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Full Length Article

Prevalence and characterization of carbapenem-resistant *Klebsiella pneumoniae* isolated from intensive care units of Mansoura University hospitals



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1. Introduction

*Klebsiella pneumoniae* (*K. pneumoniae*) is a Gram negative, facul- tative anaerobic bacilli that has great potential for significant mor- bidity and mortality in acute care settings, particularly in immunocompromised patients [[1]](#_bookmark5). Moreover, *K. pneumoniae* is one of the most common organisms showing multiple antibiotic resistance worldwide [[2]](#_bookmark20). These bacteria easily acquire and trans- fer drug resistance genes through plasmids and transposons [[3]](#_bookmark22). Acquisition of these genes leads to production of b-lactamases of which extended spectrum b-lactamases (ESBLs) are the most com- mon [[3]](#_bookmark22). ESBLs are capable to hydrolyze extended spectrum peni- cillins, cephalosporins and monobactams, leaving the carbapenem group of b-lactam antibiotics as the only choice for therapy, hence carbapenem antibiotics are used as a last resort to treat infections caused by these multidrug resistant organisms [[4,5]](#_bookmark23). However, there has been emergence of carbapenem resistant *Enterobacteri- aceae*, most commonly carbapenem resistant *K. pneumoniae* (CRKP), which have a worldwide prevalence [[6]](#_bookmark6), due to high antibi- otic use, self-medication by patients and lack of implementation of antibiotic policies in hospitals [[7]](#_bookmark6). Mechanisms described for car- bapenem resistance include, production of different classes of car- bapenemase, hyperproduction of AmpC b-lactamase with an outer membrane porin mutation, and production of ESBL with a porin mutation or drug efflux. Production of carbapenemases is the most commonly reported mechanism of carbapenem resistance in

*K. pneumoniae* [[8]](#_bookmark6). Carbapenemases are b-lactamase enzymes that

are capable of hydrolyzing all beta-lactam antibiotics, including monobactams, extended spectrum cephalosporins and car- bapenem [[9]](#_bookmark6). The most common carbapenemases include Verona integron metallo-beta-lactamases types (VIM), imipenemase (IMP) types, *Klebsiella pneumoniae* carbapenemase (KPC), oxacillinase-48 (OXA-48), and New Delhi metallo-beta- lactamase-1 (NDM-1), encoded by carbapenem resistance deter- mining genes *bla*VIM, *bla*IMP, *bla*KPC, *blaOXA-48-like*, and *bla*NDM, respectively [[10]](#_bookmark6). Phenotypic assays are used to identify activity of carbapenemase while molecular assays have developed to identify

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carbapenemase encoding genes [[10,11]](#_bookmark6). Analysis of hospital surveillance data by the Center for Disease Control and Prevention (CDC) suggested that 8% of all *Klebsiella* isolates are carbapenem resistant [[12]](#_bookmark6). Other studies showed that it accounted for 5–24% of *Klebsiella* isolates identified in hospitalized patients [[13]](#_bookmark6). In Egypt, carbapenem resistance is emerging and alarming, one study reported that carbapenem resistance was detected in 44.3% of *K. pneumoniae* isolates in Suez Canal university hospitals [[14]](#_bookmark6). The detection of carbapenem resistance is essential for the proper choice of antibiotic therapy as well as infection control measures to prevent dissemination of resistant strains in hospital settings. Therefore we set out this study to determine the prevalence of car- bapenem resistance and carbapenemase encoding genes among clinical *K. pneumoniae* isolates obtained from patients at intensive care units (ICUs) of Mansoura university hospitals (MUHs), taking in consideration that carbapenems are frequently used as an empiric therapy in ICUs at our institution.

1. Subjects and methods
   1. *Study design*

This was a cross-sectional study, between January 2015 and March 2016. All patients admitted to different ICUs of MUHs and had confirmed infection by *K. pneumoniae*, were enrolled in this study. The ICU bed numbers range from 4 to 27, with a median of 10. This study was conducted with approval from the Medical Research Ethics Committee, Mansoura University.

* 1. *Case definition*

A patient with a culture positive for *K. pneumoniae* was deemed to have an infection if *K. pneumoniae* was isolated from a sterile site (e.g., blood, peritoneal fluid, cerebrospinal fluid, or pleural fluid) in combination with clinical signs and symptoms of infection. Pneu- monia was diagnosed on the basis of clinical signs and symptoms (cough, dyspnea, fever), the appearance of infiltrate on chest radio- graphy and heavy growth of organisms in purulent tracheal secre- tions or bronchoalveolar lavage fluid (>104 colony forming units/ ml). These samples were examined after Gram staining for the detection and quantification of leukocytes and organisms.

