Jugah et al. *Afr. J. Clin. Exper. Microbiol.* 2026; 27 (3): 309 - 319 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Jul 2026; Vol.27 No.3
AJCEM/2496. https://www.ajol.info/index.php/ajcem

Copyright AJCEM 2026: https://dx.doi.org/10.4314/ajcem.v27i3.7

Original Article **Open Access**

Prevalence of New Delhi metallo-β-lactamase gene among multi-drug-resistant *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates at the University College Hospital, Ibadan, Nigeria

*[1,2]Jugah, C. C., [1,3]Makanjuola, O. B., [1]Ogunleye, V. O., [4]Azuh, V. O., [4]Kazeem, O. S., [1,4]Subulade, A. A., [4]Adesanya, O. A., [4]Adeniyi, A. V, and [1,3]Kehinde, A. O.

[1]Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, Nigeria
[2]College of Medicine, University of Ibadan, Nigeria
[3]University College Hospital, Ibadan, Nigeria
[4]Biorepository and Clinical Virology Laboratory, College of Medicine, University of Ibadan, Nigeria
*Correspondence to: christianaokiyi25@gmail.com

Abstract:

Background: Failure of antimicrobial therapy may give an insight into carriage of resistance genes among microbial pathogens. The ease of spread of these genes including beta-lactamase genes such as *bla*$_{NDM}$, *bla*$_{KPC, and}$ *bla*$_{OXA-48}$ is a major public health challenge worldwide. The study aimed to detect New Delhi metallo-β-lactamase (*bla*$_{NDM}$) gene carriage among multidrug-resistant (MDR) *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates from clinical specimens at the University College Hospital, Ibadan (UCH), Ibadan, Nigeria.
Methodology: This was a laboratory-based study of 110 presumptively identified Gram-negative bacterial isolates at the routine microbiology laboratory of UCH Ibadan, Nigeria between May and August 2021. The isolates were re-identified and confirmed as *E. coli*, *K. pneumoniae* and *P. aeruginosa* using biochemical tests, including oxidase test, cetrimide agar test, and Analytical Profile Index (API) system. Antibiotic susceptibility test of confirmed isolates to selected antibiotics was done using the modified Kirby-Bauer disk diffusion method and multi-drug resistance was defined as resistance to 3 or more antibiotic classes. The *bla*$_{NDM}$ was detected among the MDR isolates by conventional polymerase chain reaction using specific primers. Descriptive statistics were used to summarize variables, and Chi-square was used to compare categorical variables, with p value ≤ 0.05 taken to be statistically significant.
Results: Of the total 110 Gram-negative bacilli collected from the routine microbiology laboratory, 90 were confirmed as the Gram-negative bacilli of interest, with *K. pneumoniae* (58.9%, n=53), *E. coli* (31.1%, n=28) and *P. aeruginosa* (10.0%, n=9). Majority of the isolates (n=77, 85.6%) were susceptible to ertapenem, while most were resistant to ciprofloxacin (n=75, 83.3%), and 64 (71.1%) were multidrug-resistant. Among the MDR isolates, NDM carriage rate was 12.5% (n=8/64), with the carriage rate highest in *P. aeruginosa* (50.0%, n=1/2), followed by *K. pneumoniae* (13.6%, n=6/44) and *E. coli* (5.6%, n=1/18) but no significant difference in the carriage rate (x^2=3.417, p=0.1811).
Conclusion: This study shows a high prevalence of MDR Gram-negative bacilli, with *K. pneumoniae* being the highest. Resistance was mostly observed to ciprofloxacin, while most isolates were susceptible to ertapenem. *bla*$_{NDM}$ gene was detected in all the three MDR bacterial pathogens. Emphasis should be placed on rational antibiotic use and routine susceptibility testing, including resistance gene detection, to guide therapy, and prevent emergence of multidrug resistance in microbial population.

Keywords: Multi-drug resistance; New Delhi metallo-β-lactamase; Carriage; Gram-negative bacilli

Received Feb 12, 2026; Revised Apr 13, 2026; Accepted Apr 14, 2026

Copyright 2026 AJCEM Open Access. This article is licensed and distributed under the terms of the Creative Commons Attrition 4.0 International License <a rel="license" href="http://creativecommons.org/licenses/by/4.0/", which permits unrestricted use, distribution and reproduction in any medium, provided credit is given to the original author(s) and the source. Editor-in-Chief: Prof. S. S. Taiwo

Prévalence du gène de la métallo-β-lactamase New Delhi parmi les isolats cliniques d'*Escherichia coli*, *Klebsiella pneumoniae* et *Pseudomonas aeruginosa* multirésistants à l'Hôpital Universitaire d'Ibadan, au Nigéria

*[1,2]Jugah, C. C., [1,3]Makanjuola, O. B., [1]Ogunleye, V. O., [4]Azuh, V. O., [4]Kazeem, O. S., [1,4]Subulade, A. A., [4]Adesanya, O. A., [4]Adeniyi, A. V., et [1,3]Kehinde, A. O.

[1]Département de Microbiologie Médicale et de Parasitologie, Collège de Médecine, Université d'Ibadan, Nigéria
[2]Collège de Médecine, Université d'Ibadan, Nigéria
[3]Hôpital Universitaire d'Ibadan, Nigéria
[4]Biobanque et Laboratoire de Virologie Clinique, Collège de Médecine, Université d'Ibadan, Nigéria Ibadan, Nigéria
*Correspondance à: christianaokiyi25@gmail.com

Résumé:

