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Molecular Characterization of Carbapenemase Producing *Klebsiella Pneumoniae* Dominance of OXA-48, KPC, VIM and NDM Producers in Khartoum, Sudan

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Abstract

Background and Objectives: *Little is known about carbapenemase producing Klebsiella pneumoniae (CPK) in Sudan. This study aimed to determine the prevalence of CPK in a major hospital in Khartoum, Sudan between may 2015 - January 2017 and to characterize the isolates and detect the types of carbapenemase (s) they produced.*

Materials and Methods: *The study was done in the Department of Molecular Microbiology, Faculty of Medical Laboratories Science, Al-Neleen University. All the isolates were obtained from clinical samples of patients treated inside the hospitals. Strains of K. pneumoniae resistant to at least one carbapenem (imipenem or meropenem) by using disc diffusion technique according to the CLSI guidelines were included in this study. Molecular detection of carbapenemase genes was achieved using Real-Time PCR (Sacace Biotechnologie, Italie).*

Results: *A total 96 strains of K. pneumoniae of different non duplicated isolates were obtained from different hospitals. Seventy-two percent (70/96) isolates were positive for carbapenemase genes; 59.4% (57/96) were positive for blaKPC genes, 57.3% (55/96) were positive for blaNDM genes, 37.5% (36/96) were positive for blaVIM genes and 35.4% (34/96) were positive for blaOXA-48 genes. Nineteen isolates possessed four genes (bla_{KPC}, bla_{NDM}, bla_{VIM} and blaOXA-48), fourteen isolates possessed three genes{(blaNDM, blaVIM and blaOXA-48=6), (blaKPC, bla_{NDM}, and bla_{OXA-48}=3), (bla_{KPC}, bla_{NDM} and bla_{VIM} =3), (bla_{KPC}, bla_{VIM} and bla_{OXA}-48=2)}, 27 isolates possessed two genes{(bla_{KPC} and bla_{NDM} =21), (bla_{KPC}, bla_{OXA-48}=2), (bla_{NDM} and bla_{VIM} =3), (bla_{NDM} and bla_{OXA-48}=1)}, 10 isolates possessed only one gene (bla_{KPC} =8, bla_{OXA-48}=1 and bla_{VIM} =1) and the remaining 26 isolates were free from these genes.*

Conclusion & Recommendation: *In Sudan, the most common type of carbapenemase gene multidrug-resistant K. pneumoniae is KPC. Co-production of KPC, VIM, NDM and OXA-48 genes are found in K. pneumoniae. To our knowledge, this study was done for the first time in Sudan. Therefore, it is necessary to determine carbapenem resistance in K. pneumoniae isolates and take essential infection control precautions to avoid spread of this resistance.*

Introduction

Klebsiella pneumoniae is a one of the lactose fermenting Gram negative rod that have ability to grow in the presence or absent of oxygen, and it is possess high virulence factors that lead to increased their morbidity and mortality rate especially in weak immunity individuals [1]. Add to that, it is among the group of multiple antibiotic resistant organisms [2]. And through plasmids and transposons it exchange the drug resistant genes with each other and other kinds of organisms, therefore these lead to production of β-lactamase of which carbapenemase are the most common enzyme [3].

The antibacterial families of which called β-lactam antibiotics consist of Penicillins, Cephalosporins and Monobactams were used to treat infections caused by this highly virulence bacteria but due to emergence of extended spectrum β-lactamase (ESBL) which showed resistant against this families, the carbapenem family of antibiotics are being the last line of defense against the multidrug resistant organisms [4].

However, due to excessive use, uncontrolled taking of antibiotic by individuals and absent of antibiotic instruction in the hospitals, all these lead to appearance of resistant against this antibiotic family, especially carbapenem resistant *K. pneumoniae* (CRKP) which spread all over the world [5,6]. Mechanisms of resistant against this antibiotic include carbapenemase enzyme production which is the most common one, production of AmpC β-lactamase, porin and outer membrane mutation and efflux pump [7].

