

Antibiogram Profile of Bacteria Isolated from different Clinical Specimens in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka

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Abstract— The danger posed by pathogenic bacteria that are resistant to antibiotics has become a global issue and developing strategies for restoring treatment options against them is inevitable. This research aimed at determining the “Antibiogram profile of bacteria isolated from different clinical specimens in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka” was carried out in the microbiology laboratory of the Hospital between December 2018 and May 2019. A total of 707 clinical samples from 500 patients attending the Hospital were examined by streaking method of microbial culture and susceptibility tests by Agar diffusion method. A total of 491 clinical specimens had positive growth and 860 bacteria were isolated. 280 male patients and 220 female patients were tested with the P-value at .08. The frequency of the isolates from different samples showed that urine had the highest number of isolates 139 (16.16%), followed by wound with 123 (14.30%) and Nasal swab recorded the number of least isolates; 38 (4.41%). The susceptibility pattern of the isolates to various antibiotics used varied as *Staphylococcus aureus* exhibited highest sensitivity against Ofloxacin and least sensitivity range against Erythromycin. *Pseudomonas aeruginosa* was sensitive to most of the antibiotics used with greatest sensitivity against Azithromycin while *Proteus Spp.* had the least sensitivity to most of the antibiotics. However, all the isolates had the greatest resistance against Piperacillin-tozabactam and Clindamycin. The high level of resistance observed in Piperacillin-Tozabactam, Cefixime, Erythromycin, Gentamicin and Clindamycin can be attributed to the irrational use of antibiotics in the study area and a possible high level of drug abuse. There should be continuous monitoring and periodical research on antibiogram profile of these bacteria isolated from different clinical specimens before definitive treatment of bacterial infections to reduce the burden posed by multidrug resistant bacteria.

Keywords— Antibiotic Susceptibility, Bacteria, Clinical specimens, Resistance, Awka.

I. INTRODUCTION

Antibiotics are substances produced by microorganisms that inhibit the growth or kill other microorganisms (1). Though they are critical in modern medicine, but their widespread use or misuse has led to the evolution of microbial strains resistant to most of the commonly used antibiotics (2). Brooks (3) noted that antibiotics revolutionized medicine in the 20th Century, however, their effectiveness and easy access have also led to their overuse, prompting bacteria to develop resistance thereby putting the global health at high risk with multidrug-resistant bacteria observed globally. Presently, antimicrobial resistance poses a major threat to patient's treatment as it leads to increased morbidity and mortality, increased hospital stay, and severe economic loss to the patient and nation (4); (5). Due to the pacing advent of different resistance mechanisms and decrease in efficacy of antibiotics used in treating common infectious diseases, patients now endure prolonged illness, higher expenditures for health care, and an immense risk of death. Infections caused by resistant bacteria adversely affect treatment outcomes, costs, disease spread and duration of illnesses, posing a serious challenge to the future chemotherapies (6); (7).

According to Hussain (8), multidrug resistance in bacteria may be caused by any of the two mechanisms. Firstly, these bacteria may accumulate multiple genes, each coding for resistance to a single drug, within a single cell. This accumulation occurs typically on resistance (R) plasmids. Secondly, multidrug resistance may also occur by the increased expression of genes that code for multidrug efflux pumps, extruding a wide range of drugs. Antibiotic resistant bacteria are able to transfer

copies of DNA that code for a mechanism of resistance to other bacteria even distantly related species, which then are also able to pass on the resistant genes and so generations of antibiotic resistant bacteria are produced. This process is called horizontal gene transfer (8). When strains have multiple antibiotic resistance, the choice of therapy is limited, thus the tremendous therapeutic advantages afforded by the introduction of new antimicrobial agents will always be threatened by the emergence of increasingly resistant bacteria pathogen (9); (10). To overcome these difficulties, monitoring of resistance profiles in the health institutions is needed (11); (12); (13).

David and Nanette (14) described plasmids as extra pieces of genetic material found in many cells (Bacteria) that usually confer a specific property to the cell. These properties include antibiotic resistance, toxin production, and many other features. The role of plasmids in the dissemination of antibiotic resistance is increasingly worrisome to human health; allowing pathogenic bacteria to obtain multiple resistance genes in a single transfer event (15). The present study was performed to determine the antibiogram profile of bacteria isolated from different clinical specimens in Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka in order to inform the Clinicians on possible treatment options due to multidrug resistance experienced in the healthcare delivery.

II. MATERIALS AND METHODS

2.1 Study Area

Clinical specimens were collected from Chukwuemeka Odumegwu Ojukwu University Teaching Hospital (COOUTH) Awka (formerly called Amaku General Hospital) which is the only tertiary hospital in Awka the capital of Anambra State between December 2018 and May 2019. Their analyses were carried out in the Microbiology laboratory of same hospital during the study period.

2.2 Study Design

The study population includes 500 patients who attended Chukwuemeka Odumegwu Ojukwu University Teaching Hospital Awka between December 2018 and May 2019. However, only subjects sent to the bacteriology laboratory for microscopy culture and sensitivity formed the test population. A total of 707 different clinical specimens; Ear discharge, Sputum, Urethral swab, Wound swab, Urine from Catheter, Urine, Nasal swab, High vaginal swab, Stool, Eye swab and Blood were collected from patients. These samples were collected from Monday to Friday in the morning hours of 8.00 a.m-12:00 noon except on public holidays and weekends. However, ethical clearance for this study was obtained from the ethical committee of the Hospital management board.

