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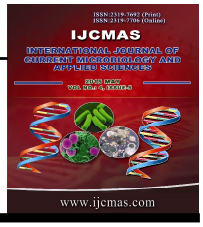

Original Research Article

Detection of *Klebsiella pneumoniae* Carbapenemase (KPC) Producing Gram Negative Superbugs: An Emerging Cause of Multidrug-Resistant Infections in General Surgery Department of Sohag University Hospital, Egypt

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A B S T R A C T

Klebsiella pneumoniae carbapenemase (KPC)-producing bacteria are emerging highly drug-resistant Gram-negative bacilli causing infections associated with significant morbidity and mortality. This study was undertaken to detect the resistance to carbapenems in clinical isolates of Gram negative pathogens obtained from patients admitted in the Department of General Surgery, Sohag University Hospital and to assess KPC production among these isolates. A total of 215 clinical isolates obtained over a period from September 2013 to March 2015. These isolates were screened for carbapenem resistance by culturing on CHROM agar KPC media. Species identification by using API 20 E test strips as a biochemical identification system and antimicrobial susceptibility testing by the disk diffusion method were carried out. Carbapenem-resistant isolates were screened for carbapenemase production by the modified Hodge test (MHT) and positive isolates were further checked for KPC production by phenylboronic acid (PBA) test. Isolates were also checked for metallo-β-lacatmase (MBLs) production by the EDTA combined disk test (CDT) and by disk enhancement test with both PBA and EDTA for the detection of co-production of KPC and MBL. Of the total 59 carbapenem resistant isolates, MHT identified 52 (88.14%) isolates as carbapenemase producers, MBLs activity was detected in 20 (33.9%) isolates by CDT, KPC in 14 (23.73%) isolates by PBA test and co-existence of both KPC and MBL in 9 (15.25%) isolates. Multidrug resistance characterized the studied isolates, with colistin being the most active agent. Emergence of KPC producing pathogens in our setting creates an important challenge for clinicians and hospital epidemiologists, because it is added to the already high burden of antimicrobial

Keywords

Carbapenem resistance, KPC, Modified Hodge test, Boronic acid test

Introduction

Carbapenems, first introduced in 1980, are the potent antibiotics for the treatment of infections due to multidrug-resistant Gram-negative bacteria, including those with extended spectrum β-lactamases (ESBLs) and AmpC β-lactamases (Yong *et al.*, 2009).

At least two mechanisms can be responsible for acquired carbapenem resistance in Gram-negative bacteria: (i) reduced outer membrane permeability by porin loss in combination with the production of an extended spectrum beta-lactamase (ESBL) or of AmpC-type beta lactamase; and (ii) production of beta-lactamases capable of hydrolysing carbapenems (carbapenemases) (Nordmann *et al.,* 2012). While the former mechanism is a result of mutation and has a low overall propensity to disseminate; acquired carbapenemases are located on integron structures that reside on mobile genetic elements such as plasmids or transposons, thus enabling widespread dissemination among the gram negative fraternity (Canton *et al.,* 2012). Among these carbapenemases, metallo-β-lactamases (MBLs; Ambler class B) and *Klebsiella pneumoniae* carbapenemase (KPC; Ambler class A) are prevailing in large geographic regions (Gomez *et al.*, 2011). The increasing incidence of KPC-producing strains worldwide has posed a challenge for diagnosing and treating such infections. They hydrolyse all β - lactam agents including penicillins, cephalosporins, aztreonam, and carbapenems, and their activity is inhibited by boronic acid and, partially by clavulanic acid and tazobactam (Bansal *et al.,* 2013). Up to date, eight different KPC variants (KPC-2 to -9) have being identified different from each other by 1 or 2 amino acid substitutions. KPC-2 and -3 are the most common variants identified in *Enterobacteriaceae* and *P. aeruginosa*. KPC-6, -7, and -8 have been identified only in *K. pneumoniae*, while KPC-9 was detected in *Escherichia coli* and KPC-5 in *P. aeruginosa* (Robledo *et al.,* 2010). Detection of KPC producers in Gram-negative bacteria is important, as they are also associated with many other resistance determinants, giving rise to multidrug resistance and even pandrug resistance. KP carbapenemases can be detected by phenotypic as well as genotypic methods (Amjad *et al.,* 2011). It is important for patient care as infections caused by KPC-producing bacteria have been associated with increased cost and length of stay as well as frequent treatment failures and death (CDC, 2009). Risk factors for such infection include advanced age (Nadkarni *et al.,* 2009), being severely ill (Gasink *et al.,* 2009), previous treatment with antibiotics (Bratu *et al.,* 2005), organ transplantation, mechanical ventilation, and long hospital stays (Patel *et al.,* 2008). Reports are mixed as to whether previous carbapenem use is associated with the development of infections caused by KPC-producing bacteria (Maltezou *et al.,* 2009; Weisenberg *et al.*, 2009). Treatment of these infections is particularly worrisome as the carbapenems are often agents of the last resort for resistant Gram-negative infections. The optimal treatment of such infections is not well established and clinical outcome data remain sparse. Thus, in order to limit the spread of these serious KPC-producing pathogens, rapid detection, followed by implementation of adequate infection control methods, is essential. Keeping in mind the above facts, the present study was undertaken to explore the occurrence of carbapenemases among the clinical isolates of Gram- negative bacteria which were further tested phenotypically for KPC and MBL production.

