



Comparative Study on Effectiveness of Commercial Antibiotic Discs in Federal Capital Territory (FCT), Nigeria

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Authors' contributions

This work was done in collaboration among all authors. Author MIA designed the study, wrote the first draft of the manuscript and performed the analysis. All the authors managed the literature search writing of the final manuscript, read and approved the final manuscript.

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ABSTRACT

Clinically, antimicrobial susceptibility testing results provide guidance in the choice of antimicrobial agents in patient care. The accuracy of results from antimicrobial susceptibility testing can be affected by multiple factors including the media, antimicrobial discs or preparations, inoculum's size, plate reading and incubation conditions. Misleading results from antimicrobial susceptibility test leads to the indiscriminate and irrational use of antibiotics and have impacted grossly to the global challenge of antimicrobial resistance. The objectives of this study were to compare the efficacy of different brands of locally and foreign manufactured multi-antibiotic discs on bacteria and assess any significant variation. Two brands each of locally and foreign manufactured multi-antibiotic discs were purchased from retail stores within the FCT. The antibacterial susceptibility of *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 9150, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* ATCC 25922 and *Streptococcus pyogenes* were carried out using agar diffusion method. There were differences between the diameter zones of inhibition produced by the local brands and the foreign brands of antibiotic discs.

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Amoxicillin/clavulanic acid (30 µg) disc produced the highest variation within the four brands with zones of inhibition range 12.0 – 20.0 mm against the test organisms. There is need for regulatory bodies like NAFDAC and SON to routinely validate and assess the qualities of these products in the market.

Keywords: Antimicrobial susceptibility test; antibiotic discs; resistance; zone of inhibition.

ABBREVIATIONS

FCT- Federal Capital Territory

ZOI- Zone of inhibition

1. INTRODUCTION

Clinically, antimicrobial susceptibility testing results provide guidance in the choice of antimicrobial agents in patient care [1]. The accuracy of results from antimicrobial susceptibility testing can be affected by multiple factors including the media, antimicrobial discs or preparations, inoculum's size, plate reading and incubation conditions [2]. Also, by the competence of the medical laboratory personnel [3]. For the results to be reliable, there is need for careful control and standardization of the various steps and components of the testing procedures [2]. Misleading results from antimicrobial susceptibility test leads to the indiscriminate and irrational use of antibiotics and other reasons have led to a global challenge of antimicrobial resistance [4]. The development of antimicrobial resistance of many bacterial species has posed a treat to the existing antibiotics [5,6]

Controlled tube dilution tests remains the most accurate method for carrying out antimicrobial susceptibility test, however, they are time-consuming and can only be used routinely in some specialized hospital laboratories [7]. The disc diffusion testing based on the Kirby-Bauer method is the simpler method and is therefore the most widely used [8]. 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 [9]. One of the most critical components of the disc diffusion method is the quality of the antimicrobial discs used and a good antimicrobial susceptibility test must conform to CLSI standard guidelines [10].

To meet the quality and performance standards required, antimicrobial discs need to be

manufactured within strict control limits and handled correctly within the laboratory [1]. 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 [7]. However the emergence of instruments for analysing zone of inhibition has brought paradigm shift to interpretation of results [11].

Variations in the paper quality of antibacterial discs that are commercially available in Nigeria have been reported by Eze et al., 2014 [7]. They observed high rates of differences in the thicknesses, weights, water absorbability and diameters of the disc-papers of all brands of antibacterial discs evaluated (both local and imported discs). These variations can affect the results of antibiotic susceptibility tests, when different brands of antibiotic discs with different paper qualities are used.

This study aims to compare the agreement between commercial antibiotics discs available in Abuja using the antibiotics common among the discs.

2. MATERIALS AND METHODS

2.1 Media

Muller hinton agar (MHA), Muller hinton broth (MHB). All media was purchased from Oxoid Ltd., UK.

2.2 Test Organisms

The test organisms used for this study include: Pure cultures of *Staphylococcus aureus* ATCC 25923, *Salmonella typhi* ATCC 9150, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli* ATCC 25922 and *Streptococcus pyogenes*.

2.3 Study Design

The study was a laboratory base study, conducted in the Federal Capital Territory, Nigeria.

2.4 Sampling

Four brands of commercial antibiotics multi-discs used in this study were purchased from retail medical stores in Abuja. Two of the brands were manufactured locally (MX, OP) while the other 2 (CE, HM) manufactured outside Nigeria.

2.5 Antibacterial Susceptibility Test

The disc diffusion method in accordance with CSLI was adopted for the study. Hundred microliters of 0.5 Macfarland culture (approximately $1-2 \times 10^8$ cfu/ml) of each organism was spread on the surface of MHA and allowed to stand for about 1 h. The multi-antibiotic discs were placed on the surface of the inoculated MHA ensuring that the antibiotic discs had complete contact with the agar surface. The plates were incubated for 24 hours at $37 \pm 2^\circ\text{C}$ in incubator after which the zones of inhibition were recorded. The experiments were done in triplicates [12].