Contexte: L'échec d'un traitement antimicrobien peut révéler la présence de gènes de résistance chez les agents pathogènes microbiens. La facilité de propagation de ces gènes, notamment les gènes de bêta-lactamases tels que *bla*$_{NDM}$, *bla*$_{KPC}$ et *bla*$_{OXA-48}$, constitue un enjeu majeur de santé publique à l'échelle mondiale. Cette étude visait à détecter la présence du gène de la métallo-β-lactamase de New Delhi (*bla*$_{NDM}$) chez des isolats d'*Escherichia coli*, *Klebsiella pneumoniae* et *Pseudomonas aeruginosa* multirésistants (MDR) provenant d'échantillons cliniques prélevés au Centre Hospitalier Universitaire d'Ibadan (UCH), à Ibadan, au Nigéria.
Méthodologie: Cette étude de laboratoire a porté sur 110 isolats bactériens à Gram négatif présumés, identifiés au laboratoire de microbiologie de routine du CHU d'Ibadan, au Nigéria, entre mai et août 2021. Ces isolats ont été réidentifiés et confirmés comme étant des espèces d'*E. coli*, *K. pneumoniae* et *P. aeruginosa* par des tests biochimiques, notamment le test de l'oxydase, le test sur gélose au cétrimide et le système API (Index du Profil Analytique). La sensibilité aux antibiotiques des isolats confirmés a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer modifiée. La multirésistance a été définie comme une résistance à au moins trois classes d'antibiotiques. Le gène *bla*$_{NDM}$ a été recherché parmi les isolats multirésistants par PCR conventionnelle à l'aide d'amorces spécifiques. Des statistiques descriptives ont été utilisées pour résumer les variables, et le test du χ^2 a permis de comparer les variables catégorielles. Un seuil de signification statistique de $p \leq 0,05$ a été retenu.
Résultats: Sur un total de 110 bacilles Gram négatifs collectés au laboratoire de microbiologie de routine, 90 ont été confirmés comme étant les bacilles Gram négatifs d'intérêt, avec *K. pneumoniae* (58,9%, n=53), *E. coli* (31,1%, n=28) et *P. aeruginosa* (10,0%, n=9). La majorité des isolats (n=77, 85,6%) étaient sensibles à l'ertapénem, tandis que la plupart étaient résistants à la ciprofloxacine (n=75, 83,3%) et 64 (71,1%) étaient multirésistants. Parmi les isolats multirésistants (MDR), le taux de portage de NDM était de 12,5% (n=8/64), avec le taux le plus élevé chez *P. aeruginosa* (50,0%, n=1/2), suivi de *K. pneumoniae* (13,6%, n=6/44) et d'*E. coli* (5,6%, n=1/18). Cependant, aucune différence significative n'a été observée entre les taux de portage (χ^2=3,417, p=0,1811).
Conclusion: Cette étude révèle une forte prévalence de bacilles Gram négatifs multirésistants, *K. pneumoniae* présentant le taux le plus élevé. La résistance était principalement observée à la ciprofloxacine, tandis que la plupart des isolats étaient sensibles à l'ertapénem. Le gène *bla*$_{NDM}$ a été détecté chez les trois bactéries pathogènes multirésistantes. Il convient de privilégier un usage rationnel des antibiotiques et la réalisation systématique de tests de sensibilité, incluant la détection des gènes de résistance, afin d'orienter le traitement et de prévenir l'émergence de la multirésistance au sein des populations microbiennes.

Mots-clés: Multirésistance; Métallo-β-lactamase de New Delhi; Portage; Bacilles Gram négatifs

Introduction:

Infections caused by multidrug resistant (MDR) organisms result in prolonged hospital stays, increased healthcare costs, and increased mortality and morbidity due to limited treatment options (1). The increasing prevalence of MDR pathogens has become a major public health challenge worldwide (2,3). In Nigeria, studies have reported high prevalence of 70-90% multidrug resistance among clinical isolates (4,5). Several factors have contributed to the increasing trend in microbial drug resistance. The ease of spread of resistance genes is a major challenge coupled with their enhanced selection as a result of drug misuse and abuse by patients and individuals (6).

Over-the-counter availability of antibiotics, lack of surveillance and routine susceptibility testing in resource-limited countries have contributed immensely to irrational antimicrobial drug usage, thus compounding the present challenge of antimicrobial resistance (AMR) (7, 8). Other factors such as overuse of antibiotics for treatment and growth promotion in animals have been noted to contribute to the development and spread of resistance (9,10). The limited scientific strategies to develop new classes of antibiotics have also partly contributed to the proliferation of resistance in MDR organisms (11).

Resistance of microbes to antimicrobial agents is attributed to their ability to produce enzymes such as extended-spectrum beta-lactamase (ESBL) that inhibit the effect of these agents (12). Of great concern are MDR organisms resistant to the carbapenems, which are considered the drugs of 'last resort' for treating infections caused by these pathogens (13). Organisms resistant to carbapenems have been isolated from various sources, including human and animal populations and the environment

(14). Outbreaks caused by carbapenemase-producing Enterobacteriaceae have also been reported from patient samples (15).

The horizontal transmission of carbapenemase genes, mediated by mobile genetic elements carrying additional resistance elements, confers resistance to various groups of antibiotics, resulting in MDR (16,17). Carbapenemases belong to molecular class A (KPC), class B (MBL including NDM, IMP and VIM) and class D (OXA-48-like carbapenemase) according to the Ambler classification system (18,19). The production of carbapenemase has been shown to be the main mechanism of carbapenem resistance in epidemiological studies (20,21). MDR isolates categorized as being susceptible to carbapenem by phenotypic tests can still carry carbapenemase resistance gene on molecular testing (22).

The occurrence of multiple resistance determinants including more than two carbapenemases in one isolate has also been frequently reported (23,24). Decreased permeability, ESBL production and efflux pump over-expression are noted to play a role in carbapenem resistance (25,26). Antibiotics such as ceftazidime/avibactam, aztreonam/ avibactam, colistin, tigecycline and fosfomycin have been recommended for the treatment of infections caused by carbapenem-resistant Enterobacterales (CRE) (27).

Two-thirds of all healthcare associated infections have been reported to originate from just six MDR organisms, including *Enterobacter* species, *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, *Staphylococcus aureus* (methicillin-resistant *S. aureus* -MRSA, vancomycin-resistant *S. aureus*-VRSA) and *Enterococcus* species (vancomycin resistant enterococci-VRE). These organisms are collectively known as the ESKAPE pathogens due to their ability to evade the mechanism of action of several antibiotics (28). *Escherichia coli*, *Klebsiella* spp and *P. aeruginosa*, identified as priority pathogens by the World Health Organization (WHO), are among the most common pathogens responsible for nosocomial infections, and their emerging resistance to a wide range of antibiotics poses a major challenge to patient treatment and public health at large (29,30).

Although there are extensive records on the phenotypic resistance patterns of these organisms, very little is known about the genes responsible for resistance in most MDR isolates in Nigeria (31). Local studies have reported susceptibility profiles of many of these organisms against antimicrobial agents without indicating the resistance genes responsible (32). Despite the existence of MDR among enteric bacteria in Nigeria, there is limited information regarding the genes responsible for resistance (33,34). This study therefore aimed to detect the presence of NDM resistance gene in three common MDR Gram-negative bacilli of medical importance (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*).

Materials and method:

Study design and site:

This laboratory-based study of selected Gram-negative bacterial isolates (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) was carried out at the University College Hospital (UCH), Ibadan, from May to August 2021. UCH is a major tertiary healthcare and referral centre in south-western Nigeria, providing specialized medical services and serving as a training and research institution for healthcare professionals.

Ethical approval:

The study was approved by the Ethics and Research Committee (UI/EC/21/0179) of the University College Hospital and University of Ibadan, Nigeria.