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A diagnosis of urinary tract infection required the isolation of at least 10000 microorganisms/ml associated with at least two of the following: dysuria; frequency; and/or pyuria (>10 white blood cells per high power field) [[15]](#_bookmark6).

* 1. *Sample collection and processing*

Samples differed according to site of infection; they included blood, peritoneal fluid, urine, respiratory secretions, wound swabs, and cerebrospinal fluid. Only one isolate per patient was included. All collected samples were processed in the Microbiology Diagnos- tics and Infection Control Unit (MDICU), within 1–2 h of collection.

* 1. *Isolation and identification of K. pneumoniae*

Bacterial identification to the species level were carried out by colonial morphology on blood and MacConkey’s agar plates (oxoid, UK), Gram stained films, biochemical reactions including oxidase, motility, indole, methyl red, voges-proskauer, citrate and urease tests and confirmed with API 20E (BioMerieux, Inc., Hazelwood, MO).

* 1. *Antibiotic susceptibility testing*

The disk diffusion method was employed to determine the sus- ceptibility profile, carried out on a Muller-Hinton agar, as recom- mended by CLSI M100-S24 [[16]](#_bookmark7). The following disks, provided by (Oxoid, UK) were used: imipenem (IPM) (10 lg), meropenem (MEM) (10 lg), ertapenem (ERT) (10 lg), amoxicillin/clavulanic acid (AMC) (30 lg), piperacillin/tazobactam (TZP) (110 lg), ceftazidime (CAZ) (30 lg), cefepime (FEP) (30 lg), ciprofloxacin (CIP) (10 lg), gentamicin (CN) (10 lg), amikacin (AK) (30 lg). CRKP was defined as *K. pneumoniae* isolates that test intermediate or resistant to ertapenem, according to breakpoints defined by the CLSI [[16]](#_bookmark7).

* 1. *Detection of carbapenemase production*

Carbapenemase production was confirmed by the following tests: a) Modified Hodge test (MHT): a 1:10 dilution of 0.5 McFar- land standard suspension of *E. coli* ATCC 25922 was made (adding 0.5–4.5 ml of saline) and swab streaked all over the plate. Then, 10 lg ertapenem disk was placed in the center of the test area. The test isolate was streaked in a straight line from the disk to the edge of the plate. Interpretation of negative and positive tests was done according to CLSI, (2014) [[16]](#_bookmark7). b) Boronic acid synergy test: was done by streaking 0.5 McFarland standard suspension of the test isolate on a plate. 10 lg ertapenem and 400 lg of phenylboronic Acid (PBA) disks were then placed on the inoculated plate 15 mm apart center to center, and incubated for 24 h. The presence of enhanced growth inhibition zone between the car- bapenem disk and boronic acid disk was considered positive for KPC enzyme production [[17]](#_bookmark8) c) Ethylene diammine tetra acetic acid (EDTA) test: 0.5 McFarland standard suspension of the test isolate was spread on the surface of a Mueller Hinton Agar plate. Two 10 lg ertapenem disks were placed on the agar 15 mm apart

center to center. 10 ll of 0.5 M EDTA was added to one of the erta- penem disk to get 750 lg concentration and incubated at 37 °C

overnight. Increase of inhibition zone diameter of more than 5 mm in the disk potentiated with the EDTA was considered posi- tive for metallo-b-lactamase production [[18]](#_bookmark9).

* 1. *Detection of carbapenemase encoding genes*

DNA extraction was performed by the boiling method using the CDC protocol [[19]](#_bookmark10). Polymerase chain reaction (PCR) was carried out

in a thermal cycler machine (MJ Research, Inc., USA). The genes *bla*IMP, *bla*VIM, *bla*KPC, *blaOXA-48-like* and *bla*NDM-1 were ampli- fied using primers and conditions as described by Karuniawati et al. (2013) [[7]](#_bookmark6). A volume of 1 lL of template DNA was added to a final volume of 25 lL PCR mixture comprising 12.5 lL of Taq PCR Master Mix (Fermentas, UK), including, 1× PCR buffer,

1.5 mmol/L MgCl2, 0.15 mmol/L dNTP, and 1.25 IU Taq DNA poly-

merase, 1 lL of 0.8 lmol/L each primer (except OXA 0.4 ll) and

9.5 lL of sterile distilled water. The primer pairs were tested in simplex PCR (only one gene screened, for blaNDM-1 gene) and with a multiplex approach. The amplicons were analyzed by elec- trophoresis in a 1.5% agarose gel.

