Phenotype Characterization of Beta-Lactamase Producing *Enterobacteriaceae* in the Intensive Care Unit (ICU) of Cipto Mangunkusumo Hospital in 2011

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ABSTRAK

Tujuan: *mengetahui karakteristik fenotip bakteri Gram negatif famili Enterobacteriaceae penghasil enzim beta-laktamase, seperti ESBL, AmpC, dan karbapenemase.* ***Metode:*** *tiga metode berbeda dilakukan untuk mengkonfirmasi karakteristik fenotip ketiga enzim tersebut, yaitu metode difusi cakram untuk konfirmasi ESBL, uji cakram AmpC (berbasis cefoxitin) untuk konfirmasi AmpC, dan uji Hodge termodifikasi untuk konfirmasi karbapenemase.* ***Hasil:*** *dengan metode difusi cakram ganda, kami dapatkan 58.42% isolat merupakan penghasil ESBL, 1.98% merupakan penghasil AmpC dengan uji cakram AmpC (berbasis cefoxitin), dan 27.59% merupakan penghasil karbapenemase dengan uji Hodge termodifikasi.* ***Kesimpulan:*** *hasil penelitian ini menunjukkan prevalensi Klebsiella pneumoniae penghasil beta-laktamase khususnya ESBL sangat tinggi, dan tentu saja AmpC dan karbapenemase tidak boleh diabaikan sebagai penyebab infeksi meskipun presentasenya kecil.*

Kata kunci: *Enterobacteriaceae, resistensi antibiotik, ESBL, AmpC, karbapenemase.*

ABSTRACT

Aim: *the goal of this study is to understand the phenotype characteristic of beta-lactamase enzymes producing Enterobacteriaceae, such as ESBL, AmpC, and carbapenemase.* ***Methods:*** *three different methods are performed to confirm those phenotypic characteristics, namely double disk diffusion method to confirm ESBL, AmpC disk test (cefoxitin-based) to confirm AmpC, and modified Hodge test to confirm carbapenemase.* ***Results:*** *using double disk diffusion method, we found 58.42% isolates are ESBL-producing, whereas the outcomes of AmpC disk test shows 1.98% are AmpC-producing. By conducting modified Hodge test (MHT), 27.59% isolates are confirmed as carbapenemase-producing bacteria.* ***Conclusion:*** *this study confirmed the prevalence of beta-lactamase producing Klebsiella pneumoniae is very high. Nevertheless, AmpC and carbapenemase should not be ignored despite their low prevalence.*

Key words: *Enterobacteriaceae, antibiotic resistance, ESBL, AmpC, carbapenemase.*

INTRODUCTION

The increasing incidence of antibiotics resistance is getting more global attention. The absence of antibiotic regulation in most Asian countries, including Indonesia, is believed to have contribution to the rapidly increasing infections of multi-resistance pathogenic bacterias.[1] Infectious disease experts and clinical microbiologists have agreed that antibiotic multi-resistant Gram negative bacterias pose the greatest risk to the public health. This happens not only because of the resistance of Gram negative bacteria is faster than those of Gram positive, but also the discovery and development of new antibiotics to fight Gram negative bacteria are far fewer.[2] Many references reported that the increasing resistancies of Gram negative is due to mobile genetic elements, thus readily spread through bacterial population.[2,3]

Among Gram negative bacteria, *Enterobacteriaceae* is the most common cause of hospital acquired infections, such as urinary tract infection, blood stream infection, pneumonia, and abdominal infection.[4] High number of antibiotic resistance of those bacterias have been reported in many countries. This should be taken into consideration when choosing proper antibiotics, especially to patients with severe or critical illness and those who need immediate therapy, for example, intensive care unit (ICU) patients.