Source of bacterial isolates:

The bacterial isolates (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) presumptively identified from clinical samples (urine, sputum, wound swabs, biopsy materials, body fluids, and blood cultures) submitted for routine diagnostic testing, were obtained by consecutive sampling from the Medical Microbiology and Parasitology Laboratory in UCH, Ibadan.

Re-identification and phenotypic confirmation of selected bacterial isolates:

A total of 110 collected Gram-negative bacterial isolates were re-characterised to confirm their identity. Gram staining technique was done for confirmation of Gram-negative bacilli. Identification of species of the Gram-negative bacilli was carried out using oxidase test, cetrimide agar test, and the Analytical Profile Index (API) system.

Oxidase test:

The oxidase test was carried out using impregnated oxidase strips. A sterile loop was used to pick a pure inoculum and smeared on the strip. A purple colour change observed within 10 seconds was recorded as positive (36). This test was used to differentiate oxidase-positive bacteria such as *Pseudomonas* spp from oxidase-negative bacteria, including *E. coli* and *Klebsiella* spp, among the selected bacterial isolates.

Cetrimide agar test:

Cetrimide agar (Oxoid, UK) was used to selectively isolate and identify *P. aeruginosa*. A

loopful of the oxidase positive isolate was streaked on the surface of the agar and incubated at 37 °C for 24–48 hours. The presence of greenish pigment (pyocyanin) and characteristic grape-like odor was considered indicative for *P. aeruginosa* growth (37).

Analytical profile index (API) system (API-20E):

The species of the presumptive fermentative Gram-negative bacteria isolates were identified using the API-20E test. A suspension of each pure isolate from 24-hour growth was prepared using normal saline and adjusted to 0.5 MacFarland standard. The bacterial suspension was used to rehydrate each of the wells on the API strip, which was then incubated for 24 hours. Colour changes were observed after incubation following manufacturer's instructions.

The positive and negative results were compiled to obtain a profile number, which was interpreted online to determine the identity of the isolate (38). This test was used to confirm the identity of members of the family Enterobacteriaceae, including *E. coli* and *Klebsiella* spp among the selected bacterial isolates.

Antibiotic susceptibility testing of the selected isolates:

The antibiotic susceptibility of the confirmed isolates was tested on Muller-Hinton agar using the modified Kirby-Bauer disc diffusion method. Seven antibiotics (Oxoid, UK); amoxicillin-clavulanic acid (30µg), gentamicin (10µg), ciprofloxacin (5µg), piperacillin-tazobactam (100/10µg), ceftazidime (30µg), aztreonam (30 µg) and ertapenem (10µg) were tested. Inoculum of each confirmed isolate was prepared and standardized to 0.5 MacFarland (~1.5 x 10^8 CFU /ml) and then inoculated on Mueller-Hinton agar plates. Antibiotic disc**s** were applied, and plates were incubated at 37ºC overnight. The inhibition zone diameters were measured and interpreted as susceptible or resistant according to the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines (39). *Escherichia coli* ATCC 25922 was used as control strain.

Isolates resistant to at least three antibiotics classes were regarded as multidrug resistant. MDR isolates were sub-cultured into Tryptic Soy Broth (TSB) and then stored at -20 ºC to ensure preservation of viable cells for molecular analysis (36).

Resistance gene detection by PCR:

The stored isolates were retrieved for molecular analysis, which was carried out in the Biorepository and Clinical Virology Laboratory, College of Medicine, UCH, Ibadan.

DNA extraction by chemical method:

Bacterial broth from pure culture was transferred into 2ml microtubes and centrifuged at 10,000 rpm for 5 minutes. Pellets were re-suspended in 520 µL of Tris-EDTA (TE) buffer and vortexed. Fifteen microliters of 20% sodium dodecyl sulphate (SDS) were added, and the mixture was incubated at 37ºC for 1 hour. A solution of 100µL of 5 M NaCl and 80 µL of 10% cetyltrimethylammonium bromide (CTAB) in 0.7 M NaCl was added and vortexed, followed by incubation at 65ºC for 10 minutes and cooling on ice for 15 minutes. An equal volume of phenol:chloroform:isoamyl alcohol (25:24:1) was added, vortexed, incubated on ice for 5 minutes, and centrifuged at 10,000 rpm for 10 minutes. The aqueous phase was transferred to a new tube, and isopropanol (1:0:6) was added to precipitate DNA, followed by incubation at -20ºC for 1 hour.

The DNA was collected by centrifugation at 12,000 rpm for 10 minutes, washed with 500 µL of 70% ethanol, centrifuged for 5 minutes at 10,000 rpm, air-dried at room temperature for 3 hours, and dissolved in 50 µL of distilled water (35)

Amplification by conventional PCR:

The NDM gene was detected by PCR assay using specific primer targeting bla_{NDM} in the MDR isolates. Specific primers for PCR (NDM forward: CCAATATTATGCACCCGGTCG; reverse: ATGCGGGCCGTATGAGTGATTG) used have been previously documented by Handal et al., (40). Reaction cocktail used for the PCR primer set included 5 x PCR SYBR Green buffer (2.5µl), $MgCl_2$ (0.75µl), 10pM dNTP (0.25µl), 10pM each forward and reverse primers (0.25µl), 8000 U of Taq DNA polymerase (0.06µl) which was made up to 10.5µl with sterile distilled water to which 2µl template was added. The primer mix, master mix, and water were added to the well plate, after which the DNA template was added to the plate.

The PCR conditions include initial denaturation at 94ºC for 5 minutes, followed by 30 cycles consisting of denaturation at 95ºC for 30 seconds, annealing at 51ºC for 40 seconds, extension at 72ºC for 40 seconds and final extension of 72ºC for 5 minutes. The PCR set up was allowed to run for at least 1 hour 45 minutes for completion of the PCR. The PCR was carried out in GeneAmp 9700 PCR system thermal cycler (Applied Biosystem Inc., USA) using the appropriate profile as designed for the primer pair.

Gel electrophoresis of amplified products:

The PCR amplicons were resolved on

2% agarose gel prepared in Tris-acetate-EDTA (TAE) buffer. Electrophoresis was carried out at a constant voltage for approximately 1 hour. DNA bands were visualized under ultraviolet light and compared with a molecular weight marker (DNA ladder). Bands corresponding to the expected size of the target gene, as observed in the positive control, were recorded as positive, while absence of such bands was recorded as negative. A *Klebsiella pneumoniae* isolate harboring the bla_{NDM-1} gene was used as the positive control.

