Molecular Detection and Characterization of Carbapenemases Among Carbapenem Resistant *Escherichia coli* and *Klebsiella pneumoniae* Isolated from Urine

Hussaini I.M [1*], Suleiman A.B [1], Olonitola O.S [1], Oyi R.A [2]

Abstract

Objective: Carbapenem resistance mediated by carbapenemases poses a significant public health threat due to its transferable nature, unlike other carbapenem resistance mechanisms. This study aimed to molecularly detect and characterize carbapenemases among carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine samples. Methods: A total of 123 non-duplicate isolates (70 E. coli and 53 K. pneumoniae) were screened for carbapenem resistance using Clinical Laboratory Standard Institute guidelines. Carbapenem-resistant isolates were further tested for the presence of carbapenemase genes (blaKPC, blaOXA, blaNDM) using PCR. Positive PCR products were sequenced, and sequence similarity was analyzed using nucleotide BLAST. Multiple sequence alignment was performed using ClustalW and BioEdit. Results: Among the 123 isolates screened, 6 (4.88%) were carbapenem-resistant, including 2 (2.86%) *E. coli* and 4 (7.55%) *K. pneumoniae* isolates. Carbapenemase genes were detected in five out of the six carbapenem-resistant isolates. The most frequently detected carbapenemase gene was blaOXA (57.14%), followed by blaNDM (42.86%). No blaKPC gene was detected. Co-harboring of blaNDM and blaOXA genes was observed in two isolates. Sequence similarity analysis showed 98–100% identity with carbapenemase genes from GenBank. Nucleotide substitutions were absent in blaNDM gene sequences, while nucleotide substitutions leading to corresponding amino acid changes were observed in blaOXA gene sequences at various positions. Conclusion: Carbapenem resistance in the studied isolates was predominantly mediated by OXA and NDM carbapenemases. These findings underscore the importance of monitoring carbapenemase spread among Gram-negative bacteria to mitigate the emergence and dissemination of carbapenem-resistant strains, which jeopardize the efficacy of carbapenems as last-resort antibiotics.

Keywords: Carbapenem resistance, carbapenemases, *Escherichia coli*, *Klebsiella pneumoniae*, PCR detection

Significance | Carbapenem resistance mediated by carbapenemases, such as blaOXA and blaNDM, poses a critical public health threat due to its high transmissibility and limited treatment options.

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Editor Md Asaduzzaman Shishir, And accepted by the Editorial Board Feb 09, 2021 (received for review Jan 05, 2021)

Introduction

Klebsiella pneumoniae and *Escherichia coli* are important pathogens, causing various infections including pneumonia, bacteremia, septicemia, purulent infections, and urinary tract

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Please cite this article.
Hussaini I.M., Suleiman A.B. et al. (2021). Molecular Detection and Characterization of Carbapenemases Among Carbapenem-Resistant *Escherichia Coli* And *Klebsiella Pneumoniae* Isolated from Urine, Journal of Primeasia, 2(1), 1-8, 20212

infections, all of which may occur in either a community or hospital setting (Yao et al., 2015). *K. pneumoniae* and *E. coli* are common causes of bacteremia, pneumonia, urinary tract infection, and liver abscess (Chiu et al., 2018).Carbapenems have a penicillin-like five-membered ring, but the sulfur at C-1 in the five-member ring is replaced with a carbon atom and a double bond between C-2 and C-3 is introduced. It has the broadest spectra of antimicrobial activity among all β-lactams and are primarily used to treat infections by aerobic Gram-negative bacteria. The emergence and spread of acquired carbapenem resistance due to carbapenemases are a major concern of public health and is considered a global sentinel event (Jeon et al., 2015).One of the most serious health threats of the 21st century is antimicrobial resistance (AMR). Antimicrobial resistance challenges effective treatment of infectious diseases now and in the future (World Health Organization [WHO], 2015). In the clinical context, the emergence of carbapenem non-susceptible isolates poses a serious threat to patient survival because infections caused by carbapenem non-susceptible isolates have limited treatment options and are associated with high mortality (Chiu et al., 2018).

