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Clinical Isolates Resistance to Commonly Used Antibiotics: A Concern in Healthcare Setting

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Abstract

Antibiotics resistance is a global issue, becoming more intensified because of the diminishing number of new antibiotics. Samples were collected aseptically from hospital surfaces with swab sticks. Isolated microorganisms from the samples collected were identified using standard microbiological methods. A total of 109 isolates were obtained *Staphylococcus aureus* (29), *Staphylococcus epidermidis* (13), *Streptococcus spp*. (16), *Escherichia coli* (8), *Klebsiella pneumonia* (7), *Proteus spp* (5), *Enterobacter aerogenes* (6), *Bacillus cereus* (10), *Micrococcus leteus* (6) and *Pseudomonas aeruginosa* (9). Gram negative bacterial isolated in the study shows multi-drug resistance to about four to five of the antibiotics tested. Most notably *E. coli, Enterobacter aerogenes, Klebsiella pneumonia,* and *Proteus mirabilis*. Although Tarivid and Perfloxacin demonstrated a high potency against these organisms. All Gram positive isolate shows 100% resistance to Ampiclox and Zannicef. *Staphylococcus epidermidis,* and *Staphylococcus. aureus* shows resistance to multiple antibiotics. The present of multidrug resistance microorganism in hospital environment is a concern in healthcare delivery.

Keywords: Antibiotics resistance; Antibiotics; Healthcare; Microorganism

Introduction

Hospitals environments provide anchorage for multi-drug resistant microorganisms borne by patients and staff. Antimicrobial resistance in diseases causing microorganisms is a well-known problem hampering quality healthcare delivery worldwide [1,2]. Antibiotics resistance is a global issue, becoming more intensified because of the diminishing number of new antibiotics produced [3]. The potency of currently available antibiotics is decreasing due to the increasing number of resistant strains causing infections and evolving of new disease causing pathogens [4]. 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 [5-7].

Material and Methods

Study site

Male surgical ward, Female surgical, Intensive care unit, Accident and Emergency ward, and Children ward of the State Specialist Hospital, Akure, Ondo State, Nigeria.

Ethical consideration

A letter of ethical consideration was collected from the Chief Medical Director who is the chairman of the ethical committee of the hospital, for sample collections

Surface sampled

Bed rail, Bed linen, Pillow case, Fan switch, Drip stand, Door knob, Chair, Sink knob, and Light-plug-ins.

Swab samples

Using swab sticks soaked in 8.5% physiological saline samples were taking by rubbing the surfaces with swab sticks. The samples were packed in airtight test tubes and taking to the laboratory for microbial analysis.

Isolation and identification of bacteria

Swab sticks were streaked on already prepared culture media in Petri dishes, the Petri dishes were then incubated for 24 hours, and isolated bacteria were identified through morphological and biochemical tests.

Antibiotic susceptibility test of Gram positive and Gram negative bacteria isolates

The Kirby-Bauer Disc Diffusion Method according to the method of (CLSI, 2003) was used to test the susceptibility of the identified Gram positive bacteria isolates to Septrin (SXT, 30 µg), Erythomycin (E, 10 µg), Pefloxacin (PEF, 30 µg), Gentamycin (GEN, 30 µg), Ampiclox (APX, 30 µg), Zinnacef (Z, 30 µg), Amoxacillin (Am, 30 µg), Rocephin (R, 25 µg), Ciprofloxacin(CPX,10 µg), Streptomycin (S, 30 µg), while Gram negative bacteria were tested to Gentamicin (CN) 10 µg, Septrin (SXT) 30 µg, Chloramphenicol (CH) 30 µg, Ciprofloxacin (CPX) 10 µg, Amoxacilin (AM) 30 µg, Augmentin (AU) 30 µg, Pefloxacin (PEF) 30 µg, Tarivid (OFX) 10 µg, Streptomycin (S) 30 µg and Sparfloxacin (SP) 10 µg. A sterile platinum loop was used to pick colonies of isolates from the culture plate and emulsified into 4 ml of sterile peptone water to match with 0.5 McFarland turbidity standards (10^5 Cfu/ml). Using a sterile swab, the surface of Mueller Hinton Agar (Oxoid, Basingstoke, UK) in a Petri dish was evenly inoculated with the suspension of the isolates. With the Petri dish lid in place, about 10 minutes was allowed for the surface of the agar to dry. A multichannel disc dispenser (Oxoid, Basingstoke, UK) was used to deposit the antibiotics discs onto the surface of the inoculated medium. The plate was then incubated at

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37°C for 18 hours each isolate tested was replicated in triplicate. The diameters of the zones of inhibition were measured in millimeters. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimeter and interpreted on the basis of CLSI guideline (CLSI, 2005).

