Molecular characterization of carbapenem resistant Gram-negative rods in Neonatal Intensive Care Unit of Mansoura University Children's Hospital

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Received 7 February, 2021; Accepted 13 May, 2021

Carbapenems are group of extended-spectrum β-lactam antimicrobials frequently used for treating multidrug-resistant Gram-negative bacilli (GNB) infections. This study aimed at detecting and characterizing carbapenem resistance (CR) genes among GNB isolated from patients treated in neonatal intensive care unit (NICU) of Mansoura University Children's Hospital (MUCH), Egypt. It is a prospective study conducted from 2015 to 2016. A total of 158 GNB isolates were examined for CR both phenotypically and genotypically. Among 158 Gram negative isolates, there were 58 (36.7%) CR strains. Extended-spectrum β-lactamase (ESBL) production was confirmed in all 58 (100%) isolates. Carbapenemase production was detected in 52 (89.5%) strains while metallo beta-lactamase (MBL) production was found in 33 (56.9%) strains. Molecular characterization of CR strains revealed that 57 (98.3%) isolates were positive for carbapenemase encoding genes. KPC gene was the most frequent detected gene (34/58). VIM, IPM, OXA and NDM genes were also detected in 15, 13, 9 and 1 isolate, respectively. Only one isolate was negative for all encoding resistance genes despite positive for ESBL phenotype. Infection with CR strains has been increasing in clinical settings which limit the use of carbapenems.

Key words: Gram-negative bacilli, carbapenem resistance, carbapenemase, metallo beta-lactamase, multiplex polymerase chain reaction (PCR), carbapenemase encoding genes, neonatal intensive care unit.

INTRODUCTION

Treating neonatal infections is challenging due to spread of multidrug-resistant bacteria (Mzimela et al., 2021). Carbapenems are final antimicrobial therapy for life threatening microbes, nevertheless, the appearance of carbapenemases in Gram negative bacteria has put the clinicians in front of restricted treatment choices.
Carbapenem resistance (CR) may be due to either carbapenemase production or other mechanisms, such as alteration of outer membrane permeability together with extended-spectrum β-lactamase (ESBL) production, over expression of AmpC type β-lactamases or activation of efflux pumps. The production of carbapenemases is generally a more potent mechanism of CR compared with the other mechanisms (Nordmann et al., 2012; Woodford et al., 2004). Ambler classification categorizes the β-lactamases into four classes (A to D). Most of carbapenemase-producing bacteria related to A class (bla_kPC), B class (bla_vim, imp, and ndm), and D class (bla_oxa-48) (Albiger et al., 2015). The Pseudomonas and Acinetobacter species releasing these enzymes have a wide geographical distribution and have been associated with hospital outbreaks (Woodford et al., 2004). Dissemination of carbapenemases is rapid and widespread in healthcare settings (Elbadawi et al., 2021).

Several methods for detecting carbapenemase production have been used. These methods include phenotypic and molecular methods (Al-Zahrani, 2018). This study aimed at molecular characterization of beta-lactamases associated with CR Gram negative neonatal infections.

**METHODOLOGY**

**Study design**

This prospective study was conducted over 2 years during 2015 and 2016 in the NICU of MUCH, which is a level III unit with 25 incubators in 5 equal-sized rooms, that admits approximately 450 neonates per/year; there are no single rooms for isolation.

**Sample collection and processing**

A total of 158 GNB were detected from clinical samples (blood, urine, wound, tracheal aspirate, abscess, etc.) of 350 admitted neonates. Collected specimens were sent to Microbiology Diagnostics and Infection Control Unit laboratory in less than 2 h. If delay in transportation is expected, the specimens (except blood culture bottles) were kept at 4°C in the refrigerator. Clinical samples were processed using the standard laboratory techniques. Microscopic examination of Gram-stained films of the different samples was carried out to find any bacterial cells. Followed by culture on Nutrient agar, Blood agar, MacConkey agar and CLED agar (for urine samples) plates (Oxoid, UK) using the streak plate technique. Plates were kept in incubator at 37°C for 24 h.

