Multiple ambler class A ESBL genes among *Klebsiella pneumoniae* isolates in a Malaysian district hospital

Mohd Helmi, U.[1,2], Mohd Desa, M.N.[3], Taib, N.M.[1], Tengku Jamaluddin, T.Z.M.[1] and Masri, S.N.[1*]
[1]Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia
[2]Department of Pathology, Sultanah Fatimah Specialist Hospital, Johor, Malaysia
[3]Department of Biomedical Science, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Selangor, Malaysia
*Corresponding author e-mail: sitinorbaya@upm.edu.my
Received 15 May 2015; received in revised form 17 June 2015; accepted 19 June 2015

Abstract. Detailed reports regarding the distribution and activity of extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* isolates are currently not widely available in the Malaysian setting. This study was conducted to determine the ESBL genes distribution rate, phenotypic detection, and antimicrobial susceptibility patterns among beta-lactam resistant *Klebsiella pneumoniae* isolated from a Malaysian district hospital.

   K. pneumoniae isolates were collected from a microbiology laboratory at Hospital Pakar Sultanah Fatimah, Malaysia. Following exclusion and inclusion criteria, 141 isolates were selected for this study. *K. pneumoniae* was identified by phenotypic method, whilst antibiotics' susceptibility patterns were determined by the Kirby-Bauer method, as described in Clinical Laboratory Standard Institute (CLSI) guidelines (Oxoid, UK; Becton-Dickenson, USA). Detection of Ambler Group A ESBL gene (bla_{SHV}, bla_{TEM}, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-8}$, $bla_{CTX-M-9}$, and $bla_{CTX-M-25}$) was done using polymerase chain reaction (PCR).

   ESBL genes were found in 85.8% of *K. pneumoniae* (121 of 141) isolates. Only bla_{SHV}, bla_{TEM}, $bla_{CTX-M-1}$, and $bla_{CTX-M-9}$ were detected among *K. pneumoniae* isolates with distribution rates of 75.2% (106 of 141), 41.1% (58 of 141), 44% (62 of 141), and 0.7% (1 of 141), respectively. There was no $bla_{CTX-M-2}$, $bla_{CTX-M-8}$, or $bla_{CTX-M-25}$ detected from any isolates in this study. Sequencing of representative amplicons revealed bla_{SHV} as SHV-12, bla_{TEM} as TEM-1, $bla_{CTX-M-1}$ as CTX-M-15, and $bla_{CTX-M-9}$ as CTX-M-18. The phenotypic detection rate of ESBL was 71.6% (101 of 141), whilst 9.2% (13 of 141) were positive for carbapenemase. AmpC beta-lactamase was detected in 22% (31 of 141) of all isolates. Antibiotic resistance was between 44.6% (netilmicin) and 97.2% (cefotaxime).

   Based on ESBL genes distribution, blaSHV was a predominant gene found in one of Malaysian district hospitals, notwithstanding having bla_{TEM}, $bla_{CTX-M-1}$, and $bla_{CTX-M-9}$. Despite carrying multiple ESBL genes, some strains were positive for carbapenemase or AmpC beta-lactamase, which resulted in high antimicrobial resistance rates.

INTRODUCTION

Extended Spectrum Beta-Lactamase (ESBL) is produced by a wide range of bacteria classes, mostly *Enterobacteriaceae* such as *Klebsiella pneumoniae*. The enzyme is either chromosomal or plasmid-mediated (Bradford, 2001). ESBL is one of the antibiotic resistance determinants commonly encountered in clinical settings that can contribute to resistance or decreased sensitivity toward several antimicrobial classes. Previously, these enzymes were categorized based on the spectrum of their antimicrobial substrate profile, enzyme inhibition profile, hydrolysis rate, binding affinity, isoelectric focusing, protein molecular weight, and amino acid composition (Paterson & Bonomo, 2005). With the advancement of molecular

techniques, classification of ESBL can be further elaborated by the nucleotide and amino acid sequences encoding the enzymes (Bush & Jacoby, 2010; Bush, 1995). To date, more than 1300 distinct beta-lactamases have been reported worldwide, and the number is increasing (Bush, 2013). Pertinent to current practice in most clinical microbiology laboratories, only ESBL groups 2be, 2f, and group 3 are screened and confirmed phenotypically by established methods (CLSI, 2011). These groups are among the ESBL enzymes that are inhibited by clavulanic acid, which is used in combination with ceftazidime or cefotaxime during ESBL confirmation tests.

