

RESEARCH ARTICLE

## Frequency of Carbapenemase Producing *Klebsiella pneumoniae* in Makkah, Saudi Arabia

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### ABSTRACT

**Objective:** Carbapenemase producing *Klebsiella pneumoniae* is emerging as an important pathogen worldwide which has alarming clinical threat with serious effects on patients' outcome. The aim of our study is to determine prevalence of carbapenemase producing *K. pneumoniae* and its antibiotic susceptibility pattern in tertiary care hospitals.

**Methods:** A prospective study was performed on 260 clinical isolates of *K. pneumoniae* collected from five different hospitals of Makkah, Saudi Arabia from January to July 2015. All clinical isolates were confirmed as *K. pneumoniae* using Vitek 2 system. These isolates were then screened for potential carbapenemase producers by determining reduced susceptibility to carbapenems using representative antibiotic disks of third generation cephalosporins and carbapenems in the disk diffusion test following guidelines of Clinical and Laboratory Standards Institute. Minimum inhibitory concentration of isolates found positive in screening test were determined for cephalosporins, carbapenems, aminoglycosides and tigecycline and colistin using Vitek-2 system. Modified Hodge test was performed to detect carbapenemase production on the isolates suspected for carbapenemase production.

**Results:** Out of 260 *K. pneumoniae* isolates, 31 (11.9%) have shown 100% resistance to all cephalosporins. Of 31 *K. pneumoniae* isolates, 15 (48.4%) were found positive for carbapenemase. All carbapenem resistant isolates were sensitive to colistin and tigecycline. However, these isolates have shown resistance to amikacin (41.9%) and gentamicin (51.6%), respectively.

**Conclusion:** This is the first report from Makkah reporting carbapenemase producing *K.pneumoniae*. Of 12% potential carbapenemase producing *K.pneumoniae*, 48.4% were found positive for carbapenemase production. Molecular characterization of these strains will help to determine the type of carbapenemase prevalent in this area. *J Microbiol Infect Dis* 2016;6(3): 121-127

**Key words:** *Klebsiella pneumoniae*; carbapenemase; drug resistance; Modified Hodge test

### INTRODUCTION

Carbapenems are usually used to treat infections caused by Gram-negative bacteria that produce extended-spectrum  $\beta$ -lactamases. Multiple drug resistant and carbapenemase producing *K. pneumoniae* is emerging as an important pathogen worldwide, which has alarming clinical threat with serious effects on patient outcomes [1]. According to global antibiotic resistance estimates published by the World Health Organization in 2014, *Klebsiella pneumoniae* and *Escherichia coli* were listed as two of the three agents of greatest concern, associated with both hospital and community acquired infections [2].

Among the *Enterobacteriaceae*, clinically significant carbapenemases are the Ambler molecular class A (KPC), class B (VIM, IMP, NDM), and class D (OXA-48) types [3]. Among them, two clinically significant carbapenemase subclasses; *K. pneumoniae* carbapenemase (KPC) and New Delhi metallo- $\beta$ -lactamase-1 (NDM-1) are of particular importance [4].

The KPC which was first identified in 2001 in USA [5] remained limited to USA till 2005. Since then, the spread of KPC has accelerated worldwide and KPC-producing strains are now emerging worldwide [6,7] and also become endemic in many parts of the world, including the United States,

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Greece, South America, and China [8]. The NDM-1 was first identified in 2008 in a *K. pneumoniae* isolate recovered from a Swedish patient who has been previously hospitalized in New Delhi, India [9]. Soon after this, NDM-1 was found throughout India, Pakistan, and in the United Kingdom [10] and are increasingly being reported worldwide [11]. Although, India was considered as the main reservoir of the NDM-1-producers, several NDM-1-producing enterobacterial isolates have been reported from the Balkan states [12] suggesting that this is another reservoir of NDM-1 producers.

Recent studies conducted in Middle East countries also suggests that this region might be an additional reservoir of NDM producers as it has been reported from Oman [13], Lebanon [14], Saudi Arabia [15,16], and Qatar [17].

The Modified Hodge Test (MHT) has been used extensively as a phenotypic method for the detection of carbapenemase activity and it is the only carbapenemase detection method recommended by the Clinical and Laboratory Standard Institute (CLSI) for screening purposes [18]. Molecular detection of carbapenemase genes using polymerase chain reaction (PCR) based methods was found to be quick, accurate and effective in the detection of specific carbapenemases genes, such as KPC, NDM-1, OXA-48, etc. [13-17]. Simple PCR assays targeting a single carbapenemase type have been used successfully in numerous studies. It has also been reported that PCR tests have the highest specificity in identification and confirmation of the carbapenemases [5,10,12].

