Plasmid Profiles of Multidrug Resistant Uropathogens and Antibacterial Efficacy of Leaf Extracts of Luffa cylindrica

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ABSTRACT

This study analyzes the antibacterial activity of Luffa cylindrica leaf extracts, the plasmid profile and molecular characterization of MDR Uropathogenic bacteria obtained from patients from selected hospitals in Asaba. Two hundred (200) samples of urine were collected, microscopic examinations, cultures, and susceptibility tests were performed. Bacterial isolates that exhibited resistance to three or more classes of antibiotics were defined, for the purpose of this study, as Multidrug-Resistant (MDR) uropathogens. Molecular identification was performed using 16S rRNA sequencing, while plasmid extraction and curing analyses were used to determine the role of plasmid-mediated resistance. Series of quantitative and qualitative tests were conducted in order to assess the phytochemistry of the leaf extracts, and the antibacterial properties of the extracts were characterized using the agar-well diffusion method, along with a determination of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). Among the 200 specimens tested, 62 (31%) tested positive in cultures, producing 70 isolates, including Staphylococcus aureus (40%), Escherichia coli (28.6%), Klebsiella pneumonia (18.6%), Proteus mirabilis (7.1%), and Pseudomonas aeruginosa (5.7%). 76% of the isolates were multi-drug resistant (MDR) and Pseudomonas aeruginosa and Proteus mirabilis had 100% resistance. Molecular identification based on 16S rRNA gene sequencing revealed that the isolates were closely related to Escherichia coli, Enterobacter cloacae, and other clinically relevant bacterial species. However, confirmation of uropathogenicity would require further characterization of specific virulence determinants associated with urinary tract infections. All MDR strains had large plasmids (>10 kbp) and in the strains from which plasmids were removed, resistance was decreased, indicating that the resistance was from plasmids. The phytochemical analysis showed that the leaves tested positive for alkaloids, flavonoids, phenols, saponins, tannins, terpenoids, glycosides and steroids. Inhibitory zones of 24-34 mm were produced from ethanolic extracts which also had significant antibacterial activity with Minimum inhibitory concentrations (MICs) of the ethanolic extract ranged from 103.75 ± 4.78 to 310 ± 8.16 mg/mL. Although antibacterial activity was observed, these MIC values are relatively high compared with conventional antibiotics such as ciprofloxacin, suggesting that further purification of active phytochemical constituents may be required to achieve clinically relevant potency. The results suggest that the Luffa cylindrica leaf-extracts have antibacterial qualities and can be used as alternative treatments for resistant strains of uropathogens.

Keywords: Multidrug-resistant uropathogens, Luffa cylindrica, Plasmid-mediated resistance, Phytochemicals, Antibacterial activity.

INTRODUCTION

One of the most common infections that humans can contract is urinary tract infections (UTIs). In the global scope of public health, this is a considerable distress. The urinary tract is made up of a number of different organs. The kidneys, ureters, bladder and urethra together make up the urinary tract. As a system, they perform the functions of the production, transport, storage and excretion of urine [1]. The urine is produced and stored through the ureters, the bladder and the urethra. Though each of the components can serve specific roles, the system is designed to work together for the more complex functions. The UTI occurs in the instance of infection of the urinary system. These infections can be initiated through a number of different microorganisms, and can be made more complex through different factors, anatomical, behavioral, hygienic, or, through a combination of

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several of these. These can include the act of engaging in sexual intercourse, the act of in a careless manner washing the perineal area, and, on the other hand, the type of sanitary facilities used. The urine that is present in the urinary system can be made up of different compositions, which can also be the cause for the infection being persistent [2].

Every year, roughly 150 million people are diagnosed with urinary tract infections (UTIs), resulting in significant illness, permanent complications, and additional costs to the healthcare system, exceeding 6 billion dollars [2]. The most recent analyses from the Global Burden of Disease continue to show a remarkably high incidence of UTIs from 1990 to 2019, especially in women, older adults, and those with weakened immune systems [3]. UTIs can present in several ways: as cystitis, pyelonephritis, or as asymptomatic bacteriuria. If these infections are not treated, they can lead to dire outcomes, including death, serious kidney damage, sepsis, and increased risk of infection in the future [4]. The prevalence of these infections in females is largely explained by the shorter length of the female urethra, which is also further away from the urethra than in males [5].

The pathogens that cause UTIs are mainly bacteria. Approximately 80–90% are caused by Escherichia coli, which is followed by Klebsiella pneumoniae, Proteus mirabilis, Staphylococcus saprophyticus, Staphylococcus aureus, Pseudomonas aeruginosa, and other Enterobacteriaceae [6]. Treatment of UTIs is largely based on the use of antimicrobial agents, such as beta-lactams, fluoroquinolones, cephalosporins, aminoglycosides, nitrofurantoin, and cotrimoxazole. However, uropathogens are increasingly difficult to treat and often exhibit variable and declining susceptibility to these agents due to geographic and temporal influences [7].

The swift emergence and distribution of multidrug-resistant (MDR) uropathogens have further worsened the challenges of UTI management. MDR bacteria are especially harmful to the efficacy of the outcomes because they are defined as resistant to three or more classes of antibiotics [8]. One of the leading factors that have caused this resistance is the rapid acquisition and dissemination of multiple resistance traits through the transfer of plasmid-mediated genes, which diminishes the available options to treat the infection and increases the likelihood of treatment failure [9]. The levels of antimicrobial resistance continue to increase, and without aggressive intervention, the number of deaths caused by drug-resistant infections is anticipated to exceed 10 million by the year 2050 [10].

