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Evaluation of the Quality of Commercial Antibacterial Discs Available in Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author COE designed and supervised the study. Author PME wrote the protocol, managed the literature searches, and performed the statistical analysis. Authors PME, EEA, PCE and RNE managed the analyses of the study. Author CCA proof read and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was undertaken to compare the quality and efficacy of six brands of antibacterial discs that are commercially available in Nigeria.
Methodology: The brands evaluated include two foreign brands (Oxoid and Abtek) and four local brands (Optudisc, Polydisc, Maxidisc and Jirehdisk). The brands were analyzed by antibiotic susceptibility testing (AST) using laboratory isolates of *Staphylococcus*

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aureus and *Escherichia coli* to measure the antimicrobial performances of the brands; and UV-Vis spectrophotometry to measure the absorbances of antibiotics extracted from antibiotic discs of the various brands.
Results: All of the brands of antibacterial discs of under study exhibited variations in their antimicrobial performances and UV-absorbances. This was observed where some of the discs with lower stated potencies produced inhibition zones and absorbances far greater than similar discs from other brands with higher stated potencies. Also, discs of the same stated potencies showed variable results in both the antibiotic susceptibility testing (AST) and UV-Vis spectrophotometric analyses. Coefficients of variation greater than 5%, which indicates high disc-to-disc variation and unsatisfactory reproducibility, were recorded highest among the local brands during the AST. All the brands with multidisc panels, except the Abtek and Polydisc brands, produced some zones of inhibition that are unreadable. Of all the zones of inhibition that were unreadable, Optudisc brand recorded the highest rate (36·7%), while 6·7% of discs of Jirehdisk brand and 6·7% of discs of Maxidisc brand produced inhibition zones that were unreadable.
Conclusion: All brands of susceptibility discs evaluated in this study except the Oxoid and Abtek brands manifested poor quality and performed below expected standard, though one of the local brands (Polydisc) performed closest to the foreign brands. With further improvement in quality, these brands may be recommended for use in Nigeria.

Keywords: Antibacterial (antibiotic) discs; antibiotic susceptibility testing; UV-Vis Spectrophotometry; quality.

1. INTRODUCTION

The testing of organisms for sensitivity to antibiotics has always presented a problem to the clinical test laboratory. This problem has increased with the introduction of every new antibiotic. The most accurate method of performing this task is by controlled tube dilution tests which unfortunately are time-consuming and can only be used routinely in some specialized hospital laboratories. The use of the dry antibiotic disc, which was introduced as a simple and accurate means of screening has received wide acceptance in hospitals and other laboratories, seemed a logical solution [1].

Antimicrobial discs need to be manufactured within strict control limits and handled correctly within the laboratory, otherwise, they cannot meet the quality and performance standards required [2]. In the developed countries, it is believed that these conditions are adequately met, but in the developing countries, this may not usually be the case [3,4].

Antibacterial discs that are commercially available in Nigeria may be fake or adulterated and may not contain the acclaimed quantity of active ingredient, which is a reflection of what goes on in many developing countries, in particular, sub-Saharan Africa. Due to the high cost of procuring imported standardized discs, some clinical laboratories, as well as private laboratories, in Nigeria have resorted to local and commercial production of antibiotic discs. It is reasonable to expect that discs contain the stated antimicrobial agent in the amount specified, yet studies have shown that many antibiotic discs either do not possess the stated active ingredient or that the active ingredient present may be less than the amount indicated by the manufacturer, or that the products may have been adversely affected by storage conditions in various retail outlets across the country.

According to Greenberg et al. [1], the manufacture of antibiotic discs and their successful use involve a number of considerations-the quality of antibiotic, the composition of the discs (paper, tablet or other construction), test performance, etc. Variations in the paper quality of antibacterial discs that are commercially available in Nigeria have been reported by Eze et al. [5]. They observed high rates of differences in the thicknesses, weights, water absorbabilities and diameters of the disc-papers of all brands of antibacterial discs evaluated (both local and imported discs). These variations will grossly affect the results of antibiotic susceptibility tests when different brands of antibiotic discs with different paper qualities are used.

