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Determination of the frequency of carbapenemase producing *Klebsiella pneumoniae* isolates in Dhaka city, Bangladesh

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Resistance of *Klebsiella pneumoniae* against carbapenem, imparted by the presence of carbapenemase, is an emerging global health problem with high morbidity and mortality. Thus, the present study attempted to detect the frequency of carbapenemase producing *K. pneumoniae* in Dhaka city of Bangladesh and thereby determine the health risk associated with their presence. A total of 647 *K. pneumoniae* isolates were detected from 2800 patients with urinary tract infection, bacterimia, wound infections and respiratory diseases. Thirty one carbapenem resistant isolates were found to harbor *K. pneumoniae* carbapenemase (KPC) through modified Hodge test. The KPC positive isolates were then subjected to the study of antibiogram and showed resistance against all the ß-lactam antibiotics along with carbapenems, while they were sensitive against colistin. Additionally, 287 isolates were found to be extended-spectrum β-lactamases (ESBLs) positive apart from the KPC positive ones.

Key words: Carbapenem; *Klebsiella pneumoniae* carbapenemase (KPC); multi-drug resistance

Enterobacteriaceae, mostly *Escherichia coli* and *Klebsiella pneumoniae*, are the most frequent inhabitants among the intestinal microflora causing infections such as cystitis, pyelonephritis, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections (1). They can spread easily among humans and cause community and hospital acquired infections. These microorganisms may acquire resistance against the common antibiotics mostly through plasmids and transposons mediated gene transfer (1-5). In cohort, during the past two decades, extended-spectrum β-lactamases (ESBLs) producing Enterobacteriaceae has been emerged (6).

K. pneumoniae are among the most predominant genera that carry ESBLs and are found to be highly resistant against antibiotics including aminoglycosides, fluoroquinolones, and sulfonamides (6). In order to achieve an effective medication, the use of cephalosporin has increased against the isolates carrying ESBLs. However, in recent years, ESBLs producing isolates, capable of hydrolyzing cephalosporins except carbapenems, have also been reported (1). Therefore, carbapenems are the preferred antibiotic for the treatment of ESBL positive *K. pneumoniae.* Unfortunately, over use of this drug creates a selective pressure to *K. pneumoniae* strains which resulted in the acquisition of resistance by the production of the enzyme carbapenemase, which destroys carbapenem (7). The emergence of *K. pneumoniae* that harbor the plasmid encoded carbapenemase, commonly known as *K. pneumoniae* carbapenemase (KPC), has now become a global public health concern (8, 9).

The prevalence of carbapenemase producers still remains obscure because of the lack of proper detection method in many countries, especially those with limited resources and poor laboratory settings (10). Notably, an early detection of carbapenemase producing bacteria in their primitive infection stage is essential to prevent the progression of hospital-based outbreaks (11). Moreover, the rapid spread of carbapenem resistant *K. pneumoniae* may limit the effectiveness of carbapenems in the treatment of multidrug-resistant infections (12).

Carbapenemases can be detected phenotypically by the modified Hodge test which demonstrate 100% sensitivity and specificity when compared to other available tests (13, 14). Another standard method of carbapenemase identification based on the detection of specific genes through PCR (15, 16). Hence, the eradication of KPC positive *K. pneumoniae* often requires the use of polymixins and tigecycline, even they have been reported to be nephrotoxic (17). The present study aimed to conduct antimicrobial susceptibility of *K. pneumonia* and to determine the prevalence of *K. pneumoniae* harboring KPC in clinical specimens.

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MATERIALS AND METHODS

The study was carried out at Square Hospital Ltd., Dhaka, Bangladesh from January 2011 to December 2011. Specimens including urine, blood, tracheal aspirates and pus were aseptically collected from hospitalized and out patients reported the hospital. All the specimens were spread over the surface of MacConkey agar, blood agar and chocolate agar media and were incubated at 37 ^{o}C for 18-24 hours. *K. pneumoniae* isolates were identified based on their typical colony characteristics followed by biochemical identification tests including the triple sugar iron agar (TSI) test, motility indole urea (MIU) test, citrate utilization test and bile esculin agar test (18).

Detection of extended-spectrum β-lactamase (ESBL). Isolates were introduced onto Mueller-Hinton agar media. Ceftazidime (30 µg) , ceftriaxone (30 µg), cefotaxime (30 µg) and aztreonam (30 µg) discs were placed peripherally away from amoxicillin clavulanic acid (20+10 µg) disc which was placed at the center of the Mueller-Hinton plate. Band formation between amoxyclav disc and any other disc was considered as ESBL positive (19).

Detection of KPC. Carbapenem (meropenem and imipenem) resistance was determined through antibiotic susceptibility test by disc diffusion method using the Kirby-Bauer technique (20-21). Finally, the detection of KPC from carbapenem resistant isolates was performed by Modified Hodge test (22). A 6 hour culture suspension of *E. coli* ATCC 25922 adjusted to 0.5 McFarland standard, was introduced on to the surface of Mueller-Hinton agar (MHA) (HI-MEDIA, Mumbai, India). After drying, 10 µg meropenem disc (HI-MEDIA, Mumbai, India) was placed at the center of the plate and the test strain was streaked from the edge of the disk to the periphery of the plate in four different directions. The plate was incubated overnight at 37 ^{o}C. The presence of a 'cloverleaf shaped' zone of inhibition indicated the production of carbapenemase by the test strain.

