Turk J Pharm Sci 2022;19(6):681-685
DOI: 10.4274/tjps.galenos.2021.29794

ORIGINAL ARTICLE

Whole Genome Sequencing of Antibiotic Resistant Genes in Isolates from Surfaces in a Science Laboratory

Christiana JESUMIRHEWE[1]*, Aisha Olamide ABDUSALAM[1], Werner RUPPITSCH[2]

[1]Igbinedion University, Prof. Dora Akunyili College of Pharmacy, Department of Pharmaceutical Microbiology, Okada, Nigeria
[2]Austrian Agency for Health and Food Safety, Institute of Medical Microbiology and Hygiene, Vienna, Austria

ABSTRACT

Objectives: Isolates obtained from laboratory surfaces were identified and characterized.

Materials and Methods: Ten consecutive isolates were obtained from 30 sample surfaces of a University Science Laboratory in Edo State Nigeria in May, 2021. Swabs of surfaces from the laboratory were obtained aseptically. The sample swabs were streaked on MacConkey, eosin methylene blue, mannitol salt, and nutrient agar plates, respectively, and incubated appropriately. Distinct colonies were randomly obtained from culture plates and characterized phenotypically. Matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) was used to analyze four isolates (40%) obtained by selection criteria. Susceptibility testing using antibiotics was performed for the identified isolates by Kirby-Bauer method for 15 antibiotics. Isolate characterization and identification of resistance determinants were determined using whole genome sequencing (WGS).

Results: Microorganisms identified included *Leclercia adecarboxylata*, *Enterobacter hormaechei*, *Atlantibacter hermanii*, and *Stenotrophomonas maltophilia*. Three identified isolates were antibiotics-resistant and were investigated by WGS. Resistance genes were found in all (100%) of the resistant laboratory isolates. The resistance determinants included β-lactamase genes, aminoglycoside modifying enzymes, *qnr* genes, sulfonamide, tetracycline, and trimethoprim resistance genes, respectively. Two isolates carried *ESBL* genes and $bla_{CTX-M-15}$ was detected.

Conclusion: Our study displays the dissemination of antibiotic resistance among isolates obtained from surface of a University Science Laboratory. To the best of our knowledge, we have reported the first genomic characterization of resistance to antibiotics in isolates obtained from surfaces of a University Science Laboratory in Nigeria.

Key words: Whole genome sequencing, antibiotic resistance, science laboratory surfaces

INTRODUCTION

Microorganisms are ubiquitous. They have been detected in several areas of the environment. Air, water, soil, and fluid from animals are carrier/vehicles of microorganisms. The quality of air is usually affected by the presence of microorganisms, which include bacteria, fungi, and viruses and people breathe in on average 14 m^3 of air *per* day.[1] Poor air quality, especially contaminated with microorganisms, can lead to severe health challenges for humans. Microorganisms are transmitted through other routes, which include contaminated food and food products, droplet contact by sneezing, coughing or contacting with contaminated surfaces or soil. The mechanisms how microorganisms attach to animate and inanimate things have been previously reported.[2] Biofilms may be found on a wide variety of surfaces. Physical forces determine how microorganisms are transmitted and attached to surfaces. Once bacteria get attached to surfaces, they start to divide, resulting in biofilms, which cause the complex structure of natural sediments.[3] The microorganisms may either be active

*Correspondence: ebarunosen2002@yahoo.co.uk, Phone: +2348034648066, ORCID-ID: orcid.org/0000-0002-8829-0713
Received: 20.10.2021, Accepted: 17.12.2021
©Turk J Pharm Sci, Published by Galenos Publishing House.

