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Microbiological Assessment and Comparison of Some Commercially Available Antibiotic Sensitivity Discs in Port Harcourt, Nigeria

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Abstract:
This study was carried out to assess and compare some antibiotic sensitivity discs commonly available in Port Harcourt, Nigeria. Antibiotic discs from MAXI, NEW IMPROVED, RAPID, ABTEK and OPTU were compared using OXOID as the standard. A total of four clinical isolates (Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, and Streptococcus pneumoniae) and two standard strains Escherichia coli, ATCC25922 and Streptococcus pneumoniae ATCC 25923 were tested for their sensitivity to Ciprofloxacin, Erythromycin, Gentamicin and Amoxicillin-clavulanic acid. Disc diffusion method was used to determine the sensitivity of the test organisms to the various discs. The data showed variations for all six manufacturers of Antibiotic Disc when compared with OXOID Disc. Laboratory survey showed that 71.9% of the laboratories used OPTU disc, 21.09% used MAXI disc and 7.01% used ABTEK disc.

Keywords: *Antibiotic sensitivity disc, OXOID Disc, E. coli, P. aeruginosa, S. aureus S. pneumonia, ciprofloxacin, erythromycin, gentamicin and amoxicillin-clavulanic acid*

1. Introduction

The discovery of antibiotics revolutionised the management of infectious diseases. The introduction of penicillin, for example, yielded dramatic results in the management of several infections due to susceptible organisms. At that time, many infections could be successfully treated empirically based on the clinician's past clinical experience. However, this is becoming more of the exception than the rule (Walker, 2007) as the overuse and misuse of antibiotics has led to the emergence of resistance to these life-saving drugs over time. Antimicrobial resistance has become a global phenomenon as it has been observed to occur to essentially all of the antimicrobial agents currently approved for use in human and veterinary medicine. This, combined with the variety of antimicrobial agents currently available, makes the selection of an appropriate agent an increasingly more challenging task. Consequently, it has become imperative for clinical microbiologists to provide clinicians with accurate information necessary for the selection of appropriate antibiotics for patient therapy and care. In order to arrive at prompt and accurate therapeutic decisions clinicians are now, more than ever before, dependent on data from in vitro antimicrobial susceptibility testing thus highlighting the importance of the diagnostic laboratory in clinical practice.

Susceptibility testing is also an important first step in providing surveillance data for use in local and national aggregate databases (Mendez et al., 2000; Matynia et al., 2005; Sandle, 2005). Susceptibility testing is performed daily in diagnostic laboratories. A number of different standard methods for antimicrobial susceptibility testing (AST) are available to determine bacterial susceptibility to antimicrobials. However, disc diffusion method has been extensively used for this objective (Jorgensen et al., 2007) and appears to be a method of choice for the clinical microbiologists for in vitro antimicrobial susceptibility testing.

Disc diffusion is described as the diffusion of antimicrobial agents of a specified concentration from discs, tablets or strips, into a solid culture medium that has been seeded with the selected inoculum isolated in a pure culture. Disc diffusion is based on the determination of a zone of inhibition proportional to the susceptibility of a bacterial isolate to the antimicrobial present in the disc. The method is simple and practical and has been well-standardised (Jorgensen et al., 2007; CLSI, 2009). This test is performed by applying a bacterial inoculum of approximately 1-2 x 10^8 cfu /ml to the surface of a large (150 mm in diameter) Mueller-Hinton agar plate. Up to 12 commercially prepared, fixed concentrations, paper antibiotic discs are placed on the inoculated agar surface. Plates are incubated for 16-24 hours at 35^0C prior to determination of results.

Quality assurance must be applied for antimicrobial susceptibility testing using internal quality control protocols for monitoring of precision and accuracy of the methods (Kahlmeter *et al*., 2002)Furthermore, quality control in AST helps in concurrently monitoring the performance of the test and ensures that the test is performed properly with resultant improvement in treatment outcome. AST result can affect both the clinician's choice of antimicrobial agent and patient outcome, since the patient may not receive optimal care if treatment is based on unreliable laboratory test report. The laboratory plays a crucial role in helping clinicians to choose appropriate antimicrobial agents for treating infections (Cunney et al., 2000). Thus, laboratories that report result for any antimicrobial agent without censoring inappropriate results may encourage inappropriate antimicrobial use and compromise programme designed to promote good antibiotic stewardship. Clinicians depend heavily on information from the clinical microbiology laboratory for treatment of their seriously ill patients. The clinical importance of antimicrobial susceptibility test results, therefore, requires that these tests be performed under optimal conditions and that laboratories have the capability to provide results for the newest antimicrobial agents. Air travel and mass movement of people from one part of the globe to another has contributed in no small measure to the spread of antimicrobial resistance. In combating this problem, it is desirable to have uniform standards with respect to concentration and variety of antimicrobial agents in antibiotic susceptibility discs. This will help to ensure that antibiotic susceptibility test results are reproducible and applicable elsewhere. For this to be achieved the need to have minimum standards of knowledge, capability and expertise of medical laboratories and standard antibiotic sensitivity discs in Port Harcourt cannot be overemphasized.