Several works have been done on evaluating the potency of antibiotic discs from different brands, by comparing their performances in susceptibility tests [2,6,7,8]. The Kirby-Bauer disc diffusion technique is the method most commonly employed in this analysis. Although many methods had been proposed to aid the quality control of antimicrobial/antibiotic susceptibility testing (AST), the applicable measures aiming at detecting the exact content of a certain agent in antibiotic discs are scarce [9]. Few or no other analytical methods have been reported for the identification and estimation of the antibiotic contents in sensitivity discs, except for the use of capillary electrophoresis as described by Xu et al. [10] and the use of High-Pressure Liquid Chromatography described by Hagel et al. [11].

Spectrophotometric techniques have been used in the quantitative and qualitative assay of antibiotics and many other drugs, but extensive literature survey reveals that they may not have been applied in the evaluation of the antibiotic contents of sensitivity discs. As Beer's law simply implies that absorbance is proportional to concentration, this research work will incorporate the use of UV-Vis spectrophotometry for the evaluation of the drug concentrations of various brands of antibiotic discs by determination and comparison of their various absorbances with that of a standard.

2. MATERIALS AND METHODS

2.1 Materials

2.1.1 Antibiotic discs

Six brands of antibiotic susceptibility discs were used in this study. Two imported brands (Oxoid and Abtek), and four locally manufactured brands (Optudisc, Polydisc, Maxidisc and Jirehdisk). All discs were stored at 2-8℃ throughout the period of the study as recommended by the manufacturers.

2.1.2 Test organisms

Pure cultures of *Staphylococcus aureus* and *Escherichia coli* obtained from the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Nigeria were used in this research.

2.1.3 Culture media and reagents

Culture media used were Nutrient Agar, Nutrient Broth and Mueller-Hinton Agar (Oxoid Limited, UK). Reagents used include McFarland 0·5 turbidity standard (prepared from barium chloride sulfuric acid and water), sodium hydroxide (Chemproha Chemicals, the

Netherlands), methanol (SIGMA-ALDRICH Inc., Germany), sodium chloride (BDH Chemicals, England), distilled water, etc.

2.2 Methods

2.2.1 Antibiotic susceptibility testing (AST)

Laboratory isolates of two organisms (*S. aureus* and *E. coli*) were used in the AST. Inhibition zone diameters (IZDs) measured in millimeters (mm) were used as a parameter for evaluating the performance of the antibiotic discs. McFarland 0·5 turbidity standard was used to adjust the inocula of the bacterial cultures grown overnight at 37℃. Disc diffusion susceptibility test (modified Kirby-Bauer method) was carried out as described by Cheesbrough [12]. This process was carried out in triplicate for every antibiotic disc contained in each brand used in the study and their mean inhibition zone diameters (IZDs) calculated and recorded. Their coefficients of variation were also calculated to demonstrate the reproducibility or disc-to-disc variation between the IZDs produced in the replicate tests with antibiotic discs from the same source.

2.2.2 UV-Vis spectrophotometry

A JENWAY 6505 single beam UV-Vis spectrophotometer (Bibby Scientific Ltd., UK) was used in this analysis.

Ten (10) single discs of each antibiotic from the respective brands of antibacterial discs under study were cut out and placed into separate bijou bottles. Also, 10mL of the appropriate antibiotic solvent was then added to the respective bottles to obtain an equivalent of one (1) disc to 1mL. These bottles were packed in flasks, and the flasks were placed in a flask shaker and shaken at 500 osc/mins for 4 hours to extract the antibiotics from the paper discs into the solution.

After shaking, the contents of the bottles were filtered through No. 1 Whatman filter papers into separate test tubes. The clear solutions of each antibiotic obtained were then analyzed in a UV-Vis spectrophotometer at their various absorption maxima to obtain their absorbances.

This process was carried out in triplicate for every antibiotic contained in each brand of the antibiotic discs used in this study and their mean absorbance calculated and recorded.

3. RESULTS

The quality of the six brands of antibacterial discs was evaluated by determining and comparing their antibacterial performances and spectrophotometric absorbances.

Table 1 shows the characteristics of the brands of antibacterial discs evaluated in the study.

3.1 Antibiotic Susceptibility Testing (AST)

Tables 2 and 3 show mean IZDs and coefficients of variation obtained when the different brands of antibiotic discs were tested against *S. aureus* and *E. coli* respectively. The mean IZDs were calculated from the triplicate zone diameters obtained for every antibiotic contained in each brand of the antibacterial discs used in the study. Also, their coefficients of variation were also calculated to demonstrate the reproducibility or disc-to-disc variation between the IZDs produced in the replicate tests with antibiotic discs from the same source.