The most existent carbapenemase genes include *Klebsiella pneumoniae* carbapenemase (KPC), Verona integron metallo-beta-lactamases types (VIM), oxacillinase-48 (OXA-$_{48}$) and New Delhi metallo-betalactamase-1 (NDM-1) which arised from the following blaKPC, blaVIM, blaOXA-$_{48}$ and blaNDM, respectively [8]. The research rolled out by a Center for Disease Control and Prevention (CDC) among many hospitals showed that resistant to carbapenem is reach 8% among isolated *K. pneumoniae* [9].

In another study, Sahin et al, 2015, they determine that of 43 strains, 35 (85 %) were carbapenemase-positive. Seven of the isolates (16.3 %) were positive for the OXA-48 gene, as detected by multiplex PCR. The NDM-1 gene was detected only in one strain (2.3 %) [10].

Study by Sonnevend et al 2015, in the Arabian Peninsula, 145 *K. pneumoniae* isolates collected in 16 hospitals of Saudi Arabia, Kuwait, Oman and the United Arab Emirates were studied. All strains were multidrug resistant. The frequency of various carbapenemases varied according to the participating countries, but in the collection, as a whole, blaNDM-1 was the most frequently encountered carbapenemase gene (53.8%) followed by blaOXA-48-like gene (29.7%). A comparatively high rate (4.1%) of multi-clonal strains carrying both blaNDM and blaOXA-48-like genes in the United Arab Emirates, representing the most resistant subgroup, was encountered [11].

Sakarikou et al, 2017 at their study on 55 consecutive non-duplicated clinical strains of *Klebsiella pneumoniae* isolated from blood culture in two hospitals of Rome, Italy. Only Of the 55 isolates investigated, 40 were found to be carbapenemase-producing strains and 15 noncarbapenemase- producing strains. Carbapenemase genes expressed as 31 (77%) isolates harbor KPC gene, 5 (12%) isolates contain both KPC and VIM genes and 4 (10%) isolates contain OXA-48 gene. NDM gene were absent in all isolates [12].

In the 2015, Abdullah and Mehmet were reported the First *Klebsiella pneumoniae* Isolate Co-Producing OXA-48 and NDM-1 in Turkey which was obtained from a patient who was transferred from Sanliurfa (on the border between Syria and Ankara) [13].

For all mention above to determine the carbapenemase producer *K. pneumoniae* is more important in the process of treatment, add to that used this data for prevention and control in hospital to prevent the spread of such isolates. So we carry out this research to decide the frequency of carbapenem resistance and carbapenemase encoding genes out off isolated *K. pneumoniae* obtained from different hospitals in Khartoum.

Material and method

Study design

This was a cross-sectional laboratory based study involving 96 *K. pneumoniae* carbapenem resistant isolates collected between May, 2015 and January, 2017 from clinical specimens in different hospitals in Khartoum, Sudan.

Sample size and sampling technique

Samples had been taken from admitted patients are collected depending on the site and type of infection. The estimated numbers of sample size were 100 Carbapenem resistant isolates of *K. pneumoniae*.

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 (Himedia, India), Gram stained films, biochemical tests including oxidase, motility, indole, methyl red, voges-proskauer, citrate and urease tests and confirmed with Chromogenic agar media (Liofilchem Co. Italy).

Inclusion and Exclusion criteria

All isolates from different types of samples (urine, blood, stool, sputum, CSF, wound and other body fluids) that identified as *K. pneumoniae* by conventional Biochemical methods and show resistance to one or more antibiotic disc of Carbapenem family , at the period of this study were included in this study. While Isolates of *K. pneumoniae* that show susceptibility to antibiotic disc from Carbapenem family were excluded from this study.

