ANTIBIOTICS ACTIVITIES AGAINST BACTERIAL ISOLATES IN SPUTUM SPECIMENS OBTAINED FROM SELECTED PATIENTS WITH RESPIRATORY TRACT INFECTIONS IN MAIDUGURI METROPOLIS, BORNO STATE, NIGERIA

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ABSTRACT

Many respiratory diseases and infections are often associated with sputum production. The knowledge of the role of pathogenic bacteria in sputum specimens and their susceptibility trends are essential in designing a comprehensive treatment plan for the desired clinical outcomes to be achieved. Most patients with these conditions often initiate self medication with antibiotic therapy at sub-optimal levels prior to seeking medical interventions, a practice that has encouraged bacteria resistance. A retrospective study to assess pathogenic bacteria in clinical sputum specimens of patients with chronic cough, respiratory diseases and infections attending clinics at the university of Maiduguri Teaching hospital was conducted. A total of 326 pathogenic bacteria were isolated from patients between March 2006 and November 2007. *Alpha-haemolytic streptococci* the predominant organisms accounted for 59% followed by *Klebsiella spp* (26%). *Proteus spp* was the least isolated agent being (2%). *Pseudomonas and Escherichia coli* each accounted for 3% but *α-haemolytic Staphylococcus* amounted to about 5% of total bacterial isolates while about 2.5% cases were recorded with *Staphylococcus aureus.* Their susceptibility results vary with the tested antibacterial agents but *α-haemolytic streptococci* and *proteus spp* indicated total resistance to ampicillin, co-trimoxazole and nalidixic acid. Partial resistance of *α-haemolytic streptococci, Pseudomonas spp and Escherichia coli* were recorded against all antibacterial agents. Low resistance trends to the fluoroquinolones were observed with most organisms except *α-haemolytic streptococci, Staphylococcus aureus and Klebsiella spp* which showed high resistance to norfloxacin. Our study indicated that resistance is widespread but the fluoroquinolones appeared to show higher activities against most pathogenic bacteria isolates in sputum specimens.

Keywords*: Antibacterial agents, Multi-drugs resistant, pathogens, Sensitivity and resistance, Sputum Specimens,*

INTRODUCTION

Diseases affecting the respiratory tract are common and the roles of bacterial infections in complicating respiratory diseases are widely acknowledged. Diseases like asthma, bronchitis, emphysema, chronic cough, pneumonia, cystic fibrosis are often associated with sputum production with likelihood of bacterial infections [1, 2, 3]. The symptoms of these diseases vary considerably and may include shortness of breath, fever, coughing, high fever, weakness and fatigue. The lower respiratory regions mainly the trachea, bronchi and pulmonary tissues are sterile area because of the efficient cleaning action of the ciliated epithelium lining the tract [4]. The mucoliary blanket lining the bronchi further swept upward any micro-organism reaching the lower respiratory tract where they are coughed, sneezed or swallowed [4]. The breakdown in cellular immunity may account for

reason for the colonization and the thriving of these opportunistic organisms at the lower respiratory tract regions. Any condition that leads to the damage of the epithelium such as bronchitis predisposes to infection [5, 6, 7]. The knowledge of offensive agents and their susceptibilities are important for the attainment of the desired therapeutic outcomes since they serve as guide to antibiotic choice [8]. It is a common practice for many patients to have used available antibacterial agents at sub-optimal doses prior to seeking medical attention. Antibiotics are also over-the-counter making them accessible to anyone. These practices have aided widespread of resistance pathogens over the years [9]. Even when on medical care, irrational drug use may further complicate resistance problems [10].

AIMS AND OBJECTIVES

To investigate bacteria pathogens in sputum specimens and determine their susceptibility to antibacterial agents

PROCEDURE/METHOD

Sputum specimens were obtained from patients who presented with respiratory tract infections or diseases particularly in those associated with productive cough. Only sputum specimens with nil or very low saliva contents were accepted for the study. The specimens were processed by performing Gram stain from the direct smear while pathogenic bacteria were isolated after culturing on a blood and chocolate agar incubated at 37^{o}C for 48 hours. Biochemical tests were further carried out to identify and characterise the isolates. Susceptibility tests were carried out by disc diffusion techniques with standard disc concentrations of 19 commonly used antibacterial agents in the zone. Multi-drugs resistant cases are defined as resistance to at least three different types of antibiotics. Chi square analysis was used to determine levels of significance difference between various agents.

