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Antimicrobial susceptibility testing of ciprofloxacin, amoxicillin and erythromycin against *Staphylococcus aureus* and *Escherichia coli* isolated from healthy students of Delta State University Abraka South South, Nigeria

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Abstract

Escherichia coli and *Staphylococcus aureus* have been identified to be amongst the key causes of hospital acquired infections worldwide. These microorganisms have been known for their high tendency to build up resistance to a wide variety of antibiotics that can render previously potent drugs ineffective. This study determined the frequency and susceptibility profile of *Escherichia coli* and *Staphylococcus aureus* nasal isolates from healthy students of Delta State University Abraka South South, Nigeria. The nasal and urine samples were collected and immediately inoculated on solidified Mannitol salt agar and on solid Mac Conkey agar respectively and incubated for 24 hours at 37°C. Ideal biochemical tests were carried out to identify each microorganism isolated. Out of one hundred (100) samples collected, 53 organisms were positively identified to be bacterial isolates. Nineteen (38%) strains of S. *aureus* and 3 (6%) other *S. spp* were isolated. Total number of Gram-negative bacteria from the urine samples is 32 bacterial isolates. Of the 32 predominant was twelve *Proteus mirabilis* (24%), followed by *Citrobacter freundii* (12%), and *Aeromonas hydrophilia* (12%), next is *Proteus vulgaris*(6%), *E. coli* isolates (4%) while *Pseudomonas aeroginosa* (4%) and one strain of *Alcaligenes feacalis* (2%) was isolated. The antimicrobial resistance pattern shows that all the bacterial isolates had no resistance to ciprofloxacin. *S. aureus* showed some level of resistance to amoxicillin (10.5%) while erythromycin is(20 %). *E coli* showed complete resistance to amoxicillin (100%) and erythromycin (100%). Thus, it can be inferred that ciprofloxacin can be more effective in treating infections caused by the test microorganisms, than amoxicillin and erythromycin.

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Introduction

The demand for new antibacterial agents is due to an increasing changing antibacterial resistance patterns (Oteo *et al.*, 2002). Due to reduction in number of new antimicrobial drugs, bacterial antimicrobial drug resistance has become a universal problem (spellberg *et al.,* 2004; Talbot *et al.*, 2006). Antibiotics are most likely one of the most winning forms of chemotherapy in the medicine history (katzung, 2013). The antimicrobial agent discovery produced a major impact on the survival rate from several infections. Resistance to many antimicrobial agents really have turn out to be a major trouble in continent of Africa and the majority of the Asian countries resulting to increase in disease burden, mortality, morbidity and increase in health cost. Unnecessary and indiscriminate use of antibiotics is regarded as the main contributing factor even, prolonged hospital stay, admission to intensive care units, use of catheters, and reduced control of infections (Jocelyn Y *et al.*, 2004 ; okeke *et al.*, 2007).

The recent dialogue on infectious disease and resistance to drug in sub-saharan Africa is linked to the pressing problems associated with the rising and re-emerging resistant organisms. Resistance really results to the therapeutic failure in the acute respiratory infections treatments, sexually transmitted diseases and diseases that are spread through the fecal-oral route such as dysentery, typhoid, cholera, and other diarrheal diseases (okeke *et al.*, 2007; okonko *et al.*, 2009). The antimicrobial resistance pattern of organisms has taken a vast step and because of the rising number of resistance strains causing infections has resulted to the reduction of effectiveness of available antibiotics (Nawaz *et al.*, 2009).

Beta-lactam antibiotics are the most commonly used antimicrobial agent in the treatment of infections that are caused by bacteria and known to been the commonest *cause* of resistance to beta-lactam antibiotics amid Gram-negative bacteria universally. Incessant exposure of bacterial strains to very many of β-lactams has decreased dynamic and continued production and the mutation of β-lactamases in these bacterial strains of bacteria and this has extended their activity even against the newly developed β-lactam antibiotics. The enzymes that known to be responsible for this resistance pattern are recognized as Extended-spectrum β-lactamases (Pitout and laupland, 2008; Peterson *et al*, 2005). They are serious concerns regarding increased prevalence of extended spectrum β-lactamases (ESBLs) in different parts of the world. The under use or overuse of antibiotics, drug prescription without proper sensitivity test and over dosing may have created this problem in the developing countries. *E.coli* and *Klebsiella pneumonia* have contributed 10 to 40 % of ESBLs (Amin *et al.*, 2009). Many other noteworthy mechanism of resistance to beta-lactam antibiotics includes the alteration of the Penicillin-binding proteins. This is responsible for the decreased sensitivity to β-lactam agents by *Strep*. *Pneumonia* and *Haemophilus influenza*. On the other hand, the most clinically important example is Meticillin-resistance *Staphylococcus aureus* (MRSA). (Hugo and Russell, 2013).

