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Antibiogram of Biofilm Producing Bacteria Isolated from Urine of Patients in Three Hospitals in Port Harcourt, Rivers State

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The aim of this study was to determine the antibiogram of biofilm producing bacteria isolated from urine of patients in three hospitals in Port Harcourt, Rivers State.
Study Design: The study employs statistical analysis of the data and interpretation
Place and Duration of Study: The study was conducted at three (3) hospitals; University of Port Harcourt Teaching Hospital (UPTH), Meridian Hospital D / line branch (MRD1) and Meridian Hospital Ikoku branch, all located in Port Harcourt, Rivers State. Sample collection was for three (3) months, analysis was carried out daily and it lasted for six (6) months.
Methodology: A total of Forty-five (45) urine samples were collected for a period of three (3) months from the three (3) hospitals. The samples were labelled properly, according to date and time of collection. The collected samples were subjected to standard microbiological procedures which includes standard plate counts, identification, biofilm screening, sensitivity testing using Kirby-Bauer disk diffusion method, Phenotypic screening of extended spectrum beta lactamase and molecular characterization of the isolates
Results: The results of the bacterial population of urine samples from the hospitals showed that the total heterotrophic bacterial counts for Meridian Hospital D/line (MRD1), Meridian Hospital Ikoku

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(MRD2) and University of Port Harcourt Teaching Hospital (UPTH) ranged from 4.93 - 6.30 x107cfu/ml. The Total coliform count ranged from 1.89-3.04 x106cfu/ml for Meridian Hospital D/line (MRD1), Meridian Hospital Ikoku (MRD2) and University of Port Harcourt Teaching Hospital (UPTH). Total faecal coliform counts ranged from 0.78-1.11 x105CFU/ml for Meridian Hospital D/line (MRD1), Meridian Hospital Ikoku (MRD2) and University of Port Harcourt Teaching Hospital (UPTH). A total of fifty-eight (58) bacterial isolates were isolated from urine of patients and 36(62.1%) isolates were identified as biofilm producers. The biofilm bacteria identified were 17.2% *Staphylococcus,*6.9% *E. coli,* 10.3% *Pseudomonas,* 6.9% *Proteus* ,10.3% *Bacillus and* 10.3% *Enterococcus* species. Biofilm forming ability of bacteria is considered a virulent factor and it is implicated to being a possible cause of increased resistance to most antibiotics. Varying susceptibility pattern was observed among biofilm isolates. Biofilm bacteria were resistant to several groups of antibiotics. Ofloxacin, Gentamycin, Imipenem and Nitrofurantoin can be used as drug of interest for most bacterial biofilm urinary tract infections. CTX-M and TET A gene were identified in the biofilm bacteria in this study to be possible factors that confer resistance to antibiotics. The presence of icaD and *pap*C gene in the isolates whose genome were studied have been found to be possible factors that confers biofilm producing ability. This study indicates the emergence and rapid spread of biofilm producing bacteria and their resistance to antibiotics. Therefore, strict infection control practices as well as therapeutic guidance for confirmed infections should be rapidly initiated.

Keywords: Biofilm; antibiogram; ESBL; MBL.

1. INTRODUCTION

A Biofilm is a biological community of microbial cells associated with the surface and embedded in the upper matrix of cells of bacterial origin. They are embedded in a matrix of extracellular polymeric substances that consist mainly of extracellular DNA, proteins and polysaccharides [1] and reflect a modified phenotype in relation to genetic growth and transcription. It is estimated that more than 65% of bacterial infections are caused by microorganisms when they grow into biofilms [2].

Biofilms are heterogeneous in nature. Bacteria of the same type, act and behave differently when in sessile form, compared to their planktonic form [3]. One of the most important of these is the increasing resistance of antibiotic agents [4]. The extracellular polymeric substance present in biofilm community limits the rate of diffusion of an antibacterial agent aimed at a biofilm [5]. The extracellular polymeric substance is able to do this by reacting chemically with antimicrobial agent or by reducing the rate of diffusion [6]. Microorganisms form biofilm to ensure survival. There are several beneficial interactions; which includes changes in the extracellular polymeric substance, use of enhanced genes, metabolic interactions, social control of genetic expression, increased antimicrobial resistance, human body responses and local dissemination in the biofilm community [7].

The ability of microorganism to develop biofilm is an important virulent property and a major cause of many chronic diseases [8]. Nosocomial infections have been reported to be high and this was associated with bacteria capable of producing biofilms. Biofilms demonstrate a significant role in the contamination of medical devices, by living in abiotic environments [5] such as artificial valves, catheters and many others surfaces.

The formation of biofilm in urinary catheters is a leading cause of infections in the urinary tract [9]. Infections caused by clinical biofilm has shown that antibiotic treatment is not an absolute remedy as symptoms often return even after continuous treatment. Antibiotic treatment eliminates planktonic cells, but sessile forms are resistant and continue to spread within biofilm [10]. In recent years, an increase in antimicrobial-resistant strains of bacteria has been observed and among the bacteria identified to show resistance to antibiotics, are the bacteria that produce biofilms. Biofilm bacteria are major causes of chronic, frequent infections worldwide. This is as a result of accelerated tolerance to antibiotics [11]. Their resistance to antimicrobials may be due to genetic mutations, resistance to phenotypes, stress adaptation, quorum sensing, genetic gradients, oxidative stress, antibiotic failure and heterogeneity [12].

Current guidelines for antibiotic treatment do not take into account differences in the ecological dynamics that exist between different bacteria [13]. The assumption that they will destroy the same type of bacteria regardless of where they are found has been a major cause of resistance to these antibiotics.

Antimicrobials when administered at a low level of inhibition, can induce the formation of biofilm in various bacterial strains [14]. This is particularly troubling as deep cells within the biofilm can be exposed to low levels of antibiotic resistance. Instead of inhibiting biofilm, antibiotic can promote biofilm formation [15].

There is therefore a need to control bacterial infections caused by these biofilms [16]. as well as new approaches to drug delivery. This study is aimed at determining the antibiogram of biofilm producing bacteria isolated from urine of patients in three hospitals in port harcourt, rivers state.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted at three (3) hospitals; University of Port Harcourt Teaching Hospital (UPTH), Meridian Hospital D / line branch (MRD1) and Meridian Hospital Ikoku branch (MRD2), all located in Port Harcourt Metropolis, Rivers State.

2.2 Sample Collection

A total of 45 urine samples were collected for a period of three (3) months from the three (3) hospitals. The samples were labelled properly, according to date and time of collection. The urine samples were collected using sterile specimen containers and transported aseptically to the Department of Microbiology Laboratory, Rivers State University for bacteriological analysis.

