Detection of bla\textsubscript{IMP4} and bla\textsubscript{NDM1} harboring Klebsiella pneumoniae isolates in a university hospital in Malaysia

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Background: Antibiotic resistance among Enterobacteriaceae posts a great challenge to the health care service. The emergence of carbapenem-resistant Klebsiella pneumoniae (CRKP) is attracting significant attention due to its rapid and global dissemination. The infection is associated with significant morbidity and mortality, thus creating challenges for infection control and managing teams to curb the infection. In Southeast Asia, there have been limited reports and subsequent research regarding CRKP infections. Thus, the study was conducted to characterize CRKP that has been isolated in our setting.

Methods: A total of 321 K. pneumoniae were included in the study. Each isolate went through an identification process using an automated identification system. Phenotypic characterization was determined using disk diffusion, modified Hodge test, Epsilometer test, and inhibitor combined disk test. Further detection of carbapenemase genes was carried out using polymerase chain reaction and confirmed by gene sequence analysis.

Results: All together, 13 isolates (4.05%) were CRKP and the majority of them were resistant to tested antibiotics except colistin and tigercycline. Among seven different carbapenemase genes studied (bla\textsubscript{KPC}, bla\textsubscript{IMP}, bla\textsubscript{SME}, bla\textsubscript{NDM}, bla\textsubscript{IMI}, bla\textsubscript{VIM}, and bla\textsubscript{OXA}), only two, bla\textsubscript{IMP4} (1.87%) and bla\textsubscript{NDM1} (2.18%), were detected in our setting.

Conclusion: Evidence suggests that the prevalence of CRKP in our setting is low, and knowledge of Carbapenem-resistant Enterobacteriaceae and CRKP has improved and become available among clinicians.

Keywords: carbapenem-resistant Enterobacteriaceae; carbapenemase; Klebsiella pneumoniae; Modified Hodge test

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attempted to determine the prevalence of the CRKP and its genotype among clinical isolates of *K. pneumoniae* in our hospital. Subsequently, knowledge of CRE and CRKP has improved and become available among clinicians.

### Methods

#### Study design and setting

This cross-sectional, descriptive study was conducted between April 2010 and June 2012 in Hospital Universiti Sains Malaysia (Hospital USM), Kelantan, Malaysia. Hospital USM is a tertiary teaching hospital as well as the referral hospital for the eastern coastal region of Peninsular Malaysia. The hospital has 700 beds and 28 wards, including medical, surgical, pediatric, and orthopedic wards. It also has general, surgical, and two neonatal intensive care units (ICUs).

#### Clinical isolates

The study was conducted in the Medical Microbiology & Parasitology Laboratory. *Klebsiella pneumoniae* isolated from various clinical specimens such as endotracheal aspirate, pus, sputum, urine, wound/tissue, and blood during the study period were collected and screened. Samples were processed according to standard laboratory procedures. Isolates were identified based on growth characteristics and basic biochemical testing and confirmed by the Vitek2 automated identification system, using the Vitek GNI card (bioMérieux Vitek, Durham, North Carolina, USA).

#### Antibiotics susceptibility testing

Antibiotics susceptibility testing was performed for all isolates using disc diffusion method as described by Clinical and Laboratory Standard Institute and interpreted accordingly (11). The antimicrobials tested include amikacin, gentamicin, piperacillin–tazobactam, amoxicillin–clavulanate, cefepime, cefotaxime, ceftazidime, cefoperazone, cefturoxime, ciprofloxacin, trimethoprim–sulfamethoxazole, ertapenem, meropenem, and imipenem. Isolates which demonstrated a reduced susceptibility toward ertapenem, imipenem, or meropenem by disc diffusion method. Results were further confirmed using E-test. The MICs for imipenem and meropenem ranged between 0.25 and ≥32 μg/mL. The majority of patients with CRKP, 9/13 (70%), were managed in the ICU. Other patients were treated in general wards: medical (2/13), surgical (1/13), and orthopedic (1/13). Seven (54%) CRKP were isolated from endotracheal aspirate, and the rest were isolated from blood (2/13, 15%), urine (2/13, 15%), and wound swab (2/13, 15%).

