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Antibiotic Susceptibility Pattern of *Staphylococcus Aureus* and *Escherichia Coli* Isolated from Urinary Tract Infection (UTI) Patients Attending Murtala Muhammad Specialist Hospital, Kano, Kano State, Nigeria

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ABSTRACT

The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria. The study was aimed to determine the antibiotic sensitivity patterns of *Staphylococcus aureus* and *Escherichia co*li isolated from Urinary Tract Infected (UTI) patients. Antibiotic susceptibility patterns of the bacterial isolates were determined using modified Kirby Bauer method. The susceptibility of antibiotic against *S. aureus* showed that most of the isolates were resistant to more than 3 antibiotics. Most of the isolates were sensitive to gentamicin, ofloxacin, Septrin and Augmentin. However, the isolates were more resistant to streptomycin, Nalidixic acid and levofloxacin. The susceptibility pattern of *S. aureus* against the antibiotics showed that 14 out of the 20 isolates were multi drug resistant. For *E. coli*, result showed that most of the isolates were resistant to more than 3 antibiotics. Most of the isolates were sensitive to Levofloxacin, ofloxacin, Streptomycin and gentamicin. However, the isolates were more resistant to Ceporex, Augmentin and ampicillin and 14 out of the 20 isolates were multi drug resistant. It is concluded that the bacteria isolated from UTI patients are mostly resistance to some classes of antibiotics.

Keywords: Urinary Tract Infection (UTI) and antibiotics

INTRODUCTION

Antibiotic resistance is a type of drug resistance where a microorganism can survive exposure to an antibiotic [(1)]. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria [(2)]. Infections from resistant bacteria are now too common and some pathogens have even become resistant to multiple types or classes of antibiotics (antimicrobials used to treat bacterial infections). The loss of effective antibiotics will undermine ability to fight infectious diseases and manage the infectious complications common in vulnerable patients undergoing chemotherapy for cancer, dialysis for renal failure, and surgery, especially organ transplantation, for which the ability to treat secondary infections is crucial [(3)].

Many human diseases are as a result of infections caused by bacteria pathogens, either external or internal of the human host.

One of such bacterial infection is the Urinary Tract Infection (UTI), involving the presence of bacteria in the urinary tract (UT) which is naturally sterile [4]. UTI mostly occurs in patients with anatomically and functionally normal UT and usually results from spontaneous ascent of bacteria from the urethra to the bladder. As the name indicates, the infected parts involve the urinary tract comprising of the upper and lower urinary tract. The infection is named after the part that gets infected and is referred to as cystitis and pyelonephritis [5]. Urinary tract infection is one of the major diseases that affect people of all age groups and sexes and can be separated into asymptomatic and symptomatic cases based on the pathogenesis of infection [6]. The symptoms associated with the bladder and kidney infections are contrasting which includes painful and frequent urination in case of cystitis as a result of bladder infection whereas conditions like high fever and flank pain are commonly experienced in case of pyelonephritis [5]. Urinary tract infection can be a consequence of poor diagnosis and is regarded as the common hospital acquired infection [7, 8]. Urinary tract infection is one of the major diseases that affect people of all age groups and sexes and can be separated into asymptomatic and symptomatic cases based on the pathogenesis of infection [6]. Bacteria colonization of the UT is predominantly caused by Gram-negative species, such as *Escherichia coli*, *Klebsiella*, *Proteus* and *Pseudomonas* and rarely, by Gram-positive organisms such as hemolytic *Streptococci* and *Staphylococcus saprophyticus* [9].

Gram positive bacteria cause 15-20% and gram-negative bacteria cause 80-85%. Among gram negative *Escherichia coli* is the most frequent pathogen [10], but in complicated UTI the prevalence of other antibiotic resistance organisms increases such as *Klebsiella, Proteus, Serratia, Enterobacter* and *Pseudomonas*. Among gram positives *S. saprophyticus, E. faecalis, S. pyrogens, and S. aureus* are usually prevalent and are resistant to variety of antibiotics [11]. UTIs are caused by both Gram-negative and Gram-positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC are followed in prevalence by *Klebsiella pneumoniae, Staphylococcus saprophytic*, *Enterococcus faecalis*, Group B *Streptococcus* (GBS), *Proteus mirabilis, Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida spp*. For complicated UTIs, the order of prevalence for causative agents, following UPEC as most common, is *Enterococcus* spp., *K. pneumoniae, Candida spp., S. aureus, P. mirabilis* and *P. aeruginosa* [12]. In complicated UTI the prevalence of other antibiotic resistance organisms increases such as *Klebsiella, Proteus, Serratia, Enterobacter* and *Pseudomonas*. Among gram positives *S. saprophyticus, E. faecalis, S. pyogenes, and S. aureus* are usually prevalent and are resistant to variety of antibiotics [4].

