



Isolation of Multi-Resistant Bacteria of Patients Suffering from Urinary Tract Infections in Owerri Metropolis, Imo State, Nigeria

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Abstract

The isolation and genetic analysis of multi-resistant bacteria of patients suffering from urinary tract infections in Owerri metropolis of Imo State, Nigeria was evaluated. A total of 50 urine samples were collected from Federal Medical Centre, Owerri. Streak plate method of cultivation of microorganisms was adopted in the different selective media used; MacConkey, *Salmonella shigella*, mannitol salt and eosin methylene blue agars. Nutrient agar was used for the sensitivity. Out of the 50 samples, 9 were male and 41 were females. The patients aged between 20 and 40 years. Results revealed the following organisms; *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Klebsiella* spp., and *Pseudomonas aeruginosa*. *E. coli* had the highest occurrence of 56%, followed by *S. aureus* with 26%, *Salmonella* with 8%, while *Klebsiella* and *Pseudomonas aeruginosa* had 6% and 4% respectively. The isolates were resistant to most of the antibiotics used. Sp (Sparfloxacin) showed the highest zone of inhibition with a diameter of 23mm, and the lowest is 1.0 mm (CN:Gentamycin). *E. coli* was mostly resistant to SXT (Septrin), CH (Chloramphenicol), CN (Gentamycin), S (Streptomycin), and GPX (Tarivid). *Klebsiella* was resistant to SXT (Septrin), CH (Chloramphenicol), CN (Gentamycin), S (Streptomycin), and GPX (Tarivid). *Pseudomonas* was resistant to SXT (Septrin), CH (Chloramphenicol) and *S. aureus* was only resistant to SXT (Septrin), AM (Amoxicillin), and R (Rocephine). The organisms show high resistance to SXT (Septrin), CH (Chloramphenicol), CN (Gentamycin), GPX (Tarivid), and S (Streptomycin). Plasmid extraction and Agarose gel electrophoresis was done to determine the presence of 16SrRNA gene, CTX-M gene, BlaTEM gene, SHV gene and OX gene on *Escherichia coli* and *Salmonella* spp and the result shows 1500bp of 16s gene at 100bp molecular ladder for 16SrRNA gene, 550bp gene bands of CTX-M at 100bp molecular ladder of 1500bp, 401bp of Bla TEM gene bands at 100bp molecular ladder of 1500bp and 293bp of SHV gene bands at 100pb molecular ladder of 1500bp, with OX gene showing no band. The bacteria studied contain antibiotic resistant genes. Therefore, indiscriminate use of antibiotics should be avoided.

Keywords: *Escherichia coli*; *Salmonella*; Isolation; Owerri Metropolis

Introduction

Antimicrobial resistance has been identified as one of the greatest threats to human health and developing countries like Nigeria are worst hit by this crisis [1]. The major cause of this crisis is the indiscriminate and widespread use of antibiotics, especially the antibiotics containing beta-lactam ring for example; Cefozopran, Cefquinome etc, in prophylaxis and treatment of bacterial diseases.

Antimicrobial resistance (AMR) has become a significant threat to the prevention and treatment of bacterial infections globally [2]. Importantly, in low- and middle-income countries, the potential for AMR to lead to increased morbidity and mortality may be greater given the higher burden of bacterial illness in low-income countries, delayed presentation, weaker access to diagnostics (particularly in Microbiology) and the reduced availability of second-line antibiotics [3].

Urinary tract infections (UTIs) are a community-acquired infection that represent a major burden for most healthcare systems, partially due to ineffective or inappropriate antibiotic treatment leading to treatment failures [4].

Urinary tract infection (UTI) is caused by the presence and growth of microorganisms within the urinary tract system [5]. Urinary tract infections are among the most common bacterial infections in humans both in the community and hospital settings, and they occur in all age groups, and usually required urgent treatment. In West Africa region, particularly in patients with UTI, a high level of AMR has been reported and it raises particular concerns over proper diagnosis and treatment with appropriate antibiotics thereafter. Urinary tract pathogens in Nigeria are associated with a moderate to high level of resistance to commonly used antibiotics such as Cefepime, Cefoxitin, Ciprofloxacin, Gentamycin etc. Studies from West Africa revealed moderate to high rates of AMR among *Escherichia coli* and *Klebsiella* spp. to ampicillin, Amoxicillin-clavulanic acid and septrin both among inpatients and outpatients [2]. In addition, AMR was unexpectedly observed among inpatients with UTIs to third generation cephalosporins, suggesting that extended-spectrum β -lactamase (ESBL)-producing organisms may be important pathogens in this clinical context [2]. The common etiologic agents of UTI include *Enterobacteriaceae* like *Escherichia coli* and *Klebsiella* spp, as well as Gram positive organisms like *Staphylococci* and *Enterococci* as well as *Candida albicans* in patients with under-

lying physiological debilitation [6]. *Escherichia coli* accounts for up to 80% of community-acquired uncomplicated UTIs because of its gene level mutation [7].

