blaCTX-M Gene as Risk Factor of Antibiotic Resistance

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bla$_{CTX-M}$ Gene as Risk Factor of Antibiotic Resistance

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Abstract

Excessive and misuse of antibiotics cause resistance for one or multidrug resistance. The β-lactam group is known as the most used type of antibiotic in the world. Clinical effectiveness of this antibiotic becomes limited by antibiotic resistance. Resistance mostly occurs in gram-negative bacteria especially in Klebsiella pneumoniae caused by enzymatic hydrolysis of antibiotics with genetically and functionally different enzymes called by extended-spectrum β-lactamase (ESBL). ESBL is a β-lactamase enzyme which causes bacterial resistance against penicillin, extended cephalosporin with oxyimino side chain (cefotaxime, ceftriaxone and ceftazidime) and oxyimino-monobactam aztreonam (but not cephamycin or carbapenem).

One of ESBL encoding genes is bla$_{CTX-M-15}$ *known as the most prevalent and causes any resistance. This cross-sectional study design aims to detect bla*$_{CTX-M}$ *gene from Klebsiella pneumoniae isolates and evaluates the relationship of the bla*$_{CTX-M}$ *gene with the incidence of antibiotic resistance in patients with bacterial infection at RSUP Dr. Hasan Sadikin Bandung. The results showed that from 45 samples which were tested, 43 samples are positive for bla*$_{CTX-M-98}$ *gene and 2 samples are positive for bla*$_{CTX-M-90}$ *gene. Both genes have a relationship with the incidence of antibiotic resistance in patients with bacterial infections at RSUP Dr. Hasan Sadikin Bandung.*

Keywords: Antibiotic resistance, *Klebsiellapneumoniae*, Extended-spectrum β-lactamase (ESBL), *bla*$_{CTX-M}$ gene.

Introduction

Antibiotic resistance has become growing public health threat of broad concern to many countries for many several decades. Antibiotic resistance is a natural phenomenon in microorganisms which is accelerated with selective pressure by use and misuse of antimicrobial agents in humans and animals[1]. It makes antibiotic become less effective or ineffective which results in an accelerating global health security emergency which exceeds existing drug.

Klebsiella pneumonia is one of the major ESBL-producing organisms isolated worldwide[2]. Based on WHO report on resistance to antibacterial drugs in selected bacteria of international concern, *K. pneumonia* was reported to be resistant to third-generation cephalosporin including resistance conferred by ESBLs and carbapenems[3]. The team of antimicrobial resistance control program at RSUP Dr. Hasan Sadikin Bandung reported bacterial map period on July until December 2013 that *K. pneumoniae* is the bacteria that mostly produces ESBL. The results of *K. pneumoniae* isolates from blood, sputum, body fluid, urine and feces specimen were: 71.4%, 43.2%, 60.7%, 54.7% and 66.7% respectively[4].

ESBL is a β-lactamase enzyme which causes bacteria resistance against penicillin; extended cephalosporin with oxyimino side chain (cefotaxime, ceftriaxone and ceftazidime) and oxyimino-monobactam aztreonam (but not cephamycin or carbapenem). ESBLs of class A mainly include TEM, SHV, CTX-M, VEB and GES enzymes. CTX-M enzymes is known to replace TEM and SHV mutants as the predominant ESBLs which are more active against cefotaxime and ceftriaxone than ceftazidime but point mutations can increase activity against ceftazidime[5].

WHO reported that resistance in *K. pneumoniae* to the third-generation cephalosporins was higher than 30% worldwide and higher than 60% in some of the countries[3]. This means treatment for severe *K. pneumonia* infection has to use carbapenems[1]. *K. pneumonia* which can hydrolyze all β-lactam including carbapenem rapidly emerged carbapenem resistance in *K. pneumoniae* already exceeding 50% in some countries of Eastern Mediterranean and Europe[3].

This research focuses on the detection of *bla*$_{CTX-M}$ gene from *K.pneumoniae* isolates and evaluates the relationship of *bla*$_{CTX-M}$ gene with the incidence of antibiotic resistance in patients with bacterial infection at RSUP Dr. Hasan Sadikin Bandung.

Material and Methods

Bacterial isolates: Forty-five isolates *K. pneumoniae* producing ESBLs were selected from patient's blood specimen at Clinical Pathology Department of RSUP Dr. Hasan Sadikin Bandung. DNA bacteria was extracted by using Purelink™ Genomics DNAKit Invitrogen. Polymerase chain reaction-amplification refractory mutation system (ARMS) method was used to detect *bla*$_{CTX-M}$ gene.