Patients and methods

Study design and patients

This study was carried out in the Departments of Medical Microbiology & Immunology and General Surgery; Faculty of Medicine, Sohag University during the period from September 2013 to March 2015. Clinical samples were collected from patients admitted in the Department of

General Surgery, Sohag University Hospital who developed health care-associated infections, especially those with multiple therapeutic failures. Specimens, such as wound swabs, pus, blood (collected in blood culture bottles containing brain-heart infusion broth) and urine were included in the study. Samples were collected after obtaining informed oral consent from the patients.

Primary isolation of carbapenem-resistant isolates

Samples were cultured on CHROMagar KPC plates (CHROMagar, Paris, France). CHROMagar KPC is a commercially prepared chromogenic solid medium supplemented with agents that inhibit the growth of carbapenem-sensitive bacteria. Dehydrated powder of the CHROMagar KPC was provided by the CHROMagar Company (CHROMagar, Paris, France). The medium was prepared according to the manufacturer's instructions and poured into petri dishes, stored at 4 to 6°C. All samples were cultured directly on the CHROM agar KPC plates. CHROM agar KPC medium not only used for screening for carbapenem resistant bacteria; but also can identify the type of the bacterial growth. As following 24h of incubation, carbapenem-resistant colonies appear with different colors according to their specific enzymatic properties: *Escherichia coli* appear as dark pink to reddish colonies, KEC (*Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp.) as metallic blue, *Pseudomonas* spp. as translucent cream colonies and *Acinetobacter* spp. as cream opaque colonies (CHROMagar^{TM} KPC, 2013) (Figure 1).

Differentiation of bacterial species

API 20E test strips (BioMerieux, France) was used as a biochemical identification system for identification of the isolated genera to the species level (Figure 2).

Antimicrobial susceptibility testing

The susceptibility testing of isolates to different antibiotics was carried out by the disc diffusion method (Modified Kirby-Bauer method) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2012). Using commercially available disks (Oxoid Ltd., Basingstoke UK): meropenem (10µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxone (30µg), cefepime (30µg), amoxycillin-clavulanic acid 20/10 µg, aztreonam (30µg), gentamicin (10µg), amikacin (30µg), piperacillin (100µg), pipercillin-tazobactam (100/10µg), ciprofloxacin (5µg), and colistin (10µg) on Mueller Hinton agar plate (Figure 3).

Detection of carbapenemase production using Modified Hodge Test (MHT)

Carbapenem resistant strains were subjected to MHT for detection of Carbapenemases production. This test was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2012 (CLSI, 2012). A 0.5 McFarland turbidity standard suspension of previously isolated carbapenem sensitive *Escherichia coli* in sterile saline was prepared and was diluted 1:10 in sterile saline. This was inoculated on a Mueller Hinton agar plate, as for the routine disk diffusion testing. The plate was dried for 5 minutes and a disk of ertapenem (10 µg) was placed in the centre of the agar plate. Using a swab; 3-5 colonies of the test organism were picked and were inoculated in a straight line, from the edge of the disk, up to a distance of at least 20mm. The plates were incubated at 37°C overnight and they were examined next day. They were checked for

an enhanced growth around the test organism streak at the intersection of the streak and the zone of inhibition. The presence of an enhanced growth of *E. coli* indicated Carbapenemase production by the tested organism that inactivated ertapenem which was no longer sufficient to inhibit *E. coli* and an indentation of the zone was noted and detected by the presence of a distorted or 'cloverleaf shaped' inhibition zone which was interpreted as positive for carbapenemase producing isolates (CLSI, 2012) (Figure 4).

Phenotypic detection of KPC producing isolates using phenylboronic acid (PBA) disk test

The stock solution of PBA was prepared by dissolving phenylboronic acid *(Sigma-Aldrich, Steinheim, Germany)* in dimethyl sulfoxide (DMSO) to a concentration of 20 mg/ml. From this solution, 20 µl (containing 400 µg of PBA) was dispensed onto commercially available meropenem disks. The disks were allowed to dry and used within 60 min.

The test was performed by inoculating Mueller–Hinton agar as given for the standard diffusion method and placing onto the agar one disc of meropenem (10 µg) without any inhibitor and a disk of meropenem (10 µg) containing 400 µg of PBA. The agar plates were incubated overnight at 37°C.

The diameter of the growth inhibitory zone around the meropenem disk with PBA was compared with that around the plain meropenem disk. Production of KPC was considered when the growth-inhibitory zone diameter around the meropenem disk with PBA was increased ≥5 mm compared with the growth-inhibitory zone diameter around the disk containing meropenem alone (Tsakris *et al.*, 2009) (Figure 5).

Phenotypic detection of metallo-β-lactamase (MBL) producing isolates using combined disk test (CDT)

The isolates were confirmed as MBLs producers by imipenem/EDTA combined disk test (CDT). To make 0.5 M Ethylenediamine tetra acetic acid (EDTA) solution was prepared by dissolving186.1 g of disodium EDTA.$2H_2O$ in 1000 mL of distilled water, and the pH was adjusted to 8 by sodium hydroxide (NaOH). Then the mixture was sterilized in the autoclave to prepare a sterile EDTA solution. To prepare EDTA-containing imipenem disks (750 µg EDTA); 10 µL of EDTA solution was added to imipenem disks (10 µg). These disks were dried immediately in a 37°C incubator and stored at 4°C in airtight vials until use. For each isolate, 1 imipenem disc and 1 EDTA containing imipenem disc were placed at a suitable distance on the surface of Mueller-Hinton agar plates inoculated with a bacterial suspension equivalent to 0.5 McFarland Standard. After 24 hours of incubation at 37°C, an increase of ≥7 mm in the inhibition zone diameter of EDTA-containing imipenem disk compared to imipenem disk alone was considered to be a positive test for the MBLs producer imipenem resistant strains (Yong *et al.*, 2002) (Figure 6).

