MULTIRESISTANCE PATTERN OF EXTENDED SPECTRUM β-LACTAMASE (ESBL) – *ESCHERICHIA COLI* AND *KLEBSIELLA PNEUMONIAE* STRAINS

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ABSTRACT

All specimens came in the Clinical Microbiology Laboratory of Dr. Soetomo Hospital Surabaya were prospectively studied since January 2005 until April 2005. The bacterial isolation and identification were performed using the standard method on the consecutive isolates. The biochemical reaction was performed by using the reagent of Microbact for Gram negative bacteria detection Microbact 12A (Medveg diagnostics). The susceptibility test was conducted by disk diffusion test according to NCCLS method. The phenotypic confirmation laboratory testing of ESBLs were conducted using ceftazidime disk (CAZ 30 ug) and ceftazidime plus clavulanic acid (CD 30ug/10ug) according to NCCLS and ESBLs determined using double disc (DD) synergy method. Prevalence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* from clinical specimens were high 34.84 % (115/330) and 35.35% (105/297). Antimicrobial resistance pattern of ESBL-producing *E. coli* and *Klebsiella pneumoniae* to third generation cephalosporin were high resistant, i.e.: cefotaxim (90.4%), ceftriaxon (95.7%), ceftazidime (55.7%); 81.9%, 94.3%, 87.8% respectively. The resistance pattern against ciprofloxacin have similar figure with thirds generation cephalosporin. The alternative sensitive drugs against ESBL-producing bacteria were meropenem and fosfomycin.

Keywords: Extended Spectrum β-Lactamase (ESBL), multiresistance, Escherichia coli, Klebsiella pneumoniae

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INTRODUCTION

The increasing of bacterial resistance to third generation cephalosporin has been observed in many countries (Bujdakova et al. 1998, Burgees & Hall 2004, Johnson et al. 1999; Peterson & Yu 1999). The Extended Spectrum β-lactamase (ESBL) is recognized worldwide as a big problem in hospitalized patient, certainly in critically ill patients. Even though Indonesia does not have a definitive data of ESBL, it is suggested that ESBL producing bacteria in Indonesia are prevalent.

Nowadays the cost of resistance to beta-lactam antibiotic is estimated to be between US $ 10 – 17 billion annually (Palumbi 2001). The usage of beta lactam drug would be still a first priority for the physicians, because the beta lactam drug has a wide range of spectrum and have very low toxicity to humans. The high volume and indiscriminate use of extended-spectrum cephalosporins is common practice in hospitals with high prevalence of ESBL producing bacteria (Ariffin et al. 2004; Rice 1999). In United States the occurrence of ESBL producing bacteria in *Enterobacteriaceae* ranges from 0 to 25% depending on the institutions. In Europe, varies greatly among the countries. In the Netherlands, was less than 1% of *Escherichia coli* and *Klebsiella pneumoniae* produced ESBL (Stobberingh et al. 1999). In France, 40% of *Klebsiella pneumoniae* isolates were ceftazidime resistant and ESBL producer (Branger et al. 1998). In Department of Urology, Dr. Soetomo Hospital Surabaya, 73.58% of hospitalized patients in Urology ward, consume beta-lactam drug as therapeutic agent (Kuntaman et al. 1996) and in Pediatric Department, 52 to 65% of hospitalized patients took up the antimicrobial agents (Soegijanto 1998).

The cephalosporin antibiotics have become a major part of the antibiotic formulary for hospitals especially in developing countries. They are prescribed for a wide variety of infections every day. Their undoubted popularity relies upon lesser allergenic and toxicity risks as well as a broad spectrum of activity. It is the latter feature, however, that encourages the selection of microorganisms that are resistant to these agents. There for long-term implications for the treatment and control of this heterogeneous group of super infections. When clinicians evaluate a septic patient, it is understandable that they choose empirical therapy with a cephalosporin while awaiting microbiology and other tests, since
bacterial identification and antimicrobial testing still usually require 24-48 h (Lee et al. 2001).

Resistance to third-generation cephalosporins through the acquisition and expression of extended-spectrum β-lactamase (ESBLs) among Enterobacteriaceae is increasing. The detection of extended-spectrum β-lactamase (ESBL) producing strains is important for optimal therapy of infected patients. The screening and confirmation of ESBL-producing isolates using the methods of the NCCLS are easily performed (Coudron 1997). It is suggested that very important to aware and conduct the surveillance of ESBLs in the hospital and the community.