2.3 Determination of Sample Size

The sample size was determined according to Araoye (16) using the formula;

$$N = \frac{z^2 Pq}{d^2}$$

$$N = 1.96^2 \times 0.5 \times 1/0.0025 = 500 \text{ (approximately)}$$

Where N is the sample size @ 500

z is the standard deviation @ 95% confidence interval (1.96),

P is the proportion to be used on estimation 0.5%, d is the degree of accuracy/precision expected (0.05),

$$q = 1 - p.$$

2.4 Specimen Collection

During the study period, about 707 clinical specimens were collected aseptically from 500 patients with the help of licensed Medical laboratory science personnel in the following wards: Male medical ward (MMW), Female medical ward (FMW), Male surgical ward (MSW), Female surgical ward (FSW), Antenatal ward (AW), Postnatal ward (PNW), children's ward (CW), Emergency ward (EW) and Outpatients Department (OPD) of Chukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka and taken to Bacteriology laboratory within 1hr for bacteriological examination by standard bacteriological methods of culture, microscopy and sensitivity. For the identification of isolates, morphological characteristics, biochemical and sugar tests were used.

2.5 Standardization of Inoculum

McFarland 0.5 turbidity standard was prepared by mixing 99.5ml of 1% dilute Sulfuric acid solution and 0.5ml of 1% Barium chloride to give a standard turbidity (17).

2.6 Agar Disc Diffusion Method Antibiotic Susceptibility Test

The antibiotics susceptibility of identified isolates was tested using Single disc diffusion method according to the Kirby-Bauer. Mueller Hinton agar plates were prepared according to the manufacture's instruction, the standardized organisms were introduced on to the agar by streaking method and the antibiotic discs were placed firmly on the surface of the agar using sterile dispenser. The plates were allowed to stand for an hour to enable the antibiotics to diffuse into the agar. The plates were then incubated at 37°C for 24 h, after which the plates were observed for development of inhibition zones (18). The diameters of zones of inhibition were measured and interpreted as susceptible, intermediate or resistant using the standardized method of National Committee for Clinical Laboratory Standards (19). A total of 12 antibiotic discs (Oxoid, England.) were used; Piperacillin-Tozabactam (P 110 µg), Erythromycin (ERY 15 µg), Ciprofloxacin (CIP 5µg), Cefixine (ZEM 5µg), Cefotaxime (GX 30µg), Ceftriaxone (CRO 45µg), Levofloxacin (LBC 5µg), Augmentin (AUG, 30µg), Azithromycin (AZN 15µg), Clindamycin (CD 2µg), Ofloxacin (OFX 5µg) and Gentamicin (CN10µg).

2.7 Statistical Analysis

All statistical analyses were carried out using the SPSS 21.0 window based program for the Analysis of Variance (ANOVA). A value of $P < .05$ was considered significant.

III. RESULTS AND DISCUSSION

TABLE 1
SOCIO-DEMOGRAPHIC CHARACTERISTICS OF STUDY PARTICIPANTS

Characteristics	Total No of Patients tested N0 (%)	Total no of specimen N0 (%)	Culture +ve specimen N0 (%)	Culture -ve specimen N0 (%)	P-value
Gender					
Male	280 (56.00)	403 (57.00)	278 (39.32)	125 (17.68)	.08
Female	220 (44.00)	304 (43.00)	213 (30.13)	91 (12.87)	
Total	500 (100)	707 (100)	491 (69.45)	216 (30.55)	
Age in Years					
0-15	45 (9.00)	100 (14.14)	53 (7.50)	47 (6.65)	.21
16-30	131 (26.20)	163 (23.05)	137 (19.38)	26 (3.68)	
31-45	155 (31.00)	182 (25.74)	149 (21.07)	33 (4.67)	
46-60	100 (20.00)	141 (19.94)	91 (12.87)	50 (7.07)	
>60	69 (13.80)	121 (17.11)	61 (8.63)	60 (8.49)	
Total	500 (100)	707 (100)	491 (69.45)	216 (30.55)	
Occupation					
Schooling	76 (15.20)	83 (11.74)	77 (10.89)	6 (0.85)	.06
Farming	140 (28.00)	181 (25.60)	130 (18.39)	51 (7.21)	
Civil Servants	105 (21.00)	166 (23.48)	98 (13.58)	68 (9.62)	
Trading	120 (24.00)	179 (25.31)	118 (16.69)	61 (8.63)	
Artisan	35 (7.00)	60 (8.49)	37 (5.23)	23 (3.25)	
Others	24 (4.80)	38 (5.38)	31 (4.38)	7 (1.00)	
Total	500 (100)	707 (100)	491 (69.45)	216 (30.55)	
Educational Status					
Tertiary	144 (28.80)	236 (33.38)	142 (20.08)	94 (13.30)	.15
Secondary	180 (36.00)	276 (39.03)	173 (24.47)	103 (14.57)	
Primary	106 (21.20)	116 (16.40)	100 (14.14)	16 (2.26)	
Uneducated	70 (14.00)	79 (11.17)	76 (10.54)	3 (0.42)	
Total	500 (100)	707 (100)	491 (69.45)	216 (30.55)	

KEY: +ve= positive, -ve=negative

TABLE 2
FREQUENCY OF BACTERIA ISOLATES

Bacteria Isolates	Total No (%)
<i>Staphylococcus aureus</i>	134 (15.58)
<i>Coagulase Negative Staphylococcus</i>	71 (8.26)
<i>Streptococcus pyogenes</i>	53 (6.16)
GRAM POSITIVE TOTAL	258 (30.00)
<i>Pseudomonas aeruginosa</i>	197 (22.91)
<i>Escherichia coli</i>	179 (20.81)
<i>Klebsiella Pneumoniae</i>	106 (12.32)
<i>Salmonella typhi</i>	79 (9.19)
<i>Proteus Spp.</i>	41 (4.77)
GRAM NEGATIVE TOTAL	602 (70.00)
GRAND TOTAL	860 (100)

