



Microbes and Infectious Diseases

Journal homepage: <https://mid.journals.ekb.eg/>

Original article

The prevalence of urinary tract infection among students of Modibbo Adama University of Technology, Yola Nigeria and the effects of plasmid curing on the antibiotic resistance profile of uropathogens

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ARTICLE INFO

Article history:

Received 30 August 2023

Received in revised form 28 September 2023

Accepted 30 September 2023

Keywords:

Plasmids
Urinary tract infections
Uropathogens
Antibiotics
Antibiotic resistance

ABSTRACT

Background and rationale: Plasmid curing is a potential strategy to combat antibiotic resistance that involves the elimination of plasmids responsible for disseminating resistance genes among bacteria. While hospital-acquired urinary tract infection (UTI) cases might showcase higher antibiotic resistance, the antibiotic profiles of UTI cases among hostel-residing university students remain largely unexplored. This study aims to investigate the rate of urinary tract infections among students at Modibbo Adama University of Technology, Yola, Nigeria. Moreover, this study also aims to determine the origin of antibiotic resistance profiles of these uropathogens by assessing whether they are chromosomal or plasmid-borne. **Methods:** Seventy-six urine samples were collected from Modibbo Adama University, Yola students. The samples were cultured on CLED agar, and identification was performed based on cultural characteristics, gram stain reactions, and sugar fermentation tests. Antibiotic resistance was assessed using the disc diffusion method on Mueller-Hinton agar, while plasmid curing was achieved through growth in 2% SDS. **Results:** The findings revealed that out of the 76 urine samples analyzed, 35(46%) exhibited significant bacterial growth, indicating the presence of UTIs. Five bacterial species, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Proteus mirabilis*, and *Enterococcus faecalis*, were isolated in this study. The results demonstrated that the Gram-negative bacteria were generally resistant to trimethoprim/sulfamethoxazole, amoxicillin/clavulanic, and pefloxacin, while the Gram-positive bacteria showed resistance to ampicillin and cefuroxime. **Conclusion:** In conclusion, the findings of this study demonstrated that although plasmid curing holds remarkable potential for combating antibiotic resistance, plasmid curing has minimal impact on the antibiotic profile of the identified uropathogens.

Introduction

Uropathogens are microorganisms that infect the urinary tract, leading to urinary tract infections (UTIs). These infections, caused by bacteria, fungi, and viruses are of significant health concern, impacting millions of individuals annually

[1]. Every year, there are over 10 million clinical cases, 1.5 million emergency room visits, and 300,000 hospital admissions attributed to UTI in the United States [2]. Urinary tract infections (UTIs) are infections that can occur in the urethra (urethritis), bladder (cystitis), or kidney (pyelonephritis). They

stand as one of the most common infectious diseases globally, affecting 150 million people annually. This widespread prevalence comes with significant morbidity and substantial medical costs; for instance, an estimated economic burden of over \$5 billion each year for recurrent UTIs in the United States [3]. Moreover, treating UTIs has become challenging due to the changing patterns of antibiotic resistance in uropathogens, rendering many antibiotics ineffective [4]. Antibiotic resistance among uropathogens has become a global issue [5]. The surge in antibiotic-resistant bacterial strains is a major concern for public health, given the widespread use of antibiotics in clinical settings for treating bacterial infections. Among the approximately 3 million cases of infections caused by these resistant bacteria in the United States, there is a growing incidence of urinary tract infections (UTIs) that are now resistant to the most commonly used antibiotics [6]. The resistance patterns vary geographically and within the same country. Urinary tract infections (UTIs) are primarily caused by uropathogenic *Escherichia coli* (UPEC) and *Klebsiella* spp., although in certain instances, other bacteria, including Gram-negative microorganisms like *Pseudomonas aeruginosa*, have been isolated [7,8]. Factors contributing to the rise of resistance include the misuse and overuse of antibiotics, non-prescribed usage, incomplete dosages, and easy accessibility to antimicrobial drugs [9]. Compounding the problem, some patients turn to traditional healers who offer herbal remedies of unknown composition and potency. These substances may enhance the fitness of pathogens and contribute to resistance development [10]. Routine analysis of antibiotic resistance is difficult due to resource limitations. The frequent and prolonged use of antibiotics exerts selective pressure on bacteria, leading to the spread of antibiotic resistance [10]. Bacteria that have advantages, such as the ability to form biofilms or possess plasmids (small circular DNA strands independent of chromosomes), can survive antibiotic treatment and pass on their advantages to create more antibiotic-resistant bacteria [11]. Plasmids often carry antibiotic resistance genes and can be transferred between bacteria through lateral and horizontal gene transfer [12]. The susceptibility of bacteria to antibiotics is not constant but changes over time and in different environments. Uropathogens like *Escherichia coli* can acquire resistance through genetic mutation or by acquiring pre-existing