Distribution of urinary pathogens and their susceptibility to antibiotics varies regionally so it becomes necessary to have knowledge of distribution of these pathogens and their susceptibility to antibiotics in a particular setting. Resistant to commonly used antibiotics in our hospitals increases at an alarming rate. Awareness of their occurrence and their potential effects in managing infectious diseases is low among the healthcare providers. Laboratory detections of these enzymes, proper reporting necessary precautions to avoid their spread is lacking in many of our hospitals. Rapid and accurate detection of multiple drug resistant bacteria is important for the use of appropriate antimicrobial therapy and for the control of nosocomial resistance strains. It was therefore important to identify the causative organisms and determine the antimicrobial sensitivity patterns to help reduce infections and ensure appropriate use of antimicrobials.

The aim of the study was to determine the antibiotic sensitivity patterns of *S. aureus*

and *E. coli* isolates from urinary tract infected patients.

METHODOLOGY

Material

This study used the following reagents: peptone water, Petri dishes, distilled water, Mueller Hinton Agar, wire loop, source of heat, incubator, autoclave, sensitivity disk, beaker, conical flask, universal bottle, swab stick,

Study Area

The study was conducted at the Laboratory of Pharmaceutical Technology, School of Technology Kano State Polytechnic. The samples for the study were collected from Murtala Muhammad Specialist Hospital Kano. Kano State is one of the states located in Northern Nigeria. It is geographically coordinated at 11^0 3' N and 8^0 3' E latitude and longitude respectively. It shares borders with Kaduna state to the west, Bauchi state to the South, Jigawa state to the East, Katsina state to the North. It has a total area of 20,131km2 (7,777sqm) and population of 13,405,300 [(13)].

Sample Collection

Forty (40) isolates samples were used in this study. The samples include twenty different *Staphylococcus aureus* and *Escherichia coli* isolates each isolated from patients with Urinary tracts infections (UTIs) from Murtala Muhammad Specialist Hospital Kano. The isolates were immediately transported under aseptic conditions to the Laboratory of Pharmaceutical Technology, School of technology Kano State Polytechnic in agar slant for further processing [(15)].

Sterilization of glass wares

All the glass wares (universal bottles, conical flask, and beakers) were first washed with tap water and detergent solution. They were then rinsed with distilled water and air dried. The glasses materials were autoclaved at 160^0 C for one hour. They were allowed to drop for 30 minutes before usage to avoid cracking [(9)].

Media preparation

The Peptone water and Mueller Hinton Agar media were used in this study, and both were prepared according to manufacturer's instructions. For peptone water, 15g was dissolved in 1000ml of distilled water while for 39g of Mueller Hinton Agar media was dissolved in 1000ml of dissolved water. The mixture was then shaken to obtain a homogeneous mixture. The mixture was then autoclaved with a pressure sterilizer at 121^0 C for 15 minutes. It was then allowed to cool down and the lid was removed. After 15 minutes, the peptone water was poured in sterile universal bottle while the media poured into sterile Petri dish and allowed to solidify at room temperature [(14)].

Preparation of Inoculum

Each of the collected isolates were labelled accordingly ($S_1 - S_{20}$ for *S. aureus* and $E_1 - E_{20}$ for *E. coli*) and inoculated into the peptone water and incubated at 37^0 C for 24 hours before use. After incubation, each inoculum was adjusted to 0.5 McFarland standards for use [(9)].

