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Antibiogram and Plasmid Profile of Bacterial Isolates from Intensive Care Units in a Tertiary Healthcare Facility in Umuahia

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ABSTRACT

Background: This study evaluated the bacterial contamination and the antibiogram of bacterial isolates in Intensive Care Units in Federal Medical Centre, Umuahia.
Methods: Sterile swab sticks moistened with sterile water were used to swab the surfaces of the fomites, hands and anterior nares of healthcare workers and the samples were sent to the laboratory for analysis. Plasmid preparations was done with a QIAprep Spin Plasmid Kit. Antibiotic discs of prior resistance were aseptically introduced into the Muller Hinton agar plates, ensuring that the discs made appropriate contact with the surface of the agar. These were incubated for 24 hrs at 37ºC after which plates were examined for cured colonies.
Results: The common bacterial isolates were *Staphylococcus aureus*, 39(43.2%), *Escherichia coli*, 16(17.9%), *Pseudomonas* spp, 10(11.2%), Coagulase-negative Staphylococci, 7(7.8%). Antibiotic sensitivity of the bacterial isolates was carried out using the disc diffusion method. Gram-negative bacterial isolates were more sensitive to Ofloxacin, Peflacine, Ciprofloxacin, and Streptomycin. Gram-positive bacterial isolates were more sensitive to Ciprofloxacin, Gentamicin, Rifampicin, Erythromycin, and Levofloxacin. However, *Enterobacter* spp. and Coagulase-negative Staphylococci were resistant to the drugs. The biofilm formation potential was observed in 41(46.0%) bacterial isolates. Extended Spectrum Lactamase (ESBL) producers among *E. coli* isolates was 56.2%, while *Klebsiella pneumoniae* and *Pseudomonas* spp. isolates were 33.3% and 40%, respectively, Plasmid profile was carried out on some of the bacterial isolates. Six *E. coli* isolates had plasmid size between 50-400 base pair; five *S. aureus* isolates had plasmid size between 35-150 base pair, two *Proteus* spp. and two *Pseudomonas* spp. isolates had no plasmid.
Conclusion: This study revealed the resistance of bacterial isolates after currying.

1. Introduction

Patients in Intensive Care Units (ICUs) are an important subgroup of all hospitalized patients, representing around 25% of all hospital infections [1]. The pervasiveness of ICU-obtained contaminations is essentially higher in developing countries than in industrialized nations, shifting somewhere in the range of 4.4% and 88.9% [2].

There has been an ongoing enthusiasm for utilizing synthetic fumigation in healthcare facilities because of concerns about the role of the environment as a cause of Hospital-acquired Infections (HAIs) and an observation that

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surface cleaning and purification strategies are ineffective [3].

Methicillin-resistant *Staphylococcus aureus* (MRSA) and other Gram-positive microscopic organisms have turned into an inexorably basic issue in healthcare environments [4].

Colonized microorganisms on the gadgets of HCWs might be transmitted to patients. These organisms, if pathogenic can be detrimental to the health of the patients, especially those in critical care units, and if the organisms transferred happen to be drug-resistant, the circumstance turns out to be significantly more severe as it ends up hard to treat on account of limited drug options available [5].

The improvement of nosocomial disease is a huge issue in every clinic. Such diseases can result because of different causes like advancement and determination of multidrug-resistant (MDR) microbes, immune-compromised conditions of patients, and mechanical transmission of microorganisms [6]. Some non-basic therapeutic gadgets routinely utilized by healthcare workers (HCWs, for example, stethoscopes, pulse sleeves, electronic thermometers, latex gloves, covers, pens, and white coats assume a critical role in the transmission of health care-associated infections (HCAIs) [6]. Among these gadgets, stethoscopes routinely utilized by HCWs represent a potential risk for HCAIs transmission in the hospital settings [6].