Macrolides display bacteriostatic activities against a wide range of bacteria, particularly by selective inhibition of synthesis of protein in a good number of bacteria by binding to the 50S subunit. Erythromycin could be used for treating infections of respiratory tract and it can be regarded as the drug of choice in treating of pneumonia caused by *Legionella pneumophilia*, *Legionella micadadei* and other species of *Legionella* (Katzung, 2013). It is equally indicated for the treatment of soft skin and skin infections and campylobacter infections etc. (Guillermo *et al.,* 2007). Resistance to macrolides (particularly erythromycin) has witnessed outstanding increase worldwide. The most noteworthy mechanism of resistance is usually the production of esterase by Enterobacteriaceae which hydrolyse macrolides. Several bacteria cells can inactivate erythromycin by production of enzymes such as macrolide 2'-phosphotransferase and erythromycin esterase. A lot of members of the enterobacteriaceae family including *Citrobacter*,

Enterobacter, *Escherchia*, *Yersinia*, *Klebsiella*, *Shigella*, *Proteus* and *Salmonella* species which usually produce these enzymes are extremely resistance to erythromycin. Some other mechanisms of resistance include active efflux or decreased permeability of the cell membrane that are commonly observed in some Gram-positive organism such as *Corynebacterium glutamicum* and modification of the Ribosomal binding sites by chromosomal mutation (Rosata *et al.*, 1999;Leclereg and Courvalin, 2012).

Ciprofloxacin is one of the members of the Fluoroquinolone antibiotics. It exerts antimicrobial activity by selective inhibition of topoisomerases II and IV. Ciprofloxacin can stop the relaxation of supercoiled (positive) DNA that is needed for the normal replication and transcription and can also interfere with the separating the replicated chromosomal DNA into several daughter cells during cell division. (Anderson *et al.*, 2006; Zhannel *et al.*, 2006).

There are three different mechanisms of resistance that have been identified which includes; mutations that alter the drug targets, protection of cells from lethal effects of ciprofloxacin and/or mutation that reduce drug accumulation. Usually mutations are usually the first selected in most sensitive targets; which include: DNA gyrase in Gram-negative bacteria and topoisomerase IV in Gram-positive organisms. Resistance to quinolones can be mediated by plasmids leading to the production of Qnrproteins (Chalkley and Koornh of, 1985 ; Jocoby, 2005).

The antimicrobial resistance epidemiological observations have become crucial for empirical treatment of some infections, implementation of measures of resistance control and preventing the wide-spread of antimicrobial-resistance microorganisms (WHOCCDSM, 1999; Oteo *et al.*, 2002). Considering data collated by European Antimicrobial resistance Surveillance Network (EARS-Net) in 2009, which reveals the susceptibility pattern to antibiotics and infections that are caused by *E coli and S. aureus* and which at same time it shows an alarming increase in antimicrobial resistance by these two species of microorganisms. Antibiotic resistance in E. *coli and S. aureus* isolates is increasing as the day goes by, making it a foremost public health concern. This study aims to establish the present trends of antimicrobial resistance pattern of these microorganisms against commonly available antibiotics such as ciprofloxacin, amoxicillin and erythromycinin in Abraka, as this will guide the medical practitioners in this area to proper and accurate prescriptions.

Materials and methods

Reagents and media

Media used include: Mueller-Hinton agar, mannitol salt agar (Oxoid Limited, England), Nutrient agar, peptone water (Titan Biotech Limited, India) .Amoxicillin, Ciprofloxacin, Erythromycin (all are procured from Oxoid Ltd, Wale Road Basing stoke, Hants Rg 24 8 PN, UK).