resistance genes present in their DNA or plasmids [13]. Plasmid curing, which is the removal of extrachromosomal DNA from bacteria cells, employs diverse methods. For instance, heat treatment induces stress, eliminating temperature-sensitive plasmids [14]. Chemical agents like acridine orange and antibiotics disrupt plasmid replication, aiding removal [15]. Silver nitrate [16] and UV light also prove effective in plasmid elimination [17]. The presence of plasmids in uropathogens can facilitate the spread of antibiotic resistance through conjugation [18]. This study aims to investigate the rate of urinary tract infections (UTIs) among students at the Modibbo Adama University of Technology, Yola, Nigeria. Moreover, this study also aims to determine the origin of the antibiotic resistance profiles of these uropathogens by assessing whether they are chromosomal or plasmid-borne. Moreover, university students living in close quarters within the hostel represents a dynamic and interconnected community, mirroring real-world scenarios where UTIs can easily spread. While hospital-acquired cases might showcase higher antibiotic resistance, our focus on the hostel-residing student population addresses a significant context where UTIs can impact daily life and the community. This research gap calls for a comprehensive study to understand how plasmid curing influences antibiotic resistance in uropathogens in this specific population, which can provide valuable insights into potential strategies for combating antibiotic resistance in UTIs in student communities. Overall, this study will allow us to contribute to the insights that are directly applicable to the challenges faced in university settings, guide interventions that align with the unique dynamics of this community, and aid in the development of targeted healthcare strategies.

Materials and methods

The study population

The study population consists of hostel-residing undergraduate students, both males and females, currently enrolled at Modibbo Adama University Technology Yola, Nigeria.

Inclusion criteria specifically include

- 1.Enrollment at Modibbo Adama University of Technology, Yola, Nigeria.
- 2.Hostel-residing students, age (15 years and above).

3. Students with or without documented cases of uropathogenic infections.

4. Willingness to provide informed consent to participate in the study.

5. Availability during the study period for sample collection and follow-up.

Exclusion criteria includes

1. Non-enrollment at Modibbo Adama University of Technology, Yola, Nigeria.

2. Students who have previously treated from urinary tract infections in the past 4 weeks.

3. Students who do not provide informed consent to participate in the study.

Sample size

The sample size was estimated using the formula $n = P(1-P) Z^2 / d^2$ [19], where n = sample size, P is the estimated prevalence value set at 5%, Z is the confidence limit of results (1.96), and d is the level of significance or precision (0.05).

Culture media used

Three culture media were majorly used in this work; triple sugar iron agar, nutrient broth, and cysteine lactose electrolyte-deficient (CLED) media. The media were prepared according to the manufacturer's instructions (**in the appendix**).

Sample collection and isolation of bacteria

A total of 76 urine samples were gathered from students at Modibbo Adama University (MAU), Yola, with 31 of them being males and 45 females. All the students participating in the study were provided with instructions on how to collect midstream urine samples using sterile bottles. The interval of time between sample collection and processing was kept to a maximum of 5 minutes. Before inoculation, each urine sample was shaken, and then 0.001ml of the sample was placed onto Cysteine Lactose Electrolyte-Deficient (CLED) media (CM301; Oxoid, Basingstoke, UK) which was prepared according to manufacturer's instructions. Utilizing a sterile wire loop, streak lines were drawn across the inoculum pool. Subsequently, the plates were incubated at 37°C for 24 hours. To determine a urinary tract infection (UTI), a sample was considered positive if a count of $\geq 10^5$ colony-forming units (CFU) per milliliter of urine was detected [20].

Identification of isolates

Bacterial isolates were identified using conventional microbiological tests such as Gram stain, oxidase test, and sugar fermentation for Gram-

negative bacilli and catalase, coagulase, and hemolysis for Gram-positive cocci, as outlined by Cheesbrough [21]. The colonies on CLED agar were carefully examined for their color, size, elevation, margin, and texture, which helped with the initial identification.

Antibiotic susceptibility testing (AST)

The Kirby-Bauer disc diffusion method was employed to conduct antibiotic susceptibility testing on each of the isolated bacteria. The preparation of Mueller-Hinton's agar followed the Clinical Laboratory Standard Institute's (CLSI) guidelines [22], adhering to the instructions provided by the manufacturer. Proper labelling was done for each plate. Using a sterile wire loop, the test organism was picked and levelly spread on the surface of the Mueller-Hinton agar, ensuring rotation of the plate by 60 degrees for uniform distribution. Sterilized forceps were used to select an antibiotic disk, which was then placed firmly on the surface of the Mueller-Hinton agar. Afterward, the plates were incubated at 37 °C for 24 hours. The zone of inhibition (ZOI) diameter was quantified using a meter rule, and the results were recorded [23]. The strains were tested for susceptibility against trimethoprim/ sulfamethoxazole (1.25/23.75µg), chloramphenicol (30µg), sparfloxacin (5µg), ciprofloxacin (5µg), amoxicillin (10µg), amoxicillin/clavulanic (20/10µg), gentamycin (10µg), pefloxacin (5µg), ofloxacin (5µg), streptomycin (10µg), ampicillin (10µg), cefuroxime (30µg), rifampicin (5µg), erythromycin (15µg). (These are commercial antibiotics prepared and marked by Optu Disc, United States of America). The strains were categorized as susceptible, moderately susceptible (intermediate), or resistant based on the guidelines established by the National Committee for Clinical Laboratory Standards (NCCLS).

