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# LOCALLY PREPARED ANTIBIOTIC SENSITIVITY DISCS: A SUBSTITUTE FOR IMPORTED COMMERCIAL DISCS

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## ABSTRACT

Antibiotic sensitivity discs were prepared from tablets of erythromycin, chloramphenicol, ampicillin, clindamycin gentamicin and kanamycin. Susceptibility of some clinical isolates which included *Escherichia coli, Staphylococcus aureus, Salmonella* Group D , *Pseudomonas aeruginosa*, Group A *Streptococcus* and type culture of *Pseudomonas aeruginosa* ATCC 27953 was investigated. Zones of inhibition were compared with those obtained from commercial antibiotic discs. Results obtained showed that discs prepared locally from antibiotic tablets, performed comparably with commercially obtained discs. There was no significant statistical difference between the two tested discs. We therefore recommend that antibiotic sensitivity discs can be prepared locally from antibiotic tablets and be used for routine laboratory susceptibility testing, hence saving scarce foreign exchange required for importation of commercial discs.

**KEY WORDS**: Locally prepared, Antibiotic, Substitute, Commercial disc.

## INTRODUCTION

The goal of clinical microbiology in diagnosis of infectious diseases is the isolation of the incriminated pathogen and the subsequent determination of the drug of choice for treatment. The drug of choice and the resistance are commonly determined by antibiotic sensitivity testing (Greenwood 2000). Routine disc diffusion method for detecting antibiotic resistant organisms has continued to be used by many workers world over (Manninen *et al* 1998). The disc diffusion method is also commonly used to compare in vitro sensitivity of different antibiotics (Obi *et al* 1999). The correlation between the minimum inhibitory concentration (MIC), of zones of inhibition and disc diffusion tests has been studied by many workers (de Castillo *et al* 1999, Jones 1999). The National Committee for Clinical Laboratory Standards (NCCLS) has set up interpretative criteria for different organisms in relation to zones of inhibition and concentration of antibiotics in the disc diffusion method (Traub *et al* 1998). In vitro antibiotic sensitivity tests however depend on the availability of commercial discs from major manufacturers. These have to be imported at high costs with foreign exchange. The present state of our economy demands an inward approach with attempts at locally preparing sensitivity discs using the parent antibiotics, which are commonly available. Such preparations if they compare favourably with their commercial counterparts can serve as alternatives in the face scarce resources for importation. This work is aimed at comparing locally produced antibiotic sensitivity discs with commercial ones against six clinical bacterial isolates.

## MATERIALS AND METHODS

### Preparation Of Antibiotic Discs

Whatman number one (no 1) filter papers were punched to a disc size of 0.5cm diameter using a standard office paper/file, perforating machine. Six hundred 0.5cm diameter discs were separated into six universal bottles of 100 each. They were all sterilized by autoclaving before impregnation of antibiotics.

The antibiotics used were each dissolved in appropriate volumes of sterilized distilled water (SDW) and diluted to give stock used for impregnation. The concentrations of the stock solutions were made to give the impregnated discs concentrations in line with commercial discs and NCCLS recommendations. The different stock solutions made from each antibiotic tablet, the dilutions made and the disc concentration achieved are summarized in table 1.

Impregnation was achieved by putting 1 ml of stock solution in a sterile plastic petri dish. The

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sterilized filter paper discs were then added to the solutions (100 discs for each antibiotic stock solution) and allowed to absorb fully for 10 minutes and then dried in the oven at 40$^0$C for 15 minutes. The prepared discs were put into labeled plastic universal containers and stored at 4$^0$C until required for sensitivity testing.

**Preparation And Seeding Of Inoculum**

Clinical isolates of *E. coli, S. aureus*, *Salmonella* Group D, *P. aeruginosa*, and Group A *Streptococcus* were obtained from the Public Health Laboratory, Harare . A type culture of *P. aeruginosa* ATCC 27953 obtained from CDC Atlanta served as control strain. A suspension of each organism in phosphate buffered saline [PBS] was made to $10^3$ CFU/ml using the McFarland's Nephelometer. This concentration we have observed from previous experiments, to give a good lawn of growth with well-defined and discernable zones of inhibition. One ml of each suspension was seeded onto isosensitest agar [Oxoid] as described by Straub *et al* (1998) Each of the organisms tested was seeded onto six different plates of the isosensitest agar.

**Sensitivity Testing (Disc Diffusion)**

Each seeded plate was divided into 4 quadrants and on each quadrant was placed an antibiotic disc. Two different antibiotics (locally prepared and commercial discs) were tested on each plate. All plates were incubated aerobically at 37$^0$C for 24 hours. Aerobic incubation was preferred as incubation at 5-10% carbon dioxide has been reported to result in smaller zones of inhibition by disc diffusion (Davis *et al* 2000) The zones of inhibition were measured for each disc and the average for each antibiotic was recorded.

The results for locally prepared discs and the commercially procured discs were compared statistically using the Analysis of Variance (ANOVA) test

## RESULTS

The zones of inhibition observed in the locally prepared antibiotic discs were comparable with those observed from discs procured commercially. The average zone of inhibition diameters for the six antibiotics on the five bacterial isolates and the one control type culture are shown in table 2.