**Bacterial identification and susceptibility testing**

Identification of the bacterial isolates was performed according to standard procedures, in reference to Mahon et al. (2007/2008). GNB were identified according to colonial appearance, microscopic evaluation and biochemical reactions including Oxidase test using Oxidase strips (Oxoid, UK), Kligler iron agar (KIA) test, Lysine iron agar (LIA) test, Motility, Ornithine production, Indole (MIO) test and Citrate utilization test (Oxoid, UK).

Antimicrobial sensitivity testing was performed by the disc diffusion test using Mueller Hinton (MH) agar (BBL, Becton Dickinson, Cockeysville, MA, USA) and CLSI 2017 M100-S27 breakpoint values were used (CLSI, 2017). Sensitivity testing was performed for the following 10 antibiotics agents: Amoxycillin clavulunate (AMC: 30 µg); Cefuroxime (CXM: 30 µg); Piperacillin-tazobactam (TPZ: 110 µg); Cefoxitin (FOX: 30 µg); Cefepime (FEP: 30 µg); Ceftriaxone (CRO: 30 µg); Ceftazidime (CAZ: 30 µg); Cefotaxime (CTX: 30 µg); Imipenem (IPM: 10 µg), and Meropenem (MEM: 10 µg). Bacterial isolates were diagnosed as CR if they were resistant (diameter ≤ 19 mm) to at least one of the used carbapenems (IPM and MEM).

Phenotypic detection of carbapenemase activity and ESBL production

The production of ESBL, carbapenemase and MBLs were tested using cephalosporin/clavulanic acid (BD Diagnostics, Franklin Lakes, NJ, USA) combination disc, The Modified Hodge test (MHT) and synergy combined disc test (CDT), respectively.

Phenotypic ESBL production was detected with the combination disc diffusion test with clavulanic acid. The inhibition zone surrounding the cephalosporin (Cefotaxime, Ceftazidime and Cefepime) discs combined with clavulanic acid is compared to the zone around the disc with the cephalosporin alone. The reaction was positive if the inhibition zone was 5 mm larger with clavulanic acid than without (Al Naiemi et al., 2012).

The Modified Hodge test

A 1:10 dilution of the Escherichia coli ATCC 25922 (NAMRU-3 Institute, Naval Medical Research Unit Three, Cairo, Egypt) was made by adding 0.5 ml of the 0.5 McFarland to 4.5 ml of saline. This dilution was streaked on the surface of MH agar plates using a swab, and allowed to dry for 3 to 5 min. Thereafter, 10 µg imipenem disc was placed in the center of the test plate. The test organism was streaked in a straight line from the disc to the edge of the plate. Plates were Kept at 35°C for 24 h. Plates were examined for a clover leaf like indentation of the test isolate and the reference strain of E. coli within the zone of inhibition of the imipenem disc (Tamma and Simmer, 2018).

**Detection of MBL (class B)**

CDT was performed according to Joji et al. (2019). Two 10 µg imipenem discs and two 30 µg ceftazidime discs (Becton Dickinson) were put on a plate inoculated with the test bacteria. 10 µL of sterilized 0.5 M EDTA solution (dissolve 186.1 g disodium EDTA in 1000 ml distilled water at pH 8.0) was supplemented to one disc of each antibiotic. After that, inhibition zones of the imipenem and imipenem + EDTA and ceftazidime and ceftazidime + EDTA discs were compared after 18 to 24 h of incubation at 35°C. A zone diameter difference between the imipenem and imipenem + EDTA discs or the ceftazidime and ceftazidime + EDTA discs ≥7 mm was
Table 1. Oligonucleotide primers.

| Gene     | Primer sequence (5’→3’)                      | Amplicon size (bp) |
|----------|----------------------------------------------|--------------------|
| bla<sub>KPC</sub> | Forward: CGTCTAGTTCTGCTGTCTTG  
Reverse: CTTGTACCTGTTAGGC | 798                 |
| bla<sub>VIM</sub>  | Forward: GATGGTGTTTGGTCGCATA  
Reverse: CGAATGCGCAGACCAG | 390                 |
| Bla<sub>IMP</sub>  | Forward: GGAATAGAGTGGCTTAAYTCT  
Reverse: CGGTTTAAYAAAACAACCAC | 232                 |
| bla<sub>OXA-48</sub> | Forward: GCGTGGTTAAGGATGAACAC  
Reverse: CATCAAGTTCAACCCAACCG | 438                 |
| bla<sub>NDM-1</sub> | Forward: GGTTCGGCTGCTGTCTGC  
Reverse: CGGAATGGCTCATCAGGC | 621                 |

defined as a positive result for MBL production.