2.6 Statistical Analysis

The data obtained were subjected to statistical analysis using two-way ANOVA-multiple comparisons options from the Graph pad prism® 6 software (6th edition) and differences between means were considered significant at $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Physicochemical Characteristics

Visual observation of the discs revealed variation in the type of the antibiotics present in the discs between the different brands (Table 1-4). Only 3 antibiotics (ofloxacin, gentamycin and amoxicillin/ clavulanic acid) was present in all four brands, one antibiotic present in three brands (cotrimoxazole) and some others found in only two brands of antibiotic discs (nalidixic acid, levofloxacin, ciprofloxacin, streptomycin).

For the antibiotics that were common among the brands, it was observed that there were marked differences in the antibiotic concentrations, making it difficult to directly compare the efficacies/ potency of the different brands. While the foreign brands of antibiotic discs contained gentamycin $10 \mu\text{g}$ and ofloxacin $5 \mu\text{g}$ (CE, HM), the locally produced discs contained $30 \mu\text{g}$ and $10 \mu\text{g}$ respectively (OP, MX).

The antibiotic discs from different manufacturers used for screening of Gram negative bacteria showed variation in the type of antibiotic present with similarity of only three antibiotics (ofloxacin, gentamycin and amoxicillin/ clavulanic acid). This on its own can lead to poor comparison in quality of the discs. Thus our study focused on the antibiotics common to all brands. Other factors that could hamper comparison and can also have impact on interpretation of susceptibility by the laboratory scientist is the variation in the concentration of the antibiotics. While the foreign brands of antibiotic discs contained gentamycin $10 \mu\text{g}$ and ofloxacin $5 \mu\text{g}$ (CE, HM), the locally produced discs contained $30 \mu\text{g}$ and $10 \mu\text{g}$ respectively (OP, MX). Thus there maybe need for regulatory bodies like National Agency for Food and Drugs, Administration and Control (NAFDAC) and The Standards Organization of Nigeria (SON) to regularly monitor these products ensuring uniformity in concentration of antibiotics in these products so as to prevent misleading interpretation of results.

The physicochemical assessment of the locally manufactured discs (OP, MX) revealed extremely poor packaging and outlook of the discs (Plate III & IV). Interestingly, OP contained disc that looked brownish in colour which could be due to poor handling and production process leading to charring of the discs. The packaging of pharmaceutical products and allies are an integral component in the quality of the product. Thus for a product to pass quality tests its presentation must be such as not to hamper or impact negatively on the integrity of the product [13].

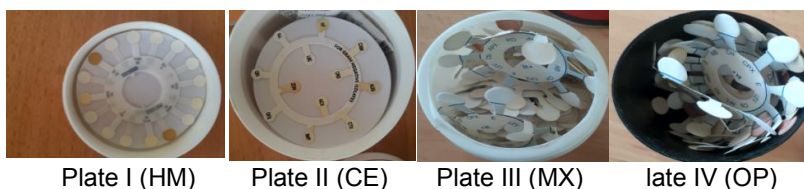


Fig. 1. Antibiotic discs in their primary packages

Table 1. Susceptibility of antibiotic discs CE on the test microorganisms

Organism	Zones of inhibition (mm)											
	OFX 5 µg	GN 10 µg	NA 30 µg	NF 30 µg	CXM 30 µg	CTX 25 µg	AUG 30 µg	IMP 10/10 µg	ZEM 5 µg	ACX 10 µg	CRO 45 µg	LBC 5 µg
<i>S. aureus</i>	19.0	27.0	14.0	23.0	13.0	13.0	16.0	-	16	16	16	-
<i>S. paratyphi</i>	15.0	15.0	16.0	20.0	24.0	19.0	13.0	10	15	15	15	15
<i>K. pneumonia</i>	32.0	23.0	24.0	16.0	19.0	27.0	15.0	14.0	15	15	15	15
<i>E. coli</i>	35.0	8.0	22.0	13.0	-	-	-	8.0	20	-	-	20
<i>P. aeruginosa</i>	30.0	21.0	11.0	-	-	14.0	-	16.0	17	-	17	17
<i>S. pyogenes</i>	24.0	21.0	19.0	-	-	-	-	-	14	10	12	15
<i>B. subtilis</i>	23.0	25.0	-	12.0	20.0	24.0	15.0	8.0	15	15	15	15