Some of the discs produced unreadable (unmeasurable) zones of inhibition, as seen amongst Optudisc, Maxidisc, and Jirehdisk. This may be as a result of the proximity of antibiotic discs in the multi-disc panels presented by these brands. These multi-discs contain ten (10) antibiotic discs as against the WHO standards which require a Petri plate to contain no more than six or seven discs per 90mm plate [13].

Discs showing unreadable inhibition zones were aseptically cut out from their multidisc panels and then used as single discs to get defined values of their antimicrobial performances against the test organisms. The IZDs produced by these discs are shown in Table 4.

Table 5 shows the variations in the compliance of brands to the Clinical and Laboratory Standards Institute (CLSI) standard for disc potencies [14]. Only the Oxoid brand showed compliance.

3.2 UV-Vis Spectrophotometric Analyses

Table 6 shows a list of some antibiotics contained in the different brands of antibiotic discs under study, their respective solvents and UV-absorption maxima used in the spectrophotometric assay of the antibiotics.

The spectrophotometric evaluation of the quality of discs was done by determining and comparing the various mean absorbances of the various antibiotics in each brand of antibiotic disc.

Table 7 shows the mean absorbances recorded by antibiotics contained in the discs of the different brands under study.

4. DISCUSSION

AST results provide guidance in the choice of antimicrobial agents in patient care. They also serve as a major source of data for surveillance of drug resistance. As such the accuracy of the results is of utmost priority [2].

In this study, some brands of discs, both imported and produced in Nigeria and used by the vast majority of routine laboratories, were evaluated by both AST and UV-Vis spectrophotometry. Comparison of discs from the different brands was somewhat limited by the fact that all manufacturers do not produce discs with the same content for all antibiotics. Although some of the antibiotic discs of the various brands under study did not have the same stated potencies, all exhibited variations in their antimicrobial performances and UV-absorbances. This is observed where some of the discs with lower stated potencies produced inhibition zones and absorbances far greater than similar discs from other brands with higher stated potencies. An example of this is seen where erythromycin from Jirehdisk brand with stated potency of 5µg produced mean absorbances of 1·767 and mean IZD of 17mm against *S. aureus*; and the same antibiotics from Maxidisc brand with stated potency of 10µg produced mean absorbances of 1.824 and mean IZDof 20mm against *S. aureus*.

Table 1. Characteristics of the different brands of antibacterial discs evaluated

Brand name	Manufacturer	Disc presentation	No. of discs per multidisc panel	Total no. of antibiotics in each brand (G+ve& G-ve)	Spatial Orientation of Discs (Distance between Discs) (mm)
Oxoid	Oxoid Ltd, UK	Single discs	NA	Single discs of the same antibiotic content per cartridge	NA
Abtek	Abtek Biologicals Ltd, UK.	Multidisc panels containing discs of different antibiotics	8	11	16
Optudisc	Optun Laboratories, Nigeria Ltd.	Multidisc panels containing discs of different antibiotics	10	17	6-8
Polydisc	Poly-Tes Med Laboratories Enugu, Nigeria.	Multidisc panels containing discs of different antibiotics	10	16	8
Maxidisc	Maxicare Medical Laboratories, Nigeria.	Multidisc panels containing discs of different antibiotics	10	14	7-10
Jirehdisk	Jireh Laboratories, Nigeria.	Multidisc panels containing discs of different antibiotics	10	15	8-10

G +ve - Gram positive
G -ve - Gram negative
NA - Not applicable

Table 2. Mean IZDs (mm) and coefficients of variation (%) produced by the various brands of antibiotic sensitivity discs against *S. aureus* in antibiotic sensitivity testing