AmpC beta-lactamase and carbapenamase, can reduce the efficiency of the ESBL detection efforts (Tsui *et al.*, 2012). Neither of the enzymes is inhibited by clavulanic acid, and they can be simultaneously produced together with ESBL group 2be enzymes (Bush and Jacoby, 2010). Adding to the challenge in ESBL detection is the potential false-negative result, due to the masking or inoculum effects (Wiegand *et al.*, 2007, Queenan *et al.*, 2004). Furthermore, most ESBL producers carry multiple ESBL enzymes simultaneously, which shows panresistant antibiotic patterns, resulting in limited treatment options (Delgado-Valverde *et al.*, 2013; Cabral *et al.*, 2012).

In the Monitoring Antimicrobial Resistance Trends Program (SMART)'s study, Hawser *et al.* (2009) reported a high rate of ESBL-producing organisms, based on data collected from Thailand, Singapore, the Philippines, and Vietnam. The ESBL-producing organisms comprised *Escherichia coli*, *Klebsiella pneumoniae*, and *Klebsiella oxytoca* with isolation rates ranging between 4.4% and 48.2%. An increasing pattern was also recorded from both health care facilities and community settings in Asia-Pacific countries. In a long-term study in Japan, steady increments of ESBL detection rates were reported to increase by 20% between 2003 and 2009 (Chong *et al.*, 2010). ESBL genes were detected in *E. coli*, *K. pneumoniae*, and *Proteus mirabilis*

isolated from health-care associated infections, and these bacteria carried multiple beta-lactamase genes simultaneously. A high prevalence was also observed in North India, where 52.3% of *K. pneumoniae* and 46.4% of *E. coli* isolates were ESBL-positive, as reported by Kaur and Aggarwal (2013).

In year 2000, 16 ceftazidime-resistant *K. pneumoniae* isolated from a pediatric oncology ward in a Malaysian tertiary hospital showed a positive ESBL phenotype (Ariffin *et al.*, 2000). However, sporadic cases from various tertiary hospitals in Malaysia showed classical patterns of ESBL genotype distribution, where SHV-type beta-lactamase is still a predominant gene produced by *K. pneumoniae* (Lim *et al.*, 2009). Nevertheless, there is still limited information regarding the distribution of ESBL genes throughout the Malaysian population, especially involving Malaysian district hospitals. Crucial information about ESBL genes' distribution and their phenotypic activities in Malaysian district hospitals must be documented to get a better understanding of their characteristics. Such information will help to detect current antibacterial resistance or emerging resistance, which will reveal its dissemination pattern, assist in antibiotic stewardship, and control its outbreak (Okeke *et al.*, 2011).

MATERIALS AND METHODS

Study Design
A cross-sectional descriptive study was carried out. Samples were taken from the Medical Microbiology Laboratory of Hospital Pakar Sultanah Fatimah (HPSF). Only one isolate per patient was accepted into this study. This 550-bed district hospital is governed by the Ministry of Health, Malaysia. It is located in the Muar district of the Johor state, which serves a population of 437,000 people (2007). This study was approved by the Ministry of Health, Malaysia (MOH) under the guidance of the Medical Research and Ethics Committee (NMRR-12-1111-13791).

Study Population and Sample Collection

One hundred and forty one *Klebsiella pneumoniae* non-duplicate samples that showed resistance to monobactam, 3rd generation cephalosporin (including cefotaxime, ceftazidime, cefoperazone and ceftriaxone) and cefepime were subjected to this study. Samples were collected between the years 2009 and 2012 and originated from inpatients of multi-disciplinary wards. All isolates were preserved and transferred to the Medical Microbiology and Parasitology Department at the Universiti Putra Malaysia for further investigation.