Phenotypic Detection of KPC and MBL coproducers using disk enhancement test with both PBA and EDTA

On Mueller Hinton agar plate inoculated with the test strain, four disks of meropenem were used. One disk of meropenem was without any inhibitor, one disk with PBA (400 µg) only, one disk with EDTA (10 µL of 0.5 M) only and fourth disk of meropenem had both PBA plus EDTA. The agar plates were incubated at 37° C overnight and the diameter of the growth inhibitory zone around these meropenem

disks with inhibitor added was compared with that around the plain meropenem disk. Production of both KPC and MBL enzymes was considered when the growth-inhibitory zone diameter around the meropenem disk with both PBA and EDTA was increased ≥5 mm compared with the growth-inhibitory zone diameter around the disk containing meropenem alone while the growth-inhibitory zone diameters around the meropenem disk with PBA and the meropenem disc with EDTA were increased <5 mm compared with the growth-inhibitory zone diameter around the disk containing meropenem alone (Tsakris *et al.*, 2010).

Statistical analysis

Results were expressed as mean ± standard deviation (SD) of the mean. Qualitative (categorical) data were represented by the number and percent (%).

Results and Dicussion

A total of 215 Gram negative bacterial isolates were obtained during the study period. Of these 215 isolates; 59 (27.4%) isolates were carbapenem resistant as detected by the growth of bacteria on the CHROMagar KPC medium. The isolated organisms were; KEC 26 (44.1%) (*Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp.), *Escherichia coli* 16 (27.1%), *Pseudomonas* Spp. 10 (16.9%), and *Acinetobacter* Spp. 7 (11.9%). (Table 1).

Of the total 59 carbapenem resistant isolates, MHT identified 52 (88.14%) isolates as carbapenemase producers by developing cloverleaf shaped inhibition zone. MBLs activity was detected in 20 (33.9%) isolates by CDT. KPC activity was detected in 14 (23.73%) isolates by PBA test and co-existence of both KPC and MBL in 9 (15.25%) isolates as detected by the disk enhancement test with both PBA and EDTA (Table 2).

Types of health care associated infections caused by KPC producing isolates

Twenty three patients were infected by KPC producing Gram negative bacteria; 14 males (60.9%) and 9 females (39.1%) with age range from 40 to 68 years (mean age 54 years). The average length of hospital stay was 30.5 days (range from 11 to 50 days). Of these cases there were: 11 (47.8%) cases of nosocomial surgical site infection, 3 (13%) cases of urinary tract infection (UTI), 7 (30.4%) cases of blood stream infection (BSI) and 2 (8.7%) cases of intra-abdominal sepsis. (Table 3)

KPC producing bacterial species identification

By using CHROMagar KPC medium for isolation of carbapenem resistant gram negative bacteria, and by using API 20E test strips as a biochemical identification system for identification of the species of the isolated genera; *Klebsiella* species were the most common bacterial isolates accounted for 34.8 % (8): *Klebsiella pneumoniae* 26.1% (6) and *Klebsiella oxytoca* 8.7% (2), followed by *Pseudomonas* species (21.7%) (5): *Pseudomonas aeruginosa* 17.4% (4) and *Pseudomonas luteola* 4.3% (1), then *Enterobacter* species (17.4%) (4): *Enterobacter sakazakii* 8.7% (2) and *Enterobacter aerogenes* 8.7% (2) and *Escherichia coli* (17.4%) (4). *Acinetobacter baumannii* was the least common species isolated (8.7%) (2) (Table 4, Figure 2).

Of the 23 patients infected by KPC producing Gram negative bacteria; 4 patients (17.4%) died during the study period. These patients shared multiple risk factors as; prolonged hospitalization, treatment with multiple antibiotic courses,

invasive procedures, and associated co-morbidities (Table 5).

Antimicrobial susceptibility pattern of KPC producing isolates

In addition to meropenem, all KPC producing isolates were found to be 100% resistant to ceftriaxone, cefotaxime, ceftazidime, piperacillin, ciprofloxacillin, and aztreonam. High resistance rates were also observed to Cefepime (91.3%), Amoxycillin-clavulanic acid (91.3%), gentamycin (87%), piperacillin-tazobactam (82.6%) and amikacin (74%). However, only (17.4%) of KPC positive isolates were found to be resistant to colistin (Table 6).

Development of resistance to carbapenem antibiotics in association with the potential of KPC production among Gram-negative bacteria have made a challenge and significant problem in the treatment of infections caused by these types of bacteria. Phenotypic detection of KPC-producing strains provides rapid, inexpensive, and accurate results that can prevent spread of this mechanism of resistance. Several phenotypic methods for detection of KPC have been suggested (Gomez *et al.,* 2011).

The early identification of KPC phenotypes often relies on indirect indicators such as reduced susceptibility to carbapenems (Moland *et al.,* 2003). CHROM agar KPC was the first commercially available chromogenic medium designed for isolation of carbapenem-resistant (CR) Gram-negative bacteria. In early studies it showed good performance when compared with in-house preparations of MacConkey agar incorporating imipenem (Adler *et al.,* 2011). In addition to that; the color and morphology characteristics on CHROMagar KPC permit easy differentiation of the bacterial colonies. Samra *et al.* (2008) compared CHROMagar KPC and direct PCR for bla_{KPC} for rapid detection of carbapenem-resistant KPC-producing *Enterobacteriaceae* from rectal swabs. The sensitivity and specificity relative to PCR were 100% and 98.4%, respectively, for CHROMagar KPC. PCR has been successfully utilized for the detection of single or multiple carbapenemase genes directly from clinical samples (Naas *et al.,* 2013).