The broad spectrum of activity and stability to hydrolysis by most beta-lactamases of the carbapenem has made them the drugs of choice for the treatment of infections caused by cephalosporin-resistant Gram-negative bacilli, especially ESBL-producing Gram-negative infections (Srinivasan et al., 2015). Carbapenem-resistant organisms (CROs) are of great significance to the medical community and are associated with higher mortality rates than carbapenem-susceptible organisms (Esterly et al., 2012). The Centers for Disease Control and Prevention (CDC) campaign to Detect and Protect against Antibiotic Resistance Initiative (known as the AR Initiative) specifically cites detection and tracking of carbapenem-resistant *Enterobacteriaceae* as a highest priority (United States Centers for Disease Control and Prevention, 2016).

Carbapenemases are the most versatile family of β-lactamases and are able to hydrolyze carbapenems and other β-lactams (Demir, Zer, & Karaoglan, 2015). Among the many mechanisms conferring resistance to carbapenems, carbapenemases can efficiently hydrolyze carbapenems and have become an important cause of antimicrobial resistance (Chiu et al., 2018). The global emergence of carbapenemase-producing *Enterobacteriaceae* (CPE) poses a threat to the achievements of modern medicine. The Centers for Disease Control and Prevention and the World Health Organization have recently classified CPE as one of the most urgent antimicrobial-resistance threats. CPE rarely arise de novo; colonization and infection occur as a result of transmission of organisms, plasmids, or transposons from person to person, with such transmission occurring predominantly in healthcare institutions (Nordmann, Naas, & Poirel, 2011; Nordmann, Dortet, & Poirel, 2012). Most worrisome, treatment of infections caused by these organisms is extremely difficult because of their multi-drug resistance, which results in high mortality rates (Fasciana et al., 2019). The spread of carbapenemase-producing strains across the world has made it necessary for us to detect and characterize carbapenemases (Gupta, Bansal, Singla, & Chander, 2013; Mlynarcik, Roderova, & Kolar, 2016).

Materials and Methods

Bacterial isolates

A total of 123 clinical isolates from urine consisting of 70 Escherichia coli and 53 Klebsiella pneumoniae isolates were included in this study. These isolates were isolated from urine of patients attending selected hospitals in Zaria, Nigeria and identified based on their colonial morphology of MacConkeyagar, Gram reaction and biochemical reactions.

Screening for Carbapenem Resistant Klebsiella pneumoniae and Escherichia coli

The isolates of *Klebsiella pneumoniae* and *Escherichia* coli were screened for carbapenem resistance as described in the manual of Clinical Laboratory Standard Institute (2015). Briefly, the isolates of Klebsiella pneumoniae and Escherichia coli were standardized by comparing their turbidity with that of 0.5 McFarland standard and subjected to antibiotics susceptibility test on Mueller Hinton agar by modified Kirby-Bauer disc diffusion technique using imipenem (10 µg) and cefotaxime (30 µg) antibiotic discs (Clinical and Laboratory Standards Institute [CLSI], 2019). Using the published 2019 CLSI guidelines, the susceptibility or resistance of the isolates to each of the antibiotics tested was determined (Clinical and Laboratory Standards Institute [CLSI], 2019). Isolates that are non-susceptible to imipenem and cefotaxime were further confirmed for carbapenemase production by PCR.

Detection of Carbapenemase Genes by PCR

Crude genomic DNA for PCR was extracted from the isolates using the heat lysis method. Briefly, colonies from overnight culture of the isolates were transferred into a test tube containing 1 mL of nuclease-free water and boiled at 100°C for 10 minutes in a water bath and subsequently frozen at -20°C for 10 minutes. This was followed by centrifugation for 10 minutes at 3000 rpm (Espinosa, Baez, Percedo, & Martinez, 2013). Five microliters (5 µL) of the supernatant was used for PCR. All isolates were screened for the resistance genes encoding KPC, NDM, and OXA by PCR assay using previously described primers (Table 1).