Identification of Isolates

All isolates were morphologically re-identified using a commercially available biochemical test, RapID™ ONE from Remel (Kansas City, USA). Identification of the isolate was performed according to the manufacturer's instructions.

Antibiotic Susceptibility Testing

Susceptibility testing was performed using the Kirby-Bauer method and followed guidelines set by the Clinical and Laboratory Standards Institute (CLSI, 2011). All *K. pneumoniae* isolates were tested against amoxicillin/clavulanic acid (20/10 µg), ampicillin/sulbactam (10/10 µg), cefoperazone/sulbactam (75/30 µg), piperacillin/tazobactam (100/10 µg), piperacillin (100 µg), aztreonam (30 µg), cefuroxime (30 µg), ceftriaxone (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), cefoperazone (75 µg), cefepime (30 µg), ertapenem (10 µg), meropenem (10µg), imipenem (10 µg), gentamicin (10 µg), netilmicin (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg) (Thermo-Fisher Scientific, UK), cefotaxime/clavulanic acid (30/10 ug) (Becton-Dickinson, USA), ceftazidime/clavulanic acid (Becton-Dickinson, USA), cefepime/clavulanic acid (Mast Group Limited, UK). *Escherichia coli* ATCC® 25922 was used as the control strain. The size of the inhibition zone was measured and compared to CLSI interpretive criteria as susceptible (S), intermediate (I), or resistant (R)(CLSI, 2011).

Phenotypic Detection of ESBL, Carbapenemase, and AmpC Beta-Lactamase

Screening and confirmation of ESBL and carbapenemase were performed as described in CLSI guidelines. Since the affinity of ESBLs enzymes for different substrates is variable, ceftriaxone, ceftazidime, cefotaxime, and aztreonam were used to increase the sensitivity of ESBL detection. ESBL production was confirmed by disc diffusion test of ceftazidime with/without clavulanic acid and/or cefotaxime with/without clavulanic acid. Ertapenem or meropenem disc was used to screen carbapenemase production whilst Modified-Hodge Test (MHT) served as a confirmation test. The phenotypic test for detecting AmpC Beta-Lactamase production was preceded with AmpC detection from Halstead *et al.* (2012), Tan *et al.* (2009), and ESBL detection from CLSI (2011).

DNA Extraction and Purification

An overnight culture grown on MacConkey media (Oxoid, UK) was used for DNA extraction using a GeneAll® Exgene™ kit. The DNA purification procedure was completed using the GeneAll® Expin™ PCR SV Protocol Handbook (GeneAll Biotechnology Co. Ltd, Seoul, South Korea).

PCR Detection of ESBL Genes (bla_{SHV}, bla_{TEM} and bla_{CTX-M})

The presence of Group A ESBL genes consisting of bla_{SHV}, bla_{TEM}, $bla_{CTX-M-1}$, $bla_{CTX-M-2}$, $bla_{CTX-M-8}$, $bla_{CTX-M-9}$, and $bla_{CTX-M-25}$ were detected by using the primers, as published by Garrec *et al.* (2011) and Woodford *et al.* (2005).

A total volume of 25 µL containing 0.5 µM for each primer, 30 µM for each dNTP, 1.5 mM $MgCl_2$, 1 U *Taq* DNA polymerase, and 1-20 ng DNA template were used in the PCR reaction. *Klebsiella pneumoniae* ATCC 700063 was used as a positive control strain. All PCR amplifications were executed using a thermal cycler (BioRad MyCycler™ Thermalcycler, USA). Initial denaturation was at 94°C for five minutes, followed by 30 cycles of denaturation at 94°C for 25 seconds, annealing at specific temperatures for 40

seconds (Table 1), and an extension at 72°C for 50 seconds. An additional extension was set for six minutes at 72°C. Four µL of amplicon of each PCR product was electrophoresed on a 1.6% agarose gel for 50 minutes at 90 V, visualized, and photographed under UV illumination with a 100-bp DNA ladder as a molecular weight marker. Representatives of each gene with specific amplicon size were purified and sequenced at First Base Laboratories (Seri Kembangan, Malaysia). Sequencing results were matched in the GenBank database for gene confirmation. A homology search was performed using the Basic Local Alignment Search Tool (BLAST) (http://www.ncbi.nlm.nih.gov/blast).