Statistical analysis:

Data were sorted, coded, and cleaned. Descriptive statistics (percentages) were used to summarize variables. Chi-square test was used to assess the association between bacterial species (*E. coli*, *K. pneumoniae*, and *P. aeruginosa*) and multidrug resistance, as well as the relationship between bacterial species and NDM gene carriage. A p value ≤ 0.05 was considered significant. Analysis was performed using SPSS version 26.0.

Results:

Frequency distribution of the selected bacterial isolates:

Of a total of 110 Gram-negative isolates collected from the routine laboratory, 90 were biochemically confirmed, with *K. pneumoniae* (n= 53; 58.9%), *E. coli* (n=28; 31.1%), and *P. aeruginosa* (n=9; 10.0%) (Fig 1). The highest frequency of Gram-negative isolates was from urine samples (n=25, 27.8%), followed by wound swab/biopsy (n=23, 25.6%), sputum (n=13, 14.4%), and blood culture (n=12, 13.3%). *Klebsiella pneumoniae* (n=13, 24.5%) and *E. coli* (n=11, 39.3%) were most frequently isolated from urine, while *P. aeruginosa* (n=3, 33.3%) was predominantly obtained from ear swabs (Table 1).

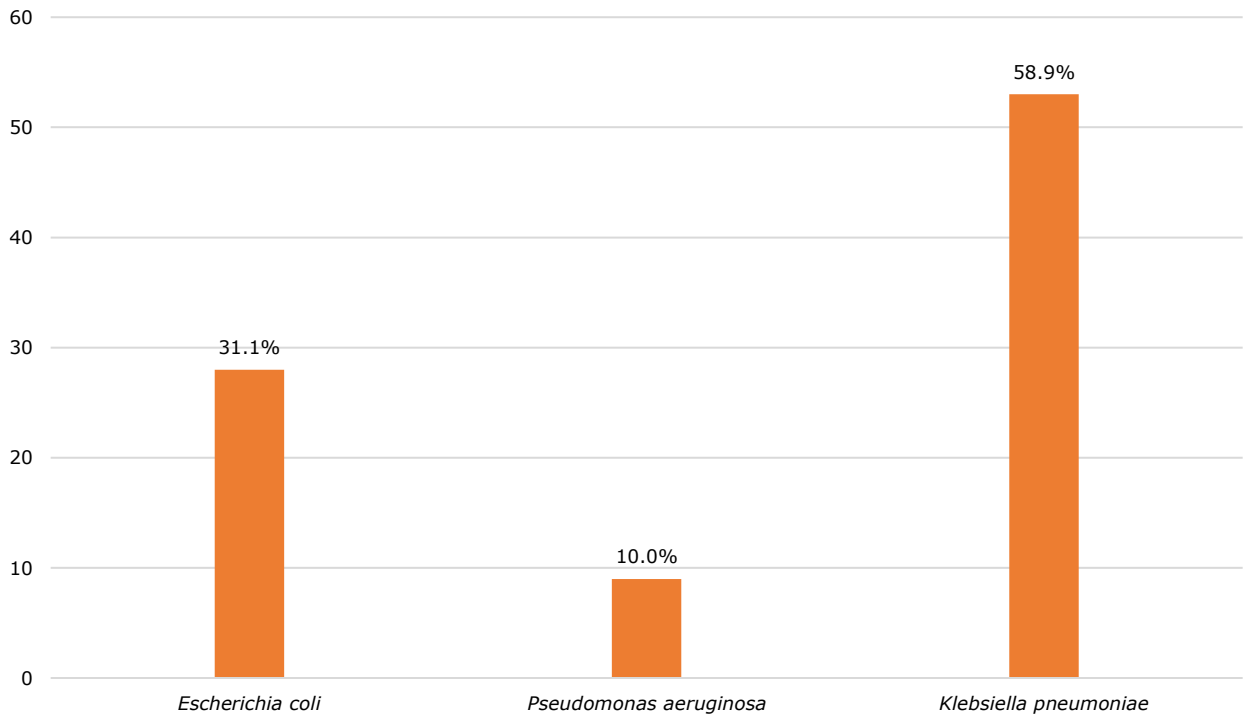

Fig 1: Frequency distribution of selected Gram-negative clinical bacterial isolates

Table 1: Distribution of *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* isolates according to clinical samples

Sample	***Escherichia coli* (%)**	***Klebsiella pneumoniae* (%)**	***Pseudomonas aeruginosa* (%)**	**Total (%)**
Sputum	1 (3.6)	11 (20.8)	1 (11.1)	13 (14.4)
Wound swab/biopsy	9 (32.1)	12 (22.6)	2 (22.2)	23 (25.6)
Tracheal aspirate	1 (3.6)	2 (3.8)	1 (11.1)	4 (4.4)
Blood culture	4 (14.3)	9 (17.0)	0	13 (14.4)
Ear swab	0	2 (3.8)	3 (33.3)	5 (5.6)
Intracervical fluid	0	1 (1.9)	0	1 (1.1)
Pleural biopsy/fluid	1 (3.6)	1 (1.9)	1 (11.1)	3 (3.3)
Pus	0	1 (1.9)	0	1 (1.1)
Tissue biopsy	1 (3.6)	1 (1.9)	0	2 (2.2)
Urine	11 (39.3)	13 (24.5)	1 (11.1)	25 (27.8)
Total	**28 (31.1)**	**53 (58.9)**	**9 (10.0)**	**90 (100.0)**

Susceptibility patterns of isolates to antibiotics:

Table 2 presents the antibiotic susceptibility of the bacterial isolates. Among *E. coli* isolates, resistance was highest to ciprofloxacin (n=25/28, 89.3%), followed by aztreonam (n= 18/28, 64.3%) and amoxicillin-clavulanic acid (n=17/28; 60.7%).

Most of the *K. pneumoniae* isolates were resistant to ciprofloxacin (n=47/53, 88.7%), ceftazidime (n=42/53; 79.3%), aztreonam (n=39/ 53, 73.6%) and gentamicin (n=39/53; 73.6%). In contrast, *P. aeruginosa* isolates showed higher susceptibility to most antibiotics tested compared to *E. coli* and *K. pneumoniae*.

Most of the isolates tested were susceptible to ertapenem with *E. coli* (n=27, 96.4%), *K. pneumoniae* (n=43, 81.3%) and *P. aeruginosa* (n=7, 77.8%).