PCR was performed in accordance with InqabaBiotec's in-house protocol using 10 µL of NEB OneTaq 2X master mix with standard buffer (Catalogue No. M0482S), 1 µL of each primer (10 µM), 7 µL nuclease-free water (Catalogue No. E476), and 1 µL of DNA

template. The PCR conditions were as follows: initial denaturation at 94°C for 5 mins, followed by 35 cycles of denaturation at 94°C for 30 secs, annealing at 50°C for 30 secs, and extension at 68°C for 1 min, and a final extension at 68°C for 10 mins (Mohammed, Zailani, & Onipede, 2015).

Agarose Gel Analysis

The PCR amplicons were visualized after running at 100 V for 90 mins on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye (Yao et al., 2015).

Carbapenemase Gene Sequencing

Sequencing was performed in accordance with InqabaBiotec's in-house protocol. Briefly, PCR products were cleaned using ExoSAP Protocol and then sequenced using the Nimagen, Brilliant Dye™ Terminator Cycle Sequencing Kit V3.1, BRD3-100/1000 according to manufacturer's instructions. Sequence chromatogram analysis was performed using FinchTV analysis software. The carbapenemase gene sequences obtained were compared with those in NCBI database. A minimum sequence percent identity of ≥ 98.00% and 100.00% coverage was used to confirm the genes. Sequences of the carbapenemase genes were edited, aligned with reference sequences from the GenBank, and translated into amino acid sequences using BioEdit version 7.2.5 (Nordmann, Dortet, & Poirel, 2012; Van der Zwaluw et al., 2020).

Results and Discussion

Out of the 123 isolates screened for carbapenem resistance, 6 (4.88%) were found to be carbapenem resistant isolates while the remaining 117 (95.12%) isolates were carbapenem susceptible isolates (Figure 1). This occurrence raises concern since carbapenems are not commonly prescribed and used in the selected hospitals in Zaria; they are usually reserved as a last resort for treating infections caused by multidrug-resistant Gram-negative bacteria (GNB). The occurrence of carbapenem-resistant Enterobacteriaceae (CRE) observed in Zaria and other regions where carbapenems are less commonly prescribed may be due to the international travel of patients from countries where CRE is endemic (World Health Organization, 2015).

However, the overall occurrence of carbapenem-resistant isolates in this study is lower than the 36.8% reported by Enwuru, Enwuru, and Adepoju-Bello (2011) in Southwest Nigeria; 15.2% reported by Oduyebo, Falayi, Oshun, and Ettu (2015) in Lagos; 7.7% reported by Anibijuwon, Gbala, and Adebisi (2018) in Ogbomoso and Osogbo, Southwest Nigeria; and 7.61% reported by Alaka, Orimolade, Ojo, and Onipede (2019) in Ile Ife. The higher occurrences observed in these studies might be due to differences in targeted bacteria, study populations, study designs, and types of screening techniques used.

The emergence and spread of CRE worldwide are of great public health concern, as there are limited antibiotics available for treating these strains. The increasing number of hospital-acquired and community-acquired infections caused by CRE, especially carbapenem-resistant *Klebsiella pneumoniae* and *Escherichia coli* isolates, is burdening the healthcare system (Centers for Disease Control and Prevention, 2013).

Out of the 70 *E. coli* isolates screened for carbapenem resistance, 2 were found to be carbapenem-resistant E. coli, giving an occurrence of 2.86%. Similarly, 4 isolates of K. pneumoniae were found to be carbapenem-resistant K. pneumoniae out of the 53 K. pneumoniae isolates screened, giving an occurrence of 7.55% (Table 2). The difference in the occurrence of carbapenem-resistant E. coli and K. pneumoniae was not statistically significant ($p \geq 0.05$). A higher occurrence of carbapenem-resistant K. pneumoniae (19.05%) was previously reported by Mukail, Tytler, Adeshina, and Igwe (2019) in Zaria. The observed difference might be due to their isolates coming from various clinical samples (urine, blood, HVS, sputum, and wound swabs).