Data Interpretation

Data analyses, including descriptive statistics, antimicrobial susceptibility patterns, and other variables, were accomplished with the SPSS program (IBM, SPSS, New York USA). The tabulation of variables was analyzed using the Chi-square test of independence and Fisher's exact test. A p value of < 0.050 was considered statistically significant.

RESULTS AND DISCUSSION

PCR detection of seven ESBL genes revealed nearly all isolates (85.8%) carried ESBL gene/genes that are blaSHV, blaTEM, blaCTX-M-1 and blaCTX-M-9. Distribution of the ESBL gene comprised 106 isolates positive for bla_{SHV}, (75.2%), 62 isolates for $bla_{CTX-M-1}$ (44.0%), 58 isolates for bla_{TEM} (41.1%), and one isolate for $bla_{CTX-M-9}$ (0.7%)(Figure 1). Most *K. pneumoniae* is known to have a chromosomally encoded bla_{SHV} gene (Bradford, 2001). This was concurrent with Lim *et al's* previous report noting that 90.2% of Malaysian *K. pneumoniae* isolates were positive for the bla_{SHV} gene. Furthermore, this ESBL gene was also detected in outbreaks in a Malaysian pediatric ward (Lim *et al.*, 2009; Palasubramaniam *et al.*, 2005). Sequencing analyses of bla_{SHV} amplicons revealed that this gene belongs to SHV-12, which was commonly found among *K. pneumoniae* isolated from a few major hospitals in Malaysia (Lim *et al.*, 2009). As one of the emerging ESBL genes reported worldwide, 62 isolates (42%) were detected carrying the bla_{CTX-M} gene (Bonnet, 2004). This is higher than that noted in a previous study, where

