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PREVALENCE AND SUSCEPTIBILITY PATTERNS OF CLINICAL ISOLATES OF *ESCHERICHIA COLI* TO VARIOUS ANTIMICROBIALS IN A CLINICAL MICROBIOLOGY LABORATORY IN SOUTH-SOUTH NIGERIA

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RUNNING TITLE: ANTIMICROBIAL SUSCEPTIBILITY OF *ESCHERICHIA COLI*

ABSTRACT

The purpose of this study is to determine the prevalence of *Escherichia coli* as an aetiologic agent in bacterial infections and its antimicrobial susceptibility patterns to ciprofloxacin, ofloxacin, norfloxacin, perfloxacin, gentamycin and cotrimoxazole as a guide for empiric therapy. A retrospective study was carried out using a clinical microbiology laboratory in Nigeria. Data retrieved include number of *E. coli* isolates, sources of the isolates and their antimicrobial susceptibility to various fluoroquinolones, gentamycin and cotrimoxazole between 2005 and 2009. Statistical analysis was carried out using SPSS version 14, Chicago IL. Out of a total of 906 bacterial isolates, *E. coli* accounted for 23 % (211) of the isolates. Thirty-eight percent (38.39 %) was isolated from urine samples, 27.96 % from high vaginal swab samples, 24.17 % from stool samples, 0.95% from urethra swabs, 1.9% from wound swabs and 6.6% from semen samples. There was poor level of susceptibility to norfloxacin (2.2%) and cotrimoxazole (23.7%), susceptibility to ofloxacin, ciprofloxacin and pefloxacin were 51.1%, 54.7% and 52.5% respectively, that of gentamycin was 51.8%. The trends across the years showed a significant increase in susceptibility to ciprofloxacin, pefloxacin and ofloxacin in 2007 after which it started reducing, while norfloxacin's susceptibility was low across the five years with maximum susceptibility at 9.1% in 2006. There was an increase in susceptibility to gentamycin as the susceptibility levels of the fluoroquinolones were reducing. There should be continuous surveillance of antimicrobial susceptibility patterns and empiric treatment with fluoroquinolones discouraged, especially for non urinary tract infections.

KEYWORDS: Antimicrobial susceptibility, Nigeria, *Escherichia coli*, fluoroquinolones, gentamycin

INTRODUCTION

Antimicrobial resistance is a worldwide problem and it is one of the greatest challenges to health care delivery[1]. There is tremendous variability in antimicrobial resistance patterns not only among pathogens causing various clinical infections but also in different geographical regions and over time [2]. Surveillance of susceptibility patterns of pathogens to antimicrobial agents is therefore, important in the monitoring and detection of increase in resistance [3-5]. It is very helpful for clinicians in prescribing antimicrobial agents especially in cases where empiric treatment is employed. In Nigeria, as is the case in many developing countries, empiric therapy is often employed as a result of inadequate staffing and laboratory facilities [6].

E.coli has been identified as a predominant pathogen for various bacterial infections especially urinary tract infections [7-11]. Fluoroquinolones have been proven to be highly effective broad spectrum agents especially against those infections caused by Gram-negative organisms.[12] Due to high resistance to cotrimoxazole, fluoroquinolones became the first drug of choice for empiric treatment of urinary tract infections and other infections caused by Gram-negative bacteria and its widespread use has resulted in the rise in their resistance.[13] Despite the regular use of empiric treatment in Nigeria, data on antimicrobial susceptibility patterns of various pathogens are scant. This study was therefore carried out to determine prevalence of *E. coli* as an aetiologic agent in community acquired bacterial infections and its antimicrobial susceptibility patterns to different fluoroquinolones, as well as gentamycin and cotrimoxazole, as a guide for empiric therapy.

