



## Bacterial isolates from swab specimens and their susceptibilities to antibacterial agents in Maiduguri Metropolitan City, Nigeria

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### ABSTRACT

The aim is to assess pathogenic bacteria from infectious swab sites and determine variation in their susceptibility and resistance to commonly utilized antibacterial agents as well as their multi-drug resistance patterns. 385 pathogenic bacteria from clinical swab specimens of ear, wounds, abscesses, eye discharges, diabetic foot ulcers, urethra and high vaginal swabs were isolated through agar culture and investigated for susceptibilities status. *Staphylococcus aureus* isolates accounted for 50.6%. Pathogens like *Escherichia coli* (17.4%), *Klebsiella spp* (11.4%), *Pseudomonas spp* (9.9%), *Proteus spp* (9.4%) and *Alpha-haemolytic streptococci* (1.3%) were similarly isolated. *Pseudomonas spp*, *Klebsiella spp* and *E. coli* showed total resistance to nalidixic acid but *Staphylococcus aureus* recorded partial resistance with all antibacterial agents. The activities of ciprofloxacin against *Pseudomonas spp* was better than other fluoroquinolones such that significant difference exists ( $P < 0.05$ ) when compared with pefloxacin. The activities of gentamicin against *Klebsiella spp* appeared not superior to streptomycin. Ciprofloxacin and ofloxacin showed uniform activities against *Proteus spp*, which showed partial resistance to all agents except sparfloxacin. Multi-drugs resistances are high with all organisms. Many pathogens cause infections in swab sites. The knowledge of causative organisms and their sensitivities are important since multi-drug-resistant organisms are widespread, thus making empirical choice difficult.

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**Keywords:** Bacterial isolates, Swab Specimens, Susceptibilities, Antibacterial Agents, Maiduguri Metropolitan City.

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### INTRODUCTION

The exploitation of micro-organisms and their products by man for several beneficial purposes are well recognized (Jonkers et al., 2009; Wassenaar, 2009). For instance, they find applications in the field of food industries and are used for the control of insect pests, production of vaccines and

antibiotics, as recombinant DNA in biotechnology industries, construction and so on (Russell, 2004; Jonkers et al., 2009; Wassenaar, 2009). Despite these applications, micro-organisms particularly bacteria constitute menace to man since they are responsible for several diseases capable of terminating man's life (Collazos, 2002).

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We have always been faced with the challenge to eradicate the pathogenic ones with antimicrobial agents since several adaptive measures are developed by microbes to evade the effects of antimicrobial agents (McManus, 1997; Anthony et al., 2002). They may cause resistance by deactivating the chemical agents and interference with nucleic acid synthesis, binding to ribosomes, inhibition of cell wall synthesis and folate metabolism, mutation and acquisition of new DNA by bacteria, alteration of drug target sites, bypassing drug metabolism and so on (McManus, 1997). Despite several improvements to sustain antimicrobial activities against pathogenic bacteria, their resistance to multiple drugs still thrive and constitute major problem in health care system (Karen, 2004), which have always justify the need for the search for new, effective and safe agents in order to resolve resistance problems.

The clinical outcomes of these effects are treatment failures, increased hospitalization and stay on health care facilities, and economic loss in terms of wages (Patrick et al., 2010). All these are putting the survival of human race at great risk. This work is the assessment of sensitivity and resistant status of bacteria isolated from swab specimens to antibacterial agents and is intended to serve useful information to clinicians and health personnel in the studied zone.

