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RESISTANCE PATTERN OF UROPATHOGENIC *E. COLI* STRAINS ISOLATED FROM A NIGERIAN HOSPITAL

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ABSTRACT

Bacterial resistance to antibiotics is a global problem which results in difficulty in treatment. Surveillance study should always be carried out in different geographical location to determine current effective antibiotics against bacterial infections. *Escherichia coli* strains were isolated from High Vaginal Swab (HVS), Endocervical Swab (ECS), and urine samples of patients visiting Igbinedion University Teaching Hospital between April and May, 2010. Identification of *E. coli* strains were based on growth on selective agar media and Gram's reaction. Susceptibility testing of these *E. coli* strains was done against an array of antimicrobials using the disk agar diffusion method and Minimum Inhibitory Concentration (MIC) determination by macrodilution method. Sixteen *E. coli* strains were isolated and identified. The percentage of resistant to Nitrofurantoin, Ciprofloxacin, Ampicillin, Amoxiclav, Chloramphenicol, Nalidixic acid, Sulphamethazole, Gentamycin were 18.75%, 18.75%, 37.5%, 18.75%, 62.5%, 12.5%, 37.5%, and 31.25% respectively. The strains were highly resistant to Chloramphenicol while susceptible to Nalidix acid. The result of disc diffusion method also agrees with MIC method. The resistance observed in this study to some antibiotics could be because of inappropriate and incorrect administration of these antimicrobial. To avoid this, there should be strict control in the use of these antibiotics.
Keywords:-*Resistance, antibiotics, uropathogens, urinary tract infections*

INTRODUCTION

Non-sexually transmitted urogenital infections which include yeast vaginitis, bacterial vaginosis, and urinary tract infections remain a major medical problem in terms of the number of women afflicted each year. The incidence of urinary tract infection, bacterial vaginosis and yeast vaginitis, is estimated to affect one billion women each year [1]. Although most patients respond to antimicrobial treatment, the recurrence rate is high and associated with side effects. *Escherichia coli* is consistently proving to be the most frequent causative agent in all parts of the world [2]. The frequency of occurrence of *E. coli* in most cases is up to 85% followed by St*aphylococcus saprophyticus, Klebsiella pneumoniae* and enterococci [3]. Therapeutic approaches to treatment of bacterial urogenital infections have remained essentially unchanged for many years. Current therapy involves long-term, low-dose antibiotic treatment which involves the active killing of bacteria that enter the bladder. Most uncomplicated UTI cases are resolved between 1 and 7 days of antibiotic therapy. In UTI, antimicrobial therapy is initiated even before the results of urine culture are available; often flouquinolones are preferred as initial agents for empiric therapy of UTIs. Ciprofloxacin is the most frequently prescribed flouroquinolones for UTIs, because it has shown excellent activity against pathogens commonly encountered in complicated UTIs [4]. Consequently the

extensive uses of antimicrobial agents have invariably resulted in the development of antibiotic resistance, which in recent years has become a major problem worldwide [5]. Bacterial isolates from UTI cases are usually characterized by steady increase in their level of resistance to commonly used antimicrobials including ampicillin, trimethoprim-sulphamethoxazole (TMP-SMX) or co-trimoxazole and the quinolones with most uropathogens being multidrug resistant [6].. There is escalating resistance to fluoroquinolones in some countries [7, 8], 30% resistance of *E. coli* to fluoroquinolones has been reported in Spain [9] while 64% resistance of *E. coli* to ciprofloxacin has been reported in Nigeria [10]. There is also increased resistance to trimethoprim-sulfamethoxazole [11, 12], (trimethoprim-sulfamethoxazole was formerly recommended daily for up to 5 years for treatment of UTI [13]. *Escherichia coli* resistance to trimethoprim/sulfamethoxazole are as high as 18% in the US and Canada [11]. The frequency and antimicrobial resistance rate of uropathogenic bacteria vary with age and geographical region and can change according to whether infections were acquired in the community or hospital [14]. Thus the aim of this study was to evaluate the resistance pattern of *E. coli* strains isolated from patients in a Nigeria hospital to various antibiotics and to determine susceptible antibiotics for treatment of uropathogenic *E. coli* infections.

MATERIAL AND METHODS

Collection and analysis of samples

Seventy three samples comprising of 12 Endocervical Swab (ECS), 18 High Vaginal Swab (HVS) and 43 urine samples were collected from patients at Igbinedion University Teaching Hospital, Okada, Nigeria by the staff of Medical Microbiology unit of the hospital between April and May, 2010. The samples were microbiologically examined for the presence of *E. coli* by inoculating on McConkey agar (Lab M, UK) media plates. Plates were incubated at 37^0C for 24 hrs. Isolates were examined for colonial morphology and lactose fermenting ability to isolate suspected *E. coli* strains.

Identification of E. coli Strains

Gram staining and growth on McConkey agar medi were performed on the isolates for preliminary identification. The following tests were done to confirm the identities of the isolates. Indole test was performed by inoculating overnight culture of the suspected isolates into peptone broth and Kovac's reagent was added to the culture .The production of a ring on the surface of the broth culture within 10 sec confirms the presence of *E. coli*. Colonial morphology on solid media was studied by streaking an 18 hr old culture of the micro-organism on Eosin Methylene Blue agar (Scharlau, Spain). The plates were examined macroscopically after incubation for 24 hr at 37^0C for the production of metallic sheen which confirms the presence of *E. coli*. Isolates were further grown on Citrate agar (Simon, Lab M, UK) by gently stabbing the agar slants on bottles with the suspected *E. coli* strains. The tubes were incubated at 37^0C for 24 hrs with the cap slightly loosed, and examined for colour production

Antibiotic susceptibility tests

The susceptibility of the bacteria to different antibiotics was tested according to a breakpoint method which agrees with standard indicated by National Committee for Clinical Laboratory Standard (NCCLS, USA), using standard antibiotic discs (Oxoid, UK). 18 hr old broth culture of *E. coli* strains of approximately 10^8 cfu1ml which is equivalents to 0.5 MacFarland standard was inoculated onto solidified and sterilized Muller Hinton agar by spread plate method. Eight different standard antibiotic disks namely: Nitrofurantoin 300 µg, Ciprofloxacin 5 µg, Ampicillin 10 µg, Chloramphenicol 30 µg, Amoxicillin 30 µg, Nalidixic acid 30 µg, Sulphamethazole 25 µg, and Gentamycin 10 µg were placed firmly at least 2 cm apart on the agar plates using a sterile forceps. The plates were kept on the bench for 30 min to allow diffusion of the antimicrobials before incubating at 37^0C for 24 hrs. The plates were then incubated for 24 hr at 37^oC and examined for clear zones of inhibition around the discs. The diameter of inhibition were measured and compared with standard zones to determine resistance as instructed by the manufacturer of the antibiotic discs (Oxoid, UK).

Determination of Minimum Inhibitory Concentration (MIC)

All isolates that shows gross resistance to Ciprofloxacin, Ampicillin, and Chloramphenicol was assayed for their MIC to the antibiotics. Stock solution of Ciprofloxacin (Bal Pharma, China), Ampicillin (Shijiazhuang Pharma, China) and Chloramphenicol (Maxheal Pharmaceutical, India) were prepared to concentration of 80 µg/ml, 160 µg/ml and 60 µg/ml respectively. The MIC of the antibiotics to the bacterial strains were determined by adding 1 ml of stock antibiotic solution to 9 ml of McConkey broth thereby reducing the concentration by 1/10. 5 ml of the medium/antibiotics solution was added to another 5 ml of McConkey broth thereby halving the antibiotic concentration. This was repeated until the least desired antibiotic concentration was obtained. 100 µl of 24 hr old bacterial culture of approximately 10^8 cfu/ml which is equivalent to McFarland standard 0.5 was inoculated into each antibiotics/medium mixture and incubated at 37^oC for 24 hr. The tubes were examined for growth. The least concentration at which no bacterial growth can be discerned is selected as the MIC.