Bacterial contamination of hospital equipment is one of the most probable causes of nosocomial infections. These contaminations are developed inside a hospital or other sort of clinical care facility and are acquired by patients while they are in the facility [7, 8]. Apart from the problem they create for patients, nosocomial infections can affect nurses, doctors, associates, guests, delivery persons, overseers, and anyone who has contact with the hospital.

This study aimed to evaluate the antibiogram of the bacterial isolates and determine the plasmid profile of some resistant isolates.

2. Materials and Methods

2.1. Study Location

This study was carried out in the Intensive Care Units of Federal Medical Centre, Umuahia, Abia State, Nigeria. The ethical committee of FMC, Umuahia approved the study, before sampling and analysis.

2.2. Sample Collection and Processing

Sterile swab sticks moistened with sterile water were used to swab the surfaces of the fomites, hands, and anterior nares of healthcare workers. The swab was rolled back and forth over each surface before being carefully capped and labeled appropriately to ensure maximal coverage of a surface area. The samples were analyzed in the laboratory. The swab samples were inoculated into suitable media (Blood agar, MacConkey agar), which were incubated for 24 h at 37°C. Suspected bacterial growths were identified by standard bacteriological methods [9].

2.3. Antibiogram

Antibiogram was performed using the disk diffusion method and was interpreted by Clinical Laboratory Standards Institute {10} on Mueller Hinton agar (Hardy Diagnostics USA). Mueller Hinton culture plates were inoculated by dipping a sterile cotton wool swab into the overnight organism growth in suspension prepared to the density of a McFarland no 0.5 opacity standard. The spread plate method was used to express excess liquid from the swab before inoculation.

Antibiotic discs that were used have the following concentrations: Streptomycin 30 µg; Ofloxacin 10µg; Norfloxacin 10 µg; Gentamicin 10 µg; Amoxil 20 µg; Ciprofloxacin 10 µg; Erythromycin 30µg; Rifampicin 10 µg; Amoxycillin/Clavulanic acid 30 µg; Cefalexin 10 µg; Nalidixic acid 30µg; Septrin 30µg.

After overnight incubation, the control and test plates were examined to ensure the growth was confluent or near confluent. The diameter of each zone of inhibition was measured in mm using a ruler on the plate's bottom. Growth starts at the endpoint of inhibition. The control strain used was Escherichia coli ATCC 25922.

2.4. Plasmid Analysis

Plasmid analysis and curing were carried out at the Nigeria Institute of Medical Research Yaba, Lagos (NIMR). According to the manufacturer's instructions, Plasmid preparations were done with a QIAprep Spin Plasmid Kit (QIAGEN, West Sussex). Plasmid DNA (10- 15 µl) was analysed by electrophoresis in agarose type II-A (Sigma) 0.8% w/v in Tris-borate-EDTA buffer (0.045 M Tris-borate, 0.001 M EDTA, pH 8.0) containing ethidium bromide 0.5 µglml [11].

2.5. Plasmid Curing

10ml of each bacterial culture was inoculated into peptone water and incubated for 24 h. The organism was introduced into a set of 20 test tubes, respectively. Ethidium bromide in various concentrations of 0, 20, 50, 100, 150, 200, 250, 300, 350, 400, 450, 500, 550, 600,650, 700, 750 and 800 µl/ml was introduced accordingly into the test tubes and incubated for 24 h at 37 ºC to determine the sub-lethal concentrations of

ethidium bromide. After 24h of incubation, 1ml aliquot from each test tube was inoculated into nutrient agar plates and incubated, after which colonies were selected and inoculated into freshly prepared Muller Hinton agar plates. Antibiotic discs of prior resistance were aseptically introduced into the plates, ensuring that the discs made appropriate contact with the surface of the agar. These were incubated for 24 h at 37ºC, after which plates were examined for cured colonies [12].