This study, therefore, set out to determine the performance, variety and concentration of antimicrobial agents in the commonly used antibiotic sensitivity discs in Port Harcourt metropolis and to compare their sensitivity/quality with imported ones using OXOID as a standard.

2. Materials and Method

2.1. Collection of Test Organisms

Clinical isolates of Staphylococcus aure us, Pseudomonas aureginosa, Escherichia coli, and Streptococcus pneunomiae were obtained from the Medical Microbiology Laboratory of the University of Port Harcourt Teaching Hospital, Port Harcourt. The organisms were collected in sterile agar slants, using a sterile loop. The slants were then incubated at 37^{0}C for 24 hours for all the organism collected. Typed cultures of Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were obtained from Department of Microbiology, Olabisi Onabanjo University Teaching Hospital, Ogun State.

2.1.1. Study Design

Fifty-Seven (57) medical laboratories around Port Harcourt were visited and information concerning antibiotic sensitivity disc, how the antibiotic discs were stored, and organisms commonly isolated and level of training of staff was obtained with the aid of a standard questionnaire.

2.1.2. Materials

These include the reagents, equipment, media and test organisms used in the course of the study. Other material includes inoculation wire loop, disposable sterile Petri dishes, universal bottles, MacCartney bottles, sterile forceps, antibiotics sensitivity discs (ABTEK, RAPID, OXOID, OPTU, NEW IMPROVED, MAXI and FON), transparent ruler, aluminium foil, cotton wool, measuring cylinder, and Bunsen burner.

2.1.3. Equipment

These include water bath (Techmel and Techmel, USA), autoclave (New Life DHG-90Z3A, England), incubator (Memmert, UK), Sensitive Balance (HCK by Dispel), and Refrigerator (LG 131).

2.1.4. Biochemical Test

Biochemical characterization of isolated organisms was done following standard methods. Isolates were tested for catalase and coagulase activity or reaction.

2.1.5. Catalase Test

A drop of 3% of hydrogen peroxide was placed on a clean greases-free glass slide. A colony of the test organism was emulsified in the drop of the reagent. Evolution of gas bubbles was an indication of a positive result while the absence of gas bubbles was a negative result.

2.1.6. Coagulate Test

About 2ml of blood from human sample was placed in a tube, followed by a loop ful of the isolate. A test that shows any degree of clotting within 24 hours of incubation at 37^{0}C is considered Coagulase positive.

2.2. Standardization of Test Organism

A sterile loop was used to inoculate the test organism into a universal bottle containing 9ml peptone water. The turbidity of the culture was adjusted to 0.5 McFarland Standard (1-2 x10^7 cfu/ml 0.5 McFarland Standards). This procedure was repeated for test organisms used.

2.3. Agar Disc Diffusion Method

Three Clinical isolates each of Staphylococcus aureus (from urine, high vaginal swab (HVS) and throat), Pseudomonas aeruginosa (from ear, sputum and wound) Escherichia coli (from wound, semen and urine) and an isolate of Streptococcus pneumonia (from throat) were used in carrying out the disc diffusion test using the modified technique commonly called Kirby Bauer Test (Bauer et al., 1959; 1966). The multidiscs (ABTEK, RAPID, OXOID, OPTU, NEW IMPROVED, MAXI and FON) were sliced aseptically and used singly.

A sterile cotton swab was placed in the bacterial suspension and the excess fluid removed by pressing and rotating the cotton against the inside of the tube above the fluid level. The swab was streaked in at least three directions over the surface of the Mueller-Hinton agar to obtain uniform growth. The plates were allowed to dry for five minutes. Using a sterile forcep, the antibiotic disk was placed on the Mueller-Hinton agar plate. The plates were allowed to stand for about 15 minutes after which they were incubated at 37^{o}Cfor 24 to 48 hours. The tests were done in triplicate. Zones of inhibition of growth were measured to the nearest whole millimetre. Following overnight incubation, the diameter of the zones of inhibition of growth obtained around each disc was used to describe the organism as resistant, intermediate, or susceptible to the antibiotics tested according to the Clinical Laboratory Standards Institute (CLSI) guideline.

2.4. Statistical Analysis

The result was expressed in mean IZD ± SEM (Standard error of Mean) and data subjected to student's t-test using SPSS Version 20. A p-value less than or equal to 0.05 was considered to be statistically significant ($p \leq 0.05$).