Antimicrobial susceptibility testing: Antimicrobial susceptibility testing was performed by VITEK according to the manufacturer's instructions.

Identification of *bla*$_{CTX-M}$ gene: All the isolates were used as templates with six primer sets as follows: forward 5'-ATGGTTAAAAAATCACTGCGCCAG-3' (F-primer-1); forward 5'-GGGGATAAAACCGGCAGCGA-3' (F-primer-2); forward 5'-GCGATGTGCAGCACCAGT AAAGC-3' (F-primer-3); forward 5'-GCGATGTG CAGCACCAGTAAAGT-3' (F-primer-4); reverse 5'-CAAACCGTCGGTGACGATTTTAGC-3' (R-primer-1) and reverse 5'-GTTGGTGGTGCCAT GCCAC-3' (R-primer-2). PCR was carried out using 2.5 μL Go Taq® Green Master Mix (Promega) according to the manufacturer's instruction. Initial denaturation at 94°C for 2 min was followed by 40 cycles of denaturation at 94°C for 30 annealing at 49.6°C (tube 1 and tube 2) dan 49°C (tube 3) for 1 min, with a final extension at 72°C for 1 min. Tube 1 consists of F1, F2, R1 and R2 primers; tube 2 consists of F1, F3, R1 and R2 primers; tube 3 consists of F1, F4, R1 and R2 primers. The products obtained were electrophoresed on 1.5% agarose gels. *bla*$_{CTX-M-98}$ gene was shown by 743 bp and 665 bp PCR product when identified using F1R2 and F4R1, *bla*$_{CTX-M-90}$ gene was shown by 665 bp PCR product when identified using F4R1.

Results

A total of 45 *K. pneumoniae* samples were obtained from patients at the hospital. All sample were isolated from patient's blood of both genders as presented in table 1.

The antibiotics susceptibility profile measured by VITEK is presented in table 2. The highest rate of resistance (100%) was shown to ampicillin, aztreonam, cephazolin, cephalothin, ceftriaxone, cefotaxime, ceftazidime and cefepime. All of the isolates showed multidrug-resistant (MDR) while resistance occurred to more than three antimicrobial families.

Table 1
Characteristic of patients

Characteristic	n	(%)
1. Sex		
Male	23	51.11
Female	22	48.89
2. Age group:		
0-27 days	20	44.44
28 days-23 months	2	4.44
2-11 years	3	6.67
12-18 years	1	2.22
19-60 years	16	35.56
>60 years	3	6.67

Table 2
Antibiotics susceptibility profile measured by VITEK

Antibiotics	n	Resistance n (%)	Intermediate n (%)	Sensitive n (%)
Ampicillin	45	45 (100)	-	-
Ampicillin/sulbactam	41	39 (95,12)	2 (4,88)	-
Piperacillin/Tazobactam	44	11 (25)	6 (13,64)	27 (61,36)
Amoxicillin/ Clavulanic acid	3	1 (33,3)	1 (33,3)	1 (33,3)
Cefoperazon/ Sulbactam	3	-	-	3 (100)
Aztreonam	31	31 (100)	-	-
Cephazolin	31	31 (100)	-	-
Cephalotin	9	9 (100)	-	-
Cefoxitin	11	1 (9,09)	-	10 (90,91)
Cefmetazole	19	3 (15,79)	-	16 (84,21)
Ceftriaxone	34	34 (100)	-	-
Cefotaxime	12	12 (100)	-	-
Ceftazidime	45	45 (100)	-	-
Cefepime	42	42 (100)	-	-
Ertapenem	31	3 (9,68)	-	28 (90,32)
Meropenem	45	3 (6,67)	-	42 (93,33)
Doripenem	3	-	-	3 (100)
Imipenem	3	-	-	3 (100)