3. Results and Discussion

3.1. Results

A total of 300 samples were collected and analyzed. The bacterial isolates recorded was 24.7%. Nine different bacterial isolates were identified. *Staphylococcus* aureus, 43.8%, *Escherichia coli*, 17.9%, *Pseudomonas spp,* 11.2% were the majority bacterial isolates, while other bacterial isolates were *Enterobacter* spp, 2.2%, *Klebsiella* pneumonia, 3.3%, *Bacillus* spp, 5.6%, Proteus spp, 3.3%, Coagulase Negative Staphylococci, 7.8% and *Streptococcus* spp, 4.4%.

As indicated by Table 1, Staphylococcus aureus, 39(43.8%), was the highest isolated bacteria, followed by Escherichia coli, 16(17.9%), while *Enterobacter* spp, 2(2.2%) was the least isolated bacteria.

In Table 2, *Klebsiella* pneumoniae, Escherichia coli, Pseudomonas spp were sensitive to Ofloxacin, Peflacine, Ciprofloxacin, and Streptomycin while Staphylococcus *aureus,* Coagulase-negative Staphylococci, *Streptococcus spp* were sensitive to Ciprofloxacin, Gentamicin, Rifampicin, Erythromycin, Chloramphenicol, and Levofloxacin.

As can be seen in Table 3, *Pseudomonas* spp had the least biofilm-forming potential, while *Klebsiella* pneumoniae had the highest biofilm-forming potential.

In Table 4, *Escherichia coli* had the highest ESBL producing isolates while *Enterobacter* spp and Proteus spp did not test positive for ESBL.

Table 5a shows the Plasmid profile of bacterial isolates, while Table 5b shows the resistance pattern of isolates before and after curing.

Figure 1 indicates the plasmid profile of bacteria isolates.

3.2. Discussion

Bacterial contamination of ICU is the major factor responsible for the increased incidence of nosocomial infections, with attendant consequential effects on patients and healthcare workers [13, 14].

Overall, the bacterial contamination rate recorded in the units was 24.7%. Of the 9 different bacterial isolates. *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas* spp were predominant in the study. Other studies have reported the *predominance* of *Staphylococcus* spp and *Bacillus* spp [6, 15, 16, 17].

Table 1: Diversity and percentage of bacterial isolates

Isolates	No	Percentage (%)
Staphylococcus aureus	39	43.82
Escherichia coli	16	17.98
Enterobacter spp	2	2.25
Proteus spp	3	3.37
Klebsiella pneumoniae	3	3.37
Streptococcus spp	4	4.49
Bacillus spp	5	5.62
CoNs	7	7.87
Pseudomonas spp	10	11.24
Total	89	100

Key: CoNS- COAGULASE NEGATIVE STAPHYLOCOCCI

This contrasts with the study from Ethiopia [18], where Coagulase Negative Staphylococci, and Iraq [19], where *Bacillus* spp and *Enterobacter* spp were the predominant bacterial isolates. The recovery of potentially clinically relevant *Staphylococcus aureus*, Coagulase-negative *Staphylococci, Escherichia coli, Klebsiella* pneumoniae, and *Streptococcus* spp from routinely used fomites, healthcare personnel, and vital areas within the units is of Infection control and prevention concern.

Klebsiella pneumoniae, *Staphylococcus aureus*, *Escherichia coli*, Coagulase-negative *Staphylococci*, *Streptococcus* spp, showed potential for biofilm formation. This is an expected result with existing literature supporting the biofilm-forming nature of Staphylococci [20].

Escherichia coli, *Klebsiella pneumoniae*, and *Pseudomonas* spp produced extended spectrum beta-lactamase (ESBL). Escherichia coli had the highest ESBL producers. This result agrees with the findings of [21] where Escherichia coli was recorded as the highest ESBL producers. These pathogens, Escherichia coli, and *Klebsiella* pneumoniae are known ESBL producing pathogens associated with multidrug resistance, with the potential of rapid dissemination and hospital-associated infections source [21].