RESULTS

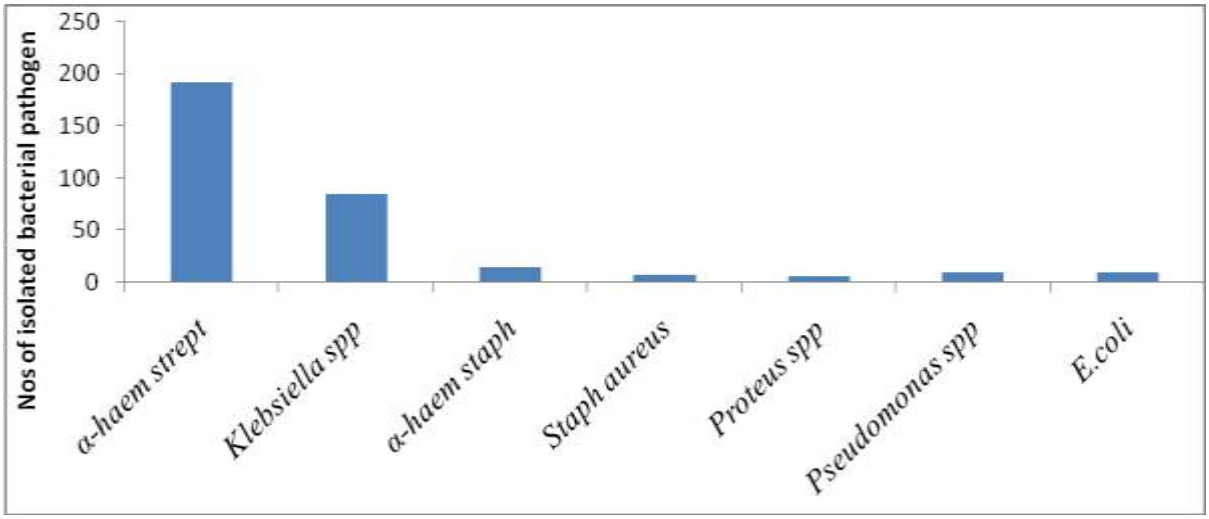

Figure 1: Distribution of bacterial isolates in sputum specimens

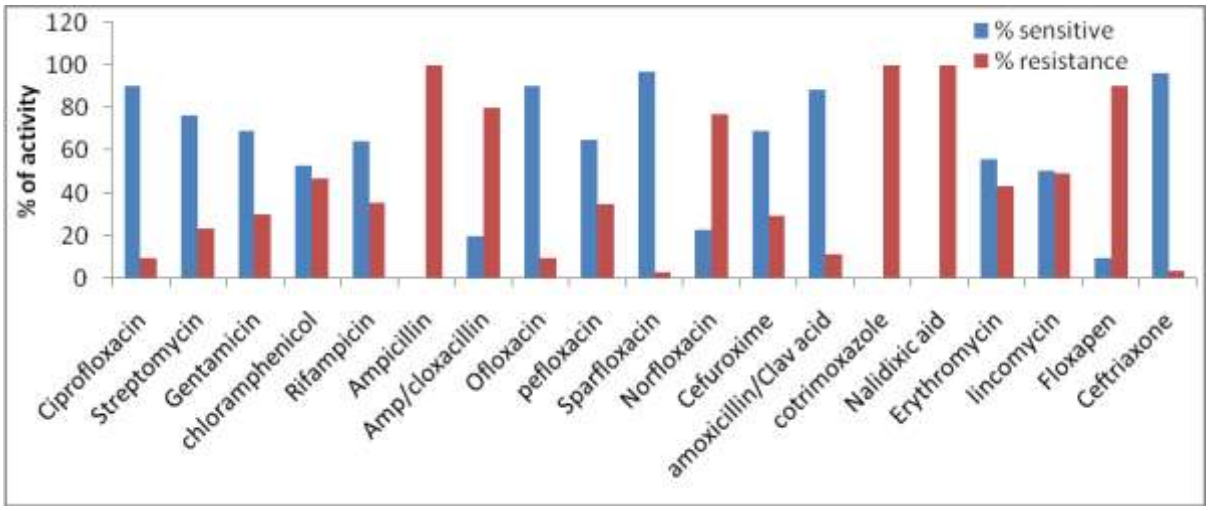

Figure 2: Percentage susceptibility of *α–haemolytic streptococci* to antibacterial agents

