



Full Length Research Paper

# Antibiotics resistance profile of bacterial isolates from surgical site and hospital environment in a University teaching hospital in Nigeria

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Abstract

Antibiotics resistance profile of bacterial isolates from surgical site and hospital environment in a University teaching hospital in Nigeria was carried out. Agar disc diffusion and Broth dilution methods were respectively used for the determination of susceptibility and Minimum Inhibitory Concentration of antibiotics on the isolates. Presence of  $\beta$ -lactamase in the isolates was determined by iodometric method, while Acridine orange was used for curing isolates with r-plasmids. The isolated bacterial species from surgical sites and the hospital environments were *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Streptococcus spp.*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterobacter spp.*, *Proteus mirabilis*, *Proteus vulgaris*, *Citrobacter freundii*, *Serratia marscesens*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Bacillus megaterium* and *Bacillus subtilis*. The antibiotics used include the following; ampicillin, penicillin, cloxacillin, chloramphenicol, streptomycin, azithromycin, erythromycin, ofloxacin, sparfloxacin, ciprofloxacin, oxytetracycline, tetracycline, doxycycline. The result revealed that all isolates showed multiple resistances to all antibiotics used. Multiple Antibiotic Resistance (MAR) index of isolates ranged from 0.6-0.8. out of 147 isolates tested for presence of  $\beta$ -lactamase, 94(64%) possessed the  $\beta$ -lactamases, and 90 out of 147 (61.2%) also harboured r-plasmids, implying that some of the  $\beta$ -lactamase enzymes produced are most likely plasmid mediated. It was concluded that presence of  $\beta$ -lactamase and r-plasmid are probably responsible for the observed multiple resistance.

**Keywords:** Resistant isolates, resistant nosocomial bacteria, nosocomial bacterial pathogens and antibiotics, antibiotics resistant pathogens, hospital acquired resistant bacteria.

## INTRODUCTION

Data on the overall patterns of antibiotic use in hospitals have frequently appeared in the literature in the last several decades. The general thrust of such data indicates that from 25% to 40% of hospitalized patients receive systemic antibiotics at any given time (Pallares

and Wenzel et al., 1993). Furthermore, a sequentially obtained data suggests that there is a trend toward increasing, rather than decreasing, antibiotic use in hospitals. Continuous and sharp increases in the use of extended-spectrum cephalosporin, vancomycin, metronidazole, and amphotericin B. have been observed (Pallares et al., 1993). Such massive use of antibacterial drugs in hospitals, whether appropriate or inappropriate, has profound effects on both the hosts

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who receive these drugs and the bacteria exposed to them.

The global antibiotic market was valued at over 20 billion dollars in 1994 (Fernandez, 1996), and this could well double in the years ahead. There is enormous pressure on the part of the pharmaceutical industry to increase antibiotic use, both to recoup the cost of new drug development, and to accomplish the very legitimate goal of making profit, and these have encouraged irrational use of antibiotics which in turn favoured development of resistance. Every major class of bacterial pathogens has so far demonstrated an ability to develop resistance to one or more commonly used antimicrobial agents (Fin, 1978; Theodore, 1998).

In the mid 1940s, shortly after the introduction of Penicillin G, it was recognized that certain strains of Staphylococci elaborated a potent  $\beta$ -lactamase, an enzymatic inactivator of penicillin, and that Penicillin G had no therapeutic activity in patients with infections caused by such staphylococci. This recognition came as a major disappointment but not a total surprise to the scientific and medical community. Since that time, it has become abundantly clear that the major nosocomial pathogens are either naturally resistant to clinically useful antimicrobial drugs or possess the ability to acquire resistance. The best known examples are the staphylococci and aerobic gram-negative bacilli, which together regularly account for most nosocomial infections (Theodore, 1998).

Origin of antibiotics resistance have been grouped into genetic and nongenetics sources. Genetic sources involve; chromosomal mutation which gives rise to change in structural receptor in the pathogen thereby making it difficult for the drug to attach, and possession of extra chromosomal elements called plasmids some of which carries resistance genes (Jawetx *et al.*, 2004). Some of these plasmids mediate for production of  $\beta$ -lactamases which are responsible for resistance in most of  $\beta$ -lactam antibiotics as well as cross resistance in other classes of antibiotics.