Table 2: Antibiotic susceptibility of *Escherichia coli, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates in University College Hospital, Ibadan, Nigeria

Antimicrobial category (Antibiotics)	***Escherichia coli* (n=28)**			***Pseudomonas aeruginosa* (n=9)**			***Klebsiella pneumoniae* (n=53)**		
	S (%)	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**	**S (%)**	**I (%)**	**R (%)**
Aminoglycoside (Gentamicin)	14 (50)	0	14 (50)	6 (66.7)	0	3 (33.3)	13 (24.5)	1 (1.9)	39 (73.6)
Fluoroquinolone (Ciprofloxacin)	1 (3.6)	2 (7.1)	25 (89.3)	6 (66.7)	0	3 (33.3)	4 (7.6)	2 (3.8)	47 (88.7)
Monobactam (Aztreonam)	4 (14.3)	6 (21.4)	18 (64.3)	6 (66.7)	2 (22.2)	1 (11.1)	11 (20.8)	4 (7.6)	39 (73.6)
Third generation cephalosporin (Ceftazidime)	8 (28.6)	5 (17.9)	15 (53.6)	7 (77.8)	0	2 (22.2)	7 (13.2)	4 (7.6)	42 (79.3)
Penicillin + β-lactamase inhibitors (Amoxicillin-clavulanic acid) (Piperacillin-tazobactam)	5 (17.8) -	6 (21.4) -	17 (60.7) -	- 5 (55.6)	- 2 (2.2)	- 2 (22.2)	9 (17) -	10 (18.9) -	34 (64.2) -
Carbapenem (Ertapenem)	27 (96.4)	0	1 (3.6)	7 (77.8)	0	2 (22.2)	43 (81.1)	3 (5.7)	7 (13.2)

S=sensitive; I=Intermediate; R=Resistant

Multidrug resistant pattern of the isolates:

Of the 90 Gram-negative bacilli tested, 64 (71.1%) were multidrug resistant (MDR), with resistance to at least three classes of antibiotics. The prevalence of MDR was highest in *K. pneumoniae* (44/53; 83.0%), followed by *E. coli* (18/28; 64.3%), and *P. aeruginosa* (2/9; 22.2%) (x^2=14.764, p=0.0006) (Table 3).

Resistance genes in clinical isolates:

Fig 2 shows the representative image of the NDM gene detected among the MDR clinical isolates on agarose gel electrophoresis with a band size of 1500bp. Positive samples were run alongside a positive control and a 100 bp molecular weight marker (MK).

Of the 64 MDR isolates, 8 (12.5%) harbored the NDM gene; 6 (9.4%) *K. pneumoniae* and 1 (1.6%) each of *P. aeruginosa* and *E. coli* (Table 4).

Table 3: Multidrug resistance among *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates in University College Hospital, Ibadan, Nigeria

Isolates	Number of isolates	Number of MDR isolates	Percentage of MDR isolates	X^2	*p* value
Escherichia coli	28	18	64.3	14.764	0.0006*
Pseudomonas aeruginosa	9	2	22.2		
Klebsiella pneumoniae	53	44	83.0		
Total	**90**	**64**	**71.1**		

*=statistically significant

MK= Marker; = Positive samples

Fig 2: Representative agarose gel electropherogram showing PCR amplified NDM gene products

Table 4: Carriage of NDM gene among multidrug *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* clinical isolates in University College Hospital, Ibadan, Nigeria

Bacterial isolate	Number of MDR isolates	Number of MDR isolates with NDM gene (%)	x^2	*p* value
Escherichia coli (n=28)	18	1 (5.6)	3.417	0.1811
Pseudomonas aeruginosa (n=9)	2	1 (50.0)		
Klebsiella pneumoniae (n=53)	44	6 (13.6)		
Total (n=90)	**64**	**8 (12.5)**		

Discussion:

The identification of the AMR patterns of bacterial isolates plays an important role in guiding rational prescription of antibiotics and management of bacterial infections. The reported organisms in this study have similarities with other studies that have been conducted on MDR organisms. From this study, *K. pneumoniae* was the most predominant, followed by *E. coli*, and *P. aeruginosa*, which agrees with the findings of Mushi et al., (41) and Aly and Balkhy (42), that reported these three bacteria as the prevalent MDR organisms. The 44.0% prevalence of *E. coli* reported by Aly and Balkhy (42) is comparable with the trend observed in the study, while their reported prevalence rates of 20.0% for *K. pneumoniae* and 18.7% for *P. aeruginosa* also support the dominance of these organisms. Other studies likewise reported *P. aeruginosa*, *K. pneumoniae*, *E. coli* and *Enterobacter* species as frequently isolated MDR organisms. The consistent isolation of these organisms across different studies confirm their importance in multidrug-resistant infections (41-44).

The predominance of *K. pneumoniae* and *E. coli* in urine samples in this study is similar to findings reported in other studies (41). Although some studies reported blood as the commonest site of infection, variations in infection sites may be influenced by differences in study settings and geographical locations (44, 45). The widespread occurrence of MDR organisms across different clinical samples further highlights the growing challenge of antimicrobial resistance in healthcare settings (46,47).

From this study, both *E. coli* and *K. pneumoniae* were resistant to ciprofloxacin, which agrees with the report of Gurung et al., (48). *Klebsiella pneumoniae* showed high resistance to ciprofloxacin, ceftazidime, aztreonam and gentamicin, which is similar to the findings of Chen et al., (49), where *K. pneumoniae* was reported to show high resistance to ciprofloxacin, ceftazidime, aztreonam, ertapenem and gentamicin among others. Ertapenem was selected as the antibiotic of choice for testing carbapenem resistance because it is the most sensitive carbapenem indicator for detecting the production of carbapenemase in Enterobacteriaceae. Unlike other carbapenems such as imipenem and meropenem, ertapenem has reduced affinity for AmpC beta-lactamases and porin mutations, which makes it suitable for differentiating carbapenemase-mediated resistance (50).

Over 70% of the isolates were multidrug resistant, indicating a high prevalence of MDR among the organisms investigated. This prevalence is comparable to the 69.6% MDR rate reported by Adenipekun et al., (5), but higher than the 42.1% reported among Enterobacteriaceae isolates by Bitew and Tsige (51), and lower than the 87.4% MDR rate reported by Eshetie et al., (52). The variation in MDR prevalence across studies may be influenced by differences in study populations, sample size, antimicrobial usage patterns, infection control practices, and geographical settings.

Klebsiella pneumoniae accounted for the largest proportion of MDR isolates in this study, constituting 68.8% of the total MDR isolates, indicating its major contribution to multidrug resistance. Furthermore, 83.0% of *K. pneumoniae* isolates were MDR, which was significantly higher than 64.3% observed in *E. coli* and 22.2% in *P. aeruginosa*. This finding supports reports that have identified *K. pneumoniae* as a predominant MDR organism, followed by *E. coli* (48). Multidrug resistance has been attributed to the accumulation and expression of multiple resistance genes on R plasmids or increased expression of genes encoding multidrug efflux pumps, which may result in complex MDR phenotypes and sometimes pan-resistance (48,53). Other studies have also reported MDR *E. coli* from drinking water sources and MDR *Pseudomonas* isolates, further confirming the widespread nature of multidrug resistance among Gram-negative bacteria (54,55).

