Research Article ISSN 2639-9458

Microbiology & Infectious Diseases

Isolation of Biofilm Producing Bacteria from Stool Samples and Their Antibiogram

Salome Ibietela Douglas*, Janet Olufunmilayo Williams, and Golden Chukwuma Onyedibia

Department of Microbiology, Faculty of Science, Rivers State University, PMB 5080, Nkpolu-Oroworukwo Port Harcourt, Rivers State, Nigeria.

***Correspondence:**

Douglas, Department of Microbiology, Faculty of Science, Rivers State University, PMB 5080, Nkpolu Oroworukwo Port Harcourt, Rivers State, Nigeria.

Received: 02 Jan 2022; **Accepted:** 26 Jan 2022; **Published:** 03 Feb 2022

Citation: Douglas SI, Williams JO, Onyedibia GC. Isolation of Biofilm Producing Bacteria from Stool Samples and Their Antibiogram. Microbiol Infect Dis. 2022; 6(1): 1-9.

ABSTRACT

Aim: *Bacterial biofilm formation is a menacing attribute that has been linked to a rise in antibiotic resistance. The aim of this study was to isolate biofilm-producing bacteria from stool samples and their antibiogram.*

Study Design: *The study involved laboratory research, statistical analysis and interpretation of the data.*

Place and Duration of Study: *The research was carried out in three (3) hospital facilities. They are University of Port Harcourt Teaching Hospital (UPTH), Meridian Hospital D/line branch (MRD1), and Meridian Hospital Ikoku branch (MDR2). Specimens were gathered within three (3) months, and analyses were performed.*

Methodology: *45 stool specimens were collected from the three (3) hospitals. The specimens were correctly labelled with the sampling date and time. Standard microbiological procedures were performed on the collected specimens, including plate counts, identification, biofilm screening, sensitivity testing, extended spectrum beta lactamase phenotypic screening, and molecular characterization of the isolates.*

Results: *The total heterotrophic bacterial counts ranged from 6.2 to 8.2 x10^7cfu/g, total coliform counts ranged from 3.2 to 4.1 x10^6cfu/g and faecal coliform counts ranged from 1.3 to 1.4 x10^5cfu/g. There was no significant difference at ($p \leq 0.05$) in the total heterotrophic bacterial counts, total and faecal coliform counts between the hospitals sampled. A total of Thirty-two (32) bacterial isolates were identified in stool specimens, with 20 (62.5%) of them being biofilm producers. Staphylococcus 30%, 35% Escherichia coli, 25% Enterococcus and 10% Bacillus species were detected among biofilm bacteria. Biofilm isolates showed a variety of susceptibility patterns and antibiotic resistance was found in biofilm bacteria. Most bacterial intestinal tract infections from patients and hospitals investigated can be treated with ofloxacin, Gentamycin, Imipenem, and Nitrofurantoin. TET A and CTX-M genes, were reported in Bacillus subtilis and Escherichia coli biofilm bacteria as possible genes that could confer antibiotic resistance.The existence of the icaD and papC genes in Bacillus subtilis and Escherichia coli has been discovered to be probable determinants that impart biofilm forming abilities, according to genomic studies.*

Conclusion: *The existence of biofilm-producing bacteria in patients' stools, as well as their antibiotic resistance, was observed in this study. Ceftazidime (third generation cephalosporin) resistance was found in both biofilm and non-biofilm bacteria. This research reveals that ofloxacin, Gentamycin, Imipenem, and Nitrofurantoin are the drugs of choice for bacterial intestinal tract infections caused by Escherichia coli, Staphylococcus, Enterococcus, and Bacillus species. As a result, proper infection control techniques and therapeutic recommendations for proven infections should be swiftly implemented.*

Keywords
Biofilm, Stool sample, Antibiogram, Genes.

Introduction
Biofilms are microbial communities that are represented in an indiscriminate manner by cells that are firmly related to one another. Biofilm formation occurs when bacteria cling to surfaces and multiply while creating external polymers that facilitate fastening and matrix formation [1]. Microorganisms are considered responsible for about 65% of bacterial infections when they form biofilms [2], and are naturally heterogeneous. When bacteria of the same type are stationary, they act and behave differently than when they are planktonic [3]. One of the most important of these is the rapid development of resistance to antibiotics that are intended to kill them [4]. The pace diffusion of an antibiotic treatment aimed at a biofilm is slowed by the extracellular polymeric material found in biofilm formation [5]. This is accomplished by the extracellular polymeric material interacting with antibiotics or limiting diffusion [6].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 are just a few of the beneficial interactions found in a biofilm coterie [7].

Swarna *et al*. [8] found that microorganism's ability to form biofilm is a significant pathogenic property and a primary cause of many chronic diseases. Biofilm-forming bacteria have been linked to chronic gastrointestinal infections [9]. Due to the huge surface area and frequent nutrition intake, the intestinal tract is a perfect environment, suitable for microorganisms and biofilms [9]. The intestinal tract is lined with viscoelastic mucus for protection, but it can be damaged by severe inflammation, Crohn's disease, ulcerative colitis and other conditions [10]. This creates the liberty for bacteria to attach to the surface and begin their formation within biofilm. The epithelium attached to it is flexible and often damaged [11]. Antibiotic treatment for infections caused by clinical biofilm has been evaluated, and it has been found that in most cases, antibiotic treatment does not give complete relief, as symptoms commonly reappear even after treatment. Free-living cells are killed by antibiotics, while sessile forms are resistant and spread within biofilms [12]. In recent years, biofilm producers have shown an increased tolerance to antibiotics [13]. Genetic mutations, phenotypic resistance, stress tolerance, quorum sensing, genetic gradients, oxidative stress, antibiotic failure and heterogeneity may all contribute to their antimicrobial resistance [14].

