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Molecular Basis for Adhesion and Biofilm Formation in Urinary Tract Infections

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ABSTRACT

Bacterial biofilms play an important role in urinary tract infections (UTIs), being responsible for persistence infections and drug resistance. Several adhesion factors are involved in attachment of bacterial cells to the urinary tract and biofilm development. Although the distribution of pathogens that cause UTIs is changing. More important is the increase in resistance to some antimicrobial agents. This study aimed to evaluate the molecular basis for adhesion and biofilm formation by the pathogens associated with UTI in ATBUTH Bauchi State, Nigeria. A random sampling method was used to select study participants. Clean catch urine samples of inpatient and outpatient with cases of UTI were collected and bacteriologically analyzed using standard microbiological procedures. Twenty-three of the bacteria isolates were detected to form biofilm which were further identified and antimicrobial susceptibility testing carried out using Vitek 2. Further analysis by PCR for biofilm and adhesion genes detected *sfa, mrpA, mrKA* and *bap* genes while *ica* gene was weakly detected. The most prevalent isolates were *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella* Typhi and *Klebsiella pneumoniae*. Our findings show that some of the isolated bacteria were susceptible to ampicillin and ceftriaxone but a significant number of them are multi-drug resistant.

Key words: biofilm, UTIs, uropathogens, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella* Typhi, *Klebsiella pneumoniae*

INTRODUCTION

Bacteriuria accompanied by urinary symptoms is referred to as a urinary tract infection (UTI), one of the most prevalent bacterial infections in clinical practice, especially in a developing nation, with a high morbidity and expense to treat if not taken care on time [1]. Urinary tract infections affect people of different ages, including young women, children and the elderly, and are one of the major causes of morbidity and health care financial burden. An estimated 40% of women have experienced a urinary tract infection at some point in their lives [2].

When bacteria first attach themselves to a surface, they multiply and produce an extracellular polymeric matrix, which leads to cell aggregation and the creation of biofilms [3]. These bacteria, known as uropathogens, have the ability to produce a wild range of adhesions for biofilm formation and attachment to solid surfaces. The primary pathogenesis and treatment failure appears to be the bacteria ability to endure and proliferate in a biofilm which is thought to be a key component in the bacterial cell long-term survival in the urinary system and their

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ability to trigger the inflammatory responses linked to UTIs [4, 5]. Moreover, a rise in medication resistance among the biofilm associated bacteria may make therapy considerably more challenging.

The most frequent pathogenic organisms associated with UTIs are gram-negative pathogens, such as *Escherichia coli* and *Klebsiella pneumoniae* as well as other Enterobacteriaceae. Furthermore, gram-positive organisms such as *Streptococcus* Group B. *Enterococci*, coagulase negative *staphylococci*, and *Staphylococcus aureus* are recognized to be the cause of UTIs. Between 0.5 and 6% of UTI cases with low fatality rates are caused by *S. aureus*. Global data show that approximately 35% of *S. aureus*-related UTI infections are symptomatic and chemotherapy is strongly advised for these individual [6]. When treating UTI cases, medication is frequently initiated empirically and is based on data gleaned from the urine bacterial pattern of antibiotic resistance [7, 8]. Despite the availability and widespread usage of antimicrobial medications, bacterial UTIs have been on the rise recently. The emergence of antimicrobial resistance in UTI has been linked to a significant portion of the increase. One significant and constantly changing public health concern is the rise in multi-drug resistance in bacterial uropathogens which is supported by biofilm formation by the bacterial [1, 7]. Globally, urinary bacteria are becoming more common place in exhibiting resistance to antibiotics [9].

MATERIALS AND METHODS

Study Design

A cross-sectional study was conducted among patients who presented with symptoms of UTI at Abubakar Tafawa Balewa University Teaching Hospital Bauchi Nigeria.

Sample Size and Sampling Techniques

The sample size was calculated using a single population proportion formula

$$n = Za^2\text{-}P\ (1-P)\ d^2,$$

where:
N = number of sample size,
Z is the statistics corresponding to a 95% level of confidence (1.96),
D = margin of error, and
P = is the assumed prevalence of uropathogenic bacteria among UTI cases (50%).

The infection prevalence was assumed to be 50% because the current status of uropathogenic bacterial infection in the area is unknown. Therefore, the sample size was adjusted to 281 UTI cases.

Inclusion Criteria

Adults and children above five (5) with symptoms of UTI attending ATBUTH were included in the study.