## MATERIALS AND METHODS

Clinical swab specimens consisting of 150 males and 235 females obtained from the ears, wounds, eye discharges, diabetic foot ulcers, urethra and high vaginal swabs were investigated for pathogenic bacteria in 2006 at Maiduguri Metropolitan City. The specimens were processed by performing Gram stain from the direct smear. Nutrient Agar and Macconkey Agar media were used to culture and isolate pathogens after inoculation and incubation at 37 °C for 48 hours. Conventional biochemical tests were used to

confirm bacterial isolates. Susceptibility tests were performed using the disc diffusion techniques and tested against the disc concentrations of ciprofloxacin (30 µg), streptomycin (30 µg), pefloxacin (10 µg), co-trimoxazole (30 µg), ampicillin (30 µg), ofloxacin (10 µg), gentamicin (10 µg), nalidixic acid (30 µg), amoxicillin/clavulanic acid (30 µg), rifampicin (10 µg), chloramphenicol (20 µg), erythromycin (30 µg), norfloxacin (30 µg) and ampicillin/cloxacillin (30 µg). Multi-drug-resistant cases are defined as resistance of isolated pathogens to antibiotics from at least three different classes.

## Statistical analysis

Chi Square Tests were used to determine levels of significant differences between the activities of two or more agents.

## RESULTS AND DISCUSSION

Assessing pathogenic bacteria from infectious sites is made possible through swab specimen collections since the procedure is simple with no associated health or safety risks (Higgins, 2008). The summary of various pathogenic bacteria isolated from swab specimens is as shown in Table 1. *Staphylococcus aureus* was the predominant pathogen accounting for 50.6% of the total bacterial isolates since the pathogen is ubiquitous and widely implicated in varieties of conditions including urinary tract infections (UTI) and skin and soft tissues infections (Nick, 2001) from where these swab specimens are obtained. This result is similar to the work of Adedeji et al. (2007) in south-west Nigeria, where *Staphylococcus aureus* was similarly reported as the major pathogen in urine and wound swabs, though *Pseudomonas spp* was the predominant isolates in their ear swabs. The slight difference is naturally due to geographical factors and variation in the study period.

The coliforms bacteria like *Escherichia coli* (17.4%), *Klebsiella spp* (11.4%), and *Proteus spp* (9.4%) were similarly isolated

particularly from the ear and high vagina swab specimens of patients who presented with otitis media and urinary tract infections, respectively but *Alpha-haemolytic streptococci* were the least isolated agents (1.3%) while *Pseudomonas spp* isolation was 9.9% (Table 1). These bacterial isolates are not in contrast with the works of other researchers (Brown and Izundu, 2004; Okon et al., 2004; Okesola and Makanjuola, 2009; Alsaimary et al., 2010) but their proportions vary due to geographical or environmental factors.

The gender distribution of isolated pathogens (Table 2) showed 39% bacterial isolates in male compared to 61% proportion in female. Gram positive bacteria accounted for 37.5% and 62.5% pathogens in male and female specimens respectively. Among the Gram negative isolates, *Escherichia coli*, *Klebsiella spp* and *Pseudomonas spp* were similarly higher in the female than the male. Only *Proteus spp* recorded higher incidence in male (63.9%) than female (36.1%). In all, 40.5% and 59.5% Gram negative pathogenic bacteria were isolated in male and female respectively. The incidence of Gram positive organisms was slightly higher than that of the Gram negative (51.9% versus 48.1%). Our results demonstrated that gender variations may contribute to the risk factors influencing infection in some swab specimens, particularly in those obtained from urogenital areas.

The *in vitro* sensitivity of *Staphylococcus aureus* indicated partial resistance to all the tested agents though at varying degrees (Figure 1). High resistance rates were recorded with the penicillins particularly ampicillin (83%), ampicillin/cloxacillin (81%) and flucloxacillin (84%). These resistance patterns are not surprising since they are easily aided through antibiotic misuse and because the organism produces enzymes that deactivate many penicillins (Herzberg and Moulton, 1987). In the UK, only 2% of *S. aureus* isolates are sensitive to penicillins (Wikipedia, 2010). Resistance of