Modern antibiotic treatment guidelines neglect differences in the ecological dynamics of different bacteria [15]. Antibiotic resistance has been attributed to the presumption that they will eradicate the same type of bacteria regardless of where they are found. Antibiotics at lower concentrations than the minimal concentration can cause biofilm development in a variety of bacterial strains [16]. This is so because cells within the biofilm may be exposed to significant amounts of antibiotics. Antibiotics, rather than limiting biofilm formation, seem to enhance it [17]. The need to control bacterial infections induced by these biofilms, as well as new drug delivery techniques, emerge [18]. The purpose of this study was to isolate biofilm-producing bacteria from stool samples and their antibiogram.

Materials and Methods
Research Area
This research was carried out in three (3) facilities within Port Harcourt Metropolis, Rivers State: University of Port Harcourt Teaching Hospital (UPTH), Meridian Hospital D/line branch (MRD1), and Meridian Hospital Ikoku branch (MRD2).

Specimen Collection
Forty five (45) stool specimens were collected from the three hospitals over the course of three (3) months. The stool specimens were labelled with the date and time of collection and transferred aseptically to the Department of Microbiology Laboratory, Rivers State University. The samples were prepared according to the Clinical Laboratory Standard Institute's Procedures [19]. Nutrient Agar, MacConkey Agar, Cysteine Lactose Electrolyte Deficient medium (CLED), Mannitol Salt Agar, Cetrimide Agar, Bile Esculin Agar, Salmonella-Shigella Agar, and Eosin Methylene Blue (EMB) Agar were used as culture media. The manufacturer's instructions were followed for preparing these media.

Bacteriological Analysis
Standard microbiological techniques were used to isolate the bacteria. Individual media were used to culture the specimens. On the stool specimens, a tenfold serial dilution was performed, in which 1g of faeces was transferred to 9ml of sterile normal saline and subsequent dilutions were made up to 10^{-6}. The spread plate technique was used in duplicate. An aliquot (0.1ml) of appropriate dilutions (10^{-4} to 10^{-6}) on Nutrient Agar, MacConkey Agar, Eosin Methylene Blue plates, Bile Esculin Agar, Cysteine Lactose Electrolyte Deficient Agar (CLED), Salmonella-Shigella Agar, and Mannitol Salt Agar plates. For 18 to 24 hours, the plates were incubated at 37°C. The colonies that developed on the plates were counted and morphologically described. Distinct colonies were sub-cultured to obtain pure isolates.

Bacterial cultures were examined for different colonies and were subcultured onto sterile solid plates. Characterization and identification of pure cultures were done using colony morphology, microscopic features, and several specific biochemical tests [6]. Matching features with existing taxa in standard guides such as The Manual for Identification of Medical Bacteria [20] and Bergy's Manual of Determinative Bacteriology were used to identify the bacteria [21]. For further identification and confirmation of the bacterial isolates identified to species level, a molecular technique (PCR) was used.

Bacterial isolates obtained from the specimens were tested for their biofilm producing capacity using the Congo red test method

[22]. Using Congo Red Agar (CRA) Medium, Freeman *et al*., [22] provided a qualitative approach for detecting biofilm development among bacterial isolates. The test organisms were inoculated onto Congo Red Agar and incubated for 24 hours at 37°C. A positive test for biofilm generation is the growth of black crystalline colonies.

The Mueller-Hinton agar was prepared according to the manufacturer's instructions and sterilized at 121°C for 15 minutes at 15 pounds per square inch in an autoclave. To avoid misleading readings of the zones of inhibition, the pH of the medium was determined to be 7.2 and put into the proper depth in the Petri dish. A sterile swab stick was immersed in the test tube containing the bacterial suspension, and the turbidity was equivalent to 0.5m McFarland Turbidity Standard. The swab was used to swab the surface of the agar in the Petri dish evenly, which contained already prepared Mueller- Hinton agar in three dimensions, while rotating the plates to about 60ºC to ensure even distribution of the organism. For about 3-5 minutes, the agar was allowed to dry. The impregnated antimicrobial discs were placed on the surface of the inoculated plates using sterile forceps. Each disc was slightly pressed down with the head of the forceps to create contact with the agar. After adding the disc, the plates were incubated aerobically at 35°C for 16-18 hours in an inverted orientation. The test plates were examined after incubation to detect the zones of inhibition [19].The diameter of each zone of inhibition was measured in mm using a ruler and documented for reference.

The presence or absence of extended spectrum beta-lactamases (ESBL) enzyme was determined by phenotyping isolates. The disc diffusion test was used to check for ESBL production in ceftazidime-resistant bacteria. ESBL positive was defined as a greater than 5mm increase in the zone diameter between ceftazidime (30µg) and ceftazidime-clavulanate (30/10µg) Yong et al., 2002.

