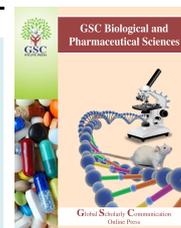


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(SHORT COMMUNICATION)



## Fluoroquinolones resistant Gram-positive cocci isolated from University of Calabar Teaching Hospital, Nigeria

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### Abstract

Eighty four clinical samples of Gram-positive cocci isolated from University of Calabar Teaching Hospital, Nigeria were examined for resistance to fluoroquinolones antibiotics using disc diffusion method on Muller Hinton agar. Forty four of the total isolates were *Staphylococcus* spp. while forty isolates were *Streptococcus* spp. Resistance rates were highest against ofloxacin 18 (41%) for *Staphylococcus* spp. and 10 (25%) for *Streptococcus* spp. followed by sparfloxacin 15 (34%) and 6 (15%) for *Staphylococcus* spp. and *Streptococcus* spp., respectively; while resistance to ciprofloxacin was the least 14 (32%) and 4 (10%) for *Staphylococcus* spp. and *Streptococcus* spp., respectively. These findings highlight the development of resistance by Gram-positive cocci to fluoroquinolones and suggest for the careful and appropriate administration of these agents as well as infection-control practices.

**Keywords:** Fluoroquinolones; Gram-positive cocci; Antibiotic resistance; *Staphylococcus*; *Streptococcus*

### 1. Introduction

The development of antibiotic resistance by bacteria is an evolutionary certainty, because of their ability to adapt to unfavorable ecological conditions. Fluoroquinolones are medications that kill bacteria or prevent their growth [1-2]. Resistance to fluoroquinolones among Gram-positive cocci has emerged as these antimicrobial agents have become extensively used in clinical medicine. Resistance is effected via changes in the bacterial target enzyme DNA gyrase and topoisomerase IV which reduce drug binding, and through movement of native bacterial membrane pumps to remove drug from the cell. In both cases, quinolones exposure selects for spontaneous mutants such as chromosomal mutations which alter the target protein or increase the level of pump expression [3-4].

When quinolones are used to treat infections caused by other bacterial pathogens, subject colonized with *staphylococcus aureus* (e.g., on their skin or mucosal surfaces) are likely exposed to sub-therapeutic antibiotic concentration and are therefore at risk becoming colonized with resistant mutants. These resident resistant strains then become the reservoir for future infections [4].

Antibiotic resistance in Gram-positive cocci is one of the major public health concerns for the beginning of the 21<sup>st</sup> century [2]. The pharmaceutical industry has responded with the development of oxalidinones, lipopeptides, injectables, streptomycins, ketolides, glycylicyclines, second generation glycopeptides and novel fluoroquinolones [5-6]. However, clinical use of these novel agents will cause new selective pressures and will continue to drive the development of resistance.

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## 2. Material and methods

### 2.1. Materials

The materials employed in this study were obtained from reputable scientific outlets (Difco Laboratories, Michigan) and were stored according to their manufacturer's instructions until required for used.

#### 2.1.1. Study site

This research was carried out in the Microbiology Research Laboratory, University of Calabar, Nigeria.

#### 2.1.2. Study samples

The samples employed in this study were *Staphylococcus* spp. and *Streptococcus* spp. isolated from different sources at the University of Calabar Teaching Hospital, Nigeria

#### 2.1.3. Culture media

The culture media employed were nutrient agar, blood agar, MacConkey agar, and Muller Hinton agar. They were all obtained in dehydrated powder form and stored at room temperature until required for use. All media were prepared according to the manufacturer's instruction [7].

### 2.2. Methods

Standard microbiological methods were employed in this study. The samples were cultured in different media. Biochemical tests were carried out for confirmation and disk diffusion technique was used in the sensitivity test according to specifications [8-10].

#### 2.2.1. Sample collection

The samples were aseptically collected from patients in University of Calabar Teaching Hospital from different sources. The samples were principally collected by the medical laboratory scientist from; skin, burns, wounds, throat washing, aspirates, sputum and blood and all samples were taken immediately to the laboratory for culturing following laid down procedures as described by Hooper [4].

#### 2.2.2. Isolation of Gram-positive cocci

The microorganisms were isolated from the clinical specimen onto solid media by streak plate method. The media employed were nutrient agar, blood agar and MacConkey agar [4, 11-12].

#### 2.2.3. Identification and confirmation of Gram-positive cocci

All microorganisms growing on the various media were characterized on the basis of gram stain reaction, microscopy, catalase and coagulase tests. The identification of each organism was based on the results of the tests. The test results were evaluated using characteristics presented in Bergey's Manual of Determinative Bacteriology [13].

#### 2.2.4. Preservation of isolates

The identified isolates of *Streptococcus* spp. and *Staphylococcus* spp. were preserved in nutrient Agar slant using bijou bottles. These bottles were properly labeled for easy identification. If not ready for use, they were stored in a refrigerator at 4 °C; since high temperature will cause profound proliferation of the isolates progeny, which was not necessary for the work [11-12].

#### 2.2.5. Fluoroquinolones sensitivity test

This test was carried out to determine the susceptibility and resistance of some fluoroquinolones (ciprofloxacin, sparfloxacin and ofloxacin) on gram-positive cocci (*Staphylococcus* spp. and *Streptococcus* spp.). In this work, a disk diffusion technique was employed [14].

The discs used in this research were laboratory prepared disc each with a concentration of 30 µg. Generally, three kinds of fluoroquinolones were used; ciprofloxacin (CPX) 500 mg, sparfloxacin (LM) 200 mg and ofloxacin (OFL) 200 mg. They were obtained at Karmel pharmacy, 90B Goldie street Calabar Nigeria. Each of these antibiotics was used to prepare a set of 200 sensitive discs.

Before preparation, the discs were separated and spread out singly in a foil paper and labeled respectively. The discs were divided into 6 and were placed into sterile bottles each containing 100 discs, which was sterilized in the oven at 160 °C for 1h and allowed to cool. The discs were then stored in sterile capped bottles at temperature of 4 °C. The dilution used for each antibiotic was dependent on the concentration of the antibiotics most suitable for the work. A control disc was used. A loopful of 2-5 discrete colonies of purified test microorganisms were inoculated into 5 ml sterile nutrient broth. The inoculated broth culture was incubated overnight and then diluted to have approximately  $10^5$  organism/ml.

The diluted broth was used to flood the entire surface of the sensitivity agar (Muller Hinton Agar) plates. Excess broth was removed by draining it aseptically into a beaker of disinfectant and the plates dried, using a sterile pair of forceps, appropriate antimicrobial discs were firmly placed onto the surface of the plate about 15 mm from the edge of the plate and 25 mm from disc to disc with each tightly pressed down to ensure contact with the agar. The plates were left at room temperature for about an hour to allow for diffusion of the antimicrobial agents. The plates were incubated overnight at 37 °C. After overnight incubation, the plates were examined for zones of inhibition. The zone diameters were measured using a transparent ruler in mm and compared with those of the control organism [5-6, 8-9, 15-17].

### 3. Results and discussion

The genus *Staphylococci* has at least 40 species. The three species used in this research were the most frequently encountered species of medical importance which are *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*. The Streptococci are a large and heterogeneous group of bacteria and no one system suffices to classify them. The species employed in this research were *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*. A total of 84 samples were collected from different sources at the University of Calabar Teaching Hospital, Nigeria. Out of the 84 samples collected, 44 were *Staphylococcus* spp. and 40 were *Streptococcus* spp.

