Original Research Article

Patterns of antibiotic resistance in Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State, Nigeria

ABSTRACT

Aim: identification of resistance pattern in some clinical isolates from Niger Delta University Teaching Hospital, Okolobiri, Nigeria
Study design: Cross-sectional and descriptive study design were utilized in this study.
Place and Duration of Study: Niger Delta University Teaching Hospital Okolobiriand Niger Delta University Molecular Laboratory CHS, Wilberforce Island, Amassoma, between February 2023 and April 2023.
Methodology: This study was specifically carried out on clinical isolates with a population figure obtained from 6 different samples collected from Niger Delta University Teaching Hospital, okolobiri in Bayelsa state. From 500 different clinical samples, 100 clinical isolates were isolated and identified using Standard Bacteriological methods. Antibiotic susceptibility testing was carried out using Modified Kirby Bauer disc-diffusion method

Results: The findings showed that out of 100 isolates identified, *Pseudomonas aeruginosa* was most prevalent, 22(22%), 20(20%) were *Klebsiella oxytoca*, and 20(20%) were *Escherichia coli.* Antibiotics susceptibility pattern of the bacterial isolates revealed high resistance rates among the isolates, with significant resistance to multiple antibiotics.
Conclusion: There was a high multi-drug-resistant Gram-negative bacteria, therefore justifying the extent of resistance observed.

Keywords: Antimicrobial Susceptibility,Clinical isolates, Niger Delta University Teaching Hospital, Okolobiri, Nigeria

1. INTRODUCTION

In every region of the world, antibiotic resistance is increasing and has reached dangerously high levels [9]. As antibiotics lose their effectiveness, new resistance mechanisms evolve and spread globally, posing a threat to our ability to treat common infectious diseases, which are becoming more challenging and difficult to cure. This results in a decline in treatment effectiveness and financial waste. The availability of antibiotics for both people and animals without a prescription hastens the development and spread of antibiotic resistance [10]. In nations without traditional medical norms, antibiotics are commonly overprescribed by medical practitioners and abused by the general people. Without rapid action, the post-antibiotic era, in which common illnesses and minor injuries can again be fatal, is about to begin.
In the past century, the discovery of antimicrobial agents became one of the most significant contributions to therapeutics and humanity, healing a wide range of communicable diseases caused by infections in both animals and humans. Antimicrobial agents are materials or composites that kill or slow the evolution of germs. Infections in the skin, heart, urinary tract, and meningitis caused by bacteria have all been treated with antibiotics. However, the indiscriminate use of currently available antimicrobial drugs in clinical therapy is encouraging

the growth of antibiotic resistance in microorganisms, creating a challenge for the future management of bacterial illnesses. Antimicrobial resistance has been ranked among the top 10 global health threats by the WHO [1], and research has shown that it can universally grow and spread to a wide range of microorganisms, leading to an increase in the number of primary care failures in the treatment of bacterial infections[1].

The World Health Organization defines antibiotic resistance as a process that takes place when a bacterium adapts over time and stops responding to antibiotics, making infections harder to treat and raising the risk of disease spread, serious illness, and death. Antibiotic resistance occurs when bacteria, no longer become susceptible to antibiotics meant to kill them. These findings suggest that the germs may resist antibiotic effects and will continue to multiply. Antibiotic-resistant sickness almost invariably necessitates a longer hospital stay, more follow-up visits to the doctor, and an expensive and riskier alternative to therapy. Antibiotic resistance does not mean that the body has become immune to antibiotics; rather, it means that germs have become resistant to the drugs used to eradicate them. Since the usage of antibiotics has increased, the issue has gotten worse. However, antibiotic usage is commonly abused and not yet regulated in almost all underdeveloped countries [2]. The aim of this study was therefore to detect antimicrobial susceptibility in some clinical isolates from Niger Delta University Teaching Hospital, Okolobiri, Nigeria.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in Bayelsa state. Bayelsa state was created out of the former old Rivers state in 1996 during the administration of the late General Sani Abacha. The state of Bayelsa is bordered by Delta state to the north, Rivers state to the east, and the Atlantic Ocean to the west and south. It is located between 4015' North and 50 and 23' South. Bayelsa state has the largest wet land in West Africa, which was also the first to discover commercial quantities of crude oil. According to census data from 2006, there are about 1.7 million people living in the state of Bayelsa.