Table 1. Oligonucleotide primers used in this study

| Primer	Primer Sequence (5' – 3')		Reference
bla_{TEM}	Forward	GAGTATTCAACATTTCCGTGTC	Garrec *et al.* (2011)
	Reverse	TAATCAGTGAGGCACCTATCTC	
bla_{SHV}	Forward	ATGCGTTATATTCGCCTGTGTATT	Garrec *et al.* (2011)
	Reverse	GCGTTGCCAGTGCTCGATCAGCGC	
$bla_{CTX-M-1}$	Forward	GGTTAAAAAATCACTGCGTC	Garrec *et al.* (2011)
	Reverse	TTGGTGACGATTTTAGCCGC	
$bla_{CTX-M-2}$	Forward	ATGATGACTCAGAGCATTCG	Garrec *et al.* (2011)
	Reverse	TGGGTTACGATTTTGCGCCGC	
$bla_{CTX-M-8}$	Forward	TCGCGTTAAGCGGATGATGC	Woodford *et al.* (2005)
	Reverse	AACCCACGATGTGGGTAGC	
$bla_{CTX-M-9}$	Forward	ATGGTGACAAAGAGAGTGCA	Garrec *et al.* (2011)
	Reverse	CCCTTCGGCGATGATTCTC	
$bla_{CTX-M-25}$	Forward	ATGATGAGAAAAAGCGTAAG	Garrec *et al.* (2011)
	Reverse	ATAACCGTCGGTGACAATTC	
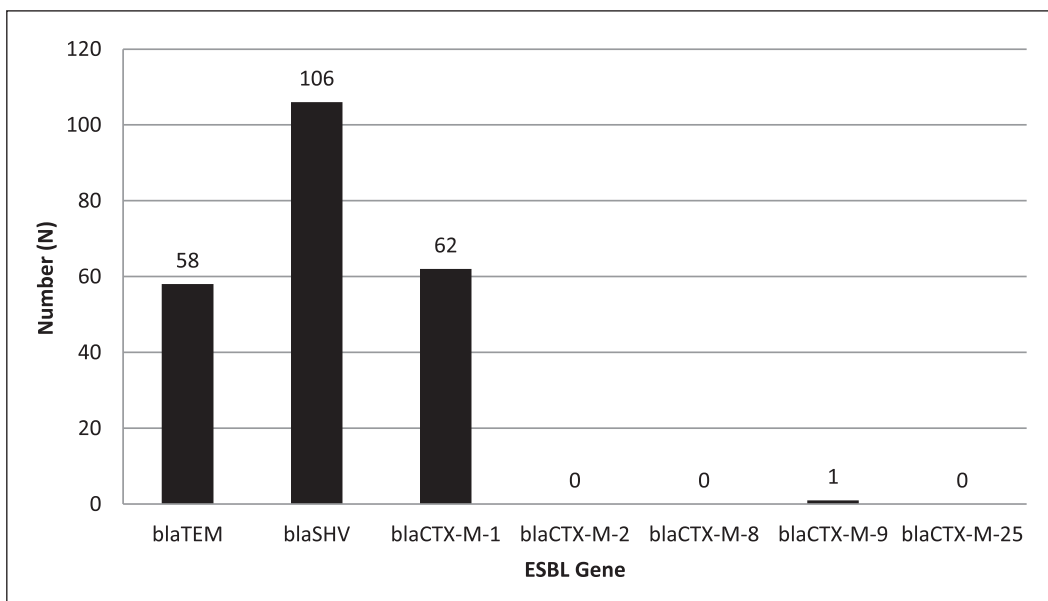
Figure 1. PCR detection of blaSHV, blaTEM, blaCTX-M-1, blaCTX-M-2, blaCTX-M-8, blaCTX-M-9, and blaCTX-M-25.

Lim *et al.* (2009) recorded a rate of 37.3% of bla_{CTX-M} gene. Therefore, this study reflects a significant distribution rate of this ESBL gene among *K. pneumoniae* isolated from a Malaysian district hospital.

Homology analyses derived from DNA sequencing revealed that bla_{CTX-M} cluster were $bla_{CTX-M-1}$ and $bla_{CTX-M-9}$. Markedly, only one isolate carried $bla_{CTX-M-9}$, a result not reported in previous studies. Figures 2 and 3 show representative images of gel-electrophoresed results of amplified ESBL genes. All amplified and purified products were confirmed by DNA sequencing. Homology analyses of ESBL genes showed these genes belong to beta-lactamase SHV-12, TEM-1, CTX-M-15, and CTX-M-18, respectively.

As shown in Table 3, phenotypic detection revealed that 101 isolates (71.6%) positive in the ESBL confirmatory test, whereas 13 isolates were positive in the Modified Hodge Test (9.2%). In addition, 31 isolates (22%) were positive for AmpC beta-lactamase. Among the 141 isolates, 20 were positive for ESBL gene/genes but negative for enzyme detection. In this circumstance, ESBL enzyme might be either sub-expressed or masked by other resistance determinants, resulting in a detection failure. The most compelling evidence in this study is that about 61.0% of total isolates carried more than one ESBL gene. This finding is in concordance with Lim *et al.* (2009) and a few studies from neighboring countries, including the Philippines, Thailand, and Indonesia (Kanamori *et al.*, 2011; Severin *et al.*, 2010; Kiratisin *et al.*, 2008). Phenotypic detection of ESBL, carbapenemase, and AmpC beta-lactamase revealed that *K. pneumoniae* isolates carry multiple resistant genes, which in this study showed up to 22% of 141 isolates positive for at least two of the phenotypic tests. However, as the molecular characterization of carbapenemase and AmpC beta-lactamase was not performed, we could not confirm the presence of any potential resistance genes.

