ORIGINAL ARTICLE

Detection of Biofilm Formation and Antibiotic Susceptibility in *Escherichia coli* Isolated From the Urine of Pregnant Women at Mnazi Mmoja Hospital, Zanzibar, Tanzania

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ABSTRACT

Background: *Escherichia coli* is one of the species mostly involved in biofilm formation, being the most important cause of relapse or chronic urinary tract infections. To develop alternative biofilm-fighting treatments, it is important to understand which types of *E. coli* form biofilms.

Study objectives: To detect biofilm formation and antibiotic susceptibility among *E. coli* isolated from the urine of pregnant women at Mnazi Mmoja Hospital, Zanzibar, Tanzania.

Methodology: Hospital-based cross-sectional study was conducted at Mnazi Mmoja Hospital in Zanzibar. A questionnaire was used to collect all the information regarding demographic characteristics. Midstream urine samples were collected and sent to the laboratory for culture, sensitivity, and biofilm tests. Positive growth culture was subjected to differential identification tests such as Motility Indole Ornithine, Urea, and citrate. The antimicrobial susceptibility test was conducted on all *E. coli* species. Biofilm production was detected using a microtitre plate assay. IBM SPSS Statistics for Windows version 20.0 (IBM Corp, Armonk, NY, USA) was used in the analysis of data.

Result: Out of 400 participants, significant growth of *E. coli* was detected in 22 (5.5%), of which 6 (27.3%) were symptomatic and 16 (72.7%) were asymptomatic. Of all *E. coli* isolates, 22 (100%) were biofilm formers, 15 (68.2%) were resistant to Amoxicillin & clavulanic acid, and 16 (72.7%) were resistant to Ampicillin. However, all isolates were sensitive to Gentamycin, Ceftriaxone, Nitrofurantoin, Norfloxacin, and Meropenem, while 21 (95.4%) were sensitive to Ciprofloxacin and Nalidixic acid.

Conclusion: We revealed that *E. coli* that formed biofilms showed significant levels of antibiotic resistance to commonly used drugs.

BACKGROUND

Urinary tract infections (UTIs) are one of the most common infections in humans, with an estimated 150 million cases reported each year around the world,[1] Likewise, UTIs are the most commonly studied health concerns in pregnancy, with prevalence ranging from 3% to 35% worldwide, with higher frequency reported in developing countries, particularly Sub-Saharan Africa (SSA), the Middle East, and Asia.[2] *Escherichia coli (E. coli)* is the most common cause of (UTIs) in humans, among the factors, due to its ability to attach to epithelial cells, urinary lavage resistance, and biofilm formation.[3]

In SSA, UTIs are among the most common infections with limited microbiological data to guide treatment decisions,[4] and have high frequency compared to developed countries.[2] A study conducted in Cameroon showed the prevalence of UTIs caused by *E. coli* to be 38% in pregnant women.[5] In Uganda, the prevalence of UTIs caused by *E. coli* was 35%;[6] in Ghana, 33.5,[7] and 15.5% in Northeastern Ethiopia.[8] This was attributed to low socioeconomic status and differences in the level of healthcare development.[9]

In Mwanza, Tanzania, it was reported that the prevalence of UTIs caused by *E. coli* among HIV-pregnant women was 21.4%.[10] In 2019, another study carried out in Mwanza, showed a prevalence of *E. coli* to be 28.0%,[11]. This study[11] compared UTIs between pregnant women with preeclampsia and those without preeclampsia, but it did not determine the biofilm-forming bacteria, which are the current worldwide threat; and data on bacterial profile and detection of biofilm-forming bacteria from UTIs among pregnant women was not assessed.

Management of UTIs requires a systematic approach to confirm the presence of infection and its type (site and either complicated or uncomplicated), assess risk factors of infection with antibiotic-resistant organisms, and select the optimal dose, route, and duration of the empiric antibiotic regimen based on a local antibiogram.[12] International guidelines recommend that Trimethoprim/sulfamethoxazole could be considered a first-line drug but only if local resistance to *E. coli* does not exceed 20%. Aminopenicillins and fluoroquinolones are no longer recommended as first-line therapies for urinary tract infections because of high resistance rates and potentially long-lasting side effects, respectively.[13] Second-line options include oral Cephalosporins, such as cephalexin or cefixime, fluoroquinolones, and β-lactams, such as amoxicillin-clavulanate.[13] For pregnant women and adolescents, Amoxicillin/clavulanic acid 500/125 mg, 12 hourly for 7 days was recommended.[14] UTIs caused by diverse populations of bacteria, including *E. coli*, adhere to one another to create a colonization surface, which results in the development of biofilm.[15] The development of biofilms by *E. coli* tends to raise the incidence of UTIs and resistance to commonly used antibiotics.[16] Therefore, this study aimed to detect biofilm formation and antibiotic susceptibility among *E. coli* isolated from the urine of pregnant women at Mnazi Mmoja Hospital, Zanzibar, Tanzania, to fill the gap of information with a view to devising appropriate control measures.

