Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine

Clinical Research

Journal home page: www.ajpcrjournal.com

EFFECT OF PAPER QUALITY OF LOCALLY MADE ANTIBIOTIC DISCS ON ANTIBIOTIC SENSITIVITY OF SOME CLINICAL BACTERIAL ISOLATES

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ABSTRACT

The effect of paper quality of four different paper types on antibiotic sensitivity of some clinical bacterial isolates was investigated using the disc diffusion method of antimicrobial susceptibility testing. Antibiotic discs were made from Whatman No.1filter paper, Copyman printing paper, Conqueror paper and Star-foolscap paper. Their difference in weight, thickness, absorbency and texture were determined and susceptibility testing was carried out with their discs against clinical isolates of*Staphylococcus aureus*,*Escherichia coli*and *Pseudomonas aeruginosa*, to determine the susceptibility and any effect in zone diameter due to difference in the paper qualities. Discs made from WhatmanNo.1 filter paper gave zone of inhibition 3mm larger than the other paper types and were also within the limits of the published standard for interpretation by the Clinical and Laboratory Standard Institute. Statistical analysis of variance showed a significant difference in zone diameters produced by the discs of thevarious paper types as indicated by high variance ratio (F-ratios, 79.73).

KEYWORDS

Antibiotic disc, Susceptibility testing, Paper quality and Zone of inhibition.

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INTRODUCTION

The early pioneers of microbiology, Pasteur, Koch and Ehrlich made many reference to and observed the actions of biological agents against the growth of microorganisms. William Roberts in 1874 observed that liquid medium in which the mold *penicilliumglaucum* was growing could not easily be contaminated with bacteria[1]. Two years later, John Tyndall observed that broth supported the growth of either bacteria or moulds but rarely both[2].Fleming

also reported the inhibitory effect of penicillin on solid media by observing an area of growth inhibition of staphylococcal colonies adjacent to a penicillin contaminant on an ager plate and this was eventually termed agar diffusion[2].The concept of attacking invading microorganisms without harming the host was first introduced by Paul Ehrlich when he discovered 'salvarsan' which he announced as a magic bullet for the treatment of syphilis[3].Antibiotic susceptibility testing developed further in 1940s when Heatley introduced the use of absorbent paper for carrying antimicrobial solutions[4].Filter paper discs incorporated with penicillin were also used by Vincent and his colleague during this period[5].After the accidental discovery of penicillin in 1928, more and more antibiotics become commercially available. Although these new antibiotics were looked at as wonder drugs initially, resistant bacteria strain soon started emerging and susceptibility test for these drugs became necessary[6].The reasons for sensitivity testing are for clinical prediction of the likely outcome of treating a patient's infection with a particular antibiotic agent and for quantitative measurement of susceptibility which can be used to monitor the emergence and prevalence of antimicrobial resistance[7]. Antimicrobial susceptibility tests measures the ability of antibiotic or other antimicrobial agents to inhibit bacterial growth *in vitro*.It is a test used to determine which antibiotic can kill the organism causing the infection[8]. Antimicrobial susceptibility testing methods may be quantitative, providing an absolute value of the minimum inhibitory concentration (MIC) or minimum Bacteriostatic or Bactericidal Concentration (MBC) of an agent that will inhibit or kill the organism respectively. Examples include Agardilution and Broth dilution methods and the commercial available E-east MIC strips. It may also be qualitative indicating whether the organism is susceptible or resistant to the antimicrobial. Examples include disc diffusion method and automated system[9-10]. The testing of sensitivity of pathogenic bacteria to antibiotics has become a necessary procedure in Hospitals and clinical laboratories because it aids the clinician in his choice of therapy[11]. The method of choice is the disc diffusion method and its acceptance has been aided by its simplicity and rapidity[12]. This method was first utilized by Beijerinck in 1889 for studying the effect of different auxins on bacterial growth[13]. It was further developed by Bauer and co-workers in their work to standardize the method[14]. It measures the qualitative action of antimicrobial agent to pathogenic organisms. In disc diffusion method of antimicrobial susceptibility testing, the antimicrobial agent diffuses from a focus or reservoir through a solid medium, inhibiting the growth of an organism to a distance depending on the sensitivity of the organism and many other factors[7, 10]. Bondi and his co-workers initially described the use of filter paper discs as a reservoir in susceptibility testing in 1947 and they are still commonly utilized today in clinical laboratories[15]. The disc diffusion method can be referred to as the Kirby-Bauer method and have been modified by most clinical laboratories due to unavailability of some required materials. For instance Mueller Hinton Agar has been replaced with Nutrient Agar which is now being used by most laboratories and researchers[16-18]. Disc diffusion test is performed according to standardized methodologies issued by a reference group, such as National Committee for Clinical Laboratory Standards (NCCLS) now known as Clinical and Laboratory Standard Institute (CLSI). Other reference groups include British society for Antimicrobial Chemotherapy (BSAC) and the Swedish Reference Group for Antibiotics (SRGA). These groups promote accurate antimicrobial susceptibility testing, develop interpretative criteria for the results as well as appropriate reporting techniques based on standard reference methods, and also establish quality control parameters for standard test methods[10, 19].The interpretative criteria for disc diffusion test fall into three categories namely Susceptible (S), Intermediate (I), or Resistant (R). The results are interpreted using the established 'interpretative criteria' for each antimicrobial and bacterial species published by the CLSI and recommended by World Health Organization[20-21]. Many clinical laboratories carry out antimicrobial