The crisis has increased interest in new antimicrobial approaches, especially those related to antimicrobial properties of medicinal plants. Therapy involving the use of plants has been practiced in traditional medicine systems, especially in Africa, and often involve the use of diverse bioactive compounds that protect against the damaging effects of microbes [11]. These phytochemicals, which include alkaloids, flavonoids, phenols, tannins, saponins, and terpenoids, are known to possess antimicrobial properties that may also work together to inhibit resistant microbes [12]

Luffa cylindrica (sponge gourd) is a member of the family Cucurbitaceae. It is a medicinal plant and is antibacterial, antifungal, and anti-inflammatory and is used in folk medicine. Various studies showed that antimicrobial activity of its leaves affect both Gram-positive and Gram-negative bacteria, and this has been attributed to the diverse bioactive phytochemicals [13]. However, the available studies are not conclusive on the extent to which the leaf extracts of Luffa cylindrica are effective on MDR uropathogens and the plasmid-mediated resistance mechanisms, if any.

For that reason, the present research focused on the plasmid profiles, molecular characterization, and antimicrobial activity of Luffa cylindrica leaf extracts on multidrug-resistant uropathogenic bacteria obtained from UTI patients. Microbiological, molecular, and phytochemical methods were integrated in this study for the first time in an attempt to provide scientific justification for the use of Luffa cylindrica as a natural adjunct or alternative therapeutics for resistant pathogen-associated UTIs.

METHODOLOGY

2.1 Study Area

This study focused on Asaba, the capital of Delta State, Nigeria, located between latitude 6°12′ N and longitude 6°44′ E. Asaba is the major urban and the primary health care hub of the state. For specimen collection, the

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Federal Medical Centre (FMC), Asaba, and St. Joseph Catholic Hospital, Asaba, were chosen. According to 2006, Asaba had 149,603 residents, while the population of the metropolitan area is estimated to be over 500,000 today [14].

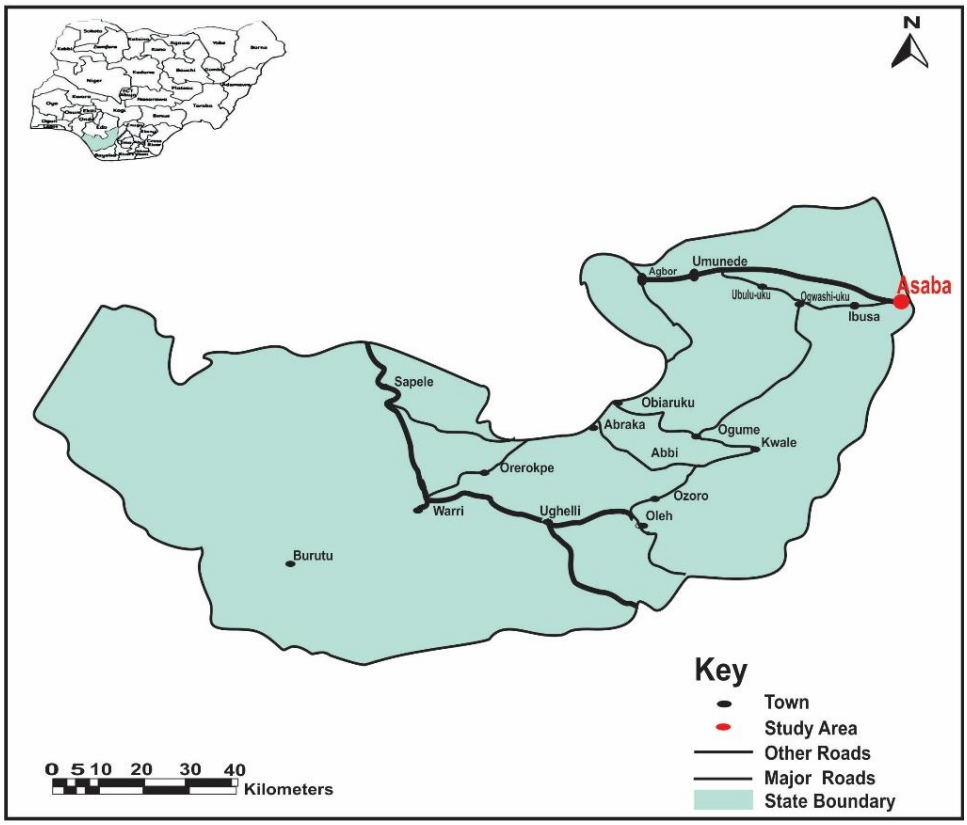

Figure 1. Map of Delta State Showing Study Area [15]

Study Design, Sample Size, and Inclusion Criteria

A hospital-based cross-sectional study design was utilized in this study. Urine specimens (200) were collected and were free from any sample collection contamination, and were taken from patients who, through clinical assessment, were found to have urinary tract infections (UTIs). In this study, adult patients between 18 and 80 years and of both genders were included, provided they consented to the study.

Ethical Approval

Prior to the onset of sample collection, the Research Ethics Committees of the respective hospitals provided the necessary ethical clearance. Participants were informed about the purpose of the study and were assured that their responses would be kept confidential.

The Collection and Processing of Urine Specimens

Samples of midstream urine were taken in sterile, wide-mouthed, and leak-proof containers using appropriate patient instructions. Sample containers were labeled and taken to the laboratory for analysis. Specimens were processed for urine microscopy, culture, and antimicrobial susceptibility testing.

Sediments for microscopy were processed by centrifuging urine samples at 3,000 rpm for 3 minutes and examining the resultant sediment for the standard constituents, which included pus cells, red blood cells, epithelial cells, yeast cells, casts, and crystals. For culture, samples were inoculated onto Cysteine Lactose Electrolyte Deficient (CLED) agar, MacConkey agar, and chocolate agar and incubated aerobically for 24 hours at 37 °C using a sterile calibrated loop. Distinct colonies were subcultured on nutrient agar to obtain pure cultures, which were subsequently identified using colonial morphology, Gram staining, biochemical tests, and molecular methods [16].