Key: spp= species

TABLE 3
FREQUENCY OF BACTERIA ISOLATES FROM CLINICAL SPECIMENS

Specimen type	Total no of specimen N0 (%)	Total no of Culture +ve Specimen N0 (%)	Total no of bacteria isolated (%)	P.value
Ear discharge	42 (5.94)	33 (4.66)	70 (8.14)	.32 Not significant
Sputum	37 (5.23)	30 (4.24)	69 (8.02)	
U/S	30 (4.24)	23 (3.25)	43 (5.00)	
Wound	123 (17.40)	76 (10.74)	123 (14.30)	
Urine from Catheter	47 (6.64)	44 (6.22)	75 (8.72)	
Urine	151 (21.31)	81 (11.45)	139 (16.16)	
Nasal swab	21 (2.97)	11 (1.55)	38 (4.41)	
HVS	90 (12.72)	65 (9.11)	89 (10.35)	
Stool	59 (8.34)	55 (7.77)	86 (10.00)	
Eye Swab	29 (4.10)	12 (1.69)	42 (4.88)	
Blood	81 (11.45)	61 (8.62)	86 (10.00)	
TOTAL	707 (100)	491 (69.44)	860 (100)	

KEY: Hvs= High vaginal swab, u/s= Urethral swab, +ve= positive, -ve= negative

TABLE 4
DISTRIBUTION OF BACTERIA IN DIFFERENT CLINICAL SPECIMENS

Specimen type	<i>S.aureus</i> 134	<i>CoNS</i> 71	<i>E.coli</i> 179	<i>P.aeruginosa</i> 197	<i>S.pyogenes</i> 53	<i>K.pneumoniae</i> 106	<i>S.typhi</i> 79	<i>Proteus spp</i> 41	Total 860 (%)
Urine	28 (3.26)	19 (2.21)	22 (2.56)	27 (3.14)	15 (1.74)	22 (2.56)	0 (0)	6 (0.70)	139 (16.16)
Nasal Swab	10 (1.60)	10 (1.60)	12 (1.40)	2 (0.23)	1 (0.12)	3 (0.35)	0 (0)	0 (0)	38 (4.44)
U/S	4 (0.47)	1 (0.12)	16 (1.86)	5 (0.58)	2 (0.23)	11 (1.28)	0 (0)	4 (0.47)	43 (5.00)
HVS	19 (2.21)	7 (0.81)	17 (1.98)	17 (1.98)	6 (0.70)	17 (1.98)	0 (0)	6 (0.70)	89 (10.35)
Catheter (urine)	9 (1.04)	8 (0.93)	6 (0.70)	42 (4.88)	3 (0.35)	6 (0.70)	0 (0)	1 (0.12)	75 (8.72)
Blood	20 (2.33)	2 (0.23)	7 (0.81)	7 (0.81)	10 (1.60)	15 (1.74)	20 (2.33)	5 (0.58)	86 (10.00)
Eye Swab	12 (1.40)	6 (0.70)	7 (0.81)	7 (0.81)	5 (0.58)	5 (0.58)	0 (0)	0 (0)	42 (4.88)
Stool	0 (0)	0 (0)	27 (3.14)	0 (0)	0 (0)	0 (0)	59 (6.86)	0 (0)	86 (10.00)
Sputum	14 (1.63)	3 (0.35)	19 (2.21)	10 (1.60)	3 (0.35)	10 (1.60)	0 (0)	10 (1.60)	69 (8.02)
Ear swab	6 (0.70)	3 (0.35)	5 (0.58)	29 (3.37)	4 (0.47)	15 (1.74)	0 (0)	8 (0.93)	70 (8.14)
Wound	12 (1.40)	12 (1.40)	14 (1.63)	51 (5.93)	4 (0.47)	2 (0.23)	0 (0)	1 (0.12)	123 (14.30)
Total	134 (15.58)	71 (8.26)	179 (20.12)	197 (22.91)	53 (6.16)	106 (12.33)	79 (9.18)	41 (4.77)	860 (100)

TABLE 5
PREVALENCE OF ISOLATED BACTERIA ACCORDING TO PATIENTS IN DIFFERENT HOSPITAL WARDS

Bacterial Isolated	Total No (%)	MMW No (%)	FMW No (%)	AW No (%)	FSW No (%)	MSW No (%)	OPD No (%)	CW No (%)	EW No (%)	PNW No (%)
<i>S. aureus</i>	134 (15.58)	31 (3.60)	28 (3.26)	7 (0.81)	4 (0.47)	11 (1.28)	16 (1.86)	19 (2.21)	13 (1.51)	5 (0.58)
<i>CoNS</i>	71 (8.26)	14 (1.62)	12 (1.40)	14 (1.62)	12 (1.40)	4 (0.47)	4 (0.47)	1 (0.12)	8 (0.93)	2 (0.23)
<i>E. coli</i>	179 (20.81)	29 (3.37)	28 (3.26)	11 (1.28)	19 (2.21)	18 (2.09)	19 (2.21)	27 (3.14)	20 (2.33)	8 (0.93)
<i>P. aeruginosa</i>	197 (20.91)	37 (4.30)	37 (4.30)	7 (0.81)	8 (0.93)	27 (3.14)	32 (3.72)	9 (1.05)	20 (2.33)	20 (2.33)
<i>S. Pyogenes</i>	53 (6.16)	15 (1.74)	11 (1.28)	1 (0.12)	0 (0.00)	10 (1.16)	10 (1.16)	1 (0.12)	3 (0.35)	2 (0.23)
<i>S. typhi</i>	79 (9.19)	17 (1.98)	15 (1.74)	1 (0.12)	0 (0.00)	14 (1.63)	14 (1.63)	2 (0.23)	9 (1.05)	7 (0.81)
<i>Proteus spp</i>	41 (4.76)	7 (0.81)	8 (0.93)	2 (0.23)	2 (0.23)	4 (0.47)	7 (0.81)	0 (0.00)	4 (0.47)	7 (0.81)
<i>K. pneumonia</i>	106 (12.33)	30 (3.49)	23 (2.67)	0 (0.00)	0 (0.00)	17 (1.98)	22 (2.56)	1 (0.12)	11 (1.28)	2 (0.23)
Total	860 (100)	180 (20.99)	162 (18.84)	43 (5.00)	45 (5.23)	105 (12.21)	124 (14.42)	60 (6.98)	88 (10.22)	53 (6.16)

KEY: Male medical ward (MMW), Female medical ward (FMW), Male surgical ward (MSW), Female surgical ward (FSW), Antenatal ward (AW), Postnatal ward (PNW), children's ward (CW), Emergency ward (EW), Outpatient department (OPD), cons= coagulase negative staphylococci, spp= species, no=number, %=percentage.