Antibacterial Agents	Oxoid			Abtek			Optudisc			Polydisc			Maxidisc			Jirehdisk		
	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV
Ciprofloxacin	5	30±0	0	-	-	-	10	u	*	5	26±1.15	6	10	32±0.00	0	10	31±0.67	3
Ofloxacin	5	23±0.33	3	-	-	-	-	-	-	5	29±0.67	3	-	-	-	10	29±1.33	7
Co-trimoxazole (SMX-TMP)	25	0±0.00	0	25	0±0.00	0	-	-	-	-	-	-	30	22±1.15	7	25	13±0.67	8
Ceftriaxone	30	10±0.00	0	-	-	-	-	-	-	30	26±1.20	7	25	25±2.67	15	30	31±0.67	3
Augmentin (Amoxy-Clav)	30	10±0.00	0	30	0±0.00	0	-	-	-	-	-	-	-	-	-	30	13±1.45	16
Gentamicin	10	20±0.00	0	10	18±0.33	3	30	30±0.88	4	10	20±0.00	0	10	23±0.58	4	10	20±1.15	8
Ampicillin	10	0±0.00	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chloramphenicol	30	0±0.00	0	30	0±0.00	0	30	u	*	-	-	-	-	-	-	-	-	-
Erythromycin	15	0±0.00	0	5	0±0.00	0	10	24±1.33	8	10	16±0.33	4	10	20±0.00	0	5	17±0.67	6
Nalidixic acid	30	11±0.00	0	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetracycline	30	10±0.00	0	10	0±0.00	0	-	-	-	-	-	-	-	-	--	-	-	-

X - Mean IZD *u - Unreadable* *SMX-TMP - Sulphamethoxazole-Trimethoprim*
SEM- Standard Error of Mean ** - Not calculated* *Amoxy-clav - Amoxycillin-Clavulanic acid*
CV - Coefficient of Variation *- - Not included*
µg - Stated potency in micrograms

Table 3. Mean IZDs (mm) and coefficients of variation (%) produced by the various brands of antibiotic sensitivity discs against *E. coli* in antibiotic sensitivity testing

Antibacterial Agents	Oxoid			Abtek			Optudisc			Polydisc			Maxidisc			Jirehdisk		
	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV	µg	X± SEM	CV
Ciprofloxacin	5	30±0.00	0	-	-	-	10	u	*	10	31±0.67	3	10	30±0.33	2	10	28±0.33	2
Ofloxacin	5	26±0.00	0	5	26±0.00	0	10	u	*	10	22±0.00	0	10	32±0.33	2	10	27±0.67	4
Co-trimoxazole (SMX-TMP)	25	24±0.00	0	25	0±0.00	0	30	u	*	-	-	-	30	u	*	25	u	*
Ceftriaxone	30	22±0.00	0	-	-	-	-	-	-	30	19±0.67	5	-	-	-	30	21±0.33	3
Augmentin (Amoxy-Clav)	30	25±0.33	2	30	34±0.00	0	30	u	*	30	31±0.67	3	30	28±1.45	7	-	-	-
Gentamicin	10	24±0.00	0	10	25±0.00	0	10	u	*	10	24±0.00	0	10	30±0.00	0	10	25±0.33	2
Ampicillin	10	11±0.00	0	-	-	-	30	30±0.33	2	30	20±1.15	8	-	-	-	25	u	*
Chloramphenicol	30	20±0.00	0	-	-	-	-	-	-	10	8±3.84	68	30	27±0.67	4	30	20±0.00	0
Erythromycin	15	13±1.15	13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Nalidixic acid	30	15±0.00	0	30	19±1.20	11	30	u	*	-	-	-	-	-	-	-	-	-
Tetracycline	30	16±0.00	0	25	24±0.00	0	-	-	-	-	-	-	-	-	-	25	28±0.00	0

X - Mean IZD *u - Unreadable* *SMX-TMP - Sulphamethoxazole-Trimethoprim*
SEM- Standard Error of Mean ** - Not calculated* *Amoxy-clav - Amoxycillin-Clavulanic acid*
CV - Coefficient of Variation *- - Not included*
µg - Stated potency in micrograms

Table 4. Mean IZDs (mm) produced by brands of antibiotic discs (Multi-discs) previously showing unreadable zones of inhibition. Each individual antibacterial agent was cut out from the multi-disc and used as a single disc to produce a clear and Measurable Zone of inhibition

