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Prevalence of *Pseudomonas aeruginosa* in Clinical Samples and its sensitivity to Citrus Extract

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ABSTRACT

The prevalence of selected pathogens in four hundred and ninety eight samples of ear, wound swabs and urine samples submitted to the Microbiological Laboratory of Wesley Guild Hospital, Ilesha, Nigeria was investigated. In ear infections, *Pseudomonas aeruginosa* predominated (50%), followed by *Staphylococcus aureus* (30%) and others (20%). In wound swabs, *S. aureus* predominated (46.3%), *P. aeruginosa* (16%). Other bacteria isolated included *Klebsiella* spp, *Proteus* spp and *Escherichia coli* accounting for 18%. In urine, *S. aureus* and *E. coli* were more frequently isolated with 49% and 23% respectively. The antibiogram studies showed that *P. aeruginosa*, was highly sensitive to Ciprofloxacin, Perfloroxacin and Gentamycin except *Pseudomonas* isolates from wounds which were resistant to Gentamycin. The isolates were resistant to Streptomycin, Ampicillin and Cotrimoxazole (septrin). Citrus juice – *C. aurantifolia* (Lime) and *C. limon* (Lemon) on *P. aeruginosa* gave positive results with lethal effects on the test organisms with zones of inhibition ranging from 7mm-22mm in diameter around the colonies.

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Keywords: *Pseudomonas aeruginosa*, prevalence, citrus extract, antibiogram

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INTRODUCTION

The genus *Pseudomonas* comprises of more than 140 species, only few of these are pathogenic to man. The others are essentially saprophytic and occur widely in nature (Crackshank *et al.*, 1975). Most infections with this organism occur in compromised hosts. The pathogenicity of these organisms is based on its ability to produce a variety of toxins and proteases. It also depends upon its ability to resist phagocytosis (Baltimore, 2000). Between 5 and 30% of normal individuals have pseudomonas in their gastro intestinal tracts but the organisms rarely predominate (Baker and Breach, 1980). The pathogens most frequently encountered from cultures of ear infections are *Pseudomonas spp*, *Staphylococcus aureus*, *Proteus spp*, *Streptococcus spp* and *Haemophilus spp*. (Keith *et al.*, 1978, Bailey *et al.*, 1974). Toda (2002) described *P. aeruginosa* as being notorious for its resistance to antibiotics and for this, it is a dangerous and dreaded pathogen. The only antibiotic agents to which strains are regularly sensitive are gentamycin, carbenicillin, and polymyxin, colistin, streptomycin, quinolones, cephalosporin but ranging degree of cross resistance between these agents have been reported.

In Nigeria, a lot of the rural populace still rely much on herbs for their health care needs, though it is used blindly as therapeutics for human and animal diseases (Peach and Tracey, 1955; Benjamin *et al.*, 1983). Some of these herbs include *Citrus aurantifolia* (lime), *Citrus limon* (lemon) and *Citrus paradissi* (grape fruit). Oboh *et al.*, (1995) reported that citrus *aurantifolia* juice has been used in various Nigerian home because of its anti microbial effect against some gram negative and gram positive bacteria. Jacob, (1972) discovered grape fruit seed extract to prevent a wide range of pathogens from building up a resistance in the body. Umezuruike (2003), reported that grape fruit seed extract is very effective in treating *Staphylococcus*, *Streptococcus*, *Salmonella*, ear infections and traveler's diarrhea. Hage's *et al.*, (2002), investigated the antimicrobial and toxicological properties of lemon and found lemon to possess significant antimicrobial activity against *Staphylococcus*, *Klebsiella*, *Escherichia coli*, *Pseudomonas* and *Candida albicans*.

This work is aimed at determining the prevalence of *P. aeruginosa* species in selected clinical samples in Wesley Guild Hospital, Ilesha, study the antibiogram profile of pseudomonas strains and determine the efficiency of citrus extract on *P. aeruginosa* isolated.

MATERIALS AND METHODS

Sample Collection Techniques

Four hundred and ninety eight samples of ear, wound swabs and urine samples submitted to the Microbiological Laboratory of Wesley Guild Hospital, Ilesa, Nigeria were used for this study

Urine samples were collected in sterile universal bottles; patients suffering from urinary tract infection were instructed on how to collect a clean-proof universal container. Specimens of ear discharge and eye were collected using sterile cotton swab sticks by the assistance of medical officer. Wounds swabs high vagina swabs (HVS) and Endocervical swabs were collected using sterile swabs sticks. Cerebrospinal fluid (CSF) samples were collected aseptically by medical officer into sterile universal containers. Blood specimens were collected from patients suffering from septicemia. Unlike other samples, blood samples were collected at the time the patients temperature began to rise from normal body temperature.