Exclusion Criteria

- Under 5 paediatric patients;
- Those with polymicrobial infections involving more than two bacterial species;
- Patients with Candida spp. as the sole pathogen or with bacteria;
- Pregnant women;
- Those who had previously been on antibiotic therapy were all excluded from the study.

Sample Collection

The study was performed among patients presented with UTI attending ATBUTH from October to December 2023. A total of 281 clean catch midstream urine samples were collected in a wide mouth sterile container from the study subjects who presented with symptoms of UTI and have not received antimicrobials within the previous fifteen days [2].

Urine Microscopy for the Detection of Bacteriuria

Detection was done microscopically using wet preparation. The performance characteristics of the test are not well-defined, because different criteria have been used to define a positive test result. In one study, the test was found to be sensitive for the detection of 10^5 cfu/mL but insensitive for the detection of lower numbers of bacteria [10]. Other investigators have found the test to be of low sensitivity for the detection of UTI [11, 12].

Isolation, Identification and Biochemical Characterization of Pathogens

The samples were processed according to a previously described methodology [13]. Only patients that presented with clinical symptoms of UTI and positive urine culture ($\geq 10^5$ CFU/mL) were studied.

Isolation of uropathogens was performed by a surface streak procedure on both blood and MacConkey agar (Oxoid Ltd. Basingstoke Hampshire, UK) using calibrated loops for semi-quantitative method and incubated aerobically at 37 ^{0}C for 24 hours, and those cultures which becomes negative at the end of 24hrs incubations were further incubated for 48 hours. A specimen was considered positive for UTI if a single organism was cultured at a concentration of $\geq 10^5$ cfu/mL. Bacterial identification was made using biochemical tests, namely indole, citrate, oxidase, H_2S production, lysine decarboxylase, lactose fermentation, urea hydrolysis, gas production, catalase, coagulase, manitol fermentation [13].

Phenotypic Assay using Congo Red for the Identification of Biofilm Forming Bacteria

The in vitro biofilm production was measured using tube method for the phenotypic assay. A qualitative method for the detection of biofilm formation was performed by inoculating 10ml of nutrient broth with a loopful of organisms from a culture plate and was incubated at overnight at 37^{0}C culture tubes were then emptied and washed with distilled water, then allow to air dry, then stained with (0.1%) Congo red for 10 minutes and washed with deionized water and allowed to air dry in inverted position. Slime production was considered positive when a visible film was observed along the inner wall or bottom of tube [14].

Genotypic Assay

A single colony of our biofilm forming isolates were inoculated in 10mL nutrient broth and incubated at 37^{o}C with shaking at 210 rpm for 24hrs. DNA extraction was performed using a Bioneer DNA extraction kit according to the manufacturer's instructions. The master mix for the PCR was prepared as follows: 3 µL of 10X PCR buffer, 3 µL of 25 mM MgCl2, 3 µL of 10 mM dNTP mix, 0.5 µL of Taq DNA polymerase, 9.5 µL of Milli-Q water, and 1 µL of each of the forward and reverse primers. Finally, 4 µL of each DNA template was added to the corresponding tubes to make up the final reaction volume of 25 µL. Amplification of mrpA, ica, mrkA, sfa and bap genes was performed using PCR. The PCR primers and condition for each gene are presented in Table 1. Finally, PCR products were mixed with 3 µL of PowerLoad DNA stain and were visible after electrophoresis in a 1% agarose gel in TBE buffer and under UV illumination. In order to ensure the accuracy of the PCR products. Out of five genes investigated only three were detected [13].

Assessment of Antimicrobial Susceptibility

Antimicrobial susceptibility testing with the Vitek 2 compact system was performed using an AST N281 card according to the Manufacturer's instructions. A panel of twenty (20) antibiotics in AST N281 card was tested. The cards were filled with inoculum (Prepared by transferring 200µl of culture suspension from the 0.5 McFarland culture suspension used for filling the identification cards into a fresh 3ml sterile saline solution obtaining a final turbidity of $8x10^6$ cfu/mL) in the filling chamber. The Vitek 2 system automatically processes the antimicrobial susceptibility cards until MIC's are obtained. The Vitek 2 compact system subsequently corrects, where necessary for MIC's in accordance with the internal database of possible phenotypes for microorganism antimicrobial agent combinations [15].

