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Original article

Beta-lactamase production among multidrug-resistant wound isolates from patients attending University College Hospital, Ibadan, Oyo State, Nigeria

***Busayo Mutiat Olowe* [*1], *Grace Iyabo Soyinka* [2]**

1- *Department of Biological Sciences, Microbiology Unit, Bamidele Olumilua University of Education, Science and Technology, P. M. B. 250, Ikere Ekiti, Ekiti State, Nigeria.*

2- *Department of Biological Sciences, Microbiology Unit, Faculty of Basic Medicals and Applied Sciences, Lead City university, Ibadan, Oyo State, Nigeria.*

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ABSTRACT

Background: Wound infections have been a problem in the field of medicine for a long time due to the emergence and re-emergence of drug resistance. The assessment of Beta-lactamase production, plasmid profiling and corresponding resistance pattern in the local terrain become important for a proper understanding of wound infection burden and its epidemiology. The study aimed to assess beta-lactamase production among multidrug-resistant wound isolates from patients attending University College Hospital (UCH), Ibadan. **Methods**: Sample size of 370 was determined using the Cochran formula. Exudates from wounds were collected with sterile swab sticks, isolation and identification were performed using standard procedures. Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disc diffusion method, Beta Lactamase Production was carried out using the starch iodide acidimetric method. Double disc synergy test and combination disk were used for extended–spectrum beta-lactamases (ESBL) and metallo-beta-lactamase (MBL) detection respectively. Plasmid curing of two beta-lactamase procedures was carried out using sodium dodecyl sulphate (SDS). After which, post-curing antibiotics susceptibility testing was performed. **Results**: Result shows 339 isolates of which 56.9% were multidrug-resistant. Beta-lactamase producers were 60.7% from which 5% and 14% were positive for ESBL and MBL test. Same resistance pattern to the antibiotics was observed in plasmid-cured isolates, meaning that their resistance was not plasmid-mediated. **Conclusion**: The establishment of beta-lactamase producing capacity in the wound isolates in this study will help clinicians in the appropriate treatment of wound infections.

Introduction

The emergence of multidrug-resistant bacteria has become a major problem. The World Health Organization has categorized antibiotic resistance as one of the three most significant severe public health problems of the 21st century [1].

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* *Corresponding author:* Busayo Mutiat Olowe

E-mail address: olowe.busayo@bouesti.edu.ng

Wound infection is a breach in the skin, and exposure of the subcutaneous tissues makes an available good and suitable environment for microbial colonization and proliferation [2]. Wound infections can be caused by different bacteria groups, which include Gram-positive and Gram-negative. The Gram-positive bacteria include: *Staphylococcus aureus*, Coagulase-negative *Staphylococcus aureus*, *Enterococci* and Gram-negative bacteria are *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter, Proteus mirabilis, Proteus vulgaris, Acinetobacter* species, *Candida* and other *Streptococci* [3].

Most of the wound samples brought to the medical laboratory for analyses of possible aetiologies come down with alarming resistance patterns to antibiotics during their antimicrobial susceptibility testing [4]. When analysed in clinical microbiological laboratories, wound samples often produce results indicative of antibiotic resistance to most antibiotics used during the susceptibility testing. This has become a great concern to laboratorians and clinicians.

The indiscriminate use of antibiotics has also led to the increase in multi-drug resistant organisms (MDRO) [5]. Other factors reported as fuelling the spread of ESBLs in developing economies include extensive self-medication, self-prescribing and non-prescription use of antimicrobials, poor hygienic conditions even in the hospital environment and very low infection control practice [6].

Among several resistance mechanisms adopted by some wound isolates is the production of hydrolytic β-lactamase enzyme, which is the most prevalent resistance mechanism towards β-lactam antibiotics [7]. Extended-spectrum beta-lactamases (ESBLs) constitute a growing class of plasmid-mediated ß-lactamases which confer resistance to broad-spectrum beta-lactam antibiotics. The infections caused by extended-spectrum beta-lactamase (ESBL) producing bacteria, constitute severe problems [8]. In addition, metallo-β-lactamases constitute a troublesome group of enzymes, since they present a broad-spectrum profile, they hydrolyse penicillin, cephalosporin and carbapenems [9].

Carbapenems are a broad-spectrum beta-lactam (β - lactam) antibiotic, developed as one of the last resort antibiotics for the treatment of serious and life-threatening infections caused by a multitude multidrug-resistant (MDR) Gram-negative bacterial infections, including extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria [10]. However, since the introduction of these antibiotics in medicine, bacteria have developed mechanisms that have resulted in the emergence of the multi-resistance.

