

## EVALUATION OF THE QUALITY OF LOCALLY MANUFACTURED ANTIMICROBIAL SUSCEPTIBILITY TESTING DISCS USED IN SOUTH EASTERN NIGERIA

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### ABSTRACT

Locally manufactured antimicrobial susceptibility discs are becoming increasingly used in both private and government hospital laboratories in Nigeria. Data on the quality of these locally manufactured antimicrobial discs are not available. In order to provide some data, we evaluated the quality of three brands of locally manufactured antimicrobial susceptibility discs in common use in southeastern Nigeria. The three brands are Brodisk, Jirehdisk and Optudisc. The performances of these brands in agar disc diffusion assay against clinical isolates of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were compared with that of Abtek, the imported brand. Un-interpretable zones of inhibition defined as large zones of inhibition that merged together or complete wiping out of bacterial growth at the time of reading of the plate, were common with Optudisc and Jiredisk brands. The imported brand, Abtek, did not produce any such results. While Gentamycin on Abtek produced a mean diameter of zone of inhibition of 15mm, 19mm and 16mm against *S. aureus*, *P. aeruginosa* and *E. coli* respectively, the same antibiotic disc with the same stated potency on Brodisk and Jiredisk produced no zone of inhibition against the test bacteria. Amoxylin and Augmentin discs on Abtek produced zones of inhibition of 13mm and 21mm against *S. aureus* respectively. Amoxylin disc on Brodisk did not produce any zone of inhibition against the bacterial strain while Augmentin disc on Jirehdisk produced un-interpretable result. Of the three locally made brands, Brodisk is the only one that conformed to the international standard of not having more than eight discs per 90mm plate. In our opinion, Brodisk can be recommended for clinical use in Nigeria with further improvement. The outcome of this study indicates the need for improved standardization in the production of these locally manufactured susceptibility discs.

### INTRODUCTION

Antimicrobial susceptibility testing 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 (1, 2). The accuracy of results of antimicrobial susceptibility testing can be affected by multiple factors which include the media, antimicrobial discs or preparations, inoculum's size, plate reading and incubation conditions (3) and the competence of the Medical Laboratory personnel (2). For the results to be reliable, there is need for careful control and standardization of the various steps and components of the testing procedures (3).

Two groups of manual method commonly used for antimicrobial susceptibility testing are the Dilution and Disc diffusion methods. The Disc diffusion

testing based on the Kirby-Bauer method is the simpler method and is therefore the most widely used (4). When this method is performed with strict adherence to the standard procedures in accordance to National Committee for Clinical Laboratory Standards (NCCLS) Method, it gives reliable results and can predict clinical efficacy of the antibiotics tested (1). One of the most critical components of the Disc Diffusion method is the quality of the antimicrobial discs. The potency and the accuracy of the antimicrobial content of the discs must be ensured. Antimicrobial discs need to be manufactured within strict control limits and handle correctly within the laboratory, otherwise, they cannot meet the quality and performance standards required.

In the developed countries, it is believed that these conditions are adequately met (4). In the

developing countries, this may not usually be the case (1). In Nigeria, for instance, imported susceptibility discs were in common use. However, due to the high cost of importation, imported discs became expensive and scarce. Some clinical laboratories resorted to in-house preparation of their antibiotic discs. With this development, some private laboratories have ventured into commercial production of antimicrobial susceptibility discs. These locally manufactured discs are considerably cheaper than the imported discs. They are also more readily available. As a result, they are becoming widely used in both private and government hospitals and laboratories. There is little or no data on the quality of these locally manufactured discs. This is the major reason for our decision to evaluate the quality and performance of the three common brands of locally manufactured antimicrobial susceptibility discs used in South-eastern Nigeria in comparison with an imported brand.

This paper presents the results of our evaluation of the performance characteristics of three brands of locally manufactured susceptibility discs. We highlight the need for improvement in the standardization of the manufacture of these local brands of susceptibility discs.