Plasmid curing

Plasmid curing was conducted following the procedure. To prepare the plasmid-curing nutrient broth, sterile test tubes were filled with 10 ml of aseptically prepared nutrient broth, following the instructions provided by the manufacturer (Oxoid Nutrient Agar, CM0003B). In each test tube, 2% concentration of sodium dodecyl sulfate (SDS) was introduced in the nutrient broth by vigorous shaking [24,25]. Subsequently, the overnight-growing culture of the isolates was inoculated into the test tubes. The test tubes were then incubated at

37 °C for 18-24 hours. Bacterial isolates recovered were preserved according to the methods modified by Marston et al. [26] and Lai et al. [27]. Freshly prepared nutrient broth was again prepared and was inoculated with the plasmid-cured tested cultures and then it was incubated at 37°C for 24 hours. To determine the antibiotic resistance profile of the uropathogens following plasmid curing, the Kirby-Bauer disk diffusion method was repeated for each of the cured isolates, and antibiotic susceptibility testing was performed.

Statistical analysis

Percentages of resistance before and after SDS treatment were compared to detect the level of significance of the change in resistance frequency using a one-way analysis of variance (ANOVA). Significant results were compared with Duncan's multiple range test using IBM SPSS version 20 software. Statistical data were presented as mean Standard error \pm SE. For all the tests, the significance was determined at the level of $p < 0.05$.

Results

Prevalence and distribution of UTI among female and male MAU students

In this study, a total of 76 urine samples were examined, and it was found that 35 of them (46%) displayed significant bacterial growth, suggesting the presence of a urinary tract infection (UTI). The data further reveals that the percentage of female participants affected (60%) was higher compared to their male counterparts (25.8%), as indicated in **Table (1)**.

Bacteria isolated from the urine samples

In this study, a total of five bacterial species were identified. Among them, three were classified as Gram-negative, while the remaining two were categorized as Gram-positive (**Table 2**). *Escherichia coli* and *Klebsiella pneumoniae* were found to be the two prevalent Gram-negative bacteria, constituting 40% and 28.6% of the total isolates, respectively. On the other hand, *Staphylococcus aureus* emerged as the most

prevalent Gram-positive bacteria, accounting for 25.7% of all the isolates (**Table 2**).

Antibiotic resistance profile of Gram-negative and Gram-positive bacteria before plasmid curing

The findings from analyzing the antibiotic resistance profile of the isolates before plasmid curing revealed that the *Escherichia coli* isolates displayed a general resistance to amoxicillin/clavulanic acid (85.7%) and pefloxacin (92.9%). Meanwhile, the *Klebsiella pneumoniae* isolates exhibited resistance to pefloxacin (80%), amoxicillin (60%), and trimethoprim/sulfamethoxazole (60%). *Proteus mirabilis* demonstrated a general resistance to the majority of the antibiotics used (**Table 3a**). In terms of the Gram-positive isolates, the analysis of their antibiotic resistance profile before plasmid curing demonstrated that *Staphylococcus aureus* isolates were resistant to amoxicillin (88.9%), cefuroxime (77.8%), and trimethoprim/sulfamethoxazole (77.8%), but sensitive to rifampicin (88.9%). *Enterococcus faecalis*, on the other hand, generally exhibited resistance to the antibiotics tested against it (**Table 3b**).

Antibiotic resistance profile of Gram-negative and Gram-positive bacteria after plasmid curing

Following plasmid curing, the analysis of the antibiotic resistance profile revealed a slight decrease in the proportion of *Escherichia coli* isolates that were resistant to amoxicillin/clavulanic acid (78.6%), pefloxacin (71.4%), and trimethoprim/sulfamethoxazole (71.4%). Similarly, there was a reduction in the number of *K. pneumoniae* isolates resistant to pefloxacin (70%) as presented in **Table (4a)**. Moreover, after plasmid curing, the examination of Gram-positive isolates demonstrated that *S. aureus* isolates exhibited resistance to amoxicillin (66.7%) and trimethoprim/sulfamethoxazole (66.7%). However, *E. faecalis* isolates continued to exhibit a general resistance to the antibiotics employed as indicated in **Table (4b)**.