* 1. *Data analysis*

Data analysis was done in STATA version 12. Out comes were presented as proportions and percentages in a tabular form.

1. Results
   1. *Patients and isolates characteristics*

This study included 125 patient with confirmed *K. pneumoniae* infections, among which 42 had CRKP infections. CRKP isolates were obtained from 23 male and 19 female patients. Patients were generally elderly (median age was 60 years, with a range of 43–77 years). Respiratory samples (62%) was the predominant source of CRKP, followed by urine (14%), wound (9.5%), blood (9.5%), and catheter tip (5%) samples.

* 1. *Susceptibility to antibiotics*

Using current breakpoints recommended by CLSI (M100-S24) for carbapenem interpretation, 42 out of 125 (33.6%) *K. pneumoniae* isolates were nonsusceptible (intermediate and resistant) to erta- penem. 25.6% and 20% were nonsusceptible to meropenem and imipenem respectively. The full antibiogram for the tested drugs is seen in [Table 1](#_bookmark1). Two isolates were resistant to all tested antibi- otics (panresistant), however these isolates clinically respond well to the last-resort antimicrobials colistin and tigecycline.

* 1. *Prevalence of carbapenemase activity based on phenotypic tests*

Carbapenamase activity was detected in 26/42 (61.9%) by MHT method, 22/42 (52.4%) by Boronic acid screen and 5/42 (11.9%) by the EDTA test. 18 of the isolates were positive for both the MHT and Boronic acid methods. Overall, 35 (83.3%) of the 42 CRKP were positive for the production of one or more carbapenemases. Details of the carbapenemase activity among the isolates are shown in [Table 2](#_bookmark2).

* 1. *Prevalence and distribution of carbapenemase genes*

Based on the PCR assays, 39/42 of the CRKP isolates were posi- tive for one or more carbapenemase genes, while none of the car- bapenemase genes tested (blaKPC, blaNDM, blaVIM-1, blaIMP, and blaOXA-48-like) were detected in three isolates tested. Further- more, these three isolates did not phenotypically express a carbapenemase, according to the MHT, Boronic acid and EDTA tests. Carbapenem resistance of these isolates is likely due to a combination of ESBLs and changes in outer membrane proteins (ESBL/Omp) [[20]](#_bookmark11). Five of the 39 (12.8%) carbapenemase gene carry- ing isolates harbored two or more genes. As shown in [Table 3](#_bookmark3), the most prevalent gene was *bla*KPC 47.8% followed by *bla*VIM-1

Table 1

Susceptibility pattern of CRKP isolates.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Antibiotic | CRKP isolates (*n*. = 42) |  | | | | | |
|  | Susceptible  *n*. | % | Intermediate  *n*. | % | Resistant  *n*. | % |  |
| Ertapenem | 0 | 0 | 3 | 7.1 | 39 | 92.9 |  |
| Meropenem | 10 | 23.8 | 2 | 4.8 | 30 | 71.4 |  |
| Imipenem | 17 | 40.5 | 0 | 0 | 25 | 59.5 |  |
| Cefuroxime | 3 | 7.1 | 7 | 16.7 | 32 | 76.2 |  |
| Ceftazidime | 1 | 52.7 | 0 | 0 | 41 | 97.6 |  |
| Cefotaxime | 0 | 0 | 0 | 0 | 42 | 100 |  |
| Cefepime | 17 | 40.5 | 12 | 28.6 | 13 | 30.9 |  |
| Piperacillin/tazobactam | 0 | 0 | 1 | 2.4 | 41 | 97.6 |  |
| Gentamicin | 31 | 73.8 | 3 | 7.2 | 8 | 19.0 |  |
| Amikacin | 19 | 45.2 | 1 | 2.4 | 22 | 52.4 |  |
| Ciprofloxacin | 2 | 4.8 | 0 | 0 | 40 | 95.2 |  |

Table 2

CRKP isolates positive for phenotypic tests.