Brands	Antibacterial agent	State Disc Potency (µg)		*S. aureus*	*E. coli*
		G+ve	G-ve	X± SEM	X± SEM
Optudisc	Ciprofloxacin	10	10	41±0.67	38±1.86
	Chloramphenicol	30	-	33±3.00	-
	Levofloxacin	10	-	33±0.33	-
	Gentamicin	30	10	-	32±0.88
	Ofloxacin	-	10	-	35±0.33
	Perfloxacin	-	10	-	34±1.76
	Augmentin (Amoxy-Clav)	-	30	-	34±1.15
	Ceporex	-	10	-	33±0.88
	Nalidixic acid	-	30	-	30±2.33
	Septrin/Cotrimoxazole (SMX-TMP)	-	30	-	39±1.00
Maxidisc	Perfloxacin	10	-	33±0.33	-
	Septrin/Cotrimoxazole (SMX-TMP)	-	30	-	35±0.88
Jirehdisk	Septrin/Cotrimoxazole (SMX-TMP)	-	25	-	24±12.20
	Ampicillin	-	25	-	24±0.88

X - Mean IZD *G +ve - Gram positive* *SMX-TMP -Sulphamethoxazole-Trimethoprim*
SEM- Standard Error of Mean *G –ve - Gram negative* *Amoxy-clav - Amoxycillin-Clavulanic acid*
- - Not included

Table 5. Variations in the compliance of brands to the CLSI standard for disc potencies of antibiotic discs by brands under study

Antibiotics	CLSI standard for disc potency (µg)*	Oxoid		Abtek		Optudisc		Polydisc		Maxidisc		Jirehdisk	
		(µg)	%	(µg)	%	(µg)	%	(µg)	%	(µg)	%	(µg)	%
Ciprofloxacin	5	5	100	-	-	10	100↑	5, 10	100, 100↑	10	100↑	10	100↑
Ofloxacin	5	5	100	5	100	10	100↑	5, 10	100, 100↑	10	100↑	10	100↑
Cotrimoxazole (SMX-TMP)	25	25	100	25	100	30	20↑	-	-	30	20↑	25	100
Ceftriaxone	30	30	100	-	-	-	-	30	100	25	16·7↑	30	100
Augmentin /(Amoxy-Clav)	20/10 (30)	30	100	30	100	30	100	30	100	30	100	30	100
Gentamicin	10	10	100	10	100	10	100	10	100	10	100	10	100
Ampicillin	10	10	100	-	-	30	200↑	30	200↑	-	-	25	150↑
Chloramphenicol	30	30	100	30	100	30	100	10	200↓	30	100	30	100
Erythromycin	15	15	100	5	66·7↓	30	100↑	10	33·3↓	10	33·3↓	5	66·6↓
Nalidixic acid	30	30	100	30	100	30	100	-	-	-	-	-	-
Tetracycline	30	30	100	10, 25	66·7↓, 167↓	-	-	-	-	-	-	25	16·7↓

Amoxy-clav - Amoxycillin-Clavulanic acid | *↑ - Increase in noncompliance* | ***%** - Percentage compliance/noncompliance*
SMX-TMP - Sulphamethoxazole-Trimethoprim | *↓ - Decrease in noncompliance* | **CLSI [14]*

Table 6. Antibiotics under comparison contained in the different brands of antibiotic discs under study, their respective solvents and UV absorption maxima used in the spectrophotometric assay of the antibiotics

	Antibiotics	Solvent	UV absorption maximaλ max (nm)	References
1	Ciprofloxacin	Water	278	[15]
2	Ofloxacin	Water	287	[16]
3	Cotrimoxazole SMX	0·1N NaOH	257	[17]
	(SMX-TMP) TMP	0·1N NaOH	287	[17]
4	Ceftriaxone	Water	330	[18]
5	Augmentin Amoxy	water	271·8	[19]
	(Amoxy-Clav) Clav	water	280	[20]
6	Ampicillin	Water	262	[21]
7	Chloramphenicol	Water	278	[21]
8	Tetracycline	0·1N NaOH	258	[21]
9	Nalidixic Acid	0·1N NaOH	385	[21]
10	Erythromycin	Methanol	285	[22]

Amoxy-clav - Amoxycillin-Clavulanic acidSMX-TMP - Sulphamethoxazole-Trimethoprim

Table 7. Mean UV-Vis Spectrophotometric absorbances of antibiotics from the various brands under study

