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Multiple-drug Resistance among Biofilm-producing Phenotypes of Nosocomial *Escherichia coli*

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Authors' contributions

This work was carried out in collaboration between all authors. Author MMI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors AI and GUM managed the analyses of the study. Author SS managed the literature searches and laboratory protocols. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: Here, we analyse biofilm formation in relation to multiple drug resistance among nosocomial isolates of *Escherichia coli.*
Study Design: Randomized study design.
Place and Duration of Study: The study was a cross sectional study conducted at the Department of Microbiology, University of Maiduguri, Nigeria, from April 2018 to May 2018.
Methodology: Ten (10) clinical isolates were collected and confirmed using standard bacteriological methodology. Congo Red Agar (CRA) was used to analyse biofilm formation among isolates. Antimicrobial susceptibility test was done using Kirby bauer disc diffusion test, where the efficacy of ten (10) selected drugs against the isolates was examined.
Results: Seventy percent (70.0%) of the isolates were found to produce biofilm. All isolates were resistant to Ofloxacin (100%; f=4.5, P<0.01). 57.1% of the biofilm producing isolates were observed to be multi drug resistant. Biofilm-producing/multi-drug resistant isolates were resistant to an

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average of ninety five percent (95.0%) of antimicrobial drugs tested. Fourty three percent (43.0%) of the biofilm producing phenotypes were Pandrug resistant.
Conclusion: In view of this, it is can be deduced that there is a relationship between biofilm formation and multiple drug resistance among nosocomial isolates of *Escherichia coli*.

Keywords: Biofilm formation; multidrug resistance; Escherichia coli; nosocomial infection.

1. INTRODUCTION

Biofilms are microbial communities characterized by an irreversible attachment of layers of cells to a surface or to each other. Enmeshed in a matrix of extracellular polymeric substances (EPS), biofilms express atypical phenotype in the form of growth rate and gene transcription compared to planktonic cells [1]. It has been established that the persistence of bacterial biofilms in the human body is a major cause of recurrent or chronic infections [2], and biofilm formation enhances the pathogenic potential of microorganisms [3]. It has been reported that microorganisms growing in a biofilm are intrinsically more resistant to antimicrobial agents than planktonic cells such that high antimicrobial concentrations are required to inactivate them, as antibiotic resistance can increase 1,000 fold [4].

A gram-negative, rod-shaped non-sporulating bacteria, *Escherichia coli* is a genetically diverse bacterial species that causes diarrhoeal diseases and a variety of extraintestinal infections which fulfill many or all of the criteria for biofilm-associated infections [5]. As a nonpathogenic member of the large intestine in vertebrates, *E. coli* appears to reside within the mucus layer without colonising the underlying epithelium [6]. Uropathogenic strains of *E. coli* are frequently isolated from biofilms formed in the lumen of catheters, where they resist antibiotic treatment and shear forces [7]. As such, it is clear that a close relationship exists between biofilm formation, antibiotic resistance and the chronicity of urinary tract infection among patients suffering from infection with uropathogenic *E. coli* [8].

Biofilm producing *E. coli* can be resistant to three or more antibiotics, giving rise to a truly multidrug resistant strain. Molecular studies have further expanded our understanding of the dynamics of *E. coli* biofilms as it relates to multidrug resistance. Several genes have been proposed to be particularly important for biofilm formation, and the importance of the rpoS gene in *E. coli* biofilm formation has been documented. Those genes whose expression gradually increased during biofilm formation (attachment>maturation) in *E. coli* include genes coding for multidrug resistance (yhiU and yhiV), anaerobic respiration (hyaABCDE, hycF, hycI, and narY) [9] and a gene reported to be upregulated in persister cells (wrbA) [10]. The level of expression of these genes increased as the level of rpoS (a 37.8 kD protein that regulates transcription in *E. coli*) expression increased during biofilm formation [11].

We, therefore, hypothesize that an association exist between biofilm formation and the ability to resist multiple antimicrobial drugs by *E. coli*. This, if proven to be true, could pose a significant therapeutic challenge and can affect the prognosis of a disease in a negative way. Hence, we analyse the interrelationship between biofilm formation and multiple drug resistance among nosocomial isolates of *E. coli*.

