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Ciprofloxacin Susceptibility Pattern in a Secondary Health Care Facility in Kebbi State, Nigeria

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***Abstract*:** Antibiotic resistance is a major challenge in management of infectious diseases globally, and particularly in developing countries. There are few studies that have analysed the impact of such abuse on the development of bacterial resistance in Nigeria and sub Saharan Africa. To this end, we retrospectively analysed ciprofloxacin susceptibility patterns in a secondary healthcare facility in Northwest Nigeria over a four year period. Three hundred and thirty six pathogens isolated from 370 patients were analysed in this study. The common pathogens isolated from wound infections were *Staphylococcus aureus* (29, 7.84%), *Pseudomonas* spp (10, 2.7%) and *Proteus* spp (7, 1.89%). In stool samples, *Proteus* (11, 2.97%), *Escherichia coli* (8, 2.2%) and *Salmonella* (6, 1.62%) were the most commonly isolated organisms respectively. While for urine samples, isolates were *S. aureus* (105, 28.37%) followed by *E. coli* from urine samples (62, 16.76%). During the study period, we observed there was a high degree of resistance to ciprofloxacin among *Proteus* spp (50%), *E. coli* (41.3%), *S. aureus* (20.6%), *Klebsiella* (20%) and *Pseudomonas* (20%). Government and stakeholders need to urgently develop antimicrobial stewardship programmes that will address the issue of antibiotic resistance in the country.

Key words: Fluoroquinolones, antibiotic resistance, ciprofloxacin, bacteria, Nigeria

Introduction

The use of antibiotics in clinical practice represents one of the tremendous achievements in the control of infectious diseases. However, the effectiveness of antibiotics has been reduced by an increasing threat of bacterial resistance. Antibiotic resistance (ABR) is the ability of bacteria to survive in antibiotic concentrations that would normally inhibit or kill the bacteria[1]. ABR has emerged as one of the greatest challenges for clinical microbiologists and healthcare practitioners worldwide today[2].Globally, deaths from antibiotic resistant bacteria are estimated to be around 700,000 annually, and projected to rise to 10 million deaths by 2050[3]. Fluoroquinolones are among the most frequently prescribed antimicrobial agents worldwide, since the expiry of the patent on this class of drugs. The European Antimicrobial Resistance Surveillance Network (EARS-Net report, indicates a significant increase in fluoroquinolone resistance across Europe since 2001, with levels ranging from 7% to 53% in 2007[4]. Fluoroquinolones, derivatives of quinolones are stable, orally administrable, broad spectrum agents used to treat a range of bacterial infections. A commonly prescribed fluoroquinolone in developing countries is ciprofloxacin which has broad spectrum activity in treatment of complicated and uncomplicated bacterial infectionsin several anatomical sites, such as respiratory tract infections, otitis media, sinusitis, eye infections, UTI and sepsis [5]. For close to two decades, fluoroquinolones were antibiotics of choice for acute respiratory, enteric and urinary tract infections as well as serious systemic infections such as bacteremia[6]. The frequent prescription of these drugs by healthcare providers, indiscriminate antibiotic use and substandard drugs in developing countries including Nigeria has contributed to the continued spread of ABR to fluoroquinolones[7].

Quinolone resistance in enterobacteria ceae is well documented, and is usually the result of chromosomal mutations leading to alterations in target enzymes or drug accumulation[8]. Single nucleotide polymorphisms in the quinolone resistance determining regions (QRDR) of *gyrA* and *parC*, two of the genes that encode DNA gyrase and topoisomerase IV respectively, can lead to conformational changes in these enzymes that prevents quinolones from binding but still preserve their enzymatic function[9,10]. Mutations in the QRDRs of *gyrA* and *parC* are the most commonly documented quinolone resistance mechanisms, but resistance is also known to be conferred by mutations in the second topoisomerase gene, *pare* [11]. Quinolone resistance can also be acquired horizontally through transferable *aac(6')-Ib* or quinolone resistance (*qnr*) DNA. AAC(6')-Ib encodes a ciprofloxacin acetylating enzyme, while the product of *qnr* inhibits quinolones binding to target proteins [12]. More recently, however, plasmid-mediated quinolone resistance has been reported in *K. pneumoniae* and *E. coli*, associated with acquisition of the *qnr* gene[8,10,13]. Other ABR mechanisms in bacteria include alteration of cell permeability, site of action of the antibiotic or the release of degrading enzymes[14,15]

In resource poor settings, poor infrastructure, lack of trained laboratory personnel and weak surveillance for ABR contributes to the spread of ABR bacteria. In Nigeria, as with most of SSA there are very few published reports on ABR to fluoroquinolones [6,16], with no published report from Northwest Nigeria. To this end, this study aimed to retrospectively analyseciprofloxacinsusceptibility patterns of commonly isolated bacteria in a secondary health care facility in Kebbi state, Northwest Nigeria.

Materials and Methods

Ethical approval: This study did not carry out any invasive procedures and patients' clinical information was not revealed to the researchers. Ethical approval for use of data was granted by the management of Aisha Muhammadu Buhari General Hospital, Jega, Kebbi State Nigeria.