**Molecular characterization of carbapenemase encoding genes**

**DNA template**

Genomic DNA was extracted using Gene JET DNA Purification Kit (Thermo Scientific, For 50 preps, Lot. 001893, The European Union (EU) Lithuania) following the manufacturer instructions.

**Multiplex PCR method**

The primers used in this study were based on primers published by Poirel et al. (2011) and are listed in Table 1. The amplification of DNA and thermal cycling conditions were done as described by Karuniawati et al. (2013). One multiplex PCR reaction was done detecting bla<sub>blaKPC</sub> and bla<sub>VIM</sub> genes. Second multiplex PCR reaction was done detecting bla<sub>KPC</sub> and bla<sub>OXA-48</sub> genes. Third reaction was done for detecting bla<sub>NDM-1</sub> gene. DNA amplification was performed through a 50 μl reaction mix having 10 mM Tris-HCL, 50 mM KCL, 1.5 mM MgCl2, 0.1% Triton X-100, 200 mM of each of the deoxynucleoside triphosphate, 5 μl of template DNA, and 0.4 mM of each primer.

The thermal cycler program was adjusted by using PTC-100TM Programmable Thermal Controller, Peltier-Effect Cycling, MJ. The amplification conditions include: initial denaturation at 94°C for 5 min; 30 cycles of a final elongation at 72°C for 5 min. For multiplex PCR of the carbapenemase genes, the annealing temperature was 55°C for amplification of bla<sub>VIM</sub>, bla<sub>IMP</sub> and bla<sub>KPC</sub> genes, 45°C for bla<sub>OXA-48</sub> genes (Karuniawati et al. 2013). The amplicons were analyzed by electrophoresis in a 1.5% agarose gel.

**RESULTS**

**Distribution of the isolated CR Gram-negative species**

During the study period, 58 CR Gram-negative species were identified from 158 GNB. The most common species were Klebsiella pneumoniae (37.9%), followed by E. coli (24.1%). Additionally, Pseudomonas aeruginosa accounted for 8.6%, Proteus mirabilis (6.9%), Serratia marcescens and Enterobacter cloacae (5.2% each), Enterobacter aerogenes and Citrobacter freundii (3.4% each), Proteus vulgaris, Klebsiella oxytoca and Acinetobacter baumannii (1.7% each). Most of the 58 strains were isolated from blood (63.8%), and from tracheal aspirates (20.7%). The remaining strains were isolated from umbilical venous catheter (5.2%), long line (3.4%), urine (3.4%), and others (3.4%) (Table 2).

**Antibiotic resistance pattern among CR Gram-negative isolates**

The overall resistance to carbapenems was high (75.9% for imipenem and 57% for meropenem). High rate of CR was detected among K. pneumoniae (86.4% for imipenem and 63.6% for meropenem) and E. coli strains (78.6% for imipenem and 71.4% for meropenem). The best susceptibility for these highly resistant strains was detected with pipracillin/tazobactem combination (K. pneumoniae resistance was 18.2% and E. coli resistance was 14.3%). Amoxicillin/Clavulinate had some activity against few strains (only one E. coli, one Enterobacter cloacae and one Enterobacter aerogenes strains) (Table 3).
Figure 1. One strain was negative for all the antimicrobial agents tested. Detection of carbapenemase production by MHT (22.4%), bla_DHA in 9 (15.5%) and bla_NDM-1 in one isolate (1.7%). Five isolates were positive for both bla_DHA and bla_KPC and 4 isolates were tested positive for both bla_OXA and bla_VIM. One strain was negative for all the tested genes despite positive ESBL phenotype (Table 4).

