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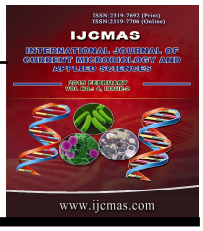

Original Research Article

Phenotypic Detection of Carbapenem Resistance among *Klebsiella pneumoniae* in Suez Canal University Hospitals, Ismailiya, Egypt

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A B S T R A C T

Klebsiella pneumoniae (KP) ranked among the top ten bacterial pathogens responsible for hospital acquired infections. The spread of KP that produce extended-spectrum β-lactamases (ESBLs) has been reported worldwide, leaving the carbapenem antibiotics as the only group of β-lactams available for therapy. Recently, carbapenem-resistant, ESBL-producing KP has been emerged as an important challenge in health-care settings. Our aim was to detect the carbapenem resistance and classes of carbapenemases in KP isolates isolated in Suez Canal University Hospitals (SCU), Ismailiya, Egypt, by using phenotypic methods. 79 KP isolates were collected from patients attending the SCU Hospitals, were screened for production of ESBLs using agar disk diffusion tests; agar disk diffusion screening and Modified Hodge test for Carbapenemase production; and carbapenemase-inhibition assay for detection of Ambler classes of carbapenemases. 44.3% of the isolates were carbapenem-resistant, 36.7% were ESBL. The majority of the carbapenemase produced was Class B (40%), followed by combined A&B, A, then class D. (Intensive Care Unit) ICU patients harbored most of carbapenem-resistant isolates**.** The SCU Hospitals showed an alarming situation of ESBL and carbapenemase producing KP, which the possibility of outbreak eruption by these difficult-to-treat pathogens, in the future.

Introduction

Klebsiella pneumoniae (KP) is a Gram-negative, facultative anaerobic rod that has great potential for hospital-acquired infection outbreaks, particularly in immune-compromised and debilitated patients. In addition to pneumonia, KP causes infection in the urinary tract, biliary tract, and surgical wound sites (Sydney, 2004).

Since 2000, the spread of KP isolates that produce extended-spectrum β-lactamases (ESBLs) has been reported worldwide. ESBLs are capable to hydrolyze extended-spectrum penicillins, cephalosporins and monobactams, leaving the carbapenem group of β-lactam antibiotics as the only choice for therapy. It is therefore mandatory

to maintain the clinical efficacy of carbapenems which have become the antimicrobial drug of the last resort (Pitout and Laupland, 2008).

The carbapenems are β-lactam antibiotics with the broadest antibacterial spectrum compared to other β-lactams. In addition, they are generally resistant to the typical bacterial enzymes, β-lactamases. Carbapenems are active against both Gram-positive and Gram-negative bacteria, including anaerobes, with the exception of intracellular bacteria such as the Chlamydiae (Pitout and Laupland, 2008). Four carbapenems are currently FDA (Food Drug Administration) approved for therapy; imipenem, meropenem, ertapenem, and doripenem. In SCU hospitals, carbapenems are frequently used as an empiric therapy for life-threatening infections as well as infections with multidrug resistant gram-negative rods (Mainardi *et al.,* 2008). Carbapenem-resistant *Enterobacteriaceae* (CRE) is emerging as new challenge in Suez Canal University (SCU) Hospitals. CRE significantly limits treatment options for critically ill patients to members of the fluoroquinolone and aminoglycoside groups. In 2004, *Klebsiella pneumoniae* carbapenemase (KPC) was first detected in New York hospitals. Over the past 10 years, a progressive increase in KPC isolates has occurred worldwide (Jean *et al.,* 2009; Mushtaq *et al.,* 2004; Deshpande *et al.,* 2010). Among 33 European countries participating in the European Antimicrobial Resistance Surveillance System (EARSS), Greece had the highest carbapenem-resistance rates among KP (46%) (Souli *et al.,* 2008). In Egypt, carbapenem resistance is emerging. In hospitalized cancer patients (Hossam and Amany, 2009), and in orthopedic ward (Khaleid *et al.,* 2010), carbapenem resistance was detected in 13.9% and 14.2% of total KP isolates**.**

A number of mechanisms have been described for carbapenem-resistance in KP: 1) production of different classes of carbapenemase, 2) hyperproduction of *Amp*C β-lactamase with an outer membrane porin mutation, 3) production of extended-spectrum β-lactamase with a porin mutation or drug efflux. Production of carbapenemases is the most commonly reported mechanism of carbapenem-resistance by KP (Jean *et al.,* 2009).