UTI is commonly treated with oral antibiotics like amoxicillin-clavulante, nitrofurantoin, cephalosporins, fluoroquinolones and trimethoprim-sulfamethoxazole [6]. However, available data indicate that Nigeria has isolates with high levels of resistance to Cephalosporins [8], thus underscoring the need for regular institutional, regional and national surveillance in order to optimize care. The antimicrobial resistance patterns of bacterial isolates from urinary tracts differ among bacterial and antibiotics, geographic regions and institutions [9]. There is a growing concern regarding antimicrobial resistance worldwide involving gram negative bacteria such as *E. coli*, *Klebsiella* spp and *Proteus mirabilis*, all known to be the dominant causative agents of UTIs compared to the gram positive such as *Staphylococci* and *Enterococci* [7].

This study will provide useful laboratory data to monitor the status of antimicrobial resistance of some uropathogens, to improve physicians' prescribing habits, treatment recommendations and encourage the national regulatory agency in checkmating antibiotic abuse within the region.

Statement of problem

According to a report by [1], African region has one of the largest gaps in data on the prevalence of AMR resulting from limited laboratory capacity and surveillance networks. An external quality assessment reported several deficits in antimicrobial susceptibility testing in many African countries [2].

The spread of these resistant bacteria isolates including the ESBLs has posed a threat to health with respect to hindering effective treatment, prolonged hospitalization and increased treatment costs [6]. The level at which patients suffer UTI is alarming and hence the need to do more research if needed considering the damages that it can cause. The main danger associated with untreated UTI is that the infection may spread from the bladder to one or both kidneys. When bacteria attack the kidneys, they can cause damage that will permanently reduce kidney function. It can also lead to infertility.

However, Urinary pathogens especially from community patients have been known to include strains that are resistant to

many of the commonly used antibiotics. Therefore, there is need for periodic monitoring of etiologic agents of urinary tract infections, and their susceptibility pattern especially in rural settings. Against this background, as well as the paucity of reports of multi-drug resistance in urinary tract infections in Owerri metropolis, this study was undertaken.

Significance of study

The rise of drug-resistant infections is worrisome. In many of these resistant urinary tract infections, it may simply be impossible to identify properly the causative agents and which patients are at risk due to poor microbiological diagnosis. These have necessitated the need for extensive research on antibiotic resistance among the patients who are on antibiotic therapy due to UTI so as to increase awareness campaign on antimicrobial resistance among etiological agents most especially in developing countries like Nigeria, where there is no or less surveillance activities and regulations guiding the use of antibiotics.

The results from this study will possibly proffer practical solutions to curtail the spread of these organisms in hospitals or communities by highlighting the best practices, infection control measures and appropriate choices of empirical antimicrobial coverage for infections due to resistant strains.

Justification

There are few data regarding etiologic agents of UTIs and their respective antibiotic sensitivity in our country, through the findings of this study, we intend to update the limited knowledge or information about common etiologic agents and respective antibiotic sensitivity which is crucial for stakeholders to search for preventive and control measures against antibiotic resistance.

Aim of the Study

The aim of this study is to investigate the prevalence of Multi-Drug resistant bacteria in patients with Urinary Tract Infection (UTI) in Owerri, South Eastern Nigeria.

The specific objectives of this study are:

- To isolate and identify bacteria associated with urinary tract infections.
- To identify the risk factors exposing patients to these resistant strains

- To determine the antibiogram of the bacteria isolates.
- To determine the multiple antibiotic resistance index (MARI).
- Genetically identify the isolates.
- To determine the molecular drug resistant markers among the resistant strains.

Materials and Methods

Study area

This study was conducted at Federal Medical Center Owerri, Imo State.

Ethical clearance

The ethical clearance for this study was obtained from Federal Medical Centre, Owerri, Imo State.

Sample and data collection

A total of 50 clinical urine samples were collected from patients who visited the Federal Medical Center Owerri (FMC) for medical care and were referred to Medical Microbiology and Parasitology Laboratories for laboratory investigations relating to Urinary tract infections. These patients did not include those who were on any antibiotic one week before the samples were collected. The urine samples were collected using labeled 20ml calibrated sterile universal containers from both inpatients and/or outpatients. The criteria for selection were based on patients seen either at the GOPD or those on admission with clinical symptoms suggestive of urinary tract infection.

The patients were instructed on how to collect clean catch mid-stream urine samples (MSU) of about (10-15 ml) in a sterile disposable container.

Demographic data and other relevant information from the patients that were recruited for this study were collected from the folder of patients.

