Microbiological Assessment of Commercial Antibiotic discs from selected Pharmacies in Bwari Area Council, Abuja, Nigeria.

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ABSTRACT:

Aims: To assess the potency of two brands of locally prepared antibiotics against two bacterial species.
Place and Duration of Study: Department of Microbiology, Veritas University, Abuja, Nigeria, between September 2021 and February, 2022.
Methodology: We purchased two brands of antibiotics and obtained our test isolates from General hospital in Bwari, Abuja. The test isolates were characterized both routinely and molecularly by Sanqer sequencing method to confirm the species. Agar diffusion method was employed to assess the effectiveness of the antibiotics on the test isolates. Finally, the **results were compared with the** Clinical and Laboratory Standards Institute (CLSI).
Results: The isolates obtained from the Bwari General Hospital were characterized routinely and molecularly to identify the species. Disc - agar diffusion modified method was employed while the potencies of the discs were compared with approved standards of the Clinical and Laboratory Standards Institute (CLSI). The two brands which are locally made and available in multidisc panels, demonstrated performances against *Staphylococcus sciuri* and *Proteus mirabilis* but showed significant variations in concentrations and in their inhibitory zone diameters. Both brands containing Tarivid (10 µg) produced only a small zone against *Proteus mirabilis*, while Streptomycin (30 µg) and Gentamycin (10 µg) exhibited unreadable zones against *Staphylococcus sciuri*. Unreadable zones of inhibition, which implies large zones that merged together and exceeded plate diameters at the time of reading; were common with both brands and can lead to mis-information, increase in the susceptibility of resistant organisms and eventually, drug abuse.
Conclusion: The result of this assessment shows, that there is gross variation in these commercially available but locally prepared antibiotic discs. The disparity in the types of antimicrobial agents and their concentrations will pose a delicate problem to clinical microbiologists and subsequently in the diagnosis, administration of antibiotics and the likelihood of drug resistance.

*Keywords: [*Antibiotics, susceptibility, discs, bacterial species, agar diffusion, inhibition zone].

1. INTRODUCTION

Antimicrobial susceptible discs aid in the identification and management of microbial infections and resistance. Clinical microbiologists must perform an antibiotic susceptibility test to determine whether a bacterial isolate is susceptible to a particular empirical antimicrobial agent or to identify resistance. According to Kahlmeter 2016 [1] d the European Committee on Antimicrobial Susceptibility Testing (EUCAST), in 2021 [2], this enables doctors to provide the best course of antibiotic treatment. This

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test is used in the drug development process to check the antibacterial activity of biological materials such as plant extracts and drug candidates. For organisms causing infectious diseases and opportunistic pathogenic species, whose susceptibilities are difficult to predict from knowledge of their identities, antimicrobial susceptibility testing (AST) is recommended.

Antibiograms, or specific susceptibility patterns, are traits of species and frequently help to identify the organisms. Antibiograms are also determined for epidemiological purposes, as the presence of uncommon antibiograms for a particular species aids in identifying the outbreak's origin and cross-infection patterns. [3]Numerous variables, including the antimicrobial preparations - discs, media, inoculum size, plate reading, incubation conditions, and the competence of the laboratory staff [4]- can affect the accuracy of susceptibility tests conducted routinely or for a specific research purpose. The accuracy of antimicrobial susceptibility test results is of the utmost importance[5] as careful control and standardization of the various steps and components of the testing procedures are required for the results to be reliable and reproducible.[6] The in vitro susceptibility of organisms to antimicrobial drugs can be assessed using a range of laboratory procedures, such as disc diffusion, broth dilution, and agar dilution techniques (Clinical and Laboratory Standards Institute.[7] Antimicrobial susceptibility testing still requires the controlled tube (broth) dilution technique, but this method is time-consuming and can only be employed regularly in a few specialist hospital laboratories. [8]

