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Preparation and standardization of locally produced antibiotic multidiscs in Benin City, Nigeria

George Akintinde Olajide[1], Helen Ogefere[2]

[1] Assistant Director, Medical Laboratory Scientist, Medical Laboratory science Council of Nigeria, South-South Zonal office, Federal Secretariat, Benin-Auchi Road, Benin City, Edo State, Nigeria

[2] Associate Professor, Department of Medical Laboratory Science, University of Benin, Benin City, Edo State, Nigeria

Abstract

Locally manufactured antibiotic sensitivity discs are becoming increasingly used in both private and public Health Institution Laboratories in Nigeria. This study aimed to prepare and standardize locally produced antibiotic multidiscs in Benin City, Nigeria. Dye cut blade with eight (8) finger holes of approximately six (6mm) diameter was constructed manually and the printed alabaster paper discs were placed on the Herzberg 13 x 18 die-cutting impression machine to bring out the multidiscs shape. Antibiotic discs named Boljid Tripple T multidiscs (Boljid) were prepared by impregnation of antibiotic solution onto 300 gram alabaster paper.

The different antibiotic tablets (Ciprofloxacin-5ug, Ofloxacin-5ug, Azithromycin-15ug, Augmentin-30ug, Pefloxacin-10ug and Levofloxacin-5ug) and injections Ceftriaxone-30ug, Gentamicin-10ug/ml, Cefuroxime-30umg, Nitrofurantoin-300ug and Ceftazidime-30ug were used for Boljid multidisc preparation. Control organisms and clinical isolates was obtained from the Lagos State University Teaching Hospital, Idi Araba, Lagos and University of Benin Teaching Hospital, Benin City, Edo State respectively. The prepared Boljid multidiscs, were standardized by comparing the efficacy with the commercially available multidiscs namely Abtek, Celtech, Divine Favour, Maxi High profile and Oxoid single discs. Triplicate plates were prepared for each of the multidiscs and tested against the test organisms using the Kirby-Bauer disc diffusion method. The test organisms investigated included clinical isolates of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella species, Proteus species* and control strains of *Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923 and Pseudomonas aeruginosa ATCC 27853.* Results obtained showed that for Ciprofloxacin, Boljid (^{25}.00± 0.58mm) and Celtech (25.50± 0.58mm) multidiscs produced similar inhibition zone diameter against ATCC *Staphylococcus aureus.* Boljid (21.67±0.33mm), Divine Favour (20.00±1.15mm) and Oxoid disc (20.00±0.00mm) produced similar zone of inhibition against *Pseudomonas aeruginosa.* For Gentamicin, Boljid, Abtek, Celtech and Maxi multidiscs produced no zone of inhibition against ATCC *Pseudomonas aeruginosa* while Divine Favour (11.67±0.58mm) and Oxoid (20.00±0.58mm) produced inhibition zones that were significantly different. Boljid, Abtek, Celtech, Divine Favour and Maxi multidiscs produced no zone of inhibition against the clinical isolates of *Escherichia coli.* Boljid (14.00±0.00mm) and Oxoid discs (14.67±0.00mm) produced similar zone of inhibition against clinical isolate of *Klebsiella species.* For Ofloxaxin, Boljid and Oxoid discs produced no zone of inhibition against the clinical isolate of *Pseudomonas aeruginosa.* Boljid multidiscs produced locally, compared favourably with Oxoid, Abtek, and Celtech used in this study and therefore recommended for product certification for routine Laboratory use.

Keywords: boljid multidiscs, control organisms, clinical isolates

Introduction

Antibiotics have been used in time memorial for the treatment of various infections. However, not all the antibiotics can be used for all infections, hence the need to check the susceptibility of the causative microorganisms implicated in an infection to antibiotics. Antibiotics are compounds synthesized naturally and artificially that have an inhibitory action on other microorganisms. Penicillin was the first identified antibiotics from the fungus known as *Penicillium notatum.* (Vineetha *et al*., 2015) [7] A good antibiotics should be effective against wide range of microbes, have less side effect, it should be highly stable and should be readily absorbed by the body tissues (Vineetha *et al*., 2015) [7]. An antimicrobial susceptibility test disc is the body tissues (Vineetha *et al*., 2015) [7]. An antimicrobial susceptibility test disc is described in the FDA regulation, 21CFR 866,1620(a) as a 'device that consists of microbic-impregnated paper discs used to measure by a disc agar diffusion technique or a disc broth elution technique in the in-vitro susceptibility of most clinically important bacterial pathogens to antimicrobial agents (FDA,1996; Eze *et al*., 2004).

Antimicrobial susceptibility testing of bacterial and fungal isolates is a common and important technique in most clinical laboratories (Vineetha *et al.,* 2015) [7]. The results of these tests are used for the selection of the most appropriate antimicrobial agents for the treatment against the infectious organisms. Till the 1950s, laboratories were lacking in the methodologies and equipment for the accurate determination of in vitro responses of organisms to antimicrobial agents. Bauer *et al*., (1966), began the development of standardized method for antimicrobial susceptibility testing using disc diffusion system. However, the susceptibility results may not always correlate with the patient's response to therapy. The response of an infected patient to antimicrobial agents is a complex interrelationship

of host responses, drug dynamics and microbial activity. Antimicrobial susceptibility tests are either quantitative or qualitative. Disc diffusion test is a qualitative test method. The National committee for clinical Laboratory standards (NCCLS), now known as Clinical Laboratory Standards Institute (CLSI) has published comprehensive documents regarding the disc diffusion systems. The agar disc diffusion test is the most convenient and widely used method for routine antimicrobial susceptibility testing. In subsequent and current practice, antimicrobial impregnated paper discs are applied onto the agar surface. Based on the Bauer-Kirby method, standardized reference procedures for the disc systems were published by World Health Organization (WHO) and Food Drug Administration (FDA) and are periodically updated by the CLSI (CLSI, 2017).