Antimicrobial Susceptibility Testing

All identified *K. pneumoniae* were tested for their antimicrobial susceptibilities by disc diffusion technique according to the CLSI guidelines [14]. The following antibiotic discs (drug concentrations in µg) were used: Meropenem (10µg) and Imepenem (10µg) (Bioanalysis Co. Italy).

Molecular detection of carbapenemase genes

Molecular techniques, primarily based on PCR, has been the reference standard for the identification and differentiation of carbapenem resistance genes depended on the excellent specificity, sensitivity, accuracy and rapidity of these methods. If identification of a carbapenemase is required for epidemiological purposes, then the PCR products must subjected to additional sequencing. These techniques generate results within 4-6 h, or even less as for this study because we used real-time PCR technique.

DNA Extraction

DNA was isolated from bacterial colonies using the boiling lysis method. Strains were streaked onto Nutrient agar and grown overnight at 37°C. A loopful of bacterial growth was suspended in 400 µl of sterile distilled water, incubated at room temperature for 5min, and then boiled for 10 min. After centrifugation at 13,200 rpm for 10 min, the pellet was discarded and the supernatant containing DNA was used directly for PCR or stored at -20°C [15].

Polymerase Chain Reaction (PCR)

A. Primers

PCR amplification was performed using published primer pairs which are as shown in (Table 1).

Table 1: Primer sets for amplification of carbapenem resistance determine genes.

Gene	Primer sequence (5/ → 3/)	TM(°C)*	Amplicons size (bp)
bla-VIM	Forward: GATGGTGT TTGGTCGCATA Reverse: CGAATGCG CAGCACCAG	54.5 57.6	390
bla-KPC	Forward: CATTCAAGGGC TTTCTTGCTGC Reverse: ACGACGGCAT AGTCATTTGC	60.3 57.3	498
bla-NDM	Forward: GGTTTGGCGA TCTGGTTTTC Reverse: CGGAATGGCT CATCACGATC	57.3 59.4	521
bla-OXA-48	Forward: GCTTGATC GCCCTCGATT Reverse: GATTTGCTCC GTGGCCGAAA	56.0 59.4	238

*TM: melting temperature of the primer.

B. Preparation of primers

For 100 pmol/ml from each primer we dissolved them in sterile DW as instructed by manufacture, then for 10 pmol/ml we dissolved 10 µl of each primer in 90 µl sterile DW.

D. Preparation of reaction mixture

The following reagents were used for each gene in the following volumes (total reaction volume was 20 µl) in 0.2 ml PCR tube;
A. 5.5 µl deionized sterile water.
B. 10 µl Master mix (iNtRON, Biotechnology, Korea).
C. 1 µl forward primer (Macrogen Company, Seoul, Korea).
D. 1 µl reverse primer (Macrogen Company, Seoul, Korea).
E. 2.5 µl DNA (template DNA).

Real-Time PCR (Sacace Biotechnologie, Italie).

Investigations were performed using amplification mixture RealMOD™ Green qRT-PCR mix (iNtRON, Biotechnology). The best results of amplification were obtained with: activation of thermostable hot-start DNA polymerase for 10 min at 94°C, followed by 40 cycles comprising: denaturation (30 s at 94°C), primers annealing (45 s at 52°C) and strand elongation (50 s at 72°C). After the end of cycling, material was cooled down to 40°C for 60 s. Fluorescence levels were measured at wavelengths: 530 nm (FAM dye) and 610 nm (LC-Red 610 dye).

Interpretation of results

A sample was considered positive by RT-PCR if it crossed the threshold before a crossing point (Cp) of 35 cycle and negative if the Cp was greater than 35. Positive result at wavelength 530 nm (FAM dye) stated the presence of bla OXA-48 or bla NDM, whereas positive result at wavelength 610 nm (LC-Red 610 dye) meant the presence of bla VIM or bla KPC.

Data collection and analysis

A questionnaire (information about isolates includes type of sample, gender and patient age) was designed and used in the study Then data were recorded and analyzed. The collected data was analyzed using statistical package for social science (SPSS) version 24.