3. Results

Fifty-seven laboratories were visited and the antibiotic sensitivity discs in common use were as shown in Fig.1 below.

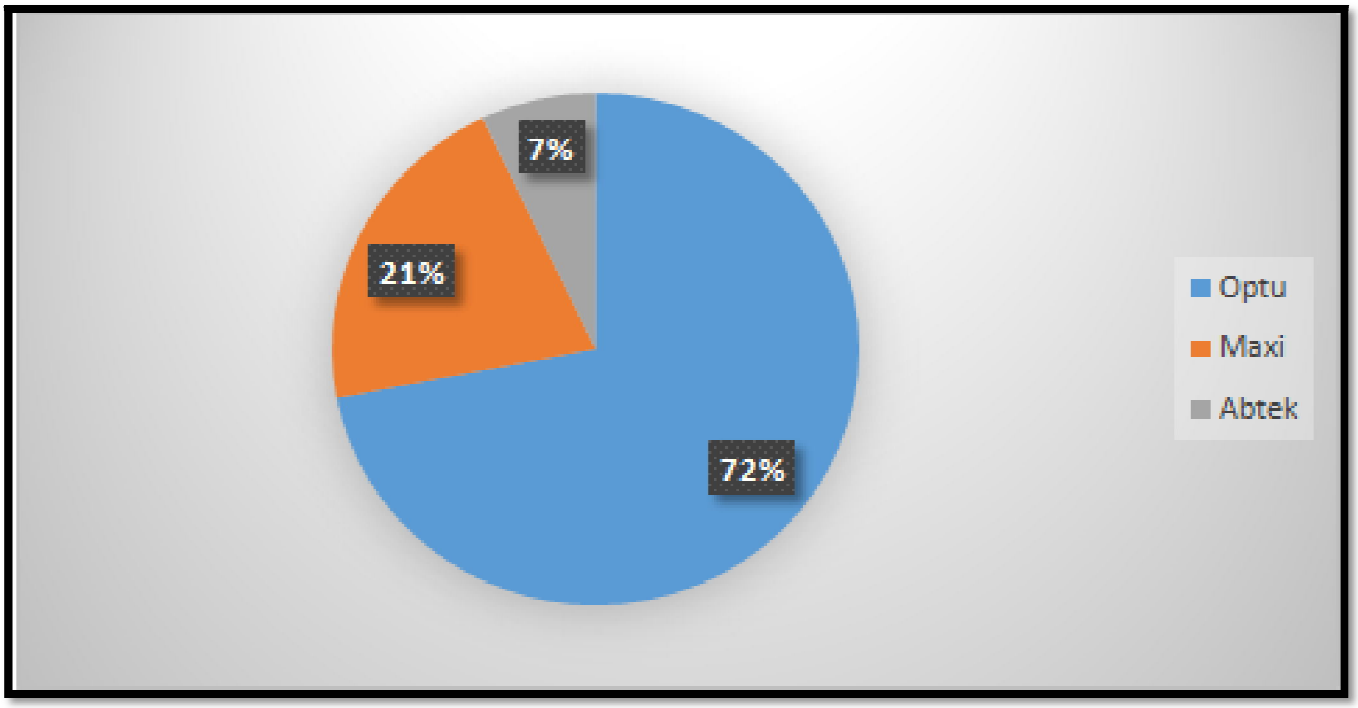

Figure 1: Percentage Distribution of Commonly Used Antibiotic Sensitivity Discsavailable in Port Harcourt