Isolation and identification of bacteria

A loopful (0.001 mL) of well mixed un-centrifuged urine was streaked on to the surface of 5% blood agar and cysteine lactose electrolyte deficient (CLED) medium. The plates will be incubated aerobically at 37°C for 24hrs. Cultures without any colony at the end of 24hrs incubation will be further incubated for 48hrs. Colony

counts of $\geq 10^5$ cfu/ml in the culture will be considered positive for UTI as suggested by [5].

Colonies from culture plates of individual patients were sub-cultured onto nutrient agar plates and incubated at 37°C to obtain pure culture characteristics. After 24hrs, pure colonies were transferred into agar slants and incubated further for 24hrs. After incubation at the appropriate temperature as stated above, the agar slants were stored in the refrigerator under 4°C until further use.

Culture plates of pure colonies of the isolates were examined for colonial appearances such as colony color, odor, consistency, opacity, size, elevation and edges and of colonies. The colonies were identified and confirmed using standard microbiological methods which include Gram staining, colonial morphology on media, and growth on selective media, lactose and mannitol fermentation.

Grams staining

The isolated bacteria were stained using Grams staining technique to differentiate between gram positive bacteria and Gram-negative bacteria. The techniques that were employed during staining process followed thus:

- From pure culture of bacteria from an individual, a smear of a bacterial colony was made on a grease free slide
- The slides were allowed to air dry and heat-fixed over a Bunsen burner
- Crystal violet stain was used to flood the slide for 1min., and afterwards rinsed with distilled water
- Gram's iodine, a mordant was used to flood the slide for 1min., and afterwards rinsed with distilled water
- 75% alcohol was applied onto the stained slides for 10sec, and rinsed with distilled water
- Safranin, a counter stain was used to flood the slide for 1min., and afterwards rinsed with distilled water.
- The stained slides were air-dried and viewed under the microscope using x 100 lens with immersion oil. The stain character and shape of the bacterial shape will be recorded.

Biochemical tests

The staining was followed by biochemical tests and culturing in selective and differential medium for suspected organisms.

Eosin methylene blue (EMB) agar

EMB agar is a differential plating medium for the detection and isolation of the Gram-negative enteric bacteria. It aids in the differentiation of lactose fermenting bacteria from non-lactose fermenting bacteria. It is used in the differentiation of *Enterobacteracea genes* and *Escherichia coli*.

Test procedures

Appropriate quantity of EMB agar was dissolved in required volume of the agar according to manufacturer's instructions. It was sterilized at 121°C for 15mins after which it was aseptically poured into sterile petri dishes and allowed to gel. Test organism was directly inoculated onto the surface of an EMB agar plate. It was incubated aerobically at 37°C. Observation for specific cultural characteristics and color changes was made after 18 - 24 hours of incubation.

Interpretations

- Coliforms that utilize the lactose and/or sucrose are blue/black with a greenish metallic sheen indicated *Escherichia coli*.
- Coliforms such as *Klebsiella pneumonia* have mucoid colonies that may be purple and/or exhibit a green metallic sheen.
- Good to excellent, colorless colonies indicated of *Proteus vulgaris*, *Salmonella* and *Shigella spp.*

Antibiotics susceptibility test

The antibiotics susceptibility procedure for bacterial isolates was carried out using Kirby-Bauer method. This method depends on the ability of disc to permit the penetration of antibiotics through the medium. 0.1ml McFarland turbidity standard of test cultures was inoculated unto a sterile solidified Mueller Hinton agar. Commercially available antibiotics sensitivity disc was aseptically placed on the surface of the medium. This was incubated at 37°C for 24hrs. The diameter of inhibition was recorded and inferences (Susceptibility criteria) were made based on Clinical and Laboratory Standard Institute [10].

Antibiotics to be used

A total of 16 antibiotics for both Gram positive and Gram negative bacteria were used in this study and they include: Amikacin (AK) 30 µg, Aztreonam (ATM) 30 µg, Cefepime (FEP) 30 µg, Cefo-

taxime (CTX) 30 µg, Cefoxitin (FOX) 30 µg, Ceftazidime (CAZ) 30 µg, Ceftriaxone (CRO) 30 µg, Chloramphenicol (C) 30 µg, Ciprofloxacin (CIP) 5 µg, Colistin Sulphate (CT) 25 µg, Gentamicin (CN) 30 µg, Kanamycin (K) 5 µg, Meropenem (MEM) 10 µg, Nitrofurantoin (F) 300 µg, Tetracycline (TE) 25 µg and Sulfamethoxazole/Trimethoprim (SXT) 30 µg.

Multiple antibiotics resistance index (MARI)

The multiple antibiotics resistance index was calculated as the ratio of number of antibiotics to which the isolates were resistant/ the total number of antibiotics against which the isolates were tested (a/b). MAR index values that are greater than 0.2 (20%) were considered to be high due to often use of antibiotic as described by [11].