The occurrence of nosocomial infection due to multidrug-resistance organisms is governed by a number of factors, including antibiotic selection pressure, the nature of the resistance determinant, whether the plasmid is conjugative or non-conjugative or is a transposon, and possible linkage with other antibiotic resistance and genetic determinant governing adhesion and pathogenicity (Davies, 1983). Although it is generally true that serious infections, such as bacteremic infection caused by drug resistant bacteria results in higher mortality than would be true of comparable serious infections caused by drug susceptible bacteria, it is usually not clear whether the increased mortality is a reflection of increased virulence, diminished effectiveness of antibiotic therapy, or both (Jassen *et al.*, 1969).

This work therefore examined resistance profile of bacterial isolates from surgical sites of patients admitted

and operated in the University Teaching hospital under surveillance and relationship between these isolates and those isolated from the hospital environment where the patients were operated and managed.

## MATERIALS AND METHODS

### Bacteriological study of the theatre air

The bacterial load of the air in the operation theater of a University Teaching Hospital in Nigeria was studied using settling plate method. Plates containing Nutrient agar, MacConkey agar, and Chocolate agar were exposed and placed in different areas of the theater at the start of each surgical operation and left exposed until the operation procedure was completed. Thereafter, they were covered and taken to the laboratory for incubation. The Nutrient and MacConkey agar plates were incubated at 37°C in an ambient incubator while plates containing Chocolate agar were incubated in a carbon dioxide incubator containing 10% CO<sub>2</sub> for 24 hrs. Colonies showing different morphological characteristics were isolated, purified into pure cultures and fully characterized. The characterized isolates were sub-cultured onto nutrient agar slant and kept in refrigerator for subsequent susceptibility test.

### Bacteriological Study of Theatre Floor

While the operation was in progress, the bacteria load of the theater floor was studied using swabbing method. The floor of theater which has already been demarcated (one meter square) was sub divided into 16 sub-squares, and one of these squares was randomly chosen and swabbed using sterile cotton swab moistened with sterile normal saline. The swab was then put into test tube containing 9ml sterile normal saline and mixed properly to discharge its contents. One milliliter (1ml) each of this mixture was then used to flood plates of Nutrient agar, MacConkey agar and Chocolate agar. Plates of nutrient agar and MacConkey agar were incubated at 37°C for 24 hrs while the plates of Chocolate agar was incubated under 10% CO<sub>2</sub> for 24 hrs (Bond and Schulster, 2004 and Favero *et al.*, 1984).

The colonies that developed after 24 hrs of incubation, were counted, streaked onto nutrient agar slants and stored at 7°C in refrigerator for further processing.

### Bacteriological Study of Operated Surgical Sites

Surgical operations in the theater were followed and the time taken to complete each surgical operation cycle (i.e. time between incision of the operation site and closure of sutured sites) was recorded. Immediately after completion of an operation and closure of the operated

site, it was swabbed using sterile swab stick. The swab was then inoculated into 9ml sterile peptone water from which 1ml each was inoculated on Nutrient agar, MacConkey agar and Chocolate agar plates. The plates were taken to the Microbiology Laboratory of the Hospital, incubated aerobically (for Nutrient and MacConkey agar plates) or under 10 % CO<sub>2</sub> (in case of Chocolate agar plates) at 37°C for 24 hours. The colonies that developed after incubation were sub-cultured, fully characterized biochemically and culturally, then kept in nutrient agar slant and stored in refrigerator for further use.

### **Determination of the Antibiotics Susceptibility Profiles of the Isolates**

Combinations of agar ditch diffusion and disc diffusion methods were used for evaluating the susceptibilities of the isolates to various test antibiotics. Where disc of antibiotics in use were available, Disc diffusion method was used for those antibiotics available in discs while agar ditch diffusion was used for the others that were not available as antibiotic discs. Susceptibilities of the isolates were tested against twenty (20) antibiotics, namely Ampicillin (10µg), Cloxacillin (5µg), Penicillin G (10µg), Cefuroxime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ceftizoxime (30µg), Ceftazidime (30µg), Gentamicin (10µg), Streptomycin (10µg), Ciprofloxacin (5µg), Ofloxacin (5µg), Perfloxacin (5µg), Spafloxacin (5µg), Azithromycin (10µg), Erythromycin (10µg), Chloramphenicol (30µg), Tetracycline (30µg), Oxytetracycline (30µg) and Doxycycline (30µg). The antibiotic discs were supplied by the department of Medical Microbiology and Parasitology, UIITH, Ilorin.