Enterobacteriaceae resistant to third generation cephalosporin are tipically caused by production of beta-lactamase enzyme, known as extended-spectrum beta lactamase (ESBL) [Ambler class A]. The genes that encode are frequently found on the same plasmid as genes that encode resistance to aminoglycoside, sulfonamide, and quinolons. These indicate that ESBL producing *Enterobacteriaceae* in hospitals, especially ICUs, are multidrug resistant.[4] Inproper empiric antibiotic to treat nosocomial infection contribute significantly to high mortality rate in ICUs.[1,4] In addition to ESBL, resistant to third generation cephalosporin may also be caused by the production of AmpC cephamycinase enzyme (AmpC) [Ambler class C] especially by *Enterobacter spp* and *Citrobacter spp*, and encoded by AmpC genes in the cromosom. However, the the AmpC encoding genes may also be mediated by plasmid [Ambler class D].[6] The AmpC producing bacteria is resistant to cephamycin (cefoxitin) too.[6] Carbapenem, like imipenem and meropenem, is the first line therapy recommended for severe infection due to ESBL producing *Enterobacteriaceae*.[7] In spite of this, since 2000, resistance to carbapenem antibiotics have also been reported.[8] Most common carbapenemase in *Enterobacteriaceae* is *K. pneumoniae* producing carbapenemase (KPC), especially in US, Asia, England, Israel, and Southern Europe.[7] In 2009, new type of carbapenemase called New Delhi Metalo-β-lactamase 1 (NDM-1) was reported [Ambler class B].[2,8,9] These findings are worrying because the choice in antibiotic therapy is getting more limited.

Taking all information above into consideration, antibiotic susceptibility test alone is not enough. Confirmation of phenotype characteristic of these beta–lactamase enzymes is important to be done, not only for epidemiology matters, also for patient's safety.

This study is a collaboration project of Clinical Microbiology Laboratory Faculty of Medicine University of Indonesia (CML-FMUI) and Intensive Care Unit, Cipto Mangunkusumo Hospital (ICU RSCM). We expect to obtain preliminary data on the prevalence of each phenotypic character with the intention that it could be the useful guidance to treat patients with antibiotics. The final outcomes would be reduction of hospital stay and treatment cost, better infection control in hospitals, and prevention of resistance spreading.

METHODS

Study Design

This study is a retrospective study with subjects consisting of all ICU patients of Cipto Mangunkusumo Hospital (RSCM) hospitalized during January to December 2011. All samples are taken based on CML-FMUI standard operational procedure.

Sample Size

By taking alpha (α) value 0.05, then Zα is 1.96 (normal curve), p is proportion of patient with *Enterobacteriaceae* producing beta lactamase infection, while q is proportion of patient with no *Enterobacteriaceae* producing beta lactamase infection. Based on SENTRY Asia-Pacific Surveillance Program that was conducted in 1998–2002, ESBL prevalence has reached 60% in several Asian countries,[1] p value 60% and q value (100%-60% = 40%), with allowable error (L) was 10%, the sample size needed is 103 isolates.

Patient samples were routinely collected for clinical purposes and then reanalyzed for the purposes of this study. During the 1-year period, from January until December 2011, all hospitalized patients at ICU Cipto Mangunkusumo Hospital were included.

Bacterial Isolates

Samples are clinical isolates from ICU patients of Cipto Mangunkusumo Hospital in 2011 which have been reserved as biological material in Laboratory of Clinical Microbiology, Faculty of Medicine. All isolates has been identified as Enterobacteriaceae using API 20E system (BioMerieux®). Those isolates have passed susceptibility test that resistant to one or more antibiotic from third generation

cephalosporin and/or resistant to aztreonam and/or intermediate or resistant to one or more antibiotic from carbapenem class.

Phenotypic Confirmation

Three different methods are performed to confirm phenotypic characteristics i.e.: (1). Double disk diffusion method to confirm the production of ESBL, (2). AmpC disk test to confirm the production of AmpC, and (3). Modified Hodge test (MHT) to confirm the production of carbapenemase.

This study has passed evaluation at the Faculty of Medicine University of Indonesia/ Cipto Mangunkusumo Hospital Ethics Committee (Letter no. 318/PT02.FK/ETIK/2012).

RESULTS

Based on ICU patients' record we found 84 patients with microbiology examination results showed infection of *Enterobacteriaceae* family bacteria. Those patients consist of 56% male and 44% female, with age ranging from 1 to 86 years. The percentage of medical and surgical cases are similar (50%:50%).

