

Full Length Research Paper

# Antibiogram of clinical isolates from a hospital in Nigeria

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**Antibiogram of clinical isolates from four wards in a government hospital in Port Harcourt, Nigeria was investigated. Swab and air samples were obtained from patients, personnel, formites and air in orthopaedic, medical, surgical and paediatric wards. A total of 56 isolates were obtained of which *Staphylococcus epidermidis* (22) was the highest. This was followed by *Staphylococcus aureus* (16), *Streptococcus* spp. (5), *Escherichia coli* (4) and *Klebsiella pneumonia* (3). *Proteus* spp., *Enterobacter aerogenes* and *Bacillus cereus* had 2 strains each isolated. The Gram positive bacteria were more resistant to norfloxacin, floxapen, and ciprofloxacin but very sensitive to gentamycin, lincocin, rifampicin and streptomycin. *S. aureus* accounted for the highest resistance to ampiclox followed by *S. epidermidis* to ciprofloxacin and norfloxacin. The Gram negative bacilli showed highest resistance to ampicillin followed by augmentin, ceporex, and nalidixic acid whereas they were more sensitive to tarivid, peflacin and streptomycin. It could be inferred from the results that patients in this hospital might be at the risk of being infected with antibiotic resistant strains during admission.**

**Key words:** Antibiogram, nosocomial, Port Harcourt, clinical isolates, gram positive bacteria, gram negative bacteria.

## INTRODUCTION

The hospital environment is uniquely suited to the spread of infections as it houses both susceptible patients and patients with difficult-to-treat infections. There is a great risk that some patients may contract hospital-associated infections other than those they were admitted for because of nosocomial pathogens around them (Esposito and Leone, 2007; Lockhart et al., 2007). The widespread use of broad-spectrum antibiotics has led to the emergence of nosocomial infections caused by drug resistant microbes (Courvalin and Weber, 2005).

The worldwide escalation in both community- and hospital-acquired antimicrobial-resistant bacteria is threatening the ability to effectively treat patients, emphasizing the need for continued surveillance, more appropriate antimicrobial prescription, prudent infection control, and

new treatment alternatives (Mulvey et al., 2004; Rhomberg et al., 2006; Zhanel et al., 2008). Antimicrobial-resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) (community-associated [CA-MRSA] and healthcare-associated [HA-MRSA]), vancomycin-resistant *Enterococcus* species (VRE), penicillin-resistant *Streptococcus pneumoniae*, extended-spectrum  $\beta$ -lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella* species (Mulvey et al., 2004), and fluoroquinolone-resistant and carbapenem-resistant members of the family *Enterobacteriaceae* and *Pseudomonas aeruginosa* are increasing in prevalence globally (Pitout et al., 2005; Lockhart et al., 2007). Available therapeutic options for antibiotic-resistant organisms are severely limited, as these organisms frequently display a multidrug-resistant (MDR) phenotype (Moland et al., 2006; Lewis et al., 2007).

Sources of antimicrobial resistant nosocomial pathogens could either be endogenous or exogenous. Endogenous sources are those that come from the patient's

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**Table 1.** Distribution of isolates in the wards.

Ward	<i>S. epid.</i>	<i>S. aureus</i>	<i>Strep.</i>	<i>E. coli</i>	<i>Kleb.</i>	<i>Proteus</i>	<i>Entero.</i>	<i>Bacillus</i>
OTW	9 (skin)	6 (skin, nasal)	-	2 (nasal)	2 (skin)	1 (air)	-	2 (air, formites)
MW	4 (skin)	3 (skin, nasal)	1 (skin)	-	1 (skin)	-	-	-
SW	4 (skin)	1 (skin)	3 (formites)	1 (nasal)	-	1 (air)	-	-
PW	5 (skin)	6 (skin, formites, nasal, air)	1 (formites)	1 (nasal)	-	-	2 (skin)	-
Total	22	16	5	4	3	2	2	2

OTW, Orthopaedic ward; MW, Medical ward; SW, Surgical ward; PW, Paediatric ward; *S. epid.*, *Staphylococcus epidermidis*; *S. aureus*, *Staphylococcus aureus*; *Strep.*, *Streptococcus* spp.; *E. coli*, *Escherichia coli*; *Bacillus*, *Bacillus cereus*; *Kleb.*, *Klebsiella pneumonia*; *Entero.*, *Enterobacter aerogenes*; *Proteus*, *Proteus* spp.

microflora while exogenous ones are from external microflora other than the patient's. Nosocomial infections have also been traced to contaminated medical equipment and devices like catheters, ventilators, oral thermometers, humidifiers and other formites (Prescott et al., 2005). The air in the hospital wards can serve as a prime repository for nosocomial pathogens that might cause respiratory infections.