MATERIALS AND METHODS

Study Design

This was a hospital-based cross-sectional study conducted from November 2022 to May 2023.

Study Site

The study was carried out at the antenatal clinic of Mnazi Mmoja Hospital (MMH) in Zanzibar. MMH serves as the main referral hospital in Zanzibar with a total bed capacity of 776, distributed across 3 campuses. The main campus of MMH is located in Stone Town, an Urban District in the Urban/Western Region of Zanzibar, and has 630 beds. Mwembeladu Maternity Home has 36 beds and Kidongo Chekundu Mental Hospital has 110 beds. Both the maternity and mental hospitals were located within the city limits of Stone Town but outside of the downtown area. About 95% of all outpatients at the hospital are self-referrals. The hospital attends an average of 74,975 outpatients, 27,185 inpatients, and 12,658 deliveries per year. The outpatient department provides diagnosis and care for patients who do not need to stay overnight; the hospital is now running 25 outpatient clinics including the Antenatal Clinic (ANC).

Study Participants

The study participants were recruited from the Antenatal Clinic (ANC) at MMH, Zanzibar.

Inclusion criteria

All consented pregnant women aged between 15 to 45 attending the ANC at Mnazi Mmoja Hospital were included in the study.

Exclusion criteria

Pregnant women, who were under antibiotics use or had taken them within the previous 2 weeks, were excluded from the study.

Sample Collection

Sterile midstream urine from each consented pregnant woman was collected by a research assistant at an antenatal clinic following Standard Operating Procedure (SOP) in a sterile container.[17] The sample was transported to the MMH laboratory for processing. Urine samples were cultured in a MacConkey Agar (MCA) plate and then incubated at 37^{0}C for 18 to 24 hours. Positive cultures and pathogens were identified according to the SOP as per the standard microbiological methods.[18] Bacteria identification tests such as Motility Indole Ornithine (MIO), Urea, Triple Sugar Iron (TSI), and citrate were used. The identified *E. coli* species were subjected to an Antimicrobial Susceptibility Test (AST) using Muller Hinton Agar (MHA).

Sample Size Estimation

Sample size (N) was calculated using the formula for precision below;

$$N = Z^2 \frac{p(1-p)}{\varepsilon^2}$$

Where;

Z=Standard normal deviation of (1.96) corresponding to a 95% confidence interval.

P=Population prevalence, 125/200 (62.5%) was the prevalence of biofilm formation from isolated *E. coli* in Uganda, [19].

ε=Precision set at 5% (0.05); and an additional 10% of non-respondents.

The minimum sample size was 400 participants.

Antimicrobial Susceptibility Test

The AST was done on MHA using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI).[20] The antimicrobial agents to be tested were Nitrofurantoin (F) (300 μg), Nalidicsic acid (NA) (30 μg), Gentamicin (GEN) (10 μg), Ciprofloxacin (CIP) (5 μg), Ampicillin (AMP) (10 μg), Ceftriaxone (CRO) (30 μg), Norflaxocin (NOR) (10 μg) and Amoxicillin & Clavulanic acid (AMC) (10 μg). Resistance was interpreted according to the National Committee for Clinical Laboratory Standards (NCCLS).[20] When isolates exhibited resistance to two or more antibiotic classes, they were classified as MDR.[21]

Detection of Biofilm Formation

The Microtitre Plate Assay (MPA) method was used for the detection of biofilm in each isolate. A flat bottom 96-well polystyrene microtitre plate with a lid was used. The freshly prepared 20 μl [concentration $5*10^6$ CFU/ml] bacterial suspension was inoculated into 180 μl of Brain Heart Infusion with 2% glucose to get roughly $5*10^5$cfu/ml as a final inoculum in a microtitre plate. Aerobically overnight incubated culture at 37 °C was washed three times with phosphate buffer saline (PBS, pH: 7.2). Then we attached bacteria through baking at 60°C for 60 min, and stained the well with 180 μl of absolute crystal violet for 1 min at room temperature. The stain was aspirated, and properly washed on the microtitre plate with PBS and the air-dried plate was resolubilized with 180 μl of 95% ethanol to detach the fixed cell from the well. The plate was left at room temperature and covered with a lid without shaking then the Optical Density (OD) of each well was measured (λmax=620 nm) using an ELISA plate reader. The cut-off value and biofilm-forming ability of isolates were reported as non-biofilm *former* and biofilm *former* but were further differentiated into weak, moderate, and strong as described by Stepanović et al.[22]

Quality Control

Reference strains of *E. coli* ATCC 25922 were used for quality control for AST. *Pseudomonas aeruginosa* ATCC 19429 was used as positive control and sterile BHI broth was used as negative control for biofilm formation test.

Data Analysis

The data was entered into Microsoft Excel 2013 for cleaning and coding, any information which was not clear was re-checked in the questionnaire. The data were transferred to IBM SPSS Statistics for Windows version 20.0 (IBM Corp, Armonk, NY, USA) for analysis. Categorical variables were presented in frequency and percentage while continuous variable data were summarized by median with interquartile range. The chi-square test was employed to determine the association between antibiotic resistance and the biofilm-forming capacity of *E. coli*. A *P* value less than 0.05 was considered statistically significant.