Molecular study on multidrug resistance

Resistant bacterial isolates were selected to test for the presence or absence of resistant genes such as quinolone resistant genes (qnr genes) and carbapenem resistant gene (blaKPC). Plasmids were isolated from the resistant bacterial isolates using the manual of extraction kit (ZymoMiniprep Kit) and resolved by electrophoresis in 1% agarose gel with ethidium bromide.

The presence or absence of resistant genes (blaKPC, qnrA, qnrB and qnrS) were carried out by PCR reaction as previously described by Elsayed and his colleagues [12].

The reaction mixture (20 µL) contained 1 µL DNA template, 2 µL 10x buffer, 2 µL dNTPs (dGTP, dATP, dCTP and dTTP), 0.2 µL Taq DNA polymerase, 1 µL of each primer (forward and reverse) and 12.8 µL water (nuclease free). The samples were gently vortexed and the PCR was performed using the thermal cycling condition including the annealing temperature for each gene.

Molecular methods of identification

DNA extraction

Five milliliters of an overnight broth culture of the bacterial isolate in Luria Bertani (LB) was spun at 14000rpm for 3 min. The cells were re-suspended in 500µl of normal saline and heated at 95°C for 20 min. The heated bacterial suspension was cooled on ice and spun for 3 min at 14000rpm. The supernatant containing the DNA was transferred to a 1.5ml micro centrifuge tube and stored at -20°C for other downstream reactions.

DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double clicking on the Nanodrop icon. The equipment was initialized with 2µl of sterile distilled water and blanked using normal saline. Two microlitre of the extracted DNA was loaded onto the lower pedestal; the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. The DNA concentration was measured by clicking on the "measure" button.

16S rRNA Amplification

The 16s rRNA region of the rRNA gene of the isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.5µM and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 130V for 30 minutes and visualized on a blue light transilluminator for a 1500bp amplicons.

Extended spectrum beta-lactamase detection

Amplification of SHV genes

SHV genes from the isolates were amplified using the SHV F: 5' CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCAC-CAGATCCT-3' primers on ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 56°C for 40 seconds; extension, 72°C for 50 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 281bp product size.

Amplification of TEM genes

TEM genes from the isolates were amplified using the TEMF: 5'-ATGAGTATTCAACATTTCCGTG-3' and TEMR: 5'-TTACCAATGCT-

TAATCAGTGAG-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream Taq Master mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 55°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 400bp product size.

Amplification of OXA genes

OXA genes from the isolates were amplified using the OXA-1F: 5'-AGCCGTAAAATTAAGCCC-3' and OXA-1R: 5'-CTTGATTGAAGGGTTGGGCG-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 47°C for 30 seconds; extension, 72°C for 40 seconds for 35 cycles and final

extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 911bp product size.

Amplification of CTX-M genes

CTX-M genes from the isolates were amplified using the CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR: 5'-ACCGGATATC-GTTGGT-3' primers on a ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 30 seconds; annealing, 52°C for 30 seconds; extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 120V for 25 minutes and visualized on a UV transilluminator for a 550bp product size.

Results

After 24 hours of incubation of the urine samples on different selective media, the morphological characteristics of the different isolates were recorded below.

S/No	Sex	Age	Nutrient Agar	MacConkey Agar	Mannitol Salt Agar	S.S.A	Eosin Methylene Blue (E.M.B)	Organism
S1	F	30	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S2	F	23	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S3	F	28	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S4	M	30	Milkish circular colonies	circular	-	Milkish circular colonies	-	<i>S. aureus</i>
S5	F	30	Milkish circular colonies	circular	-	Yellowish colonies	-	<i>S. aureus</i>
S6	F	31	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S7	M	34	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>

S8	F	26	Milkish circular colonies	-	Yellowish colonies	-		<i>S. aureus</i>
S9	F	23	-	-	-	Dark blue colony	-	<i>Salmonella sp</i>
S10	F	22	Milkish circular colonies	-	Yellowish colonies		-	<i>S. aureus</i>
S11	F	20	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S12	F	22	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S13	M	25	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S14	F	26	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S15	F	32	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S16	F	28	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S17	M	35	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S18	M	30	Milkish circular colonies		Yellowish colonies	-	-	<i>S. aureus</i>
S19	F	26	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S20	F	25	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S21	F	27	Circular greenish colonies	Bright circular pink colonies	-	-	-	<i>P. aeruginosa</i>
S22	F	21	-	-	-	Dark blue colony	-	<i>Salmonella sp</i>
S23	F	26	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>
S24	M	28	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>
S25	F	32	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>