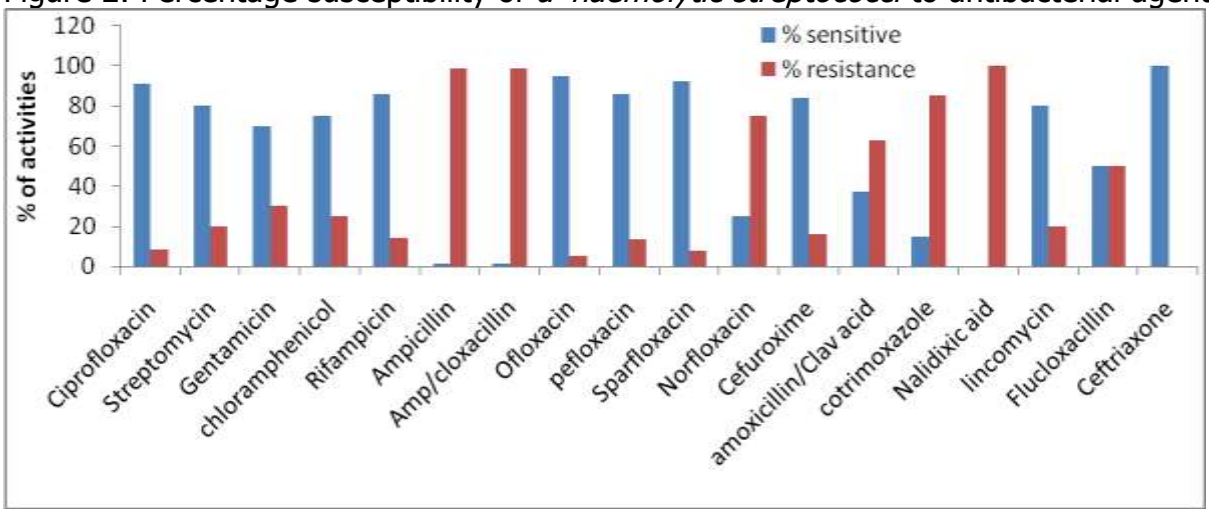

Figure 3: Percentage of susceptibility of *Klebsiella spp* to antibacterial agents

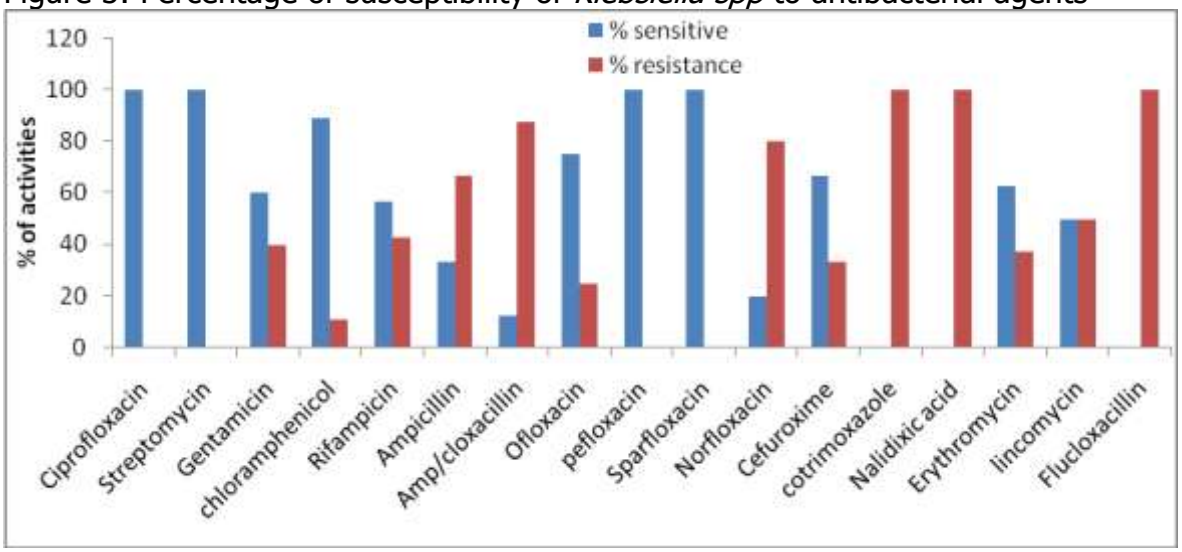

Figure 4: Percentage sensitivity of *α–haemolytic staphylococcus* to antibacterial agents