RESULTS

Sixteen *E*. coli strains were isolated from urogenital tract samples comprising 5 urine samples, 5 ECS and 6 HVS (Table 1). The *E. coli* strains were all Gram negative bacilli with large circular colonies on agar media. They grew on McConkey agar, Eosin Methylene blue agar and Citrate agar with pink, metallic green and green colouration respectively (Table 2). These characteristics confirm the bacterial strains as *E. coli*. Antibiotic susceptibility tests were performed on all *E. coli* strains by disc diffusion. Most strains were very sensitive to nalidix acid while the highest rate of resistance was observed in chloramphenicol (Table 3). Each strain had varied overall resistance pattern that ranges between 0% and 62.5%. *Escherichia coli* VHZ 100 was sensitive to all tested antibiotics while *E. coli* VHZ 050 was very resistant to most tested antibiotics (Table 3).

The Minimum Inhibitory Concentrations of the various antibiotics to some selected resistant *E. coli* strains were determined by broth macrodilution. The E. coli strains were generally not

very resistant to the tested antibiotics. Most tested strains had an MIC of 8 µg/ml to ampicillin 4 µg/ml to ciprofloxacin and 3 µg/ml to chloramphenicol (Table 4).

Table 1. Sources of isolation of E. *coli* strains

E coli strain	Source of isolation
E. coli VHZ 483	Endocervical Swab
E. coli VHZ 078	Endocervical Swab
E. coli VHZ 038	Endocervical Swab
E. coli VHZ 086	Endocervical Swab
E. coli VHZ 040	Endocervical Swab
E. coli VHZ 082	Urine
E. coli VHZ 079	Urine
E. coli VHZ 050	Urine
E. coli VHZ 084	Urine
E. coli VHZ 011	Urine
E. coli VHZ 018	High Vaginal Swab
E. coli VHZ 077	High Vaginal Swab
E. coli VHZ 012	High Vaginal Swab
E. coli VHZ 013	High Vaginal Swab
E. coli VHZ 085	High Vaginal Swab
E. coli VHZ 100	High Vaginal Swab

E. coli strain	Nitrofurantoin (300µg) R≤14	Ciproflozac-in (5µg)	Ampicilin (10µg) 11	Amoxi-clav(30µ) R ≤13	Chlorampe-nicol(30µg) R ≤12	Nalidixic acid (30µg) R ≤ 13	Sulphametazole (10µg) R ≤ 10	Gentamyc- (10µg) R ≤
E. coli VHZ 483	20 mm S	21 mm S	0 mm R	16 mm S	0 mm R	14 mm S	15 mm S	13 mm S
E. coli VHZ 078	0 mm R	0 mm R	18 mm S	16 mm S	12 mm R	15 mm S	19 mm S	14 mm S
E. coli VHZ 038	21 mm S	18 mm S	12 mm S	0 mm R	0 mm R	18 mm S	10 mm R	13 mm S
E. coli VHZ 038	21 mm S	18 mm S	0 mm R	15 mm S	16 mm S	19 mm S	11 mm S	18 mm S
E. coli VHZ 040	0 mm R	17 mm S	0 mm R	18 mm S	12 mm R	15 mm S	10 mm R	18 mm S
E. coli HZ 082	16 mm S	19 mm S	12 mm S	16 mm S	0 mm R	18 mm S	0 mm R	16 mm S
E. coli VHZ 079	30 mm S	20 mm S	14 mm S	16 mm S	13 mm S	13 mm R	15 mm S	10 mm R
E. coli VHZ 050	18 mm S	0 mm R	0 mm R	0 mm R	0 mm R	16 mm S	14 mm S	12 mm R
E. coli VHZ 084	20 mm S	24 mm S	14 mm S	0 mm R	14 mm S	19 mm S	14 mm S	10 mm R
E. coli VHZ 011	22 mm S	32 mm S	0 mm R	16 mm S	12 mm R	15 mm S	17 mm S	0 mm R
E. coli VHZ 018	14 mm R	0 mm R	15 mm S	14 mm S	14 mm S	16 mm S	14 mm S	12 mm R
E. coli VHZ 077	21 mm S	20 mm S	15 mm S	18 mm S	0 mm R	10 mm R	13 mm S	18 mm S
E. coli VHZ 012	20 mm S	28 mm S	0 mm R	15 mm S	20 mm S	20 mm S	10 mm R	15 mm S
E. coli VHZ 013	22 mm S	15 mm R	14 mm S	14 mm S	12 mm R	19 mm S	10 mm R	13 mm S
E. coli VHZ 085	45 mm S	20 mm S	16 mm S	16 mm S	0 mm R	14 mm S	0 mm R	13 mm S
E. coli VHZ 100	20 mm S	24 mm S	15 mm S	16 mm S	16 mm S	14 mm S	11 mm S	15 mm S
% resistance B	18.75%	25%	37.5%	18.75%	62.5%	12.5%	37.5%	31.25%