Antibiotic Sensitivity Test

The bacteria isolates were subjected to antibiotic susceptibility testing using the agar disc diffusion method as described by [(15)], Mueller Hinton agar (MHA) plates were inoculated with overnight culture of each isolate by streak plating. The standard antibiotic sensitivity discs were then aseptically placed at equidistance on the plates and allowed to stand for 1 hour. The plates were then incubated at 37°C for 24 hours. Sensitivity pattern of the isolates to Augmentin (30 µg/disc), Levofloxacin (10 µg/disc), Streptomycin (30 µg/disc), Amoxicillin (30µg/disc), Gentamicin (20 µg/disc), Ceporex (10µg/disc), Ofloxacin (10 µg/disc), Nalidixic acid (30µg/disc), Ciprofloxacin (10 µg/disc) and Septrin (30 µg/disc), produced by Optum Laboratories limited, were determined. Isolates were divided into three groups based on the zone of inhibition produced by the antibiotic disc; susceptible, and resistant according to the Clinical and Laboratory Standards Institute

(CLSI) guideline; Performance Standards for Antimicrobial Susceptibility Testing [16].

RESULTS AND DISCUSSION

The susceptibility of antibiotic against *S. aureus* is presented in Table 1. The result showed that most of the isolates were resistant to more than 3 antibiotics. Most of the isolates were sensitive to gentamicin, ofloxacin, Septrin and Augmentin. However, the isolates were more resistant to streptomycin, Nalidixic acid and levofloxacin.

Also, the susceptibility pattern of *S. aureus* against the antibiotics is presented in Table 2 below. The result showed that 14 out of the 20 isolates were multi drug resistant. Isolate S_6 has the highest resistant (against 9 antibiotics), followed by S_{13} (8 antibiotics) and S_5 with 7 antibiotics. The susceptibility of antibiotic against *E. coli* is presented in Table 3. The result showed that most of the isolates were resistant to more than 3 antibiotics. Most of the isolates were sensitive to Levofloxacin, ofloxacin, Streptomycin and gentamicin. However, the isolates were more resistant to Ceporex, Augmentin and ampicillin.

The susceptibility pattern of *E. coli* against the antibiotics is presented in Table 4 below. The result showed that 14 out of the 20 isolates were multi drug resistant. Isolate S_6 has the highest resistant (against 9 antibiotics), followed by S_{13} (8 antibiotics) and S_5 with 7 antibiotics

Table 1: Antibiotic Sensitivity Testing against *S. aureus*

Code	OFX	PEP	CPX	AU	CN	S	CEP	NA	SXT	PN
S_1	S	S	S	R	S	S	R	S	S	S
S_2	S	S	S	S	S	S	S	S	S	S
S_3	S	R	S	S	S	S	S	R	S	S
S_4	S	S	R	R	S	S	S	S	R	S
S_5	R	S	R	R	S	S	R	R	R	R
S_6	R	R	R	R	R	R	S	R	R	R
S_7	S	S	S	R	S	S	R	R	S	R
S_8	S	S	S	S	S	S	S	R	S	S
S_9	R	S	S	R	S	S	R	R	S	S
S_{10}	S	S	R	R	S	R	S	S	S	S
S_{11}	S	R	R	S	S	S	R	S	R	R
S_{12}	S	S	R	R	R	S	R	S	R	R
S_{13}	R	R	R	R	R	S	R	R	R	S
S_{14}	S	R	R	S	S	R	R	S	R	S
S_{15}	S	S	S	S	S	S	S	S	S	S
S_{16}	S	R	S	S	R	S	S	R	R	S
S_{17}	S	R	S	S	S	R	S	S	R	S
S_{18}	S	S	R	S	S	R	S	S	R	S
S_{19}	R	S	S	R	S	S	R	S	S	R
S_{20}	S	R	S	S	R	S	S	R	S	S

KEY: S = Sensitive > 17mm, R = Resistance < 17mm, OFX = Ofloxacin, PEP = Levofloxacin, CPX = Ciprofloxacin, AU = Augmentin, CN = Gentamicin, S = Septrin, CEP = Ceporex, NA = Nalidixic acid, SXT = Streptomycin, PN = Ampicillin,