TABLE 6
ANTIBIOTICS ZONES OF INHIBITION

ANTIBIOTICS	ERY	AZN	CRO	GX	CN	P	CIP	OFX	LBC	CD	AUG	ZEM
(POTENCY)	(15 µg)	(15 µg)	(30 µg)	(30 µg)	(10µg)	(110 µg)	(5µg)	(5 µg)	(5µg)	(2µg)	(30µg)	(5µg)
Standard	$S \leq 13$	$S \leq 13$	ENT.	ENT.	$S \leq 12$	ENT.	$S \leq 15$	ENT.	ENT.	ENT.	$S \leq 19$	$S \leq 15$
	$R \geq 23$	$R \geq 18$	$S \leq 13$	$S \leq 22$	$R \geq 15$	$S \leq 17$	$R \geq 21$	$S \leq 12$	$S \leq 13$	$S \leq 14$	$R \geq 20$	$R \geq 19$
	I = 14-22	I = 14-17	$R \geq 23$	$R \geq 26$	I = 13-14	$R \geq 21$		$R \geq 16$	$R \geq 17$	$R \geq 21$		I = 16-18
			I = 20-22	I = 23—25		I = 18-20		I = 13-15	I = 14-16	I = 15-20		
			PA/Staph.	PA /Staph.		PA		Staph.	Staph.			
			$S \leq 13$	$S \leq 14$		$S \leq 14$		$S \leq 14$	$S \leq 15$			
			$R \geq 21$	$R \geq 23$		$R \geq 21$		$R \geq 18$	$R \geq 19$			
			I = 14-20	I = 15-22		I = 15-20		I = 15-17	I = 16-18			
						Staph						
						$S \leq 17$						
						$R \geq 18$						
Result	S=505	5=768	S=820	S=680	S=572	S=466	S=796	S=787	S=687	S=442	S=684	S=516
(860 isolates)	R=355	R=92	R=40	R=180	R=288	R=414	R=64	R=64	R=73	R=418	R=176	R=314

Key: S = sensitive, R = Resistant, I = Intermediate, ENT = Enterobacteriaceae, PA = *P. aeruginosa*, Staph = *Staphylococcus*, Piperacillin-Tazobactam=P, Cefixime = ZEM, Erythromycin = ERY, Augmentin = AUG, Cefotaxime = GX Levofloxacin = LBC, Azithromycin = AZN, Gentamicin=CN, Ceftriaxone = CRO, Ciprofloxacin = CIP, Clindamycin = CD, Ofloxacin = OFX, Standard zones of inhibition (CLSI, 2014)

TABLE 7
SUSCEPTIBILITY PATTERN OF ISOLATED BACTERIA

Number of Bacteria Isolates sensitive to Antibiotics													
ISOLATE	TOTAL (%)	ERY	AZN	CRO	GX	CN	P	CIP	OFX	LBC	CD	AUG	ZEM
<i>S. aureus</i>	134 (15.58)	22 (2.56)	128 (14.77)	127 (14.77)	100 (11.62)	31 (3.60)	32 (3.72)	124 (14.42)	130 (15.12)	120 (13.95)	54 (6.28)	122 (14.19)	64 (7.44)
CoNS	71 (8.26)	69 (8.02)	70 (8.14)	70 (8.14)	66 (7.67)	61 (7.09)	54 (6.28)	69 (8.02)	68 (7.91)	70 (8.14)	67 (7.79)	70 (8.14)	60 (6.98)
<i>S. Pyogenes</i>	53 (6.16)	41 (4.77)	50 (5.70)	49 (5.70)	44 (5.12)	46 (5.35)	40 (4.65)	48 (5.58)	46 (5.35)	42 (4.88)	33 (3.84)	42 (4.88)	40 (4.65)
TOTAL G +VE	258 (30)	132 (15.34)	248 (28.83)	246 (28.60)	210 (24.42)	138 (16.05)	126 (14.65)	241 (28.2)	244 (28.37)	232 (26.98)	154 (17.91)	234 (27.21)	164 (19.07)
GRAM –VE <i>E. coli</i>	179 (20.12)	141 (16.40)	161 (18.72)	170 (19.77)	121 (14.07)	108 (12.56)	67 (7.79)	162 (18.84)	166 (19.23)	88 (10.23)	72 (8.37)	121 (14.07)	111 (12.91)
<i>P. aeruginosa</i>	197 (22.91)	135 (15.70)	184 (21.40)	188 (21.86)	157 (18.26)	132 (15.35)	86 (10)	185 (21.74)	187 (21.74)	166 (19.30)	113 (13.14)	124 (14.42)	108 (12.56)
<i>K. pneumoniae</i>	106 (12.33)	35 (4.70)	96 (11.16)	100 (0.00)	82 (9.53)	94 (10.93)	92 (10.70)	97 (11.28)	91 (10.53)	92 (10.70)	31 (3.60)	88 (10.23)	66 (7.67)
<i>S. typhi</i>	79 (9.18)	21 (2.44)	39 (4.53)	77 (8.95)	71 (8.26)	61 (7.09)	34 (3.95)	70 (8.14)	58 (6.74)	68 (7.91)	32 (3.72)	76 (8.84)	27 (3.14)
<i>Proteus spp</i>	41 (4.77)	41 (4.77)	40 (4.65)	39 (4.77)	39 (4.53)	39 (4.77)	41 (4.77)	41 (4.77)	41 (4.77)	41 (4.77)	40 (4.65)	41 (4.77)	40 (4.65)
Total GRAND TOTAL RESISTANCE (%)	602 (70) 860 (100)	373 (43.37)	520 (60.47)	574 (66.65)	470 (54.47)	434 (50.47)	320 (37.21)	555 (64.14)	543 (63.14)	455 (52.91)	288 (33.49)	450 (51.36)	352 (40.93)
		505 (58.72)	768 (89.30)	820 (95.35)	680 (79.06)	572 (66.51)	446 (51.86)	796 (92.55)	787 (91.51)	687 (79.88)	442 (51.39)	684 (79.53)	516 (60.00)
		355 (41.28)	92 (10.70)	40 (4.65)	180 (20.93)	288 (33.49)	414 (48.14)	64 (7.44)	73 (8.49)	173 (20.12)	418 (48.60)	176 (20.47)	344 (40.00)