	Discs	Rapid			Abtek			Optu			Fon			Maxi			New Improved			Oxoid		
Source		Gen 10ug	Cip 5ug	Aug 30ug	Gen 10ug	Cip 5ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 5ug	Aug 30ug
Wound	IZD in mm	16	16	-	15	20	-	23	30	30	-	-	-	20	30	11	11	24	-	15	24	14
		15	15	-	15	23	-	17	33	28	11	21	-	20	31	10	14	25	-	15	24	12
		15	15	-	13	22	-	20	25	16	10	20	-	18	30	8	10	28	-	13	25	14
	Mean ± SEM	15.33 ± 0.2	15.33 ±0.2*	-*	14.33 ± 0.4	21.66 ± 0.5	-*	20.00 ± 1.1	29.33 ± 1.5	24.66 ± 2.8	10.5 ± 0.2	20.50 ± 0.2	-*	19.33 ± 0.4	30.33 ± 0.2	9.66 ± 0.5	11.66 ± 0.7	25.6±0.7	-*	14.3 ± 0.4	24.00 ± 0.3	13.33 ± 0.4
Semen	IZD in mm	12	12	-	6	21	-	30	29	25	-	20	-	18	30	6	-	24	-	15	25	15
		11	11	-	6	21	-	25	30	19	-	24	-	18	26	11	-	25	-	8	28	10
		10	10	-	5	20	-	28	25	20	-	22	-	15	28	5	-	28	-	10	25	8
	Mean ± SEM	11.00 ± 0.3	11.00 ±0.3*	-*	5.66 ± 0.2	20.66 ±0.2*	-*	27.66 ±0.9*	28.00 ± 0.9	21.33 ± 1.2	-*	22.00 ± 0.7*	-*	17.00 ± 0.6	28.00 ± 0.7	7.33 ± 1.2	-*	25.6 ± 0.7	-*	11.0± 1.3	26.00 ± 0.6	11.00 ± 1.3
Urine	IZD in mm	15	15	-	15	31	-	25	35	20	11	26	-	30	29	22	-	24	17	16	30	12
		15	15	17	10	30	-	24	30	19	12	25	-	26	35	21	-	25	18	15	28	-
		13	13	-	11	28	-	24	32	20	10	29	-	22	36	24	-	22	15	15	25	10
	Mean ± SEM	14.33 ± 0.4	14.33 ±0.4*	17.0	12.00 ± 0.9	29.66 ± 0.5	-	24.33 ±0.2*	32.33 ± 0.9	19.66 ± 0.2	11.00 ±0.3*	26.66 ± 0.7	-	26.00 ±1.5*	33.00 ± 1.4	22.3 ± 0.5	-*	23.7 ± 0.5	16.6 ± 0.5*	15.3 ± 0.2	27.66 ± 0.9	11.00 ± 0.5

Table 1: Inhibition Zone Diameter of Gentamicin, Ciprofloxacin and Amoxicillin/Clavulanic Acid for E. coli Compared with CLSI Breakpoint for Gentamicin, Ciprofloxacin and Amoxicillin/Clavulanic Acid

*CLSI: Clinical and Laboratory Standards Institute, IZD: Inhibition Zone Diameter *: Significant Difference at P≤ 0.05, SEM: Standard Error of Mean, -: No IZD,*

Gen 10ug, ≥ 15mm=Susceptible; 13-14mm=Intermediate; ≤ 12 Mm=Resistant

CIP 5ug; ≥ 21mm =Susceptible; 16-20mm=Intermediate; ≤ 15 Mm=Resistant

Aug 30ug; ≥ 18mm=Susceptible; 14-17mm=Intermediate; ≤ 13 Mm=Resistant

Discs	Rapid			Abtek			Optu			Fon			Maxi			New Improved			Oxoid		
	Gen 10ug	Cip 5ug	Aug 30ug	Gen 10ug	Cip 5ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 10ug	Aug 30ug	Gen 10ug	Cip 5ug	Aug 30ug
IZD in mm	17	30	10	17	30	-	30	35	25	17	35	-	25	35	20	15	30	21	20	30	-
	19	28	9	18	30	10	31	35	24	21	30	4	25	36	21	22	31	20	21	30	-
	18	28	-	18	31	11	30	34	23	21	31	5	25	34	23	18	30	22	20	29	-
Mean ± SEM	18.00 ± 0.3*	28.66 ± 0.4	9.50 ± 0.2	17.66 ± 0.2*	30.33 ± 0.2	10.50 ± 0.2	30.33 ± 0.2*	34.66 ± 0.2*	24.00 ± 0.3*	19.66 ± 0.8	32.00 ± 0.9	4.50 ± 0.2	25.00 *	35.00 ± 0.3*	21.33 ± 0.5*	18.33 ± 1.3	30.33 ± 0.2	21.00 ± 0.5*	20.33 ± 0.2	29.66 ± 0.2	-

Table 2: Inhibition Zone Diameter of Gentamicin, Ciprofloxacin and Amoxicillin/Clavulanic Acid for E.Coli ATCC29922 Compared With CLSI Breakpoint for Gentamicin, Ciprofloxacin and Amoxicillin/Clavulanic Acid
*IZD: Inhibition Zone Diameter *: Significant Difference at P≤ 0.05, SEM: Standard Error of Mean, -: No IZD, Gen: Gentamicin: Gen 10ug=14-26mm, CIP: Ciprofloxacinn :Cip 5ug=30-40mm, Aug: Amoxicillin/Clavulanic Acid: Aug 30ug=18-24mm*