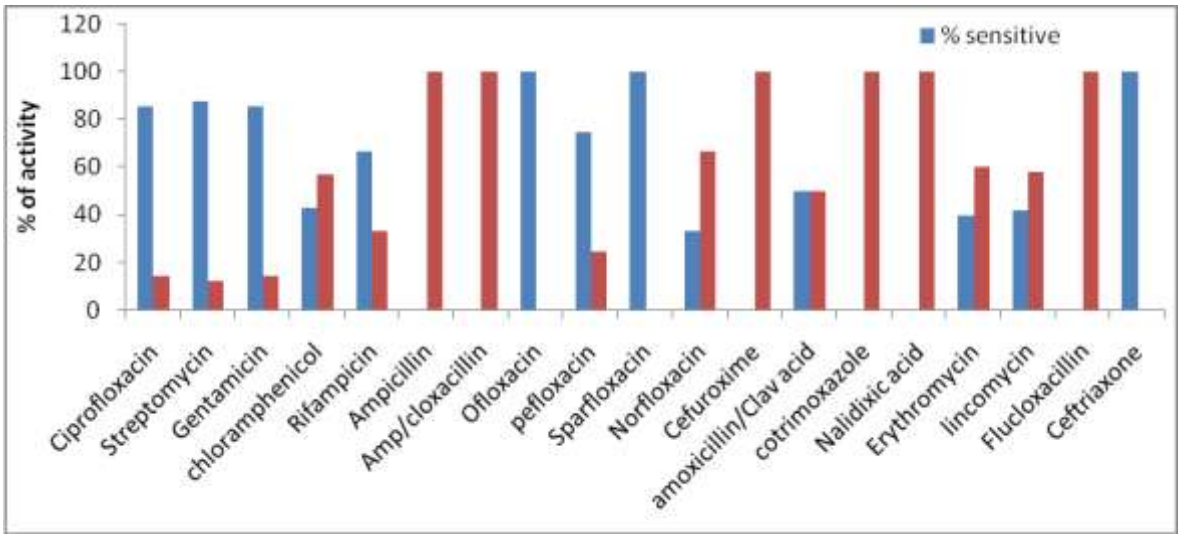

Figure 5: Percentage sensitivity of *Staphylococcus aureus* to antibacterial agents

