Molecular Characterization of Extended-Spectrum β-Lactamases-Producing Lebsiellapneumoniae Isolated from Clinical Specimens at a Tertiary-Referral Hospital in Denpasar, Bali, Indonesia

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Almost no study has been performed in Bali to characterize genotype of ESBL-producing K. pneumoniae. The aim of this study was to determine genotype of ESBL produced by K. pneumoniae isolated from clinical specimens in Sanglah General Hospital, Bali, Indonesia. Ninety-seven non-duplicative ESBL-producing K. pneumoniae isolates were examined for 16s rRNA, and then with Duplex PCR to detect blaSHV and blaCTX−M genes, and with Uniplex PCR for blaTEM gene. Half of the isolates (50.5%) had coexistences of blaTEM, blaSHV and blaCTX−M genes. Presence of two genes, blaTEM and blaSHV; blaTEM and blaCTX−M; and blaSHV and blaCTX−M were 12.4%; 15.5%; and 8.2%, respectively. This study showed the widespread of TEM-, SHV-, and CTX-M-type ESBLs in Bali. Prudent use of antibiotics can save our planet from multidrug resistance microorganisms (MDROs) threat.

Keywords: Extended Spectrum β-Lactamase, Klebsiella pneumoniae, PCR, Sanglah General Hospital Denpasar.

1. INTRODUCTION
Multidrug resistance microorganisms (MDROs) tend to increase worldwide recently. This condition is not only important for hospital setting, but also can put our planet in danger. The misuses of broad-spectrum antibiotics agents can promote higher prevalence of MDROs. One of MDROs, which is dangerous for critically ill patients in hospital, is Extended-spectrum β-lactamases (ESBL) produced by Enterobacteriaceae. Nowadays, ESBLs are more prevalent produced by Klebsiella pneumoniae. The isolates have been linked to healthcare associated infections (HAIs), which are usually difficult to be treated. ESBLs are β-lactamases that hydrolyze penicillins and oxynimo—cephalosporins, including monobactam, but are inhibited by β-lactamases inhibitor. These enzymes are resulted from point mutations of TEM or SHV genes, however, recently CTX-M type ESBLs have been reported to increase rapidly.

Several studies reported the CTX-M enzymes spread mostly in Asia, South America, Europe and Africa. Data of ESBL-producing K. pneumoniae prevalence in Indonesia is limited. Previous study reported that CTX-M-15 type ESBL was the most prevalent ESBL produced by K. pneumoniae clinical isolates from Surabaya, Indonesia. The prevalences of ESBL-producing K. pneumoniae at the tertiary-referral hospital, Sanglah General Hospital, in Bali have shown increasing based on phenotypic test. The prevalence was about as high as 35% of all K. pneumoniae isolated in January-June 2013 (unpublished data); however, almost no study has been conducted in Bali to determine molecular characterization of ESBL-producing K. pneumoniae. Therefore, the aim of this study is to investigate molecular characterization of clinical isolates of ESBL-producing K. pneumoniae at Sanglah General Hospital, Denpasar, Bali Indonesia.

2. EXPERIMENTAL DETAILS
This study has obtained Ethical Clearance Approval from Ethic Commission, Research and Development Unit of Faculty of Medicine/Sanglah General Hospital, Denpasar, Bali, Indonesia.

2.1. Bacterial Isolates and Phenotypic Characterization
Ninety-seven non-duplicative clinical isolates of K. pneumoniae were included in this study. ESBL producing K. pneumoniae were
isolated from blood (36 isolates), urine (28 isolates), pus/wound (18) sputum (10 isolates) and CSF (5 isolates). Phenotype of ESBL isolates was determined by using Disk Diffusion Synergy Test (DDST) as describe previously with slight modification. The distance between Amoxicillin-Clavulanic Acid (AMC) disk to Cefotaxime (CTX), Ceftazidime (CAZ), Cefepime (FEP), and Aztreonam (ATM) disks was 25 mm center-to-center. 