**DISCUSSION**

The emergence and global spread of acquired CR Gram-negative rods are designated a “global sentinel event” (Woodford et al., 2004). The overall rate of CR Gram-negative rods.
negative strains in our NICU during the study period was high (36.7%). This finding agrees with another Egyptian study conducted by Makharita et al. (2020) who detected 36% isolated Enterobacteriaceae were carbapenemase producers. Much lower CR isolation rates have been reported in Turkey 2.82% (Baran and Aksu, 2016) and the United States 1.4 to 4.2% (Pollett et al., 2014). Moreover, one Chinese study reported 0.9% CR isolates (28/3286) (Liao et al., 2014). The reason for higher rate of CR detected in our study could be related to the extensive use of carbapenems to treat life threatening infections in neonatal patients and absence of antibiotic stewardship program. In this study all CR strains were retrieved from the NICU in which many CR infection and colonization factors, including impaired immune status, prolonged hospital stay, and frequent use of antibiotics, were present. For this reason, proper hand hygiene and infection control measures must be emphasized. Monitoring of CR must be encouraged to reduce CR infection rate.

*K. pneumoniae* (37.9%) was the most common CR species isolated followed by *E. coli* (24.1%). This is in
agreement with other CR studies in Egypt, United States, Europe and China (Metwally and Elnagar, 2019; Centers for Disease Control and Prevention, 2013; Akova et al., 2012; Yang et al., 2018) and is consistent with the CDC’s recommendations that *K. pneumoniae*, *E. coli*, and *Enterobacter* spp. are the key health care-associated pathogens to focus on in the control of US CR epidemic (Centers for Disease Control and Prevention, 2014). That is why attention should be raised when any of these bacteria is isolated from clinical specimens.

In the present study, the overall resistance to carbapenems was high (75.9% for imipenem and 57% for meropenem). Higher rate of resistance was detected by Pollett et al. (2014); Mohamed et al. (2018), who found low rate of susceptibility to meropenem (4.3%, 3%) and to imipenem (1.7%, 3%). On the other hand, lower rate of resistance was detected by Okoche et al. (2015) who found that only 18.4% of study isolates were resistant to meropenem. The varied range in susceptibility/resistance rate of carbapenems among GNB in different studies may be due to different antibiotic usage patterns in different geographic regions.

It is difficult to compare the present results with those of others taking into consideration variations in study
Table 4. Phenotypic and genotypic characterization of CR among 58 Gram-negative clinical isolates.

| Carbapenem resistance characterization | Number of isolates (N=58) |
|---------------------------------------|---------------------------|

**Phenotypic characterization**

**Cephalosporin/Clavulanic combination disc test:**
- **ESBL positive**: 58
- **ESBL negative**: 0
- **ESBL (%)**: 100

**Modified Hodge test:**
- **MHT-positive**: 52
- **MHT-negative**: 6
- **Carbapenemase (%)**: 89.60

**Synergy combined disc test:**
- **CDT-positive**: 33
- **CDT-negative**: 25
- **MBLs (%)**: 56.9

**Genotypic characterization**
- **bla\_KPC**: 34
- **bla\_VM**: 15
- **bla\_IMP**: 13
- **bla\_OXA-48**: 9
- **bla\_NDM-1**: 1
- **bla\_OXA-48+ bla\_KPC**: 5
- **bla\_IMP + bla\_VM**: 4
- **PCR-negative**: 1
- **Carbapenemase encoding genes (%)**: 98.27

ESBL: Extended spectrum beta-lactamase, MHT: modified Hodge test, MBL: metallo beta-lactamases, CDT: combined disc test.

In the present study, all CR strains were ESBL producers and carbapenemase production was detected in 52 isolates (89.6%), while MBL production was detected in 33 (56.9%) isolates. Similarly, Fattouh et al. (2015) identified 88.14% of CR isolates as carbapenemase producers but lower MBLs activity was detected in 33.9% of the isolates by CDT. ESBL production was confirmed by Netikul and Kiratisin (2015) in 76.2% of the CR isolates and only 14.4% were positive for MHT. In accordance, Makharita et al. (2020) detected 88.14% of CR isolates as carbapenemase producers but lower MBLs activity was detected in 33.9% of the isolates by CDT. ESBL production was confirmed by Netikul and Kiratisin (2015) in 76.2% of the CR isolates and only 14.4% were positive for MHT. In accordance, Makharita et al. (2020) detected 33.2% MBLs positive isolates while only 33.6% of the CR isolates were positive MHT. On the contrary to the present results but also in Mansoura, carbapenamase activity was detected in 61.9% of CR *K. pneumoniae* isolated from ICUs of Mansoura University hospitals by MHT method (Moemen and Masallat, 2017).