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The Standardization and Maintenance of Isolates

Pure isolates were preserved on nutrient agar slants at 4 °C. Standardization and subculturing of isolates were performed prior to testing to achieve a turbidity of 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL) using regular saline which was confirmed by spectrophotometry at 600 nm [17].

Antimicrobial Susceptibility Testing

Using the Kirby–Bauer disc diffusion technique on Mueller–Hinton agar, the Clinical and Laboratory Standards Institute standards were followed. The inhibition zones were quantified in millimeters and characterized as susceptible, intermediate, and resistant. Isolates limited to three or more categories of antibiotics are recognized as multidrug-resistant (MDR) [18].

Plant Material Collection and Preparation of Extracts

Fresh Luffa cylindrica leaves were obtained from a botanical garden in Asaba and verified from the Department of Botany, Nnamdi Azikiwe University, Awka, Nigeria (voucher number: NAUH-192^A). The leaves were washed, air dried under shade for two weeks, and powderized. Twenty (20) grams of the ground plant were individually solvent extracted (with intermittent agitation) in 200 mL of ethanol, methanol (72 hours), and distilled water (24 hours). The filtrates were concentrated using a rotary evaporator at 40–50°C, and kept at room temperature until use [19]. Extracts were labeled LCE (ethanol), LCM (methanol), and LCA (aqueous).

The percentage yield of each extract was calculated using the formula:

Yield (%) = (Weight of dried extract / Weight of initial plant material) × 100

This allowed for comparison of extraction efficiency among ethanol, methanol, and aqueous solvents. Dimethyl sulfoxide (DMSO), used as the solvent for extract reconstitution, was included as a negative control during antibacterial assays to ensure that observed inhibition zones were attributable solely to the plant extracts.

Phytochemical Screening and Extractive Value

Standard methods were used to carry out qualitative and quantitative phytochemical tests for the presence of alkaloids, flavonoids, tannins, saponins, phenols, terpenoids, steroids, and glycosides. Gravimetric methods were employed to calculate the extractive values by evaporating measured quantities of individual extracts to dryness [20].

Antibacterial Action of Plant Extracts

The leaves of Luffa cylindrica were tested for antibacterial activity against multidrug resistant (MDR) isolates using the agar well diffusion method. They prepared a range of extracts from 25-400 mg/mL with dimethyl sulfoxide (DMSO). Using a 6 mm puncher, they made wells, and added 0.1 mL of their extract into the wells. The plates were incubated for 24 hours at 37 °C. The inhibition zones were measured and recorded in mm [20].

Determination of MIC and MBC

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were established for the extracts using a concentration range of 100-500 mg/mL. MIC was defined as a sample concentration with no visible growth, and MBC was defined as the first sample concentration that was able to achieve ≥99.9% death of the bacterial cells [21].

Molecular Identification of Isolates

The genomic DNA of the isolates was prepared using a ZR Bacterial DNA Miniprep Kit (Zymo Research), which is based on a manufacturer's protocol. Universal primers (27F and 1525R) were used to perform the amplification of the 16S rRNA gene. The amplified products from the PCR reactions were subjected to gel

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electrophoresis, and the desired fragments were purified and sequenced with an ABI 3130xl Genetic Analyzer. The resultant sequences were analyzed using BioEdit and MEGA X [22].

While 16S rRNA gene sequencing provides reliable taxonomic identification, it does not distinguish pathogenic strains from commensal variants. Therefore, the designation of isolates as uropathogenic was based primarily on their recovery from clinical urine samples and antimicrobial resistance patterns. Future studies should incorporate detection of virulence-associated genes such as fimH, papC, hlyA, and cnf1, which are commonly associated with uropathogenic E. coli (UPEC) and related pathogens.

Profiling and Curing of Plasmids

Using the Zyppy™ Plasmid Miniprep Kit, Plasmid DNA was extracted using the Zyppy™ Plasmid Miniprep Kit according to the manufacturer's protocol. The extracted plasmids were separated by electrophoresis on 1% agarose gel prepared in 1× TBE buffer and stained with ethidium bromide. Electrophoresis was conducted at 90 V for approximately 60 minutes, and bands were visualized under ultraviolet illumination using a gel documentation system. A 1 kb DNA molecular weight marker was included to estimate plasmid sizes. Curing of plasmids was accomplished with acridine orange (10–400 µg/mL) in MGY medium. The loss of plasmids was determined by repeated electrophoretic analysis [22].

Statistical Considerations

Statistical analyses were conducted on the results of each experiment performed in quadruplicate with results presented as means ± Standard Deviations (SD). The data was analyzed on SPSS, Version 25, and a P-value of less than 0.05 was considered statistically significant [23].

In addition to descriptive statistics, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was applied to compare inhibition zones produced by plant extracts and the standard antibiotic (ciprofloxacin). Statistical significance was considered at $p < 0.05$.

RESULTS AND DISCUSSION

Physical Appearance and Microscopic Characteristics of Urine Specimens

The findings from 200 analyzed urine specimens for microscopic and physical features are illustrated in Tables 1 and 2. The majority of specimens were amber colored (114/200), followed by pale yellow (68/200), and deep yellow (18/200). The specimen that was the cloudiest was (84/200). The following cloudy category was slightly cloudy (71/200) and colorless (45/200) was the least cloudy. 122 specimens contained white blood cells which suggested that the patient may have a urinary tract infection but the other specimen types; red blood cells, yeast cells, epithelial cells, granular casts, and calcium oxalate crystals (as well as other crystals), were absent. This suggests that the study population was likely to have a low prevalence of nephrolithiasis.