Ethical considerations

Ethical approval to conduct the study was obtained from Kilimanjaro Christian Medical University (KCMU) College and the Research Ethics Review Committee with ethical clearance number PG: 174/2022. Permission to collect data at MMH was granted by the MMH Administration. Confidentiality of participants' information was maintained. Consent to participate in the study was obtained from participants by signing consent forms. All participants were given the right of subjects to participate or reject in the study.

RESULT

Sociodemographic Characteristic of the Study Participants

Four hundred (400) pregnant women were recruited into the study, and they were aged from 16 to 43 years. The median age was 27.0 years with an Interquartile Range (IQR) of 23.00 to 30.0 years. Among the 400 participants, the majority were aged between 26 and 35 years (49.0%), resided in urban areas (60.0%), married (96.5%), and with a secondary level of education (74.2%). There was a preponderance of the participants (80.0%) with a monthly income of less than Tzs 350,000, and of being housewives (62.5%), (Table 1).

Clinical characteristic of the Study Participants

Most participants were in the second trimester (n=177, 44.3%), had no history of diabetes (n=399, 99.7%), and did not have a history of previous UTIs (n=358, 89.5%). Likewise, the most participants (285, 71.3%) were asymptomatic. Among the 115 symptomatic participants, most were feverish (78.3%), had painful urination (67.8%), had no back discomfort (58.3%), had abdominal discomfort (84.4%) and had no blood in urine (89.6%) (Table 2).

TABLE 1: Sociodemographic Characteristics of the Study Participants (N=400)

Variable	Frequency (n)	Percentage (%)
Age		
Median age (IQR) 27.00 (23.00 - 30.00)		
15-25	161	40.3
26-35	196	49.0
36-45	43	10.7
Marital status		
Single	8	2.0
Married	386	96.5
Cohabittory	3	0.7
Divorced	3	0.8
Residence		
Rural	160	40.0
Urban	240	60.0
Education		
No formal education	5	1.3
Primary	45	11.2
Secondary	297	74.2
College/university	53	13.3
Occupation		
Housewife	250	62.5
Self-employed	91	22.7
Employed	59	14.8
Monthly income		
Less than 350,000	320	80.0
350,000 to 1Million	78	19.5
Above 1 Million	2	0.5

IQR - Interquartile Range

TABLE 2: Clinical Characteristics of the Study Participants (N=400)

Variable	Frequency (n)	Percentage (%)
Trimester / gestational age		
First trimester	63	15.7
Second trimester	177	44.3
Third trimester	160	40.0
History of diabetes		
Yes	1	0.3
No	399	99.7
History of previous UTIs		
Yes	42	10.5
No	358	89.5
Symptoms		
Symptomatic	115	28.7
Asymptomatic	285	71.3
Fever		
Yes	90	78.3
No	25	21.7
Painful urination		
Yes	78	67.8

Continue

TABLE 2: Continued

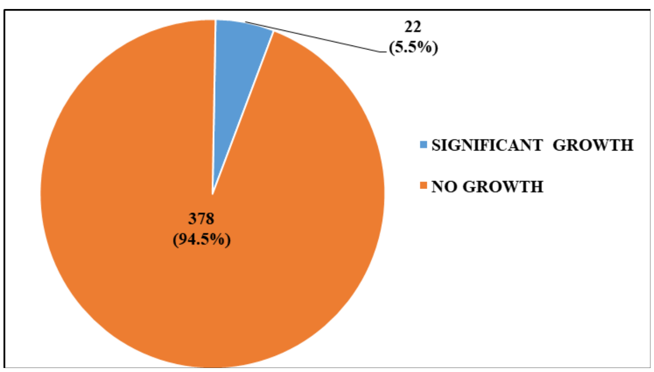
Variable	Frequency (n)	Percentage (%)
No	37	32.2
Back discomfort		
Yes	48	41.7
No	67	58.3
Abdominal discomfort		
Yes	97	84.4
No	18	15.6
Urine with blood		
Yes	12	10.4
No	103	89.6

UTIs – Urinary Tract Infections

Prevalence of *E. coli* among Pregnant Women

Out of 400 participants, *E. coli* was detected in 22 (5.5 %); and 378 (94.5%) had no growth (Figure 1). Among the 22 (5.5%) showing significant growth 6 (27.3%) were symptomatic and 16 (72.7%) were asymptomatic (Figure 2).

FIGURE 1: Proportion of Samples with *E.coli* Growth (N= 400)



Susceptibility Patterns of *E. coli* Isolated from the Urine of Pregnant Women

Of all *E. coli* isolates, 15 (68.2%) were resistant to Amoxicillin & clavulanic acid; and 16 (72.7%) were resistant to Ampicillin (AMP). All isolates were sensitive to Gentamycin, Ceftriaxone, Nitrofurantoin, Norfloxacin, and Meropenem. Only 4 (18.2%) were sensitive to ampicillin Amoxicillin & clavulanic acid (AMC), while 21 (95.5%) were sensitive to Ciprofloxacin and Nalidixic acid. Some *E. coli* isolates were intermediate to Ampicillin by 2 (9.1%) and Amoxicillin & clavulanic acid by 3 (13.6%), (Table 3). The MDR pattern of biofilm-forming *E. coli* is shown below, in which 21 (95.0%) isolates were non-MDR and 1 (5.0%) isolate was MDR to four classes of antibiotics (penicillin, fluoroquinolones, and quinolones classes) (Figure 3).