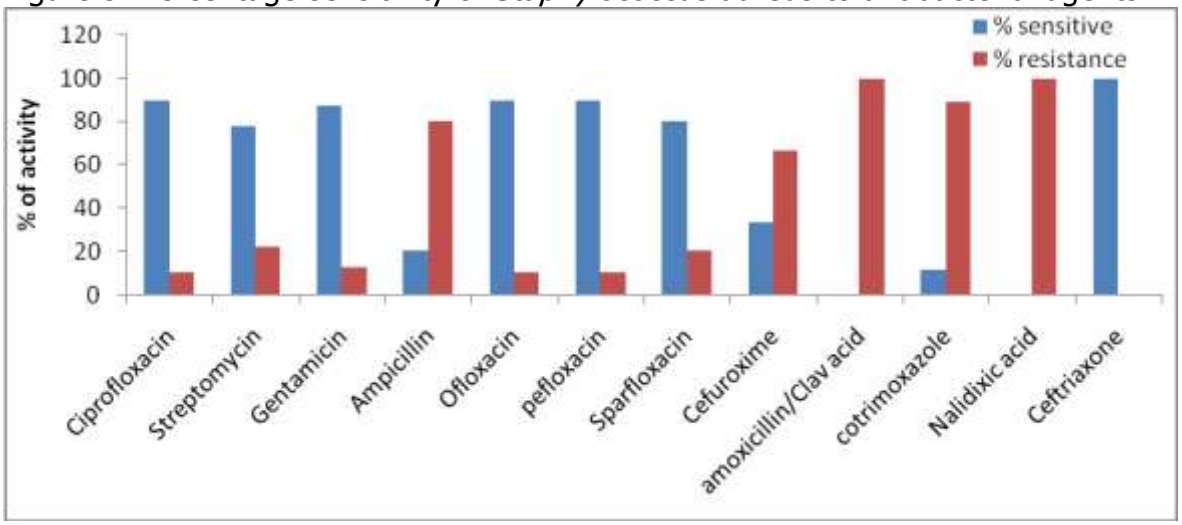

Figure 6: Percentage sensitivity of *Pseudomonas spp* to antibacterial agents

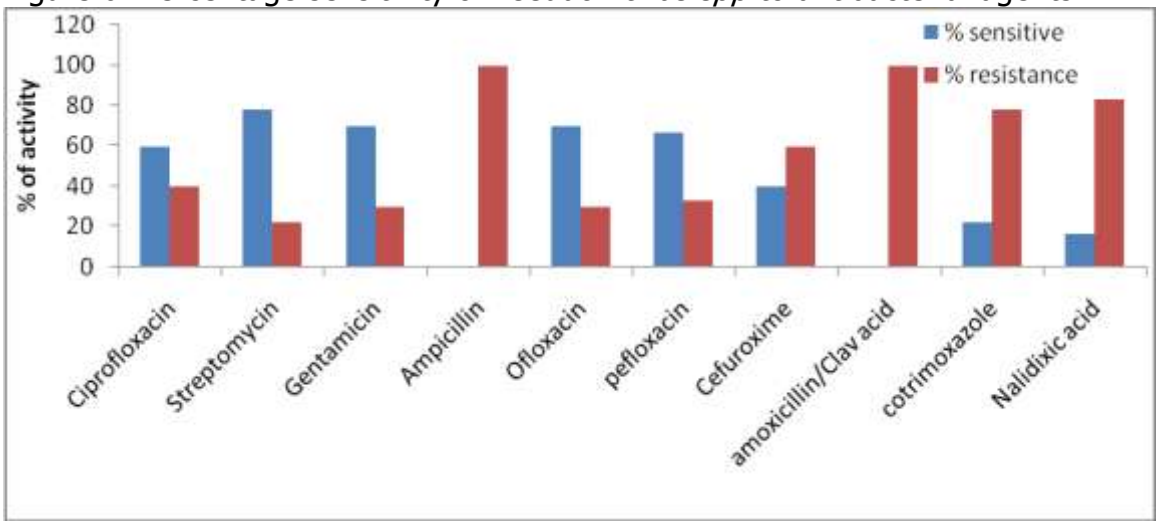

Figure 7: Percentage sensitivity of *Escherichia coli* to antibacterial agents

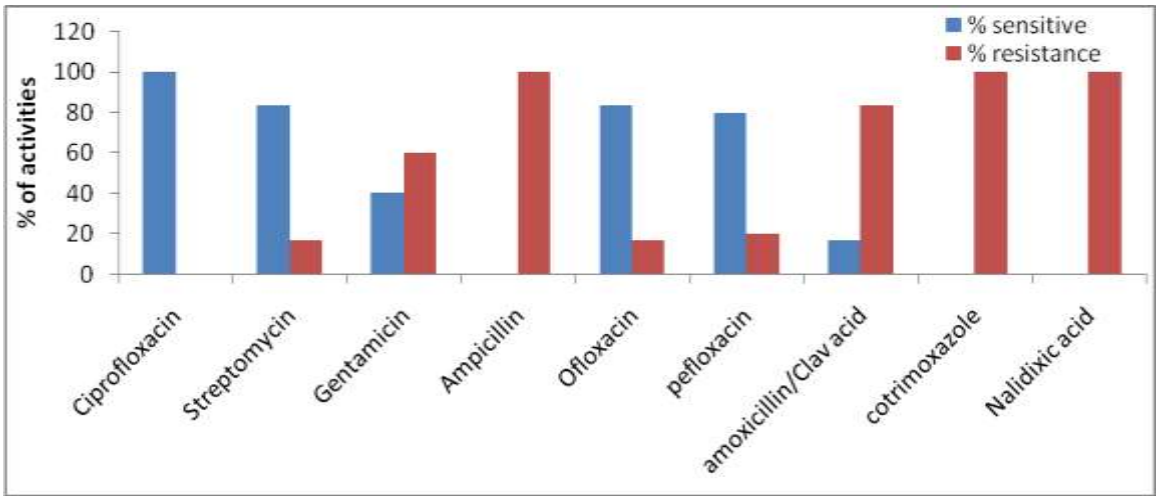

Figure 8: Percentage sensitivity of *Proteus spp* to antibacterial agents

Table 1: Multi-drugs resistant organisms and number of agents being resistant to

Isolated pathogen	Total isolated	Nos of antibiotics to which pathogens showed resistance											Total *MDR Bact.
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	
α-haem strept.	192	0	0	1	9	63	71	33	9	5	1	0	**191**
Klebsiella spp	85	0	1	0	5	19	35	14	5	5	0	1	**84**
Pseudom spp	10	0	0	0	1	1	4	2	2	0	0	0	**10**
E. coli	10	0	0	0	0	2	1	1	1	1	1	3	**10**
α-haem staph	15	0	0	0	1	6	6	1	0	0	0	0	**14**
Proteus spp	6	0	0	0	0	2	2	1	0	0	0	1	**6**
Staph aureus	8	0	0	0	0	3	0	4	0	0	1	0	**8**
Total					**16**	**96**	**119**	**56**	**17**	**11**	**3**	**5**	**323**

Key: MDR=Multidrug Resistance. *MDR is defined as resistance of pathogen to at least 3 antibiotics.

DISCUSSION

The distribution of bacteria isolated from all the sputum specimens is as shown in Figure 1. *Alpha haemolytic streptococci* was the predominant bacteria isolated being 61% but *Klebsiella* spp was the major coliform recorded being isolated in 85 sputum of patients. Other isolated pathogens include *Proteus* spp (2%) and *Escherichia coli* (3%). *Pseudomonas spp* was isolated from in 10 sputum specimens. These agents are consistent with bacteria incriminated in respiratory tract infection and diseases [11].

The susceptibilities results of tested anti-bacterial agents against various organisms are shown in Figure 2-8. The results of *α-haemolytic streptococci (Fig 2)* indicated partial resistance across the range of tested drugs. The activities of the fluoroquinolones like ciprofloxacin (90.3%), ofloxacin (90.5%), sparfloxacin (97.1%) were high except norfloxacin (23%) and pefloxacin (64.9%) which