### **Agar Ditch Diffusion Method**

All isolated bacterial species stored in slant nutrient agar were standardized for the antibiotics susceptibility tests. Isolates were sub cultured into nutrient broth, and incubated overnight. The turbidity produced was adjusted by using sterile physiological saline to match 0.5 McFarland standards [ca 10<sup>8</sup> cfuml<sup>-1</sup>] (NCCLS, 1990). This was further diluted to produce cell concentration of 10<sup>6</sup>cfuml<sup>-1</sup>.

Plates containing Mueller Hinton agar were dried and flooded with standardized inoculums of the isolates as described by Onaolapo (1997). Using sterile cork borer No.8 (8mm bore size), holes were created and the bottom partly covered with molten agar to prevent the antibiotic solution from draining away. With automatic micropipettes, 40µl of the desired concentration of different antibiotics was added, allowed to diffuse for 1 hour and thereafter incubated at 37°C for 18-24hr. Controls were equally set up.

The diameters of the resulting inhibition zones were measured using metric ruler, and recorded in milliliter. Based on the diameters of zones of inhibition and the recommendations of Kirby-Bauer (1966) and WHO (1983), the isolates were classified as sensitive or resistant.

### **Agar Disc Diffusion Test**

Antibiotics with commercially prepared antibiotics discs were tested by agar disc diffusion method. The Mueller Hinton agar was seeded as described above, and the discs containing antibiotics under test placed firmly on the surface of the agar using sterile forceps. The plates were allowed to stand for an hour to enable the antibiotics diffuse into the agar. The plates were then incubated at 37°C, after which the plates were observed for development of inhibition zones. The diameters of zones of inhibition were measured and compared as described above.

### **Determination of Multiple Antibiotics Resistance (MAR) Index**

The Multiple Antibiotic Resistance (MAR) index was determined for each of the selected bacterial isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested (Krumperman, 1983; Paul *et al.*, 1997). The higher the value of this index the higher the multiple resistance of the isolate.

### **Determination of Minimum Inhibitory Concentration (MIC) of the Isolates**

The MICs of the antibiotics used against the isolates were determined using the macrobroth serial dilution technique (Lennette *et al.*, 1990; Bary, 1976). Stock solutions of the antibiotic were prepared. For each antibiotic, twelve (12) tubes containing 1.0ml sterile peptone water were serially arranged. A 1.0 ml of antibiotic from the stock was introduced into tube 1 and 2. Serial dilutions were carried out from tube 2 to 10 such that each new dilution contains 50% less of the antibiotic contained in the preceding dilution. Standardized overnight culture of the isolates (inoculum size of ca 10<sup>6</sup> cfuml<sup>-1</sup>) were then inoculated into the tubes and incubated at 37°C for 24 hrs. Positive and negative controls were set up as recommended (Woods and Washington, 1995). After incubation, the tubes were observed for growth. Based on the pattern of growth in the tubes, the MIC of the antibiotic for the isolates was recorded.

**Table 1.** Multiple Antibiotics Resistance Profiles and Indices of Bacterial species Isolated from Theater, Surgical Ward and Wounds of Patients of a University Teaching Hospital

Isolates	MAR Profile	MAR
<i>S. epidermidis</i>	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-MA	0.7
<i>S. aureus</i>	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-CEF-CEFT-AM	0.8
<i>Strept. Pyogenes</i>	A-P-CX-C-AZ-E-PEF-OFX-T-DOX-CEF-AM	0.6
<i>Streptococcus spp.</i>	A-P-CX-C-AZ-E-OXT-T-DOX-CEF-AM	0.6
<i>Klebsiella spp.</i>	A-P-CX-C-S-E-OXT-T-DOX-CEF-AM	0.6
<i>E. coli</i>	A-P-CX-C-S-AZ-E-SPA-CIP-OXT-T-CEF-AM	0.7
<i>Enterobacter spp.</i>	A-P-CX-C-AZ-E-CIP-OXT-DOX-CEF-AM	0.7
<i>Pr. mirabilis</i>	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	0.8
<i>Cit. freundii</i>	A-P-CX-C-AZ-E-SPA-OXT-T-DOX-CEF-AM	0.6
<i>Ser. marcescens</i>	A-P-CX-C-AZ-E-OFX-CIP-OXT-T-DOX-CEF-AM	0.7
<i>Serratia spp.</i>	A-P-CX-C-S-AZ-E-OFX-SPA-OXT-T-DOX-CEF-AM	0.7
<i>Ps. aeruginosa</i>	A-P-CX-C-S-AZ-E-PEF-SPA-CIP-OXT-T-CEF-CRO-EFT-AM	0.8
<i>Pseudomonas spp.</i>	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	0.8
<i>B. cereus</i>	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-DOX-CEF-AM	0.8
<i>B. megaterium</i>	A-P-CX-C-S-AZ-PEF-CIP-OXT-T-DOX-CEF-AM	0.7
<i>B. subtilis</i>	A-P-CX-C-AZ-E-SPA-CIP-OXT-T-CEF-AM	0.6