Knowledge of local antimicrobial resistance trends among wound isolates is important not only in guiding clinicians to prescribe appropriate antibiotics but also for evidence-based recommendations in empirical antibiotic treatment of wounds and other infections [11]. The lack of comprehensive surveillance and good antibiotic stewardship programs in Nigeria has largely contributed to increasing in the disease burden of ESBL and MBL infections. Moreover, most of the routine diagnostic laboratories in Nigeria seldom test for beta-lactamase production. Hence, the knowledge and local awareness of the burden of ESBL production with its attendant antimicrobial-resistant pattern is low. It, therefore, goes unnoticed and continues to spread unabated with the associated high expenses and mortalities. This study, therefore, investigates the production beta-lactamase and resistance pattern of wound isolates in patients assessing healthcare in UCH, Ibadan, Oyo state, to contribute to the information on local antimicrobial resistance trends among wound isolates in Nigeria.

Materials and Methods

Study area

The study was carried out in the department of Medical Microbiology and Parasitology of the University College Hospital (UCH), Ibadan. This is a tertiary teaching hospital located in the South-west region of Nigeria. University College Hospital, Ibadan is a federal teaching hospital in Ibadan, Nigeria, attached to the University of Ibadan.

Sample size

This was obtained using the **Cochran** [12] formula which reads thus:

$N_{\circ} = Z^2PQ \div e^2$, where

N = required sample size

P = expected prevalence rate with reference from similar previous study.

For this purpose, the work done by **Adhikari et al.** [13] was used with a prevalence of 56.95%.≈ 0.6.

e = margin of sample error at 5% (standard value of 0.05)

Z = confidence level =1.96

Q = the desired level of precision

$N_{\circ} = 1.96^2 \times 0.6 \times 0.4 \div (0.05^2)$

$= 1.96 \times 1.96 \times 0.6 \times 0.4 \div 00025$

$= 368.7$

≈ 370 wound samples.

Samples collection

Exudates from the wound were collected with a sterile swab stick from the in-patients and out-patients units within the hospital.

Isolation and identification of isolates using biochemical characterization

Isolation was carried out following the procedure employed by **Adhikari et al.** [13] Identification was done by Gram staining techniques and biochemical tests which included: the oxidase test, indole test and coagulase test.

Antibiotics susceptibility testing to beta-lactam drugs

Antimicrobial susceptibility testing was carried out using the Kirby-Bauer disc diffusion method on Mueller Hinton agar and the resulting zone of inhibition was expressed as susceptible, intermediate or resistant according to clinical and laboratory standards institute guideline [14]. The organisms were tested against different antibiotics which were: Piperacillin/tazobactam (TZP) (100/10 µg) amikacin (AK) (30 µg), augmentin (AUG) (20µg), ceftazidime (CAZ) (30 µg), ceftriaxone (CRO) (30 µg), ciprofloxacin (CIP)(5 µg), colistin (CT) (10 µg), gentamicin (GEN)(10 µg), meropenem (MEM) (10 µg), erythromycin (ERY) (15 µg), vancomycin (VA) (30 µg), clindamycin (DA) (2 µg), cefuroxime (CXM) (30 µg), cefoxitin (FOX) (10 µg) and tigecycline (TGC) (15µg) (Oxoid, England)

Determination of beta-lactamase production

This was done using the starch iodide acidimetric method of beta-lactamase detection [15]. A solution of 0.2% soluble starch and 1% potassium penicillin G for injection was made by adding 0.1 gram of soluble starch to 45ml of sterile deionized water which was heated to properly dissolve and then allowed to cool. Five (5) millilitres of potassium penicillin G (10U/ml) were then added to attain a final concentration of 10 U/ml.

Filter paper (Whatman No 3) was cut into a strip and immersed in the starch–penicillin solution; this was drained and allowed to air dry for about 2 hours at room temperature. The strip was saturated with Gram's iodine, pouring off the excess iodine and placing it on a dry absorbent paper towel. The strip was smeared with heavy inoculums rubbing on the surface of the paper in a circular manner to cover about 5mm in diameter. A positive and negative control were done alongside using a positive strain of *staphylococcus aureus* ATCC 12981 and a negative-lactamase strain of *Escherichia coli* (*E. coli*) ATCC 12241 obtained from the department of medical microbiology and parasitology, University College Hospital Ibadan.

A positive beta-lactamase test is indicated by the decolourisation of the purple colour of the starch–iodide paper to white. A negative result shows no decolourisation.

Extended –spectrum beta- lactamases (ESBL) detection

Screening for potential ESBL-producing isolate

The microorganism that has a zone of inhibition (ZOI) of < 22mm for ceftazidime are considered potential ESBL producers and shall be considered for confirmatory tests.