Results

Isolated bacteria

The morphological and biochemical characteristics of the isolated bacteria. *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa, Proteus mirabilis and Enterobacter aerogenes* are all Gram negative bacteria and rod-like in nature. *Escherichia coli* and *Klebsiella pneumonia* are indole positive. *Proteus mirabilis* and *Klebsiella pneumonia* are urease positive. All Gram negative isolates are catalase positive but coagulase negative except *Pseudomonas aeruginosa. Proteus mirabilis* is the only spore forming and citrate positive Gramm negative isolate, while they are positive to motility test. *Escherichia coli* and *Enterobacter aerogenes* are positive to all the sugar tests. All the Gram positive isolates are cocci in shape except *Bacillus cereus. Staphylococcus aureus* and *S. epidermidis* have a cluster arrangement. *Staphylococci aureus* is the only Gram positive isolate that fermented all the sugar used. They are all indole negative except *Bacillus cereus. Staphylococcus aureus, S. pneumonia* and *M. leteus* urease positive, while *S. faecalis and S. pneumonia are* catalase negative. Coagulase positive isolates are *Bacillus cereus and Staphylococci aureus,* while *S. pneumonia* is citrate negative (Table 1).

Distribution of bacterial isolates across the wards

The distribution of bacterial isolates across the wards, *Staphylococcus aureus,* and *Escherichia coli* were predominant compared to other isolates which were less predominant. Analysis shows that distribution of bacteria species across the sampled wards is significantly different at (P=0.00) (Figure 1).

Antibiotic susceptibility pattern of Gram positive bacterial isolates

The antibiotic susceptibility pattern of Gram negative bacteria isolate in relation to inhibition zone. *Enterobacter aerogenes* shows multi

Shape	Arrangement	Gram staining	Indole test	Urease test	Catalase	Coagulase	Oxidase	Spore staining	Citrate	Motility	Sugar fermentation test Lactose	Sucrose	Glucose	Mannitol	Maltose	Probable isolates
Short Rod	singly	-	+	-	+	-	-	-	-	+	+	+	+	+	+	*E. coli rod*
Short Rod	singly	-	+	+	+	-	-	-	+	+	+	-	+	-	+	*K. pneumonia*
Small Rod	singly	-	-	-	+	-	+	-	+	+	-	-	-	+	-	*P. aeruginosa*
Cocci	cluster	+	-	+	+	+	-	-	+	-	+	+	+	+	+	*S. aureus*
Cocci	singly	+	-	-	-	-	NT	NT	-	+	+		+			*S. pneumonia*
Long Rod	singly	+	+	-	+	+	-	+	+	+	-	+	+	NT	NT	*B. cereus*
Rod	singly	-	-	+	+	-	-	+	+	+	-	+	+	-	-	*P. mirabilis*
Cocci	cluster	+	-	+	+	-	-	-	+	-	+	+	+	-	+	*S. epidermidis*
Cocci	singly	+			-	-	-	-		-						*S. faecalis*
Cocci	cluster	+	-	+	+	-	+	-	+	+	+	+	-	-	+	*M.leteus*
Rod	singly	-	-	-	+	NT	-	-	+	+	+	+	+	+	+	*E. aerogenes*

NT=Not tested; (+)=Positive; (-)=Negative

Table 1: Identification of bacteria isolates.