Table 1. Physical Appearance of Urine Specimens

Urine Colour	No of Specimens	Urine Appearance	No. of samples
Amber	114	Cloudy	84
Pale Yellow	68	Slightly cloudy	71
Deep Yellow	18	Colourless	45

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Table 2. Microscopic Examination of Urine Specimens

Parameters	No. of positive samples
WBC	122
RBC	3
Yeast cells	2
Granular casts	1
Epithelial cells	1
Calcium oxalate	-
Crystals	-

Prevalence of Urinary Tract Infection

The urine culture results for urinary tract infection showed that 62 of the 200 specimens were culture positive, achieving a prevalence of 31%. The other 138 specimens (69%) exhibited no significant growth of the urinary tract infection, indicating that no infection was present at the time of specimen collection (Table 3).

Table 3. Prevalence of Urinary Tract Infection

Culture	No. of Patients	Percentage (%)
Positive	62	31
Negative	138	69

Gender Distribution of UTI

Out of the 62 culture-positive cases, 38 (61.3%) were females and 24 (38.7%) were males, indicating that female patients have a higher occurrence of UTI, which correlates with established anatomical and physiological predispositions (Table 4).

Table 4. Gender Distribution of UTI

Gender	No. of positive specimens	Percentage (%)
Males	24	38.7%
Females	38	61.3%

Morphological and Biochemical Characterization of Isolates

Five species of bacteria were characterized and identified as Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, and Staphylococcus aureus based on culture, morphology, and biochemical characteristics (Table 5), supported through Gram reaction, motility, sugar fermentation, IMViC reactions, enzyme activity, and pigment production.

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Table 5. Morphological and Biochemical Reactions of Isolates

Biochemical Test	Isolate A	Isolate B	Isolate C	Isolate D	Isolate E
Gram Reaction	- R	- R	- R	- R	+C
Motility	-	+	+	+	-
Oxidase Test	-	+	-	-	-
Catalase Test	+	+	+	+	+
Indole Test	-	-	+	-	-
Methyl Red (MR)	-	-	+	+	-
Voges-Proskauer (VP)	+	-	-	+	+
Citrate Utilization	+	+	-	+	-
Urease Test	+	-	+	+	+
H_2S Production	-	-	-	+	
Nitrate Reduction	+	+	+	+	+
Glucose Fermentation	+ (acid and gas)	-	+ (acid and gas)	+ (acid, no gas)	+ (acid, no gas)
Lactose Fermentation	+	-	+	-	+
Sucrose Fermentation	+	-	+	-	+
Mannitol Fermentation	+	-	+	-	+
Gelatin Liquefaction	-	+	-	+	+
DNase Test	-	+	-	+	+
Growth on MacConkey Agar	Pink	Colorless	Pink	Colorless	Colorless
Pigment Production	None	Blue-green	None	None	Golden-yellow
Coagulase Test	-	-	-	-	+
Probable Organisms	Klebsiella pneumoniae	Pseudomonas aeruginosa	Escherichia coli	Proteus mirabilis	Staphylococcus aureus

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Frequency of Occurrence of Urinary Isolates

Of the constituents of bacterial isolates' frequency distribution (Table 6), Staphylococcus aureus was the highest (40%) and Escherichia coli (28.6%), followed by Klebsiella pneumoniae (18.6%), Proteus mirabilis (7.1%), and Pseudomonas aeruginosa (5.7%), both Gram-positive and Gram-negative bacteria being notable as uropathogens.

Table 6. Frequency of occurrence of Urine Isolates

Isolate	Frequency	Percentage (%)
E. coli	20	28.6
Pseudomonas aeruginosa	4	5.7
Proteus mirabilis	5	7.1
Klebsiella pneumoniae	13	18.6
Staphylococcus aureus	28	40

Age Distribution of UTI among Male and Female Patients

Age-specific analysis showed that UTI prevalence among males was maximum in the 26-32 year age group (54.2%), followed by 33-41 years (20.8%), 18-25 years (17%), and ≥42 years (8%) (Table 7). In females, the highest prevalence was also recorded in the 26-32 year age group (55%), followed by 18-25 years (21%), 33-41 years (16%), and ≥42 years (8%) (Table 7). These findings suggest a higher susceptibility in the sexually active age groups.

Table 7. Age Distribution of UTI in Male Patients

Age	No of positive specimens	Percentage (%)
18-25	4	17
26-32	13	54.2
33-41	5	20.8
42 and above	2	8

Table 8. Age Distribution of UTI in Female Patients

Age	No. of Positive Specimens	Percentage
18-25	8	21
26-32	21	55
33-41	6	16
42 and above	3	8

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Antibacterial Susceptibility Pattern of the Isolates

Ciprofloxacin (74%) was the most effective antibiotic against the Gram-negative isolates, followed by gentamicin (52.4%) and augmentin (50%), while septrin (90.5%), streptomycin (69%), and ceporex (69%) showed the highest resistance, indicating the presence of multidrug resistance (Table 9). For Staphylococcus aureus, levofloxacin showed the highest activity (93% sensitivity), followed by gentamicin (79%) and ciprofloxacin (53%). Poor efficacy was observed for ampiclox, amoxil, erythromycin, and rifampicin, confirming extensive resistance to β-lactam antibiotics (Table 10)

Table 9. Antibacterial Profile of Gram Negative Isolates

Isolate	Pattern	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
Escherichia coli (20)	S	12(60)	13(65)	16(80)	8(40)	11(65)	7(35)	13(65)	15(75)	2(10)	14(70)
	I	1(5)	2(10)	1(5)	2(10)	1(5)	1(5)	0(00)	1(5)	0(00)	2(10)
	R	7(35)	5(25)	3(15)	10(50)	8(40)	12(60)	7(35)	4(20)	18(90)	4(20)
Pseudomonas aeruginosa (4)	S	3(75)	1(25)	1(25)	1(25)	1(25)	0(00)	0(00)	0(00)	0(00)	1(25)
	I	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	R	1(25)	3(75)	3(75)	3(75)	3(75)	4(100)	4(100)	4(100)	4(100)	3(75)
Proteus mirabilis (5)	S	0(00)	0(00)	1(20)	0(00)	0(00)	0(00)	0(00)	2(40)	0(00)	1(20)
	I	0(00)	0(00)	4(80)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)	0(00)
	R	5(100)	5(100)	0(00)	5(100)	5(100)	5(100)	5(100)	3(60)	5(100)	4(80)
K. pneumonia (13)	S	10(77)	4(30.8)	13(100)	12(92.3)	10(76.9)	4(30.8)	0(00)	10(77)	2(15.4)	1(7.7)
	I	1(7.7)	1(7.7)	0(00)	1(7.7)	2(15.4)	1(7.7)	0(00)	1(7.7)	0(00)	0(00)
	R	2(15.3)	8(61.5)	0(00)	0(00)	1(7.7)	8(61.5)	13(100)	2(15.3)	11(84.6)	12(72.3)
Total (42)	S	25(60)	18(43)	31(74)	21(50)	22(52.4)	11(26)	13(31)	27(64)	4(9.5)	17(40)
	I	2(5)	1(2)	5(12)	3(7.1)	3(7.1)	2(5)	0(00)	2(5)	0(00)	2(5)
	R	15(35)	23(55)	6(14)	18(42.4)	17(40.5)	29(69)	29(69)	13(31)	38(90.5)	23(55)

Key: OFX-Tarivid (10µg), PEF- perfloxacin (10µg), CPX- Ciprofloxacin (10µg), AU- Augmentin (30µg), CN-Gentamycin (10µg), S-Streptomycin (30µg), CEP-Ceporex (10µg), NA-Nalidixic Acid (30µg), SXT-Septrin (30µg), PN- Ampicillin

Table 10. Antibacterial Profile of Gram Positive Isolates

Isolate	Pattern	CPX	NB	GN	AML	S	RD	E	CH	APX	LEV
Staphylococcus aureus (28)	S	15 (53.0)	6 (21)	22 (79)	6 (21)	8 (29)	6 (21)	8 (29)	10 (36)	0 (00)	26 (93)
	I	4 (14.3)	3 (11)	2 (7)	3 (11)	2 (7)	3 (11)	2 (7)	4 (14)	1 (3.61)	2 (7)
	R	9 (32.1)	19 (68)	4 (14)	19 (69)	18 (64)	19 (68)	18 (64)	14 (50)	27 (96.4)	0 (00)

Key: CPX-Ciproflox (10µg), NB-Norfloxacin (10µg), GN-Gentamycin (10µg), AML-Amoxil (20µg), S-Streptomycin (30µg), RD-Rifampicin (20µg), E-Erythromycin (30µg), CH-Chloramphenicol (30µg), APX-Ampiclox (20µg), LEV- Levofloxacin (20µg.

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Prevalence of Multidrug-Resistant Isolates

Of the 70 bacterial isolates that were evaluated, 53 (76%) were classified as multidrug resistant, with Pseudomonas aeruginosa and Proteus mirabilis exhibiting 100% prevalence of MDR, followed by Escherichia coli (80%), Klebsiella pneumoniae (70%), and Staphylococcus aureus (68%) (Table 11).

Table 11. Prevalence of Multidrug Resistant (MDR) Isolates

Bacterial Isolates	Total number of isolates tested	Estimated number of MDR	Percentage of MDR
E. coli	20	16	80%
Pseudomonas aeruginosa	4	4	100%
Proteus mirabilis	5	5	100%
K. pneumonia	13	9	70%
Staphylococcus aureus	28	19	68%
Total	70	53	

Antibacterial Activity of Luffa cylindrica Leaf Extracts

The antibacterial activities of the Luffa cylindrica leaf extracts are illustrated in Table 12, which shows concentration-dependent inhibition for all the organisms tested. Among the leaf extracts, the ethanolic extracts exhibited the most pronounced activity, resulting in significant inhibition zones for Klebsiella pneumoniae (34 ± 0.08 mm) and for E. coli, Proteus mirabilis, and Staphylococcus aureus (each 32 mm at 400 mg/ml). The methanolic extract showed moderate activity, while the aqueous extract exhibited the most minimal antibacterial activity.

Although the extracts demonstrated measurable inhibitory effects against the tested isolates, the relatively high MIC values observed in this study indicate lower antibacterial potency when compared with standard antibiotics. Plant extracts often contain complex mixtures of bioactive compounds at low concentrations, which may explain the higher MIC ranges. Isolation and purification of active phytochemical constituents could significantly enhance antimicrobial efficacy. Similar observations have been reported in other phytochemical screening studies where crude extracts display moderate activity but improved potency following fractionation.

Statistical analysis revealed significant differences **(**$p < 0.05$**)** between the inhibition zones produced by the plant extracts and the ciprofloxacin control, indicating that although the extracts possess antibacterial activity, their efficacy was significantly lower than that of the standard antibiotic at equivalent concentrations.