All CRKP were resistant to 2nd and 3rd generation cephalosporin tested which include cefuroxime, cefepime, cefotaxime, ceftazidime, cefoperazone, trimethoprim–sulfamethoxazole, and amoxicillin–clavulanate. A few CRKP isolates were still susceptible to aminoglycosides, whereas 5/13 (38.5%) were susceptible to gentamicin, amikacin, and netilmicyn. In addition, 4/13 (30.7%) of isolates were susceptible to ciprofloxacin and 4/30 (13.3%) to piperacillin–clavulanate. Eight isolates (61.5%) were resistant to all tested antibiotics except colistin and tigecycline. The results of antibiotic susceptibility testing for all CRKP isolates are shown in Table 1.

### Molecular detection and DNA sequencing

Detection of CRKP genes for CRKP isolates was conducted by conventional in-house polymerase chain reaction (PCR) using primers targeting 11 *bla* variants. The forward (KPC-F 5′-GCCCCTAGCTCTGCTCTT G-3′) and reverse primers (KPC-R 5′-GCCCAATCCTCGAGCCGCG-3′) detecting KPC-1 to KPC-11 were designed using Vector NTI and GeneDoc software. BLAST program searches were performed using the National Center for Biotechnology Information website to check the specificity of the primer designed (4). The detection of other resistant genes, namely *bla*~SME, bla*~IMI, bla*~MP, bla*~NDM, bla*~VIM, and bla*~OXA, was done using published primers (14–18). DNA was isolated from bacterial colonies using the boiling lysis method as previously recommended (19). Internal control (hemM: 519-bp) was incorporated in each reaction for validation. The PCR was run using a Peltier thermal cycler (MJ Research, Watertown, Massachusetts, USA). PCR products were detected by an agarose gel electrophoresis and visualized by UV transilluminator (Alpha Innotech, San Leandro, CA, USA). PCR products were sent for DNA sequencing and compared with existing databases by multiple-sequence alignment using the BLAST program.

#### Results

Altogether, 321 *K. pneumoniae* were screened in the study with 13 (4.05%) isolates showing reduced susceptibility to ertapenem, imipenem, or meropenem by disc diffusion method. Results were further confirmed using E-test. The MICs for imipenem and meropenem ranged between 0.25 and ≥32 μg/mL. The majority of patients with CRKP, 9/13 (70%), were managed in the ICU. Other patients were treated in general wards: medical (2/13), surgical (1/13), and orthopedic (1/13). Seven (54%) CRKP were isolated from endotracheal aspirate, and the rest were isolated from blood (2/13, 15%), urine (2/13, 15%), and wound swab (2/13, 15%).
databases for confirmation. Gene accession numbers for blaNDM1 and blaIMP4 are listed in Table 2. A summary of phenotypic and genotypic results is given in Table 3.

**Discussion**

This study provides the first reported data on the prevalence of CRKP among clinical isolates of *K. pneumoniae* in Kelantan, Malaysia. The prevalence of CRKP worldwide varies, partially depending on the cultural or population exchange relationship between countries and possible reservoirs of the carbapenemase producer (20). The prevalence of CRKP among *K. pneumoniae* was high in some regions: 13% in Greece, 8% in USA, 5.5% in Israel, and 5% in Argentina (4, 21). More recently, some cases of CRKP were detected in neighboring countries. For example, in Singapore the OXA-181 genotype was found to be the second most common after NDM-1 producers (24). Vietnam detected their first two cases of CRE in 2010 (25). The isolated CRE were *Escherichia coli* and *K. pneumoniae* of NDM genotype. There is a possible danger that CRE and CRKP have emerged in Malaysia, as a result of the movements of individuals unknowingly or knowingly carrying CRE or CRKP.

In this study, the proportion of CRKP (4.05%) detected was lower than those previously reported elsewhere. Concomitant with this finding, studies in Singapore and Taiwan divulged that the prevalence of CRKP was <1 and 1.2%, respectively (26). In addition, only 1.17% was found in King Aziz Medical City, Riyadh, Saudi Arabia (10). These findings indicate that although the problems do exist, the prevalence of CRKP is still low in the Asian region (7).

The majority of the study on CRE focused on patients admitted in critical care and long-term care facilities with a higher rate of antibiotic exposure (27). In contrast, our study was not focused on any specific ward, but rather on a specific organism from various departments. Thus, being non-selective of patient population might have contributed to the lower prevalence of CRKP in our study. However, looking at the isolates distribution, the majority (61.5%) of CRKP were isolated from patients admitted to the ICU.