Confirmation of ESBLs producing isolate

This was done using the double disc synergy test (DDST) [16]. A 20 ml of Mueller Hinton agar was prepared and dispensed aseptically into a Petri dish and was allowed to solidify. The plates were seeded with test organisms pre-adjusted to 0.5 McFarland turbidity standards. A disc of (amoxicillin 20 µg and clavulanic acid 10 µg) was placed at the centre of the Petri dish in between two cephalosporin antibiotics (ceftazidime 30 µg and ceftriaxone 30 µg) placed 30 mm apart. It was incubated at 37°C for 18-24 hrs after which the various inhibition zone diameters (IZDs) were measured. An enhanced zone of inhibition between any of the beta-lactam discs and the centre disc was recorded. In this study, an enhanced zone of inhibition between any of the third-generation cephalosporin antibiotic discs and an amoxicillin-clavulanic disc was confirmation of ESBL production.

Metallo-beta-lactamase (MBL) detection

This is to test for MBL Production. The isolates were subjected to MBL detection when it is resistant to carbapenems.

MBL confirmation by combination disk (CD) method

The isolates showing resistance to carbapenems were subjected to confirmation for MBL production by combined disc assay using carbapenems and carbapenems/ethylene diamine tetraacetate discs.

Two ertapenem (ETP) (Bioanalyse, Turkey) disks (10 µg) were used. In one of them, 10 µL of 0.1 mol/L (292 µg) anhydrous ethylenediaminetetraacetic acid (EDTA) was added. Then the two disks were placed 25 mm apart (centre to centre). An increase in zone diameter of > 4 mm around the ETP-EDTA disk compared to that of the ETP disk alone was considered positive for an MBL production [17].

Detection of plasmids in resistant isolates

Plasmids were isolated using the QIAGEN plasmid purification mini kit. The manufacturer's instructions were followed during the isolation process. The isolated plasmid DNA was electrophoresed on 0.8% agarose solution in a solution of ethidium bromide for 30 mins.

The integrity of the extracted plasmid was checked on a 1% agarose gel run to confirm amplification. The gel was electrophoresed at 120V for 45 minutes, visualized by ultraviolet trans-illumination and photographed. The sizes of the PCR products were estimated by comparison with the mobility of the molecular weight ladder that was run alongside experimental samples in the gel.

Plasmid curing

Plasmid curing experiment was carried out using sodium dodecyl sulphate (SDS) as the curing agent [18]. The isolates were incubated in nutrient broth at 37˚C for 24 hrs. This overnight culture was inoculated into 4.5 ml sterile nutrient broth; 0.5 ml of SDS was added. This was incubated for 48 hrs at 37˚C. Thereafter, 0.5 ml of the broth was added to 4.5 ml of sterile nutrient broth. Incubation followed for another 24 hrs at 37˚C.

Post-curing antibiotics susceptibility testing

An antimicrobial susceptibility test was again carried out on the isolates to which plasmid curing has been done. Results of antimicrobial resistance were interpreted as either plasmid or chromosomal-mediated, depending on if resistance was lost or not.

Quality control

All prepared media were checked for sterility for 24 hrs. *E. coli* ATCC 12241 was used as a quality control strain for antibacterial susceptibility testing. The *E. coli* ATCC 12241 strain was also used as a negative control in the screening and phenotypic confirmatory tests of ESBL-producing Gram-negative rods.

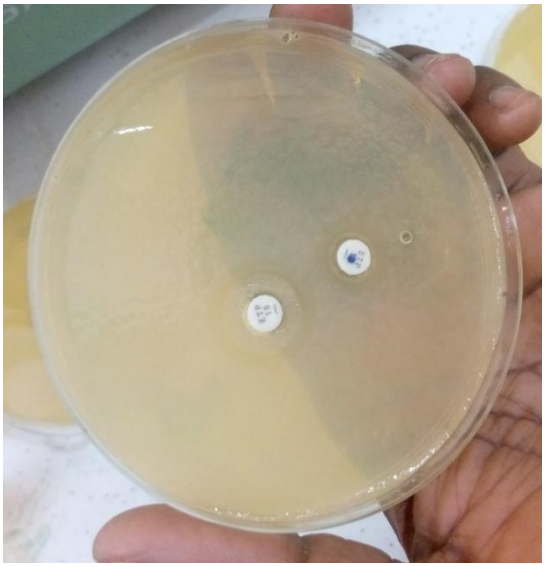
Statistical analysis

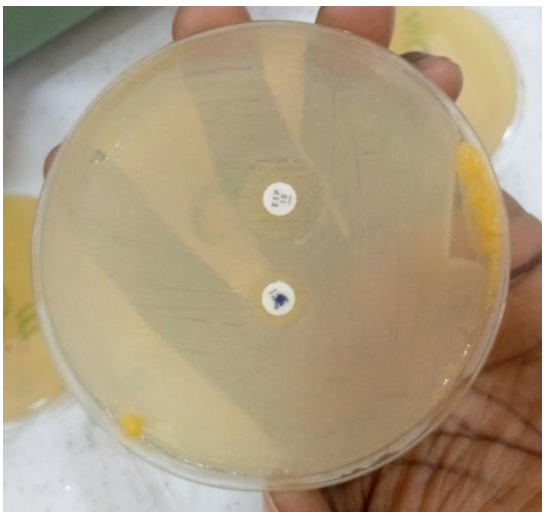
Data were presented as frequencies and/or percentages. Statistical analysis was performed using SPSS software (version 20). Proportions and the actual number of ESBL and MBL-producing isolates were used to describe frequency outputs for categorical variables. The data were presented in tables and graphs.