Ethical Consideration

The study protocol was evaluated and approved by the research ethical committee of Abubakar Tafawa Balewa University Teaching Hospital and ethical clearance was obtained. All methods were performed in accordance with relevant guidelines and regulations. After adequately explaining the objectives and purpose of the study, written informed consent was obtained from all the study subjects and/ or assent were obtained from study subject less than 18 years and/or guardians before data and sample collection. All data obtained in the course of the study was kept confidential. Positive cases were referred to the attending clinician as soon as possible for their better management.

Table 1: Primer sequence used for PCR

Target gene	Primer sequence (5-3)	Size (bp)	Annealing temp (^{0}C)
mrpA	F: TTC TTA CTG ATA AGA CAT TG	512	56
	R: ATT TCA GGA AAC AAA AGA TG		
Ica	F: TGG GTA TTC CCT CTG TCT GG	490	58
	R: TCC AGA AAC ATT GGG AGG TC		
mrkA	F: AAT GTA GGC GGC GGT CAG	351	59
	R: CTC TCC ACC GAT AAC GCC A		
Sfa	F: CTC CGG AGA ACT GGG TGC ATC TTA C	430	57
	R: CAT CAA GCT GTT TGT TCG TCC GCC G		
Bap	ATG CCT GAG ATA CAA ATT AT	498	55
	GTC AAT CGT AAA GGT AAC G		

RESULTS AND DISCUSSION

Socio-Demographic Characteristics

A total of 281 patients with clinical symptoms of UTI were recruited for this study. The mean age of the study participants was 29±11.5 years, with an age range of 15–65 years. The highest number of participants were female with 74% in the age group 25-34 majority were married with 59.1%, those with formal education 79.0%. Approximately 62.3% of the study participants had a history suggestive of UTI (Table 1).

Table 2: Socio-demographic characteristics and distribution of UTI among patients attending ATBUTH

Characteristic	No. of patients (%)
Sex	
Male	71(25.3)
Female	210(74.7)
Age group	
15-24	93(33.1)
25-34	112(39.9)
35-44	76(27.0)
45 and above	
Marital status	
Married	166(59/1)
Single	61(21.7)
Widowed and divorced	54(19)
Educational level	
Formal education	222(79.0)
No formal education	59(13.9)
History of UTI	
Yes	175(62.3)
No	106(37.7)

Two hundred and eighty-one (281) urine sample were collected from patients with clinical symptoms of UTI, only 23(8.2%) shows significant bacteriuria and positive to subsequent biochemical test for identification. *Escherichia coli* had the highest proportion of the isolates with 39.1%, followed by *Klebsiella pneumoniae* 31.7%, *Staphylococcus aureus* 17.4%, *Pseudomonas aeruginosa*, 8.7%, *Proteus mirabilis* 8.7 % and *Salmonella* Typhimurium 4.4% respectively.

Table 3: Major clinical important uropathogens isolated from patient with sepsis and symptoms of UTI in ATBUTH

S/N	Bacteria	Number (%)
1	*Staphylococcus aureus*	6 (26.1)
2	*Proteus mirabilis*	2 (8.7)
3	*Escherichia coli*	9 (39.1)
4	*Salmonella* Typhimurium	1 (4.4)
5	*Klebsiella pneumoniae*	5 (21.7)
Total		23(100)

Phenotypic Assay for Biofilm Production

Screening for biofilm production was done in-vitro by Congo red tube method. Six (6) specie of bacteria were positive (*S. aureus, P. mirabilis, E. coli, S.* Typhi and *K. pneumoniae*) and were the only one subjected to biofilm phenotypic assay using Congo red method. All the 6 species of bacteria show strong biofilm formation (Figure 1).