Table 12. Antibacterial Effects of Luffa cylinderica Leaf Extract

Isolates	Extracts	Zone of inhibition(mm) at different concentration(mg/ml)					Control 5µg Ciprofloxacin
		400	200	100	50	25	
E. coli	Ethanolic	32±0.06	25±0.08	17±0.04	11±0.03	10±0.05	25
Klebsiella pneumoniae	Ethanolic	34±0.08	28±0.07	20±0.08	17±0.04	10±0.05	15

Proteus mirabilis	Ethanolic	32±0.01	26±0.04	18±0.03	14±0.01	10±0.02	17
Pseudomonas aeruginosa	Ethanolic	24±0.04	17±0.25	15±0.03	10±0.01	8±0.04	25
Staphylococcus aureus	Ethanolic	32±0.05	24±0,07	19±0.02	16±0.04	10±0.05	25
Pseudomonas aeruginosa	Methanolic	18±0.70	7±0.30	5±0.50	3±0.04	-	20
Staphylococcus aureus	Methanolic	20±0.04	16±0.41	12±0.06	8±0.05	5±0.08	17
Klebsiella pneumoniae	Aqueous	14±0.05	10±0.04	5±0.04	3±0.05	-	10
Staphylococcus aureus	Aqueous	18±0.06	12±0.03	6±0.03	3±0.05	-	10

Results are expressed as Mean ± Standard deviation of quadruple determinations

Ciprofloxacin (5 µg disc) served as the positive control according to CLSI disc diffusion standards. Direct equivalence between crude extract concentrations (mg/mL) and antibiotic disc potency should therefore be interpreted cautiously.

Phytochemical Composition of Luffa cylindrica

Analysis of the phytochemical of Luffa cylindrica showed the presence of saponins, flavonoids, and other phytochemical constituents (Tables 13 and 14). It was evident from the results that there was a statistically significant ($p < 0.05$) difference for all the measured parameters. The highest concentration of alkaloids, flavonoids, tannins, phenols, and terpenoids was in the ethanolic extraction, while the aqueous extraction had a higher concentration of saponins and tannins. The aqueous fraction was the only fraction that did not have saponins.

Table 13. Quantitative Analysis of Phytochemical Constituents in Luffa cylindrica Leaf Extract.

Phytochemicals	**Ethanolic Extract**	**Methanolic Extract**	**Aqueous Extract**	**P-value**
Total Phenolics (mg GAE/g)	51.69±0.81	48.45±1.06	44.76±1.10	0.001
Total Flavonoids (mg QE/g)	65.36±0.47	47.56±0.92	43.42±1.35	0.001
Alkaloids mg AE/g)	71.88±1.27	49.82±0.19	26.14±0.45	0.001
Saponins (mg/g)	11.21±0.40	13.46±0.22	20.96±0.34	0.001
Tannins (mg/g)	9.39±0.39	6.60±0.40	10.24±0.39	0.001
Terpenoids (mg/g)	18.22±0.24	13.51±0.47	7.65±0.37	0.001
Glycosides (mg/g)	14.32±0.27	9.67±0.24	7.63±0.42	0.001
Steroids (mg/g)	4.75±0.16	4.07±0.05	-	0.001

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Results are expressed as Mean ± Standard deviation of quadruple determinations. Values are significant at $p < 0.05$.

Table 14. Qualitative Analysis of Phytochemical Constituents in Luffa cylindrica Leaf Extract.

Parameter	Ethanolic Extract	Methanolic Extract	Aqueous Extract
Alkaloids	+++	++	+
Flavonoids	+++	++	++
Saponins	++	++	+++
Tannins	++	+	++
Terpenoids	+++	++	+
Glycosides	++	+	+
Phenols	++	++	++
Steroids	+	+	_

Minimum Inhibitory and Bactericidal Concentrations

From table 15, it is seen that the ethanolic extracts had the lowest MIC and MBC of Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus. In contrast, higher MIC and MBC values were seen for Pseudomonas aeruginosa, Proteus mirabilis, and the methanol and aqueous extracts, signifying lower susceptibility, which correlates with the smaller inhibition zones seen in the agar diffusion tests.

Table 15. Minimum Inhibition Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Plant Extracts

Organism	**Extract**	**MIC (mg)**	**MBC (mg)**
E. coli	Ethanolic	108.75±8.53	205±4.08
K. pneumonia	Ethanolic	103.75±4.78	203.75±4.78
Proteus mirabilis	Ethanolic	203.75±4.78	307.50±9.57
Pseudomonas aeruginosa	Ethanolic	206.25±4.78	407.50±9.57
Staphylococcus aureus	Ethanolic	105±4.08	203.75±4.78
Pseudomonas aeruginosa	Methanolic	310±8.16	-
Staphylococcus aureus	Methanolic	206.25±7.50	406.25±6.29
K. pneumonia	Aqueous	307.50±9.57	-
Staphylococcus aureus	Aqueous	303.75±4.78	401.25±2.50

Results are expressed as Mean ± Standard deviation of quadruple determinations

Molecular Identification of Selected Isolates

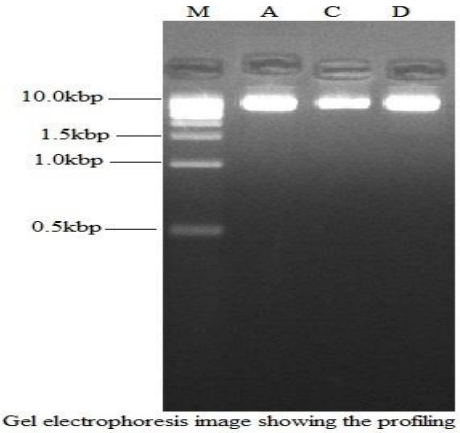
Characterization of the molecular structure using the 16S rRNA gene sequencing revealed that isolate A was nearly identical to some Enterobacter cloacae aseptically isolated variants; similarly isolate D was also nearly identical to some Enterobacter cloacae. However, isolate C was the only one which had similarity to Escherichia coli which confirms the placement of all the strains in the Enterobacteriaceae family, thus exhibiting the greatest variability of all the isolates (Table 16)

Table 16. Molecular Identification

Isolates	Closest Match	Strain Number	NCBI Accession Number	Pairwise Similarity
A	Enterobacter cloacae	CIFRI-AKSHG33	MW301108.1	93.12%
C	Escherichia coli	AT48	OP740393.1	96.72%
D	Enterobacter cloacae	RM29	MW856633.1	92.45%