METHODS

This was a single centre retrospective study done between January 2005 and December 2009 in a clinical microbiology laboratory in South-south Nigeria. Data retrieved from microbiology laboratory records included number of *E. coli* isolates, sources of the isolates and antimicrobial susceptibility to ciprofloxacin, ofloxacin, pefloxacin, and norfloxacin (fluoroquinolones) and also cotrimoxazole and gentamycin, popularly used in the country for the treatment of bacterial infections. Isolates collected from the same specimen source within seven days were excluded. Samples were inoculated on blood agar, MacConkey agar and urine samples on cystine lactose electrolyte deficient (CLED) agar. Culture plates were incubated at 37oC for 24 hours. Identification was done using Gram staining and confirmatory tests were carried out. Antimicrobial susceptibility test was carried out using antibiotic discs on Mueller Hinton agar. Antibiotic discs used were gentamycin (10mcg), cotrimoxazole 25mcg, ciprofloxacin (10mcg), ofloxacin (10mcg), norfloxacin (10mcg) and pefloxacin (10mcg). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14],. Susceptibility tests were classified into two categories – susceptible and resistant, with all intermediate susceptibility classified as resistant. For quality control of susceptibility tests, *E.coli* ATCC 27922 was used.

Analysis of data was carried out using SPSS version 14, Chicago IL. Frequency of *E.coli* and susceptibility results were expressed as percentages.

RESULTS

There was a total of 906 bacterial isolates within the time frame (2005-2009) included in this study. Of this 906, *E. coli* accounted for 23% (211). Frequency of the 211 *E. coli* isolates from various sources is shown in Fig. 1. Table 2 shows the prevalence of *E. coli* in the various sources. The total percentage susceptibility of levels of *E.coli* to the various fluoroquinolones is shown in Table 2.

The susceptibility trends of *E.coli* to pefloxacin, ciprofloxacin and ofloxacin were similar across the five years (Fig 2). The level of norfloxacin susceptibility across the five years was below 10% with the highest susceptibility level in 2006 at 9.1%. Susceptibility to gentamycin was lowest (27.3%) in 2006 and it kept increasing till it got to its highest (81.8%) in 2009.

TABLE 1: PREVALENCE OF *ESCHERICHIA COLI*

FROM THE VARIOUS SOURCES

Source	Prevalence (%)	No of *E.coli* isolates/total no of samples tested
Urine	33.37	81/243
Stool	26.56	51/192
HVS	15.86	59/372
Urethra swab	9.1	2/22
Wound	15.35	4/26
Semen	25.93	14/54

TABLE 2: ANTIBIOTIC SUSCEPTIBILITIES OF ALL *E. COLI*

ISOLATES TO ANTIMICROBIALS UNDER STUDY

BETWEEN 2005 AND 2009

ANTIMICROBIAL	% SUSCEPTIBILITY
OFLOXACIN	108/211 (51.1)
CIPROFLOXACIN	115/211 (54.7)
NORFLOXACIN	4/211 (2.2)
PEFLOXACIN	111/211 (52.5)
COTRIMOXAZOLE	50/211 (23.7)
GENTAMYCIN	109/211 (51.8)

DISCUSSION

The results show that *E. coli* is an important pathogen in community acquired bacterial infections especially in urinary tract infections, which accounts for 38.39% of all the *E. coli* isolates. Its prevalence in urine samples was also high 33.37%. Prevalence of *E.coli* in stool samples was 26.56% and prevalence in high vaginal swab samples (HVS) was 15.86%. This could be as because of the proximity of the vagina to the anus.

The results also show similar susceptibility levels among the three second generation fluoroquinolones, ciprofloxacin (54.7%), pefloxacin (52.5%) and ofloxacin (51.1%), included in the study. Susceptibility of ciprofloxacin is similar to that found in a study carried out in Tehran [15].

FIG 1: FREQUENCY OF *ESCHERICHIA COLI* FROM VARIOUS SOURCES.

