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AP-PCR TYPING OF CARBAPENEM SENSITIVE *PSEUDOMONAS AERUGINOSA* ISOLATED FROM CLINICAL SAMPLES

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ABSTRACT

In this study the antibiotic susceptibility of 51 *P.aeruginosa* strains isolated from clinical samples were detected by the disc diffusion test. The susceptibility of *P. aeruginosa* strains were found as respectively 55% amicacin, 43% aztreonam, 75% netilmycin, 68% sefepim, 73% ceftazidim, 76% ciproflaxacin, 37% gentamicin, 84% meropenem, 76% piperasillin/tazobactam, 47% tobramycin and 84% imipenem. These results show that carbapenems are the most effective antibiotics for *P. aeruginosa* strains and the efficacy of meropenem and imipenem are high for *P. aeruginosa* strains Molecular typing profiles of 43 P. aeruginosa strains which are sensitive to meropenem and imipenem antibiotics with AP 1 primary were determined in AP-PCR. As a result of AP-PCR molecular typing study of this 43 *P. aeruginosa* isolate, no correlation was found out between antibiotic sensitivities and molecular types. This situation once again reveals that reasonable antibiotic usage in absolutely

INTRODUCTION

As well as most of the *Pseudomonases* (pyocyanic) infect people, some among them form temporizer pathogen which is important especially for people having weak immunity system. In particular, *P. aeruginosa* known as temporizer pathogen has such an important place among *Pseudomonases*.It is realized that infections caused by *P. aeruginosa* in humans increase in hospital ambient lately. It is also observed that these bacterias shelter very easily and their resistance increases in hospital ambient. Generally, treatment of *Pseudomonas* infections is ratherly slow and difficult because of this bacteria is resistant to frequently used antibiotics. (1, 2, 3) Therefore, multiple antibiotic usage is recommended for treatment of *P. aeruginosa* (4,5). Particularly, in *P. aeruginosa* infections, the carbapenems are frequently used antibiotics in treatment. The reason why carbapenems are preferred in treatment is that they have wider influence spectrums than beta-lactam antibiotics. Furthermore, carbapenems are enounced as stabile against plasmid and also chromosomal betalactamases in different researches (6,7). Important infections caused by *Pseudomonases* are lung infections, respiratory infections, bacteriemia, articulation infections, urinary tract infections, gastrointestinal tract infections, burn and trauma infections, epidermic and soft tissue infections (8)

In study, AP-PCR technic was used to determine molecular typing profiles of 43 *P. aeruginosa* isolate that is sensitive to carbapenems. AP-PCR technic is frequently used in molecular typing of *P. aeruginosa* isolates which are isolated from clinical samples. (9).

MATERIALS AND METHODS

Obtaining samples: In study, 51 *P. aeruginosa* isolate, which was isolated from different samples belong to impatient in several clinics, was used.

Bacteria identification: API 20 NE (BioMeriux, Marcy l'Etoile, France) kit was used in identification of bacterias (10).

Antibiotic sensitivity test: In accordance with NCCSL standards, antibiotic sensitivity test was studied in Mueller-Hinton Agar with the method of standard disc diffusion. In disc diffusion test, amicasin 30 µg, aztreonam 30 µg, netilmicin 30 µg, sephepim 30 µg, ceftasidime 30 µg, ciproflaxacin 5 µg, gentamicin 10 µg, meropenem 10 µg, piperacillin/ tazobactam 110 µg, tobramycine 10 µg and imipenem 10 µg (Oxoid-England) antibiotics were used. In research, *P. aeruginosa* ATCC 27853 was used as control element (11). As a result of this test, sensitive *P. aeruginosa* isolates were chosen.

DNA isolation: Isolation of 51 *P. aeruginosa* isolate was performed by phenol-chloroform technic (12, 13)

AP-PCR Amplification: For AP-PCR reaction, API "5 – GTT GCG ATC -3" (Bio Thesis, Germany) primary was used. Each, including 100 µl reaction compound, was respectively calculated and put into 0,5 ml ependorph tubes.10 x Tampon (NH4)2 SO4) (Promega,USA) 10 µl, dATP, dCTP, dGTP, dTTP (Fermantes,Lithuania) 2 mM, Primary Ap 1 (Bio Thesis, Germany) 50 pmol, $MgCl_2$ (Fermantes,Lithuania) 25 mM, Taq DNA polymerase (Promega, USA) 1U and deionