

<https://doi.org/10.5281/zenodo.1119113>

## EVALUATION OF THE POTENCY OF COMMERCIAL AND LOCALLY PREPARED ANTIBIOTIC DISCS ON CLINICAL BACTERIAL ISOLATES IN CALABAR, NIGERIA

Ofonime M. Ogba<sup>1</sup>, Nsikak I. Udo<sup>1</sup>, Paul C. Inyang-Etoh, Oluwayemisi A. Olorode<sup>2</sup>

<sup>1</sup>Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, University of Calabar, Nigeria

<sup>2</sup>Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Received: 2017-08-19

Accepted: 2017-09-30

Published online: 2017-11-05

### Abstract

Antibiotic susceptibility discs are used for *in vitro* susceptibility testing of antimicrobial agents to fast growing bacteria and fastidious species by agar diffusion method. It is a semi quantitative method. This study was designed to assess the efficacy of commercial antibiotic discs over in-house prepared discs. Identified clinical isolates were obtained in house in the Department of Medical Microbiology/Parasitology, University of Calabar Teaching Hospital, Nigeria. *Staphylococcus aureus* (ATCC 7553) and *Escherichia Coli* (ATCC 25922) were obtained and used as controls. The antibiotics tested includes; Ciprofloxacin, Erythromycin, Amoxicillin, Gentamicin, Zinacef. These were obtained commercially from Pharmacy shops. The local antibiotic discs were prepared with Whatman filter paper number 3. The antibiotic susceptibility testing was done using Kirby-Bauer method. Out of the 40 clinical isolates tested, 15(37.5%) were *Staphylococcus aureus* while 10(12.5%) each were *Escherichia coli* and *Pseudomonas aeruginosa* respectively. Only 5 isolates of *Proteus vulgaris* were used. The susceptibility profile of isolates to the commonly used local antibiotic discs ranged between 66.7% to 100%, while the range for commercial discs was 53.3% to 86.7%. Locally prepared discs were found to be more effective than commercial discs. The high resistance rates (0.0% to 33.3%) of clinical isolates to the commercial discs may be attributed to prolong exposure to environmental factors such as heat, moisture, sunlight as well as humidity when transported to retailers and the final users. There is need for constant monitoring and quality controls of susceptibility testing in our laboratories for the production of quality results and efficient patient care. Antibiotic susceptibility discs can be prepared locally for routine laboratory use; this may indirectly reduce importation of commercial discs and the burden on foreign exchange.

**Keywords:** Antibiotic susceptibility testing, discs, potency.

### Background of Study

Antibiotics are substances produced by microorganisms that inhibits the growth of other microorganisms or kill the organisms. They are sometime called Antibacterial or Antimicrobials [1]. The indiscriminate and irrational use of antibiotics and other reasons have led to a global challenge of antimicrobial resistance [2]. Antimicrobial discs should be manufactured under strict compliance to standard operative procedures, otherwise, quality and performance standards will be compromised [5]. The standard procedures may not be met in the developing countries ompared to developed countries [6].

Antibacterial discs commercially available in Nigeria may not contain the required quantity of active ingredients. This picture may be seen in many developing countries. Factors to be considered in the manufacturing and successful use of antibiotic discs include: quality of antibiotics, the composition of the discs, test performance, etc [8]. Eze *et al* [9] in South Eastern Nigeria reported that commercial antibiotic discs used in their locality were made of different paper quality. They observed high rates of differences in the diameters of the disc-papers, water absorbabilities, thicknesses and weights in all brands of antibiotic discs evaluated.

The locally prepared antibiotic discs are readily available for use and are also cost effective. They seem to be more potent because they are prepared at the point of use, not exposed to excessive heat during transportation to the various retail outlets [10]. Locally prepared antibiotics are preferred because, it saves time, money, cost of transportation and delay when compared to the commercially prepared disc which is not readily available. The study was carried out to assess the potency of commercial antibiotic discs over locally prepared discs and to compare the potency of antibiotics disc from different manufacturers.

## **METHODS**

### **Study Setting**

This research was carried out in the Microbiology/Parasitology Laboratory of the University of Calabar Teaching Hospital, Calabar, Cross River State.

### **Source of bacterial isolates and antibiotics**

Identified clinical isolates were obtained in house in the same Department. *Escherichia Coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 7553) were obtained commercially and used as controls. The antibiotics tested includes; Ciprofloxacin, Erythromycin, Amoxicillin, Gentamicin, Zinacef. These were obtained commercially from Pharmacy shops.