Table 1. Prevalence and distribution of UTI among female and male students of MAU

| | Positive Number | | | |
|--------|-----------------|--------|------------|--------------------|
| | Number sampled | Hostel | Off campus | Total positive (%) |
| Female | 45 | 17 | 10 | 60 |
| Male | 31 | 1 | 7 | 25.8 |
| Total | 76 | 18 | 17 | 46 |

Table 2. Bacteria isolated from the urine samples

| Bacteria | No. of isolates (%) |
|------------------------------|---------------------|
| <i>Escherichia coli</i> | 14 (40%) |
| <i>Klebsiella pneumonia</i> | 10 (28.6%) |
| <i>Staphylococcus aureus</i> | 9 (25.7%) |
| <i>Proteus mirabilis</i> | 1 (2.9%) |
| <i>Enterococcus faecalis</i> | 1 (2.9%) |
| Total | 35 (100%) |

Table 3a. Antibiotic Resistance Profile of Gram-negative isolates before plasmid curing

| Antibiotic | <i>E. coli</i> (n=14) | | | <i>K. pneumonia</i> (n=10) | | | <i>P. mirabilis</i> (n=1) | | |
|------------|-----------------------|---|----|----------------------------|---|---|---------------------------|---|---|
| | R | I | S | R | I | S | R | I | S |
| SXT | 12 | 0 | 2 | 6 | 1 | 3 | 1 | 0 | 0 |
| CH | 5 | 1 | 8 | 2 | 2 | 6 | 1 | 0 | 0 |
| SP | 6 | 5 | 3 | 3 | 2 | 5 | 0 | 1 | 0 |
| CPX | 2 | 5 | 7 | 2 | 2 | 6 | 0 | 0 | 1 |
| AM | 8 | 2 | 4 | 6 | 0 | 4 | 1 | 0 | 0 |
| AU | 12 | 0 | 2 | 5 | 2 | 3 | 1 | 0 | 0 |
| CN | 8 | 0 | 6 | 4 | 0 | 6 | 1 | 0 | 0 |
| PEF | 13 | 0 | 1 | 8 | 0 | 2 | 1 | 0 | 0 |
| OFX | 3 | 0 | 11 | 3 | 0 | 7 | 0 | 0 | 1 |
| S | 9 | 0 | 5 | 5 | 0 | 5 | 0 | 0 | 1 |

Key: SXT: Trimethoprim/ Sulfamethoxazole, CH: Chloramphenicol, SP: Sparfloxacin, CPX: Ciprofloxacin, AM: Amoxicillin, AU: Amoxicillin / Clavulanic, CN: Gentamycin, PEF: Pefloxacin, OFX: Ofloxacin, S: Streptomycin, S: Susceptibility, I: Intermediate, R: Resistance

Table 3b. Antibiotic resistance profile of Gram-positive isolates before plasmid curing

| Antibiotic | <i>S. aureus</i> (n=9) | | | <i>E. faecalis</i> (n=1) | | |
|------------|------------------------|---|---|--------------------------|---|---|
| | R | I | S | R | I | S |
| PEF | 6 | 0 | 3 | 1 | 0 | 0 |
| CN | 7 | 0 | 2 | 1 | 0 | 0 |
| AP | 6 | 1 | 2 | 1 | 0 | 0 |
| Z | 7 | 2 | 0 | 1 | 0 | 0 |
| AM | 8 | 0 | 1 | 0 | 1 | 0 |
| R | 1 | 0 | 8 | 0 | 0 | 1 |
| CPX | 5 | 2 | 2 | 0 | 1 | 0 |
| S | 6 | 1 | 2 | 0 | 0 | 1 |
| SXT | 7 | 1 | 1 | 0 | 1 | 0 |
| E | 5 | 1 | 3 | 0 | 0 | 1 |

Key: Antibiotics: PEF: Pefloxacin, CN: Gentamycin, AP: Ampicillin, Z: Cefuroxime, AM: Amoxicillin, R: Rifampicin, CPX: Ciprofloxacin, S: Streptomycin, SXT: Trimethoprim/ Sulfamethoxazole, E: ErythromycinS: Susceptibility, I: Intermediate, R: Resistance

Table 4a. Antibiotic resistance profile of Gram-negative isolates after plasmid curing

| Antibiotic | <i>E. coli</i> (n=14) | | | <i>K. pneumonia</i> (n=10) | | | <i>P. mirabilis</i> (n=1) | | |
|------------|-----------------------|---|---|----------------------------|---|---|---------------------------|---|---|
| | R | I | S | R | I | S | R | I | S |
| SXT | 10 | 1 | 3 | 5 | 2 | 3 | 1 | 0 | 0 |
| CH | 4 | 1 | 9 | 2 | 4 | 4 | 1 | 0 | 0 |
| SP | 5 | 3 | 6 | 1 | 3 | 6 | 0 | 1 | 0 |
| CPX | 2 | 4 | 8 | 2 | 4 | 4 | 0 | 0 | 1 |
| AM | 6 | 1 | 7 | 4 | 0 | 6 | 1 | 0 | 0 |
| AU | 11 | 0 | 3 | 4 | 1 | 5 | 0 | 0 | 1 |
| CN | 7 | 1 | 6 | 4 | 2 | 4 | 0 | 1 | 0 |
| PEF | 10 | 0 | 4 | 7 | 0 | 3 | 1 | 0 | 0 |

| | | | | | | | | | |
|-----|---|---|---|---|---|---|---|---|---|
| OFX | 2 | 7 | 5 | 2 | 3 | 5 | 0 | 0 | 1 |
| S | 8 | 0 | 6 | 4 | 2 | 4 | 0 | 0 | 1 |