S26	F	20	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S27	F	24	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S28	F	26	-	-	-	Dark blue colony	-	<i>Salmonella spp</i>
S29	F	32	Extremely mucoid and raised milkish colonies	Large dark pink colonies	-	-	Large mucoid pink to purple colonies	<i>Klebsiella spp</i>
S30	F	40	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>
S31	F	38	Large thick grayish white, moist and smooth	Circular dark pink colony	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S32	F	21	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S33	F	30	Circular greenish colonies	Bright circular pink colonies	-	-	-	<i>Paeruginosa</i>
S34	M	29	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S35	F	26	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>
S36	F	23	Extremely mucoid and raised milkish colonies	Large dark pink colonies	-	-	Large mucoid pink to purple colonies	<i>Klebsiella spp</i>
S37	F	21	-	-	-	Dark blue colony	-	<i>Salmonella spp</i>
S38	F	24	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S39	F	25	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S40	F	28	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>
S41	F	28	Extremely mucoid and raised milkish colonies	Large dark pink colonies	-	-	Large mucoid pink to purple colonies	<i>Klebsiella spp</i>
S42	M	31	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S43	M	25	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>

S44	F	20	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S45	F	28	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S46	M	26	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S47	F	22	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>
S48	F	26	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S49	F	28	Large thick grayish white, moist and smooth	Circular dark pink colonies	-	-	Blue black colonies with green metallic sheen	<i>E. coli</i>
S50	M	33	Milkish circular colonies	-	Yellowish colonies	-	-	<i>S. aureus</i>

Table 1: Morphological appearance of bacteria on different selective media.

		<i>E. coli</i>	Organism				Total	
			<i>S. aureus</i>	<i>Salmonella spp</i>	<i>P. aeruginosa</i>	<i>Klebsiella spp</i>		
Sex	Male	Count	6	5	0	0	0	11
		% within Sex	54.5%	45.5%	0.0%	0.0%	0.0%	100.0%
	Female	Count	22	8	4	2	3	39
		% within Sex	56.4%	20.5%	10.3%	5.1%	7.7%	100.0%
Total		Count	28	13	4	2	3	50
% within Sex			56.0%	26.0%	8.0%	4.0%	6.0%	100.0%

Table 2: Cross-tabulation Analysis between Sex and type of Organisms.

The result of the cross-tabulation analysis presented in table 2 showed that *E. coli* was found in 54.5% males and 56.4% females have *E. coli* organism; 45.5% males and 20.5% females have *S aureus*; 10.3% females have *Salmonella spp* while none of the males has *Salmonella spp*; 5.1% females have *P. aeruginosa* while no male

has the organism, and no male has the *Klebsiella spp* organism but was found present in 7.7% female.

Also, the result revealed that *E. coli* was present in 56% of the total respondents; *S. aureus* was present in 26.0% of the total re-

spondents; *Salmonella* sp was present in 8.0% of the total respondents; *P. aeruginosa* was present in 4.0% of the total respondents, and *Klebsiella spp* was present in 6.0% of the total respondents.