FIGURE 2: Proportion of Pregnant Women with UTIs by Symptoms Category (n=22)

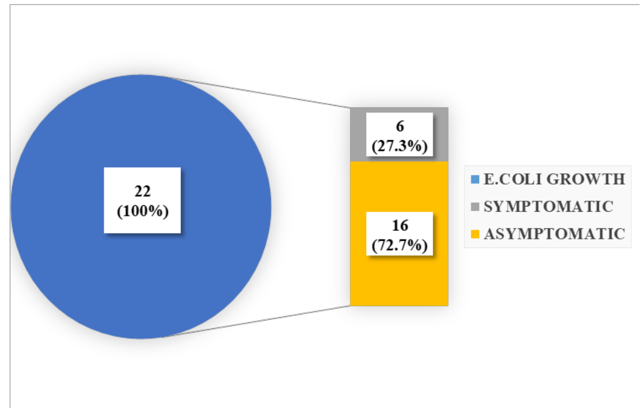
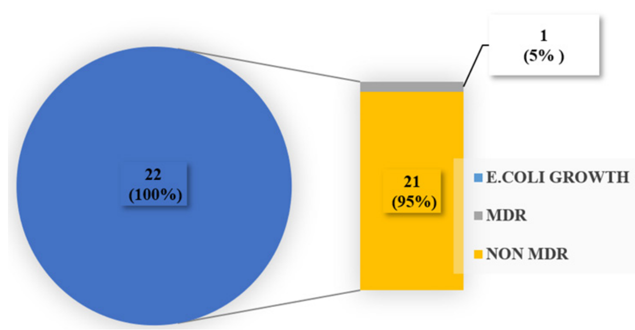


FIGURE 3: Proportion of MDR Patterns of Biofilm -Forming *E.coli* Isolates



Biofilm Formation among *E. coli* Isolated from the Urine of Pregnant Women

Among 22 *E. coli* isolates 20 (91.0 %) were found to be strong biofilm formers, 1 (4.5%) was a moderate biofilm former, 1 (4.5%) was a weak biofilm former, (Figure 4).

Association between Antibiotic Susceptibility and Biofilm-forming *E. coli*

Statistical test for association between biofilm-forming in *E. coli* isolates and susceptibility to antibiotics (that is being MDR or non-MDR) showed no statistically significant results (χ^2=0.105 and P>.05) (Table 4), which, however, were clinically relevant despite that most of *E. coli* were biofilm formers but showed high susceptibility to antibiotics drugs used.

TABLE 3: Antibiotic Susceptibility Pattern of *E. coli* isolates (N=22)

Antibiotics	Sensitive (%)	Intermediate (%)	Resistance (%)
AMP	4 (18.2)	2 (9.1)	16 (72.7)
GEN	22 (100)	0	0
AMC	4 (18.2)	3 (13.6)	15 (68.2)
CRO	22 (100)	0	0
CIP	21 (95.5)	0	1(4.5)
NAL	21 (95.5)	0	1(4.5)
F	22 (100)	0	0
NOR	22 (100)	0	0
MEM	22 (100)	0	0

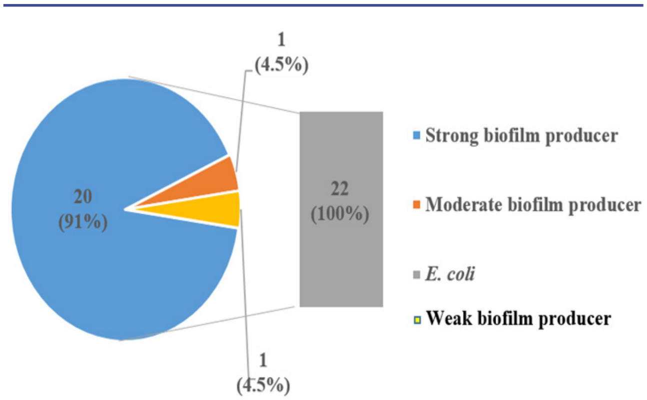
AMP, Ampicillin; GEN, Gentamycin; AMC, Amoxicillin & Clavulanic acid; CRO, Ceftriaxone; CIP, Ciprofloxacin; NAL, Nalidicsic acid; F,Nitrofurantoin; NOR, Norfloxacin; MEM, Meropenem.