The presence or absence of the Metallo beta-lactamases (MBL) enzyme was determined by phenotyping isolates. MBL generation was assessed in imipenem-resistant bacteria using a combination disc-diffusion assay with two imipenem discs, one of which had 10µl of 0.5 M EDTA added to it. The presence of an enhanced zone of inhibition of greater than7mm surrounding the imipenem-EDTA disc compared to the zone size of the imipenem disc alone was validated as positive for MBL generation [23].

Results and Discussion

The total heterotrophic bacterial counts ranged from 6.2 to 8.2 x10^7cfu/g in the stool specimen from the hospitals. The total heterotrophic bacterial counts did not differ significantly ($p \leq 0.05$) between the hospitals studied. Total coliform counts ranged from 3.2 to 4.1 x10^6cfu/g. Faecal coliform counts ranged from 1.30 to 1.36 x10^5cfu/g. Humans have a complex colony of gut microbiota living in their intestine. The bacterial burden in the stomach is substantially lower, but it exponentially increases when bacteria-contaminated substances are consumed. When microorganisms get access to the intestines, they continue to multiply and proliferate [24]. The observed variation in bacterial population in stool specimens obtained from patients across different hospitals in this study could be due to the patients' level of exposure to contaminated substances and the duration of intestinal infections, as long-term infections have a higher bacterial population [25].

The identities of isolates are revealed based on their colonial, morphological and biochemical characteristics. Thirty-Two (32) bacterial isolates belonging to the following genera were identified as; 9 (28.1) *Escherichia coli*, 2 (6.3) *Bacillus,* 8 (25) *Enterococcus*, 9 (28.1) *Staphylococcus,* 2 (6.3) *Salmonella* and 2 (6.3) *Shigella* species as. Tables 1 to 4 show the antimicrobial patterns of the individual biofilm bacterial isolates, including; *Staphylococcus, Enterococcus, Bacillus,* and *Escherichia coli* species. Antimicrobial patterns of non-biofilm bacterial isolates comprising *Staphylococcus* sp., *Enterococcus* sp., *Escherichia coli*, *Salmonella* and *Shigella* species are shown in Tables 5 to 9. The isolates antibiogram profiles were classified as sensitive, intermediate or resistant.

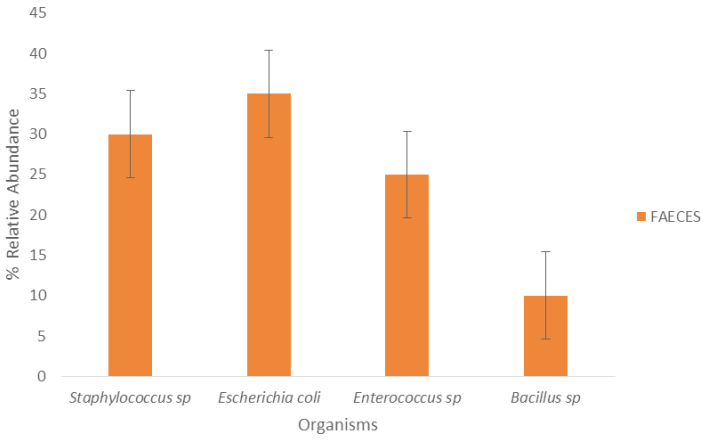

Figure 1: Percentage Relative Abundance of Biofilm Producing Bacteria Isolated from specimen.

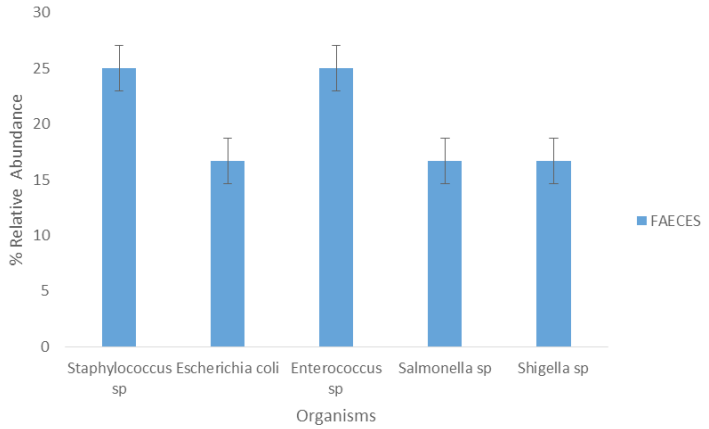

Figure 2: Percentage Relative Abundance of Non-Biofilm Producing Bacteria Isolated from specimen.

Table 1: Antibiotics Resistance Pattern of Biofilm Producing *Staphylococcus* sp Isolated from Faeces.
N=6

Antibiotics	Concentration (µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
OFL	5	2 (33.3)	0 (0.00)	4 (66.7)
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	1 (16.7)	2 (33.3)	3 (50.0)
CTR	30	6 (100)	0 (0.00)	0 (0.00)
ERY	5	4 (66.7)	0 (0.00)	2 (33.3)
CXC	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 2: Antibiotics Resistance Pattern of Biofilm Producing *Enterococcus* sp Isolated from Faeces.
N=5

Antibiotics	Concentration (µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
OFL	5	0 (0.00)	1 (20.0)	4 (80.0)
AUG	30	5 (100)	0 (0.00)	0 (0.00)
CAZ	30	5 (100)	0 (0.00)	0 (0.00)
CRX	30	5 (100)	0 (0.00)	0 (0.00)
GEN	10	1 (20.0)	0 (0.00)	4 (80.0)
CTR	30	5 (100)	0 (0.00)	0 (0.00)
ERY	5	2 (40.0)	2 (40.0)	1 (20.0)
CXC	5	5 (100)	0 (0.00)	0 (0.00)
IMP	30	2 (40.0)	1 (20.0)	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 3: Antibiotics Resistance Pattern of Biofilm Producing *Bacillus* sp Isolated from Faeces.
N=2