Apparatus

Autoclave,(Medica Instrument Manufacturing CO., India), Incubator, (Genlab, UK) Analytical balance, Ohaus Corp., USA) Hot air Oven, (Genlab, UK) Microscope,(Olympus), test-tubes, wire-loop, conical flask, Bunsen burner aluminum foil, Analytical balance, microscope, petri-dishes, microscope slide, cover glass, bijou bottles durham tubes and pipettes.

Study area

This experiment was conducted among healthy students of Delta State University Abraka, Delta State Nigeria.

Ethical consideration

Before samples were collected, information regarding the study was explained to the participants after approval was sought from the head of Institution. Oral consent for participation in the study was obtained.

Sample collection

This study was conducted in Abraka, The nasal samples were obtained with sterile pre-moistened swab sticks, which were tenderly lead into the inner area and robbed over the anterior nares of both nostrils, while the urine samples were collected using a sterile universal bottle.

Bacteriology

This experiment was conducted in Pharmaceutical Microbiology Laboratory Delta State University, Abraka, Nigeria. Samples were collected from healthy individuals. *Staphylococcus aureus* isolates were obtained via the nasal swab, while *Escherichia coli* were obtained from the urine samples. A total of 100 samples were collected.

The nasal swab samples were inoculated on Mannitol salt agar at 37°C for 24 hours while the urine samples were inoculated on solid Mac Conkey agar at 37°C for 24 hours immediately after collection. The isolates were identified with standard biochemical tests such as catalase, citrate, coagulase, hydrogen sulphide, indole, methyl red, oxidase and urease tests (Cheesbrough, 2006).

Assay of antibacterial susceptibility

Susceptibility tests were performed using the Kirby-Bauer (Bauer *et al.*, 1966) disc diffusion by using Muller-Hinton agar (CM337-oxiod).Two (2) loopful of standardized test organism (0.5 MCF) were aseptically pipetted into a 20 ml of sterile molten Mueller-hinton agar and immediately mixed.. The antibiotic disc was gently removed from the cartridge and firmly placed on the agar plate using a pair of forceps. The plates containing agar were kept at room temperature for an hour to allow for diffusion of the antibiotic into the agar medium to take place.

The agar plates were then incubated at inverted positions at 37°C for 24hrs. The zones of inhibition were measured to the nearest millimeter and recorded. The antibiotic discs used contained the following antibiotics and concentrations; Amoxicillin 30μg, Ciprofloxacin loμg, and Erytbromycin loμg. Isolates were expressed as either resistance or intermediate or sensitive according to the criteria developed by clinical and Laboratory standard Institute.

Results and discussion

Antibiotic resistance is considered to be one of the world's most important burning public health troubles. The antibiotic-resistant microorganisms can spread very fast and so pose threat to the communities with infectious disease that can be more difficult to treat and even more expensive to treat.

Failures of treatment can arise owing to the resistance presented by pathogens against highly effective broad spectrum antibiotics.

Table 1. Frequency of isolation of *Staphylococcus* species in the nasal cavity.

Microorganisms	No. of positive growth	Percentage (%)
Staphylococcus aureus	19	38
Other *Staphylococcus* species	3	6
Total	22	44

These difficult-to-treat and treatment failures infections might result in elevated death rates. (Khushal 2004).In Abraka, Delta state, despite the significant roles *Staphylococcus aureus* and *Escherichia coli* plays in the development of infections in communities only a few studies have explored emergence of resistance of *Staphylococcus aureus* and *Escherichia coli* against these commonly used antibiotics (ciprofloxacin, amoxicillin and erythromycin). Out of the one hundred (100) samples

collected from the nostrils and urine of healthy students, 53 organisms were positively identified to be bacterial isolates. Nineteen (38%) strains of S. *aureus* and 3 (6%) other *S. spp* were isolated.

Previous studies have revealed the colonization of human nostrils in Agbor by *S. aureus* as reported by Ugwu *et al.*, (2016).