Antibiotics	λ(nm)	Oxoid		Abtek		Optudisc		Polydisc		Maxidisc		Jirehdisk	
		µg	X± SEM	µg	X± SEM	µg	X± SEM	µg	X± SEM	µg	X± SEM	µg	X± SEM
Ciprofloxacin	278	5	0·278±0.017	-	-	10	1·872±0.215	5	0·527±0.020	10	1.971±0.034	10	1·986±0.042
Ofloxacin	287	5	0·459±o.o61	5	0·451±0.023	10	1·957±0.038	5	0·205±0.009	10	2.382±0.065	10	1·846±0.084
Cotrimoxazole SMX	257	25	1·300±0.014	25	1·427±0.013	30	1·984±0.079	-	-	30	2·338±0.050	25	1·951±0.156
TMP	287		1·342±0.010		1·643±0.021		2·316±0.059		-		2·674±0.038		2·560±0.282
Ceftriaxone	330	30	1·119±0.006	-	-	-	-	30	1·101±0.202	25	1·094±0.007	30	1·839±0.031
AugmentinAmoxy	271·8	30	0·590±0.028	30	0·855±0.036	30	2·014±0.010	30	0·761±0.023	30	2·648±0.069	30	2·231±0.061
Clav	280		0·332±0.016		0·690±0.026		1·694±0.006		0·594±0.019		2·397±0.035		2·188±0.084
Ampicillin	262	10	0·167±0.036	-	-	30	0·766±0.019	30	0·379±0.033	-	-	25	2·607±0.209
Chloramphenicol	278	30	0·294±0.033	30	0·615±0.065	30	2·194±0.181	10	0·276±0.025	30	2·109±0.267	30	1·765±0.146
Erythromycin	285	15	1·722±0.016	5	0·260±0.059	30	1·370±0.412	10	1·776±0.054	10	1·824±0.008	5	1·767±0.040
Nalidixic Acid	258	30	1·595±0.019	30	1·641±0.018	30	2·459±0.265	-	-	-	-	-	-
Tetracycline	385	30	0·242±0.006	25	0·148±0.003	-	-	-	-	-	-	25	0·401±0.015

X - Mean absorbance *Amoxy-clav - Amoxycillin-Clavulanic acid*
SEM- Standard Error of Mean *SMX-TMP - Sulphamethoxazole-Trimethoprim*

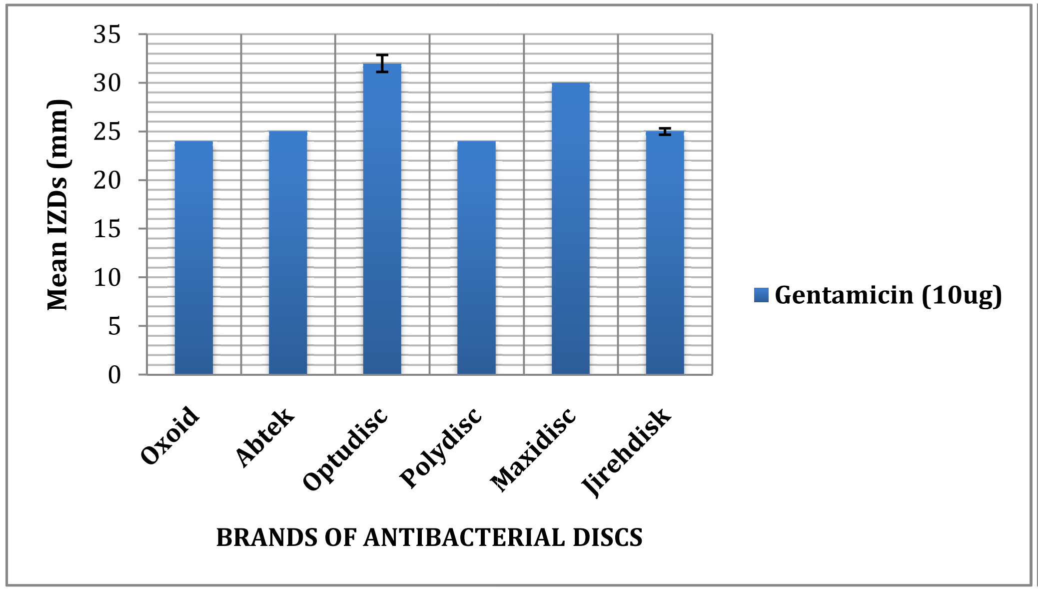

Fig. 1. Comparison of the mean inhibition zone diameters (IZDs) produced against *E. coli* by gentamicin discs of equal stated potency (10µg) from all brands of antibacterial discs evaluated