2.3 Samples and Preparations:

Samples were prepared in accordance with the guidelines of the Clinical Laboratory Standard Institute [17]. The media for bacterial culture were Nutrient Agar, MacConkey Agar, Cysteine Lactose Electrolyte Deficient medium (CLED), Mannitol Salt Agar, Cetrimide Agar, Bile Esculin Agar and Eosin Methylene Blue (EMB) were prepared according to manufacturer's instructions. normal bacteria

2.4 Bacteriological Analysis

2.4.1 Bacterial enumeration

Tenfold serial dilution was done on the urine samples, in which 1ml of urine was transferred to 9ml of normal saline and further dilutions were done up to 10^6. Aliquot (0.1ml) of appropriate dilutions (10^4, 10^5 and 10^6) were spread plated in duplicates on Nutrient Agar, MacConkey Agar, Eosin Methylene Blue plates, Bile Esculin Agar, Cysteine Lactose Electrolyte Deficient Agar (CLED), Cetrimide Agar and Mannitol Salt Agar, using the spread plate technique. The plates were incubated at 37^{o}C for 16 to 24 hours. The colonies on the plates was counted and described morphologically. The colonies formed on EMB and MacConkey was used for the enumeration of the population of coliforms. Colonies formed on Nutrient Agar was used to estimate the total heterotrophic count (THB). Bile Esculin Agar, Cysteine Lactose Electrolyte Deficient Agar (CLED), Cetrimide Agar and Mannitol Salt Agar selectively supported the growth of desired bacteria.

2.4.2 Isolation of bacteria

Isolation of bacteria was done by standard microbiological procedure. The test samples were cultured on the different media separately. Emerging cultures were examined for distinct colonies, from which inocula was subcultured onto sterile solid plates for growth. Uniformity of colonies marked purity and the pure cultures were subjected to characterization and identification. Characterization was based on colony morphology, microscopic properties and some unique biochemical tests were carried out to confirm isolates [6]. Identification was first based on matching properties with existing taxa in standard manuals including The manual for identification of medical bacteria [18] and the Bergy´s manual of Determinative bacteriology [19]. Further identification was done through Molecular technique (PCR) to confirm the identities of the isolates to species level.

2.5 Test for Biofilm Production

Bacterial isolates obtained from the samples were tested for their biofilm producing capacity using the Congo red test method [20].

2.5.1 Biofilm detection by congo red agar method

The method described by [20] is a simple qualitative way to detect biofilm production among bacterial isolates using Congo Red Agar (CRA) Medium. This method was used to determine the bacterial isolates that produce biofilms. In this regard, the test organisms were

inoculated on Congo Red Agar and incubated at 37°C for 24 hours. The formation of black crystalline colonies marks a positive test for biofilm production.

2.6 Mueller-Hinton Agar Preparation

The Mueller-Hinton agar preparation was done according to the manufacturer's specifications (38g in 1 Litre of distilled water) and sterilized in an autoclave at 121ºC for 15minutes at 15pounds per square inch. The pH of the medium was confirmed to be 7.2 and poured into the appropriate depth in the petri dish to avoid false reading of the zones of inhibitions

2.7 Antibiogram

2.7.1 Agar disk diffusion method (kirby bauer disk diffusion)

A sterile swab stick was immersed into the tube containing the bacterial suspension and the turbidity was equivalent to 0.5m McFarland Turbidity Standard and the swab was used to swab the surface of the petri dish evenly which contain already prepared Mueller Hinton agar in three dimensions and rotating the plates to about 60º to ensure even distribution of the organism. The agar was allowed to dry for about 3-5minutes. With Sterile forceps, the impregnated antimicrobial discs were placed evenly on the surface of the inoculated plate and the disc was placed 15mm away from the edge of the plate. The head of the forcep was used to Press down each disc slightly to make contact with the agar. After applying the discs, the plates were incubated in an inverted position aerobically at 35ºC for 16-18h. After incubation, the test plates were examined to determine the zones of inhibition. The diameter of each zone of inhibition was measured in mm using a ruler and recorded for reference purpose [17].

2.8 Phenotypic Detection of Extended Spectrum Beta-Lactamase (ESBL) Production

Isolates were screened phenotypically, to determine the presence or absence of extended spectrum beta- lactamases enzyme. The ceftazidime resistant strains were screened for ESBL production by using disc diffusion test. The increase in the zone of the diameter of ≥ 5-mm between ceftazidime (30µg) and ceftazidime-clavulanate (30/10µg) was considered ESBL positive [21].

2.9 Phenotypic Detection of Metallo Beta-Lactamase (MBL) Production

Isolates were screened phenotypically, to determine the presence or absence of Metallo beta- lactamases enzyme. Imipenem-resistant strains were tested for MBL production by combined disc diffusion assay using two imipenem discs, one with added 10 µl of 0.5 M EDTA. The increased zone of inhibition of > 7mm around the imipenem-EDTA disc in comparison to zone size of imipenem disc alone was confirmed positive for MBL production [21].

3. RESULT AND DISCUSSION

3.1 Bacterial Population

The results of the bacterial population of urine samples from the hospitals as presented in Table 1 showed that the total heterotrophic bacterial counts for Meridian Hospital D/line (MRD1), Meridian Hospital Ikoku (MRD2) and University of Port Harcourt Teaching Hospital (UPTH) ranged from 4.93 - 6.30 $x10^7$cfu/ml. There was no significant difference ($p \leq 0.05$) in the Total heterotrophic bacterial counts between the hospitals sampled. The Total coliform count ranged from 1.89-3.04 $x10^6$cfu/ml for Meridian Hospital D/line (MRD1), Meridian Hospital Ikoku (MRD2) and University of Port Harcourt Teaching Hospital (UPTH). Faecal coliform counts ranged from 0.78-1.11 $x10^5$cfu/ml for Meridian Hospital D/line (MRD1), Meridian Hospital Ikoku (MRD2) and University of Port Harcourt Teaching Hospital (UPTH).

3.2 Morphological and Biochemical Characteristics of the Isolates

The identities of isolates are revealed on the basis of their colonial, morphological and biochemical characteristics. Fifty-eight (58) bacterial isolates belonging to the following genera were identified as *Escherichia coli, Staphylococcus, Klebsiella, Streptococcus*, *Proteus, Serratia, Pseudomonas, Bacillus and Enterococcus* species as shown in Tables 2 and 3.