recorded lower activities against *α-haemolytic streptococci*. The recorded activities of fluoroquinolones are possibly attributed to their limited abuse in the past [12] compared with agents like ampicillin (0%), nalidixic acid (0%) and co-trimoxazole (0%) which recorded total resistance to the organism possibly due to their prior misuse in the past since they are readily available [9]. Trivial improvement were recorded in the synergistic effects of ampicillin/cloxacillin (20%) over ampicillin alone agent (0%) but high activities of amoxicillin/clavulanic acids (88.6%) were observed which suggest that acquired beta-lactamases is one of the major ways the organism may develop resistance against the penicillins. Among the cephalosporins, ceftriaxone demonstrated superior activities (100%) against *α-haemolytic streptococci* than cefuroxime (69.3%). Although 191 of the isolated 192 *α-haemolytic streptococci* were multi-drugs resistant strains, resistance to 5 antibiotics are the predominant cases (Table 1).

The sensitivity and resistance of *Klebsiella* spp to antibacterial agents is as shown in Figure 3. *Klebsiella* spp were not sensitive to nalidixic acid while norfloxacin failed to demonstrate high In-vitro sensitivity activities typical of other fluoroquinolones. Flucloxacillin showed sub-optimal activities (50%) but is still superior to other penicillins such as ampicillin (1.4%), ampicillin/cloxacillin (1.4%) and amoxicillin/clavulanic acid (37.4%). The low resistance of *Klebsiella* spp to other drugs like gentamicin (29.9%), streptomycin (19.7%), rifampicin (14.3%), chloramphenicol (25%), cefuroxime (16%) and lincomycin (20%) make these agents an effective alternative choice where the fluoroquinolones are contraindicated. However, multi-drugs resistant *Klebsiella* spp were observed in 84 out of 85 isolates with the highest proportion occurring with 5 different agents (Table 1). Multi-drugs resistant *Klebsiella spp* are similarly reported elsewhere in the world [13, 14].