TABLE 4: Association Between Antibiotic Susceptibility and Biofilm-Forming *E. coli*

Antibiotic	Susceptibility pattern	Biofilm Formation Pattern			Chi-Square	
		Weak(%)	Moderate(%)	Strong(%)	χ2	P Value
AMP	Intermediate	0 (0)	0 (0)	2 (9.1)	0.825	.935
	Sensitive	0 (0)	0 (0)	4 (18.2)		
	Resistance	1 (4.5)	1 (4.5)	14 (63.6)		
AMC	Intermediate	0 (0)	0 (0)	3 (13.6)	1.027	.906
	Sensitive	1 (4.5)	1 (4.5)	4 (18.2)		
	Resistance	0 (0)	0 (0)	13 (59.1)		
CIP	Sensitive	1 (4.5)	1 (4.5)	19 (86.4)	0.105	.949
	Resistance	0 (0)	0 (0)	1 (4.5)		
NAL	Sensitive	1 (4.5)	1 (4.5)	19 (86.4)	0.105	.949
	Resistance	0 (0)	0 (0)	1 (4.5)		

AMP, Ampicillin; AMC, Amoxicillin & Clavulanic acid; CIP, Ciprofloxacin; NAL, Nalidicsic acid; χ2, Chi-square.

FIGURE 4: Biofilm Formation Capacity Among *E.coli* Isolates



DISCUSSION

Prevalence of *E. coli* among pregnant women

The current study's observed *E. coli* prevalence is similar to prevalence reported by similar studies conducted in Ethiopia[26], Dezful City[27], and Iran.[1] The consistency of the results could be attributed to the similar study design used and the similar hot weather conditions in the study areas. On the other hand, the study's prevalence was about half as much as that of the studies conducted in Mwanza, Tanzania, which reported the prevalence of 12.8%,[10] and 16.8%.[11] Similarly, studies conducted in Ethiopia, Nigeria, and Saudi Arabia, reported higher prevalence of *E.coli* of 15.5%,[23], 11.0%,[24] and 9.3%,[25] respectively.

The observed differences could be due to that the current study involved pregnant women without any risk condition while other studies involved pregnant women with underlying risk conditions like preeclampsia and HIV/AIDS which increased the chances of developing UTIs and also due to the small sample size used with only

E. coli as determinant for UTI. These findings imply that co-morbidities, sample size, and climate conditions of hot weather do contribute to the rise in UTI cases among pregnant women.

On the other hand, the study was contrary to the study conducted in Mwanza City, which showed that the prevalence of asymptomatic UTIs (aUTIs) was lower at 27.3% compared to 46.7% of symptomatic UTIs (sUTIs) in pregnant women.[11] It also differed from a study conducted in Ethiopia, which revealed that sUTIs and aUTIs among pregnant women were much lower at 20.4% and 17.8% respectively.[28] The findings indicate that the difference in study design and characteristics of the study participants examined has a direct influence on the prevalence of sUTIs and aUTIs in pregnant women.

Susceptibility patterns of *E. coli* isolated from the urine of pregnant women

The current study was similar to the study conducted in Uganda, which revealed that the majority of *E. coli* isolates were highly susceptible to gentamycin, ceftriaxone, nitrofurantoin, and ciprofloxacin.[6] Similar results were obtained in Nigeria,[31] Iran,[32] and Spain.[33] Also, this study was relatively concurrent with a study conducted in Uganda,[6] a study conducted in Ethiopia,[30] while a study conducted in Nigeria, showed a relatively similar result of resistance to Amoxicillin & clavulanic acid by 70.45%,[37] and this study matched one conducted in India,[16] This similarity was attributed to increased consumption of these drugs, self-medication, widespread and indiscriminate use as well as its ease of accessibility over the counter in pharmacies, which can lead to a shift to increase in resistance.

Also the study was contrary to a study conducted in Northern Tanzania, which reported that the *E. coli* isolates were 100% sensitive to ceftriaxone, Nitrofurantoin, Amoxicillin-clavulanic acid, and 50% were sensitive to Gentamycin while 33.3% were resistance to Ciprofloxacin and 50% resistance to Gentamycin.[29] Similarly, this study is not in agreement with a report from in Ethiopia, in which *E. coli* isolates had resistance to Gentamycin by 78%, Ceftriaxone (55.6%), Nitrofurantoin (33.3%), and Ciprofloxacin by 38.8%.[30] Furthermore, the study was discordant with the 2013 results from Muhimbili, Tanzania, in which *E. coli* showed high resistance to Ampicillin, and Amoxicillin & Clavulanic acid by 96.0 and 88.0% respectively.[34] In a similar study conducted in South Africa, susceptibility to Amoxicillin & Clavulanic acid was 82.9%, which is also quite different from this study.[35] Resistance to Ampicillin, and Amoxicillin & clavulanic acid was 50% and 11.1% respectively in a report from Somalia.[36] Theses findings suggest that self-medication, indiscriminate use of drugs, increased consumption of these drugs, and lack of binding restrictions on antibiotics have a direct impact on the rising resistance pattern to *E. coli*.