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

Table 4: Antibiotics Resistance Pattern of Biofilm Producing *Escherichia coli* Isolated from Faeces.
N=7

Antibiotics	Concentration (µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
OFL	5	1 (14.3)	1 (14.3)	5 (71.4)
AUG	30	4 (57.1)	3 (42.9)	0 (0.00)
CAZ	30	7 (100)	0 (0.00)	0 (0.00)
CRX	30	7 (100)	0 (0.00)	0 (0.00)
GEN	10	1 (14.3)	3 (42.9)	3 (42.9)
NIT	300	2 (28.6)	1 (14.3)	4 (57.1)
CPR	5	5 (71.4)	2 (28.6)	0 (0.00)
CXM	5	4 (57.1)	2 (28.6)	1 (14.3)
IMP	30	1 (14.3)	2 (28.6)	4 (57.1)

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 5: Antibiotics Resistance Pattern of Non-Biofilm Producing *Staphylococcus* sp Isolated from Faeces.
N=3

Antibiotics	Concentration (µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
OFL	5	0 (0.00)	1 (33.3)	2 (66.7)
AUG	30	1 (33.3)	1 (33.3)	1 (33.3)
CAZ	30	3 (100)	0 (0.00)	0 (0.00)
CRX	30	0 (0.00)	1 (33.3)	2 (66.7)
GEN	10	0 (0.00)	0 (0.00)	3 (100)
CTR	30	0 (0.00)	1 (33.3)	2 (66.7)
ERY	5	1 (33.3)	0 (0.00)	2 (66.7)
CXC	5	0 (0.00)	2 (66.7)	1 (33.3)
IMP	30	1 (33.3)	1 (33.3)	1 (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.

Table 6: Antibiotics Resistance Pattern of Non-Biofilm Producing *Enterococcus* spIsolated from Faeces. **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 (66.7)
CAZ	30	3 (100)	0 (0.00)	0 (0.00)
CRX	30	1 (33.3)	2 (66.7)	0 (0.00)
GEN	10	0 (0.00)	0 (0.00)	3 (100)
CTR	30	0 (0.00)	3 (100)	0 (0.00)
ERY	5	0 (0.00)	2 (66.7)	1 (33.3)
CXC	5	0 (0.00)	2 (66.7)	1 (33.3)
IMP	30	1 (33.3)	0 (0.00)	2 (66.7)

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 7: Antibiotics Resistance Pattern of Non-Biofilm Producing *Escherichia coli* Isolated from Faeces. **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)	0 (0.00)	1 (50.0)
CAZ	30	2 (100)	0 (0.00)	0 (0.00)
CRX	30	1 (50.0)	1 (50.0)	0 (0.00)
GEN	10	0 (0.00)	0 (0.00)	2 (100)
NIT	300	0 (0.00)	1 (50.0)	1 (50.0)
CPR	5	0 (0.00)	0 (0.00)	2 (100)
CXM	5	1 (50.0)	0 (0.00)	1 (50.0)
IMP	30	0 (0.00)	1 (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 8: Antibiotics Resistance Pattern of Non-Biofilm Producing *Salmonella* sp Isolated from Faeces.

Antibiotics	Concentration (µg)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
OFL	5	0 (0.00)	0 (0.00)	4 (100)
AUG	30	2 (50.0)	1 (25.0)	1 (25.0)
CAZ	30	4 (100)	0 (0.00)	0 (0.00)
CRX	30	1 (25.0)	2 (50.0)	1 (25.0)
GEN	10	2 (50.0)	0 (0.00)	2 (50.0)
NIT	300	1 (25.0)	0 (0.00)	3 (75.0)
CPR	5	1 (25.0)	1 (25.0)	2 (50.0)
CXM	5	2 (50.0)	0 (0.00)	2 (50.0)
IMP	30	0 (0.00)	3 (75.0)	1 (25.0)

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: Antibiotics Resistance Pattern of Non-Biofilm Producing *Shigella* sp isolated from faecal Samples.

Antibiotics	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
OFL	0 (0.00)	0 (0.00)	2 (100)
AUG	1 (50.0)	1 (50.0)	0 (0.00)
CAZ	2 (100)	0 (0.00)	0 (0.00)
CRX	2 (100)	0 (0.00)	0 (0.00)
GEN	0 (0.00)	0 (0.00)	2 (100)
NIT	1 (50.0)	0 (0.00)	1 (50.0)
CPR	0 (0.00)	1 (50.0)	1 (50.0)
CXM	0 (0.00)	0 (0.00)	2 (100)
IMP	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.