## **MATERIALS AND METHODS.**

### **Brands of Antibiotic discs.**

Four brands of antibiotic susceptibility discs were used in this study. One imported brand, Abtek (manufactured by Abtek Biologicals Ltd, U.K) and three locally manufactured brands namely Optudisc (manufactured by Optun Laboratories, Nigeria Ltd, Aba), Brodisk (Bronila Diagnostic Systems, Enugu, Nigeria) and Jirehdisk (produced by Jireh Laboratories, Nigeria). Abtek multidiscs were purchased from C.C. Obi (Nig) Ltd, in Lagos, Brodisk from the manufacturer, Bronila Diagnostic Systems, in Enugu, Optudisc from MEDLABCOP,

a Medical Laboratory supply group, in Port Harcourt, and Jirehdisk from IG Enterprises, in Aba. All discs were stored at 2-8<sup>0</sup>C throughout the period of the study as recommended by the manufacturers. The study was done between August and December 2005.

### **1.1 Strains**

Clinical isolates of *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Escherichia coli* were obtained from Microbiology Laboratory of the Federal Medical Centre (FMC), Umuahia, Abia State, Nigeria. The bacterial strains were subsequently maintained on Nutrient Agar slants at the Department of Microbiology Laboratory, Michael Okpara University of Agriculture, Umudike. Culture media used were Nutrient Agar (International Diagnostics Groups, Plc, Bury, Lancashire, U.K), and Mueller Hinton Agar (Oxoid Limited, Basingstoke, Hampshire, England). Culture media were prepared according to the instructions of the manufacturers.

The preparation of McFarland turbidity standard (Tube 0.5) and the inocula of the bacterial strains were prepared as described by Cheesbrough (5). Disc Diffusion susceptibility test as originally described by Bauer et al (7) was carried out according to the procedures of the National Committee for Clinical Laboratory Standards (NCCLS) methods (6). Briefly, a sterilized wire loop was used to transfer 3-5 isolated colonies from a Nutrient agar plate into a bijou bottle containing about 4ml of physiological saline. The colonies were emulsified in the normal saline to obtain a homogenous suspension of the bacterial cells. The turbidity of the suspension was adjusted visually to that of 0.5 McFarland Standard by adding sterile physiological saline to the suspension. This was used as the inoculum. A sterile swab stick was dipped in the standardized inoculum in a bijou bottle; excess fluid was removed from the swab by pressing it against the

side of the bottle. The surface of a Mueller Hinton agar plate previously dried in an incubator was streaked with the swab. The plate was left on the bench for about 20-30 minutes. The antimicrobial discs were aseptically placed on the inoculated plates. Each disc was gently pressed on the agar surface using a sterilized forceps to ensure proper contact. Plates were inverted within 30 minutes of applying the discs and incubated aerobically at 35-37°C for 12- 18 hours. The diameter of the zone of inhibition around each disc was measured in millimetre (mm) using a plastic transparent ruler.

## RESULTS

Three brands of locally manufactured antimicrobial sensitivity discs (Optudisc, Brodisk and Jirehdisk) were evaluated for their quality by comparing their performances with that of a foreign brand, Abtek disc, in an Agar Diffusion antibiotic susceptibility testing assay. Diameter of zone of inhibition measured in millimetres (mm) was used as a parameter for evaluation of the performance of the antibiotic discs. Table 1 presents the product presentation and packaging characteristics of the brands of antibiotic sensitivity discs evaluated in the study. The performance of Abtek and Brodisk brands of antibiotic sensitivity discs against the test bacteria are presented in Table 2 and Table 3 respectively. Few antibiotics are common to both brands. The performances of discs of these antibiotics are compared in Table 4. Gentamycin discs on Abtek produced a mean diameter of zone of inhibition of 15mm, 19mm and 16 mm against *S.aureus*, *P. aeruginosa* and *E. coli* respectively. Gentamycin discs with the same stated antibiotic concentration on Brodisk produced no zone of inhibition against the test organisms. Similarly, Amoxicillin disc on Abtek produce a mean diameter of zone of inhibition of 13mm against *S. aureus* but the same disc on Brodisk did not produce any activity. The other three antibiotics common to both brands namely Nitrofurantoin, Cloxacillin and Erythromycin produced no

inhibition zone against the test organisms. The only difference being that the Abtek Nitrofurantoin disc had a potency of 300µg while the Brodisk Nitrofurantoin had a potency of 2000µg.