	Discs	Rapid		Abtek		Optu		Fon		Max		New Improved		Oxoid	
Source		Gen 10ug	Cip 5ug	Gen 10ug	Cip 5ug	Gen 10ug	Cip 10ug	Gen 10ug	Cip 10ug	Gen 10ug	Cip 10ug	Gen 10ug	Cip 10ug	Gen 10ug	Cip 5ug
Wound	IZD in mm	15	20	16	34	29	50	15	30	25	34	15	34	22	34
		10	22	17	25	25	48	16	34	25	30	13	30	20	32
		14	18	19	31	22	44	18	33	29	30	15	33	18	30
	Mean ± SEM	13.00 ±0.9*	20.00 ±0.7*	17.33 ± 0.5	30.00 ± 1.7	25.33 ± 1.3*	47.33 ±1.1*	16.33 ±0.5*	32.33 ± 0.7	26.33 ± 0.8*	31.33 ± 0.8	14.33 ±0.4*	32.33 ± 0.7	20.00 ± 0.7	32.00 ± 0.7
Sputum	IZD in mm	15	35	15	26	30	50	10	30	24	42	-	38	21	35
		15	32	15	33	29	48	10	35	25	44	-	34	20	33
		14	31	17	35	25	45	9	30	25	39	-	34	22	30
	Mean ± SEM	14.66 ± 0.2*	32.66 ± 0.7	15.66 ± 0.4*	31.33 ± 1.7	28.00 ± 0.9*	47.66 ± 0.9*	9.66 ± 0.2*	31.66 ± 1.0	24.66 ± 0.2*	41.66 ± 0.9*	-*	35.33 ± 0.8	21.00 ± 0.3	32.66 ± 0.9
Ear	IZD in mm	13	30	14	30	26	35	-	25	20	44	-	34	20	30
		10	31	12	29	30	30	-	27	21	46	-	31	18	31
		-	30	11	31	28	30	-	22	20	44	-	30	18	34
	Mean ± SEM	11.50 ± 0.8*	30.50 ± 0.2*	12.33 ± 0.5*	30.00 ± 0.3	28.00 ± 0.7*	31.66 ± 1.0	-*	24.66 ± 0.9*	20.33 ± 0.2	44.66 ± 0.4*	-*	31.66 ± 0.7	18.66 ± 0.4	31.66 ± 0.7

*Table 3: Inhibition Zone Diameter of Gentamicin and Ciprofloxacin for P. Aeruginosa Compared with CLSI Breakpoints for Both AgentsCLSI: Clinical and Laboratory Standards Institute, IZD: Inhibition Zone Diameter *: Significant Difference at P≤ 0.05, SEM: Standard Error of Mean, -: No IZD, Gen: Gentamicin: Gen 10ug, ≥ 15mm=Susceptible; 13-14mm=Intermediate; ≤ 12 Mm=Resistant, CIP 5ug; ≥ 21mm =Susceptible; 16-20mm=Intermediate; ≤ 15 Mm=Resistant*

	Discs	Rapid		Abtek		Optu			Fon			Max			New Improved			Oxoid		
Source		Gen 10ug	Ery 30ug	Gen 10ug	Ery 30ug	Gen 10ug	Ery 30ug	Cip 10ug	Gen 10ug	Ery 30ug	Cip 10ug	Gen 10ug	Ery 10ug	Cip 10ug	Gen 10ug	Ery 10ug	Cip 5ug	Gen 10ug	Ery 15ug	Cip 5ug
Urine	IZD in mm	23	-	17	12	27	35	35	15	15	24	26	29	35	17	-	30	25	25	30
		20	-	20	13	30	30	39	17	-	18	30	30	35	15	-	35	24	20	26
		20	-	18	11	25	30	36	16	-	19	25	28	33	19	-	30	20	23	27
	Mean ± SEM	21.00 ± 0.6	-*	18.33 ± 0.5	12.00 ± 0.3	27.33 ± 0.9	31.66 ± 1.0	36.66 ±0.7*	16.00 ±0.3*	15.00 *	20.33 ±1.2*	27.00 ± 0.9	29.00 ± 0.3	34.33 ±0.4*	17.00 ± 0.7*	-*	31.66 ± 1.0	23.00 ± 0.9	22.66 ± 0.9	27.66 ± 0.7
Throat	IZD in mm	24	24	20	26	30	35	35	27	19	25	25	33	30	21	21	25	21	29	28
		25	29	26	35	30	30	37	27	24	31	30	35	30	20	29	32	28	30	34
		23	25	24	24	28	27	30	25	20	30	26	30	27	24	25	29	26	31	33
	Mean ± SEM	24.00 ± 0.3	26.00 ±0.9	23.33 ± 1.1	28.33 ± 2.2	29.33 ± 0.4	30.66 ± 1.5	34.00 ± 1.3	26.33 ± 0.4	21.00 ± 0.9*	28.66 ± 1.2*	27.00 ± 0.9	32.66 ± 0.9	29.00 ± 0.6	21.66 ± 0.7	25.00 ± 1.5	28.66 ± 1.3	25.00 ± 1.3	30.00 ± 0.3	31.33 ± 1.1
HVS	IZD in mm	24	-	15	-	30	27	35	22	15	20	25	11	30	-	-	20	20	-	28
		25	-	15	-	27	31	30	18	16	20	20	20	28	-	-	22	28	-	26
		15	-	18	-	28	30	31	16	19	21	18	21	26	-	-	18	16	-	25
	Mean ± SEM	24.66 ± 0.2	-	16.00 ± 0.6	-	28.33 ± 0.5	29.33 ±0.0*	32.00 ±0.9*	18.66 ± 1.1	16.66 ± 0.7*	20.33 ± 0.2*	21.00 ± 1.3	17.33 ± 2.0	28.00 ± 0.7	-*	-	20.00 ±0.7*	21.33 ± 2.3	-	26.33 ± 0.5