susceptibility testing but various constraints result in the use of inappropriate antibiotic sensitivity discs. Majority of the Hospital laboratories procure commercial antibiotic discs, while others either prepare their own discs or use both commercial and self-prepared discs[16]. The quality of paper used in the production of sensitivity discs is an important factor as the specification of these papers varies somewhat as to weight, thickness, texture, and absorbability of water, whichmay affects the result of the test. In recent times, laboratory workers and clinicians have questioned whether difference in the quality of the paper used to produce antibiotic sensitivity disc can cause any significant changes in the result of antibiotic susceptibility testing.Paper quality as stated in this work refers to some properties of paper such as weight, thickness, texture and absorbency of water and does not include other physical, chemical and optical properties of paper.The aim of this study is to determine if there are any difference in inhibition zones produced by antibiotic discs made from papers of different quality and to determine the susceptibility pattern of some selected clinical bacteria isolate to antibiotic discs made from papers with different quality.

MATERIALS AND METHODS

Microbiological Culture Media

The media used for the study were commercially obtained and include Nutrient agar, Blood Agar, and Nutrient broth (Oxoid). They were prepared according to manufactures instructions[6].

Test Organisims

A total of three known recent clinical isolates, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* was used in the research and were obtained from the Medical Microbiology department of Federal Medical Center Owerri Imo State Nigeria. They were isolated from patients with urinary tract infections and were inoculated onto blood agar plates and transported to the laboratory in sterile polyethylene bags for microbiological analysis and biochemical tests.

Identification of Bacteria Isolates[6] All bacterial isolates were identified by their cultural and morphological characteristics on media plates, Gram reaction and biochemical tests.

Preparation of Paper Disc[22]

Four types of paper, namely Whatman No.1 filter paper (P1), Conqueror paper (P2), Copyman printing paper (P3) and Star-Foolscap paper (P4) were selected for preparing the discs. Their selection was based on theirfrequent use in discs preparation and their differentspecifications which include weight, thickness, texture and absorbency of water (Table1). WhatmanNo.1 filter paper Catalog No. 1001 was commercially obtained from OkeySurgicals stores, others were obtained from Arinze Stationery store both in Owerri City, Imo State Nigeria.To facilitate identification of discs, code names of antibiotics were printed on the sheets of paper before discs of 6mm were punched out from the four paper types using an office hole-puncher. Sterilization was done by autoclave for 15 minutes at 121^0C, and allowed to cool.

Preparation of Antibiotic Solutions[6, 22]

Standard antibiotic powders of known concentrations were commercially obtained for the study. The antibiotic powders include Ampicillin, Gentamicin, Penicillin, Ceftriaxone, Ciprofloxacin, Erythromycin, Cefotaxime, Nalixidic acid and Chloramphenicol. They were all products of Beecham pharmaceutical Germany.The antibiotic powders were dissolved in their appropriate solvents and further diluted in distilled water. The solutions were prepared to contain the desired disc potency in 0.02ml of the drug solution.The sterile disc was placed in Petri dishes and 0.02ml of the antibiotic solution was delivered to the disc using a single point pipette.Without covering the petri dishes, the discs were allowed to dry in an incubator at 37^0C for 1 hour. After drying, the discs were placed in a sterile clean, air-tight container and stored in the refrigerator at 8^0C. They were tested using known standard organisms before they were used. The container was removed from the refrigerator 1 hour before use in order to adjust the container to room temperature.