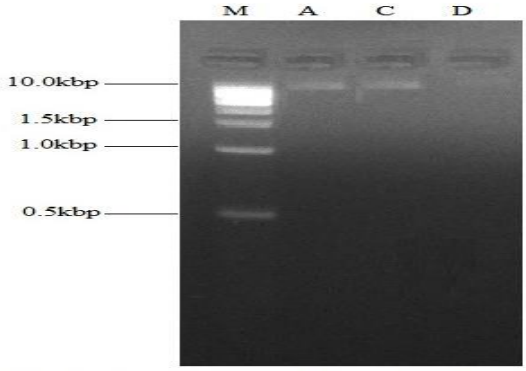
Plasmid Profiles of Isolates

Agarose gel electrophoresis of the plasmid profile revealed the presence of one or more stable plasmids in each of isolates A, C, and D. These plasmids appear to be of the large molecular weight (>10 kbp) and could be of a type that confers antimicrobial resistance or virulence factors (Plate 1).



Gel electrophoresis image showing the profiling of uncured Plasmids with high molecular weight. Lane M is a 1kbp DNA ladder

Plate 1. Gel electrophoresis image showing the profile of the uncured plasmid



Gel electrophoresis image showing the profiling of cured Plasmids.

Plate 2. Gel electrophoresis image showing the profile of the cured plasmid.

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Antibiotic Susceptibility of Cured Isolates

After the plasmid curing process was completed, Enterobacter cloacae strains CIFRI-AKSHG33 and RM29 and Escherichia coli strain AT48 were all completely susceptible to the antibiotics that were tested. Notably, large zones of inhibition appeared against fluoroquinolones, which confirms that the isolate(s) of which the plasmids were not cured show resistance due to the presence of plasmids, as shown in Table 17.

Table 17. Antibacterial Profile of Cured Isolates

Isolate	Patterns	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
Enterobacter cloacae strain CIFRI-AKSHG33	Sensitive	31mm	30mm	33mm	27mm	29mm	25mm	28mm	31mm	26mm	25mm
Escherichia coli strain AT48	Sensitive	30mm	32mm	33mm	28mm	30mm	26mm	29mm	30mm	27mm	24 mm
Enterobacter cloacae strain RM29	Sensitive	29mm	31mm	34mm	29mm	28mm	27mm	30mm	29mm	27mm	25 mm

Key: OFX-Tarivid (10µg), PEF- perfloxacin (10µg), CPX- Ciprofloxacin (10µg), AU- Augmentin (30µg), CN-Gentamycin (10µg), S-Streptomycin (30µg), CEP-Ceporex (10µg), NA-Nalidixic Acid (30µg), SXT-Septrin (30µg), PN- Ampicillin (30µg).

Using traditional microbiology practices, including Gram staining, biochemical analysis, and molecular analysis, the current study documented 70 bacterial isolates from a data set of 200 urine samples. The reliability of bacterial identification employing the phenotypic and genotypic methods enhanced the thorough identification of the study population's uropathogenic bacteria's epidemiology, resistance patterns, and therapeutic challenges [24].

The urine specimens were macroscopically and microscopically examined in accordance with established UTI guidelines [25]. The predominant urine colors, in order of frequency, were amber, pale yellow, and deep yellow. Urine specimens were predominantly cloudy. Urine cloudiness was predominantly pyogenic in association with the presence of pus (pyuria), pus-forming bacteria (bacteriuria), and inflammation of pyelonephritis (UTIs). The presence of white blood cells (WBCs) in a majority of the samples was indicative of a urinary tract infection [26, 27]. The population being studied appear not to have complications associated with UTI-related infections, such as the presence of blood in urine (hematuria), fungal infections (mycoses), or kidney stone disease (nephrolithiasis), as evidenced by the presence of red blood cells, yeast cells, casts, crystals, and calcium oxalate, all of which were absent in the samples [28, 29].

In this study, the confirmed prevalence of UTI was 31%, with even more positive cases for females (61.3%) than for males (38.7%). This difference is in compliance with the registered UTI cases for males and females, and agrees with [30], that suggested that higher UTI cases in females are due to the functional and structural attributes of the urinary system, which includes a shorter urethra and the close location of the urethra to the anus. These features, especially in concern to sexually active females, allow easier upward movement of fecal bacteria in the urinary system. Staphylococcus aureus was the first uropathogen to be isolated, and in order, the other uropathogens were Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, and Pseudomonas aeruginosa. Despite the fact that Escherichia coli is the primary recognized uropathogen in the world, especially with community-acquired UTIs, the significant isolation of Staphylococcus aureus in this study shows that Gram-positive cocci are potentially gaining prominence as a uropathogen, which may be as a result of catheterization, being in a hospital, or having other health problems [31]. The isolation of Pseudomonas aeruginosa and Proteus

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mirabilis, though as noted previously as infrequent, is of importance, as these are the uropathogens that may be encountered in uncomplicated UTIs and are more associated with complex and health system associated UTIs [1].

The fact that the antibiotic susceptibility profiles exhibited notable and widespread resistance among the isolates reflects and confirms the serious public health challenge the threats posed by the current study demonstrate. Escherichia coli showed notable resistance towards frequently prescribed antibiotics such as Septrin, Streptomycin, and Augmentin. Pseudomonas aeruginosa showed complete resistance to several antibiotics, including Ceporex, Nalidixic acid, and Septrin. Comparable resistance trends were noted within Staphylococcus aureus, especially towards the β-lactam antibiotics, Ampiclox and Amoxil. The study confirms global epidemiological resistance trends and confirms the increasing resistance among uropathogens, a resistance increasing primarily as a result of the irrational use of antibiotics, self-medication, and poor antimicrobial treatment regimens [30].