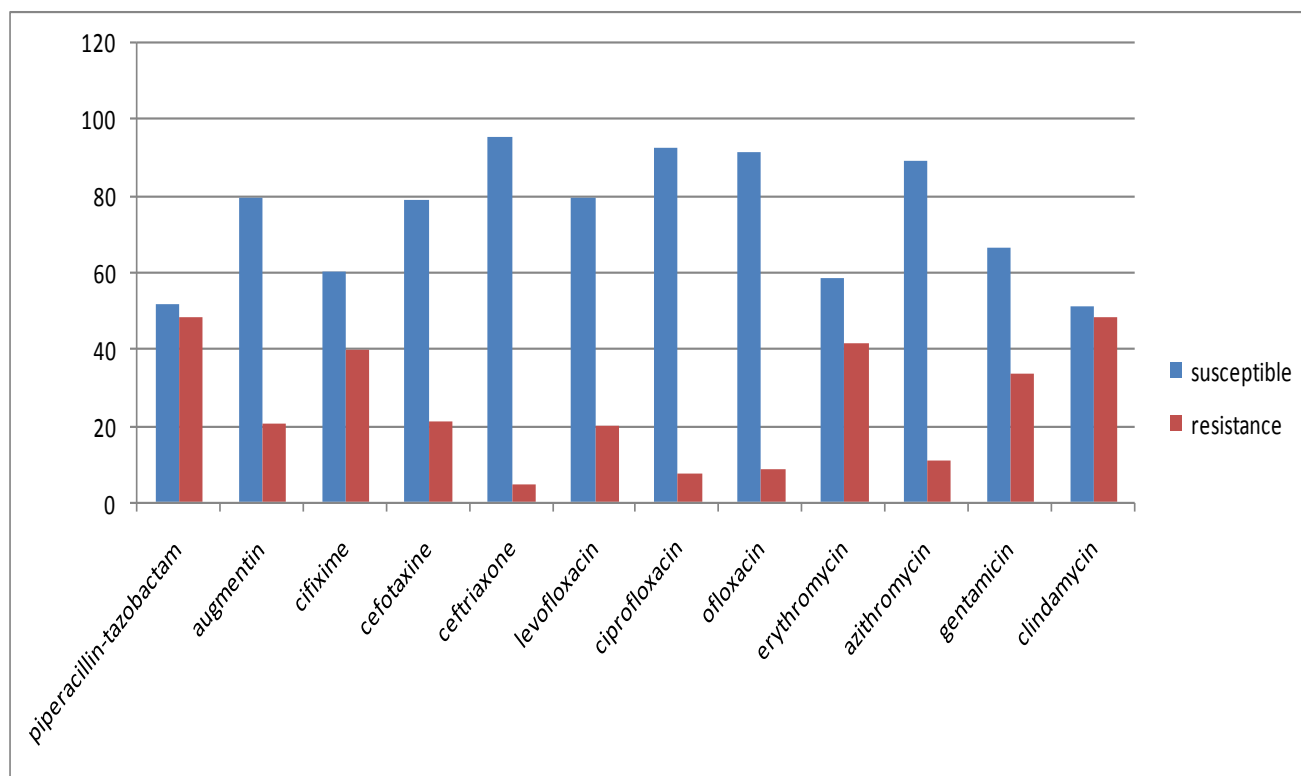


FIGURE 1: Bar chart showing percentage of the antibiotics sensitivity and resistance

TABLE 8
SUSCEPTIBILITY FREQUENCY OF THE ISOLATES TO THE ANTIBIOTICS

Isolates	Number of Isolates	Total Number of Antibiotics	Total Number of Sensitivity	Total Number of Resistance
<i>S. aureus</i>	134	1608 (15.58%)	1054 (10.21%)	554 (5.36%)
<i>CoNS</i>	71	852 (8.25%)	794 (7.69%)	58 (0.56%)
<i>S. pyogenes</i>	53	636 (6.16%)	521 (5.04%)	115 (1.11%)
<i>E. coli</i>	179	2148 (20.81%)	1488 (14.41%)	660 (6.39%)
<i>P.</i>	197	2364 (22.90%)	1765 (17.10%)	599 (5.80%)
<i>K. pneumoniae</i>	106	1272 (12.32%)	964 (9.34%)	308 (2.98%)
<i>S. typhi</i>	79	948 (9.18%)	634 (6.14%)	314 (3.04%)
<i>Proteus spp</i>	41	492 (4.76%)	444 (4.30%)	48 (0.47%)
TOTAL	806	10,320 (100%)	7664 (74.26%)	2656 (25.74%)

Key: *CoNS*= coagulase negative *Staphylococcus*, *spp*= species

Multidrug resistance by infectious agents poses a great danger and setback in a search for a suitable chemotherapy, therefore understanding the resistance pattern has become imperative in winning this war against antibiotics resistance by bacteria.