Preparation of Inoculum[23] Standard saline solution was used to prepare an inoculum with

density equivalent to a 0.5 McFarland Opacity Standard. In the preparation of the inoculum, 8 colonies of the test organisms were suspended in 5ml of saline and thoroughly mixed and adjusted to required density. To obtain a uniform growth, the bacteria suspensions were agitated a little before inoculation.

Antibiotic Sensitivity Testing[23]

For the susceptibility test, the disc diffusion method was employed in line with the CLSI guidelines under aseptic condition. For inoculation, a sterile cotton swab was dipped into the inoculum suspension and excess inoculum removed by firmly rotating the swab against the inside wall of the tube above the fluid level. The dry surface of the Nutrient agar plates were inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated twice with the rotation of the plate at an angle of 60^0each time. The preparation was allowed to dry for 5 minutes with the lid closed. A pair of sterile forceps was used to place the prepared antibiotic disc evenly and firmly onto the inoculated agar surface. The plates were incubated aerobically at 37^0C overnight.After incubation the plates were checked for pure confluent growthand the diameter of the zones of inhibition of growth were measured to the nearest millimeter with a transparent meter ruler.

RESULTS

Paper specification

The four types of papers used to produce the antibiotic discs used in this study were found to have different physical properties which include weight, thickness, texture, and absorbency. The results were summarizes in Table No. 1.

Susceptibility pattern of tested bacterial isolates

Antibiotic discs were prepared from each type of paper and placed on ager plates seeded with the test organisms namely *E. coli, S. aureus and P. aeruginosa*, so that each plate contains 9 discs, each disc representing a different drug with known concentration. Three of such plates were prepared for each bacterial isolate and for each type of paper and for all the antibiotics tested at a known concentration and the mean zone of inhibition calculated.Tables No. 2-4 summarizes the susceptibility pattern and the average diameters of zones of inhibition produced by the various paper discs against selected bacterial isolates.

Effect of paper quality

In all, 324 zones of inhibition were obtained for analysis. The complete data were subjected to statistical analysis of variance so that any significant difference in zones of inhibition due to paper type could be evaluated. Separate analyses of variance were calculated against each organism as shown in Table No. 5. The comparison of the mean zone diameter of the various prepared paper discsis illustrated in Figures No. 1-3. The statistical results of the two-way analysis of variance (ANOVA) shows significant differences in zone diameter produced by the disc of the different paper types for all the organismsand antibiotics tested and were indicated by a high variance ratio (F-ratios, 79.73).

DISCUSSION

The manufacture of antibiotic discs and their successful use involve a number of considerations which include the quality of antibiotic, the composition of the discs (paper, tablet or other construction) andthe standard of test performance.The aim of the present study was to determine the effect of paper quality of locally made antibiotic discs on some clinical bacterial isolates and their susceptibility pattern. Differences in inhibition zone diameter produced by antibiotic disc made from four different type of paper were compared. The result of this study shows that each type of paper used to produce antibiotics disc affects the diameter of inhibition produced by the disc. This finding is in agreement with the result of the work carried out by Kramer and Kirshbaum in 1961, but disagrees with that of Ostrander and Griffith carried out in 1959, which indicated that unless some other agents, such as certain dyes, were present, the paper used made no difference[24-25]. Marthand his colleges in 1963also reported that different grades of paper used for antibiotic discs affect diameter of zones of inhibition produced by the discs[26]. In this present