Obvious advantages include a greater speed of detection and potentially a higher sensitivity than that offered by culture (Singh *et al.,* 2012). Disadvantages include a higher cost for processing samples and the need for specialized equipment and/or expertise. On this background; in this study we used CHROMagar KPC medium for rapid and direct detection of CR Gram negative pathogens from clinical specimens. CHROMagar KPC demonstrated an excellent ability to detect CR Gram negative pathogens.

All bacteria isolated on CHROM agar KPC medium were confirmed as meropenem resistant as detected by the antibiotic susceptibility testing by the disc diffusion method. In our study a total of 59 isolates were carbapenem resistant; and by the color and colony morphology we could differentiate the bacterial growth up to the species level. The highest percentage of resistance to Carbapenems was seen in KEC; 26 (44.1%) (*Klebsiella* spp., *Enterobacter* spp. and *Citrobacter* spp.), followed by *Escherichia coli* 16 (27.1%), *Pseudomonas* Spp.10 (16.9%), and *Acinetobacter* Spp. 7 (11.9%). A study by Ramana *et al.* (2013) showed that, among the different *Enterobacteriaceae* members tested, *Klebsiella* spp. showed the highest percentage of carbapenem resistance at 30%, whereas *Proteus* spp. and *Citrobacter* spp

revealed comparatively low carbapenem resistance of 17% and 12%, respectively. Several tests have been described for phenotypic detection of KPC β -lactamases. A modified version of the cloverleaf (Hodge) test, originally established for detection of penicillinases, accurately detects carbapenemases (Anderson *et al.*, 2007). In our study; of the 59 carbapenem resistant isolates; 52 (88.41%) isolates were found to produce carbapenemase enzyme by MHT. This could be explained as there are other causes of carbapenem resistance among Gram negative bacteria as; overproduction of ESBL or Amp C enzyme with porin loss (Paterson, 2006) which could be the reason in bacterial strains which were carbapenem resistant but negative by MHT. The evaluation of this test showed that it was simple with minimal infrastructure and cost effective reliable assay and could be applied in routine microbiology laboratories in detecting carbapenemase producers.

Although Anderson *et al.* (2007) reported that; it was not specific for KPC production. Another study by Girlich *et al.* (2012) showed that the overall sensitivity and specificity of the MHT in detecting KPC was low (77.4% and 38.9%, respectively). This could be because MHT detects other carbapenemase enzymes in addition to KPC as it is only indicative of enzymatic activity of carbapenemase and cannot differentiate class A carbapenemases from class B MBLs. Thus, a practical, highly sensitive, and highly specific method for the detection of KPC-possessing isolates using boronic acid disk test was used. There is an enhanced affinity of the phenylboronic acid moiety to the active-site serine residue of class A KPC β-lactamase. Boronic acid tests using imipenem, or meropenem as an antibiotic substrate demonstrated an excellent ability to differentiate KPC enzymes (Tsakris *et al.*, 2009). In previous studies the results of using BA as an inhibitor for detection of KPC were completely confirmed by molecular tests (Girlich *et al.*, 2012). In our study KPC activity was detected in 14 (23.73%) isolates by Boronic acid test. The CR isolates from our study were further tested for MBL production by the CDT; and 20 (33.9%) isolates were found to be MBL producers. This study also demonstrated the co-production of KPC and MBL in 9 (15.25%) isolates by disk enhancement test with both PBA and EDTA. The production of both enzymes might contribute to their hydrolytic activity and levels of resistance to broad-spectrum beta-lactams, as well as to the possible co-migration of both enzymes. Moreover, KPC and MBL genes are often co-transferred with plasmid-mediated ESBL, fluoroquinolone and aminoglycoside resistance genes (Chmelnitsky *et al.*, 2008).

However, the remaining MHT positive isolates which were negative for KPCs and MBLs presumably had the other types of carbapenemase enzymes as the oxacillinase enzyme, as the OXA encoding gene has been found to be highly disseminated in the last few years. Not only do KPC-producing organisms hydrolyze carbapenems, but also, they are often resistant to multiple other antibiotics (Van Duin *et al.*, 2013). In our study; the antibiotic susceptibility pattern of KPC positive isolates revealed that the isolates were highly resistant to all commonly used antibiotics. This was well agreed with the observations obtained in other studies (Endimiani *et al.*, 2009). Consequently, they are invariably associated with high treatment failure rates (Tumbarello *et al.*, 2012).

Table.1 Carbapenem resistant isolates detected by the growth of bacteria on CHROM agar KPC medium

No. of isolates (%)	Carbapenem resistant isolates	SSI	UTI	BSI	Intra-abdominal sepsis
26 (44.1)	KEC *(Klebsiella, Enterobacter, Citrobacter)*	15	4	5	2
16 (27.1)	*Escherichia coli*	7	7	2	0
10 (16.9)	*Pseudomonas* Spp.	7	0	1	2
7 (11.9)	*Acinetobacter* Spp.	3	0	4	0
Total 59 (100)		32 (54.2)	11 (18.6)	12 (20.3)	4 (6.8)

Figure.1 KPC producing Gram-negative bacilli on CHROMagar KPC medium: a) Dark pink colonies of E. coli, b) White creamy opaque colonies of *Acinetobacter* spp., c) Metallic blue colonies of KEC "*Klebsiella, Enterobacter,* and *Citrobater spp.*," d) Mixed growth of metallic blue colonies of KEC and translucent cream colonies of *Pseudomonas spp*.

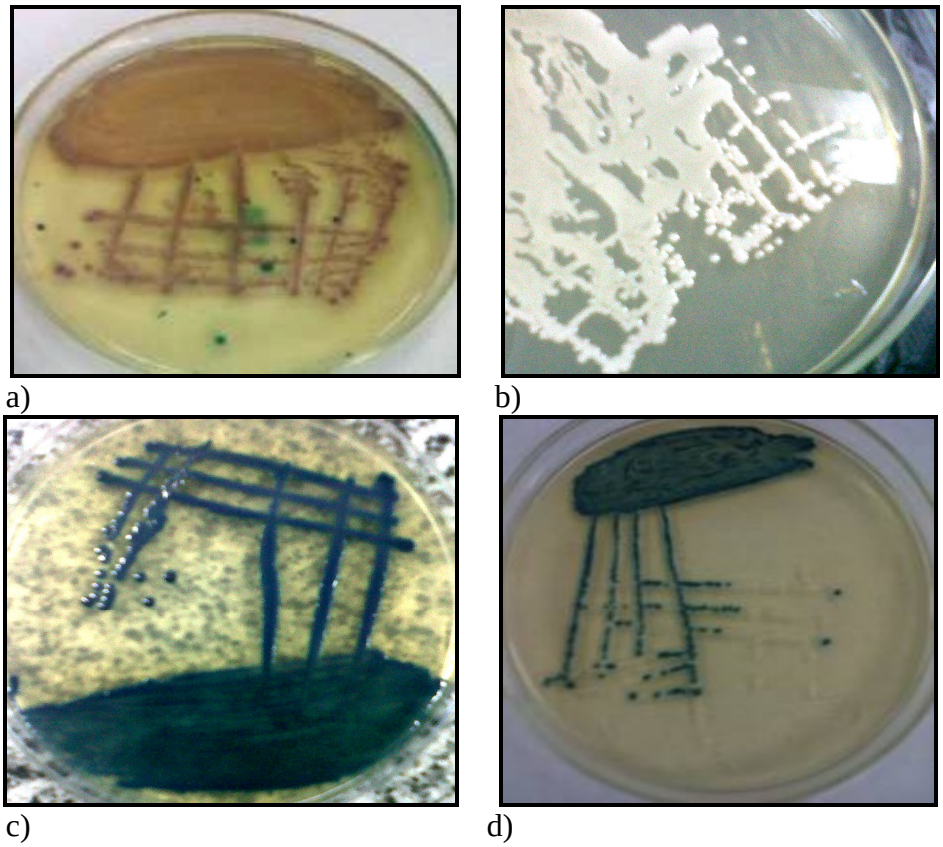


Table.2 The distribution of carbapenemases among Carbapenem resistant Gram negative isolates

Carbapenem resistant isolates	No. of isolates (%)
Carbapenem resistant isolates (Growth on CHROM agar KPC medium)	**59 (100)**
Carbapenemases producers (MHT)	**52 (88.14%)**
KPC producers (PBA test)	**14 (23.73%)**
MBLs producers (CDT)	**20 (33.9%)**
MBLs+ KPC producers	**9 (15.25%)**

Table.3 Data and results of KPC producing isolates

NO. of isolate	KPC producing isolate	Sample site	Age in years	Gender	Length of hospital stay in days
1	*K. pneumoniae*	SSI	55	M	11
2	*E. coli*	UTI	60	M	20
3	*P. aeruginosa*	SSI	47	M	14
4	*E. sakazakii*	BSI	70	F	38
5	*K. pneumoniae*	SSI	42	F	13
6	*E. aerogenes*	BSI	69	M	48
7	*P. aeruginosa*	SSI	53	F	35
8	*P. aeruginasa*	SSI	56	M	45
9	*K. pneumoniae*	SSI	58	M	25
10	*E. sakazakii*	SSI	47	F	15
11	*E. aerogenes*	BSI	72	F	50
12	*E. coli*	UTI	63	M	32
13	*K. pneumoniae*	SSI	51	F	21
14	*E. sakazakii*	BSI	62	M	33
15	*A. baumannii*	BSI	65	M	31
16	*K. oxytoca*	peritonitis	70	M	18
17	*P. aeruginosa*	SSI	64	F	15
18	*E. coli*	UTI	66	F	21
19	*P. luteola*	peritonitis	79	M	48
20	*E. coli*	BSI	61	M	23
21	*E. sakazakii*	SSI	55	F	45
22	*K. oxytoca*	SSI	47	M	39
23	*A. baumannii*	BSI	60	M	47

Table.4 The distribution of KPC- producing bacterial species

KPC producing Isolates	No. (%)	Species	No. (%)
***Klebsiella* spp.**	8(34.8)	***K. peumoniae***	6(26.1)
		K. oxytoca	2(8.7)
***Pseudomonas* spp.**	5(21.7)	***P. aeruginosa***	4(17.4)
		P. luteola	1(4.3)
***Enterobacter* spp.**	4(17.4)	***E. aerogenes***	2(8.7)
		E. sakazakii	2(8.7)
Escherichia coli	4(17.4)	***E. coli***	4(17.4)
***Acinetobacter* spp.**	2(8.7)	***A. baumannii***	2(8.7)
Total	23(100)		23(100)