Total number of urine samples = 50

Total number of female samples = 39

S/N	Organism	SXT	CH	SP	CPX	AM	AU	CN	PEF	GFX	S
S1	<i>E. coli</i>	R	R	11.0	18.0	5.0	21.0	7.0	11.0	18.0	R
S2	<i>E. coli</i>	8.0	R	13.0	15.0	10.0	18.0	R	18.0	22.0	R
S3	<i>E. coli</i>	13.0	R	17.0	10.0	8.0	13.0	R	13.0	11.08	8.0
S6	<i>E. coli</i>	R	6.0	20.0	16.0	7.0	11.0	5.0	9.0	14.0	3.0
S7	<i>E. coli</i>	R	10.0	8.0	2.0	13.0	7.0	2.0	5.0	R	R
S9	<i>Salmonella</i>	R	R	13.0	17.0	10.0	19.0	R	10.0	11.0	8.0
S 11	<i>E. coli</i>	5.0	2.0	18.0	7.0	13.0	18.0	3.0	11.0	18.0	4.0
S12	<i>E. coli</i>	13.0	5.0	23.0	17.0	11.0	19.0	R	17.0	21.0	R
S13	<i>E. coli</i>	19.0	R	15.0	13.5	8.5	13.0	19.0	R	18.0	2.0
S14	<i>E. coli</i>	7.0	13.0	19.5	11.0	6.5	18.0	R	18.0	14.5	7.5
S15	<i>E. coli</i>	13.0	17.0	13.0	13.0	11.0	17.0	R	13.0	11.0	R
S16	<i>E. coli</i>	R	R	10.0	6.0	8.0	8.0	3.0	11.0	6.0	6.0
S17	<i>E. coli</i>	3.0	8.0	11.0	19.0	7.0	18.0	R	16.0	14.0	3.0
S19	<i>E. coli</i>	8.0	10.0	14.0	11.0	17.0	13.0	8.0	15.0	13.0	6.0
S20	<i>E. coli</i>	10.0	13.0	8.0	13.0	6.0	9.0	3.5	9.0	12.0	10.0
S21	<i>P. aeruginosa</i>	8.0	11.0	13.0	8.0	17.0	17.0	1.0	21.0	9.0	R
S22	<i>Salmonella</i>	10.0	7.5	15.0	11.0	15.0	21.0	4.0	19.0	16.0	R
S25	<i>E. coli</i>	R	R	13.0	9.0	4.6	13.0	R	3.5	R	R
S26	<i>E. coli</i>	R	3.0	7.0	13.0	16.0	13.0	5.0	9.0	4.0	R
S27	<i>E. coli</i>	3.0	R	10.0	14.0	9.0	14.0	R	13.0	R	R
S28	<i>Salmonella</i>	5.0	R	13.0	10.0	11.0	10.0	R	11.0	R	R
S29	<i>Klebsiella</i>	R	R	8.0	5.0	13.0	11.0	R	8.0	R	R
S31	<i>E. coli</i>	8.0	R	9.0	11.0	13.0	8.0	R	5.0	R	R
S32	<i>E. coli</i>	3.0	5.0	3.0	7.0	7.5	5.0	3.0	8.0	3.0	R
S33	<i>P. aeruginosa</i>	R	R	7.0	4.0	3.6	8.0	2.0	10.0	6.0	3.0
S34	<i>E. coli</i>	10.0	R	5.0	8.0	10.0	11.0	R	5.0	R	5.0
S36	<i>Klebsiella</i>	8.0	10.0	9.5	5.6	9.0	11.0	4.0	8.0	4.0	R
S37	<i>Salmonella</i>	4.0	5.0	7.0	4.2	11.2	14.2	3.0	6.0	3.0	R
S38	<i>E. coli</i>	8.0	R	3.2	7.0	8.0	7.0	R	4.0	R	3.0
S39	<i>E. coli</i>	R	3.0	5.0	10.2	13.0	6.0	2.0	6.0	2.0	R
S41	<i>Klebsiella</i>	3.0	R	7.0	7.2	8.0	10.0	R	3.0	3.0	R
S42	<i>E. coli</i>	5.0	R	3.0	6.0	11.0	9.5	1.0	6.0	8.0	3.2
S44	<i>E. coli</i>	7.0	2.0	8.2	3.6	9.0	7.0	R	4.6	6.6	6.0
S45	<i>E. coli</i>	R	4.2	6.0	2.0	6.0	6.2	R	7.0	R	7.2

S46	<i>E. coli</i>	2.0	6.6	8.0	10.2	11.0	8.0	R	5.0	R	3.0
S48	<i>E. coli</i>	6.0	R	3.0	8.0	13.0	5.2	7.0	8.0	5.0	R
S49	<i>E. coli</i>	R	5.0	4.5	5.0	8.0	10.0	8.2	10.0	3.0	2.0

Table 3: Zone of inhibition of isolates on antibiotics pregnates discs.

Key
 R= Resistance
 SXT= Septrin 30ug
 CH= Chloramphenical 30ug
 SP= Sparfloxacin 10ug
 CPX= Ciprofloxacin 30ug
 AU= Augmentin 10ug
 AM= Amoxacillin 30ug
 CN= Gentamycin 30ug
 PEF= Pefloxacin 30ug
 GFX= Tarivid 10ug
 S= Streptomycin 30ug.

S/n	Organism	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
S4	<i>S. aureus</i>	25.0	10.0	12.0	18.0	20.0	23.0	19.0	R	R	5.0
S5	<i>S. aureus</i>	10.0	15.0	9.0	10.0	R	10.0	7.0	5.0	R	R
S8	<i>S. aureus</i>	19.0	13.0	14.0	17.0	5.0	13.0	15.0	R	5.0	10.0
S10	<i>S. aureus</i>	13.0	20.0	8.0	15.0	10.0	17.0	18.0	13.0	11.0	R
S23	<i>S. aureus</i>	7.0	13.5	11.5	9.0	14.0	13.0	19.5	11.0	R	5.0
S24	<i>S. aureus</i>	13.0	8.0	9.0	13.0	7.0	11.0	15.5	8.0	9.0	3.0
S30	<i>S. aureus</i>	19.0	15.2	14.8	14.0	7.5	17.0	13.5	14.0	10.5	R
S35	<i>S. aureus</i>	13.0	10.0	12.0	11.0	R	13.0	9.5	R	130	3.5
S40	<i>S. aureus</i>	20.0	14.0	11.0	16.0	6.0	20.0	17.0	9.0	11.0	2.5
S42	<i>S. aureus</i>	13.0	10.0	R	11.0	R	18.0	13.0	8.0	10.0	R
S47	<i>S. aureus</i>	10.5	8.0	6.5	13.0	R	9.0	5.0	13.0	5.0	R
S50	<i>S. aureus</i>	10.6	6.6	R	14.0	3.0	10.6	7.0	4.6	8.0	2.0

Table 4: Zone of inhibition of isolates on antibiotic impregnated discs.