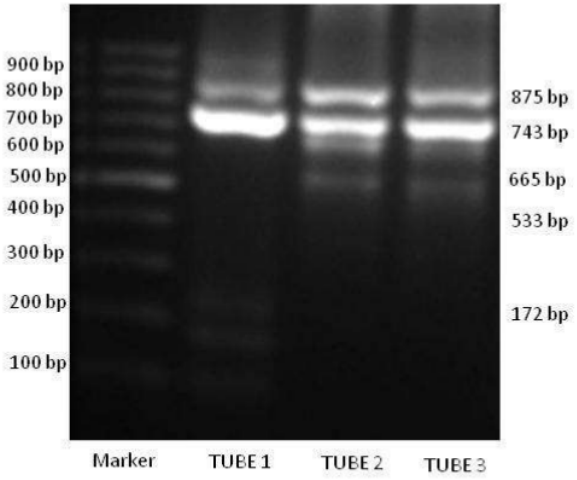

Fig. 1: UV visualization result of *bla*$_{CTX-M-98}$ and *bla*$_{CTX-M-90}$ gene. F1R2 (Mutation at codon A722G): 743 bp, F3R1 (wild-type) and F4R1 (mutation at codon C230T): 665 bp, F3R2 (mutation at codon A722G) and F4R2 (Mutation at codon C230T and A722G) 553 bp, F2R1 (wild-type): 172 bp, band showed in very low intensity.

All of the isolates, 45 samples showed positive ESBLs resistance. PCR primer indicated 43 isolates carried *bla*$_{CTX-M-98}$ gene and 2 isolates had *bla*$_{CTX-M-90}$ gene. The result of UV gel visualization is shown in fig.1. PCR product from *bla*$_{CTX-M-98}$ gene was shown by double bands in 743 bp and 665 bp when identified using F1R2 and F4R1 and PCR product from *bla*$_{CTX-M-90}$ gene was shown by a single band in 665 bp when identified using F4R1.All 45 samples showed positive ESBLs resistance, 43 samples are positive *bla*$_{CTX-M-98}$ gene shown by appearance double bands for two mutation points at A77V and D240G, 2 samples are positive *bla*$_{CTX-M-90}$ gene shown by appearance of single bands for one point mutation at A77V. This result is consistent with the result reported by Zhao and Hu[6] who reported *bla*$_{CTX-M-98}$ gene to have a point of mutation at A77V and D240G, while *bla*$_{CTX-M-90}$ gene only has one-point mutation at A77V.

Discussion

This study showed high rate resistance (100%) to cefepime the fourth generation of cephalosporins and third generation including cephalosporins ceftriaxone, cefotaxime and ceftazidime among ESBL producing *K. pneumoniae* isolates. This result is in line with the report from Raei et al[7] at Imam Hussein hospital in Tehran, ESBL K. pneumoniae isolates were resistant to cefotaxime (95.6%), ceftazidime (89.1%) and ceftriaxone (96.7%). Zhang et al[8] also report that the prevalence rate of ESBL *K. pneumoniae* causing community-onset infections in China is 31.8% comparable with nosocomially acquired ESBL *K. pneumoniae* by multiple studies across China (30.1-39.7%).[9,10]

The third generation cephalosporins of antibiotics are commonly used in Indonesian hospitals. Croatia also has high rates of resistance to the third generation cephalosporins in CTX-M producers[11]. In this study, imipenem the drug of choice for complicated infections in ESBL-producing *Klebsiella* strains and it was 100% sensitive. This result is in line with reports from Russia hospitals where 100% sensitivity to imipenem was reported[12]. Seyedpour et al[13] reported that the *K. pneumoniae* isolates were susceptible to imipenem while Du et al[14] have other findings that indicated rates of resistance to imipenem were 3.3%. The dissemination of ESBL *K. pneumoniae* in the community has become another challenge for resistance control in health-care systems. There is difference about geographically and antibiotic policies in each region, so it impacts to the difference prevalence rates of ESBL production in different countries or parts of each country.

This present study shows a high frequency of MDR among ESBL K. *pneumoniae* isolates. Similar findings were reported by Raei et al[7]. There was 96.7% multidrug resistance observed from ESBL *K. pneumoniae* isolates. This is due to mobile genetic elements such as transposons or plasmid encoding ESBLs which contain resistance genes (chromosomally located β-lactamase) to other antibiotics. All of 45 ESBL producer *K. pneumoniae* isolates in this study carried the *bla*$_{CTX-M}$ gene, the predominant ESBL bla genes in most parts of the word. High prevalence of *bla*$_{CTX-M}$ gene as an endemic was also reported in the Teaching Hospital of Kashan Iran.

Resistance to widely used and available oral antibacterial drugs has emerged and spread globally.

Conclusion

In conclusion, based on this study, high ESBL *K. pneumoniae* with CTX-M type was revealed. There are 43 isolates carrying *bla*$_{CTX-M-98}$ gene and 2 isolates had *bla*$_{CTX-M-90}$ gene. Both genes have a relationship with the incidence

of antibiotic resistance in patients with bacterial infections at RSUP Dr. Hasan Sadikin Bandung.