In this study, 8 of the 64 MDR isolates (12.5%) were positive for the NDM gene, indicating the presence of potential carbapenemase-producing strains among the MDR isolates. This finding is of clinical importance because the NDM gene confers resistance to carbapenems, which are considered drugs of 'last resort' for the treatment of severe Gram-negative infections (13). The detection of NDM among MDR isolates suggests limited therapeutic options and highlights the risk of treatment failure and rapid dissemination of resistance within healthcare settings. The detection of the NDM gene among multidrug-resistant isolates in this study highlights the presence of carbapenem resistance across the studied organisms. However, the NDM carriage rate was highest in *P. aeruginosa* (50.0%), followed by *K. pneumoniae* (13.6%) and *E. coli* (5.6%), although this difference was not statistically significant.

The overall prevalence of NDM observed in this study is comparable to previous reports, including the 10.2% carbapenemase resistance reported by Mohammed et al. and the 14.3% NDM prevalence reported by Tsai et al., (56,57). However, higher NDM prevalence of about 25% has been reported in studies from Kano and Yenagoa, Nigeria, which may be attributed to differences in antibiotic usage patterns, infec-

tion control practices, and study populations (58,59). These findings suggest that NDM-mediated resistance is present across multiple Gram negative organisms rather than being restricted to a single predominant species. The high resistance trend is indicative of high antibiotic selection pressure largely due to relatively cheap cost and easy availability of these agents, mostly used as first line or common choice of treatment in many healthcare settings in these regions (43,44).

Conclusion:

This study showed a high level of multidrug resistance, including the emergence of carbapenem resistance among clinical isolates, as reported in other studies. The highest prevalence of MDR was observed among *K. pneumoniae* isolates. Resistance was mostly to ciprofloxacin, while ertapenem was the antibiotic to which these bacteria were most susceptible.

The NDM gene was detected in *K. pneumoniae*, *E. coli*, and *P. aeruginosa*, with no statistically significant difference in carriage rates among the bacteria. Emphasis should be placed on rational antibiotic use and routine susceptibility testing, including resistance gene detection and sequencing, to guide treatment and curb multidrug resistance.

Acknowledgements:

The authors acknowledge the staff of the Medical Microbiology and Parasitology Laboratory, University College Hospital (UCH), Ibadan, particularly the Medical Laboratory Scientists, for their support and for providing the clinical isolates used in this study.

Contributions of authors:

JCC and KAO were involved in the study conceptualization and design; JCC, OVO and KOS carried out data collection and methodological activities; JCC, AOA and AVO performed the molecular work; AAV conducted the data analysis; JCC and SAA produced the initial draft; and KAO and MOB critically reviewed the initial draft. All authors were involved in findings interpretation, critical review and approval of the final draft.

Source of funding:

No funding was received for the study.

Conflict of interest:

Authors declare no conflict of interest.

References:

1. Serra-Burriel, M, Keys, M, Campillo-Artero, C, et al. Impact of multi-drug-resistant bacteria on economic and clinical outcomes of healthcare-associated infections in adults: Systematic review and meta-analysis. PLoS One. 2020; 15 (1): e0227139. https://doi.org/10.1371/journal.pone.0227139
2. Tacconelli, E, Carrara, E, Savoldi, A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect Dis. 2018; 18 (3): 318–327. https://doi.org/10.1016/S1473-3099(17)30753-3.
3. Pitout, J. D., and Laupland, K. B. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: an emerging public-health concern. Lancet Infect Dis. 2008; 8 (3): 159–166. https://doi.org/10.1016/S1473-3099(08)70041-0.
4. Adeyemi, K., Okunrounmu, P., Olagbende, A., Adedokun, O., Hassan, A., and Atilola, G. High prevalence of multiple drug-resistant enteric bacteria: evidence from a teaching hospital in Southwest Nigeria. J Infect Publ Health. 2020; 13 (4): 651–656. https://doi.org/10.1016/j.jiph.2019.08.014.
5. Adenipekun, E. O., Jackson, C. R., Ramadan, H., et al. Prevalence and multidrug resistance of *Escherichia coli* from community-acquired infections in Lagos, Nigeria. J Infect Dev Ctries. 2016; 10 (9): 920-931. https://doi.org/10.3855/jidc.7997.
6. Tenover, F. C. Development and spread of bacterial resistance to antimicrobial agents: an overview. Clin Infect Dis. 2001; 33 Suppl 3: S108–S115. https://doi.org/10.1086/321834.
7. Okeke, I. N. Diagnostic insufficiency in Africa. Clin Infect Dis. 2006; 42 (10): 1501–1503. https://doi.org/10.1086/503308.
8. Ventola, C. L. The antibiotic resistance crisis: part 1: causes and threats. P T. 2015; 40 (4): 277–283.
9. Finley, R. L., Collignon, P., Joakim Larsson, D. G., et al. The scourge of antibiotic resistance: the important role of the environment. Clin Infect Dis. 2013; 57: 704–710. https://doi.org/10.1093/cid/cit355.
10. Tang, Q., Song, P., Li, J., Kong, F., Sun, L., and Xu, L. Control of antibiotic resistance in China must not be delayed: the current state of resistance and policy suggestions for the government, medical facilities, and patients. Biosci Trends. 2016; 10: 1–6. https://doi.org/10.5582/bst.2016.01034.
11. Gupta, V., and Datta, P. Next-generation strategy for treating drug resistant bacteria: Antibiotic hybrids. Indian J Med Res. 2019; 149 (2): 97–106. https://doi.org/10.4103/ijmr.IJMR_755_18.
12. Reygaert, W. C. An overview of the antimicrobial resistance mechanisms of bacteria. AIMS Microbiol. 2018; 4 (3): 482–501. https://doi.org/10.3934/microbiol.2018.3.482.
13. Tam, V. H., Chang, K. T., Abdelraouf, K., et al. Prevalence, resistance mechanisms, and susceptibility of multidrug-resistant bloodstream isolates of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2010; 54 (3): 1160-1164. https://doi.org/10.1128/AAC.01446-09.
14. Woodford, N., Tierno, P. M Jr., Young, K., et al. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A beta-lactamase, KPC-3. Antimicrob Agents Chemother. 2004; 48 (12): 4793–4799. https://doi.org/10.1128/AAC.48.12.4793-4799.2004.
15. Lepuschitz, S., Baron, S., Larvor, E., et al. Phenotypic and genotypic antimicrobial resistance traits of *Vibrio cholerae* non-O1/non-O139 isolated