Results

Antimicrobial Susceptibility Testing

315 clinical isolates were categorized according to the susceptibility of imipenem and meropenem. 96 (30.5%) isolates were resistant to decreased susceptibility to imipenem and meropenem (zones of 21 mm or less) and 219 (69.5%) isolates were susceptible to carbapenems.

Genotypic detection of crbapenemase genes using Real-Time PCR (RT-PCR)

Polymerase Chain Reaction (PCR) was performed for isolates that show resistant or reduce susceptibility against carbapenem antibiotic to detect crbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM} and bla_{OXA-48}). Seventy two percent (70/96) were positive for carbapenemase genes; 59.4% (57/96) were positive for KPC genes, 57.3% (55/96) were positive for NDM genes, 37.5% (36/96) were positive for VIM genes and 35.4% (34/96) were positive for OXA-48 genes. Nineteen isolates possessed four genes, fourteen isolates possessed three genes, twenty seven isolates possessed two genes, ten isolates possessed only one gene and the remaining twenty six isolates free from these genes Table (2).

Table 2: frequency and distributed of Genes out of Isolated *K.pneumoniae*

No of Genes detected	Type of Genes	No of isolated organisms	Percentage %
4 Genes	KPC+NDM+VIM+OXA-48	19	19/96 (19.7%)
3Genes	NDM+VIM+OXA-48	6	14/96 (14.5%)
	KPC+NDM+OXA-48	3	
	KPC+NDM+VIM	3	
	KPC+VIM+OXA-48	2	
2 Genes	KPC+NDM	21	
	KPC+OXA-48	2	
	VIM+NDM	3	
	NDM+OXA-48	1	
1 Genes	KPC	8	10/96 (10.4%)
	VIM	1	
	OXA-48	1	
Zero gene	No gene	26	26/96 (27.3%)

Discussion

The fact detection of carbapenem resistance bacteria helps physicians not only to give the proper antibacterial treatment but also to cease their dissemination. The introductory screening for carbapenemase producers in clinical specimens is depend first on phenotypic tests, and so the confirmation tests are mainly depend on molecular tests. But, traditional phenotypic tests have some disbenefits such as time consuming, interpretation is so difficult in some cases and less sensitivity and specificity among different species [16].

In this study, we did a rapid, sensitive, and specific real-time-PCR assay for the detection of four carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM} and bla_{OXA-48}). This assay can be performed in less than 4 hours, which will reduce the chance of spreading the organism in the hospital.

As matter of fact This study showed that The most prevalent gene among the 96 CRKP isolates genes was bla_{KPC} at 59.4% which agreement with what was seen in a previous study in Egypt by Dalia & Doaa, 2017 (47.8%), in Italy by Sakarikou et al, 2017 (77%) and in study from china by Wang et al, 2012 (91%) while differ from previous study which revealed this gene as less detected as from Tanzania by Martha et al, 2014 (4%) from Serbia by Anika et al, 2016 (.8%) or completely absent as from Turkey four studies, KSA two researches, from Jordan, Morocco, Arabian peninsula, Tunisia, Grees and from Italy by Giancarlo [10-13,17-29].

As for the second gene this study reported the bla NDM gene is the second more predominant gene (57.3%) which agree with previous study from Jordan by Amin et al, 2017 (1%) and two studies from KSA by Shible et al, 2013, and Maryam et al, 2017 with (20%) and (5%) respectively [23-25].

However, another studies reported that the NDM gene is the most detected gene as from Turkey by two studies Karaby et al, 2016, and Karaaslan et al, 2015 (100%: 10%) respectively , Arabian peninsula by Sonnevend et al, 2015. (53,8%) and from Serbia by Anika et al, 2016 (27.3%) at the same time other studies reported this gene as less revealed such as Dalia & Doaa, 2017 from Egypt (4.3%), Sahin et al, 2015, from turkey (2%) and Martha et al, 2014 from Tanzania (3%) Or completely absent in studies from China and two researches from Italy [10-12,17-21,29-30].