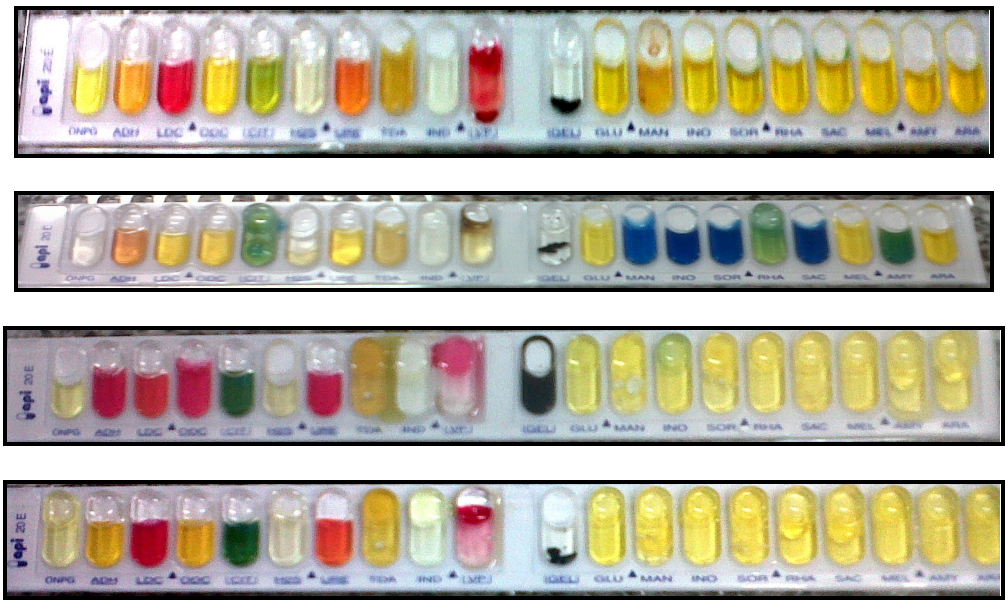
Table.5 Health care-associated infections (HAI) caused by KPC producing isolates

HAI	NO. of isolates	Outcome	KPC producing isolate
Infected surgical wound	**11**	**Recovered**	***P. aeruginosa* (4)**
			***K. pneumoniae* (4)**
			***K. oxytoca* (1)**
			***E. sakazakii* (2)**
UTI	**3**	**Recovered**	***E. coli* (3)**
Blood stream infection	**7**	**2 Died**	***A. baumannii* (2)**
		5 Recovered	***E. aerogenes* (2)**
			***E. coli* (1)**
			***K. peumoniae* (2)**
Intra-abdominal sepsis	**2**	**2 Died**	***P. luteola* (1)**
			***K. oxytoca* (1)**

Table.6 Antimicrobial susceptibility pattern of 23 KPC producing isolates

Antibiotics	Antibacterial class	Number of resistant isolates (%)
Meropenem	Carbapenems	23(100)
Piperacillin	Extended-spectrum –β lactams	23(100)
Ceftazidime	Third generation Cephalosporin	23(100)
cefotaxime	Third generation Cephalosporin	23(100)
Ceftriaxone	Third generation Cephalosporin	23(100)
Aztreonam	β - lactams	23(100)
Ciprofloxacin	Fluoroquinolone	23(100)
Amoxycillin-clavulanic acid	β-lactam/inhibitor combination	21(91.3)
Cefepime	Fourth generation Cephalosporin	21(91.3)
Gentamycin	Amino glycosides	20(87)
Piperacillin-tazobactam	β-lactam/inhibitor combination	19 (82.6)
Amikacin	Amino glycosides	17(74)
colistin	Polymixin E	4(17.4)

Figure.2 API 20E strips with a panel that was identified as: a) *Klebsiella pneumoniae*, b) Pseudomonas luteola, c) Klebsiella oxytoca, d) Enterobacter sakazakii



a)

b)

c)

d)

Figure.3 Antibiotic susceptibility testing showing KPC producing *Klebsiella pneumoniae* with extreme-drug resistance pattern

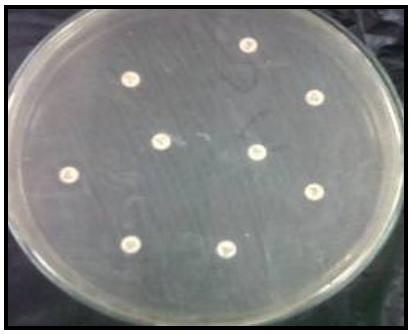
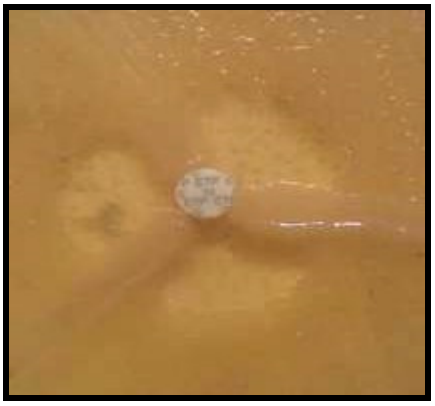


Figure.4 (a&b): Modified Hodge Test: "clover leaf"-shaped inhibition zones indicating carbapenemase production by the tested isolate

a)



b)

Figure.5 PBA disk test; with increase of growth inhibitory zone diameter around meropenem& PBA disk ≥5 mm as compared with the meropenem disk alone indicating KPC producing isolate