|  |  |  |  |
| --- | --- | --- | --- |
| Test | CRKP isolates (*n*. = 42) |  |  |
|  | *n*. | % |
| MHT | 26 | 61.9 |  |
| Boronic acid | 22 | 52.4 |  |
| MHT and Boronic positive | 18 | 42.9 |  |
| EDTA | 5 | 11.9 |  |
| Total | 35 | 83.3 |  |

Table 3

Distribution of carbapenemase encoding genes in the CRKP isolates.

|  |  |  |  |
| --- | --- | --- | --- |
| Carbapenemase-encoding genes | Total genes |  |  |
|  | *n*. | % |
| *bla*KPC | 22 | 47.8 |  |
| *bla*VIM-1 | 10 | 21.7 |  |
| *Bla*IMP | 7 | 15.2 |  |
| *blaOXA-48-like* | 5 | 10.9 |  |
| *bla*NDM-1 | 2 | 4.3 |  |
| Total genes | 46 | 100 |  |

21.7%, *bla*IMP 15.2%, *bla*OXA-48-like 10.9% and *bla*NDM-1 4.3%. Number of genes per isolate were shown in [Table 4](#_bookmark4).

* 1. *Correlation of the phenotype and genotype of carbapenem resistance*

Out of 26 MHT positive isolates, only 18 were positive for KPC by PCR and 5 were positive for OXA-48. Of the 22 Boronic acid pos- itive isolates, 18 were positive by the MHT, and 20 were positive for KPC by PCR assay. Out of the 5 isolates detected by EDTA test as positive for metallo-b-lactamases, all were positive by PCR (4 IMP,1 NDM-1). The EDTA test was more accurate in detection of metallo-b-lactamases, specially IMP and NDM-1.

Table 4

Number of genes per isolate.

1. Discussion

Our results present a worrying trend of antimicrobial resistance in the middle east region, as the prevalence of CRKP was 33.3% among *K. pneumoniae* isolates. Previous Egyptian literature showed a prevalence of 44.3% of CRKP isolates [[14]](#_bookmark6), others reported lower incidence at 13.9% in the Egyptian National Cancer Institute [[21]](#_bookmark12) and 14.2% in Al-Azhar University Hospital [[22]](#_bookmark13). Similarly other studies showed varying prevalence rate from 20 to 40% in New York and Greece [[23,24]](#_bookmark14). Higher result of 83% was shown in a study in USA [[25]](#_bookmark15). The high trend of CRKP in the current study could be attributed to the frequent use of carbapenems as an empiric ther- apy in ICUs at our institution, as well as lack of implementation of antimicrobial stewardship program. Our study subjects were ICU- admitted patients, since it was documented in previous studies that ICU stay was a major risk factor for CRKP acquisition [[26]](#_bookmark16). In this study, sputum samples were the predominant sources of CRKP. These finding are similar to those observed by a study in Indonesia in which sputum had the largest number of bacteria carrying carbapenemase-encoding genes [[7]](#_bookmark6). This may be due to cross- infection with multi-resistant clones or long-term exposure of res- piratory tract microbiota to antibiotics, causing accumulation of resistance determinants. These resistant organisms may later cause respiratory tract infection [[27]](#_bookmark17). In the current study, non sus- ceptibility to ertapenem, based on the updated CLSI criteria was used in the surveillance of CRKP isolates. The use of ertapenem has been suggested to screen for carbapenem resistance among *Enterobacteriaceae* [[28]](#_bookmark18). Endimiani et al. (2009) reported that nearly 60% of CRKP isolates are susceptible to IPM or MEM, and resistant to ertapenem [[29]](#_bookmark19). The isolates collected in this study were highly resistant to b-lactams, fluoroquinolones and variably to aminoglycosides. Antibiotic susceptibilities of CRKP showed a worrisome trend, since two patients presented with panresistant isolates. Therefore there is an urgent need to develop new antimi- crobials for CRKP, together with strict infection control measures in particular hand-hygiene to control the cross-infection with

*K. pneumoniae.* The most prevalent gene among the 42 CRKP iso- lates genes was *bla*KPC at 43.5% which differs from what was seen in a previous study in Egypt, where *blaOXA-48-like* types were the most predominant at 28.6% *and bla*KPC accounted for only for 19% [[30]](#_bookmark21). OXA-48 gene was detected in 10.9% genes among our isolates. Since the emergence of this gene in Turkey, the Middle East and