Agar dilution is most typically employed to test the efficacy of new antibiotics when a small number are tested against a big panel of various bacteria. [9] This method is used by researchers to establish the Minimum Inhibitory Concentration (MIC) of antibiotics. In order to evaluate the MIC of the antibiotic against many types of bacteria, this technique enables replicate spots of one bacterial type to be examined [10] or spots of diverse bacteria. Antibiotics are thought to have a minimum inhibitory concentration against a particular bacterium at the lowest concentration at which bacterial growth was inhibited.[11]

The most precise method of measuring bacterial resistance to antibiotics is agar dilution, which is regarded as the reference standard in susceptibility testing. [11] CLSI recommends using this method for susceptibility testing of *N. gonorrhea*[12] as well as fastidious bacteria like *Helicobacter* sp. and *Campylobacter* sp., which are anaerobic bacteria. [7] Multiple pathogen samples can be evaluated simultaneously, and the outcomes of agar dilution can be easily replicated and monitored for less money.[11]

Unlike the agar dilution method, the broth dilution method uses a liquid growth medium that is seeded with a predetermined number of bacterial cells and contains geometrically rising quantities of a twofold dilution series of the antimicrobial agent (e. g. 1, 2, 4, 8, and 16 g/ml). Whether the method is referred to as Macro-dilution when employing a total volume of 2 ml or Micro-dilution when carried out in micro-titer plates with a capacity of 500 l per well depends on the test's final volume. Small, disposable plastic trays are used in the mechanical and miniature form of broth dilution known as microdilution.

The creation of MICs, the reproducibility and convenience of having pre-prepared panels, and the economy of chemicals and space during the procedure are all benefits of the microdilution method. The main drawbacks of the broth dilution technique include the time-consuming nature of making the antibiotic solutions for each test, the potential for mistakes during setup, and the relatively high reagent and storage requirements. [6] Furthermore, the MIC values do not reveal the antimicrobial drugs' mechanism of action (bactericidal or bacteriostatic). If the antibiotic had a bacteriostatic impact on the tested bacterial species, live cells may still be present in the MIC wells or tubes with no discernible growth. [13] But the most practical approach is still the disc diffusion method, which is still the method of preference for typical laboratories. [14] The antimicrobial drug is allowed to penetrate into the media and interact with newly seeded test organisms on a plate. [14]

Susceptibility testing must now be conducted on a regular basis due to the commercial availability of numerous antimicrobial agents and medications. The various susceptibility testing techniques and their extensive application are evidence of the crucial function that resistance detection serves in susceptibility testing. The accuracy of each of these approaches must be assessed by contrasting the outcomes with those from traditional systems. The majority of processes merely classify microbes as being either highly susceptible, moderately susceptible, intermediately susceptible, or resistant to the various antimicrobial agents. All commercial techniques, however, have the same objective of identifying resistance and the affinity of isolated organisms to antimicrobial substances.

Testing for susceptibility is typically avoided when conducted on organisms that are known to be non-pathogenic and on members of the normal flora in their natural habitats. [3] The potency and effectiveness of the antimicrobial content of the discs, as well as the quality of the paper, must be guaranteed. The quality of antibiotic discs is especially important in susceptibility tests of microbial pathogens. Commercially accessible antimicrobial discs, whether they are imported or locally produced, are occasionally deficient in the quality, amount, or concentration of the purported antimicrobial agents needed to determine their test results. [15] The necessity for additional study on antimicrobial susceptibility discs therefore arises. There is no work being done on these kinds of antibiotics in Bwari Area Council, FCT, Abuja. In order to determine the microbiological potency of antibiotic discs used in antimicrobial susceptibility testing from particular pharmacies in Bwari Area Council, FCT, Abuja, this research is being conducted.

2. MATERIALS AND METHOD

2.1 Sample collection

Antibiotic susceptibility discs of two kinds were purchased from a pharmacy in Bwari Area Council, Federal Capital Territory, Abuja. In accordance with the manufacturers' recommendations, the discs were kept at 6°C throughout the duration of the study.