Table 1 shows that male participants were higher in number than female participants with 280 (56.00%) and 220 (44.00%) respectively. Majority of the samples were collected from the male 403 (57.00%) while the female had 304 (43.00%) samples, the male had more positive growth than the female of 278 (39.32%) and 213 (30.13%) respectively though not statistically significant with P-value at .08. The age bracket of 31-45 years had the greatest isolates of 155 (31.00%) with the P-value at .21. The groups that contributed the greatest number of samples are farmers, civil servants and traders, age group of 31-45years and participants with educational levels of secondary and tertiary. This could be attributed to the reason of being the workforce, full of energy and have families that depend on them. They are exposed to all manner of hazards on the course of their jobs, livelihood and endeavours. This is contrary to the findings of Amsalu (19) who reported that majority of their samples 288 (56.5%) were collected from females, their highest isolation rates were obtained from 10-19 years and the isolated bacteria was relatively higher in females 81 (28.1%) than males 62 (27.9%) with P value = .96.

A total of 258 (30%) Gram positive bacteria were isolated; *S. aureus*, CoNS and *Streptococcus pyogenes* while the Gram negative has a total of 602 (70%); *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* and *Proteus* spp as shown in Table 2. Amsalu (19) reported similar result/percentage that Gram negative bacteria accounted for 67.8% and the rest 32.2% were Gram positive bacteria. The most common bacteria isolated were *Escherichia coli* 35 (24.5%), *Staphylococcus aureus* 31 (21.7%) and *Klebsiella species* 21 (14.7%). A study in South Western Nigeria by Taiwo (20) agrees with the result as it revealed that Gram positive bacteria constituted 47.3% while Gram negative constituted 52.7%. The most common organisms were *Staphylococcus aureus* (37.3%), *Klebsiella* (30%), *Pseudomonas* (8.2%), *Proteus* (6.4%), *Escherichia coli* (5.5%) and coagulase negative staphylococci (4.6%).

This is also in agreement with Makanjuola (21) who reported that more than half of the infections occurring in their study were noted to be caused by Gram-negative bacteria. Although there is a wide variation in the distribution of these Gram-negative organisms, *Pseudomonas aeruginosa* appears to predominate globally which is consistent with these findings. However, Erbay (22) noted a preponderance of *Klebsiella* spp, especially *Klebsiella pneumoniae*, which accounted for close to half of the isolated organisms in their study is a common nosocomial pathogen whose rates of colonization is very high therefore likelihood of infection rises dramatically with hospitalization. Jroundi (23) revealed that the organisms most frequently isolated were *Staphylococcus* spp, *Escherichia coli* and *Klebsiella* but *Staphylococcus* remains the frequent germ found in most studies.

In this study, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, CoNS, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Proteus* Spp were isolated from different clinical specimens. However, the frequency of different bacteria isolates revealed that *Pseudomonas aeruginosa* had the greatest frequency of 22.91%, this is followed by *Escherichia coli* and *Staphylococcus aureus* with 20.81% and 15.58% respectively as shown in Table 2. This is in agreement with the study by Akindele and Afolayan, (24) and Church (25) who isolated *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Proteus* Spp. On the contrary, Demilie (26) reported that the most prevailing bacteria isolated from the various specimens in all the laboratories in their study were *E. coli* and *Staphylococcus aureus*. This could be attributed to the fact that most times *Staphylococcus aureus* and *Escherichia coli* can exist as normal flora of the skin but can become infectious when there is a break in the skin from a wound or surgery, or if there is a compromised or suppressed immune system.

The distribution of bacteria isolates according to different specimens indicated that Urine sample had the highest number of isolates with a total of 139 (16.16%) followed by wound with 123 (14.30) as shown in table 3. This is in agreement with Amsalu (19) who reported that the most frequently processed specimen in hospital laboratories was urine with 44.3%. This could be attributed to the fact that Urine is the most frequently demanded sample during the investigation of urinary tract infections therefore, there is high possibility of more isolates coming from urine when there is growth during culture.

The individual isolates from urine sample indicated that *Staphylococcus aureus* has the highest number of isolates 28 (3.26%). This was closely followed by *P. aeruginosa* with 27 (3.14%) isolates. However, *Escherichia coli* and *Klebsiella pneumoniae* had the same number of isolates 22 (2.56%) as indicated in table 4. This is in agreement with the report by Ekwealor (27) that *Staphylococcus aureus* was found to be the predominant and most frequently isolated urinary pathogen, followed by *Escherichia coli* in Awka. They further explained that studies had previously linked the increasing cause of urinary tract infections (UTIs) by *Staphylococcus* to increased use of instrumentation such as bladder catheterization. However, the observed high proportion of *Staphylococcus* varied with some previously published studies where *E. coli* was found to be the predominant urinary tract pathogen (27).

This is contrary with findings of Amsalu (19) who reported that predominant isolate from urine was *Escherichia coli* (42.9%) followed by *Klebsiella pneumoniae* (12.7%) and *Staphylococcus aureus* (12.7%). However, this difference might be explained by difference in geographic area and time of study; while Amsalu (19) conducted theirs between January 2012 and December 2014 in Southern Ethiopia, our research was conducted between December 2018 and May 2019 in Awka, Nigeria.

Other urinary tract samples had the following figures as shown in table 5; High vaginal swab had *Staphylococcus aureus* 19 (2.21%) while *Pseudomonas aeruginosa* and *Escherichia coli* had 17 (1.98%) isolates each. For Urethral swab, *Escherichia coli* had 16 (1.86%) while *Klebsiella pneumoniae* had 11 (1.28%) and *Staphylococcus aureus* 4 (0.47%). Urine from catheter; *Pseudomonas aeruginosa* had 42 (4.88%), *Staphylococcus aureus* 9 (1.04%) and *Escherichia coli* had 6 (0.70%) as shown in table 5. This is in line with the findings of Kolawole (28) who reported that the most common pathogenic

organisms of urinary tract infection (UTI) are *Escherichia coli*, *Staphylococcus aureus*, *Proteus spp.*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa*.