The first carbapenemase was identified in 1993**,** then a large variety of carbapenemases has been identified, and classified into three classes according to their amino acid sequence; Ambler class A (serine carbapenemases), class B (metallo-carbapenemases) (MBL), and class D (oxacillinase carbapenemases) (Queenan and Bush, 2007). These classes can be detected by the Modified Hodge Test (MHT) and the carbapenemase inhibition tests, as well as PCR detection and sequencing of specific carbapenemase production genes (Miriagou *et al.,* 2010). MHT detects the production of diffusible carbapenemase in strains of KP while the carbapenemase inhibition tests are used to distinguish between the different classes of carbapenemases, based on *in-vitro* inhibition of carbapenemase activity by addition of an inhibitor specific for a class of carbapenemase (Stuart and Leverstein-Van, 2010).

The ease of horizontal transmission of carbapenem-resistant plasmids among admitted patients, or within the commensal bacterial populations of the same patient may promote dissemination of carbapenem-resistance to new populations of *Enterobacteriaceae*, including organisms of low virulence, leading to the establishment of reservoirs of carbapenem-resistant clones in patients and/or the environment. This

process raises the spectrum of untreatable community-associated and healthcare-associated infections (Amy *et al.,* 2011). The detection of carbapenem-resistance in a hospital is essential for the proper choice of antibiotic therapy**.** It has a great impact on infection control, and can prevent dissemination of the resistant strains in hospital settings. Defining mechanism of resistance by detecting the carbapenemase classes can promote defining new resistant clones and trace its source in the hospital environment (Stuart and Leverstein-Van, 2010).

The aim of the study is to detect carbapenem resistance and classes of carbapenemase, among KP isolates in SCU Hospitals in Ismailia, Egypt, by using phenotypic methods.

Methodology

Clinical specimens (n=400) were collected from admitted patients at the urology, internal medicine, surgery wards, adult ICU and neonatal ICU, as well as patients attending different outpatient clinics. The specimens included blood, pus, urine and sputum. Specimens were processed and KP strains were identified morphologically and biochemically (Betty *et al.,* 2007). ESBL production was detected using agar disk diffusion method (Clinical and Laboratory Standard Institute (CLSI), 2013). Carbapenemase production was detected by agar disk diffusion screening test and confirmed by Modified Hodge test (Clinical and Laboratory Standard Institute (CLSI), 2013). The class of carbapenemase enzyme was determined using carbapenemase inhibition tests (the combined disk method) using meropenem disk (10μg). For Class A, KPC-type enzyme production was suspected when there is ≥5mm increase of inhibition zone diameter around the meropenem/phenylboronic acid disk (10 μg/600 μg), than meropenem disk (10μg). MBL (class B) detection method is based on the synergy between MBL inhibitors EDTA and meropenem. This was achieved when there is ≥5mm increase of inhibition zone diameter around the meropenem/EDTA disk (10 μg/292 μg), than meropenem disk (10μg). There is no specific inhibitor for class D carbapenemases, their detection depend on exclusion of the other classes (Christine *et al.,* 2011).

The carbapenem-resistant KP strains were also screened for resistance to the following antibiotics by the standard disk diffusion method (Clinical and Laboratory Standard Institute (CLSI), 2013): ciprofloxacin (5μg), levofloxacin (5μg), gentamicin (10μg), amikacin (30μg), and colistin disk (10μg).

The results of the collected data were entered into statistical package of social sciences (SPSS-17) program for statistical analysis. Descriptive data were managed according to its type and summarized by frequencies and percentages. Test of significance used was Pearson Chi-Square test.