Ethical approval

This was sought from the Ethics and Research Committee of the University of Ibadan /University College Hospital, UCH, Ibadan, Oyo state, Nigeria, for this study with full approval granted with number UI/EC/21/0135.

Diagrams of MBL confirmation by combination disk (CD) method





Results

From among all the wards, the highest influx of wound samples (24.86%) was gotten from the surgical outpatient (SOP) ward of the hospital complex followed by general outpatient (GOP) (5.41%) while the least (0.27%) came from the ear, nose and throat (ENT), Southwest 4 (SW4) and East 2 (E2). The ENT is an outpatient ward, while the SW4 and E2 are inpatient wards in the hospital **(Table 1).**

Results showed that out of the 370 wound samples, 253 showed positive growth (68.4%) while 117 yielded no growth (31.6%) **(Table 2).** The positive growth varied in the number of identified isolates totalling 339 isolates. The following were the organism identified: *Pseudomonas aeruginosa, Klebsiella pneumoniae, Klebsiella oxytoca, Enterobacter cloacae, Staphylococcus aureus, Coagulase Negative staphylococcus, Methicillin Resistant Staphylococcus aureus, Candida species, Stenotophomonas maltophilia, Escherichia coli, Providentia stuarti, proteus mirabilis, proteus vulgaris, Enterobacter cloacae, Acinetobacter baumanii, and Klebsiella aerugenes* **(Table 3).** A total of 114 of the isolated organisms were Gram-positive, coagulase-positive and confirmed *Staphylococcus aureus (S. aureus*). *Staphylococcus aureus* had the highest score of the entire organism (33.63%) followed by a Gram-negative and oxidase-positive organism confirming it as *Pseudomonas aeruginosa (*17.4%) and next in magnitude was also a Gram-negative organism, indole-negative *Klebsiella pneumoniae (*16.81%). Of the total isolates, 61.4 % were Gram-negative bacteria and 37.2 % were Gram-positive bacteria. *Staphylococcus aureus* had 90.5 % of the entire Gram-positive with coagulase-negative *Staphylococcus aureus* (CONS) taking 7.9% and methicillin-resistant *Staphylococcus aureus* (MRSA) had the least 1.6%.

The antimicrobial susceptibility testing results are reported as being susceptible (S) or resistant (R) according to the zone of inhibition (ZOI) observed on the Mueller Hinton agar. This result showed a varied degree of resistance and susceptibility as shown in **figure (1).** The organisms demonstrated varied resistance patterns to the different antibiotics used. The most effective first-line antibiotics for *S. aureus* were augmentin and cefoxitin followed by clindamycin and gentamicin but the *S. aureus* showed the highest resistance to ciprofloxacin followed by augmentin. Amongst the Gram-negative organism, *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *E. coli* had a high susceptibility rate to meropenem which is a second-line antibiotic while the resistance pattern varied, *Pseudomonas aeruginosa* had the highest resistance to ceftazidime, *Klebsiella pneumoniae* to ciprofloxacin and *E. coli* had high resistance to both ciprofloxacin and augmentin.

Among all the isolates, only 56.9% demonstrated multiple antibiotic resistance **(Table 4)**. The organisms which included *E. coli, Klebsiella oxytoca, Klebsiella pneumoniae, Pseudomonas mirabilis, Pseudomonas aeruginosa* and *S. aureus* demonstrated high multiple antibiotic resistance (MAR) **(Table 4).** Beta-lactamase testing was carried out on all MAR isolates (Gram-positive and Gram-negative) using the starch iodide paper test. All Gram-negative isolates were positive except five of the isolates which showed negative results, so also all the Gram-positive MAR isolates were negative except three of the isolates which showed positivity. ESBL testing results revealed that among all the isolates with resistance to ceftazidime, only 5% produced ESBL while others (95%) were negative for ESBL production **(Figure 2).** The MBL testing showed that all the isolates (86%) with resistance to carbapenems were negative except a few (14%) ones which were positive for MBL testing **(Figure 3).**

Two identified MAR organisms with beta-lactamase production, *Klebsiella oxytoca* (ESBL positive) and *Enterobacter cloacae* (MBL positive), were selected for this study and analysed for the detection of a plasmid. Their plasmid analysis showed that they both possessed high molecular weight plasmids 1200bp and 1886bp respectively **(Figure 4).** Thereafter, plasmid-curing was carried out and **figure 5** revealed the absence of plasmids in these organisms after plasmid curing. These plasmid-cured organisms when subjected to antimicrobial susceptibility testing following standard procedures as outlined by CLSI [14] were found to still maintain the same resistance pattern to the antibiotics as when they were not cured. Meaning that their resistances were not plasmid-mediated.