From fig1the antibiotics resistance pattern of the *Staphylococcus aureus* isolates showed that there was no resistance to ciprofloxacin which is comparable with findings of Ugwu *et al.*, (2006) in Agbor, but showed some level of resistance to amoxicillin (10.5%) while erythromycin is(20 %). *E. coli* isolates were completely resistance to amoxicillin and this present study is in line with that reported by Sabir *et al.*, (2014) where a total resistance was documented. However this finding deviates from the findings of Nkang *et al.*, (2009) that documented high sensitivity of *E. coli* to amoxicillin.

Table 2. Frequency of isolation of Enterobacteriaceae in the Urine Samples.

Microorganisms	No. of positive growth	Percentage (%)
Proteus mirabilis	12	24
Citrobacter freundii	6	12
Aeromonas hydrophilia	6	12
Proteus vulgaris	3	6
Psedumonas species	2	4
Escherichia coli	2	4
Alcaligenes feacalis	1	2
Total no of positive growth	32	
Number of samples	50	

The resistance of the test organism to penicillins indicate a careless use of these antibiotics for the treatment of urinary tract infections, nosocomial infections and other infections caused by *E. coli*. In different parts of the world, resistance of *E. coli* to penicillins group of antibiotics has been on the higher side and is rising every day (Olowu, *et al.*, 2007).

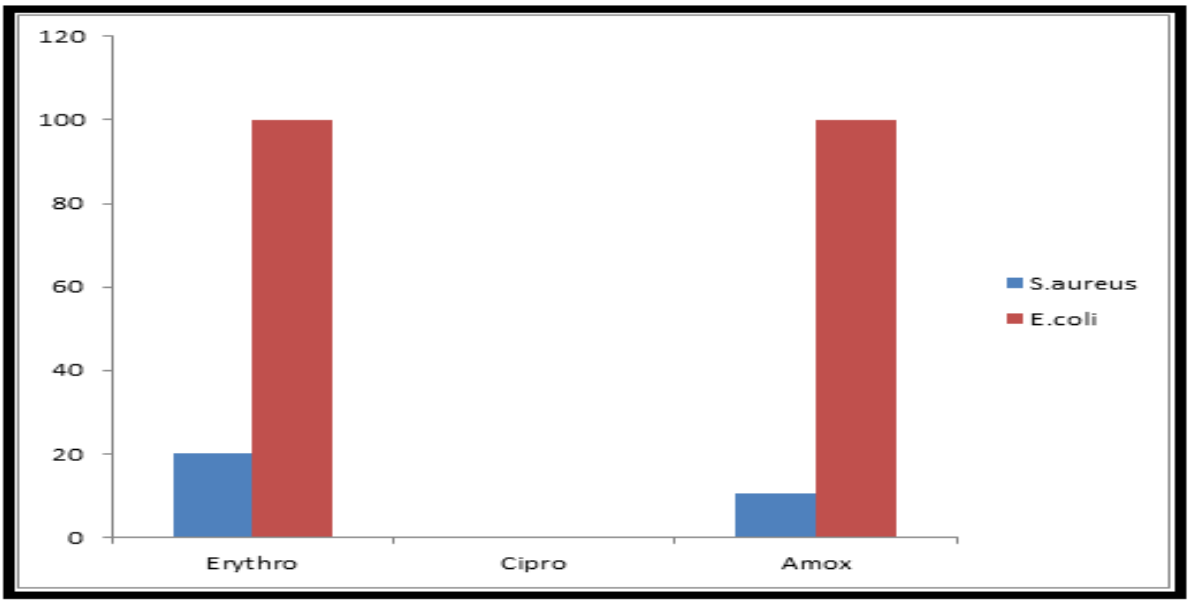

Fig. 1. Antimicrobial resistance pattern of E. coli and S. aureus against Ciprofloxacin, Amoxicillin and Erythromycin.

However, from Fig 2, *Escherichia coli* and *S. aureus* isolates recorded complete susceptibility to ciprofloxacin (100%). This is comparable with the study conducted in 2009 by Nkang *et al.*, who documented that these test organisms were highly sensitive to ciprofloxacin. However, the findings of this study deviates from the study conducted by Siddiqui *et al*, in 2013 and Sabir *et al.*, in 2014 as a susceptibility of 68 % and 44.8 % were documented respectively. *Staphylococcus aureus* was reasonably susceptible to erythromycin (80%). This present result is in agreement with that reported by Nkang *et al.*, (2009) where a high susceptibility of *S. aureus* was also observed. However, the isolates of *E. coli* were resistant to erythromycin. Though, various studies from different parts of the world have shown that the fluoroquinolones are effective in treating nocosomial infections caused by *S. aureus* and E. coli (Mavroidi, V *et al.*, 2012).