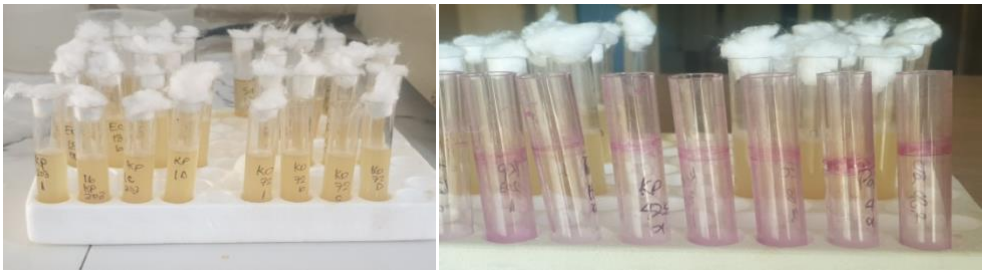
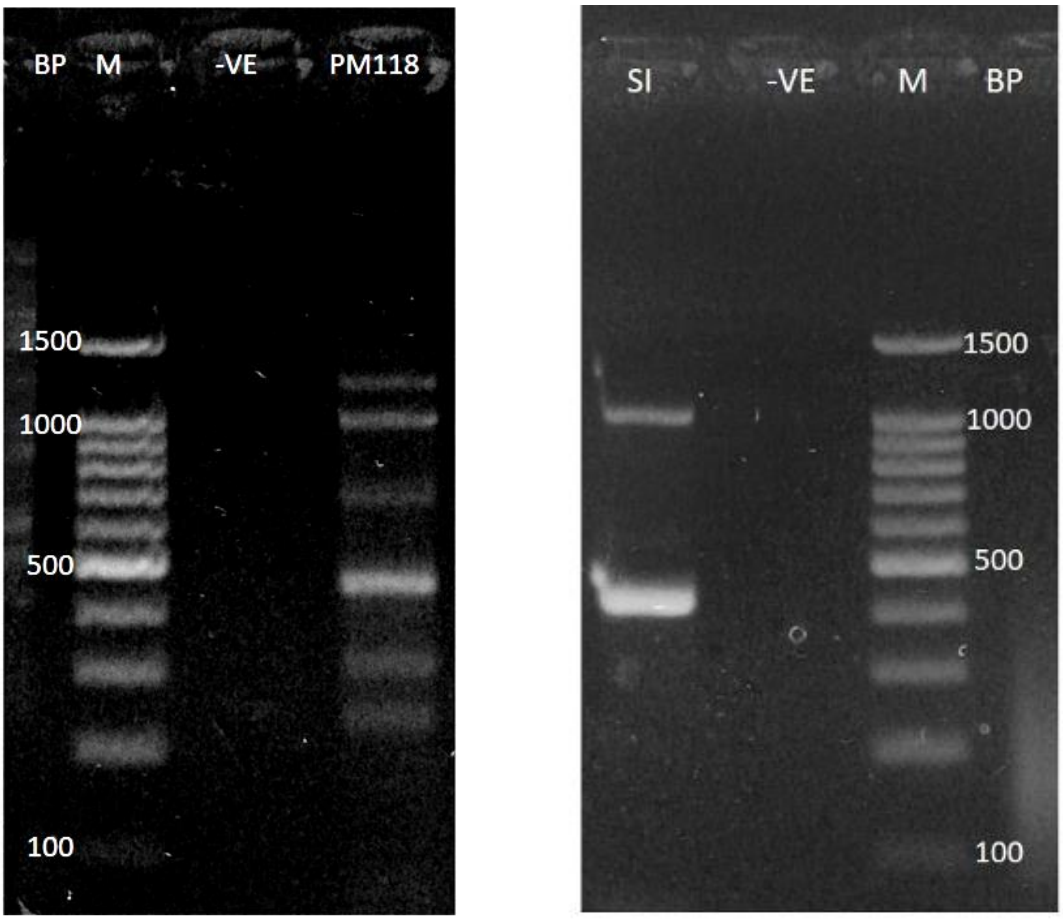


Figure 1: In-vitro assay for biofilm production

Genotypic Detection of Adhesion Factor Gene

Using a polymerase chain reaction (PCR), all biofilm-forming isolates were examined for the presence of distinct adhesion factor genes. It was examined whether the genes associated with the following adhesion factors could be found. The target genes include the fibrilla (*sfa, ica, mrkA, mrpA, and bap*) (Figure 2).