Setting: Jega local government area (LGA) is one of the twenty-one LGAs in Kebbi state belonging to the Gwandu emirate. Jega LGA has a projected population based on 2006 census of 270,517, comprising mostly muslims, farmers and other low income earners. Jega has only one General hospital (AMBGH) and two private hospitals that provide basic health care services.

Aisha Muhammadu Buhari General Hospital (AMBGH): AMBGHhas an 80 bed capacity and serves a rural population from villages and settlements within Jega LGA. The hospital provides services in diagnosis, curative, promotive and rehabilitative services in medical treatment and an outpatient clinic. AMBGH is staffed by one physician supported by fifteen nurses and midwives, and a microbiology laboratory led by a qualified laboratory scientist. The facility has minimal laboratory facilitiesand can only carry out urine, stool, sputum culture, microscopy, biochemical, immunological tests, HIV screening and tuberculosis testing.

Sample collection: This was a retrospective study of the data on fluoroquinolone resistance patterns carried out in Jega local government area (LGA), Kebbi state, Northwest Nigeria, at the department of microbiology of (AMBGH), Jega, Kebbi State Nigeria. All microbiology reports for urine, stool, vaginal swabs and wound samples between June 2014 and September 2017 were included for analysis. For this study, we excluded data with incomplete sensitivity results, and all samples for microbiological analysis were referred to the laboratory by the physician.

Microbiological analyses: Patients referred to the microbiology laboratory by the physician were provided with labelled specimen bottles and trained on how and when to provide samples for laboratory diagnosis. All samples brought to the laboratory were immediately processed following standard bacteria culture procedures. Biochemical characterization of isolates was carried out under the strict supervision of a medical laboratory scientist licensed by the Medical and Laboratory Science Council of Nigeria (MLSCN). Antibiotic susceptibility testing was carried out following the Clinical Laboratory Standards 38
(CLSI) guidelines [27]. In thi
we report the rates of susceptibility and resistant isolates. AMBGH microbiology laboratory is regularly subjected to external audits by the

MLSCN to assess the state of the facility for providing routine diagnostic services.

Statistics: Data were accumulated and analysed with Microsoft excel and Stata version 12 software (College Station, Texas, USA) to generate differential descriptive statistics including frequency tables thatshowed the frequency and percentage distribution of isolates.

Results

Demographics

In total three hundred and seventy patients were sampled at the microbiology laboratory of AMBGH between June 2014 and September 2017. For the study period reviewed, the ratio of male to female sampled was 0.47:1.0, and the median age of the children was 10 yrs (IQR, 15 yrs) and that of adults was 35 yrs (IQR, 21 yrs) respectively (Table 1).

Bacteria spectrum

In total 336 isolates were included within the study period reviewed. There were no significant differences (P=0.09) in the isolates collected among children or adults (Table 2). The most frequent isolate was *Staphylococcus aureus* from urine samples (105, 28.37%) followed by *Escherichia coli* from urine samples (62, 16.76%). From wound infections the most frequent isolate was *S. aureus* (29, 7.84%), followed by *Pseudomonas* spp (10, 2.7%) and *Proteus* spp (7, 1.89%). In stool samples, *Proteus* (11, 2.97%), *E. coli* (8, 2.2%) and *Salmonella* (6, 1.62%) were the most commonly isolated organisms respectively (Table 3). Other samples such as sputum and high vaginal swabs revealed *S. aureus* to be the main pathogenic organism isolated in culture.

Antibiotic susceptibility

Next we assessed the proportion of isolates that were not susceptible to ciprofloxacin from antibiotic sensitivity tests carried out using the disk diffusion method. During the study period, we observed there was a high degree of resistance to ciprofloxacin among *Proteus*spp (50%), *E. coli* (41.3%), *S. aureus* (20.6%), *Klebsiella* (20%) and *Pseudomonas* (20%) respectively. Susceptibility to ciprofloxacin by the isolates was highest in *Salmonella* (71.4%), *S. aureus* (65.2%), *Klebsiella* (60%), *Pseudomonas* (60%), *E. coli* (41.3%), and *Proteus* (40%) respectively. Other isolates within the study period reviewed demonstrated intermediate level susceptibility (Table 4).

Discussion

In developing countries, antibiotics use without prescription due to their availability over the counter appears to be the norm, in addition to sub-optimal health care systems that lack the capacity to provide timely laboratory results for clinicians to make decisions with. These issues and others promote the rapid increase in the spread of antibiotics resistant bacteria in resource poor settings. Here we review four year microbiological data from a secondary health care facility in a rural area in Northwest Nigeria. This was carried out in order to provide valuable laboratory information for clinicians and health care practitioners on the pattern of ciprofloxacin susceptibility, in the absence of routine antibiotic resistance surveillance activities. Our data suggests that the most commonly isolated pathogen from samples and