Key: SXT: Trimethoprim/ Sulfamethoxazole, CH: Chloramphenicol, SP: Sparfloxacin, CPX: Ciprofloxacin, AM: Amoxicillin, AU: Amoxicillin / Clavulanic, CN: Gentamycin, PEF: Pefloxacin, OFX: Ofloxacin, S: Streptomycin S: Susceptibility, I: Intermediate, R: Resistance

Table 4b. Antibiotic resistance profile of Gram-positive isolates after plasmid curing

| Antibiotic | <i>S. aureus</i> (n=9) | | | <i>E. faecalis</i> (n=1) | | |
|------------|------------------------|---|---|--------------------------|---|---|
| | R | I | S | R | I | S |
| PEF | 3 | 2 | 4 | 1 | 0 | 0 |
| CN | 5 | 0 | 5 | 1 | 0 | 0 |
| AP | 5 | 0 | 4 | 0 | 1 | 0 |
| Z | 4 | 2 | 3 | 0 | 1 | 0 |
| AM | 6 | 0 | 3 | 0 | 1 | 0 |
| R | 1 | 1 | 7 | 0 | 1 | 0 |
| CPX | 4 | 1 | 4 | 0 | 0 | 1 |
| S | 5 | 1 | 3 | 0 | 0 | 1 |
| SXT | 6 | 1 | 2 | 0 | 1 | 0 |
| E | 4 | 1 | 4 | 0 | 1 | 0 |

Key: Antibiotics: PEF: Pefloxacin, CN: Gentamycin, AP: Ampicillin, Z: Cefuroxime, AM: Amoxicillin, R: Rifampicin, CPX: Ciprofloxacin, S: Streptomycin, SXT: Trimethoprim/ Sulfamethoxazole, E: Erythromycin, S: Susceptibility, I: Intermediate, R: Resistance

Discussion

The findings of this study revealed that the overall occurrence rate of urinary tract infection (UTI) among students at Modibbo Adama University (MAU) was 46%, which comparatively corresponds to the occurrence rate documented from other studies conducted within the country and from other parts of the world, including Pakistan (52.76%) [28], Egypt (52.2%) [29], and Nepal (54.25%) [30]. Nevertheless, a significantly greater occurrence rate was documented in a study carried out in Niger State, Nigeria (75%), by **An et al.** [23]. Conversely, the findings of the study were notably greater than what **Worku et al.** [5] and **Ejerssa et al.** [1] reported; their work indicated an overall occurrence rate of 9.8% and 15.5%, respectively. This discrepancy might be due to variations in personal hygiene standards, sample size, and social behaviors [31]. This difference noted could potentially suggest that the pattern of UTIs in the sample population has changed over time, possibly due to various temporal and local factors such as changes in environmental conditions like climate [31].

Furthermore, the findings of this study revealed that UTIs are more prevalent in females (60%) compared to males (25.8%). This finding aligns with previous reports by **Odoki et al.** [32] and other researchers indicating a higher prevalence of UTIs in females. The anatomical differences between males and females, such as the shorter

female urethra and its proximity to the anus, contribute to this disparity by facilitating the entry of bacteria into the urinary tract. UTIs can be caused by both Gram-negative and Gram-positive bacteria.

In our study, *Escherichia coli* constituted the predominant isolated uropathogen associated with UTI among the study population, accounting for 14 (40%). Comparable findings were reported in different regions of Nigeria, including 50% from Afikpo, Ebony State [33], 56% from Zaria, Kaduna State [34], and 42% from Ibadan, Oyo State [35]. Studies conducted in various parts of the globe have also documented consistent outcomes comparable to our results concerning *Escherichia coli*, revealing 45.2% in Eastern Ethiopia [1], 42.4% from Khartoum, Sudan [36], and 67.6% from India [4]. The dominance of *Escherichia coli* among the study population may not be bewildering because intestinal commensals play a more significant role in urinary tract infections due to their proximity to the genito-urinary area anatomically. Moreover, *Escherichia coli* is also considered uropathogenic due to specific virulence factors (P-fimbria and S-fimbria adhesions) that enable colonization and invasion of the urinary epithelium [5]. Nevertheless, our findings differ from a study conducted in Awka, Anambra State, where *Pseudomonas aeruginosa* showed the highest occurrence rate of 38.10% [12] and a study carried out in Minna, Niger State, where *Klebsiella pneumonia* showed the highest frequency of occurrence of 39.1% [23]. The variance may arise from the endemic nature of the isolate among the

study population as well as the climatic and geographic differences in the study sites [1]. However, in this study, *Escherichia coli* remained the most prevalent isolate, while *Klebsiella pneumoniae* became the second most prevalent bacteria, accounting for approximately 29% of the isolates. These results indicate a changing pattern of uropathogens, suggesting that other bacteria are becoming increasingly important. Both *Escherichia coli* and *Klebsiella pneumoniae* are enteric bacteria, highlighting their significant role in UTI occurrence.