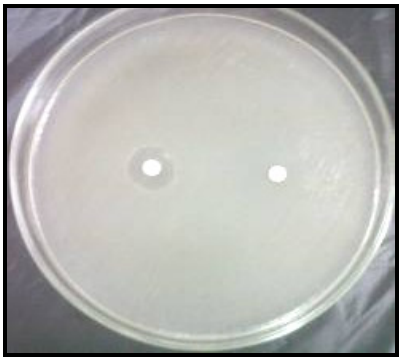
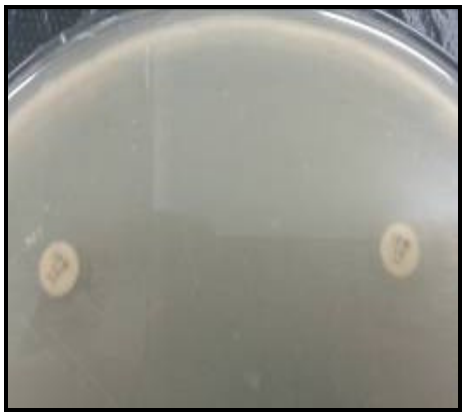


Figure.6 Combined disk test (CDT): a) Negative CDT with absence of inhibition zone around IPM plus EDTA disk. b) Positive CDT with enhanced inhibition zone of ≥7 mm around IPM plus EDTA disc indicating MBL producing isolate



a)

b)

In our study, colistin turned out to be the most effective antimicrobial against KPC producing isolates followed by amikacin. The best therapeutic approach to KPC-producing organisms has yet to be defined; however, common treatments based on in vitro susceptibility testing are the polymyxins, tigecycline, and less frequently aminoglycoside antibiotics (Arnold *et al.*, 2011). Given the limited therapeutic options, it is crucial to control the spread of these organisms. In our hospital, the implementation of infection control measures was insufficient and new cases have been reported.

Present results mandate for strong and prompt intervention in our setting. The prevalence of those pathogens in hospitals with the risk for further propagation in the community is of great concern. Hospitals must be prepared so that they can identify these organisms early and institute enhanced infection control efforts when necessary.

The present study revealed the occurrence of carbapenem resistance due to a KPC in our hospital setting and this could not be ignored. It also validated simple, cost effective and highly sensitive phenotypic methods can be employed in any laboratory to both screen for and confirm the presence of this important mechanism of antimicrobial resistance. This will further help in timely implementation of strict infection control practices as well as clinical guidance regarding the potential risks for therapeutic failure.

References

Adler, A., Navon-Venezia, S., Moran-Gilad, J., Marcos, E., Schwartz, D., Carmeli, Y. 2011. Laboratory and clinical evaluation of screening agar plates for detection of carbapenem-resistant Enterobacteriaceae from surveillance rectal swabs. *J. Clin. Microbiol.*, 49: 2239–42.

Amjad, A., Mirza, I.A., Abbasi, S.A., Farwa, U., Malik, N., Zia, F. 2011. Modified Hodge test: A simple and effective test for detection of carbapenemase production. *Iran. J. Microbiol.*, 3(4): 189–93.

Anderson, K.F., Lonsway, D.R., Rasheed, J.K. 2007. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in Enterobacteriaceae. *J. Clin. Microbiol.*, 45: 2723–2725.

Arnold, R.S., Thom, K.A., Sharma, S., Phillips, M., Johnson, J.K., Morgan, D.J. 2011. Emergence of *Klebsiella pneumoniae* Carbapenemase (KPC)-Producing Bacteria. *South Med. J.*, 104(1): 40–45.

Bansal, M., Vyas, N., Sharma, B., Maheshwari, R.K. 2013. Differentiation of Carbapenemase producing Enterobacteriaceae by Triple disc Test. *Indian J. Basic Appl. Med. Res.*, 3(1): 314–320.

Bratu, S., Tolaney, P., Karumudi, U. 2005. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, NY: molecular epidemiology and in vitro activity of polymyxin B and other agents. *J. Antimicrob. Chemother.*, 56: 128–132.

Canton, R., Akova, M., Carmeli, Y., Giske, C.G., Glupczynski, Y., Gniadkowski, M. 2012. Rapid evolution and spread of carbapenemases among Enterobacteriaceae in Europe. *Clin. Microbiol. Infect.*, 18(5): 413–31.

Centers for Disease Control and Prevention (CDC), 2009. Guidance for control of infections with carbapenem-resistant or carbapenemase-producing Enterobacteriaceae in acute care facilities. *Morb. Mortal Wkly Rep.*, 58: 256–260.

Chmelnitsky, I., Navon-Venezia, S., Strahilevitz, J. 2008. Plasmid-mediated qnrB2 and carbapenemase gene blaKPC-2 carried on the same plasmid in carbapenem-resistant ciprofloxacin-susceptible Enterobacter cloacae isolates. *Antimicrob. Agents Chemother.*, 52: 2962–5.

CHROMagar™ KPC, 2013. For detection of Gram-negative bacteria with a reduced susceptibility to most of the carbapenem agents.

Endimiani, A., Hujer, A.M., Perez, F. 2008. Characterization of blaKPC-containing *Klebsiella pneumoniae* isolates detected in different institutions in the Eastern USA. *J. Antimicrob. Chemother.*, 63: 427–37.

Gasink, L.B., Edelstein, P.H., Lautenbach, E. 2009. Risk factors and clinical impact of *Klebsiella pneumoniae* carbapenemase-producing K. pneumoniae. *Infect. Control Hosp. Epidemiol.*, 30: 1180–1185.

Girlich, D., Poirel, L., Nordmann, P. 2012. Value of the modified Hodge test for detection of emerging carbapenemases in Enterobacteriaceae. *J. Clin. Microbiol.*, 50(2): 477–9.

Gomez, S.A., Pasteran, F.G., Faccone, D. 2011. Clonal dissemination of Klebsiella pneumonia ST258 harbouring KPC-2 in Argentina. *Clin. Microbiol. Infect.*, 17: 1520–1524.