### **Preparation of Antibiotic Discs**

Whatman filter paper grade 3 was used. The whatman paper discs of 6mm in diameter were punched and sterilized. Codes and the concentration of the antibiotics were printed on each filter paper disc [4]. Antibiotic stock solutions were prepared with known concentrations of the antibiotic powder in sterile distilled water. The stock solution was stored in the refrigerator. It was diluted to a required concentration of working solution thereafter. The discs were impregnated with 0.01 $\mu$ l of the working solution of antibiotics using automatic pipette. impregnated discs were dried in an incubator at 37°C and stored in small ampoules with a desiccant at minus 20 °C [4,6].

## Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was determined by the Kirby-Bauer disc diffusion method on Muller Hinton agar, and interpreted according to the recommendations of the Clinical Laboratory Standards Institute. The antibiotic disc was applied using a sterile forceps and sufficiently separated from each other in order to prevent overlapping of the zone of inhibition. The plates were incubated at 37°C for 18-24 hours. The zone of inhibition was measured with a meter rule and it was compared with the Clinical Laboratory Standards Institute (CLSI) interpretive guide for antimicrobial susceptibility testing [11-12].

## Data Analysis

Data were analyzed using Epi Info 2012 (CDC, Atlanta, Georgia, USA) statistical software. Descriptive statistics were carried out. Frequencies were calculated for categorical variables. Interactions between specific categorical clinical variables were tested for significance using the  $\chi^2$  test. A p-value of  $\leq 0.05$  was considered statistically significant.

## RESULTS

Figure 1 shows the distribution of clinical isolates used in comparing the efficacy of locally prepared antibiotic discs with commercial discs. Out of the 40 clinical isolates used 15 (37.5%) were *Staphylococcus aureus* while 10 (25.0%) isolates each were *Escherichia coli* and *Pseudomonas aeruginosa*. Only 5 isolates of *Proteus Vulgaris* were used.

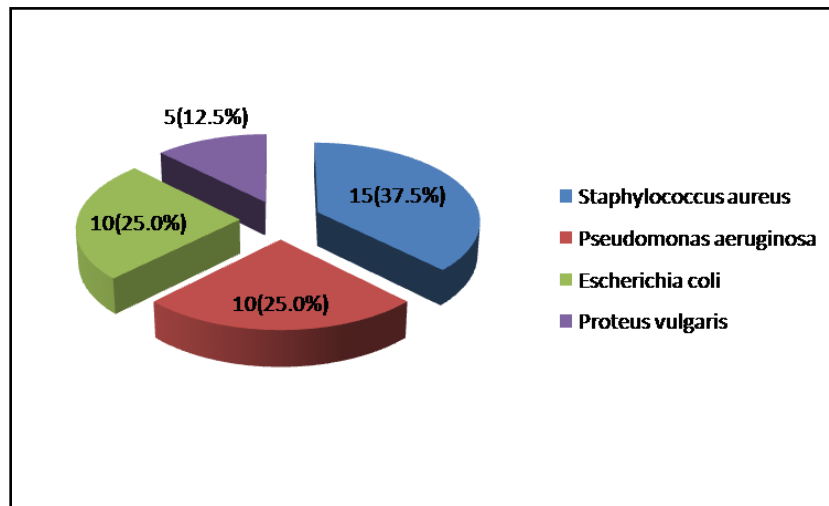


Fig. 1 Distribution of clinical isolates used for antibiotic susceptibility testing

Table 1 shows the susceptibility rate of clinical isolates to locally prepared antibiotic discs. The susceptibility profile of isolates to the commonly used local antibiotic discs ranged between 66.7% to 100%. *Staphylococcus aureus* susceptibility profile range between 66.7% - 93.3%. The organism was most susceptible to Erythromycin 93.3%, followed by Fortum 86.7%. The lowest susceptibility 66.7% was observed on ciprofloxacin and Ceftriaxone respectively.

*Escherichia coli* susceptibility profile range between 80.0% - 90.0%. The highest susceptibility rate 90.0% was observed in Gentamicin while other three antibiotics showed 80.0% susceptibility (Table 1). *Pseudomonas aeruginosa* susceptibility profile for local discs range between 70.0% - 100%. It was most susceptible to Fortum, while the lowest susceptibility (70.0%) was observed in Gentamycin. *Proteus vulgaris* was most susceptible to Ceftriaxone (100%) and least susceptible to gentamicin (60.0%) (Table 1).

Table 2 shows the susceptibility rate of clinical isolates to commercial antibiotic discs. The susceptibility profile of clinical isolates to the commonly used commercial discs ranged between 53.3% to 86.7%. Erythromycin was the most potent disc to *Staphylococcus aureus* with 86.7% susceptibility rate, while ciprofloxacin was the least potent (53.3%).