Table 2: Susceptibility Pattern of *S. aureus*

S/N	Isolate code	No. of antibiotics resistant to	Resistant to	Status
1	S_6	9	OFX, PEP, CPX, AU, CN, S, NA, SXT, PN.	MDR
2	S_{13}	8	OFX, PEP, CPX, AU, CN, CEP, NA, SXT.	MDR
3	S_5	7	OFX, CPX, AU, CEP, NA, SXT, PN.	MDR
4	S_{12}	6	CPX, AU, CN, CEP, SXT, PN.	MDR
5	S_{11}	5	PEP, CPX, CEP, SXT, PN.	MDR
6	S_9	4	OFX, AU, CEP, NA.	MDR
7	S_{16}	4	PEP, CN, NA, SXT.	MDR
8	S_{19}	4	OFX, AU, CEP, PN.	MDR
9	S_4	3	PEP, AU, SXT.	MDR
10	S_7	3	AU, CEP, NA, PN.	MDR
11	S_{10}	3	CPX, AU, S.	MDR
12	S_{17}	3	PEP, S, SXT.	MDR
13	S_{18}	3	CPX, S, SXT.	MDR
14	S_{20}	3	PEP, CN, NA.	MDR

Table 3: Antibiotic Sensitivity Testing against *E. coli*

Code	OFX	PEP	CPX	AU	CN	S	CEP	NA	SXT	PN
E_1	S	S	S	R	R	S	R	R	S	S
E_2	R	S	R	R	S	R	R	R	S	R
E_3	S	S	S	S	S	S	R	R	S	S
E_4	R	S	R	R	S	R	R	R	R	R
E_5	R	S	R	S	R	R	R	S	R	R
E_6	S	S	S	S	S	S	S	R	S	R
E_7	S	S	S	S	S	R	R	S	S	S
E_8	S	S	R	R	S	R	S	S	S	S
E_9	R	S	S	S	S	S	S	S	S	S
E_{10}	S	R	R	S	S	R	R	S	R	R
E_{11}	R	R	R	S	R	R	R	S	S	R
E_{12}	S	S	S	R	R	S	R	S	S	R
E_{13}	S	S	R	R	R	S	R	R	R	S
E_{14}	R	R	R	S	R	R	S	R	R	R
E_{15}	S	S	S	S	S	S	S	S	R	S
E_{16}	R	S	R	R	S	R	R	S	R	R
E_{17}	S	S	S	S	S	S	S	S	S	R
E_{18}	R	S	R	S	S	S	R	S	S	R
E_{19}	R	S	S	R	S	S	R	R	S	S
E_{20}	S	R	S	S	S	R	R	S	S	S

KEY: S = Sensitive > 17mm, R = Resistance < 17mm, OFX = Ofloxacin, PEP = Levofloxacin, CPX = Ciprofloxacin, AU = Augmentin, CN = Gentamicin, S = Septrin, CEP = Ceporex, NA = Nalidixic acid, SXT = Streptomycin, PN = ,

Table 4.4: Susceptibility Pattern of *E. coli*

S/N	Isolate code	No. of antibiotics resistant to	Resistant to	Status
1	E_4	8	OFX, CPX, AU, S, CEP, NA, SXT, PN.	MDR
2	E_{14}	8	OFX, PEP, CPX, CN, S, NA, SXT, PN.	MDR
3	E_2	7	OFX, CPX, AU, S, CEP, NA, PN.	MDR
4	E5	7	OFX, CPX, CN, S, CEP, SXT, PN.	MDR
5	E_{11}	7	OFX, PEP, CPX, CN, CEP, NA, PN.	MDR
6	E_{16}	7	OFX, CPX, AU, S, CEP, SXT, PN.	MDR
7	E_{10}	6	PEP, CPX, S, CEP, SXT, PN.	MDR
8	E_{13}	6	CPX, AU, CN, CEP, NA, SXT.	MDR
9	E_1	4	AU, CN, CEP, NA	MDR
10	E_{12}	4	AU, CN, CEP, PN.	MDR
11	E_{18}	4	OFX, CPX, CEP, NA.	MDR
12	E_{19}	4	OFX, AU, CEP, NA.	MDR
13	E_8	3	CPX, AU, S.	MDR
14	E_{20}	3	PEP, S, CEP.	MDR

DISCUSSION

Microbial resistance to antibiotics is one of the most serious health threats threatening human well-being today. Antibiotic resistance is a type of drug resistance where a microorganism is able to survive exposure to an antibiotic [(17)]. Infections from resistant bacteria are now too common and some pathogens have even become resistant to multiple types or classes of antibiotics. The widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria [(2)]. One such microbial resistance of much interest nowadays to the scientific world in general and medical perspective is the resistance of *S. aureus* and *E. coli* to different class of antibiotics.