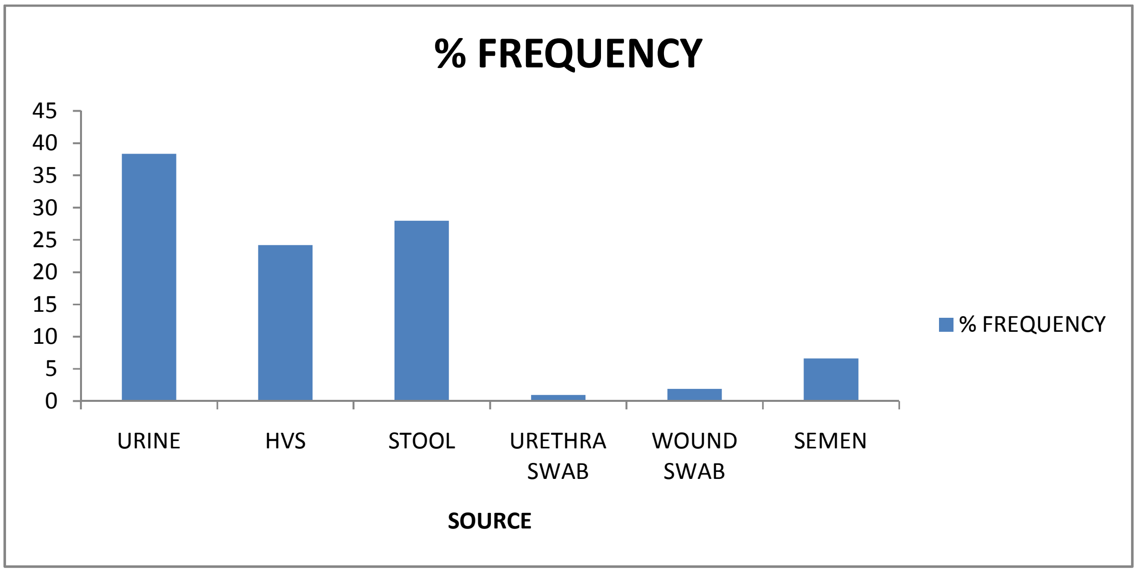
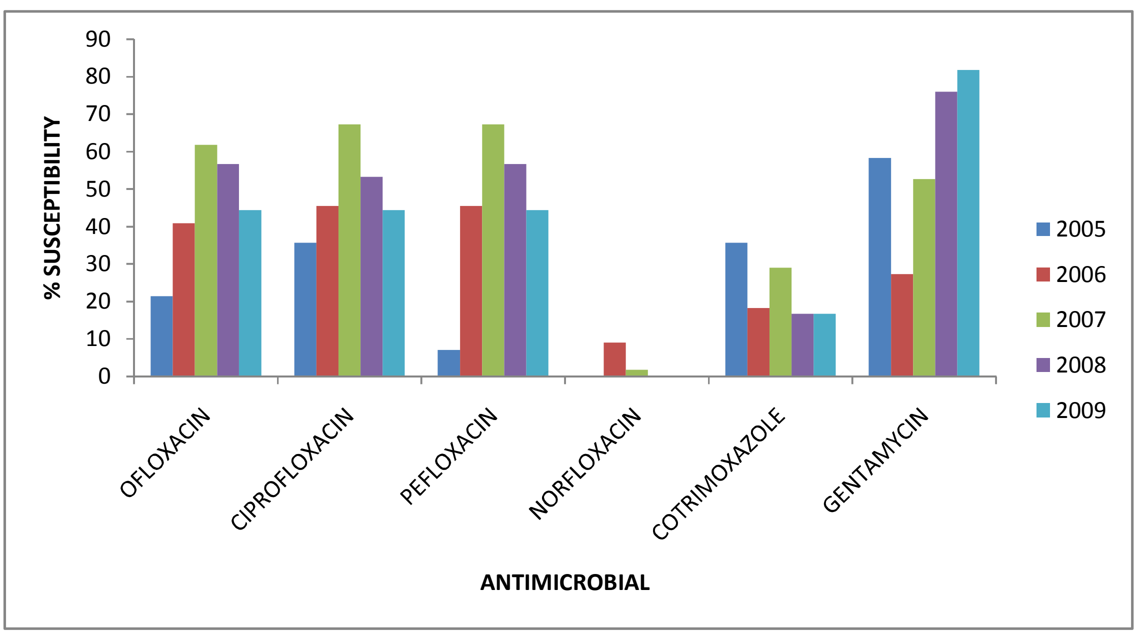