In coming years, carbapenemase producing *K. pneumoniae* can cause considerable clinical problems because they are multidrug resistant, lacking susceptibility to  $\beta$ -lactam antibiotics, fluoroquinolones, and aminoglycosides. Thus, therapy for clinically significant isolates rests on the use of tigecycline or polymyxins (colistin), both of which have been associated with the development of resistance and are potentially toxic [19].

Since carbapenemase producing *K. pneumoniae* is an emerging pathogen worldwide which has serious clinical and infection control implications, therefore, this study was designed with the objective to determine the frequency of carbapenemase producing *K. pneumoniae* in various tertiary care hospitals.

## METHODS

### **Bacterial isolates**

This prospective descriptive study was carried out from January to July 2015 at Microbiology Laboratory of Department of Laboratory Medicine, Faculty of Applied Medical Sciences, Umm Al Qura University, Makkah, Saudi Arabia after the approval of institutional Bioethical Committee. A total of 260 *K. pneumoniae* clinical isolates were collected from five different hospitals of Makkah city during the study period. The distribution of these isolates among participating hospitals was: Al-Noor Specialist hospital (n=92); King Faisal hospital (n=50); King Abdul Aziz hospital (n=45); Hera'a General hospital (n=36); Maternity and Children hospital (n=37).

### **Identification of clinical isolates as *K. pneumoniae***

All clinical isolates collected from the hospitals were identified using standard microbiological methods. Briefly, isolates were sub cultured on blood agar plates and incubated at 37 °C for 16-24 hours. Following incubation, colonies were processed for identification of *K. pneumoniae* using Vitek 2 compact system (BioMerieux, USA).

### **Screening of *K. pneumoniae* isolates for suspected carbapenemase production**

All *K. pneumoniae* isolates were first screened for reduced susceptibility to carbapenems by disk diffusion method following guidelines of CLSI [18]. Following antibiotic disks (Oxoid, UK) were used in the disk diffusion susceptibility test: ceftazidime (30  $\mu$ g), ceftriaxone (30  $\mu$ g), cefepime (30  $\mu$ g), imipenem (10  $\mu$ g) and meropenem (10  $\mu$ g). Using standard interpretive table of zone diameter breakpoint of CLSI, the test organism was classified as susceptible, intermediate or resistant.

According to CLSI guidelines *K. pneumoniae* that are resistant to one or more of the third generation cephalosporins e.g., cefotaxime, ceftriaxone, and/or ceftazidime and have reduced zones of sensitivity to carbapenem (e.g. ertapenem 19-21 mm and/or meropenem 16-21 mm) are suspected for carbapenemase production. Alternately, MICs 2  $\mu$ g/ml for ertapenem and 2-4  $\mu$ g/ml for meropenem may indicate carbapenemase production. *K. pneumoniae* isolates which fulfilled these criteria were subjected to MHT for the detection of carbapenemase production.

### Detection of carbapenemase production in suspected isolates

All suspected isolates (from screening step) of *K. pneumoniae* were tested for the production of carbapenemase by MHT as described in CLSI guidelines [18] and results were interpreted accordingly. The MHT was performed as follows: First of all bacterial suspension of the carbapenem susceptible strain of *E.coli* ATCC 25922 was prepared in 5ml sterile saline and turbidity was adjusted to 0.5 McFarland using turbidity meter Densichex plus (BioMerieux). This suspension was then diluted to 1:10 using sterile saline. This diluted suspension of *E.coli* ATCC 25922 was then swabbed onto labeled Mueller Hinton agar plate. The plate was allowed to dry for 3-5 minutes. Then, a 10µg meropenem susceptibility disk was placed in the centre of the plate. *K. pneumoniae* ATCC BAA 1705 and *K. pneumoniae* ATCC BAA 1706 were used as positive and negative control, respectively. After this, positive and negative controls and test microorganisms were streaked in a straight line from the edge of the disk to the edge of the plate. The plate was then incubated at 37°C for 16-18 hours. After this incubation period, plate was examined for a cloverleaf-like indentation at the joining of the test organism and the *E. coli* ATCC 25922, within the zone of inhibition of the carbapenem susceptibility disk.