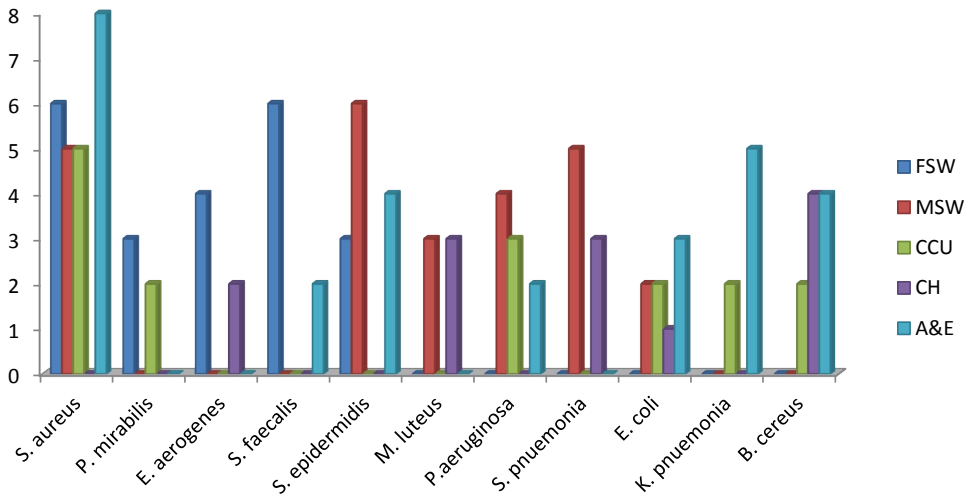

Figure 1: Distribution of bacterial isolates across the wards (X^2=0.00, P<0.05).

Antibiotic	*Proteus mirabilis*	*Enterobacter aerogenes*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Klebsiella pneumonia*
AMOXACILIN	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.20 ± 0.37^{b}	0.00 ± 0.00^{a}	3.80 ± 0.86^{b}
PEFLOXACIN	9.10 ± 0.81^{bc}	0.00 ± 0.00^{a}	11.50 ± 1.67^{c}	6.40 ± 1.03^{b}	11.00 ± 1.18^{bc}
TARIVID	4.14 ± 1.04^{ab}	0.00 ± 0.00^{a}	11.60 ± 1.57^{c}	7.20 ± 1.59^{bc}	8.90 ± 0.87^{bc}
STREPTOMICIN	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.70 ± 0.66^{b}	0.00 ± 0.00^{a}
CHLORAMPHENICOL	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
CIPROFLOXACIN	9.60 ± 0.5^{d}	1.90 ± 0.25^{b}	5.44 ± 0.73^{c}	1.70 ± 0.37^{ab}	0.00 ± 0.00^{a}
AUGUMENTIN	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.10 ± 0.10^{b}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
GENTAMICIN	1.50 ± 0.22^{a}	0.50 ± 0.32^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.50 ± 0.22^{a}
SEPTRIN	0.00 ± 0.00^{a}	2.20 ± 0.37^{a}	1.20 ± 0.20^{a}	1.30 ± 0.20^{a}	8.60 ± 1.96^{b}
SPARFLOXACIN	5.50 ± 0.86^{b}	0.00 ± 0.00^{a}	3.80 ± 0.68^{bc}	0.00 ± 0.00^{a}	1.90 ± 0.40^{ab}
A result of the mean of five replicates ± standard error. Values with different superscript within the same column are significantly different at p<0.05 using Tukey.					

Table 2: Zone of inhibition in mm of Gram positive bacteria isolates to antibiotics.

Antibiotic	*S. aureus*	*S. pneumonia*	*B. cereus*	*S. faecalis*	*M. leteus*	*S. epidermidis*
AMOXACILIN	0.00 ± 0.00^{a}	4.60 ± 0.51^{b}	4.00 ± 0.71^{b}	0 .00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
ROCEPHIN	3.80 ± 0.58^{bc}	5.00 ± 0.32^{c}	0.00 ± 0.00^{a}	4.40 ± 0.40^{c}	2.70 ± 0.37^{b}	0.00 ± 0.00^{a}
CIPROFLOXACIN	5.30 ± 0.64^{b}	9.40 ± 0.51^{c}	5.00 ± 0.71^{b}	9.00 ± 0.89^{bc}	17.40 ± 1.78^{d}	0.00 ± 0.00^{a}
AMPICLOX	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
STREPTOMYCIN	11.60 ± 0.92^{d}	5.60 ± 0.51^{c}	3.10 ± 0.51^{b}	4.00 ± 0.32^{bc}	3.60 ± 0.51^{bc}	0.00 ± 0.00^{a}
ZANNICEF	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
SEPTRIN	4.80 ± 0.60^{bc}	0.00 ± 0.00^{a}	0.10 ± 0.10^{b}	0.00 ± 0.00^{a}	3.60 ± 0.51^{b}	0.00 ± 0.00^{a}
ERYTROMICIN	6.40 ± 0.51^{c}	0.00 ± 0.00^{a}	4.40 ± 0.48^{bc}	2.80 ± 0.37^{b}	3.60 ± 0.51^{b}	4.20 ± 0.37^{b}
PEFLOXACIN	2.80 ± 0.37^{a}	7.60 ± 0.51^{c}	4.20 ± 0.37^{ab}	5.40 ± 0.75^{bc}	5.60 ± 0.68^{bc}	11.60 ± 0.68^{d}
GENTAMYCIN	0.00 ± 0.00^{a}	11.40 ± 0.60^{d}	2.80 ± 0.37^{b}	0.00 ± 0.00^{a}	8.80 ± 0.73^{c}	0.00 ± 0.00^{a}
A result of the mean of five replicates ± standard error. Values with different superscript within the same column are significantly different at p<0.05 using Tukey						