The problems of bacterial drug resistance have been globally documented particularly in healthcare-associated infections, and it has become one of the health-security concerns.[38] The finding of this study is contrary to one reported in ,in Uganda, where the MDR rate was 64%,[19] while in Ethiopia, MDR rates of 95%, and 78% have been reported.[39,40] These differences could be related to the extent of irrational use of antibiotics, with areas having a large degree of irrational use having a high prevalence of MDR.

Biofilm-forming *E. coli* isolated from the urine of pregnant women

This was a high proportion of biofilm formers compared to the study conducted in India on 100 *E. coli* strains, in which 72 of them were biofilm positive, comprised of 6% strong positive, 80% moderate positive and 14% weakly positive.[41] Likewise, a study conducted in Iran, showed that 48.4% of the *E. coli* isolates were strong biofilm formers, 15.6% were moderately potent, 21.8% were weak and 14.2% were not biofilm formers.[3] These differences may be due to having a high number of *E. coli* examined compared to that of the current study.

Association between antibiotic susceptibility and biofilm-forming *E. coli*

The current study result was relatively similar to the study conducted in Iran, which showed that there was no statistically significant relationship between antibiotic susceptibility and biofilm formation; although biofilm production increases antibiotic susceptibility in bacteria, drug resistance does not depend only on the presence of biofilm and many other factors such as the presence of degrading enzymes, the presence of effusion pumps, and changes in the site of action[3]. Furthermore, the study was contrary to the Ugandan study findings which demonstrated a significant association between antibiotic susceptibility and biofilm formation of *E. coli*.[19] In accord with that, studies conducted in India[41] and Nepal[42] showed that antibiotic susceptibility of biofilm-forming *E. coli* was significantly higher than that of biofilm-non-forming *E. coli*, ($P<.05$). The finding indicate that sample size and comparison factors are more importance in looking for association despite of positive impact observed.

Strengths and Limitations of the Study

Strengths

This was the first study to demonstrate how crucial biofilm formation was to Zanzibar's antibiotic susceptibility monitoring. Furthermore, the research revealed that the majority of antibiotic susceptible *E. coli* isolates exhibited high biofilm formation abilities.

Limitations

The virulence factors linked to biofilms, such as hemagglutinations, gelatinase productions, Extended Spectrum Beta Lactamases (ESBLs), Ampicillin-resistance group C (AmpC) beta-lactamase, and carbapenemases, which are linked to antibiotic susceptibility resistance for *E. coli*, were not examined in the current investigation. Additionally, phenotypic characterization was employed for biofilm detection; however, a molecular technique could probably have yielded a more comprehensive picture.

CONCLUSION

Resistance was found against the routinely used antibiotics of Ampicillin, Amoxicillin & clavulanic acid to the majority of the biofilm-forming *E. coli* isolates in the current investigation. Therefore, AMR surveillance is needed to monitor the effect of biofilm throughout the UTIs' causative agents.

Recommendation

In treating UTI cases in pregnant women, the screening of antimicrobial susceptibility patterns before the prescription of antibiotics is highly recommended. Furthermore, studies should be conducted especially in all UTIs causative agents to detect biofilm and its association with antibiotic susceptibility. This will, in turn, improve understanding particularly in UTIs diagnosis which has a bigger impact on treatment management.

Finally, the MPA methods should be introduced in the AMR surveillance program which is affordable and quantitative in examining the biofilm formation of every microorganism undergoing resistance.

REFERENCES

1. Amiri M, Lavasani Z, Norouzirad R, et al. Prevalence of urinary tract infection among pregnant women and its complications in their newborns during the birth in the hospitals of Dezful city, Iran, 2012 - 2013. Iran Red Crescent Med J. 2015;17(8). doi:10.5812/ircmj.26946

2. Gilbert NM, O'brien VP, Hultgren S, Macones G, Lewis WG, Lewis AL. Urinary tract infection as a preventable cause of pregnancy complications: opportunities, challenges, and a global call to action. Glob Adv Heal Med. 2013;2(5):59-69. doi:10.7453/gahmj.2013.061

3. Nikzad M, Mirnejad R, Babapour E. Evaluation of antibiotic resistance and biofilm formation ability uropathogenic E. coli (UPEC) isolated from pregnant women in Karaj. Iran J Med Microbiol. 2021;15(2):195-211. doi:10.30699/ijmm.15.2.195

4. Schmider J, Bühler N, Mkwatta H, et al. Microbiological characterisation of community-acquired urinary tract infections in Bagamoyo, Tanzania: a prospective study. Trop Med Infect Dis. 2022;7(6):100. doi:10.3390/tropicalmed7060100

5. Ndamason LM, Marbou WJT, Kuete V. Urinary tract infections, bacterial resistance and immunological status: a cross-sectional study in pregnant and non-pregnant women at Mbouda Ad-Lucem hospital. Afr Health Sci. 2019;19(1):1525-1535. doi:10.4314/ahs.v19i1.26

6. Johnson B, Stephen BM, Joseph N, Asiphas O, Musa K, Taseera K. Prevalence and bacteriology of culture-positive urinary tract infection among pregnant women with suspected urinary tract infection at Mbarara Regional Referral Hospital, South-Western Uganda. BMC Pregnancy Childbirth. 2021;21(1):159. doi:10.1186/s12884-021-03641-8