A positive MHT test had a clover leaf-like indentation of the *E. coli* ATCC 25922 growing along the test organism growth streak within the zone of inhibition because the carbapenemase produced by *K. pneumoniae* isolate inactivated meropenem that diffused into the medium. Thus there was not sufficient meropenem present to inhibit the growth of *E. coli*

ATCC 25922 and indentation of zone was noted. A positive MHT indicated carbapenemase production by the test microorganism. A negative MHT test has no growth of the *E. coli* ATCC 25922 along the test organism growth streak within the zone of inhibition. A negative test indicated no carbapenemase production by the test microorganism.

### Antibiotic susceptibility test of carbapenem resistant isolates

Minimum inhibitory concentration of isolates which were found positive in screening test were determined for ceftazidime, ceftriaxone, cefepime, imipenem, meropenem, amikacin, gentamicin, tigecycline and colistin using gram-negative antibiotic susceptibility panel AST-N292 of Vitek-2 system (BioMerieux, USA). The data of all isolates was entered and analyzed using Microsoft Excel 2007.

## RESULTS

### *K. pneumoniae* isolates screened for suspected carbapenemase production

In this study a total of 260 *K. pneumoniae* strains isolated at five different hospitals of Makkah were screened for potential carbapenemase producers using third generation cephalosporins and carbapenem disks. The drug resistance to cephalosporin ranged from 29.2% to 42.7%, whereas among carbapenems; both imipenem and meropenem showed 11.9% resistance, each (Table 1). Among cephalosporins, ceftriaxone showed highest resistance (42.7%) followed by cefepime (30.9%) and ceftazidime (29.2%). Out of 260 *K. pneumoniae* isolates 31 (11.9%) isolates showed 100% resistance to all cephalosporins and carbapenems tested.

**Table 1.** Screening results of *K. pneumoniae* isolates for potential carbapenemase production.

Hospitals	CFZ	CTX	CFP	IMP	MRN	Pan resistance
Al-Noor Specialist Hospital (n=92)	29 (31.5)	42 (45.6)	25 (27.2)	13 (14.1)	13 (14.1)	13 (14.1)
King Faisal Hospital (n=50)	15 (30.0)	29 (58.0)	11 (22.0)	3 (6.0)	3 (6.0)	3 (6.0)
King Abdul Aziz Hospital (n=45)	16 (35.5)	21 (46.6)	14 (31.1)	10 (22.2)	10 (22.2)	10 (22.2)
Hera'a General Hospital (n=36)	12 (33.3)	16 (44.4)	12 (33.3)	3 (8.3)	3 (8.3)	3 (8.3)
Maternity and Children Hospital (n=37)	4 (10.8)	3 (8.1)	3 (8.1)	2 (5.4)	2 (5.4)	2 (5.4)
Overall drug resistance (n=260)	76 (29.2)	111 (42.7)	65 (30.9)	31 (11.9)	31 (11.9)	31 (11.9)

CFZ=Ceftazidime, CTX=Ceftriaxon, CFP=Cefepim, IMP=Imipenem, MER=Meropenem, Pan resistance=Pan-resistance to pan resistance and add cephalosporins and carbapenems

**Table 2.** Minimum inhibitory concentration of carbapenem resistant *K. pneumoniae* and results of Modified Hodge Test.