Table 3: Zone of inhibition in mm of Gram negative bacteria isolates to antibiotics.

drug resistance against Amoxacilin, Pefloxacin, Tarivid, Stretomicin, and Chloramphenicol with no inhibition zone (0.00 ± 0.00), Klebsiella pneumonia also shows resistance to multiple antibiotics, Stretomicin, Chloramphenicol, Ciprofloxacin and Augumentin with no inhibition zone (0.00 ± 0.00). Pefloxacin is active against *Pseudomonas aeruginosa* (11.50 ± 1.67) and *E. aerogenes* (11.00 ± 1.18) while Tarivid has highest inhibitory zone against *Pseudomonas aeruginosa* (11.60 ± 1.57). Proteus mirabilis also shows resistance against, Chloramphenicol, Septrin, Augumentin, Streptomicin, and Amoxacilin with no zone of inhibition (0.00 ± 0.00). *E. coli* demonstrated multiple resistance has no zone of inhibition was recorded against Augumentin, Amoxacilin, Sparfloxacin and Gentamycin (Table 2).

Antibiotic susceptibility pattern of Gram negative bacterial isolates

The susceptibility pattern of Gram positive bacteria to antibiotics in relation to inhibition zone in millimetre. All Gram positive isolate shows resistance against Ampiclox and Zannicef with no zone of inhibition (0.00 ± 0.00). *Staphylococcus epidermidis* shows resistance to multiple antibiotics, Amoxacilin, Recophin, Ciprofloxacin, Ampiclox, Streptomycin, Zannicef, Septrin and Gentamycin with no zone of inhibition (0.00 ± 0.00). Ciprofloxacin is most active against Micrococcus leteus with a clear zone of 17.40 ± 1.78. Erythromycin shows potency against all Gram positive isolates except *S. pneumonia* while Pefloxacin shows potency against all Gram positive bacteria isolates. *Staphylococcus aureus* and *Streptococcus faecalis* shows were resistance Amoxacilin and Gentamycin (Table 3).

Discussion

The *in-vitro* antibiotic test reveal that most pathogens causing nosocomial infection are becoming more resistance to the commonly use antibiotics. Most of the Gram negative isolated in the study shows multi-drug resistance to about four to five commonly used antibiotics. Most notably, *E. coli, Enterobacter aerogenes, Klebsiella pneumonia,* and *Proteus mirabilis* demonstrated multiple resistant to most of the antibiotics tested. Although Tarivid and Perfloxacin demonstrated a high potency, these might be due to the low prescription trends of it in the study area. The high rate of multiple resistance rate demonstrated by the Gram negative isolate may be due to the fact that these antibiotics having been in use for much longer time or over used and/or their oral route of administration might affected their rate of absorption into blood stream as explained [8]. Multi-drug resistance of Gram negative bacteria isolates have been reported by numerous studies [9]. Reported high rate of multiple drug resistance in *Enterobacteriaceae* isolated from hospital environments.

All Gram positive isolate shows 100% resistance to Ampiclox and Zannicef. *Staphylococcus epidermidis,* and *S. aureus* shows resistance to multiple antibiotics. *S. aureus* and *S. epidermidis* showed similar susceptibility pattern being highly susceptible to Streptomycin and resistant to Augmentin. Therefore Augmentin should not be recommended for the treatment of infections caused by these organisms [10]. Susceptibility of *S. aureus* and *S. epidermidis* in this study to Erythromycin does not necessary imply that these antibiotics may represent therapeutic options for infections caused by these organisms. The susceptibility of *Staphylococcus aureus* to erythromycin was in accordance with the report of who reported 41.86% rate of susceptibility of *Staphylococcus aureus* to erythromycin [11].

Conclusion

Although the study do not look into the resistant mechanism of these bacteria isolates using molecular typing, but it is evidence in this study that disease causing microorganisms are becoming resistance

to commonly used antibiotics. Antimicrobial surfaces should be introduced in our healthcare setting to help reduces microbial load on surfaces.

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