Molecular characterization of the isolates revealed that 57 (98.3%) were positive for carbapenemase encoding genes. In accordance with the present results, Makharita et al. (2020) found 98.6% (74/75) of CR strains were carrying *KPC* gene, moreover 97.3% (73/75) were carrying *GES* gene. Also, similar to the present results, Yang et al. (2018) found that, among all CR strains *bla\_KPC* carrying rate was 60.3% and *bla\_IMP* were detected in 4.5% of total CR strains. Not so far from the present results. Pollett et al. (2014) stated that, a carbapenemase-encoding gene was found in 81.7% (94/115) of CR with *bla\_KPC* the most prevalent (78.3%).

These results were different with Elbadawi et al. (2021) who detected that the most prevalent gene was NDM-1 gene. On the other hand, Baran and Aksu (2016). reported that the OXA-48 gene was the most frequent gene as it was detected in 86/181 (47.5%) strains, NDM-1 gene in 6 (3.3%) strains, and VIM gene in 1 (0.6%) strain. IMP and KPC genes were not identified. Both OXA-48 and NDM-1 were produced by 3 strains and both OXA-48 and VIM were produced by one strain.

It is cleared that carbapenemase genes tend to be frequent in certain countries. It was found that *bla\_KPC*...
genes are dominant in USA, Greece and Egypt, while bla$_{NDM}$ genes are commonly detected in isolates recovered from India, Pakistan and Far East (Elbadawi et al., 2021).

A study in our locality conducted by Moemen and Masallat (2017) detected that 92.9% of the CR isolates were positive for one or more carbapenemase genes. Five of the 39 carbapenemase gene carrying isolates harbored two or more genes. The most prevalent gene was bla$_{KPC}$ 47.8% followed by bla$_{VIM}$ 21.7%.

One strain that was negative for all the tested genes was detected despite positive ESBL phenotype. Similarly, Moemen and Masallat (2017) found that, none of the tested carbapenemase genes tested (bla$_{KPC}$, bla$_{NDM}$, bla$_{VIM-1}$, bla$_{IMP}$, and bla$_{OXA-48}$) were detected in three isolates. Carbapenem resistance of these isolates is mostly due to a combination of ESBLs and changes in outer membrane proteins (ESBL/Omp) (Endimiani et al., 2010).

This study had several limitations. First, the study did not check tested isolates for sensitivity to last-line antimicrobials, such as colistin, tigecycline, and fosfomycin. Secondly, only isolates that showed imipenem or meropenem in the resistant were tested. Unlike, carbapenemases have been found in carbapenem-sensitive Enterobacteriaceae, specially bla$_{OXA-48}$ (Nordmann et al., 2012). Finally, clinically significant isolates were mainly examined and this undervalues the colonized patients, which may forcefully propagate CR (Viau et al., 2012). For this reason, given that CR are increasing in this region, augmentation of local infection control measures beyond core elements to active surveillance may be useful, as stressed by the CDC (Centers for Disease Control and Prevention, 2014).

**CONFLICT OF INTERESTS**

The authors have not declared any conflicts of interests.

**REFERENCES**

Akova M, Daikos GL, Tzouvelekis L, Carmeli Y (2012). Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. Clinical Microbiology and Infection 18(5):439-448.

Al Naemi N, Cohen Stuart J, Leverstein van Hall M (2012). NVMM Guideline Laboratory detection of highly resistant microorganisms (HRMO), version 2.0. 2012; Available: http://www.nvmm.nl/richtlijnen/hrmolaboratory-detection-highly-resistant-microorganisms

Albigger B, Glashner C, Struelens MJ, Grundmann H, Monnet DL (2015). European Survey of Carbapenemase-Producing Enterobacteriaceae Working Group. Carbapenemase-producing Enterobacteriaceae in Europe: assessment by national experts from 38 countries. Europe's Journal on Infectious Disease Surveillance, Epidemiology, Prevention and Control 20(45).