References

1. Francesca P., Patrizio P. and Annalisa P., Antimicrobial resistance: a global multifaceted phenomenon, *Pathog Glob Health*, **109(7)**, 309–318 **(2015)**

2. Jacoby G.A. and Munoz-Price L.S., The new *β*-lactamases, *The New England Journal of Medicine*, **352(4)**, 380–391 **(2005)**

3. World Health Organization, Antimicrobial Resistance Global Report on Surveillance: Global Report on Surveillance, Geneva, Switzerland **(2014)**

4. Clinical Pathology Department of Doctor HasanSadikin Hospital Bacterial Pattern and Sensitivity to Antibiotics in Doctor HasanSadikin Hospital Semester 2, Bandung, Indonesia **(2013)**

5. Livermore D.M., Cantón R., Gniad-kowski M., Nordmann P., Rossolini G.M. and Arlet G., CTX-M: changing the face of ESBLs in Europe, *J Antimicrob Chemother*., **59**, 165–174 **(2007)**

6. Zhao W.H. and Hu Z.Q., Epidemiology and genetics of CTX-M extended-spectrum beta-lactamases in gram-negative bacteria, *Crit Rev Microbiol.*, **39(1)**, 79–101 **(2013)**

7. Raei F., Eftekhar F. and Feizabadi M.M., Prevalence of Quinolone Resistance Among Extended-Spectrum beta-lactamase producing uropathogenic *Klebsiellapneumoniae*, *Jundishapur J Microbiol.*, **7(6)**, doi: 10.5812/jjm.10887 **(2014)**

8. Zhang J., Kai Z., Beiwen Z., Lina Z., Ping S., Jinru J., Zeqing W., Lanjuan L., Jianying Z. and Yonghong X., High Prevalence of ESBL-Producing *Klebsiellapneumoniae* Causing Community-Onset Infections in China, *Front Microbiol*., **7**, 1830 **(2016)**

9. An S., Chen J., Wang Z., Wang X., Yan X., Li J., Chen Y., Wang Q., Xu X., Li J., Yang J., Wang H. and Gao Z., Predominant characteristics of CTX-M-producing *Klebsiellapneumoniae* isolates from patients with lower respiratory tract infection in multiple medical centers in China, *FEMS Microbiol Lett*., **332(2)**, 137-45 **(2012)**

10. Yang Q., Zhang H., Wang Y., Xu Y., Chen M., Badal R.E., Wang H., Ni Y., Yu Y., Hu B., Sun Z., Huang W., Wang Y., Wu A., Feng X., Liao K., Shen D., Hu Z., Chu Y., Lu J., Cao B., Su J., Gui B., Duan Q., Zhang S., Shao H., Kong H., Hu Y. and Ye H., A 10 year surveillance for antimicrobial susceptibility of Escherichia coli and *Klebsiellapneumoniae* in community and hospital-associated intra-abdominal infections in China, *J Med Microbiol*., **62(9)**, 1343-9 **(2013)**

11. Bedenić B., Vranes J., Bosnjak Z., Marijan T., Mlinarić-Dzepina A., Kukovec T., Knezević J., Anusić M., Beader N., Barl P., Leskovar V. and Kalenić S., Emergenceof CTX-M group 1 extended-spectrum beta-lactamase-producing *Klebsiellapneumoniae* strains in the community, *Med Glas (Zenica)*, **7(1)**, 32-9 **(2010)**

12. Edelstein M., Pimkin M., Palagin I., Edelstein I. and Stratchounski L., Prevalence and molecular epidemiology of CTX-M extended-spectrum beta-lactamase-producing Escherichia coli and *Klebsiellapneumoniae* in Russian hospitals, *Antimicrob Agents Chemother*., **47(12)**, 3724–32 **(2003)**

13. Seyedpour S.M. and Eftekhar F., Quinolone Susceptibility and Detection of qnr and aac(6')-Ib-cr Genes in Community Isolates of *Klebsiellapneumoniae*, *Jundishapur J Microbiol*., **7(7)**, doi: 10. 5812/jjm.11136 **(2014)**

14. Du J., Li P., Liu H., Lu D., Liang H. and Dou Y., Phenotypic and molecular characterization of multidrug resistant *Klebsiellapneumoniae* isolated from a university teaching hospital China, *PLoS One*, **9(4)**, doi: 10.1371/journal.pone.0095181 **(2014)**.

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