The total numbers of isolates from those 84 patients are 112 because several patients had more than one examination. Sputum is the primary source of isolates with almost 60%, the rest are from 12 other sources i.e. urine, pus, blood, etc. (**Table 1**).

Table 1. Sources of specimen

Specimens	Percentage (%)
Sputum	58.3
Urine	12.0
Pus	7.4
Blood	6.5
Wound swab	5.6
Tissue	3.7
Feces	1.9
Ascites fluid	1.9
CVC tip	0.9
Pleura fluid	0.9
Lochia	0.9
Total	100

The 112 isolates are then further analyzed to identify their species and phenotypic characteristic. (**Table 2**)

Table 2. *Enterobacteriaceae* isolates

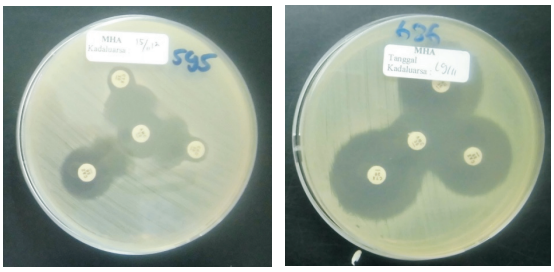
***Enterobacteriaceae* species**	**Number of isolates (%)**
Klebsiella pneumoniae	61 (54,46)
Escherichia coli	15 (13,39)
Proteus mirabilis	9 (8,04)
Serratia liquafaciens	5 (4,46)
Serratia odorifera	5 (4,46)
Enterobacter cloacae	5 (4,46)
Citrobacter freundii	3 (2,67)
Salmonella arizonae	2 (1,78)
Klebsiella oxytoca	2 (1,78)
Serratia ficaria	1 (0,9)
Serratia fonticola	1 (0,9)
Serratia marcescens	1 (0,9)
Edwardsiella tarda	1 (0,9)
Shigella A	1 (0,9)
Total	112 (100)

Not all isolates proceeded to the phenotypic confirmation test. Referring to CML-FMUI WHONET susceptibility data, 11 of 112 isolates (9.82%) are still sensitive to third generation cephalosporin, aztreonam, and carbapenem. Among 101 isolates (90.18%) that are resistant to third generation cephalosporin and aztreonam, 29 isolates (25.89%) are found to be resistant to carbapenemase too. Phenotypic confirmation test for ESBL and AmpC were conducted on those 101 isolates, while 29 of them were also tested for carbapenemase.

Later after conducting double disk diffusion and AmpC disk test to those second category isolates above, we confirm 58.42% are ESBL positive and 1.98% are AmpC positive. Meanwhile, modified Hodge test conducted to 29 isolates included to the third category, 27.59% are carbapenemase positive but not with strong indication (weak positive).

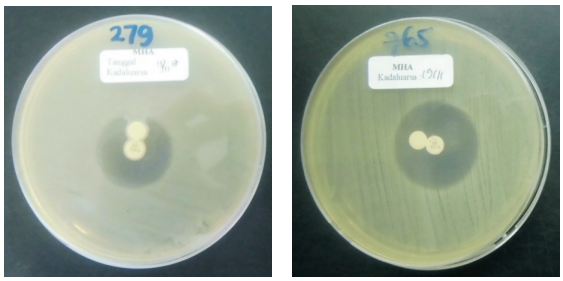
Among ESBL positive patients, 78% were clinically improving and 22% patients has died, while the percentage among ESBL negative patients are 83% and 17%, respectively.

Then we conducted the Chi-square test to analyze the statistical association between infection of beta lactamase-producing *Enterobactericeae* with age, gender, clinical manifestation (medical or surgery), and outcome (died or clinically improving). The association between those variable were found to be statistically insignificant ($p>0.05$).