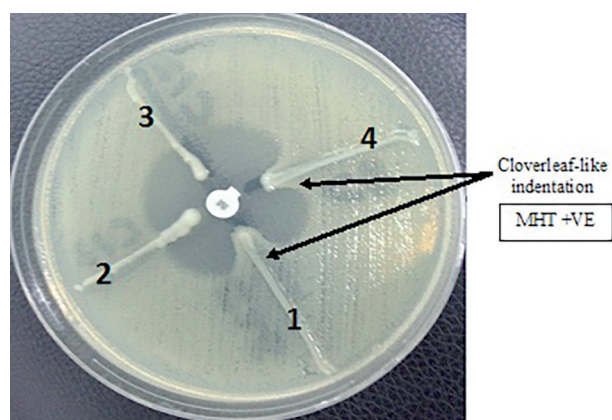
Isolate No	CFZ	CTX	CEF	IMP	MER	AK	GEN	COL	TIG	MHT
C-1	>64	>64	>64	>16	>16	16	4	<0.5	<0.5	+ve
C-2	>16	>64	>16	>8	>8	<16	>8	<0.5	<0.5	+ve
C-3	>64	>64	>64	>16	>16	16	<1	<0.5	<0.5	-ve
C-4	>64	>64	>64	>16	>16	16	<1	<2	2	+ve
C-5	>64	>64	>64	>16	>16	16	<1	<0.5	<0.5	+ve
C-6	>64	>64	>64	>16	>16	16	<1	<0.5	<0.5	+ve
C-7	>64	>64	>64	>16	>16	16	<1	<0.5	<0.5	+ve
C-8	>16	>64	>64	>16	>16	16	4	<0.5	<0.5	+ve
C-9	>16	>64	>64	>16	>16	16	4	<0.5	2	-ve
C-10	>64	>64	>64	8	>16	>64	>16	<2	2	-ve
C-11	>64	>64	>64	8	>16	>64	>16	<2	2	-ve
C-12	>64	>64	>64	>16	>16	>64	>16	<0.5	<0.5	-ve
C-13	>64	>64	>64	>16	>16	>64	>16	<0.5	<0.5	-ve
C-14	>64	>64	>64	4	>16	>64	>16	<0.5	<0.5	-ve
C-15	>64	>64	>64	>16	>16	>64	>16	2	2	-ve
C-16	>64	>64	>64	8	>16	>64	>16	<0.5	<0.5	-ve
C-17	>64	>64	>64	>16	>16	>64	>16	<0.5	<0.5	-ve
C-18	>64	>64	>64	>16	>16	>64	>16	<0.5	<0.5	-ve
C-19	>64	>64	>64	8	>16	8	>16	<2	2	+ve
C-20	>64	>64	>64	>16	>16	4	>16	<0.5	<0.5	+ve
C-21	>64	>64	>16	8	>16	16	4	<0.5	<0.5	-ve
C-22	>64	>64	>16	>16	>16	16	4	<0.5	2	-ve
C-23	>64	>64	>64	>16	>16	64	>16	<0.5	<0.5	+ve
C-24	>64	>64	>16	4	>16	8	4	<2	<0.5	+ve
C-25	>64	>64	>64	>16	>16	16	4	<2	2	+ve
C-26	>64	>64	>64	2	2	16	4	<0.5	<0.5	+ve
C-27	>64	>64	>64	>16	>16	16	4	<0.5	<0.5	-ve
C-28	>64	>64	>16	>16	>16	64	>16	<2	2	-ve
C-29	>64	>64	>16	>16	>16	64	>16	<0.5	<0.5	-ve
C-30	>64	>64	>64	8	>16	64	>16	<0.5	<0.5	+ve
C-31	>64	>64	>64	8	>16	16	4	<0.5	<0.5	+ve
	R=31	R=31	R=31	R=31	R=31	R=13 (41.9%)	R=16 (51.6%)	R=0	R=0	

CFZ=Ceftazidime, CTX=Ceftriaxone, CFP=Cefepime, IMP=Imipenem, MER=Meropenem, AK=Amikacin, GEN=Gentamicin, COL=Colistin, TIG=Tigecycline, Modified Hodge Test (MHT)

Variable drug resistance pattern for cephalosporins and carbapenems was seen in participating hospitals (Table 1). In general, low rate of drug resistance was seen at Maternity and Children Hospital both for cephalosporins (8.1-10.8%) and carbapenems (5.4%). Among cephalosporins, high resistance to ceftriaxone (44.4-58%) followed by (30-35.5%) resistance to ceftazidime was seen at all participating hospitals. Among carbapenems, the high rate of resistance both to imipenem and meropenem was seen both at King Abdul Aziz hospital (22.2%) and Al-Noor Specialist hospital (14.1%), and low rates at other three hospitals (5.4-8.3%).

#### Minimum Inhibitory concentration of carbapenem resistant *K. pneumoniae* and results of Modified Hodge Test

Out of 260 *K. pneumoniae* isolates studied, 31 (11.9%) showed 100% resistance to all cephalosporins and carbapenems tested (Table 1). Most of the strains exhibited high MIC values both for cephalosporins ( $\geq 64$ ) and carbapenems ( $\geq 8$ ) indicating that these could be possible carbapenemase producers. Among aminoglycosides, resistance to amikacin was 41.9% and 51.6% to gentamicin. None of the isolates has shown resistance either to colistin or tigecycline (Table 2). Of these 31 isolates, fifteen isolates (48.4%) were found positive for carbapenemase production (Figure 1 and Table 2) and 16 were negative using MHT.