Chi square analyses among ESBL phenotypic-genotypic positive isolates showed potential significant associations in relation to bla_{SHV}, bla_{TEM}, and bla_{CTX-M} ($p=0.002$, $p=0.003$ and $p<0.000$, respectively). These results show a statistically significant relationship ($p < 0.050$) between these detection methods among the beta-lactam
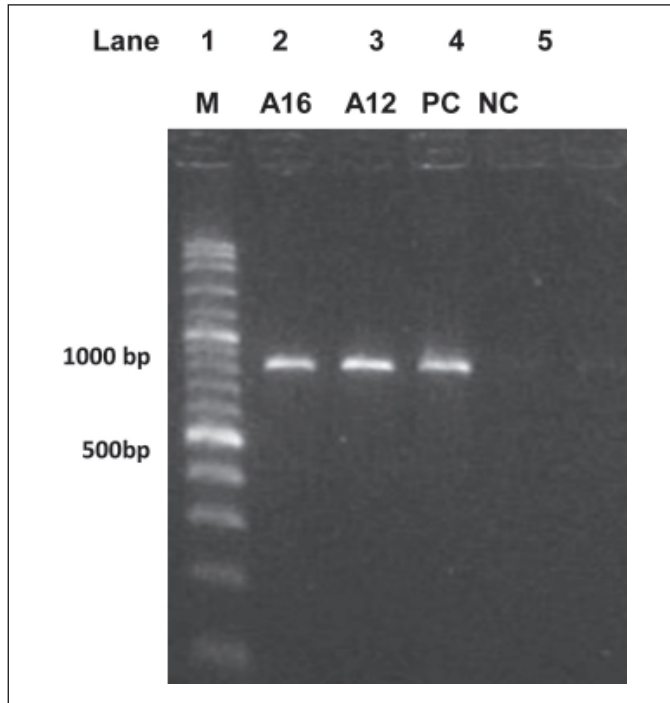
Figure 2. Presence of an ESBL gene by amplification of 848 bp bla$_{TEM}$ gene. The first lane is the Molecular Weight Marker (M) for 100 bp. PC = Postive control. NC = Negative control.
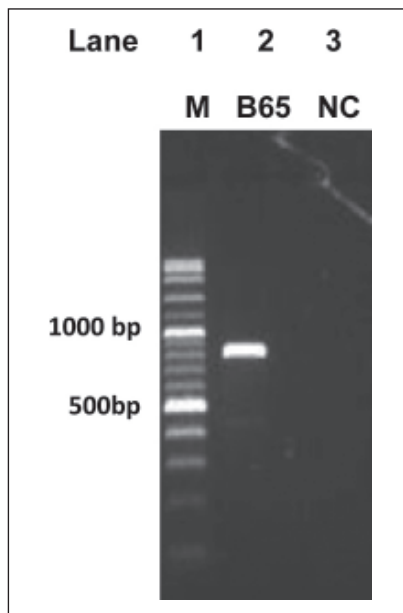

Figure 3. Presence of an ESBL gene by amplification of 870 bp bla$_{CTX-M-9}$ gene. The first lane is a Molecular Weight Marker (M) for 100 bp. NC = Negative control.

Table 3. ESBL gene distribution and phenotypic detection of ESBL, carbapenemase and AmpC beta-lactamase among *Klebsiella pneumoniae* isolates from Hospital Pakar Sultanah Fatimah, Muar, Johor, Malaysia

Phenotype Detection	Gene Detection									Total
	No Gene TEM/SHV/C1/ C2/C8/C9/C25	TEM	SHV	C1	TEM/ SHV	TEM/ C1	SHV/ C1	TEM/ SHV/ C1	TEM/ SHV/ C9	
No ESBL/AmpC/ Carbapenemase Phenotype	8	0	13	0	2	3	3	1	0	30
ESBL	7	1	13	3	3	4	17	24	1	73
AmpC	3	1	1	1	1	0	0	0	0	7
ESBL/ Carbapenamase	0	0	0	0	0	1	1	5	0	7
ESBL/AmpC	1	0	1	0	0	1	9	6	0	18
Carbapenemase/ AmpC	1	0	1	0	1	0	0	0	0	3
ESBL/AmpC/ Carbapenemase	0	0	0	0	0	0	0	3	0	3
Total	20	2	29	4	7	9	30	39	1	141