Isolation Procedures

Urine: Samples were mixed thoroughly by inverting the container several times, using a sterile wire loop, the sample was inoculated on dried CLED (Oxoid) agar plates. The plates were incubated overnight at 37°C,

Ear discharges, eye swabs and wound swabs: The swabs were streaked on sterile Chocolate agar, and MacConkey (Oxoid) agar plates by first made primary inoculum and then streaked out using sterile wire loop to give discrete colonies. The plates were incubated at 37°C for 24 hours. Suspected colonies were stored on slant for further tests.

HVS and ECS: The swabs were streaked on sterile chocolate agar and MacConkey agar plates by first made primary inoculum and then streaked out using sterile wire loop to give discrete colonies. The

plates were incubated at 37°C for 24 hours suspected colonies were stored on slant for further tests.

CSF: CSF specimens were inoculated chocolate agar plates and MacConkey agar plates. Chocolate agar plates were incubated in 10% CO₂ and MacConkey agar plates were incubated at 37°C for 24 hours and then observed for suspected colonies.

Characterization of Isolates

Characterization of isolates was carried out by employing macroscopic, microscopic, physiological, serological and biochemical tests.

Antibiotic Sensitivity Test

Preparation of Inoculum

Organisms under test (pure isolates) were inoculated into 4ml of peptone broth and incubated at 37°C for 2-5 hours and adjusted turbidity to match an opacity tube containing 0.5ml of 1% barium chloride in 1%.

Sulphuric acid (N/36) to give a standard inoculum used in seeding the plates for sensitivity testing (Clark *et al.*, 1981).

Sensitivity Testing

Susceptibility of the isolates to antimicrobial agents was determined by the agar diffusion techniques using Kirby-Bauer disc method (Prescott *et al.*, 2002) commercially prepared antibiogram doses in (microgram [mcg]) contained in 10 tipped compound susceptibility disk with there corresponding concentrations were used.

Determination of the Effect of Citrus Extracts on the Strains of *P. aeruginosa*

Preparation of Citrus Extracts: *Citrus aurantifolia* (lime) *C. limon* (lemon) and *C. paradissi* (grape fruit) were washed in running tap water in the laboratory surface sterilized with 70% alcohol and then rinsed with sterile distilled water. The fruit were peeled and cut diametrically; juice was expressed into a sterile universal container separately and then filtered into another sterile container to remove the seed and other tissues. Double fold dilution of juices was made with sterile distilled water to give ½ and ¼ strength.

Seed Extracts

The seeds of the grape fruits were air-dried for two weeks to prevent loss of active components. They were ground into powder with electric blender. Absolute ethanol and methanol were used for the extraction. Varied concentrations (0.5, 0.75, 1.0mg/ml) were made by dissolving them in one milliliter of each of the solvent ad then soaked over night.

Screening of Citrus Extracts for Antimicrobial Activities

Screening for antimicrobial activities of each citrus extract on each of the test organism used in this investigation was carried out using the methods of Clark *et al.*, (1981); and Toda *et al.*, (1989).

RESULTS

Four hundred and ninety-eight clinical samples were collected form children's welfare clinics and adults' wards including out patients clinic of Wesley Guild Hospital Ilesa, Osun State (Table 1).

The distribution of different pathogens (*P. aeruginosa*, *Staph.aureus*, *Proteus spp*, *Klebsiella spp* and *Escherichia coli*) isolated from different samples is as shown in table 2. *Pseudomonas aeruginosa* was isolated from most of the samples tested followed by *S. aureus* and *Proteus spp*, *Klebsiella spp* and *E. coli*.

Table 1: Source and number of different samples used

Samples	Children	Adults	Total
Ear swabs	33	7	40
Wound swabs	12	23	35
Urine samples	12	151	163
Blood	64	13	77
Cerebrospinal fluid (CSF)	45	0	45
Endocerviccal swab (ECS)	0	39	39
High vaginal swab (HVS)	1	29	30
Eye swab	6	1	7
Semen	0	5	5
Sputum	0	55	55
Catheter tip	0	2	2
TOTAL			498

The results of distribution of pathogens from different samples are as shown in table 2. It was observed that *P. aeruginosa* was the most incriminating pathogens of the ear infection account for 50% of the total number of isolates. Others are *S. aureus* (30%) *Proteus sp* (14%) *Klebsiella spp* (2%) and *E. coli* (2%). With 46.3%, followed by *P. aeruginosa* (16%) while *Klebsiella* account for 17% of the total number. Percentage is based on total number of isolates.