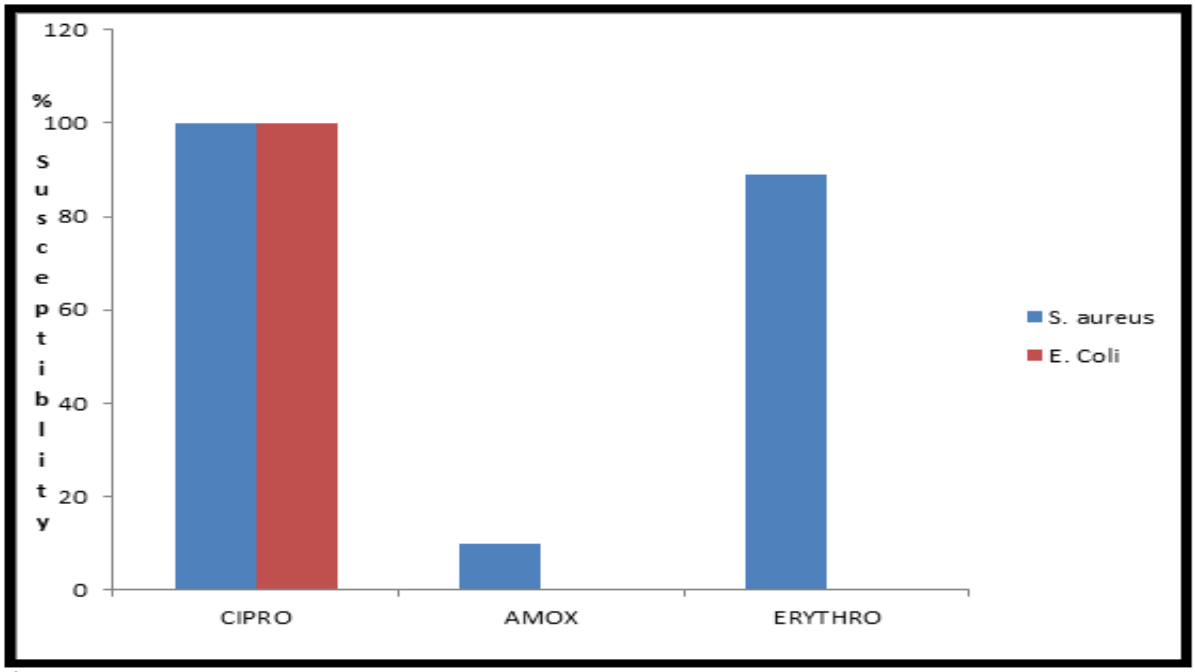

Fig. 2. Antimicrobial susceptibility pattern of *E. coli* and *S. aureus* against Ciprofloxacin , Amoxicillin and Erythromycin.

It is a fact that antibiotic resistance has been in existence for as long as antibiotics have been in use for treating infections. Nevertheless, the escalating frequency in the new millennium of infections due to the so-called 'ESKAPE' pathogens, (*Eneterococcus faecium*, *Acinetobacter baumannii, Staphylococcus aureus*, *Klebsiella* species,, *Pseudomonas aeruginosa*, and the extended spectrum β-lactamase-producing strains of *Escherichia coli* and *Enterobacter* species) together with the diminishing output of new antibiotics from pharmaceutical industries have increased the measure to preserve antibiotics.(Siritan *et al.*, 2016)

The encouraging findings in this study, was the low percentage resistance to ciprofloxacin. However, carefulness is required; the use of fluoroquinolones drugs must be restrictive and discriminative so as to prevent a rapid development of drug resistance. This study highlights the need for antimicrobial susceptibility pattern determination from time to time so that proper guidelines for hospital antibiotics policies can be developed. Thus, this recent study will be very useful for the Pharmacist and Physician for prescribing antimicrobial drug.

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