Notes: C1: CTX-M-1; C2: CTX-M-2; C8: CTX-M-8; C9: CTX-M-9; C25: CTX-M-25

resistant *K. pneumoniae*. Further *Chi* square analyses on sample type, gender, disciplines/ departments, and age group showed no significant relationship between the variables and ESBL gene detection, p> 0.05 (data not shown).

Susceptibility patterns of 20 anti-microbials against *Klebsiella pneumoniae* are shown in Table 4. Resistance to beta-lactam/beta-lactamase ranged from 84.4% (cefoperazone/sulbactam), to 87.2% (ampicillin/sulbactam), 91.5% (amoxicillin/ clavulanic acid), and 94.3% (piperacillin/ tazobactam). Resistance to cephalosporin groups was 80.1% (cefepime) and 97.2% (cefotaxime). In this study, 97.9% of isolates were resistant to piperacillin, while 81.6% of isolates were resistant to aztreonam. *K. pneumoniae* showed resistance towards aminoglycosides group between 44.7% (netilmicin) and 80.1% (amikacin). As for the carbapenem group, 59.6% of total isolates were resistant to ertapenem, 73.8% to meropenem, and 85.1% to

imipenem. Ciprofloxacin and trimethoprim/ sulfamethoxazole, which are commonly used in clinical settings, showed 78% and 76.7% resistance, respectively.

All of the antibiotics tested in this study were clinically available in Malaysian hospitals, but some of their resistance have not been reported in previous studies. Resistance rates against the antibiotics are high compared to previous studies and the 2013 Malaysian National Surveillance on Antibiotic Resistance report (http://www. imr.gov.my/report/nsar.htm) (Lim *et al.*, 2009; Loh *et al.*, 2007). The beta-lactam/ beta-lactamase inhibitor combination (amoxicillin/clavulanic acid, ampicillin/ sulbactam, cefoperazone /sulbactam and piperacillin/tazobactam) resistance rate in this study was higher (91.5%, 87.2%, 84.4%, and 94.3%, respectively) than those reported in 2003–2004 by a tertiary hospital (Loh *et al.*, 2007). Some of these antibiotics were not included in the study by Lim *et al.* (2009), except amoxicillin/clavulanic acid, which

Table 4. Antimicrobial susceptibility patterns of 20 antibiotics tested on *Klebsiella pneumoniae* isolates

Antibiotic	Number of Isolates, (%)	
	Susceptible [n (%)]	Resistance [n (%)]
Amoxicillin/clavulanic acid	12, (8.5%)	129, (91.5%)
Ampicillin/sulbactam	18, (12.8%)	123, (87.2%)
Cefoperazone/sulbactam	22, (15.6%)	119, (84.4%)
Piperacillin/tazobactam	8, (5.7%)	133, (94.3%)
Piperacillin	3, (2.1%)	138, (97.9%)
Aztreonam	26, (18.4%)	115, (81.6%)
Cefuroxime	15, (10.6%)	126, (89.4%)
Ceftriaxone	12, (8.5%)	129, (91.5%)
Ceftazidime	16, (11.3%)	125, (88.7%)
Cefoperazone	5, (3.5%)	136, (96.5%)
Cefotaxime	4, (2.8%)	137, (97.2%)
Cefepime	28, (19.9%)	113, (80.1%)
Ertapenem	57, (40.4%)	84, (59.6%)
Meropenem	37, (26.2%)	104, (73.8%)
Imipenem	21, (14.9%)	120, (85.1%)
Gentamicin	45, (31.9%)	96, (68.1%)
Amikacin	28, (19.9%)	113, (80.1%)
Netilmicin	78, (55.3%)	63, (44.7%)
Ciprofloxacin	31, (22.0%)	110, (78.0%)
Trimethoprim/sulfamethoxazole	33, (23.4%)	108, (76.6%)

showed a resistance rate of 10%. For the same antibiotics, resistance rates were recorded between 19.2%, 29.2%, 10.7%, and 8.6%, respectively in the 2013 Malaysian National Surveillance on Antibiotic Resistance report.