MATERIALS AND METHODS

The bacterial targets for the study of the ESBL were Escherichia coli and Klebsiella spp that were commonly harbor the ESBLs enzyme. All specimens came in the Clinical Microbiology Laboratory of Dr. Soetomo Hospital Surabaya were prospectively studied since January 2005 until April 2005.

The bacterial isolation and identification were performed using the standard method on the consecutive isolates. The biochemical reaction was performed by using the reagent of Microbact for Gram negative bacteria detection Microbact 12A (Medveg diagnostics). The susceptibility test was conducted by disk diffusion test according to NCCLS method. The control strains used E. coli ATCC 25922 and Klebsiella pneumoniae ATCC 700603

All isolates of Escherichia coli and Klebsiella pneumonia that resistant to cefotaxim or ceftazidime that commonly used as a routine examination for susceptibility testing were enrolled in this study. The bacterial strains included if in the diffusion method susceptibility test have an inhibition zone ≤ 22 millimeter for ceftazidime or ≤ 27 millimeter for cefotaxim.

The phenotypic confirmation laboratory testing of ESBLs were conducted using ceftazidime disk (CAZ 30 ug) and ceftazidime plus clavulanic acid (CD 30ug/10ug) according to NCCLS and ESBLs determined using double disc (DD) synergy method with 15 mm distance edge to edge. The ESBL producing bacteria is stated if the inhibition zone around the ceftazidime plus clavulanic acid disk enlarging ≥ 5 millimeter compared to the inhibition zone of ceftazidime alone (bulging appearance).

The (DD) method was done from a colony on blood agar plate culture grown overnight, as recommended for the standard disk diffusion test. Disks containing the standard CD or amoxillin-clavulanic acid (20 ug and 10 ug) put in the centre of an agar Muller Hinton (Oxoid); and then ceftaxidime, cefotaxime, ceftriaxone, cefepim, cefixime in distance 15 mm edge to edge from CD. Disk placement was expedited by melting holes in the lid of a petri plate and using the lid as a template to mark the bottom of the agar plate for proper disk location. All isolates were also susceptibility tested with amikacin, meronem, ciprofloxacin, and fosfomycin. Inoculated media were incubated overnight at 35°C. An enhanced zone of inhibition between any one of the β-lactam disks and the dicks containing clavulanic acid was interpreted as presumptive evidence for the present of an ESBL (Coudron 1997; Villegas et al. 2004).

RESULTS

In the period of 4 months (January 2005 - April 2005) have been examined 3149 clinical specimens from hospitalized patients, comprised of 231 sputum, 807 blood/ feces, 1614 urine and 479 pus/wound swab, and 18 cerebrospinal fluid. The isolates were 330 Escherichia coli, and 297 Klebsiella pneumoniae, were documented. In the confirmation test for ESBL producing, it has been identified ESBL strains of 115 (34.84 %) Escherichia coli, and 105 (35.35 %) Klebsiella pneumoniae.

Table 1. The Distribution of the Enterobacteriaceae isolates from clinical specimens in Dr. Soetomo Hospital Surabaya Indonesia (January 2005-April 2005).

<table>
<thead>
<tr>
<th>Specimen</th>
<th>E. coli</th>
<th>K. pneumonia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine</td>
<td>279</td>
<td>152</td>
</tr>
<tr>
<td>Pus</td>
<td>42</td>
<td>63</td>
</tr>
<tr>
<td>Blood/faeces</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Sputum</td>
<td>1</td>
<td>60</td>
</tr>
<tr>
<td>Total</td>
<td>330</td>
<td>297</td>
</tr>
</tbody>
</table>
Table 2. The prevalence of ESBL among *E. coli* and *K. pneumoniae* among the clinical isolates in Dr. Soetomo Hospital Surabaya Indonesia (January 2005-April 2005).

<table>
<thead>
<tr>
<th>Isolates</th>
<th>ESBL positive (% of total isolates)</th>
<th>Total isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. coli</em></td>
<td>115 (34.84%)</td>
<td>330</td>
</tr>
<tr>
<td><em>K pneumoniae</em></td>
<td>105 (35.35%)</td>
<td>297</td>
</tr>
</tbody>
</table>

Table 3. Antibiogram of ESBL-*E. coli* and *Klebsiella pneumoniae* strains in Dr. Soetomo Hospital Surabaya (January 2005 – April 2005).