Table 2:
Distribution of different pathogens

Organisms	Otitis media	Wound swab	urine
<i>P. aeruginosa</i>	25 (50)	7 (16)	9 (8.7)
<i>S. aureus</i>	15(30)	19(46.3)	51(49)
<i>Proteus spp</i>	7(14)	3(7.3)	5(4.8)
<i>Klebsiella spp</i>	1(2)	7(17)	24(23)
<i>Escherichia coli</i>	1(2)	5(12.2)	15(14.4)
No growth	1	3	71

Urine culture showed that *S. aureus* and *E. coli* were more frequently isolated with 49% and 23% respectively while *Klebsiella* account for 14.4% and *P. aeruginosa* (8.7%).

Results of comparative antimicrobial activity of citrus extracts with commercial antibiotics disc were shown on Table 3.

Table 3:
Comparative antimicrobial activity of citrus extracts with commercial antibiotics disc

	A							B				
	Inhibition Zone (mm)							GEN	COL	CIP	PER	
	Lemon juice		Lime juice		Grape juice		Seed ethanol extract	Methanol extract				
<i>P.aeruginosa</i> strains from ear	1/2	1/4	1/2	1/4	1/2	1/4						
3292	13	7	16	14	-	-	-	-	15	13	-	-
3291	17	12	17	13	-	-	-	-	13	20	-	-
1450	17	16	20	17	-	-	-	-	16	15	33	27
2374	16	12	21	14	-	-	-	-	7	-	25	20
1808	17	11	17	13	6	4	-	-	16	14	35	21
1528	15	9	17	11	-	-	-	-	14	12	22	16
2540	20	18	21	19	12	-	-	-	17	12	37	25
2791	22	20	22.5	21	-	-	-	-	-	-	33	22
351	19.5	15	20	15	-	-	-	-	-	-	30	210

Key: GEN = Gentamycin COL = Colistin- = No Inhibition; CIP = Ciprotab PER = Perflotab

The best antimicrobial activity was observed in the juice of lime and lemon. They were active against all stain of *P. aeruginosa* tested, *Citrus aurantifolia* (lime) which produced wider zone of inhibition ranging from 7mm to 21.5mm at the double dilution of 1/2 and 1/4 The grape fruit juice showed little or no significant antimicrobial activity against the test organisms.

Methanol and ethanol extracts of grape fruit seed did not show any antibacterial activity. The juice of both lime and lemon had better antibacterial activity and compared favourably to the commercial antibiotic disc.

DISCUSSION

In this study, the incidence of *P. aeruginosa* was found to be 50% in patients with Otitis media from the total number (40) investigated *Staph aureus* followed with the incidence of 30% while *Proteus* ranked third with incidence of 14%. These results are similar to the findings of Ogisi and Osamar (1982). They reported a prevalence of 31% and 24% for *Pseudomonas spp* and *Proteus spp* respectively while Colar *et. al.*, (1983) reported a prevalence of 38% and 9.8% for *Pseudomonas spp* and *Proteus spp* respectively.

The results obtained also agreed with the findings of Selina (2002) who rated *P. aeruginosa* as the most common bacteria isolated from mild to severe form of external otitis and chronic suppurative otitis media.

The values of 16% and 46.3% prevalence of *Pseudomonas* and *Staphylococcus aureus* obtained in wound swabs investigated were similar to the study, *Pseudomonas* was found to be the most commonly cultured organism (54.2%) followed by *S. aureus* (20.8%). The slight difference may be due to geographical factors and also duration of the investigation. Investigation on other samples such as blood, CSF, HVS, ECS and semen showed little or no significant involvement of *Pseudomonas*.

The sensitivity pattern of the organisms to Gentamycin, Ciprotab, Perflotab in this study were comparable to the values of 92%, 88% and 59% as respectively obtained by Ikeh *et al.* (1993). But the high in-vitro sensitivity recorded for ciprotab and perflotab (quinolones) may not serve useful purpose in children because quinolones are usually contra indicated as paediatric regimens because of side effects in children and they are expensive for adult use.

Extracts of citrus fruits especially lime and lemon showed high antibacterial activity against all the pseudomonas strains tested (Table 3). This suggests that the citrus juice contains active ingredient which qualify them for medicinal use. Lime juice has been locally applied on wounds and accidental cuts or abrasions in various Nigerian homes with the belief that this will speed up healing (Walker, 1953).

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