2.2Experimental Design

Cross sectional and descriptive study design were utilized in this study.

2.3 Study Population

This study was specifically carried out on clinical isolates with a population figure obtained from 6 different samples collected from Niger Delta University Teaching Hospital, Okolobiri in Bayelsa state.

2.4 Ethical Clearance

Ethical clearance was duly obtained from hospital ethical clearance committees of the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa state.

2.5Sample Technique

Simple random sampling technique was used to collect isolates for this research work.

2.6Sample Size Determination

Taro Yamane statistical formula was applied to determine the sample size mathematically expressed as follows[3]

$n = N/ 1 + N (e) 2$

In the formula above.
n ----- Is the required sample size from the population under study.
N ----- Is the whole population that is under study (134).
e. ----- Is the precision or sampling error (0.05).
Substituting:

n=134/〖1+134(0.05)〗^2

n = 134/(1+134(0.0025))
n = 134/(1+0.335)
n = 162/1.355
n = 100
Therefore, sample size 100respondents out of the entire 134 respondents was the lowest accepted number of responses to maintain a 95% confidence level.

2.7 Inclusion and Exclusion criteria
Inclusion: All isolates that were obtained and are multidrug resistant, were included in this research work.

Exclusion:All isolates that were obtained and are not multidrug resistant, were excluded in this research work.

2.8 Sample collection
Clinical isolateswere collected from Niger Delta University Teaching Hospital (NDUTH) Okolobiri in Bayelsa State. They were immediately transported to Niger Delta University Microbiology Laboratory for analysis.

2.9 Sample processing

2.9.1 Isolation

With the aid of sterile wire loop, the isolates on peptone broth collected were aseptically sub-cultured on Mac Conkay agar, nutrient agar, Luria-Bertani (LB) Broth respectively and incubated at 37ºc for 24 hours.

2.9.2 Identification
After 24 hours of incubation, the cultivated colonies were identified macroscopically and microscopically for pigmentation, size, texture, and degree of opacity.

2.9.2.1 Colonial Morphology

The morphology of the bacterial colonies such as size, pigmentation, surface and degree of opacity was observed macroscopically and recorded.

2.9.2.2 Gram Staining

On a clean grease free glass slide, a drop of normal saline was placed, and a colony of the test isolates was picked with the aid of a sterile wire loop and emulsified on the drop of saline. The slide was allowed to air dry and was heat fixed by passing the slide over a Bunsen flame 3 times. The smear was stained with crystal violet for 1 minute and rinsed with tap water. The slide was flooded with lugol's iodine for 1 minute and rinsed with tap water. It was then rapidly decolorized with acetone alcohol for few seconds and rinsed with tap water. The smear was counter stained with neutral red for 2 minutes and rinsed with tap water. The back of slide was

cleaned dry with cotton wool and allowed to air dry. A drop of immersion oil was placed on the smear and it was view under the microscope using 100x objective lens.

2.9.2.3 Standardization of Bacteria Isolates
Isolated colonies from pure culture plates were sub-cultured into peptone water and incubated for 12 hours. Turbidity was then adjusted by dilution with sterile peptone water until visually comparable with a MacFarland's 0.5 standard. MacFarland's 0.5 standard was prepared by adding Barium Chloride (BaCl) with Tetraoxosulphate VI acid (H_2SO_4).

H_2SO_4(1%) was prepared by measuring out 1ml of H_2SO_4 with the aid of a measuring cylinder and was added to a measured 99mls of distilled water in another beaker. Then, 99.4mls of preparation was transferred into a clean beaker with the aid of sterile pipette.

The 0.6ml of Barium chloride solution was then added to 99.4mls of H_2SO_4 Given final volume 0f 100mls of Barium sulphate. The absorbance of the turbidity standard was verified using a spectrophotometer at 625nm and an Absorbance of -0.08 to 0.10 for the 0.5 Mac farland standard was read.

2.9.2.4 Identification of bacterial isolates (API 20E)
An incubator tray was placed on a flat surface and 5ml of sterile distilled water was distributed into the tray to create a humid chamber. The API 20E strip was placed in the tray. Using a sterile disposable applicator stick, a single colony from an isolate plate was emulsified in 5ml sterile distilled water. This was adjusted to 0.5 McFarland's standard. With a sterile disposable pipette, both the tube and cupule of the test CIT, VP, and GEL were filled with the bacterial suspension, while only the tubes were filled for others. An anaerobic environment was created in the test ADH, LDC, ODC, URE, and H_2S by filling mineral oil. The tray was labeled with identification number and the lid placed. It was then incubated for 24 hours at 37oC. It was read by observing color change by comparing with the API reading scale (Color chart).