*Staphylococcus aureus* was first reported in the early 1940's followed by rapid rise in resistance trends in many regions (Franklin, 2003). Cotrimoxazole and nalidixic acid similarly showed high resistance rates (being 87% and 92% respectively) possibly due to their past misuse or overuse (Goossens et al., 2005). The sensitivity result for cotrimoxazole is higher than reported in South-eastern Nigeria, but that of ampicillin is almost similar (Ikeagwu et al., 2008) while higher resistance rates are reported in other regions (Chigbu and Ezeronye, 2003). The empiric use of these agents should therefore be avoided. However, clavulanic acid in amoxicillin leading to the inhibition of the  $\beta$ -lactamase enzymatic activities of *Staphylococcus aureus* (Kar, 1996) made significant impact ( $X^2=13.77$ ,  $P<0.005$ ) in its sensitivity pattern over the other penicillins lacking such combination.

The activities of the fluoroquinolones against *Staphylococcus aureus* range between 88-97% except in norfloxacin whose low *in-vitro* activities (36%) indicated significant difference ( $X^2=201.28$ ,  $P<0.005$ ) when compared with the rest fluoroquinolones. Although these activities are higher than recorded in South-eastern Nigeria (Ikeagwu et al., 2008), it is still necessary to regulate their indiscriminate uses in order to sustain their relevance in chemotherapy. The activities of gentamicin (53%), lincomycin (55%) and erythromycin (52%) against *Staphylococcus aureus* were just slightly above the average. 177 (90.8%) cases of multi-drug-resistant *Staphylococcus aureus* were encountered during the study (Table 3). Multi-drug-resistant *Staphylococcus aureus* reported elsewhere in other regions (Voss and Doebbellling, 1995), but cases in this study zone are much higher than recorded in the United States (Styer et al., 2006). Poor compliance with antibiotic policy may account for the differences.

The *in-vitro* sensitivity and resistance of *Pseudomonas spp* to antibacterial agents is as shown in Figure 2. The total resistance

recorded with co-trimoxazole, nalidixic acid and cefuroxime is in contrast to similar study reported in Jamaica (Brown and Izundu, 2004) possibly because of past overuse of agents and poor control measures. It is also a common practice for patient to misuse several agents before seeking medical interventions from practitioners. However, high resistance rates were observed with all other agents including some fluoroquinolones with ciprofloxacin indicating high activities (97%) that were significantly different ( $X^2=15.68$ ,  $P<0.005$ ) from ofloxacin (70%) and pefloxacin (59%) as well as sparfloxacin ( $P<0.050$ ). These results are remarkably higher than previously reported for these drugs against this pathogen in the region (Okon et al., 2004) indicating that there is an improvement over their past irrational utilization, but sparfloxacin is no longer the most active agent against *Pseudomonas spp* in the region as previously reported (Okon et al., 2004) due to its increased usage. The aminoglycoside gentamicin and streptomycin appeared to have uniform activities (Figure 2) and have similarly shown improved activities than previously reported in the zone (Okon et al., 2004). The recorded higher activities over their previous status is not because of antibiotic usage policy but is attributable to increased use of newer agents, indicating that proper antibiotic usage policy can resolve the high rate of resistance problems in the region.

Multi-drug-resistant *Pseudomonas spp* (94.7%) are recorded in (Table 3), making it one of the highest in this study. *Pseudomonas spp* are similarly reported to show multi-drug-resistant cases elsewhere in the world (Marelee et al., 2005) since several mechanisms including those of acquired  $\beta$ -lactamase resistance,  $\beta$ -lactamase production, upregulation of efflux systems and decreased outer membrane permeability are reported as ways some *Pseudomonas spp* can cause multi-drug resistance (Livermore, 2002). With ciprofloxacin serving as one of the most frequently used empiric agents in the region,

our study has not found significant resistance risk associated with the agent against *Pseudomonas spp* isolated from swab sites.