Moreover, the result of the present study revealed a high resistance pattern with the commonly used antibiotics; Amoxycillin/Clavulanic acid, Ampicilin, Cephalexin, Amoxil, Norfloxacin. A similar pattern was reported in other studies [16, 17, 18, 19]. The same pattern was seen in [22] work with amoxicillin, ampicillin-cloxacillin, and cotrimoxazole being highly resistant. None of the bacterial pathogens exhibited multidrug resistance pattern. This is in contrast to the study in Ethiopia [18], which showed multidrug resistance pattern.

Plasmid has been described as extra chromosomal elements capable of independent replications. These DNA molecules are different from the chromosomal DNA and are in bacteria [12]. Plasmid analysis was carried out on some of the bacterial pathogens. Three Escherichia coli isolates had plasmid sizes 50, 300, 400 base pair, while the remaining three Escherichia coli isolates had no plasmid, three *Staphylococcus aureus* isolates had plasmid sizes 35, 50,100,150. In contrast, the remaining *Staphylococcus aureus* had no plasmid.

Table 2: Antibiogram of the bacterial isolates

		No (%) Sensitive To									
GNB	**No of isolates**	**OFX**	**PEF**	**CPX**	**AU**	**CN**	**S**	**CEP**	**NA**	**SXT**	**PN**
Klebsiella pneumoniae	3	3 (100)	2(66.7)	3(100)	0	1(33.3)	3(100)	0	2(66.7)	2(66.7)	0
Escherichia coli	16	9(56.3)	8(50)	5(31.3)	4(25)	8(50)	8(50)	3(18.8)	1(6)	5(31)	6(38)
Pseudomonas spp	10	5(50)	5(50)	5(50)	2(20)	4(40)	5(50)	1(10)	1(10)	3(30)	4(40)
Proteus spp	3	3(100)	2(66.7)	3(100)	0	0	2(66.7)	1(33)	1(33)	2(66.7)	1(33)
Enterobacter spp	2	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	0	0	0	0
	GPB	**CPX**	**NB**	**CN**	**AmL**	**S**	**RD**	**E**	**CH**	**APX**	**LEV**
Staphylococcus aureus	39	15(39)	8(21)	19(49)	5(13)	19(49)	23(59)	19(49)	11(28)	6(15)	23(59)
CoNS	7	1(14.3)	0	1(14.3)	0	0	2(28.6)	1(14.3)	2(28.6)	0	2(28.6)
Streptococcus spp	4	3(75)	1(25)	2(50)	2(50)	3(75)	3(75)	2(50)	1(25)	1(25)	3(75)

Key: OFX-Ofloxacin, PEF-Peflacine, CPX-Ciprofloxacin, AU-Amoxycillin-Clavulanic acid, CN-Gentamycin, S-Streptomycin, CEP-Cefalexin, NA-Nalidixic acid, SXT-Septrin, PN-Ampicillin, CPX-Ciprofloxacin, NB-Norfloxacin, CN-Gentamicin, AML-Amoxil, RD- Rifampicin, E-Erythromycin, CH-Chloramphenicol, APX-Ampiclox, LEV-Levofloxacin

Table 3: Biofilm-forming potential of the different bacterial isolates

Isolates	Total No	Positive Bf	Percentage(%)
Staphylococcus aureus	39	21	53.85
Escherichia coli	16	6	37.5
Enterobacter spp	2	0	0
Proteus spp	3	2	66.67
Klebsiella pneumoniae	3	3	100
Streptococcus spp	4	2	50
Bacillus spp	5	0	0
CoNs	7	4	57.14
Pseudomonas spp	10	3	30

Key: BF Biofilm Formation

Table 4: Frequency of ESBL producing isolates

Isolates	Total No Tested	Positive Esbl	Percentage(%)
Escherichia coli	16	9	56.25
Klebsiella pneumoniae	3	1	33.33
Pseudomonas spp	10	4	40
Proteus spp	3	0	0
Enterobacter spp	2	0	0