2.10 Antimicrobial Susceptibility Test

2.10.1 Disk Diffusion Method

The antibiotic susceptibility testing of the pure isolates was carried out by an agar diffusion method (Kirby Bauer) using commercially available discs to determine the drug sensitivity and resistance pattern of the isolates. With a sterile disposable applicator stick, a single colony from an isolate plate was emulsified in 5ml sterile distilled water. This was adjusted to 0.5 McFarland's standard. This was carried out according to the standard disc diffusion technique as described by Clinical Laboratory Standard Institute [8]. Paper discs (Abtek Biologicals) contain the antibiotics: The antibiotics included ciprofloxacin (CPX) 10mcg, Gentamicin (CN) 10mcg, Norfloxacin (NB) 10mcg, Amoxil (AML) 20mcg, Chloramphenicol (H) 30mcg, Erythromycin (E) 30mcg, Ampiclox (APX)20mcg, and Levofloxacin (LEV) 20mcg for the Gram-positive Bacteria. Septrin (SXT) 30mcg, Chloramphenicol (CH) 30mcg, Sparfloxacin (SP) 10mcg, Ciprofloxacin (CPX) 10mcg, Amixacillin (AM) 30mcg, Augmentin (AU) 30mcg, Gentamicin (CN) 10mcg, Pefloxacin (PEF) 30mcg, Tarivid (OFX) 10mcg, and Streptomycin was used for Gram negative bacteria. Theantibiotic disc was taken out of the refrigerator and allowed on the bench at room temperature. A sterile cotton swab was dipped into the respective bacterial stock solution, and excess fluid from the swab was removed by pressing the swab against the wall of the tube. The Mueller-Hinton (MH) agar entire surface was then swabbed with the bacterial suspension and allowed to dry air for 15 min. Antibiotic discs impregnated with a defined quantity of antimicrobial agent was placed and then layered aseptically on the Mueller-Hinton agar surface. The Plates were then incubated at 37°C overnight and the zones of inhibition (ZI) recorded. Interpretation of results were based on

guidelines of the Clinical and Laboratory Standards.A concentration gradient of the antibiotic forms by diffusion from the disc and growth of the test organism is inhibited at a distance from the disc forming zone of inhibition. The zone of inhibition was measured with meter rule, which determines if the antibiotic used was resistance, intermediate, or sensitive to the isolates.

3. RESULTS AND DISCUSSION

Table 1: Distribution of Specimen by Gender

Specimen	Male (%)	Female (%)	Total
Stool	14 (41.17%)	20 (58.82%)	34 (34%)
Sputum	8 (80%)	2 (20%)	10 (10%)
Blood	6 (60%)	4 (40%)	10 (10%)
High-vaginal swab	0 (0%)	4 (100%)	4 (4%)
Wound swab	8 (50%)	8 (50%)	16 (16%)
Urine	12 (46.15%)	14 (53.84%)	26 (26%)
Total	48 (48%)	52 (52%)	100 (100%)

Table 2: Distribution of Bacterial Isolates by Specimen

Specimen	*K. pneumonia*	*K. oxytoca*	*P. aeruginosa*	*P. mirabilis*
Stool	2(5.88%)	10(29.41%)	2(5.88%)	2(5.88%)
Sputum	0(0%)	0(0%)	8(80%)	0(0%)
Blood	2(20%)	4(40%)	2(20%)	0(0%)
HVS	0(0%)	0(0%)	2(50%)	2(50%)
Wound swab	2(12.5%)	0(0%)	4(25%)	4(25%)
Urine	4(15.38%)	6(23.07%)	4(15.38%)	2(7.69%)
Total	10(10%)	20(20%)	22(22%)	8(10%)