Table I0: 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 9	6 (66.7)	4 (44.4)	2 (22.2)	1 (11.1)	1 (11.1)
Escherichia coli 9	7 (77.8)	5 (55.6)	3 (33.3)	1 (11.1)	1 (11.1)
Enterococcus sp 8	5 (62.5)	3 (37.5)	2 (25)	2 (25)	2 (25)
Bacillus sp 2	2 (100)	1 (50.0)	2 (100)	1 (50.0)	1 (50.0)
Total 32	20 (62.5)	13 (40.6)	9 (28.1)	5 (15.6)	5 (15.6)

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

From stool samples collected from the three (3) hospitals, a total of thirty-two (32) species of bacteria were identified. The relative abundance of *Escherichia* coli (28.1%), *Staphylococcus* (28.1%), *Enterococcus* (25%), *Bacillus* (6.3%), *Salmonella* (6.3%), and *Shigella* (6.2%) species were detected in the stools of patients. Meridian Hospital D/Line had the highest relative abundance13 (40.6%), University of Port Harcourt Teaching Hospital had the relative abundance 10 (31.3%), and Meridian Hospital Ikoku had the lowest relative abundance 9 (28.1%). The observed variation in bacterial populations in stool specimens obtained from patients across the different hospitals in this study may be due to the patients' level of exposure to contaminated substances and the duration of intestinal infections, as long-term infections have a higher bacterial population [25].

Biofilm producers were identified in 20 (62.5%) isolates. This corresponds to the findings of [26], who found 64.28% of biofilm-producing bacteria. Since biofilm, bacterial isolates had adhering features like flagella that assist movement to receptor sites, and they were able to form biofilms (substratum). The human digestive system has a diverse and abundant microbiota, which helps bacteria stick together [27]. The human digestive system offers the necessary conditions for the formation of bacterial biofilm communities, such as mucus composition, adhesion sites along the tract, and gut motility [28].This observation is likewise in line with the findings of Macfarlene *et al.,* [27]. As indicated in Figure 1, the biofilm bacterial isolates were distributed as *Escherichia coli* 21.9 %, *Enterococcus* 15.6 %, *Bacillus* 6.25 %, and 18.8 % *Staphylococcus* species. The identification of *Staphylococcus* as a biofilm bacteria isolated from faeces is in line with the findings of Ponnusamy *et al.,* [29]**.**

Non-biofilm producers were found in a total of 12 (37.5%): *Escherichia coli* 6.3 %, *Staphylococcus* 9.4 %, *Enterococcus* 9.4 %, *Salmonella* 6.3 %, and 6.3% *Shigella* species as illustrated in Figure 2, are non-biofilm producers. Biofilm and non-biofilm potential was identified in bacterial isolates such as *Escherichia coli*, *Staphylococcus*, and *Enterococcus*. The strain types and genetic makeup of bacterial isolates of the same genera differ in their potential to produce biofilms. The biofilm potential of certain bacteria is acquired by the transfer of genetic information among bacterial isolates in a biofilm community. Irrespective of their inability to produce biofilm, they are infectious to humans [30].

The results of the antibiotic pattern of *Staphylococcus* sp as shown in Table 1 indicates that greater number of the *Staphylococcus* spp were susceptible to Ofloxacin (66.7%) followed by Gentamycin and Imipenem (50%). *Staphylococcus* sp showed complete resistance to Ceftazidime, Augmentin, Cefuroxime, Ceftriaxone and Cloxacillin (100%). The observed susceptibility of *Staphylococcus* sp to Ofloxacin was also in accordance with the report of Amadi *et al.* [31] and Uwazuoke *et al*. [32]. High sensitivity to gentamycin in this present study compares favorably with the reports of Ndip *et al*. [33].Their resistance to Augmentin, Ceftazidime, Cloxacillin, Ceftriaxone and Cefuroxime was 100%. The resistance to Cloxacillin in this study contradicts with the findings of Ndip *et al*. [33], which revealed that Cloxacillin was highly recommended in staphylococcal infections.

The results of the antibiotic pattern of *Enterococcus* sp as shown in Table 2 indicates that greater number of the *Enterococcus* sp were susceptible to Ofloxacin and Gentamycin (80%).The high sensitivity to gentamycin as seen in this study is in agreement with the findings of Monika *et al*. [34]. *Enterococcus* sp showed resistance to Augmentin, Ceftazidime, Cefuroxime, Ceftriaxone and Cloxacillin (100%).

The antibiotic pattern of *Bacillus* spas shown in Table 3 indicates that greater number of *Bacillus* sp were susceptible to Ofloxacin (100%) followed by Gentamycin, Erythromycin and Imipenem (50%). *Bacillus* sp showed complete resistance to Ceftazidime, Augmentin, Cefuroxime and Ceftriaxone (100%).

Antibiotic pattern of *Escherichia coli* as shown in Table 4 indicates that greater number of *Escherichia coli were* susceptible to Ofloxacin (71.4%) followed by Nitrofurantoin and Imipenem (57.1%). Susceptibility to Ofloxacin in this study concurs with the findings of Niranjan *et al*. [35]. *Escherichia coli* showed complete resistance to Ceftazidime and Cefuroxime and (100%).

The fundamental issue with biofilm-forming bacteria-caused infections is the low susceptibility of bacteria to the antimicrobials applied [36]. The high resistance of biofilm bacteria to the beta-lactam antibiotics Ceftazidime, Cefixime, and Cefuroxime found in this study could be attributed to their abuse and the acquisition of blaCTX, blaSHV, and blaTEM [37]. The drugs of choice for intestinal tract bacteria biofilm infections caused by *Escherichia coli*, *Staphylococcus*, *Enterococcus*, and *Bacillus* species were determined to be Ofloxacin, Gentamycin, Imipenem, and Nitrofurantoin in this research.