<table>
<thead>
<tr>
<th>Antimicroba</th>
<th>ESBL-<em>E. coli</em> (Resistant)</th>
<th>ESBL-<em>K. pneumoniae</em> (Resistant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amoxyclav</td>
<td>40% (46/115)</td>
<td>43.80% (46/105)</td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>90.4% (104/114)</td>
<td>81.90% (86/105)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>95.7% (110/115)</td>
<td>94.3% (99/105)</td>
</tr>
<tr>
<td>Cefazidime</td>
<td>55.70% (64/115)</td>
<td>84.80% (89/105)</td>
</tr>
<tr>
<td>Cefepim</td>
<td>47.8% (55/115)</td>
<td>56.7% (59/104)</td>
</tr>
<tr>
<td>Meronem</td>
<td>2.6% (3/115)</td>
<td>1.9% (2/105)</td>
</tr>
<tr>
<td>Ceftixime</td>
<td>99.1% (114/115)</td>
<td>97.1% (102/105)</td>
</tr>
<tr>
<td>Amikacin</td>
<td>19.3% (22/114)</td>
<td>27.9% (29/104)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>79.10% (91/115)</td>
<td>60% (63/105)</td>
</tr>
<tr>
<td>Fosfomycin</td>
<td>8.7% (10/115)</td>
<td>13.5% (14/114)</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In our study at 2001/2002 showed that the cefotaxim resistant *Escherichia coli* were prevalent in hospitalized patients, at the time of admission (AMRIN data, was not yet published). In study 2005 of the ESBL producing bacteria, all isolates were from hospitalized patients, it was suggested these isolates were hospital origin. In this study was found that the prevalence rate of ESBL producing bacteria, in both *Escherichia coli* and *Klebsiella pneumoniae* among hospitalized patient were rather high. This prevalence rate was comparable against the data from South East Asia countries, i.e. Singapore, Malaysia, Philippine, Hongkong and Japan, but was higher than the Netherlands isolates (Table 4).

The high prevalence of ESBL producing bacteria in this hospital was suggested as an impact of the common usage of third generation cephalosporin, certainly cefotaxim in a daily practice of the therapy on infected patients. Pena *et al* showed that by routine preventive measure of infection control and restriction of oxyimino beta lactam use in Intensive Care Unit (ICU), was able to decrease the incidence rate of ESBL producing *Klebsiella pneumoniae*. During three years period of the intervention study, the carrier rate of ESBL producing *Klebsiella pneumoniae* 24.8% in the fist year, 24.4% during the second year (Pena et al. 1998).

Plasmid mediated beta lactamas ESBL that so far was documented only in Gram negative bacilli. It is commonly included in Class A beta lactamases of Bush classification. Over last 15 years, has been documented some outbreaks of infection with ESBL producing organisms (Peterson & Yu 1999; Samaha-Kfouy & Araj 2003; Spanu et al. 2002). In a survey of 369 American Clinical Microbi ology Laboratories, about 32% of *Enterobacteriaceae* tested were ESBL producer. A controversy exists as weather is an ESBL producing organisms which cephalosporin MICs are in the susceptible range (Emery & Weymouth 1997; Samaha-Kfouy & Araj 2003; Spanu et al. 2002). As many as 140 American isolates of ESBL producing *Klebsiella pneumoniae*, only 23% have been reported as cefotaxim resistant when examined using the NCCLS break points (Jacoby & Han 1996). The similar study in Europe showed 36% of the ESBL producing *Klebsiella spp* were cefotaxim resistant (Babini & Livermore 2000). The susceptibility of ESBL producing *Klebsiella pneumonia* were as follow (Paterson 2001): susceptible (S), intermediate (I) or resistant (R) to cefotetan 95.8%, 2.8% and 1.4%; for cefoxitin 80.6%, 9.7% and 9.7%; for cefepim 97.2%, 4.2%, and 16.7%; for cefotaxim 48.6%, 29.2%, and 22.2%; for ceftiraxone 36.1%, 32%, and 31.9%; for cefazidime 19.4%, 8.3% and 72.2% respectively. More than 90% of ESBL producing organisms were susceptible to cephampcin (cefoxitin and cefotetan) (Paterson 2001; Winokur et al. 2001; Xiong et al. 2002).
Extended-spectrum β-lactamases (ESBLs) are a group of plasmid-mediated enzymes with the ability to hydrolyze third-generation cephalosporins and monobactams. Most organisms harboring such enzymes remain susceptible to carbapenems, whereas the activity of ciprofloxacin, cefepime, and beta-lactam/beta-lactamase inhibitor combinations is variable. Infections caused by microorganisms producing ESBLs have become a serious problem for hospitals worldwide (Villegas et al. 2004).