Table 5 presents the results of the performance of the Optudisc brand and Table 6 presents that of Jirehdisk. Most of the discs on Optudisc brand produced un-interpretable zones of inhibition against the test bacteria. The zone of inhibition was regarded as un-interpretable when it was too large and either merged together or the whole plate was wiped out before the incubation period was reached. When this occurred in at least 2 out of 3 plates tested, it was recorded as un-interpretable. This occurred most frequently with Gram Positive discs of Optudisc tested against *S. aureus*

We compared the performances of discs of the same antibiotics common to both Abtek and Optudisc. The results are presented in Table 7. Seven antibiotics are common to Abtek and Jirehdisk. Comparing the performance of discs of these antibiotics (Table 8), Augmentin disc on Abtek produced a mean diameter zone of inhibition of 21mm against *S. aureus* while the disc of the same antibiotic on Jirehdisk produced un-interpretable results. On the other hand while Gentamycin disc against Gram Positive produced a similar result against *S. aureus* for both brands, the discs of the antibiotic on the Gram Negative pack produced different results. Abtek discs produced zone of inhibition of 16 mm and 19 mm against *E.coli* and *P. aeruginosa* respectively while the Jirehdisk produced no zone of inhibition against these organisms. Ofloxacin (Tarivid) and Tetracycline discs produced similar results against *P.aeruginosa* (see Table 8). Out of the three local brands, Optudisc produced the greatest number of un-interpretable results followed by Jirehdisk. Brodisk performed closest to the Abtek indicating that it appeared to be the most standardized of the locally manufactured antibiotic sensitivity discs.

**Table 1: Product Presentation and Packaging Characteristics of the Different Brands of Antibiotic Sensitivity Discs Evaluated**

Brand Name	Country of Manufacture	Lot No.	Format and Characteristics of disc.	No. of discs/plate (90 mm)	Total No. of antibiotics in each brand (G+ve & G-ve)
Abtek	UK.	FC05/P FC06/P	Multidisc with centre cut out for growth control. Neatly cut discs.	8	11
Brodisk	Nigeria	None	Multidisc with solid centre, no provision for growth control. Fairly neatly cut discs.	8	11
Optudisc	Nigeria	None	Multidisc with centre cut out for growth control. Discs are rough and unequal in size.	10	17
Jirehdisk	Nigeria	None	Multidisc with centre cut out for growth control. Discs are rough and uneven in outline.	10	14

**Table 2: Diameter of Zones of Inhibition (mm) Produced by Abtek Brand of Antibiotic Sensitivity Discs against the Test Organisms.**

Antimicrobial Agents	Code	Stated Disc potency		<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
		G+ve	G-ve			
Augmentin	Aug	30□g	30□g	21	0	0
Amoxicillin	Amx	25□g	25□g	13	0	0
Erythromycin	Ery	5□g	-	0	-	-
Tetracycline	Tet	10□g	30□g	0	13	0
Cloxacillin	Cxc	5□g	-	0	-	-
Gentamycin	Gen	10□g	10□g	15	19	16
Cotrimoxazole	Cot	25□g	25□g	0	0	0
Chloramphenicol	Chl	30□g	-	20	-	-
Nitrofurantoin	Nit	-	300□g	-	0	0
Nalidixic acid	Nal	-	30□g	-	8	0
Ofloxacin	Ofl	-	30□g	-	31	0

**Table 3: Diameter of Zones of Inhibition (mm) produced by Brodisk Brand of Antibiotic Sensitivity Discs against the Test Organisms**

Antimicrobial Agent	Code	Stated Disc potency		<i>S. aureus</i>	<i>P. aeruginosa</i>	<i>E. coli</i>
		G+ve	G-ve			
Ampicillin	AMP/PN	10mcg	25mcg	0	0	0
Cephalexine	Cx	10mcg	25mcg	0	0	0
Cefuroxime	Cxm	-	30mcg	-	0	0
Gentamycin	Gen	10mcg	10mcg	0	0	0
Ciproxin/Ciprofloxacin	Cip	5mcg	10mcg	21	33	0
Nitrofurantoin	F	-	200mcg	-	0	0
Aztreonam	AZM	30mcg	30mcg	0	19	*U (2of3)
Levofloxacin	Lev	-	10mcg	-	26	0
Amoxicillin	AM	20mcg	-	0	-	-
Cloxacilin	OB	10mcg	-	0	-	-
Erythromycin	E	15mcg	-	0	-	-

\*U - Un-interpretable diameter zone of inhibition; zone was too large and merged in with another zone.  
 - Not tested, antibiotic not on the G+ve or G-ve pack

**Table 4: Comparison of the performance (Diameter of zones of Inhibition (mm) of Brodisk with Abtek discs against the test Organisms.**

Antibiotics	Code		Stated disc potency				Test organisms					
			G+ve		G-ve		<i>S. aureus</i>		<i>E.coli</i>		<i>P. aeruginosa</i>	
	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek	Brodisk	Abtek
Gentamycin	Gen/GEN	Gen	10mcg	10□g	10mcg	10□g	0	15	0	16	0	19
Nitrofurantoin	F	Nit	-	-	200mcg	300□g	-	-	0	0	0	0
Amoxycillin	AM	AMX	20mcg	25□g		25□g	0	13	-	0	-	0
Cloxacilin	OB	CXC	10mcg	5□g	-	-	0	0	-	-	-	-
Erythromycin	E	Ery	15mcg	5□g	-	-	0	0	-	-	-	-

**Table 5: Diameter of Zone of Inhibition (mm) Produced by Optudisc Brand of Antibiotic sensitivity discs against the test organisms.**

Antimicrobial Agent	Code	Stated Disc potency		Diameter of zone of inhibition (mm) against Test organism		
		G+ve	G-ve	<i>S. aureus</i>	<i>P.aeruginosa</i>	<i>E. coli.</i>
Tarivid (Ofloxacin)	OFX	-	10mcg	-	U (2 of 3)*	13
Peflacin	PEF	-	10mcg	-	U (2 of 3)	0
Ciproflox	CPX	10mcg	10mcg	U (3 of 3)	U (2 of 3)	16
Augmentin	AU	-	30mcg	-	0	0
Gentamycin	CN	10mcg	10mcg	U (3 of 3)	U (2 of 3)	U (2 of 3)
Streptomycin	S	-	30mcg	-	U(2 of 3)	18
Ceporex	CEP	-	10mcg	-	0	0
Nalidixic Acid	NA	-	30mcg	-	8	0
Seprin (Co-trimoxazole)	SxT	-	30mcg	-	8	0
Ampicillin	PN	-	30mcg	-	0	0
Norfloracin	NB	-	30mcg	U (3 of 3)	-	-
Lincocin	LC	-	30mcg	U (3 of 3)	-	-
Rifampin	RD	10mcg	-	U (3 of 3)	-	-
Floxapen	FLX	30mcg	-	U (3 of 3)	-	-
Erythromycin	E	30mcg	-	U (3 of 3)	-	-
Chloramphenicol	CH	20mcg	-	U (3 of 3)	-	-
Ampiclox	APX	30mcg	-	U (3 of 3)	-	-

\*U - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.  
 - Not tested, antibiotic not on the G+ve or G-ve pack

**Table 6: Comparison of the performance (diameter of zone of inhibition (mm) of Jirehdisk discs with Abtek discs against the test organisms.**

Antibiotics	Code		Stated disc potency				Test organisms				<i>p. aeruginosa</i>	
	Jireh disk	Abtek	Jireh disk	Abtek	Jirehdisk	Abtek	Jirehdi sk	Abt ek	Jirehdi sk	Abt ek	Jireh disk	Abt ek
Augmentin	AG	Aug	30mcg	30□g	-	30□g	U	21	-	0	-	0
Seprin (cotrimoxazole)	SXT	COT	25mcg	25□g	25mcg	25□g	0	0	0	0	0	0
Gentamycin	CN	Gen	10mcg	10□g	10mcg	10□g	17	15	0	16	0	19
Ofloxacin (Tarivid)	OFX	OFL	10mcg	-	10mcg	30□g	22	-	0	0	0	31
Erythromycin	E	Ery	5mcg	5□g	-	-	0	0	-	-	-	-
Nitrofurantoin	N	Nit	-	-	200mcg	300□g	-	-	0	0	0	0
Tetracycline	T	Tet	-	10□g	25mcg	30□g	-	0	0	0	0	13