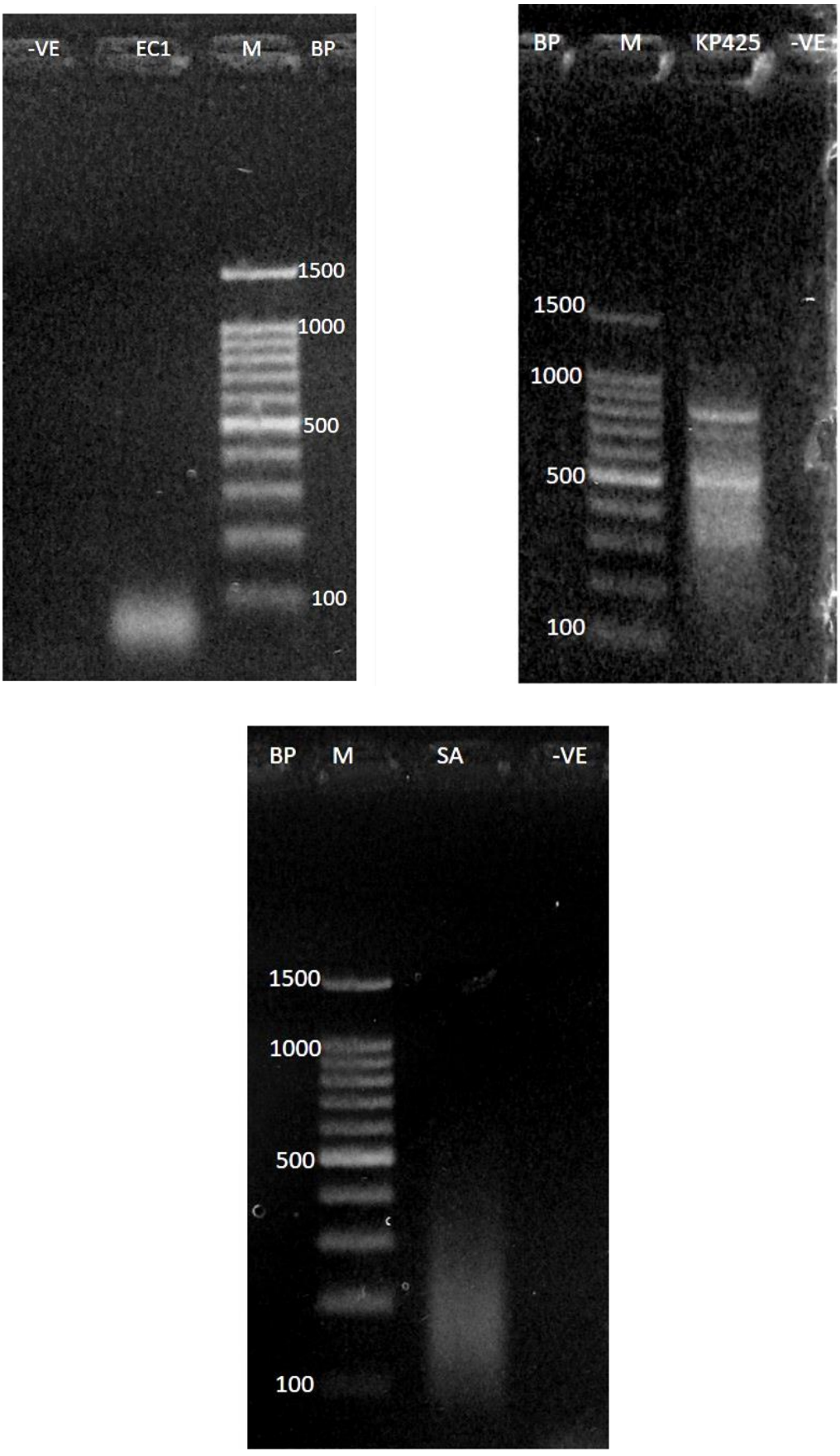


Figure 2: Gel picture of target genes

Antimicrobial Susceptibility of the Biofilm Producing Bacteria Isolated

The antimicrobial-susceptibility testing of biofilm producing bacterial isolated was assessed for 20 antimicrobial agents. The isolated bacteria from patients with UTIs revealed the presence of high levels of single and multiple antimicrobial resistances against commonly prescribed drugs (Table 3a, b, c, d and e). *Escherichia coli*, with the highest number of isolated uropathogens in this study shows to be the major causes of UTI, revealed High resistance to Gentamycin and low resistance to Cefazolin and Ceftriaxone, as indicated in Table 3c. *Staphylococcus aureus* which is the second most prevalent pathogen isolated from this study for UTI shows high resistance to Trimethoprim/Sulfamethoxazole and moderately resistance to Oxacillin, Ciprofloxacin, Clindamycin but low resistance to Levofloxacin. *Klebsiella pneumoniae* is susceptible to all other antimicrobial agent (Table 3e) but resistance to Ampicillin, Ampicillin/Sulbactam and Cefazolin. However, *Proteus mirabilis* and *Salmonella* ser. Typhimurium formed multiple resistance to 9 and 11 respectively out of the 20 antimicrobial agents. All isolates were susceptible to Levofloxacin.