The *in-vitro* antibacterial activities against *Proteus spp* isolates are as shown in Figure 3. The results indicated partial resistance to all the agents except sparfloxacin. The activities of the fluoroquinolones against *Proteus spp* range between 69% in pefloxacin to 100% in sparfloxacin. A significant difference exist ( $X^2=4.76$ ,  $P<0.05$ ) when the activities of sparfloxacin was compared with that of pefloxacin but uniform activities were recorded between ofloxacin and ciprofloxacin (91% each). Resistance rates were high with ampicillin (97%), co-trimoxazole (68%), amoxicillin/clavulanic acid (84%) and nalidixic acid (96%) against the pathogen; therefore, their empiric choices should be avoided. These values are in contrast to the resistance values for ampicillin (75%) and amoxicillin/clavulanic acid (40%) reported in Basrah, Iraq (Alsaimary et al., 2010) but ciprofloxacin activity in this study (91%) was higher than the 70% value reported for the same drug by Alsaimary et al. (2010).

The activities of ciprofloxacin (91%), ceftriaxone (75%) and amoxicillin/clavulanic acid (16%) are better than the 33%, 50% and 0% respectively recorded with these drugs in South-west Nigeria (Okesola and Makanjuola, 2009). All the isolated *Proteus spp* are multi-drug-resistant pathogens (Table 3) making it the highest in this study. Although multi-drug-resistant *Proteus spp* are similarly reported elsewhere in the world (Reslinski et al., 2005), the fluoroquinolones are still the best treatment option in the zone but these results underscore the need for urgent antibiotic utilization policy in order to maintain the current effective ones.

The *in vitro* antibacterial activities against *Escherichia coli* isolates are as shown in Figure 4. Partial resistance to all the agents were observed except nalidixic acid which recorded total resistance. The incidence is

however minimal with sparfloxacin (3%) making it the most active agent in this study. The activities of streptomycin (66%) and cefuroxime (67%) were almost uniform. However, low sensitivities of the organism to the penicillins were indicated, particularly in ampicillin (2%) and amoxicillin/clavulanic acid (14%) compare to 40% and 80% respectively reported by Alsaimary et al. (2010) in Iraq. The activities of ciprofloxacin (72%) and gentamicin (59%) are similarly slightly lower than 80% each reported for both agents (Alsaimary et al., 2010) but higher than reported by Okesola and Makanjuola (2009) in Western Nigeria for ciprofloxacin (62%), sparfloxacin (56.3%), ceftriaxone (62.3%) when compared with our study. Multi-drug resistant *Escherichia coli* are recorded in 91% cases (Table 3).

The *in-vitro* antibacterial activities against *Klebsiella spp* isolates are as shown in Figure 5. Partial resistance patterns similar to that of *E. coli* are observed with recorded high resistance rates with ampicillin (95%), cotrimoxazole (92%), amoxicillin/clavulanic acid (71%). However, while sparfloxacin (88%) and cefuroxime (57%) were observed to indicate higher activities against *E. coli* than in *Klebsiella spp*, the reverse is the case with ciprofloxacin, pefloxacin and gentamicin. The differences in activities of agents against these two pathogens are not

found to be significant but among the cephalosporins, the incidence of resistance by *Klebsiella spp* is lower in ceftriaxone (12.5%) compared with cefuroxime (43%). The recorded activities in this study are higher than those of Okesola and Makanjuola (2009) in western Nigeria. Multi-drug-resistant *Klebsiella spp* were recorded in 93.2% cases (Table 3).

The *in-vitro* antibacterial activities against  $\alpha$ -haemolytic streptococci isolates are as shown in Figure 6. The results indicated total resistance to ampicillin/cloxacillin, flucloxacillin and chloramphenicol while partial resistances were recorded with other agents except ciprofloxacin. Multi-drug-resistant  $\alpha$ -haemolytic streptococci were recorded in 80% incidences (Table 3).