OFX-ofloxacin, GN- gentamycin, NA- nalidixic acid, NF- nitrofurantoin, CXM- cefuroxime, CTX- Cefotaxime, AUG- Amoxicillin- clavulanic acid, IMP- Imipenem- cilastatin, ZEM- Cefixime, ACX- Ampiclox, CRO- ceftriaxone sulbactam, LBC- levofloxacin

Table 2. Susceptibility of antibiotic discs OP on the test microorganisms

Organism	Zones of inhibition (mm)										
	NA 30 µg	PEF 10 µg	CN 30 µg	AU 30 µg	CPX 10 µg	SXT 30 µg	S 30 µg	PN 30 µg	CEP 10 µg	OFX 10 µg	
<i>S. aureus</i>	20	20	18	14	20	17	20	20	20	20	
<i>S. paratyphi</i>	17	17	14	11	17	16	13	14	16	17	
<i>K. pneumonia</i>	20	20	20	20	20	20	20	20	20	20	
<i>E. coli</i>	20	20	20	20	20	20	20	20	17	20	
<i>P. aeruginosa</i>	20	20	20	20	20	13	20	20	20	20	
<i>S. pyogenes</i>	20	20	20	16	20	10	20	-	10	20	
<i>B. subtilis</i>	11	13	16	11	14	-	15	-	-	17	

PEF- pefloxacin, CN- gentamycin, NA- nalidixic acid, SXT- cotrimoxazole, CPX- ciprofloxacin, S- streptomycin, AU- Amoxicillin- clavulanic acid, PN- ampicillin, CEP- ceporex, OFX- ofloxacin

Table 3. Susceptibility of antibiotic discs MX on the test microorganisms

Organism	Zones of inhibition (mm)									
	SXT 30 µg	CH 30 µg	SP 10 µg	CPX 10 µg	AM 30 µg	AU 30 µg	CN 30 µg	PEF 30 µg	OFX 10 µg	S 30 µg
<i>S. aureus</i>	13	11	21	21	20	15	20	22	19	-
<i>S. paratyphi</i>	-	-	20	21	18	-	11	18	21	19
<i>K. pneumonia</i>	-	-	15	20	-	12	19	13	-	-
<i>E. coli</i>	18	14	20	21	18	-	18	22	20	11
<i>P. aeruginosa</i>	-	-	14	20	14	-	20	-	-	13
<i>S. pyogenes</i>	21	18	21	21	21	15	20	21	20	17
<i>B. subtilis</i>	21	20	21	21	20	19	18	21	21	21

OFX- ofloxacin, CN- gentamycin, S- streptomycin, SXT- cotrimoxazole, SP- sparfloxacin, CPX- Cefotaxime, AU- Amoxicillin- clavulanic acid, AM - amoxicillin, PEF- pefloxacin, CH- chloramphenicol

3.2 Susceptibility of Microorganisms to Antibiotics

In this study, comparison was done on the antibiotics common among the brands which include ofloxacin, gentamycin and amoxicillin clavulanate (Table 5-7) and the antibiotics common in 3 brands (OP,

MX and HM) which include cotrimoxazole (Table 8).

From the results all the organisms tested were susceptible to ofloxacin contained in all the brands of antibiotic discs tested with zones of inhibition (ZOI) ≥16 mm. This is in compliance to CLSI standards, [12]. However in a study by Eze

et al. [7], the mean zones of inhibition measured in OP and MX against *E. coli* were 35 mm and 32 mm respectively which was higher than what was obtained (20 mm) in our study. This could be attributed to poor quality control of the disc during production or loss of potency during storage and distribution. It was also interesting to note that the CE (foreign) containing 5 µg ofloxacin produced was larger ZOI against *S. typhi*, *K. pneumonia*, *E. coli*, *P. aeruginosa*, *B. subtilis* and *S. pyogenes* than MX and OP (10 µg). Most of the test organisms were susceptible to gentamycin with zones of inhibition ≥15 mm which is in compliance to CLSI standard. The ZOI achieved in this study by

gentamycin (OP, MX) was lower than that reported by Eze et al. [7], which can also be linked to poor quality control measures. The least inhibition was produce by amoxicillin-clavulanic acid with most of the organism being intermediate of resistant with exception to *K. pneumonia* which was susceptible.

Cotrimoxazole (Trimethoprim-ulphamethoxazole) was included only in OP, MX and HM brands. Comparatively, only HM brand complied with the CLSI standard for cotrimoxazole disc potency which is 25 µg, however, OP and MX brands each with disc potency of 30 µg did not comply with the CLSI standard.