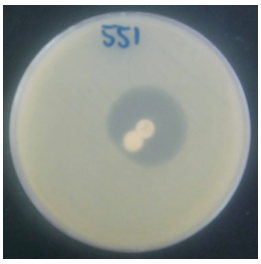
ESBL (+) ESBL (-)

Figure 1. Double disk diffusion method to confirm the production of ESBL



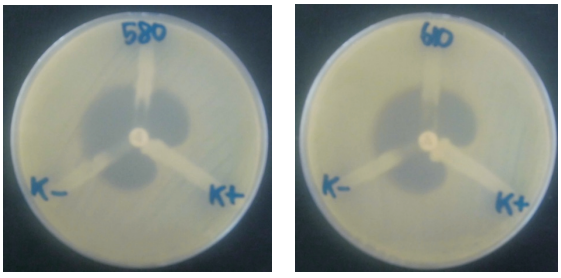
AmpC (+) AmpC (-)

Figure 2. AmpC disk test to confirm the production of AmpC



Carbapenemase (+)

Figure 3. AmpC weak positive



Carbapenemase (+) Carbapenemase (-)

Figure 4. Modified Hodge test (MHT) to confirm the production of carbapenemase

DISCUSSION

The high number of ESBL-producing bacteria found in this study is similar with the result of SENTRY Asia Pacific surveillance program study conducted during 1998 to 2002 in which the result is around 60% in several Asian countries.[1]

The considerably low AmpC might not indicate the true prevalence. Some isolates that showed the reduction of cefoxitin inhibition zone could not be concluded as AmpC positive due to the absence of positive control. We recommend collaboration with other researchers who had done AmpC before to share their positive control. Other methods, like three dimensional test and boric acid based test, may also be carried out to compare the sensitivity and specificity.

Cefoxitin has been used mostly for susceptibility test of Gram positive bacteria. It is already known that AmpC is resistant not only to third generation cephalosporin and aztreonam, it is also resistant to cefoxitin and beta lactamase inhibitor.[15] Therefore, we suggest using cefoxitin disk for routinely susceptibility test in Gram negative bacteria. This could be useful as initial screening of possible AmpC producing isolates.

Meanwhile, carbapenemase-producing *Enterobacteriaceae* are found to be relatively high, but the detection using MHT indicates weak positive result. We recommend other methods as comparison and to increase the sensitivity. Several references stated that MHT offers very good sensitivity for KPC dan OXA enzymes, but not sensitive for VIM, IMP, and NDM.[16] Another study suggests that adding ZnSO4 to MHA may increase the sensitivity for NDM producing Enterobacteriaceae.[17] Lee et al. found that the use of MacConkey agar could improve MHT performance in detecting positive result compared to MHA.[18] Pasteran et al. concluded that indicator organism *Klebsiella pneumoniae* ATCC 700603 used for MHT is more sensitive than *E. coli* ATCC 25922, especially for KPC and metallo beta-lactamase.[19]

As many studies and many attempts to modify phenotypic confirmation tests for carbapenemase producing bacteria have been done so far, we suggest conducting further research to compare each test. Thus, we could adopt the best or most appropriate method for routine examination in microbiology laboratory to support infection control surveillance program in hospitals.

Having found sputum as the major source of specimens indicated that lower respiratory tract infections are highly frequent among ICU patients and often related to ventilator-associated pneumonia (VAP).

The statistically insignificant association between infections of *Enterobacteriaceae* producing ESBL with clinical manifestations

and outcomes does not necessarily mean that it is not clinically insignificant. The result of this study confirmed that anyone could be infected by ESBL-producing *Enterobacteriaceae* regardless their age, gender, and clinical manifestation. Although there is no direct effect on patient's outcome, the infections are likely to prolong hospital stay and incur high cost.

Some published studies found that prior antibiotics administration to patients are strongly correlated with ESBL incidences.[20-22] However, such an aspect was not observed in this study. We would conduct further research to examine the connection between antibiotics prescription and other factors with infection of beta lactamase-producing bacteria.

CONCLUSION

This study confirmed the prevalence of beta-lactamase producing *Klebsiella pneumoniae* to be very high. Nevertheless, AmpC and carbapenemase should not be ignored despite their low prevalence. By understanding the resistance pattern and prevalence of the beta lactamase-producing organisms, especially *Klebsiella pneumoniae*, we would have a better infection control as well as proper and rational antibiotics prescription.

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