Number of CRKP isolates

Genes per isolate

Genes present

North Africa had become reservoirs expanding to India, Senegal, and Saudi Arabia [[31,32]](#_bookmark24). Our results detected only two (4.3%)

1 3 NDM-1, VIM-1, KPC

1 3 VIM-1, OXA-48, KPC

2 2 VIM-1, OXA-48

1 2 IMP, NDM-1

34 1 20 KPC, 6 VIM, 6 IMP, 2 OXA-48

*bla*NDM-1 genes. The low prevalence of blaNDM-1 gene observed in our study agrees with that of Morsi, et al., where it was seen in 2.4% of CRKP isolates [[30]](#_bookmark21). Moreover, the blaNDM-1 genes co-existed with other carbapenamase genes in the two isolates among which it was detected, this explained why these isolates

were pan resistant. When comparing phenotypic tests to PCR results, we noted that out of the 26 MHT positive isolates, 18 were positive for blaKPC and 5 for blaOXA-48-like by PCR. False-positive results (3 isolates) may be due to carbapenem hydrolysis by ESBLs, coupled with disrupted porin expression as reported by others [[33,34]](#_bookmark25). Conversely, false negative results (4 isolates) may be explained by presence of metallo-betalactamse producing isolates with weak carbapenemase activity as reported by Miriagou et al. (2010) [[11]](#_bookmark6). MHT was more efficient at detection of OXA-48 than KPC carbapenemases. This observation is in agreement with others in which, MHT has a high sensitivity for detection of OXA-48 car- bapenemases [[35]](#_bookmark26). As regards to Boronic acid test, out of the 22 isolates detected by Boronic acid, 20 were positive by PCR. Absence of KPC in 2 isolates could be explained by that Boronic acid detects other class A carbapenemases such as IMI, Sme, NMC, and GES as described previously [[36]](#_bookmark27). In our study, among the 5 isolates detected by the EDTA test, all were positive by PCR, whereas most of the metallo-b-lactamases detected by PCR were missed by EDTA test. This result is consistent with what was reported in a study by Khosravi. et al. (2012) [[37]](#_bookmark27), in which EDTA test showed 100% sen- sitivity, as all isolates detected by EDTA test were positive by PCR. However, they observed a low specificity of 43.1%. The main limi- tation of MHT is being time consuming and unable to distinguish blaKPC and ESBL producers with Omp changes isolates from each other. This observation has been reported by others but without clear explanation [[20,34]](#_bookmark11). Boronic acid may provide some accuracy over the MHT but still time consuming. These limitations suggest the use of multiplex PCR for optimal detection of carbapenemase and early selection of proper antibiotics.

1. Conclusion

The spread of CRKP isolates represents a serious threat to our hospitals, which prompt developing new antimicrobials for CRKP, together with strict infection control measures including hand hygiene promotion, patients’ isolation or cohorting, contact pre- cautions, environmental cleaning, active surveillance and antibi- otics stewardship programs. Using the updated CLSI breakpoints for ertapenem, susceptibility testing by disk diffusion method detected most carbapenem-nonsusceptible *K. pneumoniae* isolates without the need for other phenotypic tests. Multiplex PCR is an effective method for detection of carbapenemase genes which overcomes the limitations of the phenotypic tests.

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Competing interests

None declared.

References

1. [Sydney MF. Anaerobic gram negative bacilli. In: Sherris JC, Ryan KJ, Ray CG,](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0005) [editors. An introduction to infectious diseases. New York: McGraw Hill; 2004.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0005)

[p. 2004](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0005).

1. [Pournaras S, Poulou A, Voulgari E, Vrioni G, Kristo I, Tsakris A. Detection of the](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0010) [new metallo-b-lactamase VIM-19 along with KPC-2 CMY-2 and CTX-M-15 in](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0010) [Klebsiella pneumoniae. J Antimicrob Chemother 2010;65:1604–7](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0010).
2. [Paterson DL. Resistance in gram-negative bacteria: Enterobacteriaceae. Am J](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0015) [Infect Control 2006;34:20–8](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0015).
3. [Moquet O, Bouchiat C, Kinana A, Seck A, Arouna O, Bercion R, et al. Class D](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0020) [OXA-48 carbapenemase in multidrug-resistant enterobacteria. Senegal Emerg](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0020) [Infect Dis 2011;17:143–4](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0020).
4. [Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F. Carbapenemase genes among](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0025) [multidrug resistant gram negative clinical isolates from a tertiary hospital in](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0025) [Mwanza. Tanzania Biomed Res Int 2014:303104](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0025).
5. [Nordmann P, Cuzon G, Naas T. The real threat of *Klebsiella pneumoniae*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0030)

[carbapenemase-producing bacteria. Lancet Infect Dis 2009;9:228–36](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0030).