2. MATERIALS AND METHODS

2.1 Place and Duration of the Study

The study was a cross sectional study conducted at the Department of Microbiology, University of Maiduguri, Nigeria, from April 2018 to May 2018.

2.2 Collection of Isolates and Sub-culture

Ten (10) *E. coli* isolates (5 derived from urine samples and 5 derived from sputum samples) were collected from the Microbiology laboratory of the Department of Medical Microbiology, University of Maiduguri Teaching Hospital. They were collected aseptically and transported to the Laboratory of the Department of Microbiology, University of Maiduguri. The isolates were subjected to the Gram-staining procedure and sub-cultured onto MacConkey agar and Blood agar. Isolates were further confirmed by biochemical tests which include indole test, Urease test, Citrate utilisation, methyl red and Voges proskauer test [12].

2.3 Biofilm Formation Test

A simple qualitative method to detect biofilm production by using Congo Red Agar (CRA)

medium was used. CRA medium was prepared with brain heart infusion broth (Oxoid, UK) 37 g/L, sucrose 50 g/L, agar (Oxoid, UK) 10 g/L and Congo red indicator (Oxoid, UK) 8 g/L. First Congo red stain was prepared as a concentrated aqueous solution and autoclaved (at 121°C for 15 minutes) separately from the other medium constituents. Then it was added to the autoclaved brain heart infusion agar with sucrose at 55°C. CRA plates were inoculated with test organism (*E. coli*) and incubated at 37°C for 24 hours aerobically. Black colonies with a dry crystalline consistency indicated biofilm production. The experiment was performed in triplicate and repeated three times [13].

2.4 Antibiotic Susceptibility Test

The Kirby-Bauer disk diffusion susceptibility test was used to determine the sensitivity or resistance of *E. coli* isolates to various antimicrobial compounds. Using the published Clinical and Laboratory Standard Institute Interpretative Chart, the zone sizes of each antimicrobial compound was interpreted, reporting the organism as 'Resistant' or 'Sensitive' [14].

The susceptibility test was done on Mueller Hinton agar (Oxoid, UK) using antibiotic discs with the following concentrations: Cotrimoxazole (30 µg), Chloramphenicol (30 µg), Sparfloxacin (10 µg), Ciprofloxacin (10 µg), Amoxicillin (30 µg), Augmentin (30 µg), Gentamicin (10 µg), Pefloxacin (30 µg), Ofloxacin (10 µg), and Streptomycin (30 µg).

Multi-drug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories, Extensively-drug resistance (XDR) was defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e. bacterial isolates remain susceptible to only one or two categories) and Pandrug resistant (PDR) was defined as non-susceptibility to all agents in all antimicrobial categories [15].

2.5 Data Analysis

Data were presented as percentages and frequencies. Chi-square was used to test for association between variables and evaluations were carried out at 99% confidence level. Calculations less than p-value of 0.01 (P<0.01) was considered as statistically significant.

3. RESULTS AND DISCUSSION

3.1 Association between Biofilm Formation and Multidrug Resistance among Clinical Isolates of *E. coli*

There is an increasing interest in discerning the relationship between biofilm formation and the development of multidrug resistance among bacteria. Here, seventy percent (70.0%) of isolates examined expressed the biofilm producing phenotype, sixty percent (60.0%) were multidrug resistant and Fourty percent were of biofilm forming-multidrug resistant phenotype (Table 1). This is in agreement with reports of Sao et al. [16] while working on uropathogenic strains of *E. coli*, observed a high biofilm producing rate of sixty eight percent. Similar findings were reported elsewhere [13,8,17].

The ability of uropathogenic strains of *E. coli* to readily form biofilms on the surface of catheter materials, bladder walls and within bladder epithelial cells has been documented [18]. It interesting to know that *E. coli* isolates from the same environment can adhere differently to surfaces and create different biofilms and most bacterial cells prefer to live in a biofilm community in response to difficult environmental conditions. The mechanism of biofilm formation in *E. coli* (and other bacteria) can be correlated with other bacterial properties. This phenomenon may help explain the variation in biofilm formation inter/intra-species [19].