### Conclusion

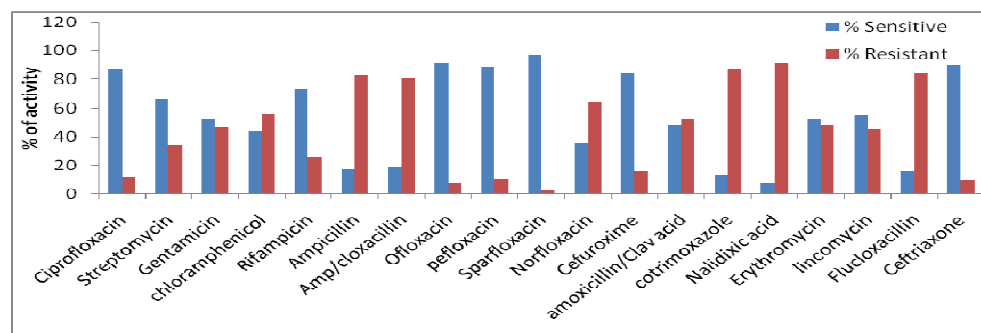
Many antibacterial agents particularly the older generation ones may have lost relevance in chemotherapy. Similarly, the high incidences of multi-drug-resistant isolated organisms occurring with several classes of antibacterial agents make their choices based on empirical consideration an uncertain venture. There is the urgent need to enhance efforts to conserve our current effective agents as well as to discover new efficacious antibiotics.

**Table 1:** Percentage distribution of isolated pathogens.

Isolated pathogens	Nos isolated	Percentage (%)
<i>Staphylococcus auerus</i>	195	50.6%
<i>Escherichia coli</i>	67	17.4%
<i>Klebsiella spp</i>	44	11.4%
<i>Pseudomonas spp</i>	38	9.9%
<i>Proteus spp</i>	36	9.4
$\alpha$ -haemolytic streptococci	5	1.3%
<b>TOTAL</b>	<b>385</b>	<b>100%</b>

**Table 2:** Gender distribution of pathogenic bacterial Isolates.

Observation	Male	Female	Total
<b>1. Gram positive pathogens</b>			
<i>Staphylococcus aureus</i>	72	123	195
<i>α-haemolytic streptococci</i>	3	2	5
Total	75 (37.5%)	125 (62.5%)	200 (100%)
<b>2. Gram negative pathogens</b>			
<i>Escherichia coli</i>	18	49	67
<i>Klebsiella spp</i>	18	26	44
<i>Pseudomonas spp</i>	16	22	38
<i>Proteus spp</i>	23	13	36
Total	75 (40.5%)	110 (59.5%)	185 (100%)
<b>Grand total</b>	<b>150 (39%)</b>	<b>235 (61%)</b>	<b>385 (100%)</b>