study, significant variations were seen among the four types of papers locally used to produce antibiotics sensitivity discs. The variation could be due to their different qualities as indicated by their specifications Table No.1. Inall the paper types tested, the zones produced by the discs made from Whatman No.1filter paper (P1) were 3mm lager than zones produced by other paper types. Discs made from Copyman printing paper (P3) gave zones closer to those of Whatman discs (P1) with an average difference of 1.2mm. The disc of Conqueror paper (P2) and Star-foolscap paper (P4) gave smaller zones of inhibition irrespective of the antibiotics and the organism tested, with discs of Conqueror paper producing the lowest zone diameter Tables No.2-4. The analysis of variance showed that there is difference in zone size due to paper type (Table 5). As mentioned earlier, this difference can be said to be due to the different specification or composition that constitute the quality of the papers and this has been indicated by variation in zone size. In view of this, Conqueror paper (P2) has high value for thickness and weight with low absorbency and this may have affected its performance in the test. In addition to this, the weight and thickness of this paper may not have allowed easy diffusion of antibiotics on the agar plates. Whatman No. 1 filter paper (P1) has the highest absorbency and this may have contributed to its good performance in the test. The zones produced by Conqueror paper (P2) and the Star-foolscap paper (P4) were smaller in size and are not within the limit of the published standard for interpretation by the Clinical and Laboratory Standard Institute (CLSI) Table No.6. These variationscould cause misinterpretation of result in antimicrobial sensitivity testing as sensitive organisms may be labeled resistant due to the effect of these types of papers. The zones produced by discs made from Whatman No. 1 filter paper (P1) were within the limit of the CLSI published standard, unlike the zones produced by Conqueror paper (P2) and Star-foolscap paper (P4).

In view of the antibiotics tested, Ampicillin (10µg), Chloramphenicol (30µg), Nalixidic acid (30µg), Erythromycin (15µg), and Penicillin (10 units)disc made from the four types of papers gave no significant zone when tested against *P. aeruginosa*. Cefotaxime (30µg), Ciprofloxacin (30µg), Ceftriaxone (30µg), andGentamicin (10µg) discs gave larger zones showing high activity against the clinical bacterial isolates Figure No.1-3. This is also in agreement with the report of Ochei and Kolhatkar[6]which stated that *pseudomonas* species are resistant to most routine antibiotics, but are susceptible to the aminoglycosides and cephalosporin.

Table No.1: Specification of the papers used for antibiotic discs

Paper Discs	Weight per disc(g)	Thickness (Inches)	Absorbency (ml/min)	Texture
Whatman No.1filter paper (P1)	0.004	0.0045	0.080	Rough
Conqueror paper (P2)	0.006	0.0051	0.013	Rough
Copyman printing paper (P3)	0.003	0.0041	0.060	Smooth
Star Foolscap paper (P4)	0.001	0.0031	0.025	Smooth

Table No. 2: Susceptibility pattern and the average diameters of zones of inhibition produced by the various paper discs against *E.coli*

Antibiotic (Concentration per discs)	Mean zones of various paper discs			
	P1	**P2**	**P3**	**P4**
Ampicillin (10 µg)	20mm	15mm	19mm	17mm
Gentamicin (10 µg)	22mm	16mm	20mm	18mm
Cefotaxime (30 µg)	29mm	26mm	29mm	28mm
Ciprofloxacin (5 µg)	34mm	30mm	33mm	31mm
Chloramphenicol (30 µg)	22mm	19mm	21mm	20mm
Ceftriaxone (30 µg)	30mm	25mm	29mm	27mm
Nalixidicacid (30 µg)	26mm	19mm	23mm	21mm
Erythromycin (15 µg)	20mm	16mm	20mm	18mm
Penicillin (10 units)	23mm	19mm	21mm	20mm

Table No. 3: Susceptibility pattern and the average diameters of zones of inhibition produced by the various paper discs against *S. aureus*

Antibiotic (Concentration per discs)	Mean zones of various paper discs			
	P1	**P2**	**P3**	**P4**
Ampicillin (10 µg)	28mm	20mm	28mm	24mm
Gentamicin (10 µg)	22mm	18mm	22mm	19mm
Cefotaxime (30 µg)	28mm	21mm	28mm	25mm
Ciprofloxacin (5 µg)	22mm	18mm	22mm	20mm
Chloramphenicol (30 µg)	20mm	15mm	18mm	15mm
Ceftriaxone (30 µg)	25mm	21mm	24mm	22mm
Nalixidicacid (30 µg)	16mm	11mm	13mm	12mm
Erythromycin (15 µg)	25mm	18mm	22mm	20mm
Penicillin (10 units)	24mm	18mm	22mm	19mm

Table No. 4: Susceptibility pattern and the average diameters of zones of inhibition produced by the various paper discs against*P. aeruginosa*

Antibiotic (Concentration per discs)	Mean zones of various paper discs			
	P1	**P2**	**P3**	**P4**
Ampicillin (10 µg)	-	-	-	-
Gentamicin (10 µg)	21mm	15mm	16mm	15mm
Cefotaxime (30 µg)	20mm	17mm	18mm	19mm
Ciprofloxacin (5 µg)	27mm	19mm	21mm	20mm
Chloramphenicol (30 µg)	-	-	-	-
Ceftriaxone (30 µg)	21mm	17mm	20mm	19mm
Nalixidicacid (30 µg)	-	-	-	-
Erythromycin (15 µg)	-	-	-	-
Penicillin (10 units)	-	-	-	-

(-): No significant zone

Table No. 5: Analysis of variance for the effect of paper types on zones of inhibition produced by the test organisms

Organisms	**Source**	**D.F**	**S.S**	**M.S**	**F**
E.coli	Zones (Blocks)	8	816.89	102.11	79.73*
	Paper	3	106.33	35.44	
	Error	24	10.67	0.4445	
S. aureus	Zones (Blocks)	8	451.39	56.64	61.85
	Paper	3	173.11	57.70	
	Error	24	22.39	0.9329	
P. aeruginosa	Zones (Blocks)	3	51.69	17.23	10.99
	Paper	3	60.69	20.23	
	Error	9	16.56	1.84	

D.F: degrees of freedom; S.S: sum of squares; M.S: mean squares;
F: variance ratio; *: highly significant

Table No. 6: Published standards for limit of inhibition zones for selected test organisms and antibiotics[27]

Antimicrobial agents	Disc potency	*E.coli*	*S. aureus*	*P. aeruginosa*
Ampicillin	10(µg)	16-22mm	27-35mm	-
Gentamicin	10(µg)	19-26mm	19-27mm	16-21mm
Cefotaxime	30(µg)	29-35mm	25-31mm	18-22mm
Ciprofloxacin	5(µg)	30-40mm	22-30mm	25-33mm
Chloramphenicol	30(µg)	21-27mm	19-26mm	-
Ceftriaxone	30(µg)	23-35mm	22-28mm	17-23mm
Nalixidic acid	30(µg)	22-29mm	-	-
Erythromycin	15(µg)	-	22-30mm	-
Penicillin	10 units	-	26-37mm	-