Table 3a: Antimicrobial susceptibility testing for *Staphylococcus aureus*

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Cefoxitin	POS	+	Clindamycin	>= 4	R
Ampicillin			Linezolid	2	S
Oxacillin	>= 4	R	Daptomycin	<= 0.12	S
Gentamycin	8	I	Vancomycin	1	S
Ciprofloxacin	>= 8	R	Doxycycline	4	S
Levofloxacin	4	R	Tetracycline	>= 16	R
Moxifloxacin	1	I	Tigecycline	<=0.12	S
Inducible Clindamycin Resistance	NEG	-	Trimethoprim/ Sulfamethoxazole	160	R
Erythromycin	>= 8	R	Nitrofurantoin	32	S

*=AES modified**=User modified

R=Resistance. S=Sensitive. I= Intermediate. MIC= Minimum Inhibitory Concentration

Table 3b: Antimicrobial susceptibility testing for *Proteus mirabilis*

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>=32	R	Ertapenem	4	*R
Ampicillin/Sulbactam	>=32	R	Meropenem	1	S
Piperacillin	>=128	R	Amikacin	4	S
Cefazolin	>=64	R	Gentamycin	8	I
Cefoxitin	32	R	Tobramycin	2	S
Ceftazidime	16	R	Ciprofloxacin	1	S
Ceftriaxone	>=64	R	Levofloxacin	1	S
Cefepime	4	S	Trimethoprim/ Sulfamethoxazole	>=320	R

*=AES modified**=User modified

Table 3c: Antimicrobial susceptibility testing for *Escherichia coli*

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>=32	R	Ertapenem		
Ampicillin/Sulbactam	>=32	R	Meropenem	=< 0.25	S
Piperacillin	=< 4	S	Amikacin	4	S
Cefazolin	4	R	Gentamycin	>=128	R
Cefoxitin			Tobramycin	=< 1	S
Ceftazidime	>=34	R	Ciprofloxacin	>=32	R

Ceftriaxone	2	R	Levofloxacin	0.25	S
Cefepime	=< 1	S	Trimethoprim/ Sulfamethoxazole		

*=AES modified**=User modified

Table 3d: Antimicrobial susceptibility testing for *Salmonella* ser. Typhimurium

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>=32	R	Ertapenem	4	*R
Ampicillin/Sulbactam	>=32	R	Meropenem	1	S
Piperacillin	>=128	R	Amikacin	4	S
Cefazolin	>=64	R	Gentamycin	>=32	R
Cefoxitin	32	R	Tobramycin	2	S
Ceftazidime	16	R	Ciprofloxacin	>=32	R
Ceftriaxone	>=64	R	Levofloxacin	1	S
Cefepime	4	S	Trimethoprim/ Sulfamethoxazole	>=320	R

*=AES modified**=User modified

Table 3e: Antimicrobial susceptibility testing for *Klebsiella pneumoniae*

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ampicillin	>=32	R	Ertapenem		
Ampicillin/Sulbactam	>=32	R	Meropenem	=<0.25	S
Piperacillin	=<4	S	Amikacin	4	S
Cefazolin	4	R	Gentamycin	=<1	I
Cefoxitin			Tobramycin	=<1	S
Ceftazidime	2	S	Ciprofloxacin	0.5	S
Ceftriaxone	2	S	Levofloxacin	0.25	S
Cefepime=<	=<1	S	Trimethoprim/ Sulfamethoxazole		

*=AES modified**=User modified

DISCUSSION

Urinary tract infections (UTIs) are one of the common and recurrent infections caused by bacteria in urinary system, the pathogenesis and antimicrobial susceptibility patterns of these organisms have led us to the study of the basis for the ability form adhesion and subsequently biofilm which enhances their survival in the host also resist antimicrobial agents thereby frustrating treatments. Our study presents several data on genetic analysis on the basis of adhesion and biofilm production by some uropathogens isolated from patients attending ATBUTH Bauchi.

In this study we present data on genetic analysis on the basis of adhesion and biofilm production by some uropathogens isolated from patients attending ATBUTH Bauchi, the pathogen virulence factors which include various surface adhesion and biofilm molecules enable it to form adhesion and biofilm in urinary tract [35]. Recently published data highlighted variability in biofilm formability among bacteria. In previous research, various results have been declared on biofilm producing bacteria and rate of biofilm production ranging from 43% to 88% [20; 21]. Our current study confirmed high ability of biofilm formation in 23 isolates (*Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Salmonella* Typhi and *Klebsiella pneumoniae).* According to a finding made by Hosseini *et al*. he noted a good and clear biofilm formation in *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Salmonella* Typhi and *Klebsiella pneumoniae* with weak, moderate, and strong biofilm makers, respectively [22].