3.3 Antibiogram Assay of the Isolates

The result of the antimicrobial pattern of the individual biofilm bacterial isolates; *Staphylococcus* sp*, Enterococcus* sp*, Bacillus* sp*, Escherichia coli, Proteus* sp *and*

Pseudomonas aeruginosa species are represented in Tables 5 to 10. The result of the antimicrobial pattern of the individual Non-biofilm bacterial isolates *Staphylococcus* sp*, Enterococcus* sp*, Escherichia coli, Streptococcus* sp, *Serratia* sp and *Klebsiella pneumonia* are represented in Tables 12 to 16. The antibiogram profile of the isolates were graded as susceptible, intermediate and resistant.

3.4 Prevalence of Bacterial Isolates from Samples and Location

A total of fifty-eight (58) bacteria were isolated from urine samples obtained from the three (3) hospitals. Table 2 shows the cultural and biochemical identities of the bacteria isolated. The findings from this study, as seen in Table 3, showed relative abundance of *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus, Enterococcus, Bacillus, Proteus, Streptococcus* and *Serratia* species, which were found in urine of hospitalised patients in Port-Harcourt, Rivers State. University of Port Harcourt Teaching Hospital had the predominant relative abundance 22(37.9%) and Meridian Hospital Ikoku had the predominant percentage occurrence 16(27.6%) as shown in Table 4. The observed variance in bacterial population observed in urine samples obtained from patients across hospitals in this study can be due to the level of exposure of patients to contaminated substances, duration of urogenital infections, as long-term infections have higher bacterial population [22,23]**.**

3.5 Prevalence of Bacteria Isolates Producing Biofilm from Urine

A total of 36(62.1%) isolates were identified to be biofilm producers. This is in line with the observation of [24] who recorded 64.28% of biofilm producing bacteria. Biofilm bacterial isolates were able to form biofilms due to the possession of adherent structures, such as flagella that aid motility to receptor sites (substratum). The presence of biofilm bacteria amongst other bacteria isolated from urine indicated possible bacteria adhesion to the uroepithelium and can cause chronic uropathogenic infections [25]. *Escherichia coli, Pseudomonas aeruginosa Staphylococcus, Enterococcus, Bacillus* and *Proteus* species were identified as biofilm producers as shown in Fig 1.

3.6 Prevalence of Non-Biofilm Producing Bacteria Isolates from Urine

A total of 22(37.9%) isolates were identified to be Non-biofilm producers; *Escherichia coli, Klebsiella pneumonia, Staphylococcus, Enterococcus, Streptococcus* and *Serratia* species were identified as non-biofilm producers as shown in Fig 2

3.7 Antibiotic Pattern of Biofilm Producing Bacteria Isolated from Various Samples

The result of the antibiotic pattern of *Staphylococcus* sp as shown in Table 5 indicates that greater number of the *Staphylococcus* sp were susceptible to Ofloxacin (80%), Gentamycin (60%), Imipenem and Erythromycin (40%). *Staphylococcus* sp showed complete resistance to Ceftazidime, Augmentin, Cefuroxime, Ceftriaxone and Cloxacillin (100%). The observed susceptibility of *Staphylococcus* sp to Ofloxacin is in accordance with the report of [26] and [27]. High sensitivity to gentamycin in this present study compares favourably with the reports of [28]. The observed resistance to Cloxacillin in this study contradicts the findings of [28] which revealed that Cloxacillin was highly recommended in staphylococcal infection.

The result of the antibiotic pattern of *Enterococcus* sp as shown in Table 6 indicates that greater number of the *Enterococcus* sp were susceptible to Ofloxacin and Gentamycin (66.6%). The high sensitivity to gentamycin as seen in this study is in agreement with the findings of [29]. *Enterococcus* sp showed a decreasing trend of resistance in the order: Ceftazidime, Cefuroxime, Ceftriaxone and Cloxacillin (100%) and Augmentin (66.6%).

Table 1. Distribution of the Bacterial Population in Urine

Hospital	THBx10^7cfu/ml	TCCx10^6cfu/ml	TFCx10^5cfu/ml
MRD1	4.93±3.46^a	1.89±1.71^a	0.78±0.49^a
MRD2	5.35±2.85^a	2.71±1.54^a	1.03±0.71^a
UPTH	6.30±3.66^a	3.04±1.85^a	1.11±0.58^a

*Key: THB (Total Heterotrophic Bacteria), TCC (Total Coliform Count), TFC (Total faecal coliform count). *Mean with the same superscript along the columns is not significantly different (p≤0.05*

Table 2. Morphological and biochemical characteristics of bacterial isolates from urine samples