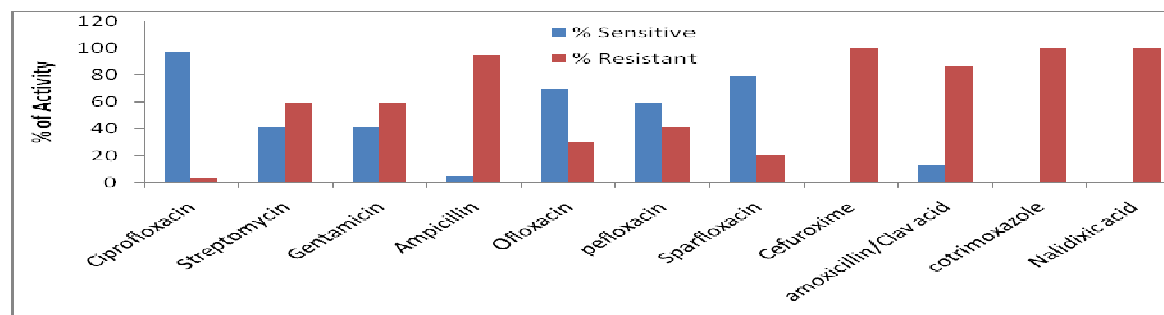


**Figure 1:** In-vitro antibacterial activities against *Staphylococcus aureus*.

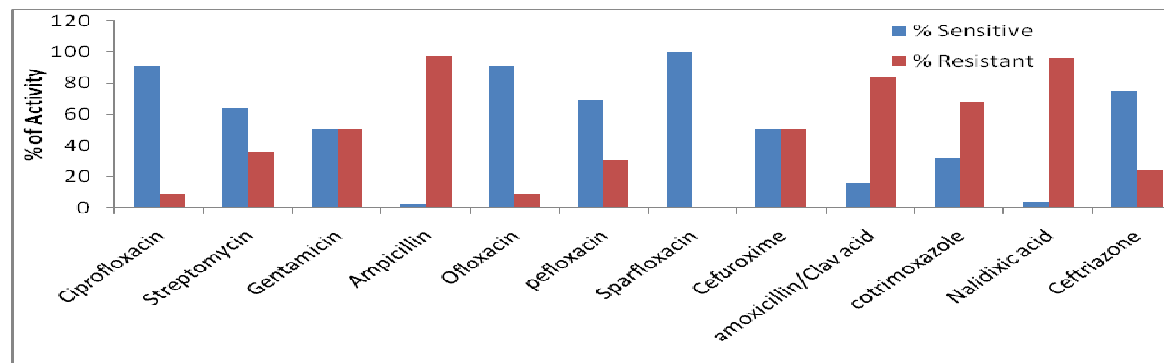
**Table 3:** Bacterial Multi-Drug Resistance Pattern\*.

Isolated pathogen	Total isolated	Nos of classes of antibiotics to which pathogens showed resistance (Multi-drug resistance (MDR) patterns)							Total MDR Bact.	% Multi-drug resistance
		1	2	3	4	5	6	7		
<i>Staph. aureus</i>	195	0	18	50	76	35	12	4	<b>177</b>	90.8%
<i>Pseudomonas spp</i>	38	0	2	8	14	12	2	0	<b>36</b>	94.7%
<i>E. coli</i>	67	0	5	16	28	17	0	0	<b>61</b>	91%
<i>Klebsiella spp</i>	44	0	3	7	24	7	3	0	<b>41</b>	93.2%
<i>Proteus spp</i>	36	0	0	11	17	8	0	0	<b>36</b>	100%
<i>α-haem strepto cocci</i>	5	0	1	1	3	0	0	0	<b>4</b>	80%
<b>Total</b>	<b>385</b>	<b>NA</b>	<b>NA</b>	<b>93</b>	<b>162</b>	<b>79</b>	<b>17</b>	<b>4</b>	<b>355</b>	<b>92.2%</b>
%				24.2%	42.1%	20.5%	4.4%	1%	92.2%	

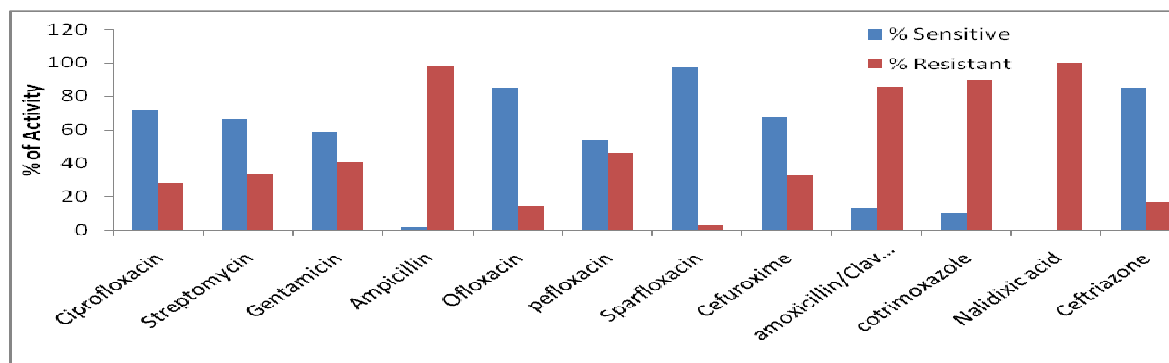
\*Resistance to pathogen is defined as bacteria resistance to antibiotics from at least 3 different classes.