### Test for the $\beta$ - lactamase production

The presence of  $\beta$ -lactamase enzyme in isolates was tested by using iodometric method (Miller and Smith, 1979). Nutrient agar containing 2% soluble starch was prepared. Overnight agar surface flooded with freshly prepared 10,000 unit/ml of Penicillin (0.06mg/ml in 0.1M phosphate buffer, pH 7.0) and left for 15-60 minutes at room temperature. Thereafter, iodine solution was added. Isolates whose colonies turned blue-black with colourless halos were considered as a  $\beta$ -lactamase producing isolates and recorded.

### Test for the Presence of Plasmids in the isolates

This acridine orange method described by Crosa *et al* (1994) was employed in this determination. The MICs value of acridine orange to twenty selected highly resistant isolates (including resistance to ampicillin and streptomycin) was determined as described in 2.8. Sub-MIC of acridine orange (750 $\mu$ g/ml) was used to cure the isolates of plasmid they might contain. Sterile Nutrient broths were inoculated with standardized overnight inoculum of the test isolates and solutions of acridine such that the final concentration of acridine in the broth became 750 $\mu$ gml<sup>-1</sup>. These were then incubated at 37°C for 18 hrs. Thereafter, growths from the tubes were sub-cultured and the MICs of the twenty isolates were re-determined as described in section 2.8. The MICs of the acridine-treated isolates were then compared with the values before plasmid curing with acridine.

### RESULTS

Antibiotic resistance profile of isolates presented in Table 1 showed that all isolates are multiple antibiotics resistant. The antibiotic resistance indices ranged from 0.6 to 0.8, with *S. aureus*, *Pr. mirabilis*, *Ps. aeruginosa*, *Pseudomonas spp.* and *B. cereus* having the highest indices (0.8 each).

Table 2 showed that out of 147 antibiotics resistant isolates tested for  $\beta$ -lactamase production, 94(64.0%) possessed  $\beta$ -lactamase enzyme. *Staphylococcus epidermidis*, produced the highest amount of the enzyme (90%), followed by *S.aureus* (84.0%), *S. marscens* (77.8%), *Cit. freundii* and *Enterobacter spp.* (75% each). The result also showed that *P.aeruginosa*, *B. cereus*, *B. megaterium* and *B. subtilis* showed low level of  $\beta$ -lactamase production (table 2).

Table 3 showed occurrence of plasmids in antibiotic resistant isolates. Out of 147 isolates tested for presence of plasmid, 90(61.2%) were positive. *Klebsiella pneumoniae* was strongly positive for plasmid harboring; it showed 100% positive for plasmids. For *Staphylococcus epidermidis*, *S. aureus*, *Enterobacter spp.*, *E. coli* and *Ps. aeruginosa* it was 85%, 80%, 75%, 62.5%, and 62,5% respectively. Low levels of plasmid production were observed in *Stept. Pyogenes*, *Serratia marcescens*, *B. cereus*, *B. megaterium* and *B. subtilis* (Table 3.0).

Resistance profile of isolates before and after plasmid curing is shown on Table 4.0. Before the curing *S.epidermidis* was resistant to 15 different antibiotics, this was reduced to 6 after curing the isolate of plasmids. Similar pattern was observed in other isolates as shown on the table.