Study of antibiogram. Antibiotic susceptibility pattern of the carbapenem resistant isolates were then determined (20-21). Meropenem (10 µg), imipenem (10 µg), cefexime (5 µg), cefuroxime (30 µg), ciprofloxacin (5 µg), ceftriaxone (30 µg), cotrimoxazole (25 µg), tetracycline (30 µg), amikacin (30 µg), gentamicin (10 µg), nitrofurantoin (300 µg), ampicillin (10 µg), chloramphenicol (30 µg), cefepime (30 µg), amoxicillin (10 µg), pipercillin tazobactam (100 µg) and coloistin (25 µg) were used for the assay.

RESULTS

Determination of the frequency of KPC. A total of 647 *K. pneumoniae* isolates were included in this study. Among the isolates, 31 (4.8%) isolates were found to be resistant against carbapenems (meropenem and imipenem). All the carbapenem resistant isolates were found to be KPC positive as revealed through the modified Hodge test.

Patients harbouring KPC positive *K. pneumoniae* were under treatment with carbapenem, cephalosporin, vancomycin, linezolid, and combination of carbapenem and cephalosporin. Ages of majority (71%) of such patients were above 45 years (Table 1). Only two patients (6.5%) were below 15 years of age. The proportion of male patients (58.1%) were higher over female ones (41.9%) (Table 1). Out of 31 patients, 27 had true infections, which were confirmed by observing patient's clinical symptoms and other supportive diagnostic reports. Remaining 4 isolates either colonized or were in unknown state. We found 287 isolates to harbor ESBL which were KPC negative.

Drug-resistance of the KPC positive *K. pneumoniae* isolates. In addition to carbapenems (meropenem and imipenem), all KPC producing isolates were found to be 100% resistant against cefepime, ceftriaxone, cefexime, cefuroxime, amoxcillin, ciprofloxacillin, ampicillin, nitrofurantoin, chloramphenicol and pipercillin-tazobactam (Table 2). Higher resistance was also observed against gentamycin (93.5%), amikacin (96.8%), cotrimoxazole (93.5%) and tetracycline (90.3). Conversely, All KPC positive *K. pneumeniae* were found sensitive against colistin sulphate (Table 2).

TABLE 1. Age and sex distribution of patients harboring carbapenemase producing *K. pneumoniae* (n= 31)

Age	Male	Female	Carbapenemase producing *Klebsiella pneumoniae* (%)
Up to 15 years	2	0	2 (6.5)
15 - 30 years	4	0	4 (12.9)
30 - 45 years	2	1	3 (9.7)
45 – 60 years	4	4	8 (25.8)
Above 60 years	6	8	14 (45.2)
Total (%)	18 (58)	13 (41.9)	31 (100)

TABLE 2. Antibiotic resistance of the KPC positive *K. pneumoniae* isolates (n=31)

Antibiotics (µg)	Number of resistant isolates (%)
Meropenem (10)	31 (100)
Imipenem (10)	31 (100)
Gentamycin (10)	29 (93.5)
Amikacin (30)	30 (96.8)
Cefepime (30)	31 (100)
Ceftriaxone (30)	31 (100)
Cefexime (10)	31 (100)
Cefuroxime (30)	31 (100)
Amoxcillin (10)	31 (100)
Ciprofloxacillin (5)	31 (100)
Nitrofurantoin (300)	31 (100)
Tetracyclin (30)	28 (90.3)
Chloramphenicol (30)	31 (100)
Cotrimoxazole (10)	29 (93.5)
Colistin (25)	0 (0)
Ampicillin (10)	31 (100)
Pipercillin Tazobactam (100)	31 (100)

DISCUSSION

The emergence and rapid dissemination of KPC producing *K. pneumoniae* is now a global health threat (23, 24). Such multi-drug resistant bacteria are difficult to treat as the treatment options are limited. The delay in microbiological laboratory confirmatory results often occur due to limitation in laboratory detection (23). Therefore, infection by KPC may accounts for an increase in the rate of morbidity and mortality of patients compared to infection by carbapenem susceptible Enterobacteriaceae (25). Importance of carbapenemase-producing pathogens underlies the fact that the genes

coding for KPC enzymes are on plasmids that can transmit resistance between organisms and between species (6, 26). Therefore, healthcare facilities must be vigilant in maintaining good infection control practices.

As revealed from the present study, out of 647 *K. pneumoniae* isolates, 4.79% were found to be resistant against carbapenems and hence were KPC positive, which is 3 times higher than that found in India, where only 1.70% KPC positive *K. pneumoniae* isolates were identified (27). The elevated frequency of KPC positive isolates in present study might be due to the increased use of carbapenem in Dhaka, Bangladesh as compared to India. Out of 31 KPC positive isolates, 27 (87.1%) showed clinical manifestation. In this study, clinical history of patients harboring KPC positive *K. pneumoniae* revealed that most patients had prior and/or prolonged hospitalization, ICU stay, and/or prior antibiotic therapy. Interestingly, from our study, none of the KPC positive isolates found to harbor ESBLs. This finding indicated that carbapenem might be the drug of choice for the treatment of ESBL positive isolates.

The antibiotic susceptibility patterns of KPC positive isolates revealed that the isolates were highly resistant against most of the antibiotics used except for colistin. Therefore, we endorse colistin to be the drug of choice for the treatment of KPC positive isolates. In another study it was found that KPC positive patients were successfully treated using tigecycline (28). However, in the present study, the effectiveness of trigecycline against KPC positive isolates was not tested.

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