(-): Not available

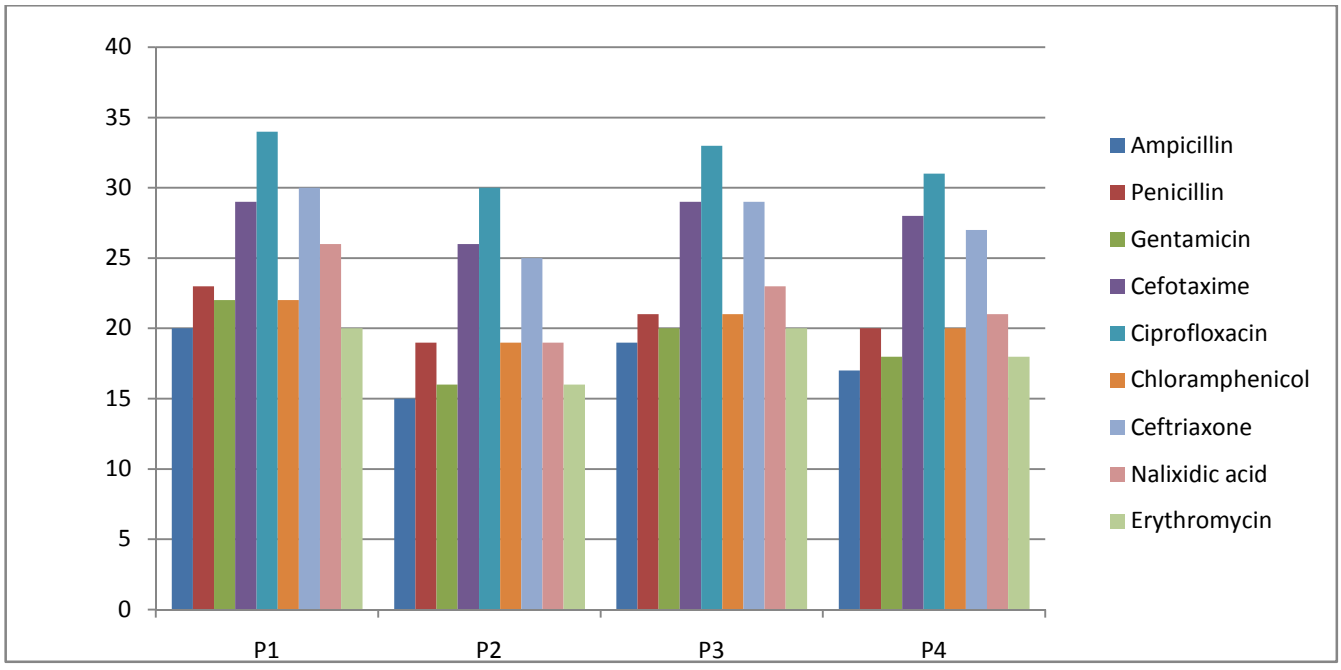

Figure No. 1: Comparison of the average zones of inhibition produced by the discs from various paper types against *E. coli*

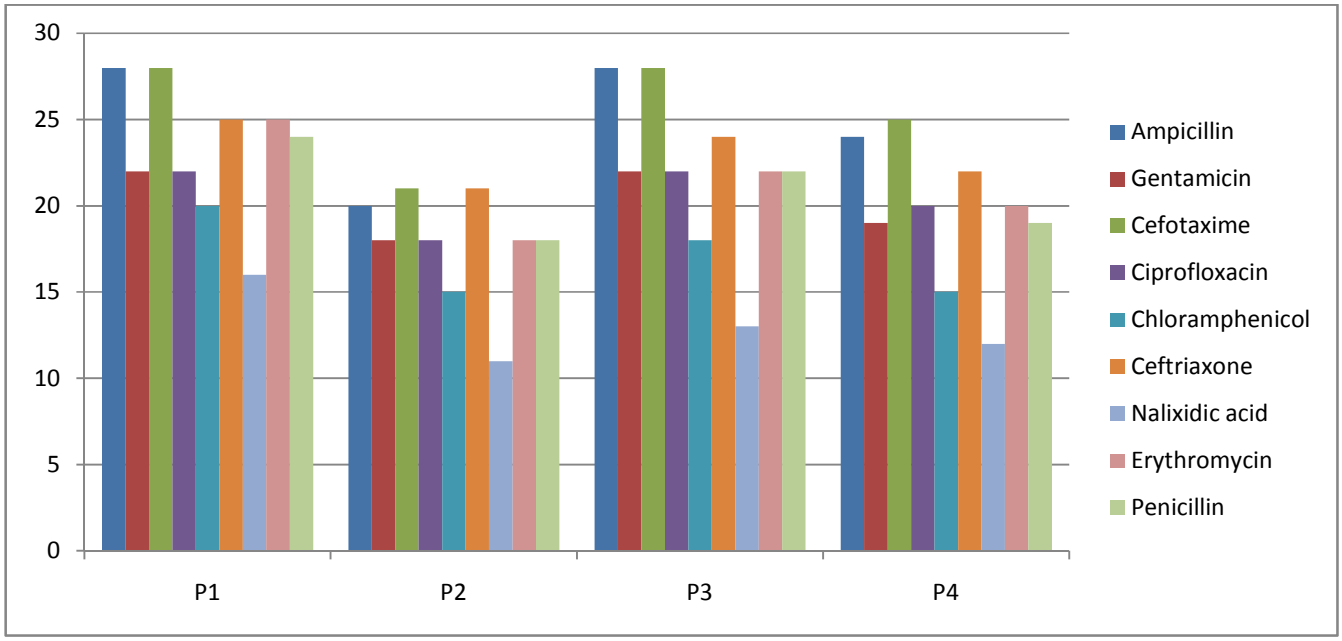

Figure No.2: Comparison of the average zones of inhibition produced by the discs from various paper types against *S. aureus*