The study was aimed to determine antibiotic susceptibility pattern of *S. aureus* and *E. coli* isolated from urinary tract infected patients attending Murtala Muhammad Specialist Hospital Kano. Findings of the present study showed that showed that most of the *S. aureus* tested were resistant to more than 3 antibiotics. Most of the isolates were sensitive to gentamicin, ofloxacin, Septrin and Augmentin. However, the isolates were more resistant to streptomycin, Nalidixic acid and levofloxacin. On the other hand, *E. coli* isolates were more sensitive to Levofloxacin, ofloxacin, Streptomycin and gentamicin but mostly resistant to Ceporex, Augmentin and ampicillin. Several studies were conducted to determine the antibiotic susceptibility pattern of bacteria associated with Urinary

Tract Infections [18, 19]. The result also showed that 14 out of the 20 isolates were multi drug resistant for both *S. aureus* and *E. coli* respectively. Antibiotic resistant isolates of *S. aureus* and *E. coli*, especially to beta lactam drugs and fluroquinolones and those producing extended spectrum ß-lactamases have increased significantly during the 2000's and in certain areas while many nosocomial and community-acquired bacteria are now resistant to several important antimicrobials [20].

In this study, the overall resistance of *S. aureus* and *E. coli* to antimicrobials was high. The result is consistent with the findings of previous studies. The resistance rate in this study was in conformity with that of [21]. High resistance rate of *E. coli* to ampicillin and Ceporex in this study justify the study conducted by [23], who reported 100% resistant of *E. coli* to beta lactam drugs. Resistance to beta lactam drugs in this study was in line with the study conducted by [19, 23] in Slovenia and Sudan respectively. One of the major reasons for this high resistance can be co-expressed resistance mechanisms in the species. Increasing rates of antimicrobial resistance among *S. aureus* and *E. coli* is a growing concern worldwide. Antimicrobial resistance in *S. aureus* and *E. coli* has been reported worldwide and increasing rates of resistance among *S. aureus* and *E. coli* is a growing concern in both developed and developing countries. The higher resistance against the above antimicrobials could be as a result of repeated or prolonged use or exposure of uropathogens to antibiotics repeated use of antibiotics can damage urethral flora, allowing uropathogens to colonize and subsequently to infect the urinary tract, leaving clinicians with very few choices of drugs for the treatment of UTI [18,19]. Moreover, this condition enables bacteria to exchange their genetic material through horizontal gene transfer resulting in resistant gene that confer resistance to antibiotics [24]. In this study, *E. coli* isolates were sensitive to gentamicin, ciprofloxacin, and streptomycin while *S. aureus* is more sensitive to gentamicin, ofloxacin, Septrin and Augmentin. High sensitivity to ciprofloxacin and gentamicin by UTI isolates was also recorded from previous studies conducted in India [25].

CONCLUSION AND RECOMMENDATIONS

Conclusion

Susceptibility pattern of *S. aureus* and *E. coli* isolated from UTI patients were determined in the present study. Findings of the study showed that most of the *S. aureus* tested were resistant to more than 3 antibiotics. Most of the isolates were sensitive to gentamicin, ofloxacin, Septrin and Augmentin. However, the isolates were more resistant to streptomycin, Nalidixic acid and levofloxacin. On the other hand, *E. coli* isolates were more sensitive to Levofloxacin, ofloxacin, Streptomycin and gentamicin but mostly resistant to Ceporex, Augmentin and ampicillin. The result also showed that 14 out of the 20 (70%) isolates were multi drug resistant for both *S. aureus* and *E. coli* respectively. In this study, the overall resistance of *S. aureus* and *E. coli* to antimicrobials was high. The higher resistance against the above antimicrobials could be as a result of repeated or prolonged use or exposure of uropathogens to antibiotics repeated use of antibiotics can damage urethral flora, allowing uropathogens to colonize and subsequently to infect the urinary tract, leaving clinicians with very few choices of drugs for the treatment of UTI.

Recommendations

Based on the finding of this study, the following recommendations were made.

1. Continue surveillance of resistant rate among bacterial isolates causing UTI is needed to ensure proper recommendation for the treatment of the disease.
2. The judicious use of antibiotic is recommended which will help to limit the increasing rate of drug resistance in the pathogens.

3. Health educational programs on preventive and control measures of the diseases should be provided

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