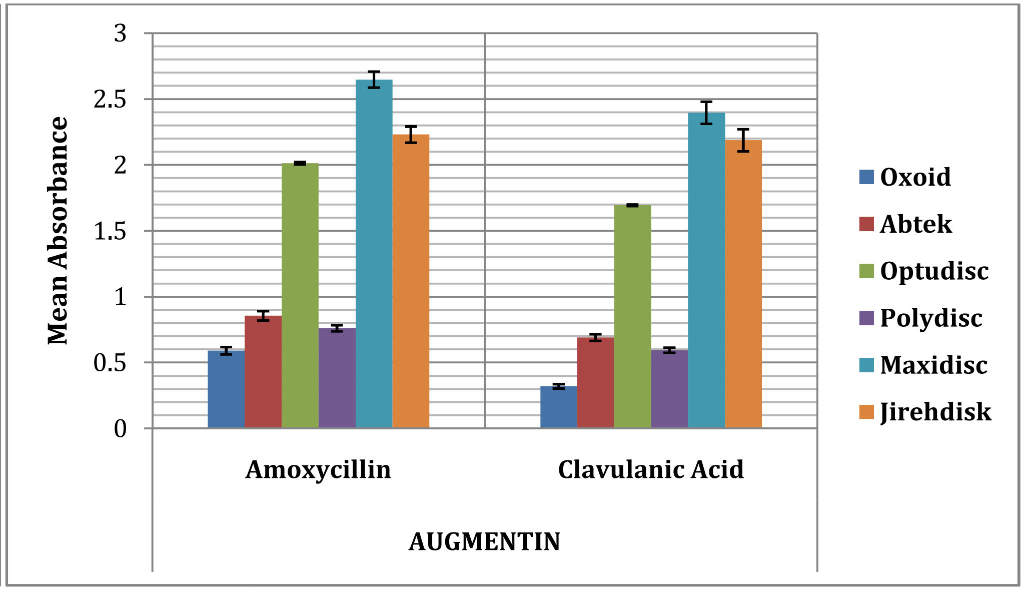

Fig. 2. Comparison of the mean absorbances recorded by augmentin (Amoxycillin-Clavulanic Acid) discs of equal stated potency (30µg) from all brands of antibiotic discs evaluated

Whereas, erythromycin of the Oxoid brand with stated potency of 15µg recorded a mean absorbance of 1·722, but had no activity against *S. aureus* in the AST with mean IZD of 0mm.

Also, discs of same stated potencies showed variable results in the ASTs and UV-V is spectrophotometric analyses. Examples are seen where gentamicin discs whose stated potency (10µg) is the same for all of the brands evaluated, showed variable results in the AST. This can be observed in Fig. 1 where against *E. coli*, the gentamicin discs from all the brands produced varied mean IZDs that ranged from 24-32 mm. Also, Augmentin (amoxycillin-clavulanic acid) whose stated potency (30µg) is the same for all of the brands evaluated, recorded variable results in the UV-spectrophotometry. Fig. 2 shows the dissimilar mean absorbances recorded by the Augmentin discs from all the brands evaluated. Absorbance values recorded for amoxycillin ranged from 0.590-2.648, and those recorded for clavulanic acid ranged from 0.332-2.397. These results reveal the discrepancies and variations in the antibiotic concentrations of the various antibiotic discs under study which may confirm the fact that the various brands of antibiotic discs under study may contain antibiotic concentrations far above or below the stated potencies/label claims.

Coefficient of variation is an indicator of disc to disc variation within a batch and would be an indicator of the reproducibility of the test results (7). According to the CLSI [23], sensitivity discs used in the microbiology laboratory should ideally have least disc-to-disc variation viz. CV less than 5%. Reproducibility was considered unsatisfactory if percent CV for a disc was more than 5%. In the AST, over 30% of discs from each of the local brands produced CV greater than 5%, but only one antibiotic disc each from the two foreign brands showed CV greater than 5% (i.e. Oxoid's erythromycin against *E. coli*-13% and Abtek's Nalidixic acid against *E. coli*-11%). Though they both did not show similar activity against the test organisms, they exhibited excellent reproducibility of IZDs produced by discs in the replicate tests.