Table 1. Number of wound samples obtained from different wards in UCH complex, Ibadan.

Name of wards	Number of wound samples obtained	Percentage (%)
General outpatient (GOP)	20	5.41
Southwest 4 (SW4)	1	0.27
EAST2 (E2)	1	0.27
ROC	6	1.62
Intensive care unit (ICU)	10	2.7
West 3	1	0.27
North west1 (NW)1	13	3.51
Accident and Emergency (A&E)	9	2.43
Surgical outpatient (SOP)	92	24.86
*EAST1 E1	5	1.35
Chief Tony Anenih Geriatric center (CTAGC)	8	2.16
SDM	2	0.54
South East3	4	1.08
West west 3 WWE3	3	0.81
Gynae	13	3.51
Southwest 1 SW1	10	2.7
North west2 Nw2	11	2.97
Haem Day care unit HDCU	4	1.08
West 4	3	0.81
East3	2	0.54
ENT	1	0.27
Gynae emerg	4	1.08
Private suites	6	1.62
ROW	6	1.62
SW3	7	1.89
NW3	5	1.35
Staff clinic	10	2.7
W3	6	1.62
SE1	12	3.24
W1	5	1.35
Total	370	100

Table 2. Growth culture of wound samples collected from UCH complex, Ibadan.

Wound sample culture	Number of samples	Percentage
Growth	253	68.4%
No growth	117	31.6%
Total samples	370	100%

Table 3. Identified organisms isolated from wound samples collected from, UCH Complex, Ibadan.

Organism name	Number isolated	Percentage (%)
Stenotophomonas maltophilia	1	0.29
Staphylococcus aureus	114	33.63
Pseudomonas aeruginosa	59	17.4
Providential stuarti	1	0.29
Proteus vulgaris	3	0.88
Proteus mirabilis	27	7.96
Morganella morgani	1	0.29
Methicillin-resistant staph aureus	2	0.59
Klebsiella pneumoniae	57	16.81
Klebsiella oxytoca	19	5.6
Klebsiella aerogenes	1	0.29
Eschericia coli	34	10.03
Enterobacter cloacae	3	0.88
Coagulase-negative staph	10	2.95
Candida spp	5	1.47
Acinetobabcter haemolyticus	1	0.29
Acinetobacter baumanii	1	0.29
Total	339	100

Table 4. Percentage distribution of multiple drug-resistant (MDR) organisms.

Organisms	MDR (%)	Non-MDR (%)	Total
Acinetobacter baumanii	1 (100)	0 (0)	1 (100)
Acinetobacter haemolyticus	0 (0)	1 (100)	1 (100)
Candida spp	0 (0)	5(100)	5(100)
Coagulase Negative Staphylococcus	5 (50)	5 (50)	10(100)
Enterobacter cloacae	2 (66.7)	1 (33.3)	3(100)
Escherichia coli	24 (70.6)	10 (29.4)	34(100)
Klebsiella aerogenes	1 (100)	0 (0)	1(100)
Klebsiella oxytoca	14 (73.7)	5 (26.3)	19(100)
Klebsiella pneumoniae	42 (73.7)	15 (26.3)	57(100)
Methicillin Resistant Staphylococcus aureus	2 (100)	0 (0)	2(100)
Morganella morganii	1 (100)	0 (0)	1(100)
Proteus mirabilis	14 (51.9)	13 (48.1)	27(100)
Proteus vulgaris	1 (33.3)	2 (66.7)	3(100)
Providencia stuartii	0 (0)	1 (100)	1(100)
Pseudomonas aeruginosa	27 (45.8)	32 (54.2)	59(100)
Staphylococcus aureus	58 (50.9)	56 (49.1)	114(100)
Stenotophomonas maltophilia	1 (100)	0 (0)	1(100)
Total	193 (56.9%)	146 (43.1%)	339 (100)

Resistance to more than 2 classes of antibiotics was taken to be multiple antibiotics resistance.

Table 5. Post plasmid-curing sensitivity result.

	Test organisms	Antibiotics							
		CAZ	CRO	TZP	AUG	FOX	GEN	CIP	MEM
ESBL	*Klebsiella oxytoca*	R	R	R	R	R	S	S	S
MBL	*Enterobacter cloacae*	R	R	R	R	R	S	S	R

Key: CAZ- ceftazidime, CRO- ceftriaxone, TZP- Piperacillin/tazobactam, AUG- Augmentin, FOX-cefoxitin, GEN- gentamicin, CIP- ciprofloxacin, MEM- meropenem

Figure 1. Percentage resistance pattern of the isolates.