The sensitivity and resistance of *α-haemolytic staphylococcus* to antibacterial agents are respectively shown in Figure 4. As observed with *α-haemolytic streptococci,* total resistance to nalidixic acid and co-trimoxazole are similarly indicated but streptomycin appears to show a better activities than gentamicin in their in-vitro activities against *α-haemolytic staphylococcus* (being 100% versus 60%). *α-haemolytic staphylococcus* demonstrated total sensitivity to all the fluoroquinolones except norfloxacin (20%) and ofloxacin (75%) . Although the activities of ciprofloxacin, pefloxacin and sparfloxacin were higher than ofloxacin, the difference was not found to be significant ($P>0.05$). The penicillins showed low activities with agents like flucloxacillin indicating total resistance. Multi-drug resistant strains are observed in 14 out of the 15 isolates.

The sensitivity and resistance patterns of *staphylococcus aureus* to antibacterial agents are respectively shown in Figures 5. *Staphylococcus aureus* is resistant to several anti-bacterial agents with total resistance occurring in over 31% of the tested agents while 21% of others showed activities that are below average. The β-lactam agents like ampicillin, ampicillin/cloxacillin, cefuroxime and flucloxacillin indicated no activities due to

the ability of the organism to produce enzymes that deactivate these drugs [15]. The inclusion of clavulanic acid with amoxicillin improved activity to 50% since clavulanic acid has inhibitory effects against the β-lactamases [16]. The activities of the aminoglycosides like gentamicin (85.7%) and streptomycin (87.5%) were equally high making them one of the most effective agents against Staph aureus in this study. All the isolates were sensitive to ceftriaxone but resistance to cefuroxime. However while its sensitivity varies with agents like chloramphenicol (42.3%), erythromycin (40%), and lincomycin (42%), all the fluoroquinolones except norfloxacin indicated good activities. All then *Staphylococcus aureus* isolates are multi-drugs resistant strains.

The sensitivity and resistance patterns of *Pseudomonas spp* to antibacterial agents are respectively shown in Figures 6. The organism indicated partial resistance to most agents except ceftriaxone and total resistance to nalidixic acid and amoxicillin/clavulanic acid. The activities of all the fluoroquinolones are uniform (90% each) except in sparfloxacin (80%) which is lower than gentamicin (87.5%). Among the cephalosporins, the activities of ceftriaxone (100%0 was superior than cefuroxime (33.3%) but high resistance were recorded with cotrimoxazole (88.9%).

The sensitivity and resistance patterns of *Escherichia coli* to antibacterial agents are respectively shown in Figures 7. The activities of the fluoroquinolones ranges from 60% in ciprofloxacin to 70% in ofloxacin while the aminoglycosides ranges from 70% in gentamicin to 77.8% in streptomycin. The organism recorded partial resistance to all agents except ampicillin and amoxicillin/clavulanic acid where total resistance were recorded. The low activities of co-trimoxazole (22.2%) and nalidixic acid (16.7%) when compared to streptomycin (77.8%) may be attributed to past misuse and variation in dosage forms since injectable antibiotics are less frequently misused than oral agents. All the E coli isolates are multi-drugs resistant strains.

The sensitivity and resistance patterns of *proteus spp* to antibacterial agents are respectively shown in Figures 8. As observed in *α-haemolytic streptococci* total resistance of Proteus spp to ampicillin, co-trimoxazole and nalidixic acid were similarly recorded. But the activities of the fluoroquinolones are high ranging from 80% to 100% while the aminoglycisides exhibited marked variation in their activities though no significant difference was indicated when the activity of streptomycin was compared with gentamicin. All the *proteus spp* isolates are multi-drugs resistant.

CONCLUSION

Pathogenic bacteria isolated in sputum varied and Resistance of isolates are widespread but the older generation antibacterial agents are the worst affected possibly because they are frequently available for misuse or abuse. The Fluoroquinolones showed partial resistance cases but to a lesser degree and have shown to be one of the most effective agents. The high incidence of multi-drugs resistant strains affecting all the pathogens though at a varying degrees calls for intense educative and campaign efforts so as to preserve our today's effective agents.

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