	Colony Characteristics							Gram Stain												
1	UR1	Circular	Raised	smooth	Moderate	GMS	translucent	-	Rod	+	-	-	+	+	-	+	+	+	+	-	-	*Escherichia coli*
2	UR2	Circular	Raised	smooth	Small	Yellow	translucent	+	Cocci	+	-	+	-	-	+	-	+	+	+	+	-	*Staphylococcus sp*
3	UR3	Circular	Raised	smooth	Moderate	White	Translucent	+	Cocci	-	-	-	-	-	+	-	+	+	+	+	-	*Enterococcus sp*
4	UR4	Circular	Raised	smooth	Moderate	GMS	Translucent	-	Rod	+	-	-	+	+	-	+	+	+	+	-	-	*Escherichia coli*
5	UR5	Circular	Raised	smooth	Small	Milky	Translucent	+	Cocci	+	-	+	-	-	+	-	+	+	+	+	-	*Staphylococcus sp*
6	UR6	Irregular	Flat	smooth	Small	Milky	Opaque	-	Rod	+	-	+	+	+	+	+	+	+	+	+	+	*Proteus sp*
7	UR7	Circular	Raised	smooth	Moderate	Red	Opaque	-	Rod	+	-	+	-	-	+	+	+	-	+	+	+	*Serratia sp*
8	UR8	Circular	Raised	smooth	Large	Pink Purple	Opaque	-	Rod	+	-	+	-	-	+	-	-	+	+	+	-	*Klebsiella sp*
9	UR9	Irregular	Raised	smooth	Small	Gray	Translucent	-	Rod	+	+	+	-	-	-	+	-	-	+	-	-	*Pseudomonas sp*
10	UR10	Irregular	Raised	Rough	Large	Milky	Opaque	+	Rod	+	+	+	-	-	+	+	-	-	-	-	-	*Bacillus sp*
11	UR11	Circular	Raised	Smooth	Small	Yellow	Opaque	+	Cocci	+	-	+	-	-	+	-	+	+	+	+	-	*Staphylococcus sp*
12	UR12	Circular	Raised	Smooth	Moderate	Pink purple	Opaque	-	Rod	+	-	+	-	-	+	-	-	+	+	+	-	*Klebsiella sp*
13	UR13	Irregular	Flat	Smooth	Small	Blue Green	Translucent	-	Rod	+	+	+	-	-	-	+	-	-	+	-	-	*Pseudomonas sp*
14	UR14	Circular	Raised	Smooth	Moderate	Red	Opaque	-	Rod	+	-	+	-	-	+	+	+	-	+	+	+	*Serratia sp*
15	UR15	Irregular	Flat	Smooth	Large	Milky	Opaque	-	Rod	+	-	+	+	+	+	+	+	+	+	+	+	*Proteus sp*
16	UR16	Circular	Raised	Smooth	Large	Milky	Translucent	+	Cocci	-	-	-	-	-	-	-	-	-	-	+	-	*Streptococcus sp*
17	UR17	Circular	Raised	smooth	Large	Milky	Translucent	+	Cocci	-	-	-	-	-	-	-	-	-	-	+	-	*Streptococcus sp*
18	UR18	Irregular	Raised	Rough	Large	Milky	Opaque	+	Rod	+	+	+	-	-	+	+	-	-	-	-	-	*Bacillus sp*
19	UR19	Irregular	Raised	Rough	Large	Milky	Opaque	+	Rod	+	+	+	-	-	+	+	-	-	-	-	-	*Bacillus sp*
20	UR20	Circular	Raised	smooth	Small	Milky	Translucent	+	Cocci	+	-	+	-	-	+	-	+	+	+	+	-	*Staphylococcus sp*
21	UR21	Circular	Raised	smooth	Moderate	White	Translucent	+	Cocci	-	-	-	-	-	+	-	+	+	+	+	-	*Enterococcus sp*
22	UR22	Circular	Raised	smooth	Moderate	Yellow	Translucent	+	Cocci	+	-	+	-	-	+	-	+	+	+	+	-	*Staphylococcus sp*
23	UR23	Circular	Raised	smooth	Moderate	White	Translucent	+	Cocci	-	-	-	-	-	+	-	+	+	+	+	-	*Enterococcus sp*
24	UR24	Circular	Raised	smooth	Small	Milky	Translucent	+	Cocci	+	-	+	-	-	+	-	+	+	+	+	-	*Staphylococcus sp*
25	UR25	Circular	Raised	smooth	Moderate	Yellow	Translucent	+	Cocci	+	-	+	-	-	+	-	+	+	+	+	-	*Staphylococcus sp*
26	UR26	Circular	Raised	smooth	Moderate	White	Translucent	+	Cocci	-	-	-	-	-	+	-	+	+	+	+	-	*Enterococcus sp*
27	UR27	Circular	Raised	smooth	Moderate	White	Translucent	+	Cocci	-	-	-	-	-	+	-	+	+	+	+	-	*Enterococcus sp*
28	UR28	Circular	Raised	smooth	Moderate	White	Translucent	+	Cocci	-	-	-	-	-	+	-	+	+	+	+	-	*Enterococcus sp*

Table 3. Percentage relative abundance of bacteria isolated from different samples

ISOLATES	URINE No. (%)
Bacillus sp.	6(10.3)
Enterococcus sp	12(20.6)
Escherichia coli	7(12.1)
Klebsiella sp	4(6.9)
Proteus sp	4(6.9)
Pseudomonas sp	6(10.3)
Serratia sp	2(3.4)
Staphylococcus sp	15(25.9)
Streptococcus sp	2(3.4)
Total No.	**58**

Table 4. Percentage relative abundance of bacteria isolates from urine based on location

LOCATIONS	No. of Isolates (%)
UPTH	22(37.9)
MRD1	20(34.5)
MRD2	16(27.6)

Table 5. Antibiotic Pattern of Biofilm Producing *Staphylococcus* sp Isolated from Urine N=10

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	2(20.0)	0(0.00)	8(80.0)
AUG	30	10(100)	0(0.00)	0(0.00)
CAZ	30	10(100)	0(0.00)	0(0.00)
CRX	30	10(100)	0(0.00)	0(0.00)
GEN	10	2(20.0)	2(20.0)	6(60.0)
CTR	30	10(100)	0(0.00)	0(0.00)
ERY	5	6(60.0)	0(0.00)	4(40.0)
CXC	5	10(100)	0(0.00)	0(0.00)
IMP	30	2(20.0)	4(40.0)	4(40.0)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 6. Antibiotic Pattern of Biofilm Producing *Enterococcus* sp Isolated from Urine N=6

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	0(0.00)	2(33.3)	4(66.6)
AUG	30	4(66.6)	2(33.3)	0(0.00)
CAZ	30	6(100)	0(0.00)	0(0.00)
CRX	30	6(100)	0(0.00)	0(0.00)
GEN	10	2(33.3)	0(0.00)	4(66.6)
CTR	30	6(100)	0(0.00)	0(0.00)
ERY	5	0(0.00)	6(100)	0(0.00)
CXC	5	6(100)	0(0.00)	0(0.00)
IMP	30	2(33.3)	2(33.3)	2(33.3)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