The most prevalent carbapenem-resistant bacteria in this study were K. pneumoniae, which might be linked to its ability to acquire and accumulate genes coding for antibiotic resistance (World Health Organization, 2017). In line with this finding, K. pneumoniae was indicated as one of the multidrug-resistant bacteria constituting an immediate threat to human health (World Health Organization, 2018; Zowawi et al., 2014). The higher occurrence of carbapenem-resistant K. pneumoniae observed in this study aligns with the report by Ssekatawa, Byarugaba, Wampande, and Ejobi (2018) and global data on carbapenem resistance in India (Nordmann, Dortet, & Poirel, 2012). Oduyebo et al. (2015) also reported a higher occurrence of carbapenem-resistant K. pneumoniae (14.5%) compared to carbapenem-resistant E. coli (7.8%).

Carbapenem resistance traits such as decreased outer membrane permeability, overexpression of β-lactamases, production of cephalosporinase, and porin loss are not transferable like carbapenemase genes. This explains why carbapenem-resistant bacteria that are not carbapenemase producers are considered of lesser public health importance compared to carbapenemase-producing carbapenem-resistant bacteria. The spread of carbapenemase producers is a significant clinical issue in controlling antibiotic-resistant GNB (Evans & Amyes, 2014).

Carbapenemase genes were detected in five out of the six carbapenem-resistant isolates (CRIs) screened, indicating that carbapenem resistance in these five isolates was mediated by carbapenemases. In the remaining one isolate, carbapenem resistance might be due to either production of other carbapenemase genes not targeted in this study, overproduction of other β-lactamases, porin loss, or reduced permeability. This finding is consistent with those of Nordmann, Naas, and Poirel (2011); Nordmann, Dortet, and Poirel (2012); and Demir, Zer, and

Table 1. Primer sequences used for the detection of carbapenemase genes.

Gene	Primer	Sequences (5' – 3')	Expected amplicon size (bp)	References
*bla*KPC	KPC-F	ATGTCACTGTATCGCCGTCT	893	Huang *et al.*, [14]
	KPC-R	TTTTCAGAGCCTTACTGCCC		
*bla*NDM	NDM-F	GGTTTGGCGATCTGGTTTTC	550	Mohammed *et al.*, [15]
	NDM-R	CGGAATGGCTCATCACGATC		
*bla*OXA	OXA-F	AACGGGCGAACCAAGCATTTT	597	Mlynarcik *et al.*, [16]
	OXA-R	GAGCACTTCTTTTGTGATGGCT		

Table 2: Occurrence of carbapenem resistant *Escherichia coli* and *Klebsiella pneumoniae*

Isolate	No. of isolates screened	No. of Carbapenem resistant isolates	Occurrence of Carbapenem resistant isolates (%)
Escherichia coli	70	2	2.86
Klebsiella pneumoniae	53	4	7.55
Total	123	6	4.88

$\chi^2 = 1.430$, $p = 0.2318$ df =1

Table 3. Distribution of carbapenemase genes among carbapenem resistant *Klebsiella pneumoniae* and *Escherichia coli*

Isolate code	Isolate identity	Carbapenemase gene(s) detected
GUM015	*Klebsiella pneumoniae*	*bla*NDM, *bla*OXA
MUF002	*Escherichia coli*	*bla*OXA
MUF012	*Escherichia coli*	*bla*NDM
AUM023	*Klebsiella pneumoniae*	*bla*NDM, *bla*OXA
GUF084	*Klebsiella pneumoniae*	*bla*OXA

Table 4. Occurrence of carbapenemase-producing isolates based on PCR

Isolate	No. positive for carbapenemase gene	Occurrence (%) of CP
Escherichia coli (n = 70)	2	2.86
Klebsiella pneumoniae (n = 53)	3	5.66
Overall (n = 123)	5	4.07