Ciprofloxacin and levofloxacin, both fluoroquinolones, exhibited the most and highly consistent antibacterial effect on both Gram-negative and Gram-positive isolates [32]. However, the fact that there are concerns regarding the total number of antibacterial agents and the associated resistance problem complicates the current treatment regimen and direction.

The leaf extracts of Luffa cylindrica exhibited dose-dependent inhibitory activity on multidrug-resistant (MDR) uropathogens, however the activity was most pronounced for the ethanolic extract. Among the tested extracts, the ethanolic extract exhibited the most potent and broadest spectrum activity against Gram-positive and Gram-negative bacteria. This activity could be related to the enhanced solubility of the active phytochemicals (flavonoids, alkaloids, tannins, and saponins) in ethanol, [12] Reported similar findings, which showed that ethanolic extracts of plants exhibited greater antimicrobial activity than aqueous extracts.

The low susceptibility of Pseudomonas aeruginosa to the extracts could be attributed to the intrinsic resistance features of the pathogen, which include resistance efflux pumps, decreased permeability of the outer membrane, and enzymatic breakdown of antimicrobial agents [33]. The lower activity of aqueous extracts also highlights the need for proper solvent choice for phytochemical extraction, as water may be inadequate in dissolving antimicrobial compounds of low polarity [34].

Assays performed to determine both the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) confirmed the results of the agar diffusion method, with the ethanolic extract characterized by the lowest MIC and MBC values, especially for, Escherichia coli and Staphylococcus aureus. This discovery reveals that the ethanolic extract in question holds the ability to provide an adequate therapeutic outcome, as the concentration required to inhibit and eliminate the respective microbial organisms is marginal [35]. In comparison, the higher values of MIC and MBC for Proteus mirabilis and Pseudomonas aeruginosa demonstrate their resistant characteristics, as well as their lowered susceptibility to antimicrobials of plant origin [36].

Molecular characterization confirms that the predominant selected isolates belong to the family Enterobacteriaceae, with Enterobacter cloacae and Escherichia coli identified as closely related. However, the pairwise similarities observed falling below the 16S rRNA species level threshold of acceptance indicates genetic distinction and the likelihood of strain level differentiation or uncharacterized components of the Enterobacter cloacae complex. This instance further demonstrates the significance of molecular approaches that supplement traditional identification routes for the purpose of epidemiological tracking and the precise identification of the organism to the correct group in the hierarchy of classification [37].

Plasmid profiling indicated the presence of large number of weight plasmids (~10 kbp) in all the tested isolates tentatively proposing the plasmid-mediated basis of the reported multidrug resistance. Such plasmids are known to carry several resistance genes including the genes for β-lactamases and aminoglycoside-modifying enzymes. After plasmid curing experiments, the isolates demonstrated decreased antibiotic resistance and consequently hinted that plasmid carry the majority of the antibiotic resistance determinants. This supports the findings of other researchers which indicated that the elimination of resistance plasmids restores the susceptibilities of bacteria to standard antibiotics [38, 39].

This study showcase the heavy burden of multuidrug resistant uropathogens. It also showcase the antibacterical activity of leaf extracts of Luffa cylindrica, especially, the ethanolic fraction. These extracts being able to act against the MDR bacteria and most possibly being able to intervene on the plasmid mediated resistance mechanisms suggests Luffa cylindrica will be very beneficial to be used as a natural antimicrobial agent. These findings are supportive of the isolation, classification and, clinical testing of the bioactive compounds as adjuncts or as alternative to standard antibiotics in the management of resistant urinary tract infections [40].

Future investigations should focus on molecular detection of virulence genes associated with uropathogenic strains to confirm pathogenic potential beyond taxonomic identification. Additionally, bioassay-guided fractionation of Luffa cylindrica extracts may facilitate identification of specific antimicrobial compounds responsible for the observed activity. Studies exploring synergistic interactions between plant extracts and conventional antibiotics could also reveal potential combination therapies capable of overcoming multidrug resistance.

CONCLUSIONS

The absence of effective antimicrobials is a persistent worldwide concern, especially in the case of infection by multidrug-resistant (MDR) uropathogens causing urinary tract infections. The ethanolic extract showed the greatest antimicrobial activity, with the most substantial zones of inhibition and the lowest values of MIC and MBC. This is most likely due to the extracts being made of bioactive phytochemicals, such as flavonoids, saponins, and other phenolic bioactive molecules, which may be in higher quantities than in other solvents. The use of plasmid profiling and curing showed that the majority of the antibiotic resistance of the isolates was due to plasmids. This reinforced the idea of using plant-derived antimicrobials to combat antibiotic resistance. The leaves of Luffa cylindrica, therefore, warrant further investigation as natural antibacterial agents. Their antibacterial properties may be beneficial as an alternative or complementary treatment for MDR urinary tract infections.

The authors recommend further study of Luffa cylindrica leaves in vivo, as well as clinical trials in humans, to determine the extracts' therapeutic potential and efficacy. The authors of this study recommend that the active ingredients of Luffa cylindrica leaves be isolated and characterized so that they may eventually be included in a formulation that is intended for use in the field of pharmaceuticals and is designed to combat antimicrobial resistance.

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AUTHOR CONTRIBUTION

The research was conceptualized by Okoye E.L and supervised by Okoye E.L and Okafor U.C. The Data collection, analysis, and experiment were carried out by Obumseli H. and Okafor U.C. The writing and arrangement of final manuscript was done by Obumseli H. and Okafor U.C. All the authors read and approved the final manuscript

COMPETING INTERESTS

The authors hereby declare that competing interests do not exist regarding the publication of this research.

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