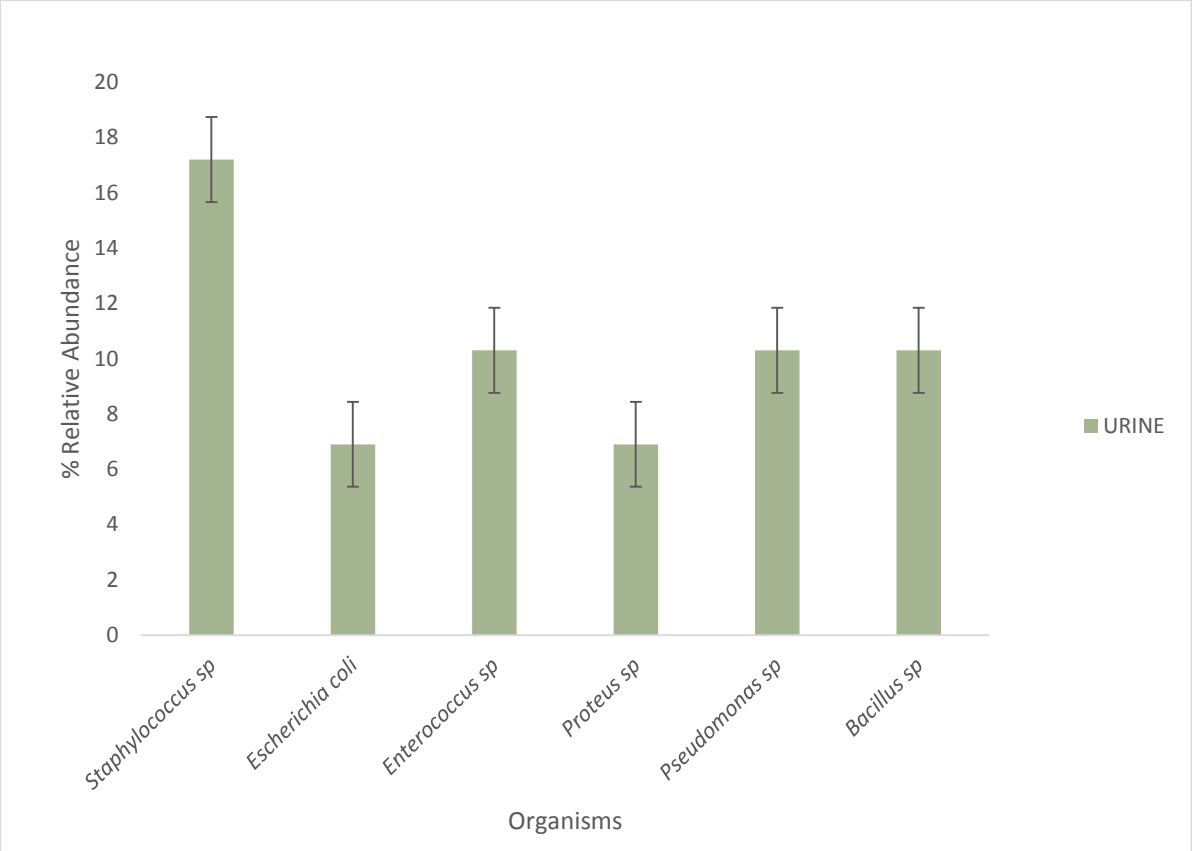

Fig. 1. Percentage Relative Abundance of Biofilm Producing Bacteria Isolated from Urine Samples

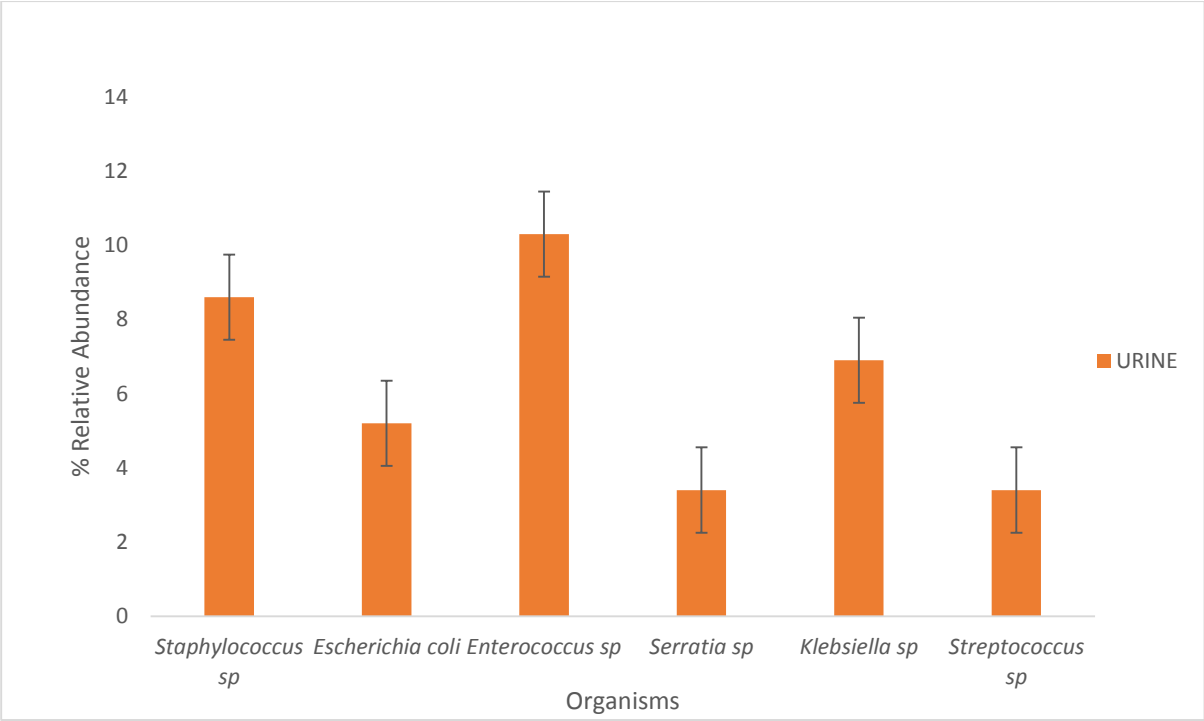

Fig. 2. Percentage Relative Abundance of Non-Biofilm Producing Bacteria Isolated from Urine

Table 7. Antibiotic Pattern of Biofilm Producing *Bacillus* sp Isolated from Urine N=6

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	0(0.00)	0(0.00)	6(100)
AUG	30	6(100)	0(0.00)	0(0.00)
CAZ	30	6(100)	0(0.00)	0(0.00)
CRX	30	6(100)	0(0.00)	0(0.00)
GEN	10	2(33.3)	0(0.00)	4(66.6)
CTR	30	6(100)	0(0.00)	0(0.00)
ERY	5	4(66.6)	2(33.3)	0(0.00)
CXC	5	6(100)	0(0.00)	0(0.00)
IMP	30	2(33.3)	0(0.00)	4(66.6)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 8. Antibiotic Pattern of Biofilm Producing *Escherichia coli* Isolated from Urine N=4

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	0(0.00)	2(50.0)	2(50.0)
AUG	30	4(100)	0(0.00)	0(0.00)
CAZ	30	4(100)	0(0.00)	0(0.00)
CRX	30	4(100)	0(0.00)	0(0.00)
GEN	10	0(0.00)	4(100)	0(0.00)
NIT	300	2(50.0)	0(0.00)	2(50.0)
CPR	5	4(100)	0(0.00)	0(0.00)
CXM	5	4(100)	0(0.00)	0(0.00)
IMP	30	0(0.00)	2(50.0)	2(50.0)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 9. Antibiotic Pattern of Biofilm Producing *Proteus* sp Isolated from Urine N=4

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	0(0.00)	0(0.00)	4(100)
AUG	30	4(100)	0(0.00)	0(0.00)
CAZ	30	4(100)	0(0.00)	0(0.00)
CRX	30	4(100)	0(0.00)	0(0.00)
GEN	10	2(50.0)	0(0.0.0)	2(50.0)
NIT	300	0(0.00)	0(00.0)	4(100)
CPR	5	4(100)	0(0.00)	0(0.00)
CXM	5	4(100)	0(0.00)	0(0.00)
IMP	30	1(50.0)	0(0.00)	1(50.0)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

The antibiotic pattern of *Bacillus* sp as shown in Table 7 indicates that greater number of *Bacillus* sp were susceptible to Ofloxacin (100%) followed by Gentamycin and Imipenem (66.6%). *Bacillus* sp showed complete resistance to Ceftazidime, Augmentin, Cefuroxime, Ceftriaxone and Cloxacillin (100%).