K.pneumoniae ATCC BAA-1706 was used as the negative control isolate in this study.

2.2. PCR Amplification for 16s rRNA, Uniplex PCR for blaTEM, and Duplex PCR for blaSHV and blaCTX-M Amplification

Bacterial genomic DNA was isolated from colonies by using Roche High Pure PCR Template Isolation Kit (Roche Life Science, Indianapolis, USA) based on manufacturer’s instruction. DNA was eluted with 50 μl of elution buffer. PCR was conducted using Go Taq® Green Master Mix (Promega, Madison, USA). Uniplex PCR to detect 16s rRNA gene was performed. It was followed by uniplex PCR to detect blaTEM gene and duplex PCR to detect blaSHV and blaCTX-M. Lists of primer sets used in this study were shown in Table I.

Protocols of PCR were described in previous study with modification for duplex PCR.5 PCR cycle was initiated with pre denaturation at 95 °C for 5 min; 35 cycles of denaturation at 94 °C for 1 min, primer annealing at 55 °C for 30 sec and extension at 72 °C for 1 min; and final extension at 72 °C for 4 min (Cy-cler, Biorad thermal cycler). Duplex PCR was performed with two primer sets of blaSHV (0.2 mM final concentration of each primer) and blaCTX-M (0.3 mM final concentration). Amplicons were electrophoresed in 0.8% agarose gel in TBE buffer, at 60 volt for 35 min. DNA was visualized with ethidium bromide and captured (Gel Doc, Biorad).

3. RESULTS AND DISCUSSION

All 97 clinical isolates were positive tested for the presence of 16s RNA gene (Fig. 1(a)) and all isolates were K. pneumoniae. Gene of blaTEM was detected using Uniplex-PCR, while blaSHV and blaCTX-M genes by Duplex-PCR (Figs. 1(b) and 1(c)).

Amplicons were electrophoresed in 0.8% agarose gel and visualized with ethidium bromide. (M = marker, 100 bp DNA Ladder, Invitrogen). It was found that most of isolates had coexistences of more than one gene. As shown in Table II, 49 (50.5%) ESBL-producing K. pneumoniae were positive harboring blaTEM, blaSHV, and blaCTX-M. Coexistences of two genes were also observed in this study. Twelve (12.4%) of isolates had blaTEM and blaSHV, 14 (15.5%) had blaTEM and blaCTX-M and 8 (8.2%) had blaSHV and blaCTX-M.

This study showed that K. pneumoniae that produce ESBL mostly possessed multiple genes, which were also reported in previous study conducted by Goyal et al. This group found that 57.1% of ESBL-producing K. pneumoniae had multiple genes of ESBL. Several studies also showed that K. pneumoniae isolates had more than one type of ESBLs, although it was not as high as that of this study. Higher prevalence of ESBLs detected in this study might be caused by misuse of antibiotics, or better screening method of MDROs in the hospital laboratory. Severin et al. reported that most of ESBL type found in Surabaya is CTX-M ESBL. Other studies in Singapore, Vietnam and Thailand also showed prominent spreading of CTX-M ESBL type. In this study, CTX-M type prevalence was also found to be high but still SHV type was predominantly found among the isolates, suggesting there might be spreading of both SHV and CTX-M in Denpasar, Bali.

4. CONCLUSION

This study showed ESBL produced by K. pneumonia isolated from clinical specimens in Bali are mostly encoded by blaTEM, blaSHV, and blaCTX-M. The molecular characterization of these types ESBL is noteworthy for clinical microbiologist to perform early and prompt laboratory detection, and also to plan infection prevention and control program in preventing spread of multidrug resistance organisms. It can act as an alarm for the clinicians in management of patients with serious K. pneumoniae infections, especially to use antibiotics prudently. At the end, we can save our planet from spreading of multidrug resistant microorganism (MDROs). Further investigation of molecular epidemiology of hospital- or community-ESBLs with bigger number of samples and involving several health care enter would be promising to get good database for ESBLs-producing microorganisms in Bali.
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References and Notes

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