The pattern of organisms isolated from urinary tract samples had *Pseudomonas aeruginosa* as the highest isolate. This is followed by *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Coagulase Negative Staphylococcus (CoNS)* and *Proteus Spp.* as the least isolates. This is similar to the findings of Muhammed (29) who reported that *Escherichia coli* and *Staphylococcus aureus* were the main isolates from all the laboratories in their research. Amin (30) also reported that the most predominantly isolated bacteria were *Staphylococcus aureus* and *Escherichia coli* however *Escherichia coli* has been the most frequently reported isolate causing urinary tract infections in similar studies. This is also in line with the confirmation by Demilie (26) who reported that *Escherichia coli* is the primary etiologic agent causing urinary tract infection, accounting for 90% of the cases in their study. Rachid (31) identified that the predominant organisms were *Staphylococcus* (18.7%) followed by *Escherichia coli* (14.7%) and *Klebsiella pneumoiae* (14.7%). The site of infection most frequently affected by *Staphylococcus* was urinary tract (42.9%). However, the urinary tract samples (Urine 16.16, HVS 10.35%, U/S 5.00% and urine from catheter 8.72%) when combined had the highest frequency of 30.23% isolates. This is closely related to the work of Unegbu (32) who reported that urinary tract infection has the highest prevalence of 36.33%. The reason for the high frequency of urinary tract infection could be attributed to poor hygiene in the hospital environment especially for the admitted patients who had no choice but to share the rest rooms with other patients who might take issues of hygiene for granted.

Wound sample had a total of 123 (14.30%) isolates with *Pseudomonas aeruginosa* at 51 (5.93%) as the highest isolate as indicated in table 5. This is followed by *Escherichia coli* with 14 (4.7%), *Staphylococcus aureus* had 12 (1.40%) and CoNS 12 (1.40%). This is in line with the findings of Motayo (33) who reported that high prevalence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* infections maybe because of an endogenous source of the infection. Infection with these organisms may also be due to contaminations from the environment. With the disruption of the natural skin barrier, *Staphylococcus aureus* may easily find their way into wounds. Basu (34), suggested that the relatively high number of *Pseudomonas aeruginosa* isolates is suggestive of the high level of nosocomial infections particularly in hospitalized patients, again, bringing up the need for strict infection control practices and good hygiene such as frequent hand washing and sterilization of wounds cleaning instruments by wound care givers. The study by Ehiaghe (35) revealed that wound swab had the highest number of clinical isolates of *Pseudomonas aeruginosa*, this may be due to surgical wounds are exposed to many ubiquitous environmental pathogens which include unsterile surfaces, water and soil.

On the contrary, Shittu (36) reported that their microbiological analysis of wound samples revealed that *S. aureus* was the leading etiologic agent of wound infection in many health institutions in their study area.

A total of 86 (10.00%) bacteria were isolated from blood samples. These include *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, CoNS, *Streptococcus pyogenes* and *Klebsiella pneumoniae*. In the study, we found out that *staphylococcus aureus* and *Salmonella typhi* had the highest number of isolates 20 (2.33%), *Klebsiella pneumoniae* had 15 (1.74%) and *Streptococcus pyogenes* had 10 (1.60%). This is closely related to the report by Mehta (37) that *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi*, CoNS and *Klebsiella pneumoniae* as the commonest pathogens implicated in septicemia. On the contrary, Alam (38) reported that Gram-positive pathogens; Coagulase-negative staphylococci, *Staphylococcus aureus* and Gram negative *Salmonella typhi* were the major causative organisms of septicemia.

A study by Falagas (39) agrees with this as they reported coagulase-negative staphylococci the commonest cause of septicemia. However, Onile (40) reported *Salmonella typhi* as the predominant pathogen. This difference could be attributed to the changing etiology of blood stream infection with the selection of drug resistant bacteria isolates that were better adapted for survival according to Taiwo (20).

We found out that Stool samples had 86 (10.00%) isolates as indicated on table 5, however, only two organisms were isolated *Salmonella typhi* 59 (6.86%) and *E. coli* 27 (3.14%). Amsalu (19) slightly agree with this as they isolated only *Salmonella typhi* 41.70% and *Shigella spp* 58.39%. On Nasal swab, we had 38 (4.44%) isolates which included the following organisms were; *E. coli* 12 (1.40%), *Klebsiella pneumoniae* 3 (0.35%), *S. aureus* 10 (1.60%), *P. aeruginosa* 2 (0.23%). This is in agreement with Amsalu (19) isolated; *E. coli* 16.7%, *Klebsiella pneumonia* 58.3%, *P. aeruginosa* 8.3% and *Proteus Spp* 8.3%.

Our findings on isolates in relation to different hospital wards as indicated in Table 5 revealed that the male medical ward (MMW) had the highest number of isolates 180 (20.99%) followed by female medical ward (FMW) with a total of 162 (18.84%) isolates and Outpatients Department (OPD) with 124 (14.42%) isolates. The Antenatal ward (AW) with 43 (5.00%) has the least number of isolates. In general, the medical wards (MMW and FMW) had the highest number of isolates 342 (39.77%), this is followed by the surgical wards (FSW and MSW) which has a total of 150 (17.44%) isolates. This is in line with Rachid (31) who reported that medical and surgical wards had the highest number of infections 392 (32.8%) and 379 (31.7%) respectively. However, Lizioli (41) reported the Surgical site infections appear higher compared to what is reported in several regions of the world.