$\chi^2 = 0.608$, $p = 0.4356$ df =1

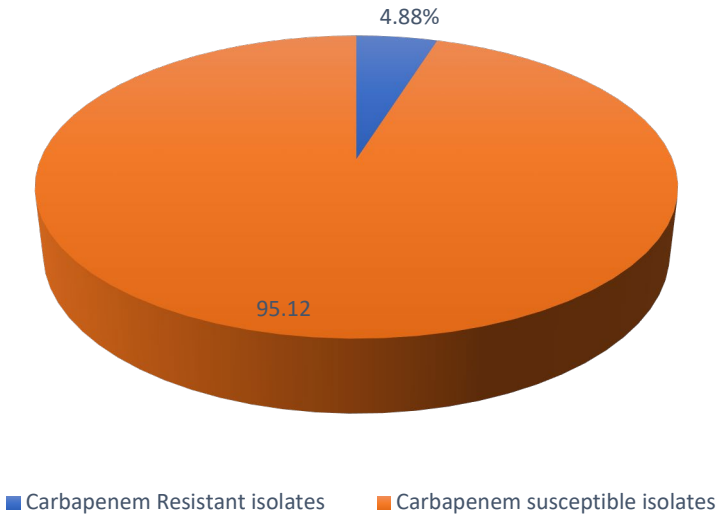

Figure 1. Overall occurrence of carbapenem resistant isolates among *Escherichia coli* and *Klebsiella pneumoniae* isolated from urine of patients attending selected hospitals in Zaria

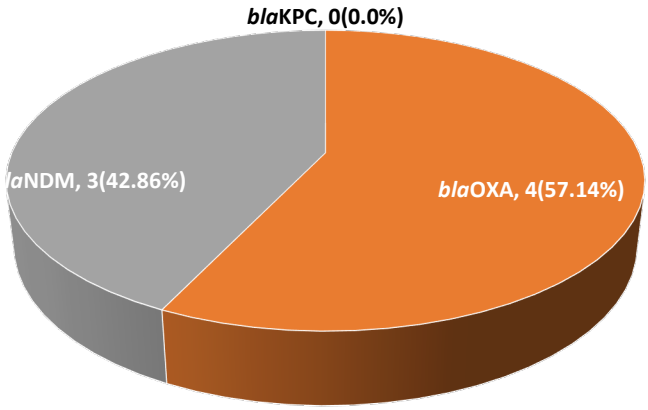

Figure 2. Percentage distribution of carbapenemase genes among the isolates

Karaoglan (2015), who reported that carbapenem resistance among Enterobacteriaceae is primarily due to the production of carbapenemases, while less frequent mechanisms include the overproduction of AmpC-mediated β-lactamases or extended-spectrum β-lactamases (ESBLs) in organisms with porin mutations. The carbapenem resistance determinants in this study were blaOXA (57.14%) and blaNDM (42.86%), while blaKPC was not detected in any of the CRIs (Figure 2). A similar phenomenon where OXA and NDM were the dominant carbapenemases in E. coli and K. pneumoniae was reported by Nordmann, Dortet, and Poirel (2012); Zowawi et al. (2014) in the Gulf Cooperation Council countries; and Al-Agamy, Aljallala, Radwana, and Shibl (2018) in Riyadh, Saudi Arabia.

The most frequently detected carbapenemase gene was the blaOXA (57.14%), which codes for OXA carbapenemase. Detection of OXA carbapenemases in Enterobacteriaceae is of major public health concern due to their ability to mutate rapidly, thereby resulting in an expanded spectrum of activity (Evans & Amyes, 2014) [33]. This finding is supported by Evans and Amyes (2014), who reported that the OXA gene is the predominant mechanism and major contributor to carbapenem resistance in *Enterobacteriaceae.*

Two isolates co-harbored blaOXA and blaNDM genes (Table 3), indicating the carriage of multiple carbapenemase genes on a plasmid which can serve as a source of multidrug resistance and may represent an emerging threat. A similar finding was also reported by Zowawi et al. (2014) in the Gulf Cooperation Council countries, Protonotariou et al. (2019) in Greece, and van der Zwaluw et al. (2020) in the Netherlands.

The overall occurrence of carbapenemase genes was 4.07%, while the occurrence of these genes in E. coli and K. pneumoniae was 2.86% and 5.66%, respectively (Table 4). The higher occurrence of carbapenemase genes in K. pneumoniae might be due to its permeability to mobile genetic elements, hence the high frequency and diversity of resistance genes observed in it. The higher occurrence of carbapenemase genes in K. pneumoniae compared to E. coli was also reported by van der Zwaluw et al. (2020) [35] in the Netherlands.