Biofilm formation has been linked with a high propensity for the development of drug resistance by bacteria. Here, we reveal that a significant proportion of the biofilm producing *E. coli* isolates examined were multidrug resistant. This is similar to the report of Bajpai et al. [20] who observed a significant correlation between biofilm formation and multidrug resistance while working on clinical isolates of *E. coli*.

Several explanations (including the failure of antibiotic penetration into biofilms) have been proposed to explain biofilm resistance to antibiotics, where some suggest that the biofilm is acting as a physical barrier [21,22]. However, others suggest that the exopolysaccharide matrix does not always act as an impenetrable barrier to the diffusion of antibiotics (especially to ß-lactams or tetracycline) and thus, other factors must account for the resistance [23,24,25].

3.2 Antibiogram Profile of *E. coli* Isolates Examined

Investigation into the antibiogram profile of isolates revealed that Ofloxacin (0.00%) was the least sensitive antimicrobial drug compared to Ciprofloxacin and Sparfloxacin (60.0% respectively), to which isolates demonstrated the highest sensitivity. All isolates were resistant to Ofloxacin but an even split (50.0%/50.0%) in resistance and sensitivity was observed against the Amoxicillin/Clavulanic acid conjugate, Augmentin (Table 2). Lower resistance rate of 6.6% by *E. coli* against Ofloxacin was observed by Rasheed et al. [26] in a study conducted on *E. coli* isolated from food sources in Hyderabad, India. Contrary findings have been reported elsewhere [27,28,29].

3.3 Antibiogram Profile of Biofilm Producing Phenotypes of *E. coli* Examined

The antibiogram profile of biofilm forming phenotypes of *E. coli* examined has shown that strains were completely resistant to Ofloxacin. But an average resistance rate was observed against other antimicrobial drugs tested (Fig. 1).

Table 1. Association between biofilm formation and multidrug resistance among clinical isolates of *Escherichia coli*

Sample	No of isolates tested	Biofilm formation test		Fisher's exact Test/*p* value
		Positive (%)	Negative (%)	
Urine	5 (50.0)	3 (30.0)	2 (20.0)	1.0000/
Sputum	5 (50.0)	4 (40.0)	1 (10.0)	0.4901
Total (%)	10 (100)	7 (70.0)	3 (30.0)	(*p<.01*)
		Multidrug resistance		Fisher's exact Test/*p* value
		Positive (%)	Negative (%)	
Urine	5 (50.0)	2 (20.0)	3 (30.0)	0.5238/
Sputum	5 (50.0)	4 (40.0)	1 (10.0)	0.1967
Total (%)	10 (100)	6 (60.0)	4 (40.0)	(*p<.01*)
		Biofilm formation and multidrug resistance		Fisher's exact Test/*p* value
		Positive (%)	Negative (%)	
Urine	5 (50.0)	1 (10.0)	4 (40.0)	1.0000/
Sputum	5 (50.0)	3 (30.0)	2 (20.0)	0.1967
Total (%)	10 (100)	4 (40.0)	6 (60.0)	(*p*<.01)

Table 2. Antibiogram profile of *E. coli* isolates examined

Antimicrobial drugs tested	CLSI break points	Number of isolates tested	Antimicrobial susceptibility test	
			Sensitive (%)	Resistance (%)
Augmentin (AU)	≤16 >	10	5 (50.0)	5 (50.0)
Gentamicin (CN)	≤12 >	10	4 (40.0)	6 (60.0)
Pefloxacin (PEF)	≤15 >	10	4 (40.0)	6 (60.0)
0Streptomycin (S)	≤11 >	10	4 (40.0)	6 (60.0)
Cotrimoxazole (SXT)	≤15>	10	4 (40.0)	6 (60.0)
Chloramphenicol (CH)	≤17 >	10	4 (40.0)	6 (60.0)
Sparfloxacin (SP)	≤15 >	10	6 (60.0)	4 (40.0)
Ofloxacin (OFX)	≤14 >	10	0 (0.0)	10 (100)
Amoxicillin (AMX)	≤16 >	10	5 (50.0)	5 (50.0)
Ciprofloxacin (CPX)	≤15 >	10	6 (60.0)	4 (40.0)