**Table 7: Comparison of the performance (Diameter of zones of Inhibition (mm) of Optudisc with Abtek discs against the test bacteria.**

Antimicrobial Agents	Code	Stated disc potency				Test organisms				P.		
		G+ve		G-ve		S. aureus		E.coli		aeruginosa		
	Optu disk	Abt ek	Optudi sc	Abtek	Optudisc	Abtek	Optu disc	Abt ek	Optu disc	Abt ek	Optudi sc	Abt ek
Augmentin	AU	Aug	-	30µg	30mcg	30µg	-	21	0	0	0	0
Gentamycin	CN	Gen	10mcg	10µg	10mcg	10µg	U	15	U	16	U	19
Tarivid (Ofloxacin)	OFX	Ofl	-	-	10mcg	30µg	-	-	13	0	U	31
Nalidixic acid	NA	Nal	-	-	30mcg	30µg	-	-	0	0	8	0
Septrin (co-trimoxazole)	SXT	Cot	-	25µg	230mcg	30µg	-	0	0	0	8	0
Erythromycin	E	Ery	30mcg	5µg	-	-	U	0	-	-	-	-
Chloramphenicol	CH	Chl	20mcg	30µg	-	-	U	20	-	-	-	-

\*U - Un-interpretable diameter zone of inhibition: zone was too large and merged in with another zone.

- Not tested, antibiotic not on the G+ve or G-ve pack

## DISCUSSION

The results of our evaluation of the three brands of locally manufactured antimicrobial susceptibility discs in common use in the South eastern parts of Nigeria indicate the need for further standardization of these brands of antimicrobial susceptibility discs. The Three 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. Furthermore, while one brand includes the same antibiotic on the Gram Positive pack, another includes the same antibiotic on the Gram Negative Pack. The situation reflects a state of much confusion, which makes it difficult to make a direct comparison of the performance of the different brands. 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. Antimicrobial agents included for susceptibility testing should conform to a national antibiotic usage policy. Furthermore, the number of discs on

a multidisc panel for 90mm Petri dish should conform to International Standard such as the recommendation of the WHO of six or not more than 8 discs per such plate. In the present study, two of the local brands had 10 discs per plate. This probably contributed to the problem of un-interpretable zones of inhibition very common to discs on these brands.

The problem of un-interpretable zone of inhibition produced by some discs on the various local brands needs further attention. In some cases, the zones of inhibition were too large and either merged together or the whole plate was wiped out before the required incubation period. This occurred in at least 2 out of 3 plates tested before it was so recorded. We want to believe that this was due to poor standardization in the preparation of the discs. The discs probably contained antibiotic concentration above the stated potency. In comparison, Abtek, the imported brand did not produce a single case of un-interpretable result. Only a case of such results was observed with

Brodisk's Aztreonam disc against *E. coli* (see Table 3).

The clinical implication of higher concentration of antibiotic beyond the required stated concentration in sensitivity discs is that misleading results might be sent to the clinicians based on the wrong results being produced by the discs. A bacterial strain may be recorded as sensitive while in actual case it is resistant. The wrong result could be used as the basis for antibiotic prescription. This will be of no benefit to the patient and can actually delay treatment with the right drug. It can further contribute to the problem of misuse of antibiotics (8) that favours the emergence of drug resistant strains of bacteria (9).

All the three locally made brands of susceptibility discs evaluated in this study manifested poor quality and performed below expected standard in comparison to the imported brand. Brodisk performed closest to the imported brand and appeared to be the most standardized. With further improvement in quality, this brand can be recommended for use in Nigeria.

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