Sequence similarity analysis revealed that the carbapenemase genes were similar to those in GenBank, showing 98-100% identity. The blaNDM genes detected in isolates GUM015 and MUF012 were 100% similar to blaNDM detected in E. coli F070 from Myanmar (Accession number: AP023238.1) and K. pneumoniae KJ10 from India (Accession number: MT462582.1). However, the blaNDM gene detected in isolate AUM023 was 99.46% similar to these strains in GenBank. The blaOXA genes detected in isolates GUM015 and MUF002 were 99.54% similar to blaOXA detected in K. pneumoniae N83 from Egypt (Accession number: MK341123.1), 98.47% similar to K. pneumoniae KPTR1-18 from Russia (Accession number: MK867763.1), and 98.47% similar to E. coli LAU-OXA from Lebanon (Accession number: CP045282.1).

Analysis of the carbapenemase gene sequences revealed some level of polymorphism in the blaOXA genes; however, this was not observed in the blaNDM genes. In line with this finding, Diene and Rolain (2014) [36] reported that OXA carbapenemases are the most variable carbapenemases. Furthermore, analysis of blaOXA gene sequences revealed that these variations resulted from preferential alteration due to antibiotic selective pressure. Despite the polymorphism observed in the blaOXA sequences, the active site regions are relatively conserved (Evans & Amyes, 2014) [33].

The fast rate at which blaOXA genes are evolving, coupled with their diversity, suggests that a number of these blaOXA genes may rapidly evolve to be resistant to new carbapenemase inhibitors (Evans & Amyes, 2014) [33].

Conclusion

In this study, out of 123 clinical isolates of Klebsiella pneumoniae and Escherichia coli from urine samples, 6 (4.88%) were found to be carbapenem-resistant. This low prevalence is notable given that carbapenems are seldom prescribed in the studied hospitals, suggesting possible international transmission of resistant strains. Among the resistant isolates, carbapenemase genes were detected in five, with blaOXA and blaNDM identified as predominant. The presence of multiple carbapenemase genes on plasmids highlights a significant concern for multidrug resistance. The genetic analysis revealed high sequence similarity to known carbapenemase genes, with notable polymorphism in blaOXA genes. These findings underscore the growing challenge of carbapenem resistance, necessitating ongoing surveillance and preventive measures to address the spread of resistant strains and protect the efficacy of existing antibiotics.

Author contributions

H.I.M. conceptualized and developed the methodology, S.A.B. and O.O.S. prepared the original draft and collected, O.R.A. reviewed and edited the writing.

Acknowledgment

The author thanks the Department of Microbiology, Faculty of Life Sciences, Ahmadu Bello University, Zaria, Nigeria.

Competing financial interests

The authors have no conflict of interest.

References

Al-Agamy, M. H., Aljallala, A., Radwana, H. H., & Shibl, A. M. (2018). Characterization of carbapenemases, ESBLs, and plasmid-mediated quinolone determinants in

carbapenem-insensitive Escherichia coli and Klebsiella pneumoniae in Riyadh hospitals. Journal of Infection and Public Health, 11(1), 64-68.

Alaka, O. O., Orimolade, E. A., Ojo, O. O., & Onipede, A. O. (2019). The phenotypic detection of carbapenem-resistant organisms in orthopedic wound infections in Ile-Ife, Nigeria. Acta Scientific Microbiology, 2(2), 35-42.

Anibijuwon, I. I., Gbala, I. D., & Adebisi, O. O. (2018). Carbapenem-resistant Enterobacteriaceae among in-patients of tertiary hospitals in Southwest Nigeria. Notulae Scientia Biologicae, 10(3), 310-317.

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

Chiu, S. H., Ma, L., Chan, M. C., Lin, Y. T., Fung, C. P., Wu, T. L., Chuang, Y. C., Lu, P. L., Wang, J. T., Lin, J. C., & Yeh, K. M. (2018). Carbapenem nonsusceptible Klebsiella pneumoniae in Taiwan: Dissemination and increasing resistance of carbapenemase producers during 2012–2015. Scientific Reports, 8, 8468.