In the present study, swab/air samples were collected from patients, hospital personnel, air and formites in four wards in a Government hospital in Port Harcourt, Nigeria in order to evaluate the antibiogram (antibiotic susceptibility pattern) of the bacteria isolated from these sources.

## MATERIALS AND METHODS

Samples used were obtained from paediatric (PW), medical (MW), orthopaedic (OTW), and surgical (SW) wards from personnel, patients, beds, cannula, oral thermometer, tables and air. The air samples were collected by exposing nutrient agar plates in the air for 5 min, while sterile swab sticks were used to collect formite, nasal and skin swabs. The samples were streaked on nutrient agar, MacConkey agar and eosin methylene blue (EMB) agar. The plates were incubated at 37°C for 24 h.

Isolates obtained after incubation were subcultured using isolation media and identified using Gram stain and biochemical tests such as catalase, coagulase, indole production, citrate utilization, triple iron sugar utilization and methyl red-Voges Proskauer as described by Cheesbrough, (1992).

## Antibiogram test

The antibiotic susceptibility patterns of the isolates to common antibiotics used in the hospital were determined using the agar-disk diffusion method on Mueller-Hinton agar as described in Department of Microbiology Laboratory Manual (2004) and Manual of Antimicrobial Susceptibility Testing (Coyle, 2005). An overnight broth culture of each isolate was uniformly spread onto the surface of the Mueller-Hinton plates. The appropriate antibiotic multi-discs (either Gram positive or negative) were aseptically placed on the agar using sterile forceps. The plates were then incubated at 37°C for 24 h. The degree of susceptibility of the test isolate to each antibiotic was interpreted as either sensitive (S) or resistant (R) by measuring the zone diameter of inhibition. The Gram positive antibiotic discs contained the following antibiotic concentrations: Tarivid 10 mcg, Peflacin 10 mcg, Ciproflox 10 mcg, Augmentin 30

mcg, Gentamycin 10 mcg, Streptomycin 30 mcg, Ceporex 10 mcg, Nalidixic acid 30 mcg, Septrin 30 mcg, and Ampicillin 30 mcg; whereas the Gram positive antibiotic discs had the following concentrations: Ciprofloxacin 10 mcg, Norfloxacin 10 mcg, Gentamycin 10 mcg, Lincocin 20 mcg, Streptomycin 30 mcg, Rifampicin 20 mcg, Erythromycin 30 mcg, Chloramphenicol 30 mcg, Amplicox 20 mcg, and Floxapen 20 mcg.

## RESULTS

A total of 56 isolates comprising 45 Gram positive and 11 Gram negative bacteria were obtained. It was observed that 62.5% of the isolates were from the skin while 16, 14.2 and 7.1% were from the nasal, formites and air samples respectively. The bacteria were identified as *S. epidermidis*, *S. aureus*, *Streptococcus* spp., *E. coli*, *Klebsiella pneumonia*, *Proteus* spp., *Enterobacter aerogenes* and *Bacillus cereus*. Orthopaedic ward had the highest occurrence of the isolates obtained. *S. epidermidis* was the most common bacterium from this ward followed by *S. aureus*. The distribution of these isolates in the different wards is shown in Table 1.

The antibiograms for the Gram positive and Gram negative isolates are shown in Tables 2 and 3. The susceptibility patterns obtained revealed varying degrees of resistance and sensitivity to the antibiotics used in the screening. The Gram positive isolates were more resistant to Norfloxacin (71.1%). This was closely followed by Floxapen (57.8%), Ciprofloxacin (51.1%) and Erythromycin (44.4%) respectively. They were sensitive to Gentamycin, (93.3%), Rifampicin (93.3%), Streptomycin (82.2%) and Lincocin (80%). On the other hand, the Gram negative isolates were more resistant to Ampicillin (81.8%), Ceporex (72.7%), Nalidixic acid (72.7%) and Augmentin (63.6%). They were very sensitive to Tarivid (100%), Peflacin (100%), Gentamycin (100%), Streptomycin (90.9%) and Ciproflox (63.6%).