2.1.1 Sources of test organisms

Culture plates of *Staphylococcus* sp. and *Proteus* sp. were obtained from the medical laboratory of General Hospital, Bwari Area Council; FCT, Abuja and transported to the Microbiology laboratory, Veritas University, Abuja. Pure cultures of the bacterial species were prepared and the isolates were characterized both routinely and molecularly to confirm each of the bacterial species.

2.2 Characterization of test isolates by routine analysis

A wet smear of the culture on the agar plate was made on a clean slide. The smear was allowed to air dry and then was heat-fixed. Gram staining according to the method described by [16] was used, and smear was observed at low magnification by the oil immersion objective of the microscope. The isolate was identified using culture morphology, microscopic examination, carbohydrate fermentation, and other biochemical assays. [17] For bacterial identification, [18] was used.

2.3. Molecular characterization

The test organisms that were suspected to be Staphylococcus sp. (SLM 003) and Proteus sp. (PTV 002) were subjected to molecular characterization using 16S rDNA sequencing. With the help of the Quick-DNATM Fungal/Bacterial Miniprep Kit (Zymo Research, Catalogue No. D6005), genomic DNA was recovered from the cultures that were provided. Using the primers and OneTaq® Quick-Load® 2X Master Mix (NEB, Catalogue No. M0486), the 16S target area was amplified. Following a run on an agarose gel, the PCR products were extracted using the ZymocleanTM Gel DNA Recovery Kit (Zymo Research, Catalogue No. D4001) by Zymo Research.

The isolated fragments were purified using Zymo Research's ZR-96 DNA Sequencing Clean-up KitTM, Catalogue No. D4050, before being sequenced both forward and backward (Nimagen, BrilliantDyeTM Terminator Cycle Sequencing Kit V3.1, BRD3- 100/1000). For each reaction and each sample, the purified fragments were examined using the ABI 3500XL Genetic Analyzer (Applied Biosystems, ThermoFisher Scientific). The ABI 3500XL Genetic Analyzer's.abl files were analyzed using CLC Bio Main Workbench v7.6, and the findings were retrieved using a BLAST search (NCBI). (Image courtesy of Inqaba Biotec West Africa Ltd.

2.4 Antimicrobial susceptibility testing (AST)

The Kirby-Bauer *et al*., modified methodology for disc diffusion susceptibility by the Clinical and Laboratory Standards Institute [7] was employed.
Three (3) to 5 colonies of the test organisms were transferred from an agar plate into a bijou bottle containing 4 ml of physiological saline using a sterilized wire loop. To create an even suspension of the bacterial cells, the colonies were emulsified in saline. By adding sterile physiological saline to the suspension, the turbidity was changed to match the 0.5 McFarland Standard.
A sterile swab stick was dipped into the bijou bottle's standardized culture before being pressed against the bottle's interior above the solution to drain any surplus liquid. The swab was used to leave streaks on the surface of a Mueller Hinton agar plate that had previously dried in an incubator. The antimicrobial discs were aseptically placed on the inoculation plates after the plate had been on the bench for 20 minutes. To ensure good contact, each disc was gently pressed onto the agar surface using sterile forceps. After placing the discs, the plates were immediately inverted and incubated aerobically at 370 C for 12 to 18 hours.
This procedure was conducted in triplicates for each antibiotic disc against the respective test organisms. Their inhibitory zone diameters inhibitory zone diameters (IZDs) were measured in mm and recorded accordingly. The Zone diameters were compared with those of the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guide, for the respective test organisms as reference.

3. RESULTS AND DISCUSSION

The quality of the two brands of antibacterial discs - Maxidisc and Optudisc were evaluated by determining their antibacterial performances in agar-disc diffusion susceptibility testing assay. The mean inhibitory zone diameters (IZDs) measured in millimeters (mm), were calculated from the triplicate zones obtained for every disc. The mean IZDs were used as the yardstick for evaluating their potency.

The table 1 below shows the characteristics of the two brands oantibacterial sensitivity discs employed in the study maxidisc and optudisc. Both are multi-panel having 10 different panels per disc with 14 antibiotics for maxidisc and 17 for optudisc per panel.

Table 1: Characteristics of the brands of antibiotic discs

Brand Name	Manufacturer	Disc Presentation	No. of discs per panel	Total no. of Antibiotics in each brand packet (G+ve & G-ve)	Spatial distance in-between discs (mm.)
Maxidisc	Maxicare Medical Laboratories, Nigeria.	Multidisc panels of different antibiotic discs	10	14	7
Optudisc	Optun Laboratories, Nigeria, Ltd.	Multidisc panels of different antibiotic discs	10	17	7

G+ = Gram positive; G- = Gram negative

Molecular characterization

Molecular characterization of the test isolates by Sanger sequencing confirmed the two isolates to be *Staphylococcus sciuri* and *Proteus mirabilis* [S1].

Table 2: Mean diameters of zone of inhibition (mm) produced by maxidisc brand of G-ve antibiotics against *Proteus mirabilis*

Antimicrobial Agent	Code	Stated potency (µg)	CLSI (µg)	Diameter of zone of inhibition (mm)	
Tarivid	OFX	10	5	11	
Pefloxacin	PEF	30	5	0	
Gentamycin	CN	30	10	11	
Augmentin	AU	10	30/20/10	0	
Amoxacillin	AM	30	30/20/10	0	
Ciprofloxacillin	CPX	30	5	11	
Sparfloxacillin	SP	10	5	7	
Chloramphenicol	CH	30	30	13	
Septrin	SXT	30	25	U	
Streptomycin	S	30	10	11	

Tarivid (OFLOXACIN) - OFX
CLSI - Clinical and Laboratory Standards Institute
U - unreadable. Zone diameter was too large, merged with another zone and exceeded plate diameter.
Augmentin* - Available in 30µg, 20µg and 10µg
Amoxacillin* - Available in 30µg, 20µg and 10µg

Table 3: Mean diameters of zone of inhibition (mm) produced by optudisc brand of G-ve antibiotics against *Proteus mirabilis*

Antimicrobial Agent	Code	Stated potency (µg)	CLSI (µg)	Diameter of zone of inhibition (mm)
Tarivid	OFX	10	5	13
Nalidixic Acid	NA	30	30	0
Pefloxacin	PEF	10	5	5
Gentamycin	CN	10	10	17
Augmentin	AU	30	30/20/10	11
Ciproflox	CPX	10	5	15
Septrin	SXP	30	30	U
Streptomycin	S	30	10	0
Ampicillin	PN	30	10	0
Ceporex*	CEP	10	30	0

Tarivid (OFLOXACIN) - OFX
CLSI - Clinical and Laboratory Standards Institute
Ceporex* - Also known as Cefalexin or Cephalexin
U - unreadable. Zone diameter was too large, merged with another zone and exceeded plate diameter.

Table 4: Mean diameters of zone of inhibition (mm.) produced by maxidisc brand of G+ve antibiotics against *Staphylococcussciuri*.

Antimicrobial Agent	Code	Stated potency (µg)	CLSI (µg)	Diameter of zone of inhibition (mm)
Pefloxacin	PEF	10	5	U
Gentamycin	CN	10	10	U
Ampilclox*	APX	30	-	19
Zinacef*	Z	20	30	25
Amoxacillin	AM	30	30/20/10	19
Rocephin	R	25	30	25
Ciprofloxacin	CPX	30	5	U

Streptomycin	S	30	10	U
Septrin	SXP	30	25	U
Erythromycin	E	10	15	U

Ampiclox* (Not enlisted) - A combination of Ampicillin (10µg) and Cloxacillin (30µg) or Penicillin (10µg) and Oxacillin (30µg).
Zinacef* - Also known as Cefuroxime
CLSI - Clinical and Laboratory Standards Institute
U - unreadable. Zone diameter was too large, merged with another zone and exceeded plate diameter.

Table 5: Mean diameters of zone of inhibition (mm) produced by optudisc brand of G+ve antibiotics against *Staphylococcussciuri*.

Antimicrobial Agent	**Code**	**Stated potency (µg)**	**CLSI (µg)**	**Diameter of zone of inhibition (mm)**
Ciproflox	CPX	10	5	U
Norfloxacin	NB	10	10	23
Gentamycin	CN	10	10	U
Amoxil*	AML	20	20/10	33
Streptomycin	S	30	10	U
Rifampicin*	RD	20	5	U
Erythromycin	E	30	15	U
Chloramphenicol	CH	30	30	U
Ampiclox*	APX	20	-	33
Levofloxacin	LEV	20	5	U

Amoxil* - Also known as Amoxicillin. Enlisted as Amoxicillin-Clavulanate
Rifampicin* - Also known as Rifampin
Ampiclox* - A combination of Ampicillin (10µg) and Cloxacillin (30µg) or Penicillin (10µg) and Oxacillin (30µg).

U – unreadable. Zone diameter was too large, merged with another zone and exceed plate diameter

Inhibitory zone diameters exceeding the edges of plates were recorded as unreadable. Maxidisc produced greater number of unreadable zones against the Gram -ve organism while Optudisc and Maxidisc produced seven and six unreadable zones against the Gram +ve organism respectively. The relative susceptibility behaviour of the antimicrobial agents common to both brands against the test organisms, are compared in Tables 6 and 7 below.

Table 6: Comparison of mean diameters of zone of inhibition (mm) of antimicrobial agents common to maxidisc and optudisc (G -ve) brands against *Proteus mirabilis*

Antimicrobial Agent	Code	Stated potency (µg)			
		Maxidisc	Optudisc		
				Max	Opt
Tarivid	OFX	10	10	11	13
Pefloxacin	PEF	30	10	0	5
Gentamycin	CN	30	10	11	17
Augmentin	AU	10	30	0	11
Ciprofloxacin	CPX	30	10	11	15
Septrin	SXP	30	30	U	U
Streptomycin	S	30	30	11	0

Tarivid (OFLOXACIN) - OFX
U – unreadable. Zone diameter was too large, merged with another zone and exceed plate diameter.

Table 7: Comparison of mean diameters of zone of inhibition (mm) of antimicrobial agents, common to maxidisc and optudisc (G +ve) brands against *Staphylococcussciuri*

Antimicrobial Agent	Code	Stated potency (µg) Maxidisc	Optudisc	Max	Opt
Streptomycin	S	30	30	U	U
Ampiclox	APX	30	20	19	33
Gentamycin	CN	10	10	U	U
Ciproflox	CPX	30	10	U	U
Erythromycin	E	10	30	U	U

U – unreadable. Zone diameter was too large, merged with another zone and exceed plate diameter.

Discussion

As a low-cost and flexible approach for AST, disc diffusion may cause undesirable variances and impotency if disc quality is not managed. [19] Both of the AST brands used in this study were produced locally and contained various antibiotics on multidisc panels that were packaged collectively in a plastic container. Similar observations about the physicochemical characteristics and subpar packaging of locally produced antimicrobial discs were reported. [8] This packaging method may cause nearby antimicrobial compounds to cross-diffuse or become contaminated, which could lead to uneven zone widths. In order for a product to pass quality standards, it must be presented in a way that does not compromise or adversely affect the product's integrity. [20]

Furthermore, the World Health Organization [21] and Clinical Laboratory Standards Institute [7] limit the number of discs on a panel to seven (7) for a 90 mm petri dish plate utilized in this investigation, as opposed to ten (10) on the brands evaluated (Table 2). This exacerbated the issue of overlapping zones and murky dimensions. According to [22], the development of illegible zones of inhibition is a severe concern and is clearly caused by a lack of standardization in the disc fabrication process. The various antimicrobial agents were represented by various codes, however the bulk of these drugs included higher concentrations than were required by standards. Additionally, this argument was made in the research of [8 ; 22] .

Optudisc brand contained 10 g of Tarivid, Pefloxacin, and Ciprofloxacin while Streptomycin and Ampicillin are both 30 g each (Tables 2; 3 and 4) for the Gram -ve discs, and Gram +ve discs from both manufacturers. Maxidisc contained 30 g of Pefloxacin, Gentamycin, Ciprofloxacin, Septrin, and Streptomycin. The implication is, an increased inhibition diameter and possible immeasurable zones in AST, which results in false positive sensitivity readings. This grave danger was also succinctly expressed in the discussion of [22] in their quality assessment of commercial discs in Nigeria. Seven antibiotics are common to both brands of Gram -ve discs and five, common to Gram +ve discs. Maxidisc produced higher number of unreadable zones against the Gram -ve organism *Proteus mirabilis*. This is in disagreement with [23] who noted that Optudisc produced more unreadable zones against the Gram -ve organisms *E. coli,* than did Maxidisc. All the antibiotics common in both brands of Gram +ve discs (except only in the case of Apiclox - APX), showed unreadable zones against the Gram +ve organism – *Staphylococcus scturi* (Table 5). This is in line with the reports of [22].

Although they bear the same codes, variations occur in their stated potencies as well as their antimicrobial performances.

Some antibiotics with lower stated potencies, were observed to produce inhibition zones greater than similar discs from the other brand with a higher stated potency (Table 5). Maxidisc contained Pefloxacin (30 µg), Gentamycin (^{30} µg), Augmentin (10 µg) and Ciprofloxacin (30 µg), potencies; but the same drugs on the Optudisc brand bear 10µg, 10µg, 30 µg and 10 µg respectively. Maxidisc brand of Pefloxacin (30µg) and Augmentin (10µg) against *Proteusmirabilis* produced no zones, whereas the same antimicrobial agents on Optudisc brand bearing potencies of 10 µg and 30 µg respectively; produced zones of 5 mm and 11 mm respectively. It was also observed that Erythromycin with different concentrations, produced unreadable diameters against *Staphylococcussciuri*. These reports agree with the comments of [22] and in the comparative study of [24] , about the impossibility of comparing the performances of different brands of commercial antibiotic discs. Both brands of antimicrobial discs differ significantly from CLSI approval on stated potency [8] however; where the manufacturers comply on concentrations, the zone diameters are almost the same with few exceptions. Streptomycin (30µg) and Gentamycin (10µg) both produced unreadable zones against *Staphylococcussciuri*, Septrin (30µg) also produced unreadable and large zones against *Proteusmirabilis* while Streptomycin produced similar zones but with exception against *Proteusmirabilis.* The problem of unreadable zones produced by both brands against *Staphylococcussciuri* is of serious clinical concern. The works of [23] also observed the problem of unreadable zones among locally produced discs, but remarked that such occurrences rarely happened with imported brands.

4. CONCLUSION

The result of this assessment shows, that there is gross variation in these commercially available but locally prepared antibiotic discs. The disparity in the types of antimicrobial agents and their concentrations will pose a delicate problem to clinical microbiologists and subsequently in the diagnosis, administration of antibiotics and the likelihood of drug resistance. The importance of this finding is to emphasize the significant role of maintaining improved standardization of antimicrobial discs manufacturing and handling, for susceptibility tests. Furthermore, to aid clinical microbiologists and researchers in the choice of antibiotics; in therapeutic management of pathogens, infections and drug discovery.

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