Clinical and Laboratory Standards Institute (CLSI). (2015). Performance standards for antimicrobial susceptibility testing (25th informational supplement, M100-S25). Journal of Clinical Microbiology, 35(3), 112-126.

Clinical and Laboratory Standards Institute (CLSI). (2019). Performance standards for antimicrobial susceptibility testing (29th supplement, M100). Journal of Clinical Microbiology, 39(3).

Codjoe, F. S., Eric, S., & Donkor, E. S. (2018). Carbapenem resistance: A review. Medical Science, 6(1), 1-28.

Demir, Y., Zer, Y., & Karaoglan, I. (2015). Investigation of VIM, IMP, NDM-1, KPC, and OXA-48 enzymes in Enterobacteriaceae strains. Pakistan Journal of Pharmaceutical Sciences, 28, 1127–1133.

Diene, S. M., & Rolain, J.-M. (2014). Carbapenemase genes and genetic platforms in Gram-negative bacilli: Enterobacteriaceae, Pseudomonas, and Acinetobacter species. Clinical Microbiology and Infection, 20(9), 831–838.

Enwuru, N. V., Enwuru, C. A., & Adepoju-bello, A. (2011). Metallo-beta-lactamase production by Escherichia coli and Klebsiella species isolated from hospital and community subjects in Lagos, Nigeria. Natural Sciences, 9, 1–9.

Espinosa, I., Baez, M., Percedo, M. I., & Martinez, S. (2013). Evaluation of simplified DNA extraction methods for Streptococcus suis typing. Revista de Salud Animal, 35(1), 59-63.

Esterly, J. S., Wagner, J., McLaughlin, M. M., Postelnick, M. J., Qi, C., & Scheetz, M. H. (2012). Evaluation of clinical outcomes in patients with bloodstream infections due to gram-negative bacteria according to carbapenem MIC stratification. Antimicrobial Agents and Chemotherapy, 56, 4885–4890.

Evans, B. A., & Amyes, S. B. G. (2014). OXA β-lactamases. Clinical Microbiology Reviews, 27(2), 241–263.

Fasciana, T., Gentile, B., Aquilina, M., Ciammaruconi, A., Mascarella, C., Anselmo, A., Fortunato, A., Fillo, S., Petralito, G., Lista, F., & Giammanco, A. (2019). Co-existence of virulence factors and antibiotic resistance in new Klebsiella pneumoniae clones emerging in south of Italy. BMC Infectious Diseases, 19, 928.

Gelband, H. N., Miller-Petrie, M., Suraj, P., Gandra, S., Levinson, J., Barter, D., White, A., & Laxminarayan, R. (2015). The state of the world's antibiotics. Washington DC: CDDEP; The Centre for Disease Dynamics, Economics and Policy.

Gupta, V., Bansal, N., Singla, N., & Chander, J. (2013). Occurrence and phenotypic detection of class A carbapenemases among Escherichia coli and Klebsiella pneumoniae blood isolates at a tertiary care center. Journal of Microbiology, Immunology and Infection, 46, 104-108.

Huang, S. R., Liu, M. F., Lin, C. F., & Shi, Z. Y. (2014). Molecular surveillance and clinical outcomes of carbapenem-resistant Escherichia coli and Klebsiella pneumoniae infections. Journal of Microbiology, Immunology and Infection, 47, 187-196.

Jeon, H., Lee, J. H., Lee, J. J., Park, K. S., Karim, A. M., Lee, C. R., Jeong, B. C., & Lee, S. H. (2015). Structural basis for carbapenem-hydrolyzing mechanisms of carbapenemases conferring antibiotic resistance. International Journal of Molecular Sciences, 16, 9654-9692.

Mlynarcik, P., Roderova, M., & Kolar, M. (2016). Primer evaluation for PCR and its application for detection of carbapenemases in Enterobacteriaceae. Jundishapur Journal of Microbiology, 9(1), e29314.

Mohammed, Y., Zailani, S. B., & Onipede, A. O. (2015). Characterization of KPC, NDM, and VIM type carbapenem-resistant Enterobacteriaceae from North Eastern Nigeria. Journal of Biological Sciences and Medicine, 3, 100-107.