Table 4: Inhibition zone diameter of Gentamicin, Ciprofloxacin, and Erythromycin for S. aureus compared with CLSI breakpoint for Gentamicin, Ciprofloxacin, and Erythromycin

IZD: Inhibition zone diameter; HVS: High vagina swab; Gentamicin: Gen 10ug, ≥ 15mm=Susceptible; 13-14mm=intermediate; ≤ 12 mm=Resistant Erythromycin: Ery 15ug; ≥ 23mm=Susceptible; 14-22mm=intermediate; ≤ 13 mm=Resistant, Cip 5ug; ≥ 21mm =Susceptible; 16-20mm=intermediate; ≤ 15 mm=Resistant

Discs	Rapid		Abtek		Optu			Fon			Max			New Improved			Oxoid		
	Gen 10ug	Ery 30ug	Gen 10ug	Ery 30ug	Gen 10ug	Ery 30ug	Cip 10ug	Gen 10ug	Ery 30ug	Cip 10ug	Gen 10ug	Ery 10ug	Cip 10ug	Gen 10ug	Ery 10ug	Cip 5ug	Gen 10ug	Ery 15ug	Cip 5ug
IZD in mm	-	22	-	22	21	35	28	-	20	-	11	35	19	-	27	11	5	32	10
	-	21	-	24	30	34	26	-	18	-	12	34	18	-	25	9	5	30	10
	-	22	-	22	24	34	26	-	20	-	12	32	19	-	25	11	6	30	9
Mean ± SEM	-*	21.66 ± 0.2*	-*	22.66 ± 0.4*	25.00 ±1.7*	34.33 ± 0.2*	26.66 ± 0.4*	-*	19.33 ±0.4*	-*	11.66 ±0.2*	33.66±0.5*	18.66±0.2*	-*	25.66 ±0.4*	10.33 ± 0.4	5.33 ± 0.2	30.66 ± 0.4	9.66 ± 0.2

Table 5: Inhibition Zone Diameter of Gentamicin, Erythromycin and Ciprofloxacin for S. Aureus ATCC25923 and CLSI Breakpoint for Gentamicin, Erythromycin and Ciprofloxacin
*IZD: Inhibition Zone Diameter; *: Significant Difference at P≤ 0.05, SEM: Standard Error of Mean,-: No IZD, Gen: Gentamicin: Gen 10ug=14-26mm; Erythromycin: Ery 15ug; ≥ 23mm=Susceptible; 14-22mm=Intermediate; ≤ 13 Mm=Resistant; Cip 5ug; ≥ 21mm =Susceptible; 16-20mm=Intermediate; ≤ 15 Mm=Resistant*

Discs	Rapid	ABTEK	OPTU	FON	MAX	New Improved	OXOID
	Ery 30ug	Ery 30ug	Ery 30ug	Ery 30ug	Ery 10ug	Ery 10ug	Ery 15ug
IZD in mm	20	21	33	21	29	19	22
	18	20	35	24	25	20	23
	16	19	32	25	25	21	23
Mean ± SEM	18.00 ± 0.7*	20.00 ± 0.3*	33.33 ± 0.5*	23.33 ± 0.7	26.33± 0.8*	20.00 ± 0.3*	22.66 ± 0.2

Table 6: Inhibition Zone Diameter of Erythromycin for S. Pneumoniae and CLSI Breakpoint for Erythromycin
*IZD: Inhibition Zone Diameter; *: Significant Difference at P≤ 0.05, SEM: Standard Error of Mean, -: No IZD, Erythromycin: Ery 15ug; ≥ 23mm=Susceptible; 14-22mm=Intermediate; ≤ 13 Mm=Resistant*

4. Discussion

Table 1 shows inhibition zone diameter of Gentamicin, Ciprofloxacin and Amoxicillin-Clavulanic acid for E.coli and the CLSI breakpoint for the three antibiotics. While all isolates were readily susceptible and met the CLSI breakpoints to the Optu, Maxi, and New Improved discs the response to Abtek and Fondisc discs fell below the CLSI breakpoints of susceptibility for E.coli. Furthermore, while isolates from urine responded well to the Oxoid discs, those from wound responded favourably to the Rapid discs. This finding seems to be partially at variance with that of Umolu and colleagues who found that Gentamicin is not effective for E. coli isolated from wound and semen (Umolu et al., 2006).