Results and Discussion

Isolation rate of KP was 19.75%, as 79 strains were isolated from 400 clinical specimens taken from different wards in the hospitals. The sources of the isolates were: urine (35%), pus (29%), blood (20.5%), and sputa (15%). Carbapenem resistance was detected in 35 KP strains (44.3%) by agar disk diffusion screening and confirmed by Modified Hodge test confirmatory tests (figure 1). Twenty-nine strains (36.7%) of *K. pneumoniae* isolates were ESBL-producing (Table 1). Among 35 carbapenem-resistant KP isolates, 45.7% were ESBL- producing strains, 65.7%

showed a resistance to ciprofloxacin, 68.6% were levofloxacin-resistant, 57.1% were resistant to gentamycin, 57.1% were resistant to amikacin, and 28.6% were resistance to colistin (Table 1). Different carbapenemase classes were detected in 35 CR-KP isolates; class A carbapenemase was the only carbapenemase detected in 5 isolates, Class B carbapenemase was detected alone in 14 strains, Both class A and B were found in 11 isolates (figure 2), while class D carbapenemase was assumed to be produced by 5 isolates (Table 2). The highest percentage of carbapenem-resistant *K. pneumoniae* isolates was detected in ICU followed by the neonatal ICU, the surgical ward, the urology ward, the internal medicine ward, and the least percentage was found among outpatients.

This study aimed to detect carbapenem resistance among KP isolates (CR-KP) in SCU Hospitals, using phenotypic methods. The isolation rate of KP was 19.7% (79 isolates from 400 infections) including both community-acquired and healthcare-associated infections. In Al-Mansoura University Hospital, Egypt, KP represented 15.4% of total nosocomial pathogens (Kandeel, 2000), and in Assiut General Hospital, Egypt, it was 10.7% (Mandour, 2002); while in the orthopedics department of Al-Azhar University Hospital, Cairo, Egypt, KP represented 14.2% of 93 infecting pathogens (Khaleid *et al.*, 2010).

In the current study, 35 KP isolates (44.3%) were carbapenem-resistant, 14.3% of these produced carbapenemase class A enzyme, 40% produced class-B, 14.3% produce class-D and 31.4% produced combination of class-A and B enzymes, making a total of 71.4% of the strains producing class B enzyme and a total of 44.7% of the strains producing class A enzyme (Table 2). These results were markedly high when compared to *Bratu et al.* (2005a,b); who reported that only 3.3% of isolated KP was carbapenem-resistant, after testing 602 samples assembled from a citywide surveillance study conducted in Brooklyn, New York**.** In a study performed in Tel Aviv Sourasky Medical Center, it was shown that only 1.2% of 4149 KP isolates were carbapenem-resistant (Leavitt *et al.,* 2007). Other Egyptian studies also showed lower incidence (13%) of carbapenem-resistant KP, in the Egyptian National Cancer Institute (Hossam and Amany, 2009) and 14.2% in the orthopedics department, Al-Azhar University Hospital (Souli *et al.*, 2008).

However, in an outbreak caused by KP in Brooklyn City General Hospital, New York, 24% of 257 KP isolates were carbapenem-resistant (producing class-A carbapenemase enzyme) (Mooty *et al.,* 2005) and 83% of 814 KP isolates showed carbapenem resistance in a study which included 69 laboratories all over Los Angeles area, USA (Marquez *et al.*,2013). Another high incidence of CR-KP (46%) was reported in Greece (Khaleid *et al.,* 2010). As regards to carbapenemase classes produced by CR-KP, a study in Greece University Hospital found that 26% of 100 KP isolates produced class B carbapenemase (Maria *et al.,* 2010). In Tunisia, *Sonia et al.* (2011); found 13.7% of 153 isolates were class D-producing CR-KP strains**.**

In the Asia-Pacific region, the SMART (Study for Monitoring Antimicrobial Resistance Trends) global surveillance program found that between 2008 and 2009, 42.7% of 110 CR-KP strains produced class A, 23.6% produced class B, and 11.8% produced class D carbapenemases (Christine *et al.*, 2012).

Table.1 Antibiotic resistance among KP isolates

Resistance profile:	**No.**	**%**
ESBL production	29/79	36.7
Carbapenem resistance (CR)	35/79	44.3
CR + Ciprofloxacin R	23	65.7%
CR + Levofloxacin R	24	68.6%
CR + Gentamycin R	20	57.1%
CR + Amikacin R	20	57.1%
CR + Colistin R	10	28.6%
CR + ESBL-production	16	45.7%

Table.2 Mechanism of carbapenem resistance in 35 CR-KP strains

Classes of Carbapenemase	No.	%
Class A only	5	14.3%
Class B only	14	40%
Class D only	5	14.3%
Class A+B	11	31.4%
TOTAL	35	100%