Al-Zahrani IA (2018). Routine detection of carbapenem-resistant Gram-negative bacilli in clinical laboratories: A review of current challenges. Saudi Medical Journal 39(9):861-872.

Baran I, Aksu N (2016). Phenotypic and genotypic characteristics of carbapenem-resistant Enterobacteriaceae in a tertiary-level reference hospital in Turkey. Annals Clinical Microbiology Antimicrobials 15:20.

Centers for Disease Control and Prevention (2014). Guidance for control of carbapenem-resistant Enterobacteriaceae. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/hai/pdfs/cre/cre-guidance-s27.pdf.

Centers for Disease Control and Prevention (2013). Vital signs: carbapenem-resistant Enterobacteriaceae. Morbidity and Mortality Weekly Report (MMWR) 62:165-170.

Choudhury DD, Singh NP, Rai S, Batra P, Manchanda V (2018). Carbapenem resistant Enterobacteriaceae neonatal gut colonization: A future concern in healthcare settings. Indian Journal Microbiology Research 5(3):349-354.

Clinical and Laboratory Standards Institute, CLSI (2017). Performance standards for antimicrobial susceptibility testing: Twenty-seven informational supplements; document (M100-S27), Wayne, PA: CLSI.

Elbadawi HS, Elhag KM, Mahgoub E, Altayb HN, Ntoumi F, Elton L, Struelens MJ, Grundmann H, CLSI (2012). Interventional.

Elhag KM, Mahgoub E, Altayb HN, Ntoumi F, Elton L, Struelens MJ, Grundmann H, CLSI (2012). Os GL, Tzouvelekis L, Carmeli Y (2015). Genetic Characterization of Carbapenem Resistant Enterobacteriaceae neonatal gut colonization: A future concern in healthcare settings. Indian Journal Microbiology Research 5(3):349-354.

Expression of Carba-NTR gene in Acinetobacter baumanii isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in Jakarta. Indonesian Journal of Biomedical Research 5(3):349-354.

Fattouh M, Nasr El-din A, Omar MA (2015). 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. International Journal of Current Microbiology and Applied Science 4(5):1-15.

Fattouh M, Al-Rashed N, Saeed NK, Bindayna KM (2019). Detection of VIM and NDM-1 metallo-beta-lactamase genes in carbapenem-resistant Pseudomonas aeruginosa clinical strains in Bahrain. Journal of Laboratory Physicians 11(2):138-143.

Kamrujwatt A, Saharan YR, Lestarci DC (2013). Detection of carbapenemase encoding genes in Enterobacteriaceae, Pseudomonas aeruginosa and Acinetobacter baumanii isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in Jakarta. Indonesian Journal of Biomedical Research 5(3):349-354.

Liao K, Chen Y, Huang H, Guo P, Chen D, Liu M, Zeng Y (2014). Molecular characteristics of carbapenem-resistant Enterobacteriaceae isolates from a Chinese Tertiary hospital in Guangdong. Journal of Microbiology and Infectious Diseases 4(4):145-151.

Mahan CR, Lehman DC, Manuselis G (2007/2008). Textbook of Diagnostic Microbiology 2007. 2008. 3rd ed. St. Louis, Mo: Saunders Elsevier.

Makharia RR, EI-Kholy I, Hetta HF, Abdelaziz MH, Hagagy FI, Ahmed AA, Algammal AM (2020). Antibiogram and Genetic Characterization of Carbapenem-Resistant Gram-Negative Pathogens Incriminated in Healthcare-Associated Infections. Infection and Drug Resistance 13:3991-4002.

Metwally WS, Elnaggar WM (2019). Multidrug Resistant Uropathogens among Egyptian Pregnant Women. American Journal of Infectious Diseases 15(4):115-122.

Moemen D, Masallat DT (2017). Prevalence and characterization of carbapenem-resistant Klebsiella pneumoniae isolated from intensive care units of Mansoura University hospitals. Egyptian Journal of Basic and Applied Science 4(1):37-41.

Nordmann P, Robert A, Thirion F, Piddock LJV, Poirel L, Naas T, Rozenberg S, Carattoli A, Aitken JA, Goosney E (2010). Carbapenemases of the metallo-beta-lactamase (MBL) family. Future Microbiology 5(10):1299-1308.