and reproduce immediately or remain inactive on surfaces for long periods, making it difficult to identify the contamination source.[4] Antibiotic resistance is a serious health challenge not only among human pathogens, but also in isolates found in other habitats. Many resistant pathogenic bacteria and commensals are found in different hosts or in the environment at large with the potential of causing infections that are usually difficult to treat.[5] *Brucella* species, *Shigella* species, *Salmonella* species, *Mycobacterium tuberculosis*, and *Neisseria meningitidis* have been reported as the most common microorganisms causing laboratory-acquired infections (LAI). Infections because of the hepatitis virus, human immunodeficiency virus, and fungal infections caused by dimorphic fungi have also been commonly reported.[6] Laboratory-acquired and nosocomial infections pose an important challenge globally and the characterization of microorganisms causing such infections is important as it provides possible therapeutic solutions for some LAI. It is important to characterize microorganisms causing LAIs to devise procedures to prevent subsequent outbreaks.[7] This study aimed to identify isolates, revealing resistance to antibiotics in isolates from surfaces at Pharmaceutical Microbiology Laboratory, Igbinedion University Okada, and characterizing the resistance mechanisms using whole genome sequencing (WGS).

MATERIALS AND METHODS

Materials

Collection of samples

Thirty samples were obtained aseptically from the surfaces of work benches, tables, fridges, sinks, equipment, windows, and doors in pharmaceutical microbiology laboratory using sterile cotton swab sticks. One sample was obtained *per* surface. Sterile cotton swab sticks were soaked in sterile peptone water before sampling.

Media preparation and sterilization

Four culture media, *e.g.* eosine methylene blue agar, mannitol salt agar, Macconkey agar, and nutrient agar, were used in this study. All culture media were prepared and sterilized based on manufacturer's instructions.

Isolation and identification

Ten consecutive isolates were obtained in May, 2021 from 30 sample surfaces of work benches, tables, fridges, sinks, equipment, windows, and doors in the pharmaceutical microbiology laboratory of Igbinedion University Okada in Edo State, Nigeria. Samples were aseptically collected and immediately inoculated on agar plates. Inoculated plates were incubated at 37°C for 24 h. Distinct colonies formed were randomly obtained from culture plates. Pure cultures were obtained afterwards on agar slants maintained at 4°C in the refrigerator throughout the study. Identification of isolates was carried out using standard microbiological techniques.[8] Identities of randomly selected four isolates were subsequently confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS) (Bruker Daltonik GmbH, Bremen, Germany) analysis.

Antimicrobial susceptibility tests

The Kirby-Bauer susceptibility testing technique (Bauer et al.)[9] was performed and results were analyzed using European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.[10] The isolates were tested using 15 antibiotics: ampicillin, meropenem, ertapenem, ceftazidime, cefotaxime, amoxicillin/clavulanic acid, cefoxitin, cefepime, cefpodoxime, tigecycline, ciprofloxacin, amikacin, piperacillin/tazobactam, cefuroxime, and gentamicin (Oxoid, Basingstoke Hampshire, UK).

Whole genome sequencing

WGS was carried out for four randomly selected isolates, whose identities were confirmed by MALDI-TOF mass spectrometry (Bruker Daltonik GmbH, Bremen, Germany) analysis. Genomic DNA (gDNA) extraction was carried out using the MagAttract HMW DNA extraction kit (Qiagen, Hilden, Germany). Quantification of gDNA was performed on a Qubit® 2.0 fluorometer using the dsDNA BR Assay kit (Invitrogen by Thermo Fisher Scientific, Waltham, MA, USA) and diluted to 0.2 ng/μL based on the manufacturer recommendations (Illumina sample preparation guide, Illumina Inc, San Diego, CA, USA). Preparation of fragment libraries of the bacterial genomes was carried out using the Illumina Nextera XT DNA library preparation kit (Illumina Inc, San Diego, CA, USA). A DNA fragment library preparation was carried out using 1 ng of gDNA (Illumina sample preparation guide). Paired end sequencing using a read length of 2 x 300 bp on an Illumina Miseq (Miseq ver. 3.0, Illumina Inc) was performed using Miseq reagent kit v3 containing the reagent cartridge and flow cell. Pooled libraries were loaded on the reagent cartridge. Samples were sequenced to obtain a minimum average coverage of 100 fold based Illumina's recommended standard protocols.