from a large Austrian lake. Front Microbiol. 2019; 10: 2600. https://doi.org/10.3389/fmicb.2019.02600.
16. Hirsch, E. B., and Tam, V. H. Detection and treatment options for *Klebsiella pneumoniae* carbapenemases (KPCs): an emerging cause of multidrug-resistant infection. J Antimicrob Chemother. 2010; 65 (6): 1119–1125. doi:10.1093/jac/dkq108
17. Kumarasamy, K. K., Toleman, M. A., Walsh, T. R., et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect Dis. 2010; 10 (9): 597–602. https://doi:10.1016/S1473-3099(10)70143-2.
18. Queenan, A. M., and Bush, K. Carbapenemases: the versatile beta-lactamases. Clin Microbiol Rev. 2007; 20: 440–458. https://doi:10.1128/CMR.00001-07.
19. van Duin, D., and Doi, Y. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence, 2017; 8 (4): 460-469. https://doi:10.1080/21505594.2016.1222343.
20. Munoz-Price, L. S., Poirel, L., Bonomo, R. A., et al. Clinical epidemiology of the global expansion of *Klebsiella pneumoniae* carbapenemases. Lancet Infect Dis., 2013; 13 (9): 785–796. https://doi:10.1016/S1473-3099(13)70190-7.
21. Patel, G., and Bonomo, R. A. "Stormy waters ahead": global emergence of carbapenemases. Front Microbiol., 2013; 4: 48. https://doi:10.3389/fmicb.2013.00048.
22. Doi, Y., and Paterson, D. L. Carbapenemase-producing Enterobacteriaceae. Semin Respir Crit Care Med, 2015; 36: 74-84. https://doi:10.1055/s-0035-1544208.
23. Cheng, L., Cao, X. L., Zhang, Z. F., et al. Clonal dissemination of KPC-2 producing *Klebsiella pneumoniae* ST11 clone with high prevalence of oqxAB and rmtB in a tertiary hospital in China: results from a 3-year period. Ann Clin Microbiol Antimicrob. 2016; 15: 1. https://doi:10.1186/s12941-015-0109-x.
24. Guducuoglu, H., Gursoy, N. C., Yakupogullari, Y., et al. Hospital Outbreak of a Colistin-Resistant, NDM-1- and OXA-48-Producing *Klebsiella pneumoniae*: High Mortality from Pandrug Resistance. Microb Drug Resist. 2018; 24 (7): 966–972. https://doi:10.1089/mdr.2017.0173.
25. Tsai, Y. K., Fung, C. P., Lin, J. C., et al. *Klebsiella pneumoniae* outer membrane porins OmpK35 and OmpK36 play roles in both antimicrobial resistance and virulence. Antimicrob Agents Chemother. 2011; 55 (4): 1485–1493. https://doi:10.1128/AAC.01275-10.
26. Hamzaoui, Z., Ocampo-Sosa, A., Maamar, E., et al. An Outbreak of NDM-1-Producing *Klebsiella pneumoniae*, Associated with OmpK35 and OmpK36 Porin Loss in Tunisia. Microb Drug Resist. 2018; 24: 1137–1147. https://doi:10.1089/mdr.2017.0165.
27. Zhou, H., Zhang, K., Chen, W., et al. Epidemiological characteristics of carbapenem-resistant Enterobacteriaceae collected from 17 hospitals in Nanjing district of China. Antimicrob Resist Infect Contr. 2020; 9 (1): 15. https://doi:10.1186/s13756-019-0674-4.
28. Boucher, H. W., Talbot, G. H., Bradley, J. S., et al. Bad bugs, no drugs: no ESKAPE! An update from the Infectious Diseases Society of America. Clin Infect Dis. 2009; 48 (1): 1–12. https://doi.org/10.1086/595011.
29. Weiner-Lastinger, L. M., Abner, S., Edwards, J. R., et al. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: summary of data reported to the National Healthcare Safety Network, 2015–2017. Infect Control Hosp Epidemiol. 2020; 41 (1): 1–18. https://doi.org/10.1017/ice.2019.296.
30. Abebe, M., Tadesse, S., Meseret, G., and Derbie, A. Type of bacterial isolates and antimicrobial resistance profile from clinical samples at a referral hospital, Northwest Ethiopia: five years data analysis. BMC Res Notes. 2019; 12 (1): 568. https://doi.org/10.1186/s13104-019-4604-6.
31. Ogbolu, D. O., and Webber, M. A. High-level and novel mechanisms of carbapenem resistance in Gram-negative bacteria from tertiary hospitals in Nigeria. Int J Antimicrob Agents. 2014; 43 (5): 412-417. https://doi.org/10.1016/j.ijantimicag.2014.01.014
32. Kehinde, A. O., Ademola, S. A., Okesola, A. O., Oluwatosin, O. M., and Bakare, R. A. Pattern of bacterial pathogens in burn wound infections in Ibadan, Nigeria. Ann Burns and Fire Disasters. 2004; 17 (1): 1-7. https://repository.ui.edu.ng/handle/123456789/10061
33. Olowe, O. A., Okanlawon, B. M., Olowe, R. A., and Olayemi, A. B. Antimicrobial resistant pattern of *Escherichia coli* from human clinical samples in Osogbo, southwestern Nigeria. Afr J Microbiol Res. 2008; 2: 8–11.
34. Nsofor, C. A., Iroegbu, C. U., Call, D. R., and Davies, M. A. Detection of antibiotic resistance genes of *Escherichia coli* from domestic livestock in southeast Nigeria with DNA microarray. J Cell Anim Biol. 2013; 7 (12): 149–163.
35. Trindade, L. C., Marques, E., Lopes, D. B., and Ferreira, M. A. S. V. Development of a molecular method for detection and identification of *Xanthomonas campestris* pv. *viticola*. Summa Phytopathologica. 2007; 33 (1): 16-23. https://doi.org/10.1590/S0100-54052007000100002
36. Cheesbrough, M. Microbiological Tests. In: District Laboratory Practice in Tropical Countries. Cambridge University Press. 2010: 64-65.
37. Ezeador, C. O., Ejikeugwu, P. C., Ushie, S. N., and Agbakoba, N. R. Isolation, Identification and Prevalence of *Pseudomonas aeruginosa* Isolates from Clinical and Environmental Sources in Onitsha Metropolis, Anambra State. Euro J Med Health Sci. 2020; 2 (2). https://doi.org/10.24018/ejmed.2020.2.2.188.
38. Holmes, B., Willcox, W. R., and Lapage, S. P. Identification of Enterobacteriaceae by the API 20E system. J Clin Pathol. 1978; 31: 22 - 30.
39. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. VET01S. 30th ed. Wayne (PA) 2020: Clinical and Laboratory Standards Institute.
40. Handal, R., Qunibi, L., Sahouri, I., et al. Characterization of Carbapenem-Resistant *Acinetobacter baumannii* Strains Isolated from Hospitalized Patients in Palestine. Int J Microbiol. 2017; 2017: 8012104. https://doi.org/10.1155/2017/8012104.
41. Mushi, M. F., Mshana, S. E., Imirzalioglu, C., and Bwanga, F. Carbapenemase genes among multidrug resistant gram-negative clinical isolates from a tertiary hospital in Mwanza, Tanzania. BioMed Res Int. 2014; 2014: 303104. https://doi.org/10.1155/2014/303104
42. Aly, M., and Balkhy, H. H. The prevalence of antimicrobial resistance in clinical isolates from Gulf Corporation Council countries. Antimicrob Resist Infect Control. 2012; 1 (1): 26. https://doi.org/10.1186/2047-2994-1-26.
43. Eltahawy, A. T., and Khalaf, R. M. Antibiotic resistance among Gram-negative non-fermentative bacteria at a teaching hospital in Saudi Arabia. J Chemother. 2001; 13 (3): 260-264. https://doi.org/10.1179/joc.2001.13.3.260.