This research has shown that fluoroquinolones are very active against Gram-positive cocci with ciprofloxacin 30 (68%) being the most active member on *Staphylococcus* spp. and sparfloxacin 29 (59%) is the second most active antibiotic in the group, while Ofloxacin 26 (59%) has limited activity on *Staphylococcus* spp. This susceptibility rate is low when compared with a research work carried out by Franklin [6] on antimicrobial resistance: the example of *Staphylococcus aureus* at the University of Chicago USA who employed non-clinical samples. The resistance pattern of Gram-positive cocci was observed in the study. *Staphylococcus* spp. were mostly resistant to ofloxacin 18 (41%) while ciprofloxacin 14 (32%) and sparfloxacin 15 (34%) were less resistant (Table 1). This finding is in agreement with reports of previous studies in this area by Blondeau [5] at the University of California, and Hooper [4] at the Oxford University College of Medicine.

**Table 1** Mean sensitivity of *Staphylococcus* species to fluoroquinolones

Antibiotics	Number of sensitive	Number of resistant
Ciprofloxacin	30 (60%)	14 (32%)
Sparfloxacin	29 (66%)	15 (34%)
Ofloxacin	26 (59%)	18 (41%)

The sensitivity test carried out on *Streptococcus* spp. reveals that the isolates were highly sensitive to fluoroquinolones: ciprofloxacin 36 (90%), sparfloxacin 34 (85%) and ofloxacin 30 (75%). Minimal resistance was observed among the isolates: ciprofloxacin 4 (10%), sparfloxacin 6 (15%) and ofloxacin 10 (25%). This supports the findings of Bisno [1] who reported that *Streptococcus* spp. is highly susceptible to fluoroquinolones.

**Table 2** Mean sensitivity of *Streptococcus* species to fluoroquinolones

Antibiotics	Number of sensitive	Number of resistant
Ciprofloxacin	36 (90%)	4 (10%)
Sparfloxacin	34 (85%)	6 (15%)
Ofloxacin	30 (75%)	10 (25%)

Table 3 reveals the cumulative sensitivity profile of Gram-positive cocci to fluoroquinolones. Significantly important is the low level of bacterial resistance associated with fluoroquinolones used (ciprofloxacin, sparfloxacin and ofloxacin) making it an essential treatment in critical situations. The resistance profile of some *Staphylococcus* spp. and *Streptococcus* spp. as observed in this study is in accordance with the findings of Lipsky who reported that since the emergence of penicillinase-producing *Staphylococcus aureus* in the 1940s, *Staphylococci*, *Enterococci* and *Streptococci* have proved themselves as multi drug resistance micro-organisms. [9]. The findings in this study of a high-level inhibition against Gram-positive cocci as observed in the cumulative susceptibility profile (Table 3) strictly conform to the work of Walker who reported that Gram-positive cocci may show a greater level of inhibition to fluoroquinolones [14].

**Table 3** Cumulative sensitivity profile of Gram-positive cocci to fluoroquinolones

Microorganisms	Number of sensitive	Number of resistant
<i>Staphylococcus</i> spp.	28 (64%)	16 (36%)
<i>Streptococcus</i> spp.	33 (83%)	7 (17%)

Mark et al. documented that fluoroquinolones exhibit a high level of antibacterial activity *in-vitro* and *in-vivo* and showed that although the newer agents offer a much broader spectrum of action, ciprofloxacin remains the most effective fluoroquinolones against Gram-positive and Gram-negative pathogens after researching on the prevalence of quinolones resistance among Gram-positive and Gram-negative pathogens at the University of Calabar Teaching Hospital [8]. The susceptibility and resistance profile shows that clinical isolates are more resistant to fluoroquinolones than non-clinical isolates for the reason being that clinical isolates are predisposed to several antimicrobial agents.

#### 4. Conclusion

This study enables us to evaluate the antimicrobial activity of these antibiotics on Gram-positive cocci. For the result obtained, fluoroquinolones (ciprofloxacin, sparfloxacin and ofloxacin) have high activity against Gram-positive cocci, while minimal resistance was observed. This study also reveals that ciprofloxacin is the most active fluoroquinolone against Gram-positive cocci followed by sparfloxacin, while ofloxacin has limited activity.

#### Compliance with ethical standards

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##### Disclosure of conflict of interest

Authors have declared that no competing interests exist.

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