1. [Karuniawati A, Saharman YR, Lestari DC. Detection of carbapenemase encoding](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0035) [genes in *Enterobacteriace, Pseudomonas aeruginosa*, and *Acinetobacter baumanii*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0035)[isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0035) [2011. Acta Med Indones 2013;45:101–6](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0035).
2. [Jean B, Patel J, Kamile R, Brandon K. Carbapenemases in *Enterobacteriaceae:*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0040)[activity, epidemiology, and laboratory detection. J Clin Microbiol](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0040) [2009;8:55–60](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0040).
3. [Queenan AM, Bush K. Carbapenemases: the versatile beta-lactamases. Clin](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0045) [Microbiol Rev 2007;20:440–58](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0045).
4. [Nordmann P, Naas T, Poirel L. Global spread of Carbapenemase-producing](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0050)

[*Enterobacteriaceae*. Emerg Infect Dis 2011;17:1791–8](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0050).

1. [Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, et al.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0055) [Acquired carbapenemases in Gram-negative bacterial pathogens: detection](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0055) [and surveillance issues. Clin Microbiol Infect 2010;16:112–22](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0055).
2. [Centers for Disease Control and Prevention. Guidance for control of infections](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0060) [with carbapenem-resistant or carbapenemase producing *Enterobacteriaceae* in](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0060) [acute care facilities. MMWR Morb Mortal Wkly Rep 2009;58(10):256–60](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0060).
3. [Weiner-Well Y, Rudensky B, Yinnon A, Kopuit P, Schlesinger Y, Broide E, et al.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0065) [Carriage rate of carbapenem-resistant *Klebsiella pneumoniae* in hospitalized](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0065) [patients during a national outbreak. J Hosp Infect 2010;74:344–9](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0065).
4. [El-Sweify MA, Gomaa NI, El-Maraghy NN, Mohamed HA. Phenotypic detection](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0070) [of carbapenem resistance among *Klebsiella pneumoniae* in Suez Canal](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0070) [University Hospitals, Ismailiya. Egypt Int J Curr Microbiol App Sci](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0070) [2015;4:10–8](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0070).
5. [Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0075) [care-associated infection and criteria for specific types of infections in the](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0075) [acute care setting. Am J Infect Control 2008;36:309–32](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0075).
6. Clinical Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty-Fourth informational supplement. Document M100–S24. Wayne, PA:CLSI; 2014.
7. [Tsakris A, Kristo I, Poulou A, Themeli-Digalaki K, Ikonomidis A, Petropoulou D,](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0085) [et al. Evaluation of boronic acid disk tests for differentiating KPC-possessing](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0085) [*Klebsiella pneumoniae* isolates in the clinical laboratory. J Clin Microbiol](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0085) [2009;47:362–7](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0085).
8. [Nagdeo NV, Kaore NM, Thombare VR. Phenotypic methods for detection of](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0090) [various b-lactamases in Gram-negative clinical isolates: need of the hour.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0090) [Chronic Young Sci 2012;3:292–8](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0090).
9. Centers for Disease Control and Prevention. Multiplex real-time PCR detection of *Klebsiella pneumoniae* carbapenemase (KPC) and New Delhi metallo-B- lactamase (NDM-1) genes. Centers for Disease Control and Prevention, Atlanta, 2011. GA: <[http://www.cdc.gov/HAI/settings/lab/kpc-ndm1-lab-protocol.](http://www.cdc.gov/HAI/settings/lab/kpc-ndm1-lab-protocol.html) [html](http://www.cdc.gov/HAI/settings/lab/kpc-ndm1-lab-protocol.html)>.
10. [Endimiani A, Perez F, Bajaksouzian S, Windau AR, Good CE, Choudhary Y, et al.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0100) [Evaluation of updated interpretative criteria for categorizing *Klebsiella*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0100)[*pneumoniae* with reduced carbapenem susceptibility. J Clin Microbiol](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0100) [2010;48:4417–25](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0100).
11. [Hossam MA, Amany E. Species distribution and antimicrobial susceptibility of](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0105) [gram-negative aerobic bacteria in hospitalized cancer patients. J Trans Med](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0105) [2009;7:7–14](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0105).
12. [Khaleid M, Ibraheim Z, Eman M. Surgical site infections and associated risk](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0110) [factors in Egyptian orthopedic patients. J Am Sci 2010;6:272–80](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0110).