With regard to the VIM gene this study said it is the third most common gene by existence of (37.5%) which agree with the two studies from KSA by Maryam et al, 2017 and Shible et al, 2013 with percentage of (2.9% : 1.6%) respectively [23,24]. However the studies by Sakarikou et al, 2017 from Italy, Dalia & Doaa, 2017 from Egypt and Koksal et al, 2016 from Turkey found this gene as second existence gene by (12% , 21.7% and 5%) respectively [12,17,22]. At the same time our study disagree with two studies which reported VIM gene as most common such as in Tanzania by Martha et al, 2014 and Italy by Giancarlo et al, 2017 (16% and 91%) respectively while the remaining studies which reviewed show absent of these gene such as Turkey, Serbia, Jordan and Arabian peninsula [10,11,20,25].

The class D carbapenemase gene OXA-48 was the less common detected one out of primers used in this study (35.4%) and this result agreed with studies from Tanzania by Martha et al, 2014, Italy by Sakarikou et al, 2017, Serbia by Anika et al, 2016 and Egypt by Dalia & Doaa, 2017 (5%, 10% ,9% and 11%) respectively while disagree with the other studies that consider OXA-48 gene as the most dominant one like studies from Turkey by Sahin et al, 2015, another one from Turkey by Koksal et al, 2016 another two studies from KSA by Shible et al, 2013 and Maryam et al, 2017, and Jordan by Amin et al, 2017 (16%, 43%, 78%, 65% and 2%) respectively While The studies from Turkey by Karaby et al, 2016 (25%), Arabian peninsula by Sonnevend et al, 2015 (30%) and Serbia by Anika et al, 2016 (8.3%) were reported this gene as second more common gene [10-12,17,19-25].

Dissemination of *K. pneumoniae* isolates harboring carbapenemase resistance genes continues unrelieved. To our knowledge this is the first research in Sudan that revealed Nineteen isolates (19.7%) possessed four genes (bla_{KPC}, bla_{NDM}, bla_{VIM} and bla_{OXA-48}) and this is unique result out of all reviewed papers, fourteen isolates possessed three genes (14.5%){(bla_{NDM}, bla_{VIM} and bla_{OXA-48}=6), (bla_{KPC}, bla_{NDM}, and bla_{OXA-48}=3), (bla_{KPC}, bla_{NDM} and bla_{VIM} =3), (bla_{KPC}, bla_{VIM} and bla_{OXA-48}=2)} which agree with one study from Morocco by Barguigua et al, 2013 they reported the first isolated carbapenemase producer *K.pneumoniae* from urine of elderly man that coproducing three genes NDM-1, VIM-1 and OXA-48, twenty seven isolates possessed two genes (28.1%){(bla_{KPC} and bla_{NDM} =21), (bla_{KPC} and bla_{VIM} =2), (bla_{NDM} and bla_{VIM} =3), (bla_{NDM} and bla_{OXA-48} =1)}these result agree with result from Turkey by Koksal et al, 2016 where they isolated on *K.pneumoniae* harbor bla_{VIM} and blaOXA-48, study from Colombia by Rojas et al, 2013 their *K.pneumoniae* carry bla_{KPC} and bla_{VIM} and the same result obtained by Sakarikou et al, 2017 in Italy their organism carry bla_{KPC} and bla_{VIM} too [12,22,26,31].