Table 1. Genome assembly statistics of the recovered isolates

Isolate	Isolation source	Genome size	Genome coverage	N_{50} (bp)	Number of contigs	Accession number
Atlantibacter hermannii	Laboratory sink	4.7	28	175,641	114	JAJNEI000000000
Stenotrophomonas maltophilia	Laboratory bench	4.6	24	38,623	220	JAJNEH000000000
Leclercia adecarboxylata	Laboratory bench	4.9	51	142,618	158	JAJNEK000000000
Enterobacter hormaechei	Laboratory sink	4.7	85	244,270	88	JAJNEJ000000000

Raw reads (FASTQ files) were trimmed at their 5' and 3' ends until an average base quality of 30 was reached in a window of 20 bases, and assembly was performed using Velvet version 1.1.04 Zerbino[11], using optimized k-mer size and coverage cut values based on the average length of contigs with >1.000 bp. Species identification *via* MALDI-TOF MS was confirmed using ribosomal multilocus sequence typing (rMLST) (https://pubmlst.org/species-id). Identification of antimicrobial resistance genes (ARGs) was carried out using the comprehensive antibiotic resistance database-resistance gene identifier (RGI).[12] ARGs were identified on the basis of a minimum cut-off of 98% nucleotide identity for perfect or strict hits predicted by RGI. Sequences were analyzed for their plasmid replicon types using PlasmidFinder and their MLST using MLST 1.8 software, both available from the Center for Genomic Epidemiology.[13,14] No statistical analysis method was used to analyzing results.

Nucleotide sequence accession numbers

This whole genome shotgun project has been deposited at DDBJ/ENA/GenBank under the accession numbers JAJNEH000000000-JAJNEK000000000. The version described in this paper is version JAJNEH010000000-JAJNEK010000000. Table 1 shows the genome assembly statistics of the recovered isolates.

Table 2. Identity of the isolates

Bacterial isolates	Identities
510509	*Atlantibacter hermannii*
510510	*Stenotrophomonas maltophilia*
510507	*Leclercia adecarboxylata*
510508	*Enterobacter hormaechei*

RESULTS AND DISCUSSION

Species identification using MALDI-TOF-MS and ribosomal MLST assigned 4 isolates to 4 different species (Table 2). Identified laboratory isolates include *Atlantibacter hermanii, Stenotrophomonas maltophilia, Enterobacter hormaechei,* and *Leclercia adecarboxylata* (Table 2). Out of 4 randomly selected identified isolates, 3 isolates revealed antibiotic resistance and were further analyzed by WGS (Table 3). No information about antibiotics is obtained in the EUCAST table for *S. maltophilia.* The laboratory isolates were ampicillin-resistant (100%), cefotaxime (67%), amoxiclav (100%), cefepime (67%), ceftazidime (67%), cefpodoxime (67%), cefuroxime (67%), ciprofloxacin (67%), gentamicin (67%), and tigecycline (67%). Two isolates were positive in the phenotypic testing of ESBLs and had *ESBL* gene $bla_{CTX\text{-}M\text{-}15}$ detected (Table 3).

WGS revealed that 3 out of 4 identified isolates harbored more than one resistance gene. Resistance genes were detected in *S. maltophilia*, but did not pass the minimum cut-off 98% nucleotide identity for perfect or strict hits predicted by RGI. The resistance determinants in the isolates included β-lactamase genes, $bla_{TEM\text{-}1}$, $bla_{ACT\text{-}24}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$; aminoglycoside modifying enzymes, *aac (3)-IId, aph(6)-Id, aph3")-Ib, aac(6')-Ib-cr4*; *qnr* gene, *qnr*B1; sulfonamide resistance gene, *sul2*; tetracycline resistance

Table 3. Antibiotic susceptibility test results of the isolates

Resistance testing *via* disk diffusion

ID no	Source	Identity	R-I						R-II						ESBL
			CTX 5-cefotaxime	FEP 30-cefepime	AMP 10-ampicillin	AMC 30-amoxicillin/ clavulanic acid	TZP 36-piperazillin/ tazobactam	CXM 30-cefuroxime	MEM 10-meropenem	ETP 10-ertapenem	CN 10-gentamicin	AK 30-amikacin	CIP 5-ciproflaxin	TGC 15-tigecyclin	FOX 30-cefoxitin
510507	Laboratory	*Leclercia adecarboxylata*	S	S	R	R	S	I	S	S	S	S	S	S	S
510508	Laboratory	*Enterobacter hormaechei*	R	R	R	R	S	R	S	R	S	S	R	R	R
510509	Laboratory	*Atlantibacter hermannii*	R	R	R	R	S	R	S	S	R	S	R	S	S
510510	Laboratory	*Stenotrophomonas maltophilia*													