The escalating antimicrobial resistance among uropathogens to commonly prescribed antibiotics has severely constrained the available drug options for UTI treatment. Notably, the results of this study before plasmid curing demonstrated that Gram-negative bacteria exhibited resistance to trimethoprim/sulfamethoxazole (76%), amoxicillin/clavulanic (72%), and pefloxacin (88%), while Gram-positive bacteria showed resistance to ampicillin (75%) and cefuroxime (83.33%). These findings are consistent with a similar study conducted by **Ejerssa et al.** [1] indicating high resistance rates among Gram-negative bacterial uropathogens to the aforementioned antibiotics. The resistance observed to amoxicillin/clavulanic acid, ampicillin, and cefuroxime could be attributed to extensive use or misuse of these drugs within the sample population since they are available over the counter.

In terms of the impact of plasmid curing on antibiotic resistance, the results indicated that subjecting the isolates to plasmid curing holds the potential for eradicating antibiotic resistance, although the findings of our study did not show a substantial effect of plasmid curing on antibiotic resistance profiles in some of the isolates. For instance, the resistance pattern of *Escherichia coli* after plasmid curing reduced to 78.57% and 71.42% resistance to amoxicillin/clavulanic and Pefloxacin, respectively, while that of *Klebsiella pneumoniae* slightly decreased to 70% for Pefloxacin. The resistance rates to trimethoprim/sulfamethoxazole (76%), amoxicillin/clavulanic (72%), and pefloxacin (88%) among Gram-negative bacteria remained high, and 75% and 83.33% of Gram-positive bacteria showed resistance to ampicillin and cefuroxime, respectively. The varied outcome of the antibiotic resistance profile after plasmid curing might stem from a variety of influencing factors. First, it is suggested that plasmid curing occurs spontaneously in different ways, which include the

treatment of plasmid with chemicals [37]. Moreover, the effectiveness of these curing agents varies significantly, and this difference could be attributed to the efficacy of the curing agent itself and the specific organism undergoing curing [13]. Ultimately, the findings of this study suggest that although plasmid curing holds remarkable potential for combating antibiotic resistance, it is crucial to emphasize that although numerous plasmids are resistant or challenging to eliminate, this does not always imply that the resistant trait is not encoded by plasmid [38]. Nevertheless, research has indicated that plasmid curing can enhance antibiotic effectiveness due to the increased susceptibility observed after curing compared to pre-plasmid curing [39,40]. Hence, this assertion aligns with the outcome of our study and thus demonstrates that the antibiotic resistance developed by some of the isolated organisms was chromosomally mediated rather than plasmid-mediated.

Conclusion

The bacteriologic agents of UTI among students of Modibbo Adama University (MAU) are *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus* species, and *Enterococcus faecalis*. The commonest agent was still *Escherichia coli*, but it accounted for a lower percentage than usually observed. *Klebsiella pneumoniae* and *Staphylococcus* species were the second most common bacterial agents for UTI in the sampled population. The results also show that while the Gram-negative bacteria were generally resistant to trimethoprim/sulfamethoxazole, amoxicillin/clavulanic acid, and pefloxacin, the Gram-positive bacteria showed resistance to ampicillin and cefuroxime. The results further demonstrated that although plasmid curing holds remarkable potential for combating antibiotic resistance, plasmid removal has minimal impact on the antibiotic profile of the identified uropathogens. This finding suggests that other mechanisms, potentially chromosomal or intrinsic resistance factors, might play a more prominent role in shaping the antibiotic resistance patterns of these uropathogens. Further research is needed to delve into these alternative mechanisms in order to provide a comprehensive understanding of the factors influencing antibiotic resistance in urinary tract infections. Additionally, our results underscore the complexity of combating antibiotic resistance, emphasizing the need for multifaceted approaches in addressing this critical public health issue.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Ethical approval

The study was conducted following the Declaration of Helsinki. The design of this study was approved by the Ethics Committee of Modibbo Adama University, Yola, Adamawa State, Nigeria, where the idea for the study was conceived. Furthermore, informed consent was obtained from all study subjects who participated in this study.

Data availability

All the data relevant to this study are available in the body of the manuscript as supporting tables. We the authors do not have any ethical or legal considerations for not making our data publicly available.