The high prevalence of CR-KP in the current study could be attributed to the excessive empirical use of carbapenems in our hospital, and the improper application of the infection control measures by the hospital personals that together have lead to multiple incidence of horizontal spread of CR-KP strains among patients. Also, the percentage of class B (MBL) was higher (25/35, 71.4%) than that of other classes; this can be explained by the dramatic increase in the detection and spread of the transferable families of metallo-enzymes which are situated within a variety of integrons, facilitating their transfer between bacterial clone in the hospital (Queenan and Bush, 2007). On the other hand, the production of class A enzyme in the present study is considered high (16/35, 45.7%). This can be explained by the great potential for spread of this enzyme due to its location on plasmids in CR-KP strains, which is known for its ability to accumulate and transfer resistance determinants (Landman *et al.,* 2005). This study showed that 36.7% of all KP isolates (n=29/79) were ESBLs-producing strains (Table 1). Closer results were found by El-Sonbaty, 2001, who reported that ESBL-production among nosocomial KP was 29.8% in Al-Mansoura University Hospitals**,** Egypt, and in Riyadh (Saudi Arabia) one study reported 27% of 125 KP strains isolated from ICU and NICU patients (El-Sweify and El-Zayat, 2008). Higher rates were also reported; in Assuit University Hospital**,** Egypt, it was (51%) (El-Gendy, 2006); in São Paulo University Hospital, Brazil (51% of 108 isolates) **(**Marra *et al.,* 2006); in Brooklyn, New York (45% of 602 KP isolates) (Bratu *et al.,* 2005a,b); and in Abbottabad medical center, Pakistan it was 57% (Ali *et al.,* 2004).

In this study, CR-KP strains also showed

other resistance patterns. Resistance to levofloxacin and ciprofloxacin was 68.6% and 65.7%, respectively. These two fluoroquinolones are the most popular oral antibiotics being used in our hospital for treatment of serious infections by gram-negative rods. In addition, 57.1% of CR-KP was resistant to both gentamycin and amikacin; while 45.7% was resistant to all β-lactam antibiotics, as they produced carbapenemases as well as ESBLs (Table 2). *Bratu et al.* (2005a,b) found 55% of 96 KP isolates to be resistant to amikacin. Also *Sanchez et al.* found 36.8% resistance to amikacin and 96.4% resistance to ciprofloxacin among a large sample of carbapenem-resistant KP, derived from the antibiogram results from The Surveillance Network Database, USA (Sanchez *et al.*, 2013). *Livermore et al.* (2011); examined a sample of 81 carbapenem resistant *Enterobacteriaceae* isolates in the UK, 75% of which showed resistance to ciprofloxacin and all isolates were resistant to gentamycin. The multidrug resistance patterns detected in our CR-KP isolates could be attributed to R-genes located on plasmid that can subsequently and quickly spread in different bacterial species, perhaps through genetic recombination.

This plasmid is known to harbor genes conferring resistance to other antibiotics in the aminoglycoside and fluoroquinolone groups. The present study showed that ICU contained the highest percentage of CR-KP clinical isolates, followed by the neonatal ICU. This result is alarming that proper infection control precautions are not well implemented in such critical care units. Similar results were reported by Carrër *et al.* (2008) on investigating an outbreak of carbapenem-resistant KP in a Turkish hospital. The most common hospital location was the ICU followed by the urology and general surgery department, equally (Carrër *et al.*, 2008). The high prevalence of CR-KP isolates in our ICU units is also attributed to the nature of patients admitted in this section; a major sector of our IUC patients are complicated or end-stage cases, referred from other hospital wards, or a different hospital within the city, where they had prolonged and various antibiotic therapies, with long periods of hospital stay. They are mostly immune-compromised, with disturbed consciousness, mostly subjected to invasive procedures and devices, and receiving combination of antibiotic therapy that included carbapenems, adding to their long stay in ICU. On the other hand, out-patients who are not exposed to the intravenous carbapenem antibiotic therapy, or to the hospital environment, are less prone to such high rates of resistance.

The present study concluded that Suez Canal University Hospitals in Ismailia showed an alarming situation of rising number of CR-KP as well as multidrug-resistant KP that can initiate future outbreak, which could be difficult to manage by the available set of antibiotics. This requires more attention to revising the antibiotic policy and strengthening the application of infection control precautions in our hospital.

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