The susceptibility test conducted on all the 860 isolates as compared to the standard, their antibiotics zones of inhibition as shown in Table 6 indicate Erythromycin exhibited susceptibility against 505 isolates and resistance against 355 against isolates. Ofloxacin had susceptibility against 787 isolates and resistance against 64 isolates. Augmentin was susceptible against 684 isolates while resistant against 178 isolates.

The following were the percentage of resistance exhibited by Piperacillin-Tozabactam (P) (48.14%), Clindamycin (CD) (48.60%), Erythromycin (ERY) (41.28%), Cefixime (ZEM) (40.00%) and Gentamicin (CN) (33.49%) as shown in figure 7.

Since a greater percentage of the isolates in this study were sensitive to Ceftriaxone, ciprofloxacin and Azithromycin as shown in Figure 1, they would be a good choice for empiric therapy of most bacterial infections within the study area while waiting for the result of culture and sensitivity tests. It is worthy of note that, majority of the isolates were resistant to Clindamycin, Erythromycin and Piperacillin-tozabactam. This high level of resistance observed against the drugs may be attributed to the irrational use of drug in this locality. The increasing level of abuse of drugs by the public, where patients indulge in antibiotic self-medication to treat all kinds of infections, has been recorded as one significant way of promoting antibiotic resistance according to Ugwu (42).

Madigan (43) reported that Clindamycin was most commonly used within the clinic due to its higher bioavailability, higher oral absorption and efficacy within the target organism spectrum. It is also the first-choice use antibiotic in veterinary microbiology, this indiscriminate use of Clindamycin both for human and veterinary purposes may as well be the reason for high resistant development. Schlünzen (44) suggested that the high resistance could be attributed to the inability of some of the antibiotics to pass through the porins of Gram-negative isolate therefore may not get to their target site. Inappropriate use of antibiotics, use of substandard brands of antibiotics may also be a factor in antibiotic resistance.

Pseudomonas aeruginosa is the most sensitive isolate in this study, having good sensitivity to most of the antibiotics tested as indicated in table 8. It was also found that some of the organisms were susceptible at varying degrees to the antibiotics used. Ceftriaxone (CRO) which is a cephalosporin has the highest sensitivity of 95.35%. This is followed by a fluoroquinolone ciprofloxacin (CIP) with sensitivity of 92.55%, a macrolide; Azithromycin (AZN) has sensitivity of 89.30%. This slightly agrees with the report of Orhue (45) which observed the Ciprofloxacin has the greatest sensitivity of 80%. The predominant isolates in this study showed different degrees of resistance to most drugs used including *Pseudomonas aeruginosa* (5.80%), *Escherichia coli* (6.39%), *Staphylococcus aureus* (5.36%) and *Klebsiella pneumoniae* (2.98%) as shown in table 8.

Augmentin also exhibited resistance to an extent and it is worthy of note that Augmentin (AUG) (amoxicillin-clavulanic acid) (20.47%) and piperacillin-tozabactam (P) (48.14%) belong to penicillin family. According to Hitchings (46), some penicillins are naturally resistant to certain beta-lactamases and are called penicillinase-resistant penicillins. Others, such as amoxicillin, ampicillin, and piperacillin can have their activity extended by combining them with a beta-lactamase inhibitor like clavulanate, sulbactam, and tozabactam. However, Brooks (1) pointed out that resistance exhibited by penicillins may be because of the absence of some penicillin binding proteins which occurs as a result of chromosomal mutation or failure of the B-lactam drug to activate autolytic enzymes in the cell wall which inhibits the organisms such as in *staphylococci* and *streptococci*.

Ekwealor (27) explained that Clavulanic Acid present in the Amoxicillin-Clavulanic Acid complex is meant to afford protection to the -lactam chemical ring nucleus present in the Amoxicillin, and this protection should be expected to enhance the activity of Amoxicillin. Hence, the Amoxicillin-Clavulanic Acid complex should demonstrate clearly significant susceptibility rates over the isolates. Ugwu (42) further explained that observed resistance against Amoxicillin-Clavulanic and piperacillin-tozabactam could be related to permeability and absorption factors influencing antibiotic transfer across the

microbial cells. Thus, the Amoxicillin-Clavulanic Acid complex (Augmentin) being a large molecule possibly would experience great difficulty in permeability and overall transport across the microbial cell wall. As a result, high resistance may be due to the relatively limited quantity available to exert an antimicrobial effect.

IV. CONCLUSION

This work on antibiogram profile of bacteria from different clinical specimens revealed that an increase in antibiotics resistance has made it necessary for the updating of information on antibiotics susceptibility pattern of bacterial isolates in order to determine appropriate empirical and definitive therapy. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Klebsiella pneumoniae* were the predominant bacteria isolates from different clinical specimens in this study.

The relatively high number of *P. aeruginosa*, *S. aureus* and *E. coli* isolates is suggestive of the high level of nosocomial infections particularly in hospitalized patients making the spread of infectious agents very easy among them. This again brings up the need for strict infection control practices and good hygiene.

The high level of resistance observed with piperacillin-Tozabactam, Cefixime, Erythromycin, Gentamicin and clindamycin can be attributed to the irrational use of drug in the study area. This is also a pointer to a situation where patients indulge in antibiotic self-medication to treat all kinds of infections. Reducing the length of stay in the hospital and duration of invasive devices like catheter can equally reduce the rate of the infection since most of them can be nosocomial.

There should be continuous monitoring, periodical research on antibiotics susceptibility pattern of these bacteria implicated and isolated from different clinical samples and conducting of microscopy, culture and sensitivity (MCS) before definitive treatment of bacteria related infections.

COMPETING INTERESTS

The authors have declared that no competing interests exists regarding the publication of this paper.

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among the authors. Author OEL designed the study and managed the analyses while Author KCC wrote the protocol, the first draft of the manuscript, managed the literature searches and also performed the statistical analysis. All authors read and approved the final manuscript.

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