Elsewhere, data from the Study for Monitoring Antimicrobial Resistance Trends (SMART) showed that ESBL-producing *K. pneumoniae* have 94% and 43% resistance rates for ampicillin/sulbactam and piperacillin/tazobactam, respectively (Hawser *et al.*, 2009). These resistance rates were literally in concordance with this study. High resistance can be due to other resistance determinants that have resistance to beta-lactamase inhibitors, for instance inhibitor-resistance TEM-type ESBL (IRT-ESBL) (Bradford, 2001). This beta-lactamase group is resistant to clavulanic acid, which is used in ESBL confirmatory testing.

High resistance rates were also observed in the cephalosporins group, including cefuroxime, ceftriaxone, ceftazidime, cefoperazone, cefotaxime, and cefepime. Resistance rates for this antibiotic group were 89.4%, 91.5%, 88.7%, 96.5%, 97.2%, and 80.1%, respectively. These rates were drastically higher when compared to Lim *et al.* (2009), Loh *et al.* (2007), or national data, which were below 42%. This can be explained by the inclusion criteria applied in this study, which called only for *K. pneumoniae* isolates that have resistance to any beta-lactam antibiotic to be used as subjects. The resistance rate for trimethoprim/sulfamethoxazole in this study was 76.6%, which was higher compared to the national rate (28.7%). On the contrary, ESBL-producing *K. pneumoniae* isolated from Surabaya, a city in a neighboring country, showed a slightly higher rate (81.9%) than this study (Kiratisin *et al.*, 2008).

Carbapenems groups, which are one of the options in treating ESBL infections, also showed low efficiency, with 59.6% (ertapenem), 73.8% (meropenem), and 85.1%

(imipenem). A Modified Hodge Test revealed 13 isolates (9.2%) as potential carbapenemase producers. On the contrary, there was no carbapenems resistance reported by Hawser *et al.* (2009) and Lim *et al.* (2009). Even so, national data revealed the emergence of carbapenem resistance, which was reported at 1.7% and 1.5% for meropenem and imipenem, respectively. The discrepancy is between 9.2% positive-carbapenemase isolates compared to the high resistance rate to carbapenems (60%-85%). This circumstance urges confirmation by determining their minimum inhibitory concentration and further molecular investigation on carbapenems-related genes.

An alternative agent for combating ESBL is the aminoglycosides group, which in this study showed 44.7%, 68.1%, and 80.1% resistance to netilmicin, gentamicin, and amikacin, respectively. Notably, the aminoglycosides resistance rate in this study was considerably higher than that of the national level, which ranged between 3% and 15.3%. A good agreement was shown in comparing data in this study and the data reported by Severin *et al.* (2010), which showed a 72.2% overall aminoglycosides resistance rate.

In conclusion, the *K. pneumoniae* isolates within the clinical environment of this Malaysian district hospital harbored considerably high numbers of ESBL gene/ genes. These isolates were characterized by high antimicrobial resistance with high potential for carrying carbapenemase or AmpC beta-lactamase. Therefore, further investigation of carbapenemase or AmpC beta-lactamase gene distribution should be explored, especially among multi-drug resistant *Enterobacteriaceae*. Equally important, high carbapenems and colistin resistance should be further clarified by minimal inhibitory concentrations. This finding warrants a wider coverage of study throughout Malaysian hospitals to determine the epidemiological status of the ESBL genes' distribution in Malaysia.

Conflict of Interest

All authors report no conflicts of interest relevant to this article.

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