Table 3. Continued

Resistance testing *via* disk diffusion

ID no	Source	Identity						ACI						XAMA			
			CPD 10-cefpodoxime	CAZ 30-ceftazidime	FEP 30-cefepime	CTX 5-cefotaxime	AMC 30-amoxicillin/ clavulanic acid	TZP 110-piperacillin/ tazobaactam	MEM 10-meropenem	TOB 10-torabmycin	AK 30-amikacin	SXT 25-trimethoprim/ sulfamethoxazole	CIP 5-ciprofloxacin	LEV 5-levofloxacin	MH 30-minocyclin	STX 25-trimethoprim/ sylfamethoxazole	CIP 5-ciprofloxacin
510507	Laboratory	*Leclercia adecarboxylata*	S	S	S	S	R										
510508	Laboratory	*Enterobacter hormaechei*	R	R	R	R	R										
510509	Laboratory	*Atlantibacter hermannii*	R	R	R	R	R										
510510	Laboratory	*Stenotrophomonas maltophilia*												No info	No info	I	No info

R: Resistant, I: Intermediate, S: Sensitive, ?: (-) no breakpoints, IE: Insufficient evidence that the organism or group is a good target for therapy with the agent, No info: No information about AB in EUCAST table for *Stenotrophomonas maltophilia*

gene, *tet*(D); phenicol resistance gene, *catII* and trimethoprim resistance gene, *dfrA14*. Other resistance determinants, which included the regulatory systems modulating antibiotic efflux CRP, antibiotic target alteration gene EF-Tu were also detected in the antibiotic resistant isolates. Table 4 shows the characteristics of the antibiotic resistant laboratory isolates. *E. hormaechei* isolated was of the sequence type ST 78. *A. hermanii, S. maltophilia,* and *L. adecarboxylata* had previously unknown sequence types (Table 4). Plasmids from the incompatibility group detected among the isolates were predominantly of Inc F and Col family types (Table 4).

Microbial contamination in a laboratory varies in different laboratories based on their geographical location and measures used to control infection, which poses an important challenge. Few reports have been made on this issue, especially in developing countries like Nigeria, which is really a drawback. Most of the previous studies were based on the phenotypic characterization of microbial isolates from surfaces in the laboratory. In a previous Nigerian study by Isola and Olatunji[15], bacterial isolates obtained from laboratory surfaces were characterized and identified. The results showed that the most frequent microorganisms from laboratory surfaces were *Bacilli*. Others included *Salmonella typhae* and *Staphylococcus aureus*. Another previous study identified *Staphylococcus epidermis* and aerobic spore bearers, *i.e. Bacillus subtilis,* a common microorganism contaminating working areas in a microbiology laboratory.[16] Strains identified were possible pathogens and could cause LAIs. In another study by Veena Kumari et al.[17], laboratory surface samples were assessed for microbial contaminants. Out of the 60 surface samples assessed, coagulase- negative *Staphylococci* were the most frequent contaminant, followed by Gram-positive bacilli (*Corynebacterium* spp.). In our study, isolates obtained were on a relatively small scale compared to previous studies. Significantly, no Gram-positive isolate was identified among the isolates obtained in this study. All isolates were Gram-negative bacteria. More than one resistance determinant was found on the draft genome sequences of the resistant isolates, which showed that they are potential pathogens that may cause LAIs.

Resistance mediated by $bla_{CTX-M-15}$ has been reported globally, including in Nigeria.[18-20] Recently, the first report on $bla_{CTX-M-15}$ in clinical isolates of *Providencia* spp., *Citrobacter freundii*, and *Atlantibacter hermannii* isolated from humans in Nigeria was published.[21] To the best of our knowledge, we report the first genomic characterization of resistance to antibiotics in isolates obtained from surfaces of a University Science Laboratory in Nigeria. This suggests the circulation of the gene $bla_{CTX-M-15}$ in a different setting. Significantly, resistance genes were detected in *L. adecarboxylata* observed to be sensitive to most of the antibiotics in the susceptibility testing. This shows the importance of studying antibiotic resistance not using phenotypic methods only but further genotypic and molecular characterization techniques like WGS.