The Multiple Antibiotic Resistance Index of biofilm forming bacteria isolated from stool specimen revealed that *Staphylococcus* sp, *Escherichia coli, Enterococcus* sp and *Bacillus* sp, had multidrug resistance index of 100%. Multidrug resistance index values greater than 0.2 indicate a high risk where antibiotics are often used [38].This surge in resistance could be due to the poorly guided and frequent use of antibiotic prophylaxis and empiric cephalosporin therapy in recent years, which has likely contributed to the rise in Cefuroxime, Ceftazidime, and Ciprofloxacin resistance [39].

Antibiotics resistance pattern of non-biofilm producing bacteria Isolated from faecal samples revealed that the results of the antibiotic pattern of *Staphylococcus* sp as shown in Table 5 indicates that greater number of *Staphylococcus* sp were susceptible to Gentamycin (100%), Ofloxacin, Cefuroxime, Ceftriaxone and Erythromycin (66.7%), followed by Imipenem, Augmentin and Cloxacillin (33.3). *Staphylococcus* sp showed complete resistance to Ceftazidime (100%).

The antibiotic pattern of *Enterococcus* sp as shown in Table 6 indicates that greater number of *Enterococcus* sp were susceptible to Gentamycin and Ofloxacin (100%), followed by Imipenem and Augmentin (66.7%). *Enterococcus sp* showed complete resistance to Ceftazidime (100%).

Escherichia coli isolatesas shown in Table 7 indicates that greater number of *Escherichia coli* were susceptible to Ofloxacin, Cefexime and Gentamycin (100%). Followed by Nitrofurantoin and Augmentin (50%).The efficacy of gentamycin, which belongs to the aminoglycosides group, is not surprising because it has been shown to function against gram-negative bacteria, such as *Escherichia coli*, by attaching to their ribosomes and suppressing protein synthesis [40]. Ceftazidime resistance was demonstrated in *Escherichia coli* (100%).

The antibiogram pattern of *Salmonella* sp as shown in Table 8 indicates that *Salmonella* was susceptible to Ofloxacin (100%) and Nitrofurantoin (75%).The susceptibility of *Salmonella* sp to Nitrofurantoin correlates with the findings of Pogue *et al.* [41]. Resistance to Augmentin, Gentamycin and Cefexime (50%) was observed in this study.

The sensitivity pattern of *Shigella* sp as shown in Table 9 indicates that a greater number was susceptible to Ofloxacin, Gentamycin and Cefexime (100%). *Shigella* sp showed resistance to Cefuroxime and Ceftazidime (100%).

The Multiple antibiotic resistance Index of non-biofilm forming bacteria isolated from faecal samples shows that *Staphylococcus, Enterococcus*, *Escherichia coli, Salmonella* and *Shigella* species had multidrug resistance index of 66.7%, 66.7%, 50%, 100% , 50%, respectively. Multiple antibiotic resistance index values in this study were greater than 0.2, indicating a high risk as antibiotics is indiscriminately used by patients whose samples were taken.

Extended spectrum beta-lactamase (ESBL) and metallo beta-lactamase (MBL) were screened in 20 (62.5%) of the bacterial isolates that tested positive for biofilm formation. Out of which 13 (40.6%) and 9 (28.1%) were confirmed as ESBL and MBL producers, respectively. *Escherichia coli* were detected as ESBL producers showing comparatively higher incidence of (55.6%), followed by *Bacillus* sp (50%), *Staphylococcus* sp (44.4%), and

Enterococcus sp (37.5%). *Bacillus* sp were identified to produce the highest MBL (100%), followed by *Escherichia* coli (33.3%). Other bacterial isolates, such as *Enterococcus* and *Staphylococcus* sp, phenotypically expressed MBL enzyme at 25% and 22.2%, respectively It was revealed that biofilm and both beta-lactamase-producing bacteria were found to be (15.6 %). The expression of resistance genes such as beta-lactamases is boosted by the biofilm matrix. This is in line with the observations of Donlan, [42]. The integration of ESBL with MBL production and biofilm production revealed that high ESBL producers were biofilm bacteria and there was a high correlation between ESBL and biofilm formation (P-value = 0.001). This contradicts the findings of [43]. Since most biofilm-producing bacteria isolated in this study were positive for extended spectrum beta-lactamases production, there was a notable link between ESBL and bacterial biofilm formation. A statistically significant correlation was revealed between MBL production and biofilm production. (P-value = 0.002, 2 = 2.36).

Bacillus subtilis and *Escherichia coli* were identified using molecular techniques. In the genomic DNA of *Bacillus subtilis* and *Escherichia coli*, the genes PapC, CTX-M, ICAD, and TET A were identified. Tetracy cline resistance is provided by TET A gene variation of the bacteria. Tetracycline resistance is common in both gram-positive and gram-negative bacteria, and it may be caused by bacteria effluxing the drug before it reaches its target, which protects the ribosome-binding site and lowers drug binding [44]. CTX-M enzymes are a subset of class A ESBLs that impart higher levels of resistance to beta- lactam antibiotics such as cefotaxime, ceftriaxone, and ceftazidime when present in bacteria. The detection of the CTX-M gene in genomic examinations of biofilm bacteria corresponds to the existence of the gene in ESBL phenotypic screening. Krumperman, [45] reported a similar finding. The ICAD gene for biofilm polysaccharide intercellular adhesion production is an adhesion gene that was found in the genomic DNA of biofilm-producing *Bacillus subtilis*.