Table 5a: Plasmid profile of bacterial isolates

No	Isolates	Plasmid size (base pair)
1	*Escherichia coli*	Nil
2	*Escherichia coli*	300
3	*Escherichia coli*	Nil
4	*Escherichia coli*	50, 300
5	*Escherichia coli*	Nil
6	*Escherichia coli*	400
7	*Proteus* spp	Nil
8	*Proteus* spp	Nil
9	*Pseudomonas* spp	Nil
10	*Pseudomonas* spp	Nil
11	*Staphylococcus aureus*	50
12	*Staphylococcus aureus*	100, 150
13	*Staphylococcus aureus*	Nil
14	*Staphylococcus aureus*	35, 100
15	*Staphylococcus aureus*	Nil

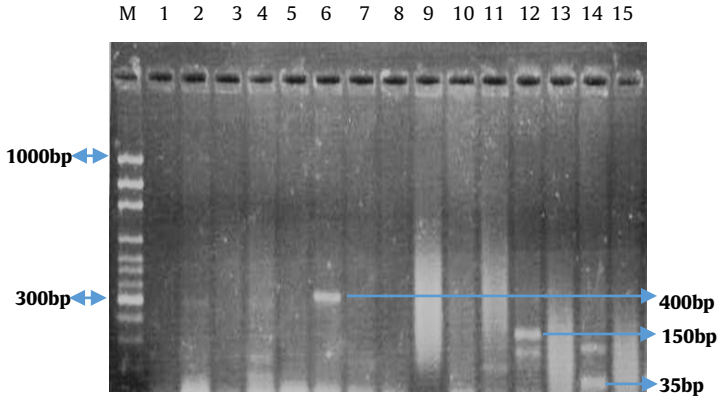

Figure 1: Plasmid analysis of some bacterial isolates

Table 5b: Resistance pattern of Isolates before and after curring

Isolates	Resistance Pattern Of Isolates Before Curring	Resistance Pattern Of Isolates After Curring
Escherichia coli	CN, OFX, AU, S, CEP, NA, PN	CN, OFX, CPX
Escherichia coli	OFX, S, CN, CEP, PEF	S, CN
Escherichia coli	CPX, AU, S, NA, CN	CPX, S
Staphylococcus aureus	LEV, S, APX, AML, CN, PEF	S
Staphylococcus aureus	APX, CPX, CN, S, OFX, NA	CN, APX
Staphylococcus aureus	AML, S, RD, CH, NB, OFX	AML, S

Hence the remaining *Staphylococcus aureus* had no plasmid, the resistance is plasmid-mediated; therefore, the isolates that were cured of plasmids were re-exposed to the antibiotics that they were resistant to initially, it was discovered that they became sensitive. The efficiency of curing has been reported to vary depending on the type of plasmid and the bacterial host harboring it [23]. The susceptibility of most of the isolates to antimicrobial agents previously resistant to them may be due to the curing action

of ethidium bromide against these isolates and as reported by [12].

4. Conclusion

The outcome of this study is of ultimate importance to the hospital infection control and prevention unit. Based on the findings of this study, the recovery of pathogens with clinical significance from fomites and crucial areas that are frequently used is of serious concern because of their clinical implication. The findings of this study have given an overview of the resistance pattern of bacterial isolates. It has formed the template to formulate intervention measures.

The hospital infection control and prevention units should adopt periodic surveillance, effective cleaning of fomites before and after use, and adhere to simple basic standard infection procedures, especially hand washing.

Authors' Contributions

Ebubechi Uloma Okey-kalu: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Resources; Visualization; Writing-original draft; Writing-review and editing. **Emmanuel Nwankwo:** Supervision; Methodology; Investigation. **Joan James-Onyekwere:** Investigation. **Mary Kalu:** Investigation.

Conflicts of Interest

The Authors declare that there is no conflict of interest.

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