Table 2. Identification of *E. coli* strains by different reactions.

Confirmatory Tests for all *E. coli* strains				
Gram Reaction	Morphology on agar media			
Gram negative bacilli	Characteristics	EMB	Citrate agar	McConkey agar
	Size	2-3 mm	0.1-0.2 mm	3-4 mm
	Colour	Metallic Green	Green	Pink
	Shape	Circular	Circular	Circular
	Consistency	Sticky	Not Sticky	Sticky

Note:- EMB is Eosin Methylene Blue agar

TABLE 3. Antibiotic susceptibility pattern of *E. coli* strains.

Note: S-Susceptibility, R-Resistance

% Resistance A- Percentage resistance of an *E. coli* strain to all tested antibiotics
% Resistance B- Percentage resistance of all tested *E. coli* strains to particular antibiotics

TABLE 4. Analysis of Minimum Inhibitory Concentration of three antibiotics to selected resistant *E. coli* strains

E. coli strains	Growth of *E. coli* at different antibiotic concentrations				MIC
	Ampicillin B.P. 2 µg/ml				
	16 µg/ml (80 µg)	8 µg/ml (40 µg)	4µg/ml or 20µg	2µg/ml or 10µg	
E. coli VHZ 084	–	–	+	+	8 µg/ml R
E. coli VHZ 050	–	–	+	+	8 µg/ml R
E. coli VHZ 011	–	–	+	+	8 µg/ml R
E. coli VHZ 012	–	+	+	+	16 µg/ml R
E. coli VHZ 483	–	–	+	+	8 µg/ml R
E. coli VHZ 040	–	–	+	+	8 µg/ml R
	Ciprofloxacin B.P. 0.015 µg/ml				
	8 µg/ml (40 µg)	4 µg/ml (20 µg)	2 µg/ml (10 µg)	1 µg/ml (5 µg)	
E. coli VHZ 015	–	–	+	+	4 µg/ml R
E. coli VHZ 050	–	–	–	+	2 µg/ml R
E. coli VHZ 078	–	–	+	+	4 µg/ml R
	Chloraphemnicol B.P. 2 µg/ml				
	6 µg/ml (30 µg)	3 µg/ml (15 µg)	1.5 µg/ml (7.5 µg)	0.75 µg/ml (3.75 µg)	
E. coli VHZ 077	–	–	+	+	3 µg/ml I
E. coli VHZ 085	–	–	+	+	3 µg/ml I
E. coli VHZ 050	–	–	+	+	3 µg/ml I
E. coli VHZ 048	–	+	+	+	6 µg/ml R
E. coli VHZ 038	–	–	+	+	3 µg/ml I
E. coli VHZ 082	+	+	+	+	>6 µg/ml R

Note:-B.P. – Breakpoint, R-Resistant, I-Indeterminable. The breakpoint for MIC was adapted from Andrew [15].