Methodology

Study Area

This study was carried out in Benin City, Edo State and in Yaba, Lagos, Nigeria. The Department of Medical Laboratory Science Laboratory, University of Benin and Public Health In-vitro Diagnostic Laboratory, Yaba, Lagos were used for this study. The Public Health in-vitro Diagnostic Laboratory is a special investigative laboratory for in-vitro diagnostic (IVD) products. It is own by the Medical Laboratory Science Council of Nigeria.

Research Design

This study is a comparative based study.

Inclusion criteria

All the multidiscs with the same disc concentration with the prepared Boljid multidiscs

Exclusion criteria

All the multidiscs with different disc concentration outside the prepared Boljid multidiscs.

Ethical Approval

Approval for this study was obtained from Edo State Ministry of Health, Benin City, Edo State, Nigeria with letter referenced HA-737/42

Test Organisms

Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923, Pseudomonas aeruginosa ATCC 27853, clinical bacterial isolates *of Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Proteus species* and *Klebsiella species* were used. The test organisms were obtained from the University of Benin Teaching Hospital Benin City, Edo State and Lagos University Teaching Hospital, Idi-Araba, Lagos, Lagos State.

Preparation of Multidiscs

Dye cut blade with eight (8) finger holes of approximately six (6mm) diameter was constructed manually to produce an eight rings paper multidiscs. Alabaster paper of 300 grams was used for the production of the multidiscs and for ease of identification, code names and disc concentrations of the different antibiotics used were coloured printed on the Alabaster paper to fit into the eight finger holes on the dye cut blade. The printed alabaster paper discs were placed on the Herzberg 13 x 18 die-cutting impression machine to bring out the multidiscs shape. The printed multidiscs were separated manually ensuring the finger rings were not cut off during separation procedure. The paper multidiscs were sterilized at 121^0C for 30 minutes. (Vineetha *et al.*, 2015) [7].

Preparation of antibiotic stock solution

Standard antibiotic drugs (Viz:- Ciprofloxacin-500mg, Ofloxacin-200mg, Azithromycin-500mg, Augmentin-625mg, Pefloxacin-400mg, Levofloxacin-500mg, Ceftriaxone-1g, Gentamicin-80mg/ml, Cefuroxime-500mg, Nitrofurantoin-100mg and Ceftazidime-1g) were obtained commercially from Lyn-Edge Pharmaceutical Limited, Lagos. Known weight of the antibiotic drugs was dissolved in sterile distilled water to obtained the stock solution. The stock solution was diluted at the time of disc preparation to obtain the working solution. A paper disc of 6mm diameter can absorb 20µl of solution. The concentration of the antibiotic solution was expressed in microgram.

Impregnation of the multidiscs.

The Boljid multidiscs used for the study were placed in sterile petri dishes. A micropipette was used to deliver 20µl of the appropriate antibiotic solution onto the designated finger ring of the multidiscs and then allowed to absorb for 2 minutes. The multidiscs were allowed to dry in a clean incubator at 37ºC for 2 hours (Melecia Antonio-Velmonto, *et al*., 1988) [5].

After drying, 25 multidiscs were placed in a sterile air tight labeled plastic containers with desiccant ($CaCl_2$). The multidiscs were stored in a refrigerator at 2-8^0C (Epoke, *et al*., 2003) [2]. The multidiscs were removed from the refrigerator 1 hour prior to usage, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts cold discs. (Lalitha, 2004) [4].

Standardization of Prepared Multidiscs

The prepared multidiscs was assessed along with the commercial multidiscs (Oxoid, Abtek, Celtech, Maxi high profile and Divine favour) for their efficacy using the Kirby-Bauer disc diffusion method. Each of the test organisms was sub-cultured onto fresh plates of Mueller Hinton Agar for 18-24 hours at 37ºC. Emergent colonies from these were suspended in sterile normal saline to a turbidity matching 0.5 McFarland standard containing 1 x 10^8cfu/ml for the test organisms (George *et al.*, 2018) [8]. Each labeled medium plates was uniformly seeded with a test organism by means of sterile swab rolled in the suspension and streaked on the plate surface. The multidiscs and other discs used for comparison were placed on the seeded plates with marked identification. (Shaidi-Bunjar, 2004) [6]. Each of the multidiscs was tested against the test organisms in triplicates and the plates were incubated at 37^0C overnight. As a control measure for the Boljid multidiscs, a blank Boljid multidiscs was placed on the seeded plates for the different test organisms. The zones of inhibition around the disc rings were measured in millimeter. The zone of inhibition is paramount in identifying an isolate as either sensitive or resistant, which is interpreted based on Clinical Laboratory Science Institute guidelines (CLSI, 2015) [1].

Statistical Analysis

Statistical analysis was carried out using the statistical

package (Graph pad Prism). All values were expressed as mean ± S.E (Mean standard error of mean). The analysis of variance (ANOVA) was used to determine significant difference in test and control strains using multidiscs. Statistical significance was set at $P \leq 0.05$.

FINDINGS

The findings in this study were as follows:

1. boljid multidiscs produced a significant zone diameter of inhibition when compared with other commercial multidiscs against all the test organisms.
2. maxi high profile multidiscs produced insignificant zone diameter of inhibition when compared to other tested multidiscs against all the test organisms.
3. divine favour and Boljid multidiscs which are locally produced compared favourably with foreign multidiscs antimicrobial disc tested.

Conclusion

This study has shown that locally prepared Boljid multidiscs is as effective as commercial foreign antibacterial multidiscs, and therefore antibacterial susceptibility multidiscs can be prepared locally for routine laboratory use in Benin City, Nigeria.

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