Antibiotic pattern of *Escherichia coli* as shown in Table 8 indicates that greater number of

Escherichia coli were susceptible to Ofloxacin, Nitrofurantoin and Imipenem (50%). Susceptibility to Ofloxacin in this study concurs with the findings of [30]. *Escherichia coli* showed complete resistance to Ceftazidime, Augmentin, Cefuroxime and Ciprofloxacin (100%).

The result of the antibiotic pattern of *Proteus* sp as shown in Table 9 indicates that greater number of *Proteus* sp were susceptible to Nitrofurantoin and Ofloxacin (100%). *Proteus sp* showed complete resistance to Ceftazidime, Augmentin, Cefexime, Cefuroxime, Ciprofloxacin and (100%).

Table 10. Antibiotic Pattern of Biofilm Producing *Pseudomonas* sp Isolated from Urine N=6

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	0(0.00)	2(33.3)	4(66.6)
AUG	30	6(100)	0(0.00)	0(0.00)
CAZ	30	6(100)	0(0.00)	0(0.00)
CRX	30	6(100)	0(0.00)	0(0.00)
GEN	10	0(0.00)	0(0.00)	6(100)
NIT	300	2(33.3)	1(16.7)	3(50.0)
CPR	5	4(66.6)	2(33.3)	0(0.00)
CXM	5	6(100)	0(0.00)	0(0.00)
IMP	30	1(16.7)	2(33.3)	3(50.0)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 11. MAR index of biofilm producing bacteria isolated from urine

MAR	*Staphylococcus* sp (N=10)	*Escherichia* coli (N=4)	*Enterococcus* sp (N=6)	*Bacillus* sp (N=6)	*Pseudomonas* sp (N=6)	*Proteus* sp (N=4)
0.1	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
0.2	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
0.3	0(0.00)	0(0.00)	0(0.00)	1(16.7)	0(0.00)	0(0.00)
0.4	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
0.5	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(16.7)	0(0.00)
0.6	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(16.7)	0(0.00)
0.7	0(0.00)	2(50.0)	4(66.7)	2(33.3)	2(33.3)	2(50.0)
0.8	10(100)	0(0.00)	2(33.3)	2(33.3)	0(0.00)	2(50.0)
0.9	0(0.00)	2(50.0)	0(0.00)	1(16.7)	2(33.3)	0(0.00)
1.0	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
MAR(%)	**100**	**100**	**100**	**100**	**100**	**100**

Table 12. Antibiotic Pattern of Non-Biofilm Producing *Staphylococcus* sp Isolated from Urine N=5

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate	n(%)	Susceptible n(%)
OFL	5	0(0.00)	1(20.0)		4(80.0)
AUG	30	2(40.0)	2(40.0)		1(20.0)
CAZ	30	5(100)	0(0.00)		0(0.00)
CRX	30	0(0.00)	2(40.0)		3(60.0)
GEN	10	0(0.00)	0(0.00)		5(100)
CTR	30	0(0.00)	2(40.0)		3(60.0)
ERY	5	1(20.0)	0(0.00)		4(80.0)
CXC	5	0(0.00)	2(40.0)		2(40.0)
IMP	30	1(20.0)	2(40.0)		2(40.0)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 13. Antibiotic Pattern of Non-Biofilm Producing *Enterococcus* sp Isolated from Urine N=6

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	1(16.7)	0(0.00)	5(83.3)
AUG	30	2(33.3)	2(33.3)	2(33.3)
CAZ	30	6(100)	0(0.00)	0(0.00)
CRX	30	3(50.0)	1(16.7)	2(33.3)
GEN	10	0(0.00)	0(0.00)	6(100)
CTR	30	3(50.0)	3(50.0)	0(0.00)
ERY	5	0(0.00)	1(16.7)	5(83.3)
CXC	5	3(50.0)	2(33.3)	1(16.7)
IMP	30	2(33.3)	1(16.7)	3(50.0)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 14. Antibiotic Pattern of Non-Biofilm Producing *Escherichia coli* Isolated from Urine N=3

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	0(0.00)	0(0.00)	3(100)
AUG	30	1(33.3)	0(0.00)	2(66.6)
CAZ	30	3(100)	0(0.00)	0(0.00)
CRX	30	1(33.3)	2(66.6)	0(0.00)
GEN	10	0(0.00)	0(0.00)	3(100)
NIT	300	0(0.00)	1(33.3)	2(66.6)
CPR	5	0(0.00)	0(0.00)	3(100)
CXM	5	1(33.3)	0(0.00)	2(66.6)
IMP	30	0(0.00)	1(33.3)	2(66.6)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 15. Antibiotic Pattern of Non-Biofilm Producing *Streptococcus* sp Isolated from Urine. N=2

Antibiotics	Concentration (µg)	Resistant n(%)	Intermediate n(%)	Susceptible n(%)
OFL	5	0(0.00)	0(0.00)	2(100)
AUG	30	1(50.0)	1(50.0)	0(0.00)
CAZ	30	2(100)	0(0.00)	0(0.00)
CRX	30	1(50.0)	0(0.00)	1(50.0)
GEN	10	0(0.00)	1(50.0)	1(50.0)
CTR	30	1(50.0)	1(50.0)	0(0.00)
ERY	5	0(0.00)	1(50.0)	1(50.0)
CXC	5	1(50.0)	0(0.00)	1(50.0)
IMP	30	0(0.00)	0(0.00)	2(100)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

The result of the antibiotic pattern of *Pseudomonas* sp as shown in Table 10 indicates that greater number of *Pseudomonas* sp were susceptible to Gentamycin (100%) followed by Ofloxacin (66.6%) and Imipenem (50%). *Pseudomonas* sp showed a decreasing trend of resistance in order: Ceftazidime, Augmentin, Cefuroxime and Cefexime (100%) respectively.