From sequencing analysis, only two genes, blaNDM1 and blaIMP4, were detected. These carbapenemase genes were increasingly isolated at rapid velocity. A study in Cipto Mangunkusumo Hospital in 2011 reported that 5% of CRKP were either blaIMP or blaNDM producers (28). In Malaysia, the first documented carbapenem-resistant case was blaNDM in 2010 and the distribution was as low as <0.2% (29). Other carbapenemase genes were not discovered in our study as blaSME and blaIMI were not.

**Table 1.** Antibiotic susceptibility patterns of Carbapenem-resistant *Klebsiella pneumoniae* (CRKP)

| Isolate | ETP | MEM | IPM | SXT | AK | CN | NET | TZP | AMC | CIP | FEP | CTX | CAZ | CXM |
|---------|-----|-----|-----|-----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|
| 1       | 15  | 14  | 19  | 6   | 22 | 20 | 21  | 24  | 12  | 19  | 13  | 6   | 6   | 6   |
| 2       | 14  | 15  | 18  | 6   | 20 | 18 | 17  | 19  | 11  | 16  | 10  | 6   | 6   | 6   |
| 3       | 7   | 11  | 10  | 6   | 13 | 6  | 8   | 6   | 6   | 10  | 6   | 6   | 6   |
| 4       | 15  | 15  | 19  | 6   | 23 | 19 | 17  | 22  | 11  | 19  | 10  | 6   | 6   | 6   |
| 5       | 14  | 14  | 15  | 6   | 19 | 17 | 15  | 21  | 11  | 14  | 8   | 6   | 6   |
| 6       | 17  | 14  | 18  | 6   | 21 | 15 | 19  | 25  | 14  | 20  | 16  | 9   | 6   |
| 7       | 11  | 13  | 15  | 6   | 17 | 6  | 6   | 6   | 9   | 6   | 6   | 6   | 6   |
| 8       | 14  | 15  | 18  | 26  | 6  | 6  | 6   | 6   | 10  | 6   | 11  | 6   | 6   | 6   |
| 9       | 7   | 7   | 7   | 6   | 6  | 6  | 6   | 6   | 6   | 6   | 6   | 6   | 6   |
| 10      | 12  | 14  | 17  | 6   | 6  | 6  | 6   | 6   | 8   | 6   | 9   | 6   | 6   | 6   |
| 11      | 10  | 12  | 14  | 6   | 6  | 10 | 6   | 6   | 6   | 9   | 6   | 6   | 6   | 6   |
| 12      | 12  | 16  | 17  | 6   | 6  | 6  | 6   | 6   | 6   | 6   | 6   | 6   | 6   | 6   |
| 13      | 14  | 14  | 17  | 6   | 6  | 6  | 6   | 6   | 6   | 6   | 6   | 6   | 6   | 6   |

ETP, ertapenem; IMP, imipenem; MEM, meropenem; SXT, trimethoprim–sulfamethoxazole; AK, amikacin; CN, gentamicin; NET, netilmicyn; TZP, piperacillin–tazobactam; AMC, amoxicillin–clavulanate; CIP, ciprofloxacin; FEP, cefepime; CTX, cefotaxime; CAZ, ceftazidime; CXM, cefuroxime.

**Table 2.** List of GeneBank accession numbers for blaNDM1 and blaIMP4 reference

| Beta lactamase genes | GenBank Accession No. |
|----------------------|-----------------------|
| blaNDM1              | KC539432.1; KC539430.1; KC310727.1; JF798499.1; KF016990.1; AP012055.1; JN157804.1 |
| blaIMP4              | JX517203.1; KF250428.1; KF184388.1; JX457479.1; JN106667.1; AJ609296.3; FJ384365.1 |

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In conclusion, the present study indicated that the prevalence of CRKP in our hospital setting was low. The data presented showed \textit{bla}\textsubscript{NDM1} and \textit{bla}\textsubscript{MP4} were mainly responsible for the carbapenem resistance in our \textit{K. pneumoniae} isolates. Further studies are warranted to determine other resistant mechanisms and also the genetic relatedness among these isolates.

**Authors’ contributions**

All authors contributed equally to this work. All authors contributed and participated in the study, preparation of the manuscript, and statistical analysis, and read and approved the final manuscript.

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**Conflict of interest and funding**

The authors declare that they have no competing interests. There are not any non-financial competing interests involved in this publication.

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