DISCUSSION

Urogenital infections remain a major medical problem in terms of the number of patients afflicted each year. Urogenital infections constitute a worldwide problem that affects >300 million women per year; these infections are a common reason for a woman's visit to a family practitioner or urologist. The main clinical outcome is morbidity and discomfort amongst a large percentage of the female population, in addition to enormous costs to the health care system for treatment. Many patients will experience a recurrence of symptoms, particularly within the first year of the original infection. Of more concern are infections that are complicated by kidney involvement because hospitalization is usually required and kidney damage or even death can occur. *Escherichla coli* is the causative agent in most cases (up to 85%) followed by St*aphylococcus saprophyticus, Klebsiella pneumoniae* and enterococci [3]. In this study, 16 isolates identified as *Escherichia coli* from urine, HVS and ECS were employed. The organisms isolated in this study are multiple drug resistant microorganisms based on the fact that most of the isolates are resistant to at least 2 antibiotics. This trend is

similar to another study carried out in another part of Africa where >68% of all isolates were resistant to 2 or more antimicrobial [16]. Many factors contribute to occurrence of multi-drug resistant uropathogens in Africa including misuse of antibiotics, counterfeit drugs, shortfall in infection control, public health and also, the fact that many of the resistance genetic determinants are plasmid borne, and thereby providing resistance to several other classes of antibiotics and transmission to other microorganisms. The result of disc diffusion agrees with MIC result. The greater percentage of the bacteria used in this study were susceptible while some strains were resistance. Nitrofurantoin and Nalidixic acid remains the drugs of choice for treatment and management of asymptomatic bacteriuria in pregnant women and symptomatic UTIs in general [17] because of its activity against several different types of E. coli, *Klebsiella aerogenes* etc. This agrees with the findings of this study as Nitrofurantoin and Nalidixic acid had excellent activity against *E. coli* from the various sources, followed by ciprofloxacin and Amoxiclav. Ciprofloxacin is the drugs of choice for treating UTI in many regions of the world but some *E. coli* strains from this study were resistant to the antibiotics as observed by the MIC result. Adeniyi and Amajoyi [18] have also reported ciprofloxacin resistance in South West Nigeria. Green and Tillotson [19] reported that increased resistance to ciprofloxacin in developing countries could be due to an increased selective pressure caused by the use of more ciprofloxacin.

Drugs resistance is one of nature's never ending process whereby organisms develop a tolerance for new environmental condition. These may be due to a pre-existing factor in the organism or it may result from acquired factors. Some naturally susceptible strains of bacteria may acquire resistance [20]. Although ampicillin is frequently suggested as the agent of choice, *E. coli* is now commonly resistant to ampicillin [21], this agrees with this study as *E. coli* strains has 37.5% resistance rate to ampicillin. Although there have been reports of resistance of sulphametazole to *E. coli* in other studies [22, 23]. Isolates from this study are relatively susceptible to the antibiotics. Moreover, the tested *E. coli* strains were highly resistant to chloramphenicol. Chloramphenicol is no longer a first line agents for any indications in developed nation due to problem of resistance, but in low income country, it is still widely used because it is inexpensive and readily available.. These worldwide problem of resistance of bacteria to chloramphenicol could arise from it widespread use in infections in developing countries. The general problem of resistance could be attributed to frequent use or abuse of antimicrobials by patients, selling of antibiotics over the counter without prescribtion and addition of antibiotics to feeds of livestocks, administering antibiotics for common cold, inappropriate dose, and inapropriates compliance to antibiotics therapy. The potential crisis at hand is the increasing prevalence of resistant bacteria and the infectious disease physicians are alarmed by the prospect that effective antibiotics may not be available to treat seriously ill patients in the near future. From this study, *E. coli* strains are susceptible to Nalidixic acid. Therefore, the antibiotics can be use for treatment of urogenital infections caused by *E. coli* strains while the observed resistance to chloramphenicol makes the antibiotics unsuitable for treatment of gurogenital infections caused by E. coli strains.

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