f = 4.5. p>0.01

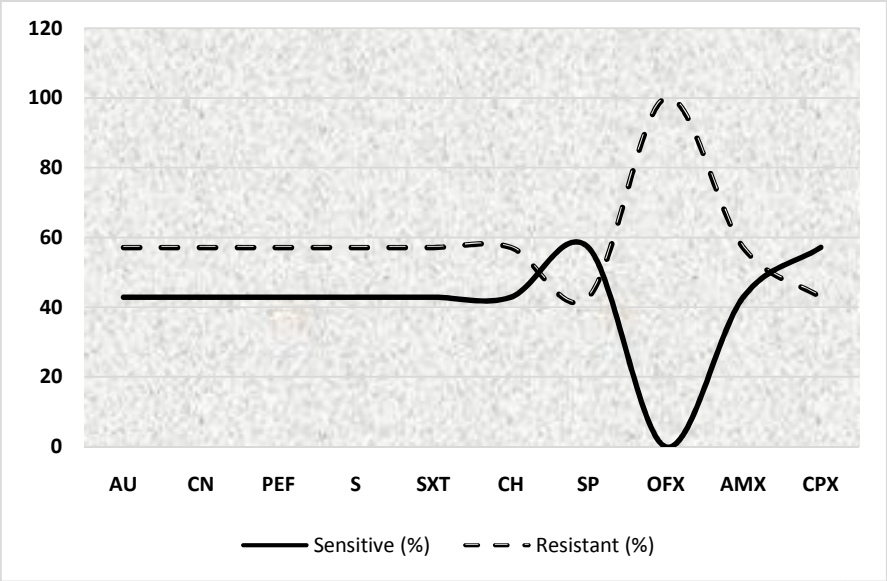

Fig. 1. Antibiogram profile of biofilm producing strains of *E. coli* examined
Key: AU:Augmentin;CN:Gentamicin;PEF:Perfloxacin;S:Streptomycin;SXT:Cotrimoxazole; CH:Chloramphenicol;SP:Sparfloxacin;OFX:Ofloxacin;AMX:Amoxicillin;CPX:Ciprofloxacin

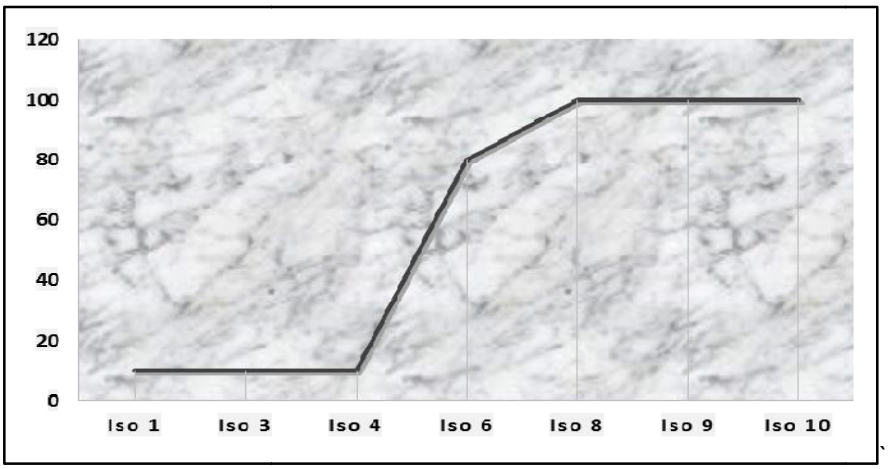

Fig. 2. Percentage resistance rate of biofilm producing *E. coli* isolates examined against antimicrobial drugs tested
Iso=Isolate no.