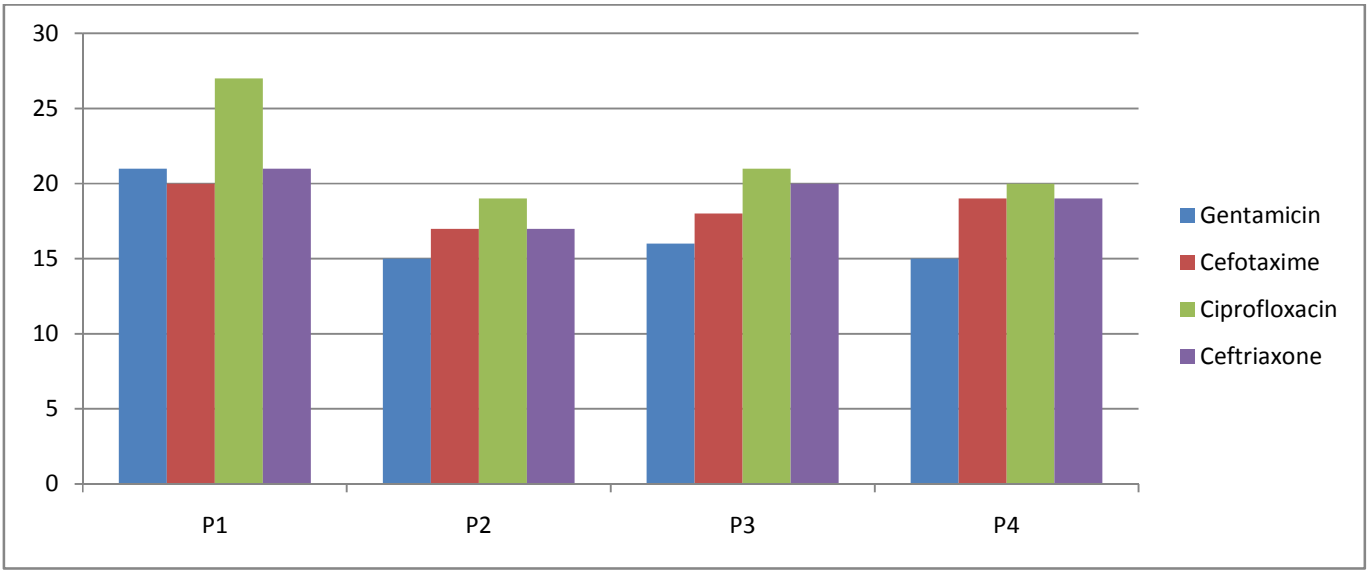

Figure No. 3: Comparison of the average zones of inhibition produced by the discs from various paper types against *P. aeruginosa*

CONCLUSION

The differences in the specifications of some types of papers used locally to produce antibiotic discs clearly outline the need for one specified paper for the preparation of antibiotic disc. More importantly the availability of affordable quality discs is indispensable in antimicrobial susceptibility surveillance of commonly encountered clinical bacterial isolates. The result of this study has shown that in trying to procure antibiotic discs locally, the different paper types used produce a significant effect on the result of susceptibility test. Therefore efforts should be made in the choice of paper, to bring to standard the result of the susceptibility testing which should also be in line with the published standards by the CLSI. The use of good quality papers for discs in susceptibility testing of common organisms encountered will not only improve the result of susceptibility testing but would also guide medical practitioners in their choice of appropriate antimicrobial agents and facilitate appropriate antimicrobial treatment.

ACKNOWLEDGEMENT

The authors are sincerely thankful to the staffs and management of Imo State University Medical Laboratory and the staffs and management of the Medical Microbiology Department of Federal Medical Centre Owerri, Imo State, Nigeria for providing the facilities to carry out this research work. We are grateful to OHANAEZE-NDI IGBO PORT ELIZABETH Chapter South Africa for financial support to publish this work