In Latin America some prior data suggest that in many centers in different countries, about 8.5% of *Escherichia coli* and 45% *Klebsiella pneumoniae* have phenotypes consistent with ESBL production, whereas in the United States, only 3.3% of *E. coli* and 8% of *K. pneumoniae* have such a phenotype (Villegas et al. 2004).

This increasing use of broader-spectrum cephalosporins in the first half of the 1990 has become one of the major factors responsible for the high rate selection of extended-spectrum β-lactamases (ESBL)-producing microorganisms in Polish hospitals (Palucha et al. 1999). An ESBL variant may be selected de nova in a given hospital, or it may be introduced from another centre. Its further spread a within the hospital can be a consequence of plasmid transmission among non-related *Enterobacteriaceae* strains, or clonal dissemination of the enzyme-producing microorganism. Parallel outbreaks caused by ESBL-expressing strains, resulting from both plasmid transfer and clonal spread have also been reported. Often, more than one ESBL variant is present in the microflora of a given hospital environment. Persistence and outbreaks of ESBL producers have been convincingly correlated with intensive use of third-generation cephalosporins (Palucha et al. 1999).

Co-resistance of beta-lactam and non-beta-lactam antibiotics was common. Almost half (48.1%) of ESBL producing *K. pneumoniae* isolates tested displayed resistance against amikacin compared to 27% of ESBL producing *E. coli*. Trimethoprim/sulfamethoxazole was affected in both *E. coli* and *K. pneumoniae*, although resistance in *E. coli* was much higher, reaching 73%. There is a strikingly high resistance of ESBL-producing *E. coli* to ciprofloxacin (95.9%) whereas ESBL-producing *K. pneumoniae* remains largely susceptible (88.6%) (Villegas et al. 2004).

The resistance pattern of ESBL-producing bacteria was remarkable for the high rate of co-resistance to other classes of antibiotics. Approximately 60% of ESBL-producing *E. coli* was resistant to quinolones. The association of ESBL production and resistance to quinolones has been reported to be 40-60% in Europe and the United states, but mostly among ESBL-producing strains *K. pneumoniae*. The use of fluoroquinolones has been identified as a risk factor for the emergence of fluoroquinolone-resistance ESBL-producing strains. The resistance of ESBL-producing *K. pneumoniae* to amikacin almost reached 50%, whereas the susceptibility to cefepime was much better, over 80% (Villegas et al. 2004).

There has been a major shift in the etiology of hospital acquired infections during the 1980s in contrast to the 1970s, that is, an increase in the laboratory isolation of...
CNS, candida, S. aureus, enterococci, P. aeruginosa and Enterobacter between 1980 and 1986-1989. Taken as a whole, the shifts are away from more easily treated pathogens towards more resistant pathogens with fewer options for therapy (Dancer 2001). An additional study shows that if cephalosporin usage is reduced as part of an overall reduction in antimicrobial prescribing, there is a decrease in hospital-acquired infections, namely, enterococcal and selected Gram-negative bacteremia, and MRSA and S. maltophilia colonization or infection (Dancer 2001). It appears that cephalosporin usage selects for and encourages propagation of these organisms. It is even possible that the cephalosporin antibiotics play a role in the molecular initiation of resistance for some (Dancer 2001).

Multiply-resistant coliforms are associated with high-level usage of cephalosporin, particularly cefotaxime, ceftiraxone and ceftazidime. These antibiotics induce and select for ESBL coliforms (ESBLC). If cephalosporins are avoided, there is less chance of selecting these highly resistant bacteria, and coliform susceptibility rates rise. At a hospital in New York, multiply-resistant E. cloacae isolates from the intensive care unit increased dramatically between 1988 and 1990. As a response, use of ceftaxidime was severely restricted in favour of piperacillin in combination with aztreonam. Though the MIC rose from 105 to 107 cfu/mL, an effect attributed to clonal dissemination, the bacterial load may exceed such high inoculum levels, reaching colony counts of 109-1010 cfu/mL (Giamarellou 2005; Mathai 2002). The inoculum effect demonstrated by many ESBL-producing strains should also be of great concern. Medeiros and Crellin found that the MIC rose dramatically when the inoculum in the susceptibility tests was increased from 105 to 107 cfu/mL, an effect also demonstrated in animal models of intra-abdominal abscesses and infectious endocarditis. In addition, in many human infections, such as meningitis, endocarditis, septic arthritis, osteomyelitis and various abscesses, the bacterial load may even exceed such high inoculum levels, reaching colony counts of 109-1010 cfu/mL (Giamarellou 2005).