Key:
 R= Resistance
 PEF= Pefloxacin 10ug
 CN= Gentamycin 10ug
 APX= Ampiclox 30ug
 Z= Zinnacef 20ug
 AM= Amoxacillin 30ug
 R= Rocephin 25ug
 CPX= Ciprofloxacin 10ug
 S= Streptomycin 30ug
 SXT= Septrin 30ug
 E= Erythromycin 10ug.

Escherichia coli = 22

Staphylococcus aureus = 8

Salmonella spp = 4

Klebsiella spp = 3

Pseudomonas aeruginosa = 2

Total number of male samples = 11

Staphylococcus aureus = 5; *Escherichia coli* = 6

Total number of isolates

Escherichia coli = 28

Staphylococcus aureus = 13

Salmonella spp = 4

Klebsiella spp = 3

Pseudomonas aeruginosa = 2

Frequency occurrence of bacteria isolate

$$\frac{28 \times 100}{50} = 56\%$$

$$\frac{13 \times 100}{50} = 26\%$$

$$\frac{4 \times 100}{50} = 8\%$$

$$\frac{3 \times 100}{50} = 6\%$$

$$\frac{2 \times 100}{50} = 4\%$$

Statistical data analysis

Statistical analysis of all the data will be done using statistical package for social sciences (SPSS) version 20. The statistical tool used in the study includes percentage distribution, Chi-square test, Spearman Correlation test and One-way Analysis of Variance.

Discussion

The significant rise in urinary tract infection (UTI) among the adult as carried out in this dissertation is as a result of antimicrobi-

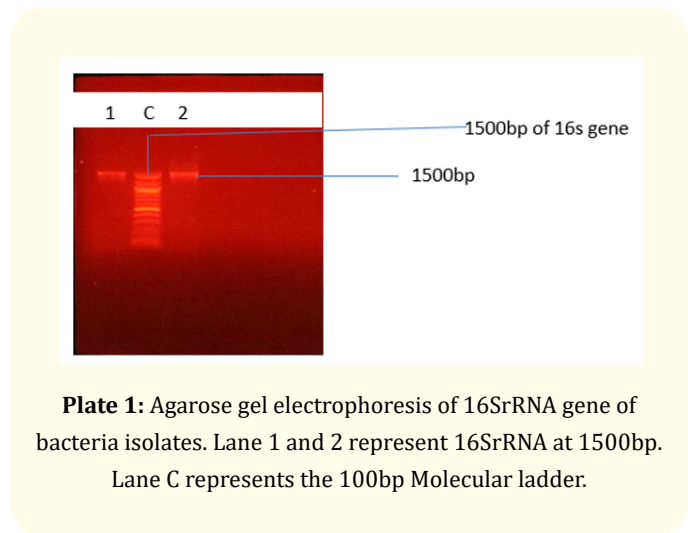


Plate 1: Agarose gel electrophoresis of 16SrRNA gene of bacteria isolates. Lane 1 and 2 represent 16SrRNA at 1500bp. Lane C represents the 100bp Molecular ladder.

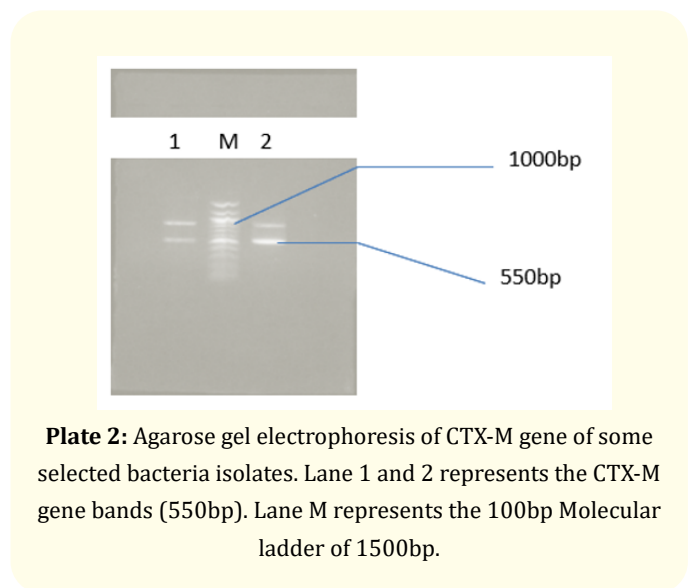


Plate 2: Agarose gel electrophoresis of CTX-M gene of some selected bacteria isolates. Lane 1 and 2 represents the CTX-M gene bands (550bp). Lane M represents the 100bp Molecular ladder of 1500bp.

al resistance developed by these bacteria, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*, *Shigella spp*, and *Klebsiella spp* and this is in line with the report of [2].

Table 1 show that 54.4% of *E. coli* was isolated from male urine samples and 56.4% was isolated from female urine samples and this in line with the report of [5] whose work revealed that 63.3% of *E. coli* was isolated from female urine sample and 42.5% from male urine sample, hence, consolidating the result obtained from table 1.

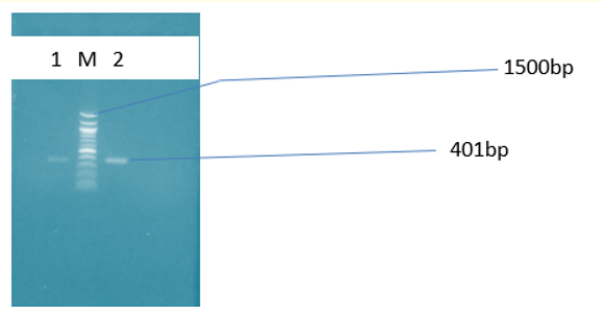


Plate 3: Showing Agarose gel electrophoresis of BlaTEM gene of some selected bacteria isolates. Lane 1 and 2 represents the SHV gene bands (401bp). Lane M represents the 100bp Molecular ladder of 1500bp.

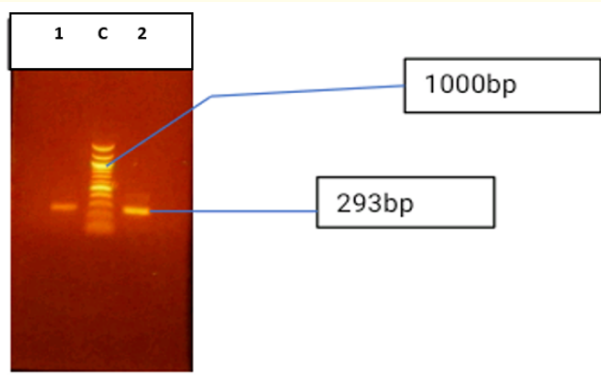


Plate 4: Agarose gel electrophoresis of SHV gene of some selected isolates. Lane 1 and 2 represents the SHV gene band (293bp). Lane C represents the 100bp molecular ladder of 1500bp.

More so [6], had a high value of *Klebsiella spp* of 37% isolated from urine sample of female cultured as compared with 7.7% of *klebsiella spp* isolated from female urine cultured as reported in table 1, proving that a lower rate of *Klebsiella spp* and *Staphylococcus aureus* was 26% when compared to [6] report which was 68.8%.

In table 3, *E. coli* was mostly resistance to Septrin, Chloramphenicol, Gentamycin, Streptomycin and Taravid whereas *Klebsi-*

ella spp and *Salmonella spp* were resistant to Gentamycin, Streptomycin, Amoxicillin as compared to [6], whose report shows that *E. coli* was mostly resistant to Amikam rather than mentioned drugs, which is contradicting but does was in correspondence with [13] report. Table 4 show the antibiotics that are resistant to *Staphylococcus aureus* to include; APX, AM, S, SXT and E.

The presence of CTX-M plasmid resistance in *Escherichia coli*, and *Salmonella spp* make them to be resistance determinants against most antimicrobial agent and this is in accordance with the report of [14] on CTX-M plasmid resistance gene of *Escherichia coli* and among other plasmid resistance gene seen in plate 1,3 and 4 respectively.

Conclusion

Some of the bacteria isolated and identified from the urinary tract infection are of public health importance, thus, when they develop resistance to antibiotics continue to be considered as a major cause of persistent urinary tract infection which could result to waist pain, incomplete menstrual cycle and eventual infertility occur. Therefore, this calls for regular surveillance and improved antibiotic stewardship.

Recommendation

- National Agency for food and Drug Administration (NAFDAC) and National Drug Law Enforcement Agency (NDLEA), should set up a local body nationwide to monitor the usage of Antibiotics especially when it involves in the treatment of Urinary tract infection in order health hazard as regards to the rate of resistance caused by the said organisms.
- Pharmacy and other Patients medical shops should always insist on seen the sensitivity of the patient laboratory result before carrying out any treatment.
- Urinary tract infection should not be treated with a thorough investigation and if approved should administer a normal and complete dose without interruption as this may lead to drug resistance by the organisms.
- There exist need to educate people to avoid drug abuse especially antibiotics at it may be detrimental to their health.
- Adequate measurement and proper hygiene should be employed by individual living in public and private facilities.
- Public health educative program should be of necessity to enlighten the general public about health implications of consuming drugs without proper or adequate check-up.

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