**Table 2.**  $\beta$ -lactamase Production in some Antibiotic Resistant bacteria Species Isolated from a University Teaching Hospital in Nigeria

Isolates	No. Tested	$\beta$ -lactamase Producers	
		n	%
<i>S. epidermidis</i>	20	18	90.0
<i>S. aureus</i>	25	21	84.0
<i>Strept. pyogenes</i>	6	5	83.3
<i>Strept. spp</i>	2	0	0.0
<i>E. coli</i>	8	6	75.0
<i>Kl. pneumoniae</i>	10	8	80.0
<i>Kl. spp.</i>	5	3	60.0
<i>Pr. mirabilis</i>	12	7	58.3
<i>Cit. freundii</i>	4	3	75.0
<i>Ent. spp</i>	4	3	75.0
<i>Serratia marcescens</i>	9	7	77.8
<i>Serratia spp.</i>	3	2	66.7
<i>Ps. aeruginosa</i>	16	6	38.0
<i>Ps. spp</i>	3	1	33.3
<i>B. cereus</i>	5	2	40.0
<i>B. megaterium</i>	5	1	20.0
<i>B. subtilis</i>	5	1	20.0
Total	147	94	64.0

**Table 3.** Occurrence of Plasmids in some Antibiotic resistant bacteria Species Isolated from a University Teaching Hospital in Nigeria

Isolates	No tested	Plasmid Carrying Strains	
		n	%
<i>S. epidermidis</i>	20	17	85.0
<i>S. aureus</i>	25	20	80.0
<i>Strept. Pyogenes</i>	6	2	33.3
<i>Streptococcus spp.</i>	2	1	50.0
<i>Klebsiella spp.</i>	5	3	60.0
<i>E. coli</i>	8	5	62.5
<i>Kl. pneumoniae</i>	10	10	100.0
<i>Enterobacter spp.</i>	4	3	75.0
<i>Pr. mirabilis</i>	12	7	58.3
<i>Cit. freundii</i>	4	2	50.0
<i>Ser. marcescens</i>	9	3	33.3
<i>Serratia spp.</i>	3	1	33.3
<i>Ps. aeruginosa</i>	16	10	62.5
<i>Ps. spp</i>	3	1	33.3
<i>B. cereus</i>	5	1	20.0
<i>B. megaterium</i>	5	2	40.0
<i>B. subtilis</i>	5	2	40.0
Total	147	90	61.2

**Table 4.** Resistant pattern of some Antibiotic Resistant Bacteria Species Isolated from a University Teaching Hospital in Nigeria, before and After Plasmid Curing

Isolates	Resistance Profile before curing	Resistance profile after curing
<i>S. epidermidis</i>	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-MA	C-E-OFX-SPA-OXT-DOX
<i>S. aureus</i>	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-CEF-CEFT-AM	C-OFX-SPA-OXT-DOX
<i>Str. pyogenes</i>	A-P-CX-C-AZ-E-PEF-OFX-T-DOX-CEF-AM	OFX-DOX-T
<i>Streptococcus spp.</i>	A-P-CX-C-AZ-E-OXT-T-DOX-CEF-AM	AZ-DOX
<i>Klebsiella spp.</i>	A-P-CX-C-S-E-OXT-T-DOX-CEF-AM	E-OXT-T
<i>E. coli</i>	A-P-CX-C-S-AZ-E-SPA-CIP-OXT-T-CEF-AM	AZ-SPA-OXT
<i>Enterobacter spp.</i>	A-P-CX-C-AZ-E-CIP-OXT-DOX-CEF-AM	AZ-OXT-DOX
<i>Pr. mirabilis</i>	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	AZ-E-OFX-SPA-OXT-DOX
<i>Cit. freundii</i>	A-P-CX-C-AZ-E-SPA-OXT-T-DOX-CEF-AM	AZ-OXT-DOX
<i>Ser. marcescens</i>	A-P-CX-C-AZ-E-OFX-CIP-OXT-T-DOX-CEF-AM	AZ-OFX-CIP-OXT-DOX
<i>Serratia spp.</i>	A-P-CX-C-S-AZ-E-OFX-SPA-OXT-T-DOX-CEF-AM	OFX-SPA-OXT-DOX
<i>Ps. aeruginosa</i>	A-P-CX-C-S-AZ-E-PEF-SPA-CIP-OXT-T-CEF-CRO-CEFT-AM	C-AZ-SPA-OXT
<i>Pseudomonas spp.</i>	A-P-CX-C-S-AZ-E-OFX-SPA-CIP-OXT-T-DOX-CEF-CRO-AM	AZ-E-OFX-SPA-OXT-DOX
<i>B. cereus</i>	A-P-CX-C-S-AZ-E-PEF-OFX-CIP-OXT-T-DOX-CEF-AM	C-AZ-PEF-OXT-DOX
<i>B. megaterium</i>	A-P-CX-C-S-AZ-PEF-CIP-OXT-T-DOX-CEF-AM	C-AZ-PEF-OXT
<i>B. subtilis</i>	A-P-CX-C-AZ-E-SPA-CIP-OXT-T-CEF-AM	AZ-SPA-CIP-OXT