Rashed MA, Elhag KM, Ntwari FM, Elton L, Struelens MJ, Grundmann H, CLSI (2012). Interventional.

Rashed MA, Elhag KM, Ntwari FM, Elton L, Struelens MJ, Grundmann H, CLSI (2012). Os GL, Tzouvelekis L, Carmeli Y (2015). Genetic Characterization of Carbapenem Resistant Enterobacteriaceae neonatal gut colonization: A future concern in healthcare settings. Indian Journal Microbiology Research 5(3):349-354.

Nokland DL (2015). Carba-NTR gene in Acinetobacter baumanii isolated from patients at Intensive Care Unit Cipto Mangunkusumo Hospital in Jakarta. Indonesian Journal of Biomedical Research 5(3):349-354.

Rashed MA, Elhag KM, Ntwari FM, Elton L, Struelens MJ, Grundmann H, CLSI (2012). Interventional.

Rashed MA, Elhag KM, Ntwari FM, Elton L, Struelens MJ, Grundmann H, CLSI (2012). Os GL, Tzouvelekis L, Carmeli Y (2015). Genetic Characterization of Carbapenem Resistant Enterobacteriaceae neonatal gut colonization: A future concern in healthcare settings. Indian Journal Microbiology Research 5(3):349-354.

Mizmel BW, Nkwanyana NM, Singh R (2021). Clinical outcome of neonates with Carbapenem-resistant Enterobacteriaceae infections at the King Edward VII hospital's neonatal unit, Durban, South Africa. Research in Southern African Journal of Infectious Diseases 36(1):a223.

Netkul T, Kiratlis P (2015). Genetic Characterization of Carbapenem-Resistant Enterobacteriaceae and the Spread of Carbapenem Resistant Klebsiella pneumoniae ST340 at a University Hospital in
Thailand. PLOS ONE 10(9):e0139116.
Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V (2012). Identification and screening of carbapenemase producing Enterobacteriaceae. Clinical Microbiology and Infection Journal 18(5):432-438.

Okoche D, Asiimwe BB, Katabazi FA, Kato L, Najjuka CF (2015). Prevalence and Characterization of Carbapenem-Resistant Enterobacteriaceae Isolated from Mulago National Referral Hospital, Uganda. PLOS ONE 10(8):e0135745.

Poirel L, Walsh TR, Cuvillier V, Nordmann P (2011). Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic Microbiology and Infectious Disease Journal 70(1):119-123.

Pollett S, Miller S, Hindler J, Uslan D, Carvalho M, Humphries RM (2014). Phenotypic and molecular characteristics of carbapenem-resistant Enterobacteriaceae in a health care system in Los Angeles, California, from 2011 to 2013. Journal of Clinical Microbiology 52(11):4003-4009.

Tamma PD, Simner PJ (2018). Phenotypic Detection of Carbapenemase-Producing Organisms from Clinical Isolates. Journal of Clinical Microbiology 56(11):e01140-18.

Vading M, Samuelsen O, Haldorsen B, Sundsfjord AS, Giske CG (2011). Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing Klebsiella pneumoniae with the EUCAST and CLSI breakpoint systems. Clinical Microbiology and Infection Journal 17(5):668-674.

Viau RA, Hujer AM, Marshall SH, Perez F, Hujer KM, Briceno DF, Dul M, Jacobs MR, Grossberg R, Toltzis P, Bonomo RA (2012). “Silent” dissemination of Klebsiella pneumoniae isolates bearing K. pneumoniae carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. Clinical Infectious Disease Journal 54(9):1314-1321.

Woodford N, Tierno PM, Young K, Tysall L, Palepou MFI, Ward E, Painter RE, Suber DF, Shungu D, Silver LL, Inglima K, Komblum J, Livermore DM (2004). Outbreak of Klebsiella pneumoniae producing a new carbapenem-hydrolyzing class A b-lactamase, KPC-3, in a New York Medical Center. Antimicrobial Agents Chemotherapy 48(12):4793-4799.

Yang Y, Chen J, Lin D, Xu X, Cheng J, Sun C (2018). Prevalence and drug resistance characteristics of carbapenem resistant Enterobacteriaceae in Hangzhou, China. Frontiers of Medicine 12(2):182-188.