Maltezou, H.C., Giakkoupi, P., Maragos, A., Bolikas, M., Raftopoulos, V., Papahatzaki, H., Vrouhos, G., Liakou V., Vatopoulos, A.C. 2009. Outbreak of infections due to KPC-2-producing *Klebsiella pneumoniae* in a hospital in Crete (Greece). *J. Infect.*, 58: 213–219.

Moland, E.S., Hanson, N.D., Herrera, V.L., Black, J.A., Lockhart, T.J., Hossain, A., Johnson, J.A., Goering, R.V., Thomson, K.S. 2003. Plasmid-mediated, carbapenem-hydrolysing β-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. *J. Antimicrob. Chemother.*, 51: 711–4.

Naas, T., Cotellon, G., Ergani, A., Nordmann, P. 2013. Real-time PCR for detection of blaOXA-48 genes from stools. *J. Antimicrob. Chemother.*, 68: 101–4.

Nadkarni, A.S., Schliep, T., Khan, L. 2009. Cluster of bloodstream infections caused by KPC-2 carbapenemase-producing *Klebsiella pneumoniae* in Manhattan. *Am. J. Infect. Control*, 37: 121–126.

Nordmann, P., Dortet, L., Poirel, L. 2012. Carbapenem resistance in Enterobacteriaceae: here is the storm. *Trends Mol. Med.*, 18(5): 263–72.

Patel, G., Huprikar, S., Factor, S.H. 2008. Outcomes of carbapenem-resistant Klebsiella pneumonia infection and the impact of antimicrobial and adjunctive therapies. *Infect. Control Hosp. Epidemiol.*, 29: 1099–1106.

Paterson, D.L. 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. *Am. J. Infect. Control.*, 34: 20–28.

Ramana, K.V., Rao, R., Sharada, V., Kareem, M.A., Reddy, L.R., Ratna Mani, M.S. 2013. Modified Hodge test: A useful and the low cost phenotypic method for detection of carbapenemase producers in Enterobacteriaceae members. *J. Natural Sci. Biol. Med.*, 4(2): 346–48.

Robledo, I.E., Aquino, E.E., Sante´, M.I., Santana, J.L., Otero, D.M., Leo´n, C.F., Va´zquez, G.J. 2010. Detection of KPC in *Acinetobacter* spp. in Puerto Rico. *Antimicrob. Agents Chemother.*, Pp. 1354–1357.

Samra, Z., Bahar, J., Madar-Shapiro, L., Aziz, N., Israel, S., Bishara, J. 2008. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant Enterobacteriaceae. *J. Clin. Microbiol.*, 46: 3110–1.

Singh, K., Mangold, K.A., Wyant, K., Schora, D.M., Voss, B., Kaul, K.L. 2012. Rectal screening for *Klebsiella pneumoniae* carbapenemases: comparison of real-time PCR and culture using two selective screening agar plates. *J. Clin. Microbiol.*, 50: 2596–600.

The Clinical and Laboratory Standards Institute, 2012. Performance Standards for Antimicrobial Susceptibility testing, twenty second informational supplement, M100-S22, Clinical and Laboratory Standards Institute.

Tsakris, A., Kristo, I., Poulou, A. 2009. Evaluation of boronic acid disk tests for differentiating KPC-possessing *Klebsiella pneumoniae* isolates in the clinical laboratory. *J. Clin. Microbiol.*, 47: 362–367.

Tsakris, A., Poulou, A., Pournaras, S., Voulgari, E., Vrioni, G., Themeli-Digalak, K., Dimitra Petropoulou, D., Sofianou, D. 2010. A simple phenotypic method for the differentiation of metallo-beta-lactamases and class A KPC carbapenemases in Enterobacteriaceae clinical isolates. *J. Antimicrob. Chemother.*, 65: 1664–1671.

Tumbarello, M., Viale, P., Viscoli, C., Trecarichi, E.M., Tumietto, F., Marchese, A., Spanu, T., Ambretti, S., Ginocchio, F., Cristini, F., Losito, A.R., Tedeschi, S., Cauda, R., Bassetti, M. 2012. Predictors of mortality in bloodstream infections caused by *Klebsiella pneumoniae* carbapenemaseproducing K. pneumoniae: importance of combination therapy. *Clin. Infect. Dis.*, 55: 943–950.

Van Duin, D., Kaye, K.S., Neuner, E.A., Bonomo, R.A. 2013. Carbapenem resistant Enterobacteriaceae: a review of treatment and outcomes. *Diagn. Microbiol. Infect. Dis.*, 75: 115–120.

Weisenberg, S.A., Morgan, D.J., Espinal-Witter, R. 2009. Clinical outcomes of patients with *K. pneumoniae* carbapenemase-producing K pneumoniae after treatment with imipenem or meropenem. *Diagn. Microbiol. Infect. Dis.*, 64: 233–235.

Yong, D., Lee, K., Yum, J.H., Shin, H.B., Rossolini, G.M., Chong, Y. 2002. Imipenem EDTA disc method for differentiation of metallo beta lactamase producing clinical isolates of *Pseudomonas spp* and *Acinetobacter spp*. *J. Clin. Microbiol.*, 40: 3798–3801.

Yong, D., Toleman, M.A., Giske, C.G., Cho, H.S., Sundman, K., Lee, K., Walsh, T.R. 2009. Characterization of a new metallo-β-lactamase gene, blaNDM-1, and a novel erythromycin esterase gene carried on a unique genetic structure in Sequence Type 14 from India. *Antimicrob. Agents Chemother.*, 53: 5046–5054.