Conclusion

The significant increase in biofilm strains and antibiotic resistant bacteria in this study portends a future menace. As a result, general monitoring of biofilm generation and beta-lactamases, as well as a strong implementation of infection control and prevention actions, might be advised in clinical laboratories. Antibiotic resistance was shown to be higher in biofilm-producing bacteria than in non-biofilm-producing bacteria, according to the study. The presence of biofilm and non-biofilm bacteria in stools of patients shows that biofilm-producing bacteria may cause intestinal tract infections, which can be symptomatic or asymptomatic depending on the severity. Ceftazidime (third generation cephalosporin) resistance was found in both biofilm and non-biofilm bacteria. This research reveals that ofloxacin, Gentamycin, Imipenem, and Nitrofurantoin are the drugs of choice for bacterial intestinal tract infections caused by *Escherichia coli*, *Staphylococcus*, *Enterococcus*, and *Bacillus* species.

This study also found that the results of extended spectrum beta lactamases phenotypic screening correlate well with the genomic approach of detecting extended spectrum beta lactamases genes. As a result, this approach for detecting extended spectrum beta lactamases can be used in resource-constrained settings.

Recommendations

In cases of infection, antibiotic susceptibility testing should be performed on all clinical samples prior to therapy. This will make it easier to administer correct prescriptions and prevent bacterial resistance. To counteract the rapid spread of antibiotic-resistant bacteria, ongoing efforts should be undertaken to monitor hospitals, infection control, and clinical trials. In order to prevent further spread of bacterial biofilm infections among patients, sterile conditions between health care professionals and patients should be ensured.

References

1. Banerjee S, Rai S, Sarma B, et al. Bacterial Biofilm in Water Bodies of Cherrapunjee: The Rainiest Place on Planet Earth. Advanced Microbial. 2012; 2: 465-475.
2. Cook LC, Dunny GM. The Influence of Biofilms in the Biology of Plasmids. Microbiology Spectrum. 2014; 5: 88-82.
3. Bjarnsholt T, Kirketerp Moller K, Kristiansen S, et al. Silver Against Pseudomonas Aeruginosa Biofilms. Pathological Microbiology and Immunological Scandinavica. 2017; 115: 921-928.
4. Mah TC, O'Toole GA. Mechanisms of Biofilm Resistance to Antimicrobial Agents. Trends in Microbiology. 2001; 1: 34-39.
5. Williams I, Venables WA, Lloyd D. The Effects of Adherence to Silicone Surfaces on Antibiotic Susceptibility in Staphylococcus Aureus. Microbiology. 1997; 143: 2407-2413.
6. Washington CW, Stephen DA, William MJ, et al. Koneman's Colour Atlas and Textbook of Diagnostic Microbiology. 2006; 145: 234-239.
7. Hall-Stodley L, Costerton JW, Stoodley P. Bacterial Biofilm from the Natural Environment to Infectious Diseases. National Revised Microbial. 2004; 2: 95-108.
8. Swarna SR, Madhavan R, Gomathi S, et al. A Study of Biofilm on Diabetic Foot Ulcer. International Journal of Pharmaceutical Biological Science. 2012; 4: 1809-1814.
9. Abraham C, Medzhitov R. Interactions between the Host Innate Immune System and Microbes in Inflammatory Bowel Disease. Gastroenterology. 2011; 140: 1729-1737.
10. Mardini HE, Kip KE, Wilson JW. Crohn's disease A Two Year Prospective Study of the Association between Psychological Distress and Disease Activity. Digital District Science. 2004; 49: 492-497.
11. Braun V, Patzer SI. Intercellular Communication by Related Bacterial Protein Toxins: Colicins, Contact Dependent Inhibitors and Proteins Exported by the Type VI Secretion System. FEMS Microbiology Letter. 2013; 345: 13-21.