Although all the discs purportedly had the same concentration of gentamicin 10µg, the inhibition zone diameter (IZD) for the Optu and Maxi discs varied significantly when compared with OXOID disc ($p \leq 0.05$). This could be as a result of higher concentration of Gentamicin in OPTU and MAXI discs which were not specified in the label (Brown et al., 1971). The E.coli isolated from urine met the CLSI breakpoint for susceptibility to Gentamicinfor OXOID, MAXI, and OPTU discs. This finding appears to be in agreement with a study done in Niger Delta University Bayelsa State, Nigeria (Ngwai et al., 2010). The inability of the RAPID, ABTEK, NEW IMPROVED and FONdiscs to meet the CLSI breakpoint for E. colisusceptibility to Gentamicin could be due to incorrect concentration of Gentamicin in the disc and the temperature and humidity condition at which the discs were stored (Erricson & Sherris, 1971).

The inhibition zone diameter of Ciprofloxacin for E.coli and CLSI breakpoint for Ciprofloxacin was also compared amongst the different discs

The concentration of ciprofloxacin for OPTU, FON, MAXI and NEW IMPROVED discs was twice that in the imported discs such as the OXOID, Abtek and Rapid disc. In spite of the specified higher concentration of ciprofloxacin in the local discs, their IZDs were not significantly greater than those of the imported ones except in the case of Rapid discs. This finding seems to deny the fact that IZD is usually directly proportional to the concentration of antibiotic in the disc as it was observed that FON disc with a concentration of 10ug gave an IZD lower than that of OXOID. Similarly, the RAPID disc with same concentration as OXOID, also gave IZD lower than OXOID. The reason for these variations could be attributed to the type of paper in which ciprofloxacin was impregnated as Erricson and Sherris, found out that the paper may contain some dye which inhibits the concentration of antibiotics in the Disc (Erricson & Sherris, 1971)..

Similar variations were seen in the IZD across the different antibiotic sensitivity discs used for all the organisms and antibiotics tested.

The RAPID and NEW IMPROVED discs, however, did not meet the required CLSI break-point and this difference could be as a result of the paper in which the Gentamicin was impregnated as Erricson and Sherris, found out that the paper may contain some dye which inhibit the concentration of antibiotic in the disc (Erricson & Sherris, 1971). The IZD of P. aureuginosa from sputum and ear was significantly different for Gentamicin from RAPID, ABTEK, OPTU, MAXI, FON and NEW IMPROVED discs when compared with OXOID disc ($p \leq 0.05$).

The concentration of Ciprofloxacin among the various discs differs. The IZD of MAXI, OPTU, NEW IMPROVED and FON discs containing a concentration of 10ug of Ciprofloxacin was found to be lower than that of OXOID disc contain 5ug, which should not be so under normal circumstances

This variation could be due to the improper storage of the discs and also the concentration of Ciprofloxacin might be lower than the specified amount in the label (Brown et al., 1971).The IZD of various discs, except RAPID disc for wound isolate met the CLSI breakpoint for P. aeruginosa sensitivity of Ciprofloxacin. This finding is in line with other report that shows that Ciprofloxacin is effective for P. aureginosa isolated from wound, sputum and ear (Ansary et al., 1994; Bertrand et al., 2001, Yow et al., 2007; Mohammedet al., 2014).The IZD of P. aureuginosa from wound was significantly different for Ciprofloxacin from RAPID and OPTU discs, when compared with OXOID disc ($p \leq 0.05$).The IZD of P. aureuginosa from sputum was significantly different for Ciprofloxacin from MAXI and OPTU Disc, when compared with OXOID disc ($p \leq 0.05$). While, the IZD of P. aureuginosa from ear was significantly different for Ciprofloxacin from MAXI and FON discs, when compared with OXOID disc ($p \leq 0.05$).