**Figure 2:** In-vitro antibacterial activities against *Pseudomonas spp.*

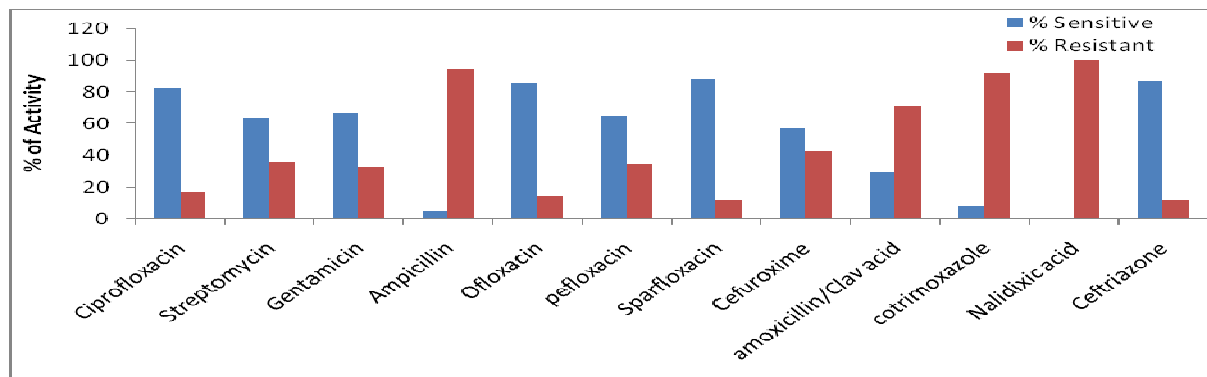


**Figure 3:** *In-vitro* antibacterial activities against *Proteus spp.*

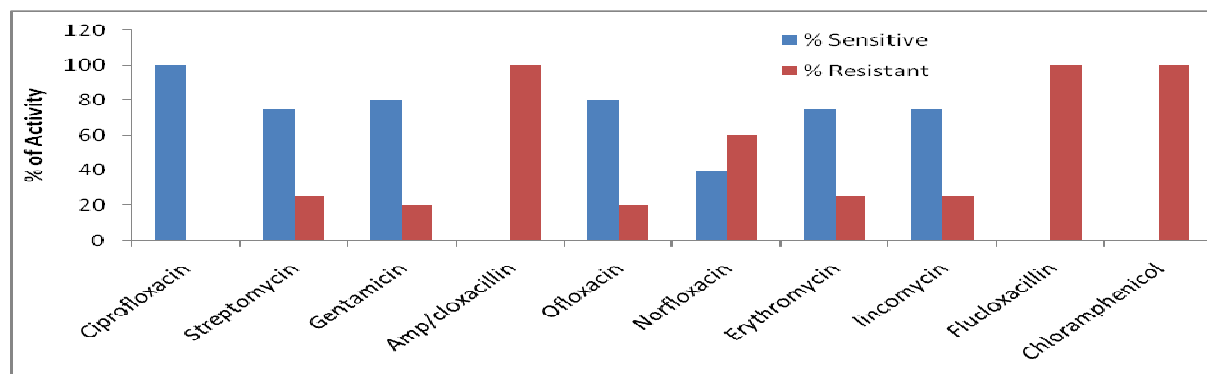


**Figure 4:** *In-vitro* antibacterial activities against *Escherichia coli.*





**Figure 5:** *In-vitro* antibacterial activities against *Klebsiella* spp.



**Figure 6:** *In-vitro* antibacterial activities against  $\alpha$ -haemolytic streptococci.

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