Enterobacter cloacae ST66, ST78, ST108, and ST114 strains are known to be extended spectrum cephalosporin-resistant spread internationally as high-risk clones.[22] *E. hormaechei* ST78 isolate detected in this study belonged to the high-risk clone known particularly for the nosocomial spread carbapenemases and ESBLs.[22] Jesumirhewe et al.[21], in a recent report, detected *E. hormaechei* ST78 isolates in a Nigerian Hospital. *E. hormaechei* ST78 identified in this study suggests the circulation of this high-risk lineage in a different setting. The detection of the predominant plasmid replicon types (Inc F and Col) among the resistant isolates in this study displayed their importance in the transmission of antibiotic resistance. A recent report indicated that the IncF plasmid type is the most prevalent among human ESBL Enterobacteriaceae isolates in Nigeria.[21]

CONCLUSION

Microorganisms are ubiquitous including the laboratory environment. Techniques for reducing contamination should be employed, which include the use of soaps to clean laboratory surfaces, pre- and post- treatment of hands in disinfectant before conducting any experiment in the laboratory, the use of protective clothing, when working in the laboratory. Laboratory coats must be strictly used in the laboratory and unworn outside the laboratory. Measures should be aimed at eliminating/significantly reducing these microorganisms from laboratory

Table 4. Details of the resistant isolates

Isolates	Sequence type	Plasmid replicon type	Antibiotic resistance genes/determinants detected
Enterobacter hormaechei	78	Col(pHAD28), Col3M	MD-regulatory system modulating antibiotic efflux CRP, antibiotic target alteration gene Ef-Tu ,BL- $bla_{ACT-24,}$ $bla_{CTX-M-15,}$
Atlantibacter hermanii	Unknown	IncFIB(pECLA), IncFII(pECLA)	*A-aac*(3)-*lld*, *aph*(6)-*ld*, *aac*(6')-*lb-cr*4, P-*catII*, *T-dfrA14,* MD-regulatory system modulating antibiotic efflux CRP,TE- tet(D), S-sul2, BL-bla_{TEM-1}, bla_{OXA-1}, $bla_{CTX-M-15}$ FQ-*QnrB1*
Leclercia adecarboxylata	Unknown	Col(pHAD28), IncFII(pCTU2)	*A-aph*(6)-*ld*, *aph*(3")-*lb*, T-*dfrA14,* MD-regulatory system modulating antibiotic efflux CRP,S-*sul*2, BL-bla_{TEM-1}
Stenotrophomonas maltophilia	Unknown	No plasmid replicon type	No resistance gene detected

A: Aminoglycosides, BL: *Beta*-lactams, MD: Multi-drug, P: Phenicol, T: Trimethoprim, TE: Tetracycline, S: Sulphonamide, FQ: Fluoroquinolones

using proper procedures. Frequent assessments of surfaces of the laboratory should be carried out not using phenotypic methods; only but molecular methods to identify and explore the genetic mechanisms of resistance to antibiotics in isolates, which is important to understand the dissemination of resistant isolates. Further molecular studies must characterize the prevailing clonal lineages and plasmids that harbor resistance mediating genes in the isolates. Frequent assessments would assist laboratories in either avoiding or eliminating most microbial contaminants found in the laboratory.

Ethics

Ethics Committee Approval: Not required for the study.

Informed Consent: Not required for the study.

Peer-review: Externally peer-reviewed.

Authorship Contributions

Concept: C.J., Design: C.J., Data Collection or Processing: C.J., A.O.A., Analysis or Interpretation: C.J., W.R., Literature Search: C.J., A.O.A., Writing: C.J.

Conflict of Interest: No conflict of interest was declared by the authors.

Financial Disclosure: The authors declared that this study received no financial support.