Table 3: Distribution of Bacterial Isolates by SpecimenCont`

P. vulgaris	*C. freundii*	*C. koseri*	*E. coli*	Total
0(0.0%)	12(35.3%)	2(5.9%)	4(11.8%)	34(34.0%)
0(0.0%)	0(0.0%)	0(0.0%)	2(20.0%)	10(10.0%)
0(0.0%)	0(0.0%)	0(0.0%)	2(20.0%)	10(10.0%)
0(0.0%)	0(0.0%)	0(0.0%)	0(0.0%)	4(4.0%)
0(0.0%)	0(0.0%)	0(0.0%)	6(37.5%)	16(16.0%)

2(7.69%)	2(7.69%)	0(0%)	6(23.07%)	26(26%)
6(2%)	14(14%)	2(2%)	20(20%)	100(100%)

Table 4: Distribution of Bacterial Isolates by Age and Gender (Male)

AGE RANGE	*K. pneumoniae*	*K. oxytoca*	*P. aeruginosa*	*P. mirabilis*
< 20	4(40%)	4(40%)	0(0%)	0(0%)
21-30	0(0%)	0(0%)	0(0%)	0(0%)
31-40	0(0%)	0(0%)	4(50%)	2(25%)
41-50	0(0%)	4(50%)	2(25%)	0(0%)
51-60	0(0%)	0(0%)	0(0%)	0(0%)
> 60	0(0%)	0(0%)	4(66.66%)	0(0%)
Total	4(11.11%)	8(22.22%)	10(27.77%)	2(5.55%)

Table 5: Distribution of Bacterial Isolates by Age and Gender (Male)Cont

P. vulgaris	*C. freundii*	*C. koseri*	*E. coli*	Total
0(0%)	0(0%)	0(0%)	2(20%)	10(27.77)
0(0%)	2(100%)	0(0%)	0(0%)	2(5.55%)
0(0%)	2(25%)	0(0%)	0(0%)	8(22.22%)
0(0%)	2(25%)	0(0%)	0(0%)	8(22.22%)
0(0%)	0(0%)	0(0%)	2(100%)	2(5.55%)
0(0%)	0(0%)	0(0%)	2(33.33%)	6(16.66%)
0(0%)	6(16.66%)	0(0%)	6(16.66%)	36(100%)

Table 6: Distribution of Bacterial Isolates by Age and Gender (Female)

AGE RANGE	*K. pneumonia*	*K. oxytoca*	*P. aeruginosa*	*P. mirabilis*
< 20	0(0%)	4(25%)	2(12.5%)	0(0%)
21- 30	0(0%)	0(0%)	2(25%)	4(50%)
31-40	4(33.33%)	2(16.66%)	0(0%)	0(0%)
41-50	0(0%)	0(0%)	0(0%)	0(0%)
51- 60	0(0%)	4(50%)	2(25%)	0(0%)
>60	6(30%)	2(10%)	8(40%)	2(10%)
Total	10(15.62%)	12(18.75%)	14(21.87%)	6(9.37%)

Table 7: Distribution of Bacterial Isolates by Age and Gender (Female)Cont

P. vulgaris	*C.freundii*	*C.koseri*	*E. coli*	Total
2(12.5%)	2(12.5%)	0(0%)	6(37.5%)	16(25%)

0(0%)	0(0%)	0(0%)	2(25%)	8(12.5%)
2(16.66%)	2(16.66%)	0(0%)	2(16.66%)	12(18.75%)
0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
0(0%)	0(0%)	2(25%)	0(0%)	8(12.5%)
0(0%)	2(10%)	0(0%)	0(0%)	20(31.25%)
4(6.25%)	6(9.37%)	2(3.12%)	10(15.62%)	64(100%)

Table 8: Susceptibility Pattern of Bacterial Isolates for Abtek Sensitivity Disk

Isolate	NE	GEN (R%)	AUG (R%)	CIP (R%)	OFL (R%)	CXM (R%)	NIT (R%)	CAZ (R%)
K.Pneumoniae	10	3(30%)	5(50%)	3(30%)	2(20%)	4(40%)	4(40%)	5(50%)
K.oxytoca	20	8(40%)	9(45%)	8(40%)	8(40%)	9(45%)	9(45%)	9(45%)
P.aeruginosa	22	10(45.45%)	11(50%)	7(31.81%)	8(36.36%)	11(50%)	11(50%)	11(50%)
P.mirabilis	10	3(30%)	4(40%)	3(30%)	3(30%)	4(40%)	4(40%)	4(40%)
P.vulgaris	2	2(100%)	3(150%)	2(100%)	2(100%)	3(150%)	3(150%)	3(150%)
C.Freundii	14	6(42.85%)	7(50%)	6(42.85%)	5(35.71%)	5(35.71%)	6(42.85%)	7(50%)
C.koseri	2	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)
E.Coli	20	5(25%)	9(45%)	6(30%)	6(30%)	9(45%)	9(45%)	9(45%)
Total	100	38(38%)	49(49%)	36(36%)	35(35%)	46(46%)	47(47%)	49(49%)