Mukail, A., Tytler, B. A., Adeshina, G. O., & Igwe, J. C. (2019). Incidence of carbapenemase production among antibiotic-resistant Klebsiella isolates in Zaria, Nigeria. BMC Research Notes, 36(1), 138-145.

Nordmann, P., Dortet, L., & Poirel, L. (2012). Carbapenem resistance in Enterobacteriaceae: Here is the storm! Trends in Molecular Medicine, 18, 263–272.

Nordmann, P., Naas, T., & Poirel, L. (2011). Global spread of carbapenemase-producing Enterobacteriaceae. Emerging Infectious Diseases, 17, 1791-1798.

Oduyebo, O., Falayi, O., Oshun, P., & Ettu, A. (2015). Phenotypic determination of carbapenemase-producing Enterobacteriaceae isolates from clinical specimens at a tertiary hospital in Lagos, Nigeria. Nigerian Postgraduate Medical Journal, 22(4), 223–227.

Olowo-okere, A., Abdullahi, M. A., Ladidi, B. K., Suleiman, S., Tanko, N., Ungokore, H. Y., & Aliyu, A. (2019). Emergence of metallo-β-lactamase producing gram-negative bacteria in a hospital with no history of carbapenem usage in Northwest Nigeria. Ife Journal of Science, 21(2), 323-331.

Protonotariou, E., Meletis, G., Chatzopoulou, F., Malousi, A., Chatzimitriou, D., & Skoura, L. (2019). Emergence of Klebsiella pneumoniae ST11 co-producing NDM-1 and OXA-48 carbapenemases in Greece. Journal of Global Antimicrobial Resistance, 19, 81-82.

Srinivasan, R., Bhaskar, M., Kalaiarasan, E., & Narasimha, H. B. (2015). Prevalence and characterization of carbapenemase-producing isolates of Enterobacteriaceae obtained from clinical and environmental samples: Efflux pump inhibitor study. African Journal of Microbiology Research, 9(17), 1200-1204.

Ssekatawa, K., Byarugaba, D. K., Wampande, E., & Ejobi, F. (2018). A systematic review: The current status of carbapenem resistance in East Africa. 11(629), 1-9.

United States Centers for Disease Control and Prevention, Department of Health and Human Services. (2016). Detect and protect against antibiotic resistance: CDC's initiative to outsmart this threat. Retrieved from http://www.cdc.gov/drugresistance/pdf/ar_initiative_fact_sheet.pdf

Van der Zwaluw, K., Witteveen, S., Wielders, L., van Santen, M., Landman, F., de Haan, A., Schouls, L. M., & Bosch, T. (2020). Molecular characteristics of carbapenemase-producing Enterobacterales in the Netherlands: Results of the

2014-2018 national laboratory surveillance. Clinical Microbiology and Infection, 26(1412), 7-12.

World Health Organization. (2015). Global action plan on antimicrobial resistance (Report no. WHA68/2015/REC/1). Geneva: The Organization.

World Health Organization. (2017). Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics (1–7). Retrieved from World Health Organization

World Health Organization. (2018). Factsheet on antimicrobial resistance. Retrieved from http://www.who.int/mediacenter/factsheets/fs194

Yao, B., Xiao, X., Wang, F., Zhou, L., Zhang, X., & Zhang, J. (2015). Clinical and molecular characteristics of multi-clone carbapenem-resistant hypervirulent (hypermucoviscous) Klebsiella pneumoniae isolates in a tertiary hospital in Beijing, China. International Journal of Infectious Diseases, 37, 107–112.

Zowawi, H. M., Sartor, A. L., Balkhy, H. H., Walsh, T. R., Al Johani, S. M., Al Jindan, R. Y., Alfaresi, M., Ibrahim, E., Al-Jardani, A., Al-Abri, S., Al Salman, J., Dashti, A. A., Kutbi, A. H., Schlebusch, S., Sidjabat, H. E., & Paterson, D. L. (2014). Molecular characterization of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in the countries of the Gulf Cooperation Council: Dominance of OXA-48 and NDM producers. Antimicrobial Agents and Chemotherapy, 58(6), 3085–3090.