44. Balkhair, A., Al-Farsi, Y. M., Al-Muharrmi, Z., et al. Epidemiology of Multi-Drug- Resistant Organisms in a Teaching Hospital in Oman: A One-Year Hospital-Based Study. Sci World J. 2014; Article ID 157102, 6 pages http://dx.doi.org/10.1155/2014/157102
45. Jaggi, N., Sissodia, P., and Sharma, L. Control of multidrug resistant bacteria in a tertiary care hospital in India. Antimicrob Resist Infect Control. 2012; 1 (1): 23. https://doi.org/10.1186/2047-2994-1-23.
46. El-Saed, A., Balkhy, H. H., Al-Dorzi, H. M., Khan, R., Rishu, A. H., and Arabi, Y. M. *Acinetobacter* is the most common pathogen associated with late-onset and recurrent ventilator-associated pneumonia in an adult intensive care unit in Saudi Arabia. Int J Infect Dis. 2013; 17 (9): e696-701. https://doi.org/10.1016/j.ijid.2013.02.004.
47. Chang, Y. J., Yeh, M. L., Li, Y. C., et al. Predicting hospital-acquired infections by scoring system with simple parameters. PLoS One. 2011; 6 (8): e23137. https://doi.org/10.1371/journal.pone.0023137.
48. Gurung, S., Kafle, S., Dhungel, B., et al. Detection of OXA-48 Gene in Carbapenem-Resistant *Escherichia coli* and *Klebsiella pneumoniae* from Urine Samples. Infect Drug Resist. 2020; 13: 2311-2321. https://doi.org/10.2147/IDR.S259967.
49. Chen, D., Li, H., Zhao, Y., et al. Characterization of carbapenem-resistant *Klebsiella pneumoniae* in a tertiary hospital in Fuzhou, China. J Appl Microbiol. 2020; 129 (5): 1220-1226. https://doi.org/10.1111/jam.14700
50. Mohapatra, S., Kumar, A., Bir, R., et al. Use of Ertapenem as a Marker for Detection of Carbapenem Resistance for Enterobacteriaceae. J Clin Diagn Res. 2020; 14 (12): DC05-DC08. https://www.doi.org/10.7860/JCDR/2020/46140/14271.
51. Bitew, A., and Tsige, E. High prevalence of multidrug-resistant and extended-spectrum β-lactamase-producing Enterobacteriaceae: a cross-sectional study at Arsho Advanced Medical Laboratory, Addis Ababa, Ethiopia. J Trop Med. 2020; 2020: 6167234. https://doi:10.1155/2020/6167234.
52. Eshetie, S., Unakal C., Gelaw A., Ayelign, B., Endris, M., and Moges, F. Multidrug resistant and carbapenemase producing Enterobacteriaceae among patients with urinary tract infection at referral Hospital, Northwest Ethiopia. Antimicrob Resist Infect Contr. 2015; 4: 12. https://doi:10.1186/s13756-015-0054-7.
53. Nikaido, H. Multidrug resistance in bacteria. Annu Rev Biochem. 2009; 78 (1): 119–146. https://doi.org/10.1146/annurev.biochem.78.082907.145923
54. Odonkor, S. T., and Addo, K. K. Prevalence of Multidrug-Resistant *Escherichia coli* Isolated from Drinking Water Sources. Int J Microbiol. 2018; 2018: 7204013. https://doi.org/10.1155/2018/7204013.
55. Olowo-Okere, A., Ibrahim, Y. K. E., Nabti, L. Z., and Olayinka, B. O. High prevalence of multidrug-resistant Gram-negative bacterial infections in Northwest Nigeria. Germs. 2020; 10 (4): 310-321. https://doi.org/10.18683/germs.2020.1223.
56. Mohammed, Y., Zailani, S., and Onipede, A. Characterization of KPC, NDM and VIM Type Carbapenem Resistance Enterobacteriaceae from North Eastern, Nigeria. J Biosci Med. 2015; 3: 100-107. https://doi.org/10.4236/jbm.2015.311013.
57. Tsai, Y. M., Wang, S., Chiu, H. C., Kao, C. Y., and Wen, L. L. Combination of modified carbapenem inactivation method (mCIM) and EDTA-CIM (eCIM) for phenotypic detection of carbapenemase-producing Enterobacteriaceae. BMC Microbiol. 2020; 20 (1): 315. https://doi.org/10.1186/s12866-020-02010-3.
58. Aminu, A., Daneji, I. M., Yusuf, M. A., et al. Carbapenem-resistant Enterobacteriaceae infection among patients admitted to intensive care units in Kano, Nigeria. Sahel Med J. 2021; 24: 1-9. https://doi.org/10.4103/smj.smj_14_20.
59. Abdurasheed, A. B., Alade, T. O., Olorode, O. A., and Joy, P. A. Prevalence of Metallo-β-lactamase (*bla*$_{IMP}$, *bla*$_{VIM}$, and *bla*$_{NDM}$) Genes among Clinical Isolates from Two Tertiary Hospitals in Yenagoa, Bayelsa State, Nigeria. Int J Res Innov Appl Sci. 2019; 4 (12): 153.