Table 4. Susceptibility of antibiotic discs HM on the test microorganisms

Organism	Zones of inhibition (mm)							
	CTR 30 µg	GEN 10 µg	COT 25 µg	LE 5 µg	NET 30 µg	TE 30 µg	AMC 30 µg	OF 5 µg
<i>S. aureus</i>	22	27	20	26	25	24	15	26
<i>S. paratyphi</i>	-	23	-	26	-	22	-	27
<i>K. pneumonia</i>	25	20	-	23	16	9	-	19
<i>E. coli</i>	10	11	21	23	10	13	-	30
<i>P. aeruginosa</i>	-	25	-	19	18	9	-	20
<i>S. pyogenes</i>	15	20	20	23	22	-	12	19
<i>B. subtilis</i>	22	20	21	21	19	22	16	24

OF- Ofloxacin, GEN- Gentamycin, COT- cotrimoxazole, AMC- Amoxicillin- clavulanic acid, NET- Netilmicin sulphate, CTR- Ceftriaxone, TE- tetracycline, LE- levofloxacin

Table 5. Susceptibility of ofloxacin from antibiotic discs against test organisms

Organism	Zones of inhibition (mm)			
	CE 5 µg	OP 10 µg	MX 10 µg	HM 5 µg
<i>S. aureus</i>	19	20	19	26
<i>S. paratyphi</i>	17	17	-	20
<i>K. pneumonia</i>	32	20	21	24
<i>E. coli</i>	35	20	20	30
<i>P. aeruginosa</i>	30	20	-	19
<i>S. pyogenes</i>	24	20	21	27
<i>B. subtilis</i>	23	17	20	19

Table 6. Susceptibility of gentamycin from antibiotic discs against test organisms

Organism	Zones of inhibition (mm)			
	CE 10 µg	OP 10 µg	MX 30 µg	HM 10 µg
<i>S. aureus</i>	27	18	20	27
<i>S. paratyphi</i>	15	14	20	25
<i>K. pneumonia</i>	23	20	18	20
<i>E. coli</i>	18	20	18	15
<i>P. aeruginosa</i>	21	20	19	20
<i>S. pyogenes</i>	21	20	15	23
<i>B. subtilis</i>	25	16	20	20

Table 7. Susceptibility of amoxicillin/clavulanic acid from antibiotic discs against test organisms

Organism	Zones of inhibition (mm)			
	CE 30 µg	OP 30 µg	MX 30 µg	HM 30 µg
<i>S. aureus</i>	16	14	15	15
<i>S. paratyphi</i>	13	11	-	-
<i>K. pneumonia</i>	15	20	19	16
<i>E. coli</i>	-	20	-	-
<i>P. aeruginosa</i>	-	20	12	-
<i>S. pyogenes</i>	-	16	-	-
<i>B. subtilis</i>	15	11	15	12

Table 8. Susceptibility of cotrimoxazole from antibiotic discs against test organisms

Organism	Zones of inhibition (mm)		
	OP 30 µg	MX 30 µg	HM 25 µg
<i>S. aureus</i>	17	13	20
<i>S. paratyphi</i>	16	-	-
<i>K. pneumonia</i>	20	21	21
<i>E. coli</i>	20	18	21
<i>P. aeruginosa</i>	13	-	-
<i>S. pyogenes</i>	10	-	-
<i>B. subtilis</i>	-	21	20

Table 9. Clsi standard

Organisms	Resistance (mm)	Intermediate (mm)	Susceptibility (mm)
Enterobacteriaceae (<i>Salmonella</i> , <i>E. coli</i> , <i>Klebsiella</i> sp), <i>P. aeruginosa</i> and <i>Staphylococcus</i> spp	≤10	11-15	≥16
<i>Streptococcus</i>	≤15	16-18	≥19

Using the CLSI standard inhibition diameter for cotrimoxazole indicating susceptibility, intermediate or resistance result for different test organisms, the zones of inhibition exhibited by cotrimoxazole disk in the three different brands against *Staphylococcus aureus*, *S. paratyphi*, *K. pneumonia*, *E. coli* indicates susceptibility as the zones of inhibition were ≥16 mm with exception of MX with inhibition diameter of 13 mm against *Staphylococcus aureus*, which indicates intermediate effect. This disagrees with the study by Eze et al. 2014 [7], who reported inhibition diameter of 22 mm for *S. aureus* indicating susceptibility. Also, no zone of inhibition was observed for *S. pyogenes* in MX and HM, however, for OP, zone of inhibition of 10 mm was observed indicating resistance.

There is therefore, need to ensure uniformity in the content and concentration of

antibiotics in the disc for commercial use. This is to prevent false positive or false negative results due to inaccuracy in interpretation or validity of the the results. This will have a negative impact in the fight against antibiotic resistance.

4. CONCLUSION

In conclusion, the results from this study show a variability in the concentration and type of antibiotics in the commercial antibiotic susceptibility discs. The zones of inhibition produced by the locally produced and foreign antibiotic disc also showed variations. There is need for proper regulation and quality control of these products to prevent misinterpretation of results which will have a negative impact on antibiotic resistance.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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