The main problem associated with infections caused by biofilm forming bacteria is the low

sensitivity of the bacteria to the antimicrobials used [31]. The resistance to beta-lactam drugs in this study is in line with the work of [32]. Most organisms were resistant to Ceftazidime which is a cephalosporin group antibiotic. The high resistance of biofilm bacteria to the beta-lactam antibiotics Ceftazidime, Cefixime and Cefuroxime as observed in this study can possibly be due to the extreme use of these antibiotics and the acquisition of blaCTX, blaSHV and blaTEM [33]. Ofloxacin, Gentamycin, Imipenem and Nitrofurantoin has been found from this study to be the drug of choice for urinary tract bacteria biofilm infections.

The Multiple Antibiotic Resistance Index of biofilm forming bacteria isolated from urine samples as shown in Table 11, revealed that *Staphylococcus* sp, *Escherichia coli, Enterococcus* sp, *Bacillus* sp, *Pseudomonas* sp and *Proteus* sp had multidrug resistance index of 100% respectively. multidrug resistance index values greater than 0.2 indicate a high risk where antibiotics are often used [34,23]. This increase in resistance might result from poorly guided and frequent use of antimicrobial prophylaxis and empiric therapy with cephalosporin in the last few years contributing to this likely rise in Cefuroxime, Ceftazidime and Ciprofloxacin resistance [35].

3.8 Antibiotic Pattern of Non-Biofilm Producing Bacteria Isolated from Urine Samples

The result of the antibiotic pattern of *Staphylococcus* sp as shown in Table 12 indicates that greater number of *Staphylococcus* sp were susceptible to Gentamycin (100%), Ofloxacin and Erythromycin (80%), followed by Cefuroxime and Ceftriaxone (60%). *Staphylococcus* sp showed complete resistance to Ceftazidime (100%)

Table 16. Antibiotic Pattern of Non-Biofilm Producing Bacteria Isolated from Urine

Antibiotics	Conc.	*Serratia* sp			*Klebsiella* sp		
		R	I	S	R	I	S
	(µg)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)
OFL	5	1(50.0)	0(0.00)	1(50.0)	0(0.00)	2(50.0)	2(50.0)
AUG	30	0(100)	2(100)	0(0.00)	1(25.0)	1(25.0)	2(50.0)
CAZ	30	2(100)	0(0.00)	0(0.00)	4(100)	0(0.00)	0(0.00)
CRX	30	1(50.0)	0(0.00)	1(50.0)	0(0.00)	2(50.0)	2(50.0)
GEN	10	0(0.00)	1(50.0)	1(50.0)	2(50.0)	0(0.00)	2(50.0)
NIT	300	1(50.0)	1(50.0)	0(0.00)	0(0.00)	1(25.0)	3(75.0)
CPR	5	0(0.00)	1(50.0)	1(50.0)	1(25.0)	2(50.0)	1(25.0)
CXM	5	1(50.0)	0(0.00)	1(50.0)	2(50.0)	0(0.00)	2(50.0)
IMP	30	0(0.00)	2(100)	0(0.00)	1(50.0)	0(0.00)	3(75.0)

KEY: (AU) Augmentin, (CAZ) Ceftazidime, (CRX) Cefuroxime, (CTR) Ceftriaxone, (ERY) Erythromycin, (CXC) Cloxacillin, (NIT) Nitrofurantoin (CXM) Cefixime, (OFX) Ofloxacin, (GEN) Gentamycin, (CPX) Ciprofloxacin, (IMP) Imipenem

Table 17. MAR Index of Non-Biofilm Producing Bacteria Isolated from Urine

MAR	*Staphylococcus* sp N=5	*Enterococcus* sp N= 6	*Serratia* sp N=2	*Klebsiella* sp N=4	*Streptococcus* sp N=2	*Escherichia coli* N=3
0.1	2(40.0)	1(16.7)	0(0.00)	1(25.0)	0(0.00)	1(50.0)
0.2	2(40.0)	0(0.00)	1(50.0)	0(0.00)	0(0.00)	0(0.00)
0.3	1(20.0)	2(33.3)	0(0.00)	0(0.00)	2(100)	0(0.00)
0.4	0(0.00)	3(50.0)	1(50.0)	1(25.0)	0(0.00)	1(50.0)
0.5	0(0.00)	0(0.00)	0(0.00)	2(50.0)	0(0.00)	0(0.00)
0.6	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
0.7	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
0.8	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
0.9	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
1.0	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
MAR%	**60**	**83.3**	**100**	**50**	**100**	**50**

Table 18. Distribution of biofilm formers and ESBL and MBL producers

Organisms No.(%)	Biofilm formers No. (%)	ESBL Producers No. (%)	MBL Producers No. (%)	ESBL and MBL Producers No. (%)	ESBL/MBL and Biofilm Producers No. (%)
Staphylococcus sp 15	10(66.7)	7(70.0)	4(40.0)	2(20.0)	2(20.0)
Escherichia coli 7	4(57.1)	2(50.0)	3(75.0)	1(25.0)	1(25.0)
Enterococcus sp 12	6(50.0)	4(66.7)	2(33.3)	2(33.3)	2(33.3)
Bacillus sp 6	6(100)	4(66.7)	2(33.3)	1(16.7)	1(16.7)
Proteus sp 4	4(100)	4(100)	1(25.0)	1(25.0)	1(25.0)
Pseudomonas sp 6	6(100)	3(50.0)	2(33.3)	2(33.3)	2(33.3)
Total 50	36(62.1)	24(66.7)	15(41.7)	9(25)	9(25)

Key: Extended spectrum beta-lactamase (ESBL), Metalo beta-lactamase (MBL)

The antibiotic pattern of *Enterococcus* sp as shown in Table 13 indicates that greater number of *Enterococcus* sp were susceptible to Gentamycin (100%), Ofloxacin and Erythromycin (83.3%) followed by Imipenem (50%), Augmentin and Cefuroxime (33.3). *Enterococcus sp* showed a decreasing trend of resistance in the order: Ceftazidime (100%) followed by Cloxacillin, Cefuroxime and Ceftriaxone (50%).

Escherichia coli isolates as shown in Table 14 indicates that greater number of the *Escherichia coli* were susceptible to Ofloxacin, Ciprofloxacin and Gentamycin (100%) Followed by Cefexime, Nitrofurantoin, Augmentin and Imipenem (66.6%). The effect of gentamycin belonging to aminoglycosides group is not surprising because it is known to work against gram negative bacteria including *E*. *coli*, by binding to their ribosomes and inhibiting protein synthesis [36]. *Escherichia coli* showed complete resistance to Ceftazidime (100%).