However, *K. pneumoniae* positive for both NDM and OXA-48 genes have been reported in only four cases around the world. The first one *K. pneumoniae* that coharbor NDM-1 and OXA-48 was isolated from an elderly male's urine sample in Morocco by Barguigua et al, 2013 [26]. The second *K. pneumoniae* was detected in Tunisia by Ben Nasr et al, 2013, where studies showed that OXA-48 gene are endemic same like Turkey. And the third *K. pneumoniae* identified in the screening rectal swab of a patient converted from the intensive care unit of a hospital found in Serbia to Bern University Hospital in Switzerland Anika et al, 2016 The forth one was reported in Arabian Peninsula which was obtained from a patient who was transferred from Sanliurfa locality by Sonnevend et al, 2015 (on the border between Syria and turkey) [11,20,27]. However , in this study we were reported the fifth *K.pneumoniae* isolate that coproduced the OXA-48 and NDM carbapenemases, which was obtained from sputum sample of elderly male patient in Ribat Teaching Hospital.

In the Asia-Pacific area, the Study for Monitoring Antimicrobial Resistance Trends (SMART) global survey scheme by Christine et al, 2012 found that by phenotypic study between 2008 and 2009, 42.7% of 110 CR-KP strains produced class A, 23.6% produced class B, and 11.8% produced class D carbapenemases [32]. The high prevalence of carbapenem resistant producing *K.pneumoniae* in the current study could be due to the excessive use of carbapenems in our hospitals, and the improper application of the infection control measures by the hospital quality control managers that simultaneously lead to multiple occurrence of horizontal spread of CR-KP strains among patients. At the same time, the incidence of class B (MBL) was higher (57% & 37.5%) than that of other classes; this can be justified by the theoretic increase in the detection and disseminate of the mobilized families of metallo enzyme which are located within a variety of integrons, increasing their mobilization between different species and strains in the hospital [33]. On the other hand, the production of class A and class D enzymes in this study is reported with high rate (59%, 35%). This can be justified by the situation of these enzymes where it exists on plasmids in CR-KP strains.

By the way all the four genes used in this study were located on the plasmid. In this study 26 isolate out of 96 isolates (27%) with resistant to reduce susceptibility to Meropenem and Imepenem by antimicrobial susceptibility testing exhibited no gene detection for all the four genes (KPC, NDM, VIM and OXA-48) used in this research and this may be due to β-lactamases enzymes that did not cover by this study or other mechanisms of resistance such as efflux pumps or mutations that occure to PBPs or alter the expression of porins on the cell wall. Of these mechanisms can cause high levels of resistance to carbapenems in certain bacterial species, such as *Klebsiella pneumoniae* [34].

All isolates with carbapenemase genes were resistant to Meropenem, suggesting that meropenem susceptibility might be an indicator for carbapenemase production among *K. pneumoniae* the same result obtained in Serbia by Anika et al, 2016 when they were studied CRE especially *K. pneumoniae* and E. coli [20].

In conclusion the dissemination of such organisms in community may initiate future outbreak, which would be difficult to deal with by the available set of antibiotics. This requires more attention to revising the antibiotic policy and strengthening the application of infection control precautions in our hospitals. So we recommend that Healthcare facilities in the country need to be aware of the emergence of these multidrug-resistant isolates, as these are of significant public health concern both in the hospital and community setting.