3.4 Percentage Resistance Rate of Biofilm Producing Phenotypes of *E. coli* Examined against Antimicrobial Drugs Tested

The biofilm forming strains were resistant to an average of 58.6% of all antimicrobial drugs tested. Four isolates (6, 8, 9, and 10) were resistant to atleast 80.0% of all drugs tested, giving rise to a typical multidrug resistant strain (Fig. 2).

3.5 Percentage Resistance Rate of Biofilm Forming/Multidrug Resistant Phenotypes of *E. coli* Examined against Antimicrobial Drugs Tested

The resistance profile of the biofilm forming isolates that are termed multidrug resistant was analysed. Isolates were resistant to an average of 95.0% of antimicrobial drugs tested. Fourty three percent of biofilm producing *E. coli* isolates examined were completely resistant (100%) to all

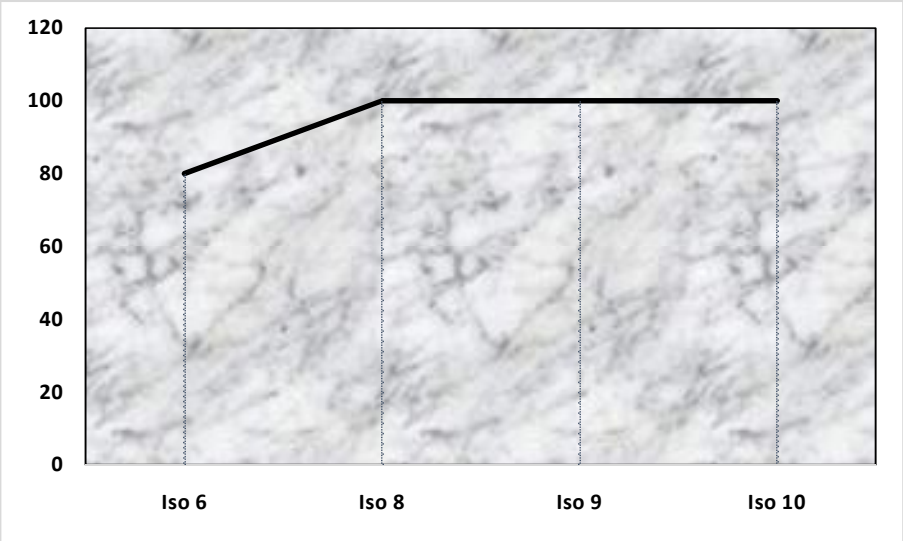

Fig. 3. Percentage resistance rate of biofilm producing/multidrug resistant *E. coli* isolates examined

Iso=Isolate no.

antimicrobial drugs tested, giving rise to a typical Pandrug-resistant strain (Fig. 3). It has been corroborated that the spread of antibiotic resistance among bacteria is mainly due to the aggregation of antibiotic resistance marker genes on plasmids [30,31] and a link has been identified between the antibiotic marker genes on the plasmid and biofilm formation by bacteria. May et al. [32] concluded that marker genes on plasmids played an important role in both resistance of biofilm cells to antibiotics and in formation of mature biofilms, as they could trigger specific chromosomal resistance mechanisms to confer a high-level resistance during biofilm formation.

Results from this study have highlighted the varying nature of drug resistance among *E. coli* isolates. From those strains that are sparingly resistant to one or two drugs to those that are resistant to more than three drugs. The third group comprises of isolates that are completely resistant to all antimicrobial drugs tested. These two latter groups are found chiefly among the biofilm producing isolates, which stresses the possible role of biofilm as a phenomenon that enhances multiple drug resistance among bacteria.

4. CONCLUSION

In conclusion, we suggest that there is an association between biofilm formation and multiple drug resistance by nosocomial isolates of *Escherichia coli*. We reveal that more than half of the biofilm forming strains were multidrug resistant and indeed, some strains were observed to be Pandrug resistant.

ETHICAL APPROVAL

Authors hereby declare that collection of isolates have been approved by the ethics committee of the University of Maiduguri Teaching Hospital, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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