## DISCUSSION AND CONCLUSION

The antibiogram revealed that out of the total Gram positive isolates that were resistant to Ampiclox and Lin-

**Table 2.** Antibiogram of the Gram positive isolates.

Isolate	Cipr	Norf	Gen	Linco	Strept	Rifamp	Erythro	Chloram	Ampi	Floxa
<i>S. aureus</i> <sup>a</sup>	S	R	S	S	S	S	S	S	R	S
<i>S. aureus</i> <sup>a</sup>	R	S	S	S	S	S	S	S	S	R
<i>S. aureus</i> <sup>b</sup>	R	S	S	S	S	S	S	S	R	R
<i>S. aureus</i> <sup>c</sup>	R	S	S	S	S	S	S	S	S	R
<i>S. aureus</i> <sup>a</sup>	S	S	S	R	S	S	S	R	S	S
<i>S. aureus</i> <sup>a</sup>	S	S	S	R	S	S	S	S	R	S
<i>S. aureus</i> <sup>b</sup>	S	R	S	R	S	S	S	R	R	R
<i>S. aureus</i> <sup>c</sup>	S	R	S	R	S	S	S	R	R	R
<i>S. aureus</i> <sup>a</sup>	S	R	S	S	R	S	R	S	S	S
<i>S. aureus</i> <sup>b</sup>	S	R	S	S	S	S	S	R	S	S
<i>S. aureus</i> <sup>d</sup>	S	R	S	S	S	S	S	S	R	R
<i>S. aureus</i> <sup>a</sup>	S	R	S	R	S	S	S	S	R	R
<i>S. aureus</i> <sup>a</sup>	R	R	S	S	S	S	R	S	S	R
<i>S. aureus</i> <sup>b</sup>	R	R	S	S	S	S	R	S	S	R
<i>S. aureus</i> <sup>a</sup>	R	R	S	S	S	S	R	S	S	R
<i>S. aureus</i> <sup>c</sup>	S	S	S	R	S	S	S	S	R	S
<i>S. epid</i> <sup>a</sup>	S	R	S	R	R	S	R	R	S	R
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	S	S	S	R
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	S	S	S	R
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	R	S	S	R
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	R	S	S	R
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	R	S	S	R
<i>S. epid</i> <sup>a</sup>	S	R	S	S	R	S	R	S	S	S
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	R	S	S	S
<i>S. epid</i> <sup>a</sup>	S	R	S	S	S	S	R	R	S	S
<i>S. epid</i> <sup>a</sup>	S	R	S	S	S	S	R	R	S	S
<i>S. epid</i> <sup>a</sup>	S	R	S	S	S	S	R	R	S	S
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	S	S	S	R
<i>S. epid</i> <sup>a</sup>	S	S	R	S	S	S	S	S	R	S
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	R	R	S	R
<i>S. epid</i> <sup>a</sup>	S	R	S	S	S	S	R	R	S	S
<i>S. epid</i> <sup>a</sup>	S	R	S	S	S	S	R	R	S	R
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	S	S	S	R
<i>S. epid</i> <sup>a</sup>	R	R	S	S	S	S	S	S	S	R
<i>Strept</i> <sup>a</sup>	R	S	R	S	S	R	S	S	R	R
<i>Strept</i> <sup>f</sup>	S	S	R	S	R	S	R	S	R	S
<i>Strept</i> <sup>f</sup>	R	S	S	S	R	S	S	R	S	S
<i>Strept</i> <sup>f</sup>	S	R	S	S	S	R	R	R	S	S
<i>Strept</i> <sup>f</sup>	R	R	S	R	S	R	S	S	R	R
<i>Bacillus</i> <sup>c</sup>	R	S	S	S	S	S	S	R	S	R
<i>Bacillus</i> <sup>d</sup>	R	S	S	S	R	S	R	S	R	S

Cipr, Ciprofloxacin; Norf, Norfloxacin; Gen, Gentamycin; Linco, Lincocin; Strept, Streptomycin; Rifamp, Rifampicin; Erythro, Erythromycin; Chloram, Chloramphenicol; Amp, Ampiclox; Floxa, Floxapen; R, resistant; S, sensitive.  
Isolate origin: <sup>a</sup>skin, <sup>b</sup>nasal, <sup>c</sup>formite, and <sup>d</sup>air.

**Table 3.** Antibiogram of the Gram negative isolates.

Isolate	Ampicil	Tarivid	Pefla	Cipro	Augumen	Genta	Strept	Ceporex	Nalid	Septrin
<i>E. coli</i> <sup>b</sup>	R	S	S	R	S	S	R	R	R	R
<i>E. coli</i> <sup>b</sup>	R	S	S	S	S	S	S	R	R	S
<i>E. coli</i> <sup>b</sup>	R	S	S	S	S	S	S	R	R	S
<i>E. coli</i> <sup>b</sup>	R	S	S	S	R	S	S	R	R	R
<i>Kleb</i> <sup>a</sup>	R	S	S	R	R	S	S	R	S	S
<i>Kleb</i> <sup>a</sup>	R	S	S	S	R	S	S	R	S	S
<i>Kleb</i> <sup>a</sup>	R	S	S	S	R	S	S	R	S	S
<i>Entero</i> <sup>a</sup>	S	S	S	R	R	S	S	S	R	R
<i>Entero</i> <sup>a</sup>	S	S	S	R	R	S	S	S	R	R
<i>Proteus</i> <sup>d</sup>	R	S	S	S	R	S	S	S	R	R
<i>Proteus</i> <sup>d</sup>	R	S	S	S	S	S	S	R	R	S

Ampicil, Ampicillin; Pefla, Peflacin; Ciprofl, Ciprofloxacin; Augumen, Augumentin; Genta, Gentamicin; Strept, Streptomycin; Nalid, Nalidixic acid; R, resistant; S, sensitive.

Isolate origin: <sup>a</sup>skin, <sup>b</sup>nasal, <sup>c</sup>formite, and <sup>d</sup>air.

cocin, *Staphylococcus aureus* were predominant. Outbreaks of *S. aureus* resistant to  $\beta$ -lactam antibiotics have been frequently associated with devastating nosocomial infections (Depardieu et al., 2007; Buhlmann et al., 2008). In this investigation, *S. aureus* showed marked resistance to Ampiclox which is a  $\beta$ -lactam antibiotic. *S. epidermidis* were more resistant to Ciprofloxacin, Erythromycin, Norfloxacin and Floxapen. *S. epidermidis* is a major cause of nosocomial infections as well because of its ability to form biofilms on the surface of medical devices. According to Cloete (2003) and Villain-Guillot et al. (2007) bacterial biofilms are inherently resistant to antibiotics and host defenses and this could explain the reason for the high resistance seen in the strains isolated. *Streptococcus* spp. showed varying degrees of resistance (3 to 6 of the antibiotics) as shown in Table 1. The two strains of *Bacillus cereus* isolated showed minimal resistance when compared with the other isolates.

The Gram negative isolates were mostly resistant to Ampicillin followed by Ceporex, Nalidixic acid and Augumentin. *E. coli* accounted for the highest resistance to the above antibiotics. It is well documented that Gram negative bacilli harbour series of antibiotic resistant genes which can be transferred to other bacteria horizontally (Piddock, 2006; Depardieu et al., 2007; Leavitt et al., 2007; Lockhart et al., 2007). All the Gram negative bacilli isolated in the present study namely *E. coli*, *Enterobacter aerogenes*, *Klebsiella pneumoniae* and *Proteus* spp. have been shown to cause different nosocomial infections by other researchers (Pitout et al., 2005; Moland et al., 2006; Lewis et al., 2007; Leavitt et al., 2007; Lockhart et al., 2007; Mendonca et al., 2007).

Resistance to antibiotics in bacteria can result from mutations in housekeeping structural or regulatory genes. Alternatively, resistance can result from acquisition of

foreign genetic information (Courvalin and Weber, 2005). Other mechanisms of antibiotic resistance in bacteria include production of plasmid- or chromosomally encoded enzymes that hydrolyze the drugs, inability of the antibiotics to penetrate the cell wall of the bacteria because of cell membrane alterations, diversion to safer alternative metabolic sites, reduced affinity of target sites/ decreased intracellular availability of the drugs and multi drug resistance efflux pumps (Cloete, 2003; Courvalin and Weber, 2005; Prescott et al., 2005; Piddock, 2006; Depardieu et al., 2007). Inappropriate practices like misuse and abuse of antibiotics and unskilled practitioners can also lead to emergence of resistance in bacteria. Expired antibiotics, self-medication, counterfeit drugs, inadequate hospital control measures can as well promote the development of resistance in clinical isolates (Prescott et al., 2005). In developing countries like Nigeria, self medication is a common practice and this might probably be a major cause of antibiotic resistance in clinical isolates since patients only think of going to the hospitals when they are unable to treat themselves. The community acquired resistant strains on admission exchange genetic information with nosocomial isolates resulting in the emergence of 'super bugs' that could cause difficult-to-treat infections (Muvley et al., 2007).

It is apparent from the results of the antibiogram that the hospital investigated could be a potential reservoir of nosocomial pathogens. The high incidence of antibiotic resistant strains isolated from patients, hospital personnel, formites and air is worrisome and as such this issue needs to be addressed properly. We highly recommend the development of infection control programmes for the surveillance of nosocomial infections and epidemiologic typing of clinical isolates in hospitals in order to check the emergence and spread of antibiotic resistant infections in patients. The use of molecular biology techniques would

also enhance the molecular identification of resistance genes (Emori and Gaynes 1993; Singh et al., 2006; Turnidge and Paterson, 2007).

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