12. Costerton JW, Stewart PS, Greenberg EP. Bacterial biofilms: a common cause of persistent infections. Science. 1999; 284: 1318-1322.
13. Simoes LC, Simoes M, Oliveira R, et al. Potential of The Adhesion of Bacteria Isolated from Materials. Journal of Basic Microbiology. 2007; 47: 174-183.
14. Hoiby N, Bjarnsholt T, Givskov M, et al. Antibiotic Resistance of Bacterial Biofilms. International Journal of Antimicrobial Agents. 2010; 35: 322-332.
15. Geli P, Laxminarayan R, Dunne M, et al. One-Size-Fits-All. Optimizing Treatment Duration for Bacterial Infections. 2012; 7: 298-312.
16. Strelkova EA, Zhurina MV, Plakunov VK, et al. Antibiotics Stimulation of Biofilm Formation. Mikrobiologiia. 2012; 81: 282-285.
17. Lewis K. Persister Cells and the Riddle of Biofilm Survival. Biochemistry. 2005; 70: 267-274.
18. Freitas AR, Faria AR, Pinto TC, et al. Distribution of Species and Antimicrobial Resistance Among Enterococci Isolated from the Feacal Microbiota of Captive Blue-Fronted Parrot. Brazil Science of the Total Environment. 2018; 6: 1428-1437.
19. Clinical and Laboratory Standard Institute. Performance Standards for Antimicrobial Susceptibility Testing, Twenty-first Informational Supplement. 2017; 30: 68-70.
20. Cowon ES, Steel KJ. Identification of Medical Bacteria 3rdEdition, University Press, University Of Cambridge, Edinburgh, Cambridge, United Kingdom. 1993; 78-84.
21. Buchman RE, Gibbons NE. Bergey's Manual of Determinative Bacteriology. American Journal of Science. 2004; 21: 529-563.
22. Freeman J, Falkiner FR, Keane CT. New Method for Detecting Slime Production by Coagulase Negative Staphylococci. Journal Clinical Pathology. 2009; 42: 872-874.
23. Yong D, Lee K, Yum JH, et al. Imipenem-EDTA Disk Method for Differentiation of Metallo-B-Lactamase-Producing Clinical Isolates of Pseudomonas Spp and Acinetobacter Spp. Journal of Clinical Microbiology. 2002; 40: 3798-3801.
24. Sender R, Fuchs S, Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. Plos Biology. 2016; 14: 45-53.
25. Hopkins MJ, Macfarlane GT. Changes in Predominant Bacterial Populations in Human Faeces with Age and with Clostridium Difficile Infection. Journal of Medical Microbiology. 2002; 51: 448-454.
26. Allam NG. Correlation between Biofilm Production and Bacterial Urinary Tract Infections: New Therapeutic Approach. Egypt Journal of Microbiology. 2017; 52: 39-48.
27. Macfarlane S, Macfarlane GT. Composition and Metabolic Activities of Bacterial Biofilms Colonizing Food Residues in the Human Gut. Applied Environmental Microbiology. 2006; 72: 6204- 6211.
28. Mahida YR, Rose F, Chan WC. Human Terminal Mucosa Expresses Novel Antimicrobial Activity Gut. 1997; 36: 64-68.
29. Ponnusamy P, Natarajan V, Sevanan M. In Vitro Biofilm Formation by Uropathogenic Escherichia Coli and Their Antimicrobial Susceptibility Pattern. Asian Pacific Journal of Tropical Medicine. 2012; 5: 210-213.
30. Fux CA, Costerton JW, Stewart PS, et al. Survival Strategies of Infectious Biofilms. Trends in Microbiology. 2005; 13: 34-40.
31. Amadi ES, Ikeagwu IJ, Iroha IR. Antibiotic Sensitivity Pattern of Staphylococcus aureus in Abakiliki, Nigeria. Journal Medical Science. 2008; 24: 231-235.
32. Uwazuoke JC, Aririatu LE. A Survey of Antibiotic Resistant Staphylococcusaureus Strains from Clinical Sources in Owerri. Journal Applied Science Environment Management. 2004; 8: 67-68.
33. Ndip RN, Ebah LME, Onile BA. Antibiogram of Staphylococcus aureus Isolated from Clinical Syndromes in Ilorin, Nigeria. Journal of Medical Science. 1997; 6: 24-26.
34. Monika Y, Rohan P, Shan D, et al. Microbial Spectrum of Urinary Tract Infections and Its Antibiogram in A Tertiary Care Hospital. International Journal of Research in Medical Sciences. 2017; 6: 2719- 2722.
35. Niranjan V, Malini A. Antimicrobial Resistance Pattern in EscherichiaColi Causing Urinary Tract Infection Among Inpatients. Indian Journal Medical Research. 2014; 6: 139-145.
36. Ruzicka F, Hola V, Votava M, et al. Biofilm Detection and the Clinical Significance of Staphylococcus Epidermidis Isolates. Folia Microbiology. 2004; 49: 596-600.
37. Gourmelon M, Petit F, Clermont O, et al. Change in the Structure of Escherichia coli Population and the Pattern of Virulence Genes along a Rural Aquatic Continuum. Frontier Microbiology. 2017; 8: 609-610.
38. Davis R, Brown PD. Multiple Antibiotic Resistance index, Fitness and Virulence Potential in Respiratory Pseudomonas aeruginosa from Jamaica. Journal of Medical Microbiology. 2016; 65: 261-271.
39. Mshana SE, Kamugisha E, Mirambo M, et al. Prevalence of Multiresistant Gram-Negative Organisms in A Tertiary Hospital in Mwanza, Tanzania. Biomedical Centre. 2009; 2: 49-51.
40. Vakulenko SB, Mobashery S. Versatility of Aminoglycosides and Prospects for Their Future. Clinical Microbiology Reviews. 2003; 16: 430-450.
41. Pogue JM, Thaden JT, Kaye KS. Role of Newer and Re-Emerging Older Agents in the Treatment of Infections Caused by Carbapenem-Resistant. Enterobacteriaceae. Virulence. 2017; 8: 406-416.
42. Donlan RM. Biofilm Formation: A Clinically Relevant Microbiological Process. Clinic Infections and Diseases. 2001; 33: 1387-1392.

43. Emami S, Eftekhar F. The Correlation between Bioflm Formation and Drug Resistance in Nosocomial Isolates of Acinetobacter Baumannii. Avicenna Journal Clinical Microbiology Infections. 2015; 2: 2-3.

44. Wang W, Guo Q, Xu X, et al. High-level tetracyclin resistance mediated by efflux pumps. Tet (A) and Tet (A)-1 with two start codons. Journal of medical microbiology. 2014; 63: 1454-1459.

45. Krumperman PH. Multiple Antibiotic Indexing of Escherichiacoli to Identify High Risk Sources of Fecal Contamination of Foods. Applied and Environmental Microbiology. 1985; 46: 165-170.