**Figure 1.** Modified Hodge Test for Carbapenemase Detection in Enterobacteriaceae.

## DISCUSSION

In the last decade, the frequent occurrence of infections due to gram-negative organisms, particularly by *K. pneumoniae* resistant to third generation cephalosporins has resulted in the use of carbapen-

ems for the treatment [20]. In the current study, 260 *K. pneumoniae* isolates showed drug resistance to third generation cephalosporin ranging from 29.2% to 42.7%. These results are comparable to a study done in Latin America where resistance rate to third generation cephalosporins was found to be between 30-50% [21]. A study from Saudi Arabia also showed similar results of drug resistance: ceftriaxone (31%), ceftazidime (28%) and cefotaxime (27%) and cefuroxime (27%) [22].

Imipenem is frequently used to treat infections caused by multidrug-resistant *K. pneumoniae*; in particular those resistant to third generation cephalosporin. In our study, resistance to both imipenem and meropenem was 11.9%. The resistance to meropenem showed in our study is comparable with the studies carried out in Turkey [23] where resistance to meropenem was 9%. Regarding imipenem resistance, this study showed 11.9% resistance which is similar to reported earlier from Saudi Arabia (9%) [22].

Production of carbapenemases is an increasing problem among clinical isolates of gram-negative bacilli. In particular, the spread of carbapenemase producing *K. pneumoniae* represents a serious threat to treatment protocols and has infection control implications [1]. Studies reported an increasing trend in the prevalence of carbapenemase producing *K. pneumoniae* from 2006 to 2010 [24] and continue to rise [7].

In Saudi Arabia, although carbapenem resistance remained uncommon among *Enterobacteriaceae*, outbreak due to carbapenem resistant organism has been reported. The first outbreak of carbapenems-resistant *K. pneumoniae* in Saudi Arabia was reported during 2010 in an intensive care unit of a tertiary care hospital, which involved 20 patients. The molecular basis of resistance was not reported [25]. However, in another study, investigators [15] detected OXA-48 and NDM enzymes in carbapenem resistant *K. pneumoniae* isolated at Riyadh hospitals in 2011. Recent study from Riyadh confirmed the emergence of OXA-48 and NDM-1 among *K. pneumoniae* in which 12 out of 60 *K. pneumoniae* strains were NDM-1 producers and 47/60 strains were positive for OXA-48. No strain was positive for KPC [16]. Compared to above mentioned studies, current study reports a high percentage of *K. pneumoniae* (48.4%) isolates to be carbapenemase producers determined by Modified Hodge Test (MHT). The limitation of this study is that the type of carbapenemase produced by these isolates cannot be determined and further characterization using

molecular methods will detect the type of carbapenemase produced by these strains. However, it indicates that there is an upsurge of carbapenemase producing *K. pneumoniae* in these settings.

The antibiotic susceptibility profile of carbapenem resistant *K. pneumoniae* isolates of this study is similar to those reported for multidrug-resistant organisms producing carbapenemase from Saudi Arabia [15,16] and other countries e.g., USA [26], Brazil [27] and China [28]. Patients infected with carbapenemase producing organisms are difficult to treat due to limited treatment options currently available e.g., tigecycline, colistin and fosfomycin, but these also have limitations [29]. Fortunately, our all carbapenemase producing strains were sensitive to both colistin and tigecycline. However, the resistance to colistin has recently been reported in *K. pneumoniae* [19]. These limitations warrant the need to search for new alternate treatment options.

The present study appears to be first from Makkah, Saudi Arabia to report carbapenemase producing *K. pneumoniae* from this area. Out of 31 potential carbapenemase producers, fifteen strains were found positive for carbapenemase production by MHT and of these; one strain (C-26) has exhibited reduced susceptibility to carbapenems (2 µg/ml) and was also found positive by MHT. This validates the findings that the isolate with reduced susceptibility to carbapenems can produce carbapenemase [30].

In conclusion, the present study reported that about 12% *K. pneumoniae* isolates were 100% resistant to both 3<sup>rd</sup> generation cephalosporins and carbapenems and 48.4% were positive for carbapenemase production using MHT. Reporting of carbapenemase producing *K. pneumoniae* in this study necessitates the initiation of surveillance study both at local and national level to determine the actual prevalence of carbapenemase producing *K. pneumoniae* in Makkah and Saudi Arabia, respectively. Further characterization of these strains by molecular methods will determine the prevalent type of carbapenemase in this area.

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