The result of the antibiotic pattern of *Streptococcus* sp as shown in Table 15 indicates that greater number of *Streptococcus* sp were susceptible to Ofloxacin and Imipenem (100%) Followed by Erythromycin, Cloxacillin, Cefuroxime and Gentamycin (50%). *Streptococcus* sp showed a decreasing trend of resistance in order: Ceftazidime (100%), Augmentin, Cefuroxime, Ceftriaxone and Cloxacillin (50%). The observed resistance to Ceftazidime contradicts the findings of [37], Were *Streptococcus* sp was identified to be susceptible to Ceftazidime.

Serratia sp as shown in Table 16 indicates that greater number of the *Serratia sp* were susceptible to Ofloxacin, Ciprofloxacin, Cefexime Gentamycin and Cefuroxime (50%). *Serratia sp* showed resistance to: Ceftazidime (100%), Ofloxacin, Cefuroxime, Nitrofurantoin and Cefexime (50%).

The susceptibility pattern of *Klebsiella* sp as shown in Table 16 indicates that greater number of *Klebsiella* sp were susceptible to Imipenem and Nitrofurantoin (75%), Ofloxacin, Gentamycin and Cefexime (50%). *Klebsiella* sp showed resistance to: Ceftazidime (100%), followed by Gentamycin and Cefexime (50%).

The Multiple antibiotic resistance Index of non-biofilm forming bacteria isolated from urine samples as shown in Table 17 shows that *Staphylococcus* sp*, Enterococcus* sp, Serratia sp, *Klebsiella* sp, *Streptococcus* sp and *Escherichia coli* had multidrug resistance index of 60%, 83.3%, 100%, 50% , 100%, 50%, respectively. Multiple antibiotic resistance index values in this study were greater than 0.2, indicating a high risk as antibiotics are indiscriminately used by patients whose samples were taken.

3.9 Distribution of Biofilm Formers, ESBL and MBL Producers

A total of 36(62.1%) bacterial isolates that tested positive to biofilm production were screened for extended spectrum beta- lactamase(ESBL) and metallo beta-lactamase (MBL) as shown in Table 18. Out of which 24(66.7%) and 15(41.7%) were confirmed as ESBL and MBL producers. *Proteus* sp was detected as ESBL producers showing comparatively higher incidence of (100%) followed by *Staphylococcus* sp (70%), *Enterococcus* sp and *Bacillus* sp (66.7%), *Pseudomonas* sp (50%) and *Escherichia coli* (50%). *Escherichia coli* was identified to be the highest MBL producers i.e., 75% closely followed by *Staphylococcus* sp (40%) which is in conformity with the data of [38]. Other bacterial

isolates such as *Bacillus* sp, *Enterococcus* sp, *Pseudomonas* sp and *Proteus* sp phenotypically expressed the presence of MBL enzyme as (33.3%), (33.3%), (33.3%) and (25%), respectively. The coexistence of biofilm along with both beta-lactamases producing strains was found to be (25%). The biofilm matrix enhances the expression of resistant genes like beta-lactamases. This is in accordance with the findings of [39]. The result of ESBL integration with MBL production and biofilm production revealed that high ESBL producers were biofilm bacteria and there was significant relationship between ESBL and biofilm formation ($\chi 2 = 3.08$, P-value = 0.002). This is contrary to the findings of [40]. The significant association between ESBL and bacterial biofilm production observed in this study is probably because most biofilm producing bacteria isolated in this study were positive to extended spectrum beta-lactamases production. The link between MBL production and biofilm production was found to be statistically significant. ($\chi 2 = 3.83$, P-value = 0.001).

3.10 Molecular Identification of Resistant and Biofilm Gene in Biofilm Producing Bacteria

Molecular studies confirmed the identification of isolates as *Escherichia coli*, *Pseudomonas aeruginosa* and *Enterococcus faecalis* respectively. The results revealed the gene identification of PapC, CTX-M, ICAD and TET A in the genomic DNA of *Escherichia coli, Pseudomonas aeruginosa* and *Enterococcus faecalis*. TET A gene variant of the bacteria provides resistance to the antibiotic Tetracycline. Tetracycline resistance is widespread among gram-positive and gram-negative bacteria and can be the result of drug efflux by bacteria, before reaching its site of action, protecting the ribosomal binding site, which reduces drug binding [41]. CTX-M enzymes are a group of class A ESBLs which when present in bacteria, generally confer higher levels of resistance against beta lactam antibiotics such as Cefotaxime, Ceftriaxone and Ceftazidime. Identification of CTX-M gene in the genomic studies of biofilm bacteria is in line with the ESBL phenotypic screening which revealed its presence. A similar result was recorded in [42]. Presence of CTX-M of ESBLs is often associated with phenotypes of resistance especially to fluoroquinolones and aminoglycosides. Biofilm polysaccharide intercellular adhesin synthesis protein ICAD gene is an adhesion gene and it was prevalent in all the genomic DNA, of the biofilm Production bacteria analysed in genomic studies.

4. CONCLUSION

The significant increase in biofilm strains and antibiotic resistant bacteria in this study provides a glimpse of the future threat. General monitoring of biofilm production and beta-lactamases; therefore, can be recommended in clinical laboratories as well as strong implementation of infection control and prevention activities. The study revealed that a higher number of antibiotics were resistant to biofilm producing bacteria as compared with non-biofilm producers. The prevalence of biofilm and non-biofilm bacteria isolated from patients urine suggests possible urinary tract infections which can be symptomatic or asymptomatic depending on the severity. Biofilm and non-biofilm bacterial were 100% resistant to Ceftazidime (third generation cephalosporin). Ofloxacin, Gentamycin, Imipenem and Nitrofurantoin have been found from this study to be the drug of choice for bacteria biofilm urinary tract infections whose etiological agents are *Staphylococcus* sp*, Escherichia coli, Enterococcus* sp*, Proteus* sp*, Bacillus* sp and *Pseudomonas.* This study also showed that the result revealed from the phenotypic screening of extended spectrum beta lactamases correlates well with the genomic method of extended spectrum beta lactamases gene detection. Thus this method can be adopted in resource limited settings for the detection of extended spectrum beta lactamases.

5. RECOMMENDATION

Antibiotic susceptibility testing should be done on all clinical samples in cases of infection before treatment. This will help proper prescriptions and reduce the level of bacterial resistance.

Ongoing efforts should be made to monitor hospitals, infection control and clinical trials to combat the rapid development of antibiotic resistant bacteria.

Hygienic conditions should be ensured between health care providers and patients to prevent the transmission of bacterial biofilm infections among patients.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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