Fig 2: SUSCEPTIBILITY TRENDS OF *E. COLI* TO VARIOUS ANTIMICROBIALS BETWEEN 2005 AND 2009



Ciprofloxacin susceptibility was the highest. This is also in line with a study carried out in France [16] . Susceptibility to gentamycin (51.8%) was also similar to that of the fluoroquinolones. However, there was poor susceptibility to cotrimoxazole and norfloxacin. Susceptibility levels for cotrimoxazole in this study was 23.7%. This is similar to other studies carried out in other places [11,17]. Poor susceptibility of *E.coli* to cotrimoxazole could possibly be as a result of the fact that it has been in use for a long time, hence giving organisms time to develop resistance mechanisms towards it. Susceptibility of *E. coli* to norfloxacin was quite low, 2.2% with the highest susceptibility of norfloxacin being 9.1% in 2006. This is contrary to the result obtained from a similar study carried out in rural Tamilinadu where susceptibility to norfloxacin was 94.44% [18]. According to reports published in 2000, quinolone susceptibility was greater than 95% [12,19]. Generally, the susceptibility levels got in this study is lower than that got from previous studies carried out in developed countries [20] . This could possibly be because of the ease of access to antibiotics over the counter in Nigeria, which is also the case in many other resource poor settings with lax policies, resulting in high and uncontrolled consumption of antibiotics. There has been a steady increase in the level of resistance of commonly used antibiotics. Ciprofloxacin, ofloxacin and pefloxacin were almost at par with susceptibility of just over 50%.. In the rural Tamilinadu study, antimicrobial susceptibility of *E.coli* from urine isolates to ciprofloxacin was 77.7% [18].

The susceptibility trends across the five years show an increase in susceptibility levels of ciprofloxacin, ofloxacin and pefloxaciin. In 2007, susceptibility of ciprofloxacin increased from 35.7% in 2005 to 67.3% in 2007; that of ofloxacin increased from 21.4% in 2005 to 61.8% in 2007 while that of pefloxacin increased from 7.1% in 2005 to 67.3% after which the susceptibility levels started dipping again. This could possibly be due to a reduction in its use prior to 2007 and the subsequent increase in resistance after 2007 might have arisen as a result of increased use. However, this cannot clearly be ascertained from this study. It was also observed that the susceptibility levels of gentamycin, an aminoglycoside, increased (27.3% in 2006 and 81.8% in 2009) as susceptibility levels of the fluoroquinolones were reducing. It should be recommended that antibiotic use be rotated. Antibiotic cycling which is the rotation of antibiotics over a stipulated time period is an important aspect in antibiotic stewardship. A study in Greece [1] showed significant increase in the susceptibility of three important Gram negative pathogens to ciprofloxacin following an eighteen month restriction on the empirical use of fluoroquinolones in the intensive care units.

High level resistance to these fluoroquinolones in this setting might be because of the high level of empiric use of antibiotics as a result of inadequate laboratory facilities, incomplete and wrong usage of antibiotics, high level of abuse as a result of over the counter acquisition of antibiotics and the possibility of having substandard drugs in the market [21]. More studies should be carried out to determine the relationship between level of consumption of these fluoroquinolones and the level of resistance.

There is need for constant antimicrobial surveillance to detect emerging antibiotic resistance patterns. This is also important in the development of hospital antibiograms in order to facilitate the use of the right antibiotics for the treatment of bacterial infections at the local level. Also in Nigeria, where over the counter acquisition of antibiotics have been implicated as one of the reasons for the development of resistance [6], policies should be put in place to control this. This will help curb the scourge of bacterial resistance.

The introduction of electronic laboratory databases which can be analysed with the help of simple software packages such as the WHONET software which can be obtained free from World Health Organisation [22], will greatly help in antibiotic surveillance.

Conclusion

This study has shown that *E.coli* is a predominant bacterial pathogen in community acquired bacterial infections. There is also a high level of resistance of *E.coli* to fluoroquinolones in the community. Inasmuch as there is reasonable level of resistance to fluoroquinolones, they will still be useful in the management of urinary tract infections, because they are excreted in the urine unchanged, hence enhancing their effects and *E.coli* has been indicated as the most common pathogen in urinary tract infections. However, for other sites, it is recommended that fluoroquinolones should not be used for empiric treatment. Proper laboratory tests should be carried out to determine pathogenic organism and sensitivity profile. Furthermore, there is need to set up proper antimicrobial surveillance systems in health institutions through which hospital antibiograms will be developed. Antibiotic cycling should also be implemented to help slow the rate of resistance development. Further studies should be carried out in the region to evaluate the effect of antibiotic utilization on bacterial resistance.

COMPETING INTERESTS: None

AUTHORS' CONTRIBUTIONS: COE and NCO designed the study protocol, NCO and OIE carried out the analysis and interpretation of data. NCO drafted the manuscript. All authors read and

approved the final manuscript. NCO and OIE are the guarantors of the paper.

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