In the High Vaginal Swab (HVS), new improved disc did not meet the CLSI break point and this may be due to incorrect concentration of Gentamicin incorporated in the disc. The IZD of S. Aureus from urine was significantly different for Gentamicin from FON and New Improved discs, when compared with OXOID disc ($p \leq 0.05$). The IZD of S. Aureus from HVS was significantly different for Gentamicin from new improved disc, when compared with OXOID disc ($p \leq 0.05$).Urine isolate of S. Aureus, Fon, Abtek And RAPID discs containing 30ug of Erythromycin did not meet the CLSI break point. This was not the same for OPTU disc, which gave a higher IZD. This difference can be attributed to probably higher concentration of Erythromycin in OPTU Disc. For throat isolate of S. Aureus, OXOID, RAPID, ABTEK, OPTU, NEW IMPROVED and MAXI discs were above the CLSI breakpoint for S. Aureus susceptibility to Erythromycin. These finding sagree with the work done by Wakode and colleagues, which showed that throat isolate of S. Aureus was susceptible to Erythromycin (Wakode et al., 2003). However, the FON disc did not meet the breakpoint and the reason could be the temperature condition at which the disc was stored.

For HVS isolate of S. Aureus, OXOID, RAPID, NEW IMPROVED, MAXI, ABTEK, and FON discs, did not meet the CLSI break point except OPTU disc. This shows that OPTU disc probably contained more Erythromycin than as specified in the label. The IZD of S. aureus from urine and HVS was significantly different for Erythromycin from FON, MAXI, ABTEK, RAPID, OPTU and NEW IMPROVED discs, when compared with OXOID disc ($p \leq 0.05$). The IZD of S. aureus from throat was significantly

different for Erythromycin from FON disc when compared with OXOID disc ($p \leq 0.05$). However, for S. aureus isolated from urine, OXOID, RAPID, ABTEK, NEW IMPROVED, MAXI and OPTU discs met the CLSI breakpoint for S. aureus susceptibility to Ciprofloxacin. This finding agrees with previous studies conducted which showed that Ciprofloxacin was effective for S. aureus isolated from urine (Mava et al., 2012; Onanuga & Awhowho, 2012). FON disc with a concentration of 10ug gave IZD lower than that of OXOID that has a concentration of 5ug of Ciprofloxacin. This could be due to incorrect concentration of Ciprofloxacin in FON disc. For throat isolate of S. aureus, the various discs gave IZD that met the CLSI break point for S. aureus sensitivity to Ciprofloxacin. This is similar to a report which showed that Ciprofloxacin was effective for S. aureus isolated from HVS (Anyadoh-Nwadike et al., 2013).

FON disc with concentration of 10ug of Ciprofloxacin gave a smaller zone of inhibition when compared with OXOID that has a concentration of 5ug. This difference could be that the concentration of FON disc is less than 10ug. The IZD of S. aureus from urine was significantly different for Ciprofloxacin from OPTU, FON and MAXI discs, when compared with OXOID disc ($p \leq 0.05$). Similarly, the IZD of S. aureus from HVS was significantly different for Ciprofloxacin from OPTU, FON and NEW Improved discs when compared with OXOID disc ($p \leq 0.05$).The Rapid, Abtek, New Improved and FON discs gave no IZD, while OXOID and MAXI discs gave IZD that deviated from the limit/range specified by CLSI guidelines. This may be indicative of resistance exhibited by S.aereus to Gentamicin. The results also showed that the IZD of ATCC25923 S. aureus was significantly different for Gentamicin from Optu, Fon, Rapid, Abtek, Optu, New Improved and Maxi discs when compared with OXOID disc ($p \leq 0.05$). The IZD for Rapid, ABTEK was lower than that of OXOID, although the concentration of RAPID and ABTEK disc had a higher concentration than OXOID. These differences may be due to incorrect concentration of Erythromycin impregnated in these discs. The IZD of S. pneumonia was significantly different for Ciprofloxacin from OPTU disc, RAPID, ABTEK, and MAXI disc when compared with OXOID disc ($p \leq 0.05$).

5. Conclusion

The result of antimicrobial susceptibility testing assists clinicians in making decision about therapeutic agents used in treatment of patient with infection and as such, the result should be reliable and dependable. When antimicrobial susceptibility testing is not performed correctly (due to poor materials/reagents), erroneous result may be reported that are potentially harmful to the patient and also increase the emerging trend of resistance of the organisms due to the misuse of antibiotics. The study found wide variations in quality of the antibiotic sensitivity discs as seen in the IZD when compared against the OXOID disc and CLSI breakpoints for the various test organisms. The result obtained from this study shows that result of AST is not reliable and cannot be depended upon by clinicians in making decision for the best therapeutic agent for treating patients. These findings from this study showed that result of AST was not very reliable and cannot necessarily be depended upon by clinicians to make decision for the best therapeutic agent for treating patients. This study, therefore, recommends stringent quality control testing of antibiotic sensitivity discs each time a new disc was introduced in the clinical laboratories on weekly basis as recommended by CLSI since the importance of sensitivity test in clinical practice just cannot be underestimated.

6. References

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