*Escherichia coli* was most susceptible to gentamicin and ceftriaxone (70.0%) respectively. Ceftriaxone was also the most potent disc for *Pseudomonas aeruginosa* (80.0%) and *Proteus vulgaris* (80.0%) respectively (Table 2). The resistant profile for the commercial discs range between 0.0% to 33.3%. *Staphylococcus aureus* was the most resistant isolates (33.3%) to ciprofloxacin discs (Table 2).

**Table 1 In vitro susceptibility patterns of selected clinical isolates on locally prepared antibiotic discs.**

Antibiotics	Antibiotics susceptibility in percentage (%)					
		CIP	CN	CFTR	CAZ	E
<i>Staphylococcus aureus</i> (n=15)	S	10(66.7)	12(80.0)	10(66.7)	13(86.7)	14(93.3)
	M	3(20.0)	3(20.0)	4(26.7)	2(13.3)	1(6.7)
	R	2(13.3)	0(0.0)	1(6.7)	0(0.0)	0(0.0)
<i>Escherichia coli</i> (n=10)	S	8(80.0)	9(90.0)	8(80.0)	8(80.0)	NA
	M	1(10.0)	1(10.0)	1(10.0)	2(20.0)	NA
	R	1(10.0)	0(0.0)	1(10.0)	0(0.0)	NA
<i>Pseudomonas aeruginosa</i> (n=10)	S	8(80.0)	7(70.0)	9(90.0)	10(100)	NA
	M	2(20.0)	3(30.0)	1(10.0)	0(0.0)	NA
	R	0(0.0)	0(0.0)	0(0.0)	0(0.0)	NA
<i>Proteus vulgaris</i> (n=5)	S	4(80.0)	3(60.0)	5(100)	4(80.0)	NA
	M	1(20.0)	0(0.0)	0(0.0)	1(20.0)	NA
	R	0(0.0)	2(40.0)	0(0.0)	0(0.0)	NA

**KEY**

n = number of isolates examined

S = Sensitive; M = moderately sensitive; R = Resistant

NA = Not applicable

**Table 2 In vitro susceptibility patterns of clinical isolates on commercial antibiotics.**

Antibiotics		Antibiotics susceptibility in percentage (%)				
		CIP	CN	CFTR	CAZ	E
<i>Staphylococcus aureus</i> (n=15)	S	8(53.3)	11(73.3)	9(60.0)	12(80.0)	13(86.7)
	M	2(13.3)	3(20.0)	3(20.0)	0(0.0)	1(6.7)
	R	5(33.3)	1(6.7)	3(20.0)	3(20.0)	1(6.7)
<i>Escherichia coli</i> (n=10)	S	6(60.0)	7(70.0)	7(70.0)	6(60.0)	NA
	M	3(30.0)	2(20.0)	1(10.0)	4(40.0)	NA
	R	1(10.0)	1(10.0)	2(20.0)	0(0.0)	NA
<i>Pseudomonas aeruginosa</i> (n=10)	S	7(70.0)	6(60.0)	8(80.0)	8(80)	NA
	M	2(20.0)	3(30.0)	2(20.0)	2(20.0)	NA
	R	1(10.0)	1(10.0)	0(0.0)	0(0.0)	NA
<i>Proteus vulgaris</i> (n=5)	S	3(60.0)	3(60.0)	4(80.0)	3(60.0)	NA
	M	2(40.0)	1(20.0)	1(10.0)	1(20.0)	NA
	R	0(0.0)	1(20.0)	0(0.0)	1(20.0)	NA

**KEY**

n = number of isolates examined

S = Sensitive; M = moderately sensitive; R = Resistant

NA = Not applicable

**Table 3 Standard charts values for zone of inhibition (mm)**

Antibiotics	Test Organism			
	<i>Staphylococcus aureus</i>	<i>Escherichia coli</i>	<i>pseudomonas aeruginosa</i>	<i>Proteus Vulgaris</i>
Ciprofloxacin	27-35	30-40	28-35	29-35
Gentamicin	24-32	21-28	19-24	25-30
Ceftriaxone	19-28	25-32	23-30	24-35
Fortum	29-33	25-31	18-22	19-30
Erythromycin	22-30	-	-	-

Source: (CLSI, 2010b) [13].

**Discussion**

The study recorded a susceptibility profile of 66.7% to 100% for locally prepared antibiotic discs while the range for commercial discs was 53.3% to 86.7%. The local antibiotic discs were more potent than commercial discs. This is in agreement with the work of Ayiegoro *et al* [14] who reported that locally prepared antibiotics are more potent than the commercially prepared disc. Waksman *et al* [15] also reported a higher susceptibility result using locally prepared antibiotics when compared to the commercially prepared antibiotics. Thus may be due to high temperature that these commercially prepared antibiotics are exposed to during transportation from the manufacturer to the end users, time of production, storage facility.

