gene. From those 7 specimens, only urine that indicated positive with 14.3% while the remaining specimens showed negative result.

Table 4. Distribution of OXA-48 Gene in Sample

Variable	OXA-48 (+)	OXA-48 (-)	Total
K. pneumoniae	1	14	15
E. coli	0	8	8
Total	1	22	23

Table 5. Sensitivity pattern of *K.pneumoniae* toward *Carbapenem* on antibiotics

Antibiotic	Positive OXA-48 Gene						Negative OXA-48 Gene					
	R	%	I	%	S	%	R	%	I	%	S	%
Ampisilin	1	100	0	0	0	0	14	100	0	0	0	0
Aztreonam	1	100	0	0	0	0	12	85.7	0	0	2	14.3
Sepefim	1	100	0	0	0	0	12	85.7	0	0	2	14.3
Amikasin	1	100	0	0	0	0	7	50	1	7.1	6	43.9
Seftriakson	1	100	0	0	0	0	12	85.7	0	0	2	14.3
Sefazolin	1	100	0	0	0	0	12	85.7	0	0	2	14.3
Siprofloksasin	1	100	0	0	0	0	8	57.2	3	21.4	3	21.4
Seftazidim	1	100	0	0	0	0	12	85.7	0	0	2	14.3
Gentamisin	0	0	1	100	0	0	10	71.4	0	0	4	28.6
Nitrofurantoin	1	100	0	0	0	0	9	64.3	4	28.6	1	7.1
Ertapenem	1	100	0	0	0	0	14	100	0	0	0	0
Meropenem	1	100	0	0	0	0	14	100	0	0	0	0
Ampisilin-Sulbaktam	1	100	0	0	0	0	13	92.9	0	0	1	7.1

DOI: 10.5281/zenodo.4657761

Table 6. Sensitivity pattern of *E.coli* toward *Carbapenem* on antibiotics

Antibiotic	Negative OXA-48 Gene					
	R	**%**	**I**	**%**	**S**	**%**
Ampisilin	8	100	0	0	0	0
Aztreonam	4	50	0	0	4	50
Sepefim	1	12.5	0	0	7	87.5
Amikasin	1	87.5	0	0	7	87.5
Seftriakson	3	37.5	0	0	5	62.5
Sefazolin	3	37.5	0	0	5	62.5
Siprofloksasin	4	50	0	0	4	50
Seftazidim	3	37.5	0	0	5	62.5
Gentamisin	3	37.5	0	0	5	62.5
Nitrofurantoin	0	0	3	37.5	5	62.5
Ertapenem	8	100	0	0	0	0
Meropenem	8	100	0	0	0	0
Ampisilin-Sulbaktam	8	100	0	0	0	0

Table 4 indicated that there was a positive OXA-48 isolate. After implementing electrophoresis, seen with UV light, and identified based on base pair 477bp, the result showed there was one isolate that indicated positive in *K.pneumoniae Carbapenem*. The sensitivity pattern against anti micro bacteria based on blaOXA-48 gene can be seen in table 5 and 6 below;

From both table 5 and 6, it can be seen that there was significant differentiation in *K.pneumoniae* bacteria. The OXA-48 gene indicated positive in *Gentamisin* antibiotic. Whereas in *E.coli* bacteria, there was no antibiotics with positive OXA-48 gene. This showed that *Gentamisin* can be said as succeed in medical treatment although the success level was intermediate.

Discussion

Since 2000, the *Ambler Carbapenem* was similar to class A of KPC type or class B (enzyme IMP, VIM, and NDM). It considered as the most important *Carbapenem* in *Enterobacteriae*, because (1) the hydrolytic activity, including *Carbapenem* and *Sefalosporin* spectrum is wide, (2) the *Carbapenem* activity is significant, and (3) the gene has been identified in whole wide world.

In the opposite, OXA-48 considered less in problem, as (1) the hydrolytic spectrum does not include as wide *Sefalosporin* spectrum, (2) the *Carbepenem* activity is lower than the class A and B enzyme, (3) the spread of blaOXA-48 gene is limited only in Turkey for years. BlaOXA-48 is a part of functional transition which its spreading fit with plasmid support deployment and not from transposon.

Apart from the reality that blaOXA-48 gene is a part of functional transition, its spreading is in accordance with the spread of plasmid support and not from transposon. The fact is the entire of blaOXA-48 is identified in relationship with insertion sequence while most of the D class of β-lactamase is often being identified. Even though, it has strong relationship in nucleotide sequence, blaOXA-48 gene is identified in different genetic context. This indicated that both of the gene do not evolved through mutation but through two separated events (8). From the observation above, it is found that one sample is positive (4.7%) with blaOXA-48 gene. Meanwhile, the blaOXA-48 is rarely found as the cause of *carbapenem* resistance case in United States of America (9). It is found that the cause of OXA-48 in *K.pneumoniae* because of the nosocomial infection in Turkey. It has higher epidemiology level in 5 with endemic situation (10). While in Spain, France, Belgium and Romania the epidemiology level only reach 4 with interregional spreading status.

The clinical consequence related with the spread of OXA-48 type is quite important since it is classified as susceptible on *carbepenem* based on EUCAST or CLSI guidance (11). The recommendation suggested by the guidelines reported vulnerable toward *carbapenem*, apart from whether the isolate create a new *carbapenem* or not. Nowadays, it is reported that imipenem could be used to cure bacteremia because *K.pneumoniae* produce OXA-48 (12). The weight of antibacterial resistance between gram-negative pathogens, especially *Enterobacteriaceae* increased rapidly in the entire world.

Conclusion

It can be indicated that among 24 patients in Dr. Mohammad Hosein Hospital, one patient was identified as positive with blaOXA048 gene, while the other 23 patients

DOI: 10.5281/zenodo.4657761

showed negative. Therefore, OXA-48 gene was only identified in *K.pneumoniae* bacteria.

Carbapenem Resistance of OXA-48 Gene Coding in Klebsiella Pneumoniae and Escherichia Coli

Nita Parisa, Muhammad Fitrizal, Ella Amalia

Introduction: Bacteria that treated with antibiotics has ability to adapt or mutate due to form a defend mechanism. OXA-48 produces isolate that have ability as a resistance to drugs and all β-lactam, including *cephalosporin*, *cephamycin*, *monobactone*, and *carbapenem*. This study aims to identify the availability of OXA-48 gene as *Carbapenem* resistance in *Klebsiella pneumonia (K.pneumoniae)* and *Escherichia coli (E.coli)* from patient in Dr. Mohammad Hosein Hospital in Palembang, Indonesia. **Method:** The isolate bacteria from patients in Dr. Mohammad Hosein Hospital who infected with *K.pneumoniae and E.coli* in September until November 2017 were identified using Vitek 2 Compact. Polymerase Chain Reaction (PCR) used to detect the presence of bla OXA-48 to compare the pattern of antibiotic resistance. **Result:** The result showed that from 24 samples, there was 1 sample (4.7%) who positive with OXA-48 gene from *K.pneumoniae* bacteria and no positive gene found in *E.coli* bacteria. While the rest of the samples (95.3%) had negative OXA-48 gene. **Conclusion:** Therefore, the OXA-48 gene was only identified in *K.pneumoniae*.

Keywords: Klebsiella pneumonia, Escherichia coli, OXA-48, Carbapenem resistance, PCR.

References

1. Jawetz, Melnick, Adelberg. Mikrobiologi Kedokteran, Edisi I. Diterjemahkan oleh Bagian Mikrobiologi Fakultas Kedokteran Universitas Airlangga. 2001.
2. Tenaillon O, Skurnik D, Picard B, Denamur E. The population genetics of commensal Escherichia coli. Nature Reviews Microbiology. 2010.
3. Rhomberg PR, Jones RN. Summary trends for the Meropenem Yearly Susceptibility Test Information Collection Program: a 10-year experience in the United States (1999–2008). Diagn Microbiol Infect Dis. 2009;65(4):414–26.
4. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. J Antimicrob Chemother. 2012;67(7):1597–606.
5. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis. 2011;70(1):119–23.
6. Meletis G. Carbapenem resistance: overview of the problem and future perspectives. Ther Adv Infect Dis. 2016;3(1):15–21.
7. Spanu T, Sanguinetti M, Ciccaglione D, D'Inzeo T, Romano L, Leone F, et al. Use of the VITEK 2 System for Rapid Identification of Clinical Isolates of Staphylococci from Bloodstream Infections. J Clin Microbiol. 2003;41(9):4259–63.
8. Potron A, Poirel L, Rondinaud E, Nordmann P. Intercontinental spread of OXA-48 beta-lactamase-producing Enterobacteriaceae over a 11-year period, 2001 to 2011. Eurosurveillance. 2013;18(31):20549.
9. Lyman M, Walters M, Lonsway D, Rasheed K, Limbago B, Kallen A. Notes from the Field : Carbapenem-resistant Enterobacteriaceae Producing OXA-48-like Carbapenemases — United States, 2010–2015. MMWR Morb Mortal Wkly Rep. 2015;64(47):1315–6.
10. Poirel L, Héritier C, Tolün V, Nordmann P. Emergence of Oxacillinase-Mediated Resistance to Imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother. 2004;48(1):15–22.
11. Malbruny B, Le Marrec G, Courageux K, Leclercq R, Cattoir V. Rapid and efficient detection of Mycobacterium tuberculosis in respiratory and non-respiratory samples [Technical note]. Int J Tuberc Lung Dis. 2011;15(4):553–5.
12. Rousseau C, Poilane I, De Pontual L, Maherault A-C, Le Monnier A, Collignon A. Clostridium difficile Carriage in Healthy Infants in the Community: A Potential Reservoir for Pathogenic Strains. Clin Infect Dis. 2012;55(9):1209–15.

DOI: 10.5281/zenodo.4657761

Carbapenem Resistance of OXA-48 Gene Coding in Klebsiella Pneumoniae and Escherichia Coli

ORIGINALITY REPORT

3%	3%	0%	0%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	ee.zntu.edu.ua Internet Source	3%

Exclude quotes	On	Exclude matches	< 3%
Exclude bibliography	On		