Adhesion and biofilm encoding genes strictly associated with adherence to the host components and biofilm formation.

Out of the 23 confirmed biofilm producing bacteria isolated in this study, *E. coli* was found to be the predominant and most frequently uropathogen biofilm forming bacteria isolated. Similar studies carried out in Southwest of Nigeria reported *E. coli* as predominant urinary tract pathogen [27; 28; 29]. In contrast, Henry *et al.* 2021 reported that *S. aureus* was the predominant isolated uropathogen from patients with signs and symptoms of UTI [30]. This discrepancy clearly indicates that the distribution of microorganisms that cause UTIs, as well as their pattern of antibiotic susceptibility, differs periodically and from location to location [31]. The ability to build biofilms and UTIs has been linked in the past [36; 37]. Through its ability to shield bacteria from the effects of hydrodynamic pressures, antibacterial drugs, and host defense mechanisms, biofilm development encourages bacteria persistence in the urinary tract. Analyzing the elements that lead to the production of biofilms may be useful in developing novel therapeutic approaches for these infections.

Among the gram-negative bacteria isolated *K. pneumonia*e was found to be the most sensitive isolate to antibiotics test, *K. pneumoniae* exhibited significant susceptibility to 9 out of 17 antibiotics used on it (Table 3e). Also it is the only bacteria isolated in the study that is susceptible to Ceftriaxone, this is in agreement with previously study published by Prakash and Saxen [27; 28; 29]. They all reported that Ceftriaxone showed effective activity against all the isolates. Since other uropathogen apart from *K. pneumoniae* isolated in this study confirmed to be susceptible to ampicillin, and Ceftriaxone, it would be a perfect choice for UTI empiric therapy pending the result of culture and susceptibility testing is available.

In this study, our isolates were resistant to ampicillin followed by Cefazolin, Trimethoprim/ Sulfamethoxazole and Ceftazidime this agrees with work of Abejew, Denboba and Mekonnen and others [30, 31, and 33]. Prolonged exposure and repeated use of antimicrobial drug could have led to higher resistance of isolates [33]. More so, continuous use of antimicrobial can also predispose to UTIs by damaging peri-urethral flora in both immune competent and immune-compromised persons, allowing colonization by uropathogens [34], as the condition permits transfer of genetic material horizontally. *Staphylococcus aureus* as the only gram-positive bacteria isolated in the study was observed to resist Clindamycin, Tetracycline, Ciprofloxacin, Levofloxacin and Levofloxacin. *Staphylococcus aureus* forming resistance to various antimicrobial agents is a major concern and seen as most frequently occurring resistant bacteria identified in many countries which makes treatments difficult [19]. However, the data shows a good relationship between biofilm producing bacteria isolated in this study and a high tendency to exhibit the multi- drug resistance pattern. We found that all biofilm producing isolates were multi-drug resistant. This agreed with a study performed by Azmi *et al*. [20].

The role of *mrpA, ica, mrkA, sfa* and *bap* genes in *Proteus mirabilis, Staphylococcus spp,* and *Pseudomonas spp, Klebsiella spp, E. coli and Salmonella spp* respectively for adhesion, colonization and the capability of biofilm production [22; 23; 24]. It was found that all the isolated bacteria contain the respective genes as mentioned above. The same finding has been recorded in a study made by Yousefi *et al.* They indicated biofilm production in *S. aureus, P. mirabilis, E. coli, S.* Typhi *and K. pneumoniae* isolated from UTI and the existence of *mrpA, ica, mrkA, sfa* and *bap* genes were confirmed in the bacteria [24] Similar to the present study, previous works have shown the existence of *mrpA, ica, mrkA, sfa* and *bap* genes was in uropathogens with the ability of biofilm production [25; 23]. For biofilm production, there are a lot of disputes on ideology of synthesis of polysaccharide intercellular adhesion (PIA) and ability to produce biofilm that is under the control of the *ica* operon. An *ica* was weakly noticed in the current study. Similarly, the low prevalence of ica gene was noted by Azmi *et al.* [20]. This implies that the *ica*- dependent system may not be an absolute mechanism in biofilm