References

- 1- **Ejerssa A, Gadisa D, Orjino T.** Prevalence of bacterial uropathogens and their antimicrobial susceptibility patterns among pregnant women in Eastern Ethiopia: hospital-based cross-sectional study. *BMC Women's Health* 2021;21:291. doi:<https://doi.org/10.1186/s12905-021-01439-6>
- 2- **Behzadi P, Urban E, Gajdacs M.** Association between Biofilm production and antibiotic resistance in uropathogenic *Escherichia coli* (UPEC): an in vitro study. *Diseases* 2020;8(2):17. doi:10.3390/diseases8020017
- 3- **Mancuso G, Midiri A, Gerace E, Marra M, Zummo S, Biondo C.** Urinary tract infections: the current scenario and prospects. *Pathogens* 2023;12(4):623. doi:10.3390/pathogens12040623
- 4- **Sharma N, Gupta A, Walia G, Bakhshi R.** Patterns of antimicrobial resistance of *Escherichia coli* isolates from urinary tract infection patients: a three retrospective study. *Journal of Applied Pharmaceutical Science* 2016;6(1):62-65. doi:10.7324/JAPS.2016.600110
- 5- **Worku G, Alamneh Y, Abegaz W.** Prevalence of bacterial Urinary Tract Infection and Antimicrobial Susceptibility Patterns among Diabetes Mellitus Patients attending Zewditu Memorial Hospital, Addis Ababa, Ethiopia. *Infection and Drug Resistance* 2021;14:1441-1454.
- 6- **Harris M, Fasolino T, Ivankovic D, Davis N, Brownlee N.** Genetic factors that contribute to antibiotic resistance through intrinsic and acquired bacterial genes in urinary tract infections. *Microorganisms* 2023;11:1407. doi:10.3390/microorganisms11061407
- 7- **Bader M, Loeb M, Brooks A.** An update on the management of urinary tract infections in the era of antimicrobial resistance. *Postgrad Med* 2017;129:242-258. doi:10.1080/00325481.2017.1246055
- 8- **Mancini A, Pucciarelli S, Lombardi F, Barocci S, Pauri P, Lodoliln S.** Differences between community and hospital-acquired urinary tract infections in a tertiary care hospital. *New Microbiol* 2020;43:17-21.
- 9- **Mohapatra S, Venugopal S, Kalaivani M, et al.** Antibiotic resistance of uropathogens among the community-dwelling pregnant and nonpregnant female: a step towards antibiotic stewardship. *BMC Infectious Diseases* 2022;22:939. doi:<https://doi.org/10.1186/s12879-022-079141>
- 10- **Dache A, Dona A, Ejeso A.** Inappropriate use of antibiotics, its reasons and contributing factors among communities of Yirgalem town, Sidama regional state, Ethiopia: A cross-sectional study. *SAGE Open Medicine* 2021;9. doi:10.1177/20503121211042461
- 11- **Orlek A, Anjum M, Mather A, Stoesser N, Walker A.** Factors associated with plasmid

- antibiotic resistance gene carriage revealed using large-scale multivariable analysis. *Sci Rep* 2023;13:2500.doi:https://doi.org/10.1038/s41598-023-29530-y
- 12-**Okoye E, Kemakolam C, Ugwuoji E, Ogbonna I.** Multi-drug resistance Tracing by plasmid Profile Analysis and the curing of Bacteria from different Clinical Specimens. *Advanced Gut and Microbe Research* 2022;3170342.doi:https://doi.org/10.1155/2022/3170342
- 13-**Nmesirionye B, Ugwu C, Nworie K.** Antibiotic Susceptibility and plasmid profile of multidrug-resistant uropathogenic *Serratia marcescens*. *Journal of Microbiology and Infectious Diseases* 2022;12(1):12-18. doi:10.5799/jjmid.1085914
- 14-**Dashti A, Jadaon M, Abdulsamad A, Dashti H.** Heat treatment of bacteria: A simple method of DNA extraction for molecular techniques. *Kuwait Medical Journal* 2009;41(2):117-122.
- 15-**Nordstrom K.** Plasmid R1-Replication and its control. *Plasmid* 2006;55(1):1-26. doi:10.1016/j.plasmid.2005.07.002
- 16-**Bhattacharya M, Wozniak D, Stoodley P, Hall-Stoodley L.** Prevention and treatment of *Staphylococcus aureus* biofilms. *Expert Rev Anti Infect Ther* 2015;13(12):1499-1516. doi:10.1586/14787210.2015.1100533
- 17-**Rai M, Deshmukh S, Ingle A, Gade A.** Silver nanoparticles: the powerful nano weapon against multidrug-resistant bacteria. *Journal of Applied Microbiology* 2012;112(5):841-852. doi:10.1111/j.1365-2672.2012.05253.x.
- 18-**Pfeifer E, Moura de Sousa J, Touchon M, Rocha E.** Bacteria have numerous distinctive groups of phage-plasmids with conserved phage and variable plasmid gene repertoires. *Nucleic acids research* 2021;49(5):2655-2673.
- 19-**Ogunlusi E.** Phenotypic and molecular characterization of multiple resistant Gram-negative bacteria in the urine of pregnant women in Ondo mother and child hospital, Ondo, Nigeria. Unpublished Masters Thesis 2016:48-69.
- 20-**Prasada Rao, CMM, Vennila T, Kosanam S, et al.** Assessment of Bacterial Isolates from the Urine Specimens of Urinary Tract Infected Patients. *Biomed Res Int* 2022;27:4088187. doi:doi: 10.1155/2022/4088187
- 21-**Cheesbrough M.** District Laboratory Practice in Tropical Countries. Vol Part 2. 2nd Edition. Cambridge University Press 2006; 2010.
- 22-**CLSI.** M100S Performance Standards for Antimicrobial Susceptibility Testing. 33rd Edition. 33rd ed. Wayne; 2023.
- 23-**An U, Momoh J, Abdulrahman A, Abubakar M.** The prevalence of bacterial urinary tract infection (UTI) among women attending antenatal clinics in the General Hospital, Minna in Niger state. *Journal of Biomedical Research* 2012;3(3):171-173. doi:https://doi.org/10.7439/ijbr.v3i3.404.
- 24-**Devi R, Surendran P, Chakraborty K.** Antibiotic resistance and plasmid profiling of *Vibrio parahaemolyticus* isolated from shrimp farms along the southwest coast of India. *World Journal of Microbiology and Biotechnology* 2009;25:2005-2012. doi:10.1007/s11274-009-0101-8
- 25-**Letchumanan V, Chan K, Lee L.** An insight into traditional plasmid curing in *Vibrio* species. *Front Microbiol* 2015;6:735. doi:10.3389/fmicb.2015.00735
- 26-**Marston C, Hoffmaster A, Wilson K, et al.** Effects of long-term storage on plasmid stability in *Bacillus anthracis*. *Appl Environ Microbiol.* 2005;71(12):7778-7780.