REFERENCES

1. Wemedo SA, Ede PN, Chuku A. Interaction between building design and indoor airborne microbial load in Nigeria. Asian J Biol Sci. 2012;5:183-191.
2. Costerton JW, Geesey GG, Cheng KJ. How bacteria stick. Sci Am. 1978;238:86-95.
3. Donlan RM. Biofilms: microbial life on surfaces. Emerg Infect Dis. 2002;8:881-890.
4. del Valle Mendoza J, Silva Caso W, Tinco Valdez C, Pons MJ, del Valle LJ, Oré VC, Michelena DC, Mayra JB, Gavidea VZ, Vargas M, Ruiz J. Diagnosis of Carrion's disease by direct blood PCR in thin blood smear negative samples. PLoS One. 2014;9:e92283.
5. Taylor LH, Latham SM, Woolhouse ME. Risk factors for human disease emergence. Philos Trans R Soc Lond B Biol Sci. 2001;356:983-989.
6. Singh K. Laboratory-acquired infections. Clin Infect Dis. 2009;49:142-147.
7. Porte L, Soto A, Andrighetti D, Dabanch J, Braun S, Saldivia A, Flores JC, Wozniak A, García P, Weitzel T. Catheter-associated bloodstream infection caused by *Leifsonia aquatica* in a haemodialysis patient: a case report. J Med Microbiol. 2012;61:868-873.
8. Cheesbrough M. District laboratory practice in tropical countries. (2nd ed). Cambridge University Press, UK; 2006:178-187.
9. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol. 1966;45:493-496.
10. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. 2021 version 11.0.
11. Zerbino DR. Using the Velvet *de novo* assembler for short-read sequencing technologies. Curr Protoc Bioinformatics. 2010;11:11.5.
12. Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Powlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. Nucleic Acids Res. 2017;45:566-573.
13. Larsen MV, Cosentino S, Rasmussen S, Friis C, Hasman H, Marvig RL, Jelsbak L, Sicheritz-Pontén T, Ussery DW, Aarestrup FM, Lund O. Multilocus sequence typing of total-genome-sequenced bacteria. J Clin Microbiol. 2012;50:1355-1361.
14. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, Møller Aarestrup F, Hasman H. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895-3903.
15. Isola OB, Olatunji EO. Isolation and identification of microorganisms from surfaces in science laboratories. SAU Sci-Tech J. 2016;1:80-87.
16. Ghayoor M, Qadoos A, Bahadar S, Hayat A, Daud M, Hassan A, Ali F, Zeb A, Rahman KU, Wahab A, Khattak ZF, Khattak B. Isolation and identification of common contaminants bacteria from working area in microbiology laboratory. J Biomol Sci. 2015;3:74-78.
17. Veena Kumari HB, Nagarathna S, Reddemma K, Lalitha K, Mary B, Sateesh VL. Containment of case-file contamination-- infection control. J Evol Med Dent Sci. 2012;1:1166-1171.
18. Cantón R, Coque TM. The CTX-M beta-lactamase pandemic. Curr Opin Microbiol. 2006;9:466-475.
19. Aibinu I, Pfeifer Y, Peters F, Ogunsola F, Adenipekun E, Odugbemi T, Koenig W. Emergence of bla(CTX-M-15), qnrB1 and aac(6')-Ib-cr resistance genes in *Pantoea agglomerans* and *Enterobacter cloacae* from Nigeria (sub-Saharan Africa). J Med Microbiol. 2012;61:165-167.
20. Raji MA, Jamal W, Ojemeh O, Rotimi VO. Sequence analysis of genes mediating extended-spectrum *beta*-lactamase (ESBL) production in isolates of Enterobacteriaceae in a Lagos Teaching Hospital, Nigeria. BMC Infect Dis. 2015;15:259.
21. Jesumirhewe C, Springer B, Allerberger F, Ruppitsch W. Whole genome sequencing of extended-spectrum β-lactamase genes in Enterobacteriaceae isolates from Nigeria. PLoS One. 2020;15:e0231146.
22. Izdebski R, Baraniak A, Herda M, Fiett J, Bonten MJ, Carmeli Y, Goossens H, Hryniewicz W, Brun-Buisson C, Gniadkowski M; MOSAR WP2, WP3 and WP5 Study Groups. MLST reveals potentially high-risk international clones of *Enterobacter cloacae*. J Antimicrob Chemother. 2015;70:48-56.