13. [Bratu S, Landman D, Haag R, Recco R, Eramo A, Alam M, et al. Rapid spread of](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0115) [carbapenem resistant *Klebsiella pneumoniae* in New York City: a new threat to](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0115) [our antibiotic armamentarium. Arch Intern Med 2005;165:1430–5](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0115).
14. [Giakkoupi P, Iakkoupi P, Pappa O. Polemis M. Emerging *Klebsiella pneumoniae*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0120)[isolates coproducing KPC-2 and VIM-1 carbapenemases. Antimicrob Agents](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0120) [Chemother 2009;53:4048–50](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0120).
15. [Marquez P, Terashita D, Dassey D, Mascola L. Population based incidence of](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0125) [carbapenem resistant *K. pneumoniae* along the continuum of care, Los Angeles](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0125) [County. Infect Cont Hosp Epidemiol 2013;34:144–50](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0125).
16. [Kandeel A. Epidemiology of carbapenemase producing *Enterobacteriaceae* in a](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0130) [general hospital. J Microbiol Infect Dis 2015;5:57–62](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0130).
17. [Tian B, Fadhil NH, Powell JE, Kwong WK, Moran NA. Long-term exposure to](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0135) [antibiotics has caused accumulation of resistance determinants in the gut](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0135) [microbiota of honeybees. MBio 2012;3(6)](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0135).
18. [Anderson KF, Lonsway DR, Rasheed JK, Biddle J, Jensen B, McDougal LK, et al.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0140) [Evaluation of methods to identify the Klebsiella pneumoniae carbapenemase](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0140) [in Enterobacteriaceae. J Clin Microbiol 2007;45:2723–5](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0140).
19. [Endimiani A, Hujer AM, Perez F, Bethel CR, Hujer KM, Kroeger J, et al.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0145) [Characterization of blaKPC-containing *Klebsiella pneumoniae* isolates detected](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0145) [in different institutions in the Eastern U.S.A. J Antimicrob Chemother](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0145) [2009;63:427–37](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0145).
20. [Morsi SS. Comparative Evaluation of Phenotypic and Genotypic Methods for](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0150) [Detection of Carbapenemases in Clinically Significant *Klebsiella pneumoniae*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0150)[Isolates. EJMM 2016;25:109–16](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0150).
21. [Poirel L, Bonnin RA, Nordmann P. Genetic features of the widespread plasmid](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0155) [coding for the carbapenemase OXA-48. Antimicrob Agent Chemother](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0155) [2012;56:559–62](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0155).
22. [Memish ZA, Assiri A, Almasri M, Roshdy H, Hathout H, Kaase M, et al.](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0160) [Molecular characterization of carbapenemase production among gram-](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0160) [negative bacteria in Saudi arabia. Microbial Drug Resistance 2015;21:307–14](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0160).
23. [Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0165) [disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0165)[spp. clinical isolates from the UK. J Antimicrob Chemother 2009;63:659–67](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0165).
24. [Carvalhaes CG, Picao RC, Nicoletti AG, Xavier DE, Gales AC. Cloverleaf test](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0170) [(modified Hodge test) for detecting carbapenemase production in *Klebsiella*](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0170)[*pneumoniae*: be aware of false positive results. J Antimicrob Chemother](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0170) [2010;65:249–51](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0170).
25. [Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0175) [of emerging carbapenemases in *Enterobacteriaceae*. J Clin Microbiol](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0175) [2012;50:477–9](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0175).
26. [Pasteran F, Mendez T, Guerriero L, Rapoport M, Corso A. Sensitive screening](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0180) [tests for suspected class A carbapenemase production in species of](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0180) [*Enterobacteriaceae*. J Clin Microbiol 2009;47:1631–9](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0180).
27. [Khosravi Y, Loke MF, Chua EG, Tay ST, Vadivelu J. Phenotypic detection of](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0185) [metallo-beta-lactamase in imipenem-resistant Pseudomonas aeruginosa. Sci](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0185) [World J 2012:654939](http://refhub.elsevier.com/S2314-808X(16)30131-2/h0185).