References

1. Sydney M F (2004) Anaerobic gram negative bacilli. In: Sherris JC, Ryan KJ, Ray CG, editors. An introduction to infectious diseases. New York: McGraw Hill.
2. Pournaras S, Poulou A, Voulgari E, Vrioni G, Kristo I et al. (2010) Detection of the new metallo-b-lactamase VIM-19 along with KPC-2 CMY-2 and CTX-M-15 in *Klebsiella pneumoniae*. J Antimicrob Chemother 65: 1604-1607.
3. Paterson DL (2006) Resistance in gram-negative bacteria: Enterobacteriaceae. Am J Infect Control 34: 20-28.
4. Mushi MF, Mshana SE, Imirzalioglu C, Bwanga F (2014) Carbapenemase genes among multidrug resistant gram negative clinical isolates from a tertiary hospital in Mwanza. Tanzania. Biomed Res Int 303104.
5. Karuniawati A, Saharman YR, Lestari DC (2013) Detection of carbapenemase encoding genes in Enterobacteriace, Pseudomonas aeruginosa, and Acinetobacter baumanii isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in 2011. Acta Med Indones 45: 101-106.
6. Nordmann P, Cuzon G, Naas T (2009) The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect Dis 9: 228-236.
7. Jean B, Patel J, Kamile R, Brandon K (2009) Carbapenemases in Enterobacteriaceae: activity, epidemiology, and laboratory detection. J Clin Microbiol 8: 55-60.
8. Nordmann P, Naas T, Poirel L (2011) Global spread of Carbapenemase-producing Enterobacteriaceae. Emerg Infect Dis 17: 1791-1798.
9. Centers for Disease Control and Prevention. Guidance for control of infections with carbapenem-resistant or carbapenemase producing Enterobacteriaceae in acute care facilities. MMWR Morb Mortal Wkly Rep (2009) 58: 256-260.
10. Sahin K, Tekin A, Ozdas S, Akin D, Yapislar H, et al. (2015) Evaluation of carbapenem resistance using phenotypic and genotypic techniques in Enterobacteriaceae isolates. Ann Clin Microbiol Antimicrob 14: 44.
11. Sonnevend Á, Ghazawi AA, Hashmey, Jamal W, Rotimi VO, et al. (2015) Characterization of Carbapenem-Resistant Enterobacteriaceae with High Rate of Autochthonous Transmission in the Arabian Peninsula. PLoS ONE 10.
12. Christina S, Marco C, Camilla D, Silvia A, Cartesio F (2017) Rapid detection of carbapenemase producing Klebsiella pneumonia strains derived from blood cultures by Matrix-Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). BMC Microbiology 17: 54.
13. Abdullah K and Mehmet B (2015) The First *Klebsiella pneumoniae* Isolate Co-Producing OXA-48 and NDM-1 in Turkey. Ann Lab Med 35: 382-383.
14. CLSI (2012) Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard, 9th edn, M7- A9. Wayne, PA: Clinical and Laboratory Standards Institute.
15. Chen L, Mediavilla JR, Endimiani A, Rosenthal ME, Zhao Y (2011) Multiplex real-time PCR assay for detection and classification of *Klebsiella pneumoniae* carbapenemase gene (bla KPC) variants. J Clin Microbiol 49: 79-585.
16. Doyle D, Peirano G, Lascols C, Lloyd T, Church D, et al. (2012) Laboratory detection of Enterobacteriaceae that produce carbapenemases. J. Clin. Microbiol 50: 3877-3880.
17. Dalia M, Doaa TM (2017) Prevalence and characterization of carbapenem-resistant *Klebsiella pneumoniae* isolated from intensive care units of Mansoura University hospitals. Egyptian Journal of Basic and Applied Sciences 4: 37-41.
18. Lijun W, Haitong Gu, Xinxin Lu (2012) A rapid low-cost real-time PCR for the detection of *Klebsiella pneumoniae* carbapenemase genes, Annals of Clinical Microbiology and Antimicrobials 11: 9.
19. Martha FM, Stephen E, Mshana, Can Imirzalioglu, Freddie B (2014) Carbapenemase Genes among Multidrug Resistant Gram Negative Clinical Isolates from a Tertiary Hospital in Mwanza, Tanzania. Hindawi Publishing Corporation BioMed Research International 303104, 6.
20. Anika T, Zora J, Mira Mihajlović-Ukropina, Deana M, Branka Z, et al. (2016) Carbapenemase Production In Hospital Isolates Of Multidrug-Resistant Klebsiella Pneumoniae And Escherichia Coli In Serbia. Vojnosanitetski Pregled. Military-Medical and Pharmaceutical Review.
21. Oguz Karabay, Mustafa A, Mehmet K, Onur K, zlem A, Aydemirand A et al. (2016) The carbapenem-resistant Enterobacteriaceae threat is growing: NDM-1 epidemic at a training hospital in Turkey. Ann Clin Microbiol Antimicrob 15: 6.
22. Koksal C, Gonullu N, Kalayci F, Kiraz N (2016) Detection of carbapenemase genes OXA-48, VIM, IMP, KPC and NDM in carbapenemase-producing *Klebsiella pneumoniae* isolates from blood cultures of hospitalized patients in Istanbul, Turkey, 17th International Congress on Infectious Diseases / International Journal of Infectious Diseases 45: 1-477.
23. Atef Shibl AB, Mohamed A, Ziad M, Abiola S, Shamshad Abdul Khader d, et al. (2013) The emergence of OXA-48- and NDM-1-positive *Klebsiella pneumoniae* in Riyadh, Saudi Arabia, International Journal of Infectious Diseases 17: 1130-1133.
24. Maryam A, Ali A, Manal A, Hisham A (2017) Comparison of phenotypic and PCR methods for detection of carbapenemases production by Enterobacteriaceae, Saudi Journal of Biological Sciences 24: 155-161.
25. Amin A Aqela, b Panagiota Giakkoupic, Hamed Alzoubia, Ibrahim Masalhad, Matthew J Ellingtone, et al. (2017) Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study, Journal of Infection and Public Health 10: 150-155.
26. Barguigua A, El Otmani F, Lakbakbi EF, Talmi M, Zerouali K, et al. (2013) First report of a *Klebsiella pneumoniae* strain coproducing NDM-1, VIM-1 and OXA-48 carbapenemases isolated in Morocco. APMIS 121: 675-677.
27. Ben Nasr A, Decré D, Compain F, Genel N, Barguellil F, et al. (2013) Emer-gence of NDM-1 in association with OXA-48 in *Klebsiella pneumoniae* from Tunisia. Antimicrob Agents