7. Laari JL, Anab M, Jabong DP, Abdulai K, Alhassan AR. Maternal age and stage of pregnancy as determinants of UTI in pregnancy: a case of Tamale, Ghana. Infect Dis Obstet Gynecol. 2022;2022. doi:10.1155/2022/3616028

8. Belete MA. Bacterial profile and ESBL screening of urinary tract infection among asymptomatic and symptomatic pregnant women attending antenatal care of Northeastern Ethiopia Region. Infect Drug Resist. 2020;Volume 13:2579-2592. doi:10.2147/IDR.S258379

9. Ankur G, Namita S, Sapna G, et al. Prevalence of asymptomatic urinary tract infections in the three trimesters of pregnancy. Int.J.Curr.Microbiol.App.Sci. 2015;Special Issue1:110-117. Accessed on 12 February 2023.

10. Chaula T, Seni J, Ng'walida N, et al. Urinary tract infections among HIV-positive pregnant women in mwanza city, Tanzania, are high and predicted by low CD4+ count. Int J Microbiol. 2017;2017:1-7. doi:10.1155/2017/4042686

11. Kaduma J, Seni J, Chuma C, et al. Urinary tract infections and preeclampsia among pregnant women attending two hospitals in Mwanza City, Tanzania: A 1:2 Matched case-control study. Biomed Res Int. 2019;2019:3937812. doi:10.1155/2019/3937812

12. Kaye KS, Gupta V, Mulgirigama A, et al. Antimicrobial resistance trends in urine Escherichia coli isolates from adult and adolescent females in the United States from 2011 to 2019 : rising ESBL strains and impact on patient management. 2021;73(11):1992-1999 doi:10.1093/cid/ciab560

13. Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C. Urinary tract infections: the current scenario and future prospects. Pathogens. 2023;12(4). doi:10.3390/pathogens12040623

14. Bader MS, Loeb M, Leto D, Brooks AA. Treatment of urinary tract infections in the era of antimicrobial resistance and new antimicrobial agents. Postgrad Med. 2020;132(3):234-250. doi:10.1080/00325481.2019.1680052

15. Dang H, Lovell CR. Microbial surface colonization and biofilm development in marine environments. Microbiol Mol Biol Rev. 2016;80(1):91-138. doi:10.1128/mmbr.00037-15

16. Karigoudar RM, Karigoudar MH, Wavare SM, Mangalgi SS. Detection of biofilm among uropathogenic Escherichia coli and its correlation with antibiotic resistance pattern. J Lab Physicians. 2019;11(01):017-022. doi:10.4103/JLP.JLP_98_18

17. Balows A. Manual of clinical microbiology 8th edition. Diagn Microbiol Infect Dis. 2003;47(4):625-626. doi:10.1016/S0732-8893(03)00160-3

18. Garcia L. Clinical Microbiology Procedures Handbook,. p. 3.2.1.2.; 2010.

19. Katongole P, Nalubega F, Florence NC, Asiimwe B, Andia I. Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic Escherichia coli isolated from clinical isolates in Uganda. BMC Infect Dis. 2020;20(1):453. doi:10.1186/s12879-020-05186-1

20. CLSI. Performance standard for antimicrobial susceptibility testing. 32 ed. CLIS supplement M100. J Serv Mark. 2022;32nd Ed. https://clsi.org/media/wi0pmpke/m100ed32_sample.pdf

21. Karigoudar RM, Karigoudar MH, Wavare SM, Mangalgi SS. Detection of biofilm among uropathogenic Escherichia coli and its correlation with antibiotic resistance pattern. J Lab Physicians. 2019;11(01):017-022. doi:10.4103/jlp.jlp_98_18

22. Stepanović S, Vuković D, Hola V, et al. Quantification of biofilm in microtiter plates: an overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. Apmis. 2007;115(8):891-

899. doi: 10.1111/j.1600-0463.2007.apm_630.x.

23. Willy Fred N, Gichuhi JW, Mugo NW. Prevalence of urinary tract infection, microbial aetiology, and antibiotic sensitivity pattern among antenatal women presenting with lower abdominal pains at Kenyatta National Hospital, Nairobi, Kenya. Open Access J Sci Technol. 2015;3. doi:10.11131/2015/101115

24. Aminu KY, Aliyu UU. Asymptomatic bacteriuria in pregnant women in the antenatal booking clinic at Aminu Kano Teaching Hospital, Kano, Nigeria. Open J Obstet Gynecol. 2015;5(5):286-297. doi:10.4236/ojog.2015.55042

25. Ali MA, Majed SA, Saad AA, Mohammad. Prevalence of urinary tract infection and antibiotic resistance pattern in pregnant women, Najran region, Saudi Arabia. African J Microbiol Res. 2019;13(26):407-413. doi:10.5897/AJMR2019.9084

26. Wabe YA, Reda DY, Abreham ET, Gobene DB, Ali MM. Prevalence of asymptomatic bacteriuria, associated factors and antimicrobial susceptibility profile of bacteria among pregnant women attending Saint Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. Ther Clin Risk Manag. 2020;16:923-932. doi:10.2147/TCRM.S267101

27. Ejerssa AW, Gadisa DA, Orjino TA. Prevalence of bacterial uropathogens and their antimicrobial susceptibility patterns among pregnant women in Eastern Ethiopia: hospital-based cross-sectional study. BMC Womens Health. Published online 2021;21(1):291. doi:10.1186/s12905-021-01439-6

28. Gessese YA, Damessa DL, Amare MM, et al. Urinary pathogenic bacterial profile, antibiogram of isolates and associated risk factors among pregnant women in Ambo town, central Ethiopia: a cross-sectional study. Antimicrob Resist Infect Control. 2017;6(1):1-10. doi:10.1186/s13756-017-0289-6

29. Ngowi BN, Sunguya B, Herman A, et al. Prevalence of multidrug-resistant UTI among people living with HIV in Northern Tanzania. Infect Drug Resist. 2021;14:1623-1633. doi:10.2147/IDR.S299776

30. Awoke N, Kassa T, Teshager L. Magnitude of biofilm formation and antimicrobial resistance pattern of bacteria isolated from urinary catheterized inpatients of Jimma University Medical Center, Southwest Ethiopia. Int J Microbiol. 2019;2019. doi:10.1155/2019/5729568

31. Olowe OA, Adefioye OJ, Ajayeoba TA, et al. Phylogenetic grouping and biofilm formation of multidrug-resistant Escherichia coli isolates from humans, animals and food products in South-West Nigeria. Sci African. 2019;6:e00158. doi:10.1016/j.sciaf.2019.e00158

32. Boroumand M, Sharifi A, Manzouri L, Khoramrooz SS, Khosravani SA. Evaluation of pap and sfa genes relative frequency P and S fimbriae encoding of uropathogenic Escherichia coli isolated from hospitals and medical laboratories; Yasuj City, Southwest Iran. Iran Red Crescent Med J. 2019;21(8). doi:10.5812/ircmj.89499

33. Ballén V, Cepas V, Ratia C, Gabasa Y, Soto SM. Clinical Escherichia coli: from biofilm formation to new antibiofilm strategies. MDPI. 2022;10(6):1103. doi:10.3390/microorganisms10061103

34. Fredrick F, Francis JM, Fataki M, Maselle SY. Aetiology, antimicrobial susceptibility and predictors of urinary tract infection among febrile under-fives at Muhimbili National Hospital, Dar es Salaam-Tanzania. 2013;7(12):1029-1034. doi:10.5897/AJMR12.1866

35. Bhola P, Mvelase NR, Balakrishna Y, Mlisana KP, Swe Swe-Han K. Antimicrobial susceptibility patterns of uropathogens isolated from pregnant women in KwaZulu-Natal Province: 2011 - 2016. South African Med J. 2020;110(9):872. doi:10.7196/SAMJ.2020.v110i9.14468

36. Mohamoud H, Tadesse S, Derbie A. Antimicrobial resistance and ESBL profile of uropathogens among pregnant women at Edna Adan Hospital, Hargeisa, Somaliland. Ethiop J Health Sci. 2021;31(3):645-652. doi:10.4314/ejhs.v31i3.22

37. Amadu DO, Nwabuisi C, Yunusa T, Nasir I. Prevalence and associated risk factors of uropathogenic Escherichia coli isolates from catheterized persons at Ilorin. 2019;9(2):119-128. doi: 10.21608/AEJI.2019.11367.1019

38. Report S. Antimicrobial resistance and healthcare-associated infections. J Hosp Infect. 2016;94(4):412. doi:10.1016/j.jhin.2016.10.014

39. Alemu A, Dagnew M, Alem M, Gizachew M. Uropathogenic bacterial isolates and their antimicrobial susceptibility patterns among HIV/AIDS patients attending Gondar University Specialized Hospital Gondar, Northwest Ethiopia. J Microbiol Res Rev. 2013;1(4):42-51. www.resjournals.org/JMR. .Accessed on 23 March 2023.

40. Fenta GM, Legesse MH, Weldearegay GM. Bacteriuria and their antibiotic susceptibility patterns among people living with HIV attending Tikur Anbessa Specialized and Zewditu Memorial Hospital ART Clinics, Addis Ababa, Ethiopia. J Bacteriol Parasitol. 2016;7:292. doi:10.4172/2155-9597.1000292

41. Ponnusamy P, Natarajan V, Sevanan M. In vitro biofilm formation by uropathogenic Escherichia coli and their antimicrobial susceptibility pattern. Asian Pac J Trop Med. 2012;5(3):210-213. doi:10.1016/S1995-7645(12)60026-1

42. Neupane S, Pant ND, Khatiwada S, Chaudhary R, Banjara MR. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended-spectrum beta-lactamase production in uropathogenic Escherichia coli isolated from the patients suspected of urinary tract infections visit. Antimicrob Resist Infect Control. Published online 2016;5:5. doi:10.1186/s13756-016-0104-9

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