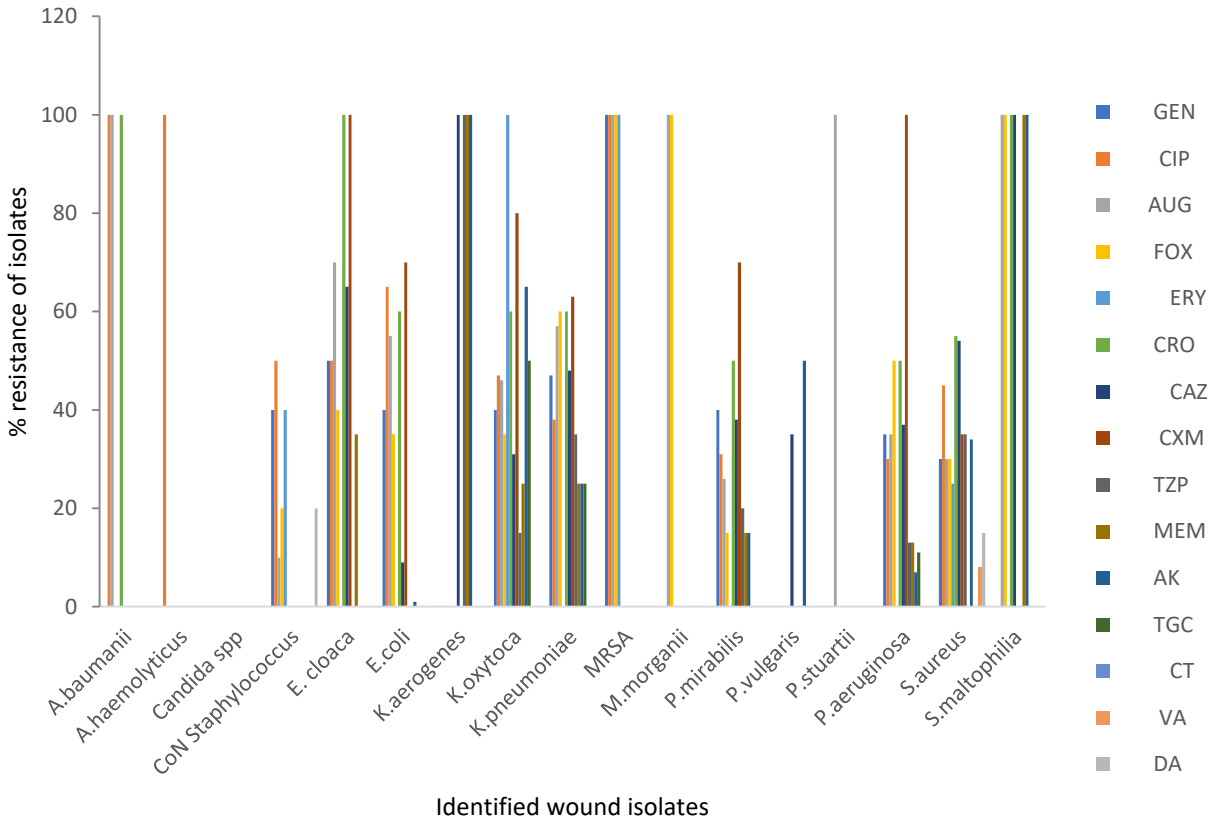


Figure 2. The percentage of ESBL producing organisms.

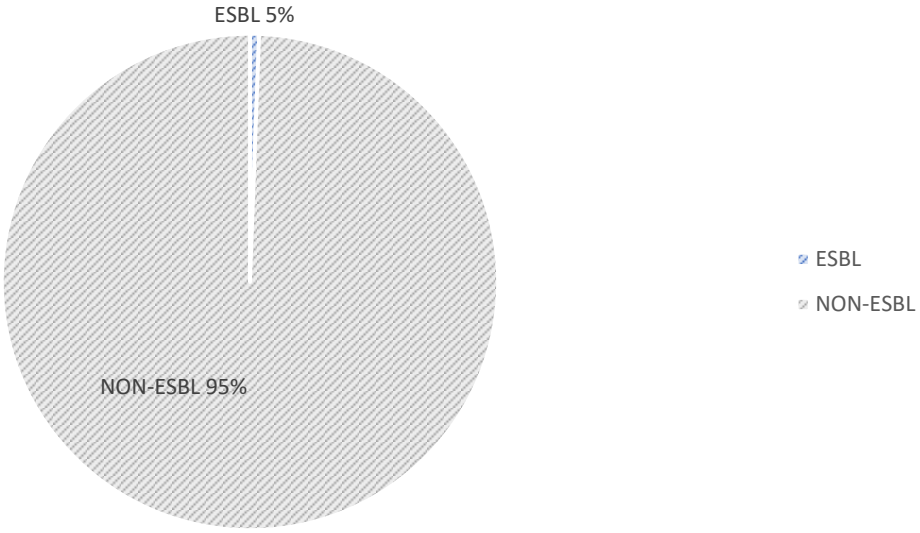


Figure 3. The percentage of MBL producing organisms.