Some resistant coliforms merely colonize patients; other invade to cause infection. Yet other epidemic strains spread to cause outbreaks of virtually untreatable disease. The location of antibiotic resistance mechanisms on plasmids facilitates easy spread between species and genera, and is most likely to occur in the gastrointestinal tract (Dancer 2001).

Hospitals contain a concentrated reservoir of resistant coliforms but a dilute version exists in the community. The path between the hospital and the community runs both ways. Even patients with no prior hospital contact can display clinically significant infection with multi resistant coliforms. Critically ill patients in intensive care units rapidly acquire such organism for time to allow the patient to recover from initial pathology before succumbing to hospital acquired resistant microbes (Dancer 2001).

The steadily increasing prevalence of ESBL-producing Enterobacteriaceae worldwide has created a great need for accurate techniques of laboratory testing for identification (Giamarellou 2005; Mathai 2002). The double-disc approximation test and the broth dilution MIC reduction method are the easiest and most cost-effective for application by clinical laboratories.

According to the NCCLS guidelines, and as already mentioned, many ESBL-producing isolates are not always phenotypically resistant to oximinocephalosporins. However, patients suffering from infections caused by ESBL-producing organisms are at risk of treatment failure if an ESC is prescribed. Therefore, it is imperative for the clinical microbiology laboratory to identify isolates that possess increased MICs $\geq 2 \mu g/mL$ to oximino-cephalosporins, even though they may be equal to or below the susceptibility breakpoint (MIC $\leq 8 \mu g/mL$), and subsequently to implement one of the reported methods to detect ESBLs. On the other hand, in order to avoid misleading results for the clinician, and according to NCCLS recommendations, it is very important that any Klebsiella spp. or E. coli that is reported as being resistant to all cephalosporins, penicillins and aztreonam, regardless of the susceptibility test result (Giamarellou 2005; Mathai 2002).

The inactivation effect demonstrated by many ESBL-producing strains should also be of great concern. Infections caused by ESBL-producing E. coli or K. pneumoniae were associated with a significantly longer duration of hospital stay and greater hospital charger. ESBL-producing and K. pneumoniae infections have a significant impact on several important clinical outcomes, and efforts to control outbreaks of infection with similar strains should emphasise the judicious use of all antibiotics as well as barrier precaution to reduce their spread (Giamarellou 2005). Treatment options and outcomes for serious infections are caused by ESBL-producing organism. Therefore, appropriately organized prospective trials in centers with endemic ESBL-producing bacteria are urgently required (Giamarellou 2005; Kim et al. 2002). The epidemiological characteristics of ESBL outbreaks indicate that the problem may be attributed to clonal dissemination, antibiotic selective pressure on plasmid dissemination.
amongst distinct clones, or both (Bradford 2001; Giamarellou 2005).

Therefore, both isolation procedures (e.g., gloves, gowns) and application of hand hygiene, according to the recent CDC guidelines, as well as appropriate antibiotic utilization policies, should be considered. Reasons to control ESBL producers in particular include: the potential transfer of multiple antibiotic resistance as a result of genes found on large plasmids that may also carry resistance determinants to other classes of antibiotics, such as aminoglycosides and sulphonamides; their association with nosocomial outbreaks characterized by high morbidity and mortality rates; the need to limit outbreaks by controlling resistance. In addition, as we face the so-called ‘End of Antibiotics’ era, the application of various methods to control antimicrobial use, in an effort to reverse resistance, is of extreme importance. Selective removal, control or restriction and, recently, cycling of antibiotics, have been suggested. Antibiotic restriction was the most important in the clonal outbreak. Raymond et al. concluded that the implementation of a quarterly empirical antibiotic rotation schedule in an ICU was feasible, and was associated with significant reductions in the incidence of infection, antibiotic-resistant organism infection and infectious mortality without an increase in antibiotic cost (Giamarellou 2005).

CONCLUSION

Prevalence of ESBL producing *Escherichia coli* and *Klebsiella pneumoniae* from clinical specimen in Dr. Soetomo Teaching Hospital Surabaya were high 34.84% (115/330) and 35.35% (105/297) since January 2005 until April 2005. Antimicrobial resistance pattern of ESBL-producing *E. coli* and *Klebsiella pneumoniae* to third generation cephalosporin were high resistant, i.e.: cefotaxim (90.4%), ceftriaxon (95.7%), ceftazidime (55.7%); 81.9%, 94.3%, 87.8% respectively. The resistance pattern against ciprofloxacin have similar figure with thirds generation cephalosporin. The alternative sensitive drugs against ESBL-producing bacteria were meropenem and fosfomycin.

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