swabs analysed was *S. aureus*, followed by *E. coli*. Our findings also revealed a high degree of ciprofloxacin resistance in the isolates, with *Proteus* spp demonstrating most resistance (50%), followed by *E. coli*(41.3%). In a five year retrospective study carried out in Bahia, Brazil a high degree of ciprofloxacin resistance was observed in community acquired urinary tract infection isolates from the participants. Ciprofloxacin resistance was highest in *E. coli* isolates, with up to 36% of the isolates in a particular year being resistant[17]. In a retrospective study in Gabon, ciprofloxacin resistance of 24.1% was also reported in *E. coli*[18]. Quinolone resistance has also been reported in Cameroon from S. Typhi and Ghana in*Vibrio cholerae* respectively [19,20]. Studies on fluoroquinolones resistance in Africa, including Nigeria are very scarce. The few published papers on fluoroquinolones resistance in Nigeria clearly suggest the circulation of ciprofloxacin resistant bacteria in the country[16,21–25]. In a study carried out in Minna, north central Nigeria, the authors found that *Salmonella enterica* serovar Typhi were resistant to commonly prescribed antibiotics ceftriaxone, cefuroxime, amoxicillin, ampicillin, ciprofloxacin, and augmentin[26]. The increase in treatment failures to β-lactams and sulfamethoxazole+trimethoprim over the past years led to the use of fluoroquinolones as the first choice, hence putting pressure on the use of this drug as first line therapy for most bacterial infections.

The review of microbiological data from the secondary health care facility in this study reveals an alarming trend of ciprofloxacin resistance in the population. Our study also provides local ABR data which could be used by clinicians in the region to guide clinical decisions. Ciprofloxacin, a broad spectrum antibiotic is widely used in management of infections in developing countries. Our study suggests the need to establish surveillance for ABR in Nigeria and Africa in general, replicating the approach of the European Antimicrobial Resistance Surveillance Network (http://ecdc.europa.eu/en/activities/surveillance/ EARS-Net/Pages/index.aspx), which monitors antibiotic consumption in comparison to spread of resistance. In developing countries, the challenge of managing the spread of ABR is enormous, owing to lack of good sanitation practices, lack of potable water, poor hygiene, high poverty levels, illiteracy and sub-optimal healthcare systems. Hence, there is a need for African governments to have short term goals that will produce significant results in dealing with the menace of ABR.

This study has some limitations, first is the inability of the health facility considered in this study to carry out blood culture to isolate pathogens in the blood of patients. Lack of blood culture facility is one of the major challenges facing diagnostic laboratories in Africa, and even when present, the approach used might be sub-optimal or lack the required sensitivity. Inability to carry out blood culture in this facility has potentially excluded bacteria that might be important in our understanding of the ciprofloxacin resistant bacteria circulating in the population. Second, incessant power cut offs also made it impossible for the facility to preserve

multidrug resistant isolates for molecular studies. Hence the underlying molecular mechanisms of resistance in the isolates are unclear. Third, the isolates described here do not distinguish between community or hospital acquired infections based on the records made available by the health facility. Lastly, it is possible that the number of isolates reported in this study might be below the true number of isolates actually reported due to poor record keeping which is common to most African hospitals, but that might not be the case in our study.

To conclude, it is very likely that fluoroquinolones resistance emerged with increase in their indiscriminate use [6], hence there is an urgent need for establishing an antimicrobial stewardship programme that will address antibiotic use in humans and animals. This programme should be designed to complement improving the quality of laboratories, and establishing a national surveillance programme to detect, report and monitor multidrug resistant bacteria strains.

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Table 1. Demographics of patients

	Children (≤ 18 yrs)	Adult (> 18 yrs)
	54	316
Median age (IQR)	10	35
	Male	Female
Sex, n (%)	119 (32.2%)	251 (67.8%)

Table 2. Distribution of isolates from children and adults

Isolates	*Proteus spp*	*Klebsiella*	*Pseudomonas*	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella*
Children (≤18 yrs)	7	-	3	28	14	2
Adults (>18 yrs)	23	10	7	176	61	5
Total	30	10	10	204	75	7

Table 3. Source of isolates from patients' samples

Samples	*Proteus spp*	*Klebsiella*	*Pseudomonas*	*Staphylococcus*	*Escherichia coli*	*Salmonella*
Wound infections	7	3	10	29	-	-
Urine infections	21	7	-	105	62	1
High vaginal swab	-	-	-	20	4	-
Sputum	-	-	-	50	1	-
Stool	11	-	-	-	8	6
Total	30	10	10	204	75	7

Table 4. Susceptibility pattern of bacterial isolates to ciprofloxacin

Samples	*Proteus spp*	*Klebsiella*	*Pseudomonas*	*Staphylococcus*	*Escherichia coli*	*Salmonella*
Susceptible	12 (40%)	6 (60%)	6 (60%)	133 (65.2)	31 (41.3%)	5 (71.4%)
Intermediate	3 (10%)	2 (20%)	2 (20%)	29 (14.2%)	13 (17.4%)	1 (14.3%)
Resistant	15 (50%)	2 (20%)	2 (20%)	42 (20.6%)	31 (41.3%)	1 (14.3%)
Total	30 (100%)	10 (100%)	10 (100%)	204 (100%)	75 (100%)	7 (100%)