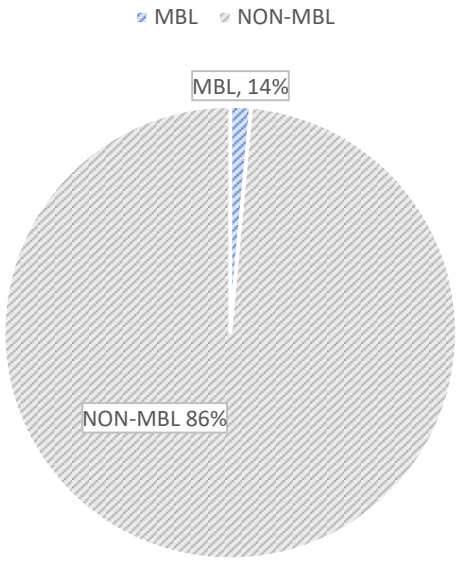
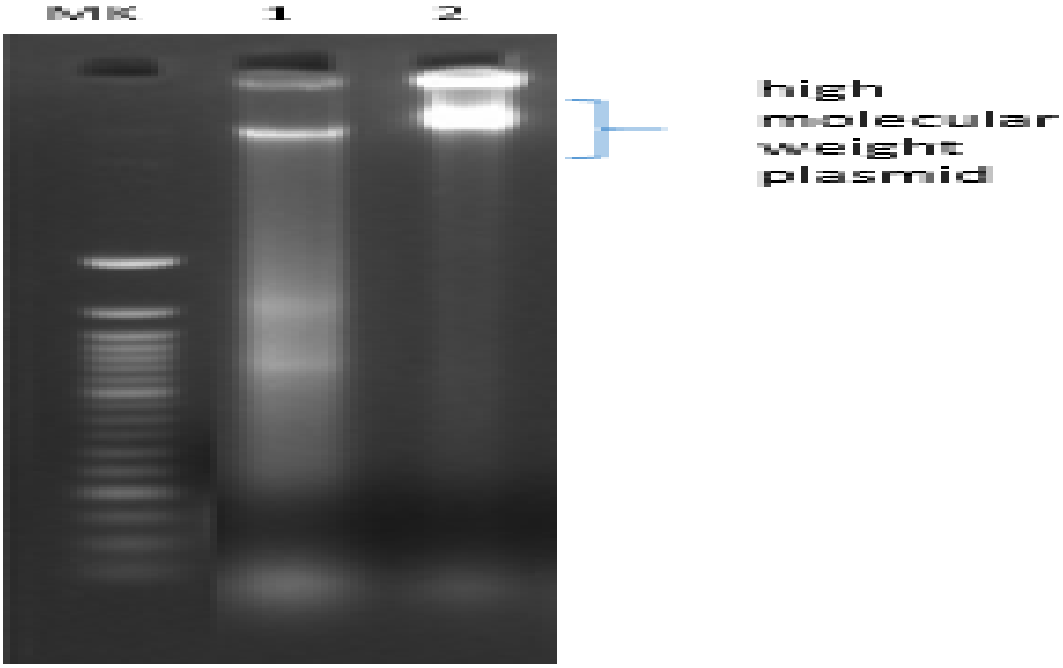
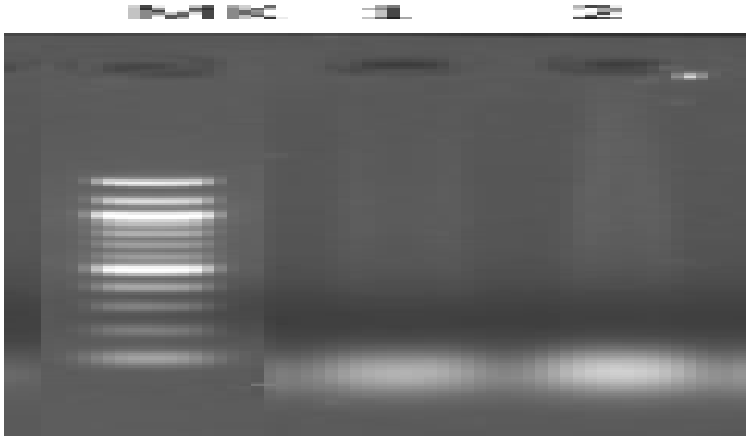


Figure 4. Agarose gel shows the presence of high molecular weight plasmid.