Key: GEN—Gentamycin, AUG—Augmentin, CIP—Ciprofloxacin, OFL—Ofloxacin, CXM—Cefixime, NIT—Nitrofurontion, CAZ—Ceftazidime, CRX—Cefuroxime, LYN—Lyncomycin, MRP—Meropenem, PEF—Pefloxacin, CEP—Ceporex, S—Streptomycin, NA—Nalidixic acid, STX—Septrin, PN—Ampicilin.

Table 9: Susceptibility Pattern of Bacterial Isolates for Abtek Sensitivity DiskCont

CRX (R%)	LYN (R%)	MRP (R%)	PEF (R%)	CEP (R%)	S (R%)	NA (R%)	STX (R%)	PN (R%)
5(50%)	4(40%)	4(40%)	5(50%)	5(50%)	3(30%)	5(50%)	5(50%)	5(50%)
10(50%)	9(45%)	3(15%)	9(45%)	10(50%)	6(30%)	10(50%)	10(50%)	10(50%)
11(50%)	9(40.90%)	8(36.36%)	10(45.45%)	10(45.45%)	8(36.36%)	11(50%)	11(50%)	11(50%)
4(40%)	4(40%)	3(30%)	4(40%)	4(40%)	4(40%)	4(40%)	4(40%)	4(40%)
2(100%)	2(100%)	0(0%)	1(50%)	3(150%)	3(150%)	3(150%)	3(150%)	3(150%)
7(50%)	7(50%)	6(42.85%)	7(50%)	7(50%)	6(42.85%)	7(50%)	7(50%)	7(50%)
1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)	1(50%)
9(45%)	9(45%)	8(40%^)	8(40%)	9(45%)	9(45%)	9(45%)	9(45%)	9(45%)
49(49%)	45(45%)	33(33%)	45(45%)	49(49%)	40(40%)	50(50%)	50(50%)	50(50%)

Key: GEN—Gentamycin, AUG—Augmentin, CIP—Ciprofloxacin, OFL—Ofloxacin, CXM—Cefixime, NIT—Nitrofurontion, CAZ—Ceftazidime, CRX—Cefuroxime, LYN—Lyncomycin, MRP—Meropenem, PEF—Pefloxacin, CEP—Ceporex, S—Streptomycin, NA—Nalidixic acid, STX—Septrin, PN—mpicilin.

In this study, stool specimens had the highest distribution of bacterial isolates, with 20 (58.82%) for females being more common. This may be because women are more likely to

engage in kitchen-related activities and/or because of the length of their nails, which may attract or carry gems that could be introduced into food during preparation and may result to food poisoning. *Pseudomonas aeruginosa* and *Klebsiella oxytoca* had the highest frequency of bacterial isolates by specimen in this investigation. These results are like those of the study by Mi et al.[4], which identified *Pseudomonas aeruginosa* as one of the most significant pathogens in the nosocomial setting. This pathogen not only has a high level of natural resistance to many antimicrobial agents, but it also has the unique ability to develop resistance to almost all commercially available antibiotics and disinfectants via a variety of mechanisms. In this study, the age group > 60 (female) had the greatest bacterial isolates, which is in accordance with the study by Linlinet al.[5], in which the highest bacterial isolates were found in the > 60age group. Most of the women in this group work as food vendors (commonly called `mama put`), restaurant owners, and other people who are involved in the kitchen, which may be why there is a high incidence of bacterial isolates in this population. Other possible causes include a lack of clean water for cooking, the location (a dirty environment), the temperature (eating contaminated cold food or improperly cooked food), and several other factors, including personal hygiene.