REFERENCE

1. Poupard J, Rittenhouse S & Walsh L. The evolution of antimicrobial susceptibility testing methods, *Plenum press New York*, 1994, 1-144.
2. Philip I. Re-evaluation of antibiotic breakpoints, *Clinical Infectious Disease*, 33(3), 2001, 230-232.
3. Kerr JR. Antibiotic treatment and susceptibility testing, *Journal of Clinical Pathology*, 58, 2005, 786-787.
4. Heatley N. Method for the assay of Penicillin, *Biochemical Journal*, 38, 1944, 61-65.
5. Vincent JG & Vincent HW. Filter paper disc modification of the Oxford cup Penicillin, *Proceeding of the Society for Experimental Biology & Medicine*, 55, 1944, 162-164.
6. Ochei J &Kolhatkar A. Antimicrobial Susceptibility tests. In: Textbook of Medical Laboratory Science Theory and Practice, Tata *McGraw-Hill New Delhi*, 7, 2000, 1-1284.
7. Ojiegbe GC. Laboratory control of antibiotic agents. In: Textbook of Antibiotic Drugs and their Laboratory Control, Cel-Bez publisher and *Co. Owerri*, 1, 2006, 1-76.
8. Jacquelyn GB. Antimicrobial Therapy. In: Textbook of Microbiology Principle and Exploration, *John Wiley and Sons New York*, 4, 1999, 1-756.
9. Cormican M, Whyte T &Hanahoe B. Antimicrobial susceptibility testing in Ireland: an introduction to the methods of National Committee for Clinical Laboratory Standards (NCCLS), *Medical Microbiology and Bacteriology*, 1, 2001, 1-47.
10. Julianna E. Comparison of the Swedish reference group of Antibiotics and the Clinical Laboratory Standard Institute sensitivity testing methods. School of Biological Science, Dublin, 2006, 1-43.
11. Joseph G, Sinata K, Pierre N, Dieudonne A & Peter N. Antimicrobial activity against gram negative bacilli from Yaounde Central Hospital Cameroon, *African Health Science*, 6 (4), 2006, 232-235.
12. Joshi A, Iyer V, Blasubramaniau U, Kagal A &Bharadwaj R. Comparison of efficacy of three commercially available antibiotic discs, *Indian Journal of Medical Microbiology*, 26 (2), 2008, 160-162.
13. Dufrenoy J. Les methods auxanographiquesetleur application au dosage des antibiotiques, *Annales de parasitology Humaineet compare*, 22, 1947, 449-479.
14. Bauer AW, Kirby WM, Sherris JC &Turch M. Antibiotic susceptibility testing by a standardized single disc method, *American Journal of Clinical Pathology* , 45, 1966, 493-496.

15. Bondi A, Spaulding E, Smith D & Dietz C. Routine method for rapid determination of susceptibility to Penicillin and other antibiotics, *American Journal of the Medical Sciences*, 213, 1947, 221-225.
16. Sudha V, Prasad A, Khara S &Bhafia R. Antimicrobial susceptibility testing in India: a status survey, *Indian Journal of Medical Microbiology*, 17, 2001, 222-223.
17. Joseph O. Antibiotic susceptibility patterns of urine bacteria isolates in Zaria, Nigeria, *Tropical Journal of Pharmaceutical Research*, 2(2), 2003, 223-228.
18. Deeni Y &Taura A. Antimicrobial susceptibility of clinical isolates of enteric bacteria in Kano Nigeria, *Orient Journal of Medicine*, 6(1&2), 1994, 22-29.
19. WIPO. Method for calibrating antibiotic disc diffusion testing of microorganisms. World Intellectual Property Organization, 2002.
20. Lalitha MK. Manual on antimicrobial susceptibility testing, *Indian Association of Medical Microbiology*, 1, 2003, 1-47.
21. Jorgensen JH & Ferraro MJ. Antimicrobial susceptibility testing: general principles and contemporary practice, *Journal of Clinical Infectious Diseases*, 26(1), 1998, 973-980.
22. Melecia A, Antonio J & Cristina U. Local production of low cost quality antibiotic susceptibility discs for the Philipians, *Philipine Journal of Microbiology Infectious Diseases*, 17 (2), 1988, 66-75.
23. CLSI. Performance standards for Antimicrobial susceptibility testing: fourteenth information supplement. *Clinical and Laboratory Standard Institute*, 24(1), 2004, M100-S14.
24. Kramer J &Kirshbaum A. Effect of paper on the performance assay in the control of antibiotic sensitivity discs, *Journal of Applied Microbiology*, 9(4), 1961, 334-336.
25. Ostrander WE & Griffith LJ. An evaluation of different types of paper concerning their suitability for sensitivity disc, *Antibiotic Annals*, 7(58), 1959, 813-817.
26. Marth EH, Alexander FJ &Hussong RV. Detection of levels of Penicillin in milk using different grades discs, *Journal of Milk Food Technology*, 26, 1963, 150.
27. NCCLS. Performance standards for antimicrobial disc susceptibility test: Approved standard, Wayne, 8th edition, 2003, M02-A8.