Lane 1*: Klebsiella oxytoca* (ESBL positive) (1886 bp) and Lane 2: *Enterobacter cloacae* (MBL positive)(1200bp), Mk- Molecular weight

Figure 5. Agarose gel shows the absence of plasmid after curing.



Discussion

Out of 370 samples in this study, 256 (68.5%) showed culture positivity which is in accordance with the study done by **Jain et al.** [19] which showed a culture positivity of (62%). Another study by **Chaudhary et al.** [20] on wound samples revealed a culture positivity of (77.6%) which was quite higher than the one obtained in this study. Also, **Bastola et al.** [21] showed (48.6%) culture positivity which was lower than that obtained in this study

The predominance of *S. aureus* followed by *Pseudomonas* is supported by the study of **Chaudhary et al.** [20]. **Adhikari et al.** [13] in their work on infected wounds in Nepal established that the most common bacterial isolates were *E. coli* followed by *S. aureus* which is not the same as obtained in this work. *Pseudomonas aeruginosa* was the highest Gram-negative bacteria isolated in this study followed by *Klebsiella pneumoniae* and then *E. coli* and it is similar to the work done by **Azene et al.** [22]. This study showed that the most susceptible first-line antibiotic for *S. aureus* was cefoxitin followed by augmentin and clindamycin this was in contrast to the findings of **Mabrouk et al.** [23] who obtained a 100% resistant rate to cefoxitin against *S. aureus.* Resistance to the selected antimicrobials was very high. The overall multiple drug resistance of the isolates in this study was (58%), which was a little bit lower compared to 66.7% and 68.3% obtained from the study carried out by **Adhikari et al.** [13] and **Raza et al.** [24] respectively. But the study by **Pirvanescu et al.** [25] obtained only (27.6%) MDR. High resistance of the isolates to antibiotics may be due to practising self-medication or unavailability of guidelines regarding the selection of drugs thereby which lead to inappropriate use of antibiotic [26].

In a study from Uganda, the ESBL-producing Gram-negative bacteria in wound swabs was 100% [13], this value is quite higher than the 5% obtained in this study. Varying prevalence figures of MBLs have been reported in Nigeria, **Yusuf et al.** [27] while working on some clinical isolates reported 16.7% in *Klebsiella. pneumoniae*, 16% in *Proteus* and 13.5% in *E. coli*. They also observed MBL producers in both hospital and community isolates. Many metallo-beta-lactamase genes constitute reservoirs in the environment. During the course of evolution, some environmental bacteria such as *Bacillus cereus*, and *Bacilus anthracis* produced metalloenzymes to protect themselves against beta-lactams produced naturally by some soil-dwelling bacteria (*Streptomyces* sp.) or fungi. The dissemination of carbapenemase genes, therefore, proceeds in two directions: environmental sources may provide the genetic source of the enzyme, and clinical strains may spread these enzymes both within the hospital and into the environment.

In the past, all ESBLs were sensitive to carbapenems such as imipenem or meropenem. It was actually used to define ESBLs. Carbapenems were the last resort for infections caused by ESBLs. This is changing as revealed in this study. Though a

very low prevalence figure of ESBL resistant to the carbapenem, imipenem was observed, it is emerging. The control of spread is now or never because cumulative experience with ESBL supports the idea that once the prevalence goes beyond a critical level, their eradication from bacterial communities is nearly impossible. Bearing the frightening properties of metalloenzymes in mind, it is important that a great effort should be made in the pursuit of preventive medicine.

This study further reveals that the isolates were still maintaining the resistance pattern as when not cured which is in agreement with the work by **Costa et al.** [28] where the isolate resistance is said to be chromosomally mediated after plasmid-curing. Study by **Reboucas et al.** [29] reported a rather different dimension were two of the five isolates become totally susceptible to all the antibiotics they were exposed to indicating, the resistance was plasmid-mediated.

Conclusions

This study had showcased the various organisms causing wound infections in the University College Hospital as a tertiary hospital, out of these organism some strains are somehow rare and they are not in the majority. Example of such includes *Stenotophomonas maltophilia, Klebsiella aerogenes, Acinetobacter baumannii*. The most predominant organism however was found to be Gram-positive followed by the Gram-negatives. This study had established the existence of beta lactamase producing capacity in the isolates obtained from wound infections in the University College Hospital Ibadan (UCH). It is therefore, necessary for every medical practitioner to have better knowledge of causative agent of wound infections and their sensitivity pattern. This is to control wound infections and to minimize the rate of mortality, morbidity and prolonged hospital stay due to wound infections. A proper control of antibiotic usage will prevent the emergence of resistant strains of bacteria.

It is therefore necessary that individuals and stake holders in implementation of health practices should be made aware of the menance of non-prescription, misuse of antibiotics or self-prescription of these drugs from man to man and as a result a proper advocacy on right and proper administration of drugs in all dosage forms should begin on a high pedestal.

Conflicts of interest

The authors declare no conflict of interest.

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