Chemother 57: 4089-4090.
28. Meletis G, Tzampaz E, Protonotariou E, Sofianou D (2010) Emergence of *Klebsiella pneumoniae* carrying blaVIM and blaKPC genes. HIPPOKRATIA 14,2: 139-140.
29. Ripabelli G, Sammarco ML, Flocco R, Scutellà M, Recchia L, et al. (2017) Klebsiella Pneumoniae Isolated From Intensive Care Unit Patients with Respiratory Tract Infections: Characterization by Pulsed-Field Gel Electrophoresis, Antimicrobial Resistance and Pcrs for Carbapenemase Genes Detection. J Respir Med Lung Dis 2: 1008.
30. Karaaslan A, Soysal A, Altinkanat Gelmez G, Kepenekli Kadayifci E, Syletir G, et al. (2015) Molecular characterization and risk factors for carbapenem-resistant Gram-negative bacilli colonization in children: emergence of NDM producing Acinetobacter baumannii in a newborn intensive care unit in Turkey. J Hosp Infect 92: 67-72.
31. Rojas MF, Mojica AV, Blanco M, Correa A, Montealegre MC, et al. (2013) Emergence of *Klebsiella pneumoniae* Coharboring KPC and VIM Carbapenemases in Colombia, Antimicrobial Agents and Chemotherapy p 57: 1101-1102.
32. Christine L, Gisele E, Meredith H, Kevin B, Johann D (2012) Surveillance and Molecular Epidemiology of *Klebsiella pneumoniae* isolates that produce Carbapenemases: First report. Antimicrob. Agents Chemother 57: 130-136.
33. Queenan AM and Bush K (2007) Carbapenemases: the versatile betalactamases. Clin Microbiol Rev 20: 440-458.
34. Rodriguez-Martinez JM, Poirel L, Nordmann P (2009) Molecular epidemiology and mechanisms of carbapenem resistance in Pseudomonas aeruginosa. Antimicrob. Agents Chemother 53: 4783-4788.