## DISCUSSION

Antibiotic resistant profiles of bacteria isolated from the theatre, surgical ward and surgical wounds of patients in a University Teaching hospital in Nigeria in the period under study showed that isolated bacteria were multiple antibiotic resistant isolates. They exhibited multiple resistance to the majority of antibiotics tested. Multiple antibiotic resistances have been reported to occur through different mechanisms:

(i) Modification of drug target site or reduction of cell permeability to the drug. These mechanisms have been established with development of resistance to aminoglycoside of *Enterobacteriaceae*, *Pseudomonas spp.*, *Staphylococci*, especially when Gentamycin and Amikacin are used (Shaw *et al.*, 1993).

(ii) Production of  $\beta$ -lactamase enzymes which destroy  $\beta$ -lactam ring of  $\beta$ -lactam antibiotics, thereby rendering the drug useless. *Streptococci*, *Staphylococci*, *Pseudomonas spp* and *Enterococci* are recognized as organisms that normally develop resistance by these mechanisms.

(iii) Efflux pump to reduce antibiotics concentration in the cell is another mechanism used by *Streptococci*, *Staphylococci* and *Pseudomonas spp.* to develop resistance to tetracycline, especially cloxycycline (Neu, 1989., Spear *et al.*, 1992).

Prevalence of these multidrug resistant isolates in the theatre, surgical wards and wounds is a cause of concern because of its attendant effect. It implied that;

- Most of the commonly used antibiotics will not be useful in the management of wound and surgical sites infected with such pathogens
- Infected wounds would take longer to heal
- Cost of treatment such as cost of drugs, test etc would increase
- Patients will stay longer in the hospitals
- Higher likelihood of transmitting such pathogens to other patients.

Resistance to multiple classes of antimicrobial agents such as chloramphenicol, tetracycline and quinolones in gram – negative and gram-positive bacteria have been described to be more often due to efflux of the drugs out of the bacterial cell (Nikaido, 1991 and Levy, 1992).

Therefore, it is important that the hospital management adopt rational use of antibiotics to avoid epidemic out break of drug resistant bacterial infections in the hospital.

The result of this work also showed that 64% of the 147 Multiple Antibiotics Resistant (MAR) isolates were  $\beta$ -lactamase producers. That most strains of the isolated bacterial species (*S. epidermidis*, *S. aureus*, *Strep. pyogen*, *E. coli*, *Kl. pneumoniae*, *Citrobacter freundii*, *Ser. Marcescens*) were  $\beta$ -lactamase producers imply that

substantial number of the MAR isolates developed resistance to the drugs as a result of  $\beta$ -lactamases production. Most isolates that produced  $\beta$ -lactamase enzymes were also found positive for plasmid harbouring.

Relationship between  $\beta$ -lactamase production and presence of plasmid in some of the MAR isolates showed that all the isolates except one (*Pr.mirabilis*) that produced  $\beta$ -lactamase also have plasmids. Also, one strain of the isolates that produced  $\beta$ -lactamase had no plasmid. These findings confirmed that most of the  $\beta$ -lactamases produced by some of these isolates were plasmid encoded, and most likely responsible for the resistance of the isolates to the antibiotics. Plasmid – mediated resistance to  $\beta$ -lactam agents is most often a result of  $\beta$ -lactamases (Fred and Denis, 1998). Resistance of these isolates to some 2<sup>nd</sup> and 3<sup>rd</sup> generation cephalosporins is an indication that some of the  $\beta$ -lactamase enzymes produced are likely to be Extended Spectrum Beta lactamase enzymes which are known for causing inter-resistance to other classes of antibiotics.

This finding is in agreement with the conclusion of some researchers that high percentage of many commonly encountered gram- positive and negative nosocomial pathogens have at least one and frequently multiple plasmids (Chang *et al.*, 1988, Costello *et al.*, 1980, Chow *et al.*, 1985, Cynamon and Palmer, 1983; Fred and Denis, 1998).

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