From Table 1, we see the spatial orientation of discs of brands having multidisc panels. This reveals the distances between discs ranging from 16mm for Abtek, and 6-10mm for the different local brands. This is contrary to the specifications which require discs to be spaced at a minimum of 25mm [24]. Apart from the Oxoid brand which came as single discs, discs of the foreign brand Abtek and all four local brands were presented in multidisc panels having more than 7 discs of different antibiotics per panel. This also does not comply with WHO recommendation of not using more than seven discs per 90 mm plate [13]. All of the local brands contained ten discs per panel and this proximity of one antibiotic disc to another may be responsible for the merging of the IZDs produced by the antibiotic discs to produce zones of inhibition that are unreadable, or that the discs probably contained antibiotic concentration above the stated potency. This problem of discs producing unreadable zones of inhibition is apparently, due to poor standardization in the preparation of the discs. This was confirmed by Ekundayo and Omodamiro [2] who evaluated the quality of locally manufactured antimicrobial susceptibility testing discs used in South Eastern Nigeria.

All the brands with multidisc panels, except the Abtek and Polydisc brands, produced some zones of inhibition that are unreadable. Of all the zones of inhibition that were unreadable, Optudisc brand recorded the highest rate (36·7%), while 6·7% of discs of Jirehdisk and 6·7% of discs of Maxidisc brand produced inhibition zones that were unreadable. The foreign brand Abtek and the four local brands contained different types and number of antibiotics on the multidisc panel. Different manufacturers also use different codes and in some cases different concentrations for the same antibiotics and in Table 5, it can be seen that only the Oxoid brand gave 100 percent compliance to the CLSI standard for antibiotic disc potencies/concentration [14]. The marked differences in the antibiotic contents within brands makes it quite impossible to directly compare the performances of the different brands and according to Ekundayo and Omodamiro [2], the situation may further be a reflection of lack of clear policy guidelines on antibiotic usage in the country or the failure of the manufacturers to comply with such guidelines.

Another factor that may seriously affect the activity of the antibiotic discs is the effect of storage of discs in inappropriate temperatures. Manufacturers and retailers of these products may not have given special attention to the maintenance of cold chain from their warehouses to the end users. Also, marketers of discs who may wish to store these products under the recommended temperature conditions may be faced with the problems of irregular and fluctuating power supplies.

These discrepancies and variations in the antimicrobial performances and antibiotic concentrations recorded by the different brands of antibacterial discs calls for concern, as these discs may produce false positive or negative results when employed in the laboratory for susceptibility testing. This presents a grave danger in clinical practice. A bacterial strain may be recorded as sensitive while in actual case it is resistant, or it might be the other way round. These erroneous results could be used as the basis for antibiotic prescription and this will be of no benefit to the patient, and may also have a powerful negative influence on antibiotic usage and hence on the factors that facilitates the emergence of antimicrobial drug resistance.

All the brands of susceptibility discs evaluated in this study expressed varying performances. The Oxoid brand recorded the best performance, followed by the Abtek brand. Polydisc performed closest to the foreign brands and of all local brands, the Polydisc brand appeared to be the most standardized. Jirehdisk was next in line in terms of performance after Polydisc. With further improvement in quality, these brands can be recommended for use in Nigeria.

5. CONCLUSION

Good quality of antibiotic discs is a fundamental prerequisite to accurate antibiotic susceptibility tests (10). The brands of discs, both imported and produced in Nigeria and used by the vast majority of routine laboratories, were evaluated by both AST and UV-Vis spectrophotometry. This study also proved the potential of UV-Vis spectrophotometry as an easy choice for performing disc quality control under appropriate conditions.

The results of this study indicate that susceptibility discs used in Nigeria are of low quality, possibly reflecting the lack of control of quality in the production and/or storage of the products prior to their distribution. These data point to the need for deployment of effective systems of supervision of the marketing of these products and insightful programmes of quality control on the part of the laboratories that use them. It is therefore important that regulatory agencies and manufacturers should take cognizance of these problems associated with the quality and performances of antibiotic discs during susceptibility testing and consequently proffer solution towards the standardization of discs used in antibiotic susceptibility testing.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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