Statistical analysis of the result using ANOVA test showed no significant difference between locally prepared antibiotic discs and their commercial counterparts ($p<0.05$).

Wide spread resistance was observed with *E. coli, S. aureus* and *Pseudomonas*. Zones of inhibition diameter breakpoint of 15 mm were used to determine sensitivity as suggested by Andrews *et al* (1999). Zones of inhibition less than 15mm were regarded as resistant.

## DISCUSSION

Sensitivity testing still remains a cornerstone in clinical bacteriology diagnosis. Isolation of a pathogen without knowledge of its antibiogram in face of widespread resistance to commonly used drugs is valueless. The multi-resistance of our isolates in this work attests to this fact. A comparison of our locally prepared antibiotic sensitivity discs with commercially imported ones gave similar zones of inhibition without any

**TABLE 1: PREPARATION OF STOCK ANTIBIOTIC SOLUTIONS FOR FILTER PAPER DISC IMPREGNATION**

| Antibiotic | Tablet Conc. | Volume of SDW to dissolve Tablet | Conc. of Solution | Dilution Needed | Stock* to Impregnate 100 discs | Disc Conc. |
|---|---|---|---|---|---|---|
| Erythromycin | 250 mg | 25ml | 10,000ug/ml | $10^{-1}$ | 1000ug/ml | 10ug |
| Clindamycin | 150mg | 75ml | 20,000ug/ml | $10^{-2}$ | 200ug/ml | 2ug |
| Chloramphenicol | 250mg | 10ml | 25,000ug/ml | $10^{-1}$ | 2,500ug/ml | 25ug |
| Ampicillin | 250mg | 10ml | 25,000ug/ml | $10^{-1}$ | 2500ug/ml | 25ug |
| Gentamycin | 80mg | 8ml | 10,000ug/ml | $10^{-1}$ | 1000ug/ml | 10ug |
| Kanamycin | 1mg | 3.3ml | 300ug/ml | $10^{-2}$ | 3,000ug/ml | 30ug |

* 1 ml of stock solution was used to impregnate 100 discs

**TABLE 2 : AVERAGE DIAMETERS OF INHIBITION ZONES OBSERVED WITH LOCALLY PREPARED AND COMMERCIAL ANTIBIOTIC SENSITIVITY DISCS**

| Bacterial Isolates | Amp. | | Chloramph. | | Eryth. | | Clind. | | Kanam. | | Genticin | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | C | L | C | L | C | L | C | L | C | L | C | L |
| *E. coli* | - | - | - | - | - | - | - | - | 18 | 16 | - | - |
| *S. aureus* | 10 | 7 | - | - | - | - | - | - | 6 | 8 | 10 | 12 |
| *Salmonella* | 25 | 26 | 26 | 28 | - | - | - | - | 22 | 24 | 20 | 18 |
| *P. aeruginosa* | - | - | - | - | - | - | - | - | - | - | 8 | 6 |
| Gp A. *Strept.* | 35 | 32 | 28 | 23 | 22 | 20 | 25 | 27 | - | - | - | - |
| *P. aeruginosa* **[ATCC27953]** | - | - | - | - | - | - | - | - | - | - | **22** | **18** |

**Key**

| | | | |
|---|---|---|---|
| Amp. | Ampicillin | C | Commercially imported antibiotic disc |
| Chloramph. | Chloramphenicol | L | Locally prepared antibiotic disc |
| Eryth. | Erythromycin | - | No discernable inhibition zone observed |
| Clind | Clindamycin | | Diameters are in millimetres |
| Kana | kanamycin | | |

statistical significant difference when tested on six bacterial isolates from clinical specimens. Zone of inhibition size by disc diffusion has been shown to correlate well with minimum inhibitory concentration [MIC] (Wang et al 1999). Disc diffusion thus remains a very useful tool for antibiotic sensitivity testing.

The commercially available sensitivity discs are produced on the antibiotics manufactured by the Drug Company that distributes them. Some locally available, useful and sometimes cheaper antibiotics may not have commercial discs for them, making their sensitivity testing difficult. Local preparation of antibiotic sensitivity discs will introduce greater flexibility with minimum wastage. Multiresistant organisms like *P. aeruginosa* need not be tested with commercial multidiscs containing many ineffective drugs but tested with locally prepared known effective drugs.

Disc diffusion test, which was found to be inexpensive, quick and to give results within 18 hours (Menon and Ponnuvel 2000) have now become expensive in our setting. Procurement of commercial sensitivity discs like most other reagents depend on foreign exchange. At the current unstable exchange rate a container of 100 multi-discs costs US $100.This brings the cost of one sensitivity test to about US $2. In this work, the local production of 100 discs each for about six antibiotics was US $10. This brings the cost of one sensitivity test using locally prepared disc to about US $0.1. This can generate a lot of savings, which can be channeled into other needy areas of the health care delivery. In conclusion, locally produced antibiotic sensitivity discs can be used in place of commercially imported ones for routine sensitivity testing. Further work is needed to determine the shelf life and optimum storage conditions of locally prepared antibiotic sensitivity discs to guide its usage.

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