- 27-**Lai M, Shaffer S, Panopoulos N.** Stability of plasmid-borne antibiotic resistance in *Xanthomonas vesicatoria* in infected tomato leaves. *Phytopathology* 1977;67:1527-1530.
- 28-**Zubair K, Shah A, Fawwad A, Sabir R, Butt A.** Frequency of urinary tract infection and antibiotic sensitivity of uropathogens in patients with diabetes. *Pakistan Journal of Medical Sciences*2019;35(6):1664-1668. doi:10.12669/pjms.35.6.115
- 29-**El-Nagar MA, Dawood ADS, Gabr H, El-Naby ED.** Prevalence of urinary tract infection in Damietta diabetic patients. *Menoufia Medical Journal* 2015;28(2):559-564. doi:10.4103/1110-2098.163918
- 30-**Kumar Jha P, Baral R, Khanal B.** Prevalence of uropathogens in diabetic patients and their susceptibility patterns at a tertiary care center in Nepal- a retrospective study. *International Journal of Biomedical Laboratory Science* 2014;3(2):29-34.
- 31-**Wilfred N, Gichuhi J, Mugo N.** 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. *Journal of Sci Technol* 2015;3:6. doi:https://doi.org/10.11131/2015/101115
- 32-**Odoki M, Aliero A, Tibyangye J, et al.** Prevalence of bacterial urinary tract infections and associated factors among patients attending Hospitals in Bushenyi District, Uganda. *int J Microbiol.* 2019;2019:4246780. doi:10.1155/2019/4246780
- 33-**Onuoha S, Fatokun K.** Prevalence and antimicrobial susceptibility pattern of urinary tract infection (UTI) among pregnant women in Afikpo, Ebonyi state, Nigeria. *Am J Life Sci* 2014;2(2):46-52. doi:https://doi.org/10.11648/j.ajls.20140202.12
- 34-**Giwa F, Ige O, Haruna D, Lamido T, Usman S.** Extended-spectrum beta-lactamase production and antimicrobial susceptibility pattern of uropathogens in Tertiary Hospital in Northwestern Nigeria. *Annals of Tropical Pathology*2018;9(1):11-16. doi:10.4103/atp.atp_39_17
- 35-**Okonko I, Ijandipe L, Ilusanya O, et al.** Incidence of urinary tract infection (UTI) among pregnant women in Ibadan, South-western Nigeria. *African Journal of Biotechnology.* 2009;8(23):6649-6657.
- 36-**Hamdan H, Ziad A, Ali S, Adam I.** Epidemiology of urinary tract infections and antibiotics sensitivity among pregnant women at Khartoum North Hospital. *Ann Clin Microbiol Antimicrob* 2011;10:2. doi:https://doi.org/10.1186/1476-0711-10-2
- 37-**Ineta B, Madu E, Abdulhadi A, Ibrahim H.** Antibiotic susceptibility and plasmid profile of clinical isolates of *Escherichia coli*. *Biomedical Research* 2018;29(17):3303-3310.
- 38-**Trevors J.** Plasmid curing in bacteria. *FEMS Microbiology Letters* 1986;32:3-4.
- 39-**Ebele L, Christian K, Emmanuel T, Ifeoma O.** Multidrug Resistance Tracing by Plasmid Profile Analysis and the Curing of Bacteria from Different Clinical Specimens. *Advanced Gut & Microbiome Research* 2022;3170342. doi:https://doi.org/10.1155/2022/3170342
- 40-**Caro L, Churchward G, Chandler M.** 5 Study of Plasmid replication in vivo. *Methods in Microbiology* 1984;17:97-122