production and these strains of bacteria may use other systems to produce biofilm. In conformity with the research conducted by Sharma *et al.* [26], who reported a very low of *ica* gene in *Staphylococcus aureus* isolates. On the other hand, bap gene is said have important function in biofilm formability in *Salmonella spp*. In conformity with the research conducted by Sharma *et al*. [26] who reported a presence of bap gene in *Salmonella* isolates, in our study we also recorded the presence of *bap* gene in *Salmonella spp* isolated. Similar to Ghasemian *et al.*, who demonstrated the high presence of bap gene [25]. *sfa* adhesion binding to soluble elastin and promotes binding of *Escherichia coli* to the extracellular matrix leading to colonization of microorganisms on human tissues. In our study *sfa* was found weakly in *E. coli* isolated from our samples and was compatible with the study reported by Azmi *et al*. [20]. Furthermore, *mrpA* is mostly known with the binding of *Proteus spp* to the extracellular matrix and implanted biomaterials, and was identified in *P. mirabilis* isolated in this study. A study reported the presence of *mrpA* gene among MRSA isolates [20], *mrpA* promote biofilm formation and have a major function in bacterial extra-cellular-membrane interaction and development of ica-independent biofilms. *mrkA* gene was confirmed in *Klebsiella spp* isolated from our samples. This finding is similar to the data of Uribe-Garcı'a *et al*. who detected the presence of *mrkA*, in most of the uropathogens isolated in his study [25]. The same finding has been reported from a study made by Chen *et al.* which revealed the presence of *mrkA* in *K pneumoniae* isolates under study [26].

Both recent report and the result of current study have demonstrated disparities in the presence of adhesion and biofilm related genes that could likely attribute to the distribution of various molecular types of bacteria in different area. It is presumably due to irrational use of antibiotics in the past with insufficient doses and duration.

CONCLUSIONS

While a great deal of researches have been done on the biofilm formation of both gram-positive and gram-negative bacteria, little is known about the molecular underpinnings for the adhesion and biofilm formation, as these bacteria have recently developed a high resistance to the majority of traditional antibacterial agents. One important factor in the development of UTIs is the uropathogens' ability to produce biofilms. In this work, we assess the uropathogen's capacity to form biofilms and report the correlation between the *mrpA, ica, mrkA, sfa* and *bap* genes and biofilm forming ability. Several adhesion factor genes linked to biofilms may be targets for UTI treatment and prevention. Although we found biofilm producer bacteria with a low biofilm expressing genes, suggesting that these strains could use other systems to build biofilm, even though the majority of the biofilm-producing isolates in the current study were positive for biofilm adhesion genes. Among the uropathogens examined, *Proteus mirabilis, Staphylococcus* species, *Pseudomonas, Klebsiella, Escherichia coli,* and *Salmonella spp*. were the most common. In order to guarantee the best possible care, it is necessary to regularly assess the antibiotic susceptibility profile of bacteria linked to UTIs before prescribing antibiotics, as evidenced by the high levels of multi-drug resistance seen in the bacteria isolated from ATBUTH patients. The proposed regulations of the Infectious Diseases Society of America state that patient history, drug accessibility, and geographic susceptibility data should all be taken into consideration when administering empirical antibiotic treatment for UTIs [38]. Our findings show that our isolates were susceptible to ampicillin and ceftriaxone, but resistant to cefazolin, trimethoprim / sulfamethoxazole, and ceftazidime, and less effective for the management of UTI among our study participants. The majority of the bacterial isolates have been shown to have an extremely high level of multi-drug resistance.

RECOMMENDATIONS

Based on the finding, we therefore recommend the following;

- Further research should examine the role and expression level of adhesion factor genes involved in biofilm formation and link them to the genetic makeup of uropathogenes as well as the development of various UTI types.
- The first course of action for UTIs in the research region should be the empirical treatment with ampicillin and ceftriaxone.
- The government ought to enact legislation mandating the careful use of antibiotics in order to prevent the spread of antibiotic resistance in microorganisms.

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AUTHOR CONTRIBUTIONS

Abdurrahman A. S., Mudathir Q. and Adamu M.B. conceived and designed the research the research. Shuaibu Y. U., Dauda E. S., and A. K. Jimoh edited and revised manuscript. Zailani S. B. and Mudathir Q. analyzed data and interpreted results of the study. Abdurrahman A. S., Mudathir Q., Adamu M.B and Balogun S.A. participated in sample collection, performed experiments and drafted manuscript.

DATA AVAILABILITY

The corresponding author has made available the data analysed during this study accessible on request.

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