For the treatment of invasive bacterial infections in humans, the prevalence of bacteria like *Pseudomonas aeruginosa, Klebseillaoxytoca,* and *Escherichia coli* that are resistant to antibiotics has emerged as a new concern to public health. The preferred medications include third generation cephalosporins, fluroquinolones, and aminoglycosides. Most of the bacterial isolates in this study were resistant to the effects of antibiotics, and overall, the incidence of antimicrobial susceptibility of the bacterial isolates was low. It is understood that *Pseudomonas aeruginosa* and Klebsiella species are multidrug resistant organisms because the susceptibility pattern revealed that (38%) were resistant to Gentamicin, Augmentin (49%), Ciprofloxacin (36%), Ofloxacin (35%), Cefixime (46%), Nitrofurotion (47%), Ceftazidine (45%), Cefuroxime (49%), Lyncomycin (45%), Meropenem (33%), Peflox (45%). This is consistent with Azimi et al.[6], who found high levels of antibiotic resistance among Klebsiella and Pseudomonas species. Consumption of foodstuffs or products containing antibiotic residues, improper use of antibiotics, frequent use of over-the-counter medications without adequate hospital drug supervision, the presence of efflux pumps and other drug-resistance genes, and specific mutations in chromosomally located genes that can result in cross resistance are all factors that contribute to the high rate of resistance. Our investigation also showed that third generation cephalosporins such CAZ, CXM, CRX, as well as others like NA, STX, and PN, have substantial levels of resistance. The fact that these medications are less expensive and mostly prescribed for the treatment of bacterial infection may be the cause of the development of high resistance to them. Despite the high levels of resistance to IMP, some isolates were found to be sensitive to it. Since carbatenem, including IMP, are the main class of antibiotic for treating infections caused by multidrug-resistant Gram-negative bacteria, it is the treatment of choice when other isolates turn out to be resistant [7].

4. CONCLUSION

The most prevalent organism isolated from clinical isolates was *Pseudomonas aeruginosa, f*ollowed by *Klebsiella oxytoca.* There was a high multi-drug-resistant Gram-negative bacteria, therefore justifying the extent of resistance observed.

CONSENT

All authors declare that 'written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the

written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

Ethical clearance was duly obtained from hospital ethical clearance committees of the Niger Delta University Teaching Hospital, Okolobiri, Bayelsa State, Nigeria.

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

REFERENCES

1. World Health Organisation. (2021). Antimicrobial resistance: global report on surveillance 2021. *World Health Organization.*RetrievedSeptember, 2021.https://www.who.int/publications-detail-redirect/ 9789240027336.
2. World Health Organisation. (2020). Antimicrobial resistance: global report on surveillance 2020. World Health Organization.Retrieved September, 2021.https://www.who.int/publications-detail-redirect/ 9789240027336.
3. Seyed, M., Niloofar, S., Azad, K., Seyed, F. and Davoud, E. Evaluate the relationship between class1 integrons and drug resistance genes in clinical isolates of *Psuedomonas aeruginosa*. *The Open Microbiology Journal,* 2016;10: 188-96.
4. Mi, L., Jie, M., Wei, J. and Wan, X. Antimicrobial Resistance and Molecular characterization of gene cassettes from class 1 integrons in *Pseudomonas aeruginosa* strains. *Microbial drug Resistance,* 2020; 6:670–6.
5. Linlin, X., Xiaotong, W., Nana, K., Mei, C., Long, Z., Quhao, W. and Weiwei, L. Polymorphisms of gene caseettes promoters of the class 1 integrons in clinica*Proteus* isolates. *Frontiers in Microbiology,* 2019; 10:790-9.

6. Azimi, L., Reza, A., Mahla, A., Faranak, A. and Abdolaziz, R. Multidrug resistance *Pseudomonas aeruginosa* and *Klebsiella pneumonia* circulation in a burn hospital Tehran, Iran. *German Medical Science Hygiene and infection control,* 2019;14: 1-9.
7. Chau-chyun, S., Ya-Ting, C., Shang-Yi, L., Yen-Hsu, C. and Po-Ren, H. Infections caused by carbapenem-resistant Enterobacteriaceae; An update on therapeutic options. *Frontiers in Microbiology,* 2019; 10:80-9.
8. Clinical and Laboratory Standard Institute, Methods for Antibacterial Diffusion and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. 2014.
9. Antimicrobial Resistance Collaborators. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The Lancet; 399(10325): P629-65. DOI: https://doi.org/10.1016/S0140-6736(21)02724-0, 2022.
10. World Health Organization. Key Facts: Antimicrobial resistance, 2023.