There was no all-round most potent commercial disc as the four clinical isolates displayed different susceptibility rates to the antibiotics tested. The high resistance rate (0.0% to 33.3%) of clinical isolates to the commercial discs may be attributed to the mode of storage of these antibiotic discs, prolong exposure to other environmental factors such as heat, moisture, sunlight as well as humidity when transported to retailers and the final users [11].

This study has demonstrated a significant difference between the potency of locally prepared and commercial antibiotic susceptibility discs. This is not in agreement with the work of Epoke *et al* [16] in Calabar, Nigeria, who reported that discs prepared locally from antibiotic tablets, performed comparably with commercially obtained discs. The variation in results may be due to the sources of isolates tested. Epoke *et al* [16] used the American Type Culture Collection (ATTC) isolates while our isolates were locally sourced.

### Conclusion

Locally prepared discs seem to be more effective than commercial discs. Antibiotic susceptibility discs can be prepared locally for routine laboratory use, this may indirectly reduce importation of commercial discs and the burden on foreign exchange.

### Recommendation

There is need for constant monitoring and quality controls of susceptibility testing in our laboratories for the production of quality results and efficient patient care.

### References

1. Daniel, P. N., Kitshbaum, A., Kramer J. (2014): The assay and control of antibiotic discs, *Antibiotic chemo-therapy*: 10:249-258
2. Silva, E., Díaz, JA., Arias, MJ., Hernández, AP., Torre, A. (2010). *BMC Clinical Pharmacology* 2010, 10:3 <http://www.biomedcentral.com/1472-6904/10/3>
3. Center for Disease Control (2011) measurements and significance of antibiotic activity in the urine. P.. 41-49
4. Vineetha N, Vignesh RA, Sridhar D. (2015). Preparation, Standardization of Antibiotic Discs and Study of Resistance Pattern for First-Line Antibiotics in Isolates from Clinical Samples. *International Journal of Applied Science*; 1(11): 624-631
5. Ekundayo EO, Omodamiro OD. (2008). Evaluation of locally manufactured antimicrobial susceptibility testing discs in South Eastern Nigeria. *African Journal of Clinical and Experimental Microbiology*. 2008;99(3):122-128.
6. Sudha V, Prasad A, Khare S, Bhatia R. (2001). Antimicrobial susceptibility testing in India – A status survey. *Indian Journal of Medical Microbiology*;19:222-223.
7. Greenberg L, Fitzpatrick KM, Branch A.(1957). The Status of the antibiotic disc in Canada. *Canadian Medical Academic Journal*. 1957;76:194-198.

8. Eze PM, Ajaegbu EE, Esimone CO. (2014). Evaluation of the paper quality of antibacterial discs commercially available in Nigeria. *Journal of Scientific Research and Reports*; 3(8):1079-1087.
9. John FA & Rippers RA. (2012). Preparation and control of antibiotic susceptibility discs and other devices containing antibiotic. Pp. 41-48
10. Clinical and Laboratory Standards Institute (CLSI) (2010a). Approved standard M2-A10. Performance standards for antimicrobial susceptibility tests, 10th ed. CLSI, Wayne, Pa.
11. Ogba OM, Mandor BI & Omang HM. (2014). Antibiotic susceptibility pattern of *Serratia marcescens* isolates from wound infections in a tertiary health institution in Calabar, Nigeria. *El Mednifico Journal*, (S.I.) 2(3): 223-226. July 2014. <http://www.mednifico.com/index.php/elmedj/article/view/201/136>
12. Clinical and Laboratory Standards Institute (CLSI) (2010b). CLSI document M100-S19. Performance standards for antimicrobial susceptibility testing, 20th informational supplement, Wayne, Pa.
13. Ayiegoro, JI, Daniel UV & Thomas VO. (2008). Efficacy of different antibiotics on gram negative organisms. Pp. 41-48.
14. Waksman, P. E., Shrivatsava, S. Chaudhary, M. (2009) ceftriaxone sulbactam combination: Microbial analysis by variation of ratios and comparative disc diffusion. *Current Resistant in Bacteriology* 2: 505.
15. Epoke, J., Igumbor, E. O., Asuquo, A. E. & Tichawowona, C. (2003). Locally prepared antibiotic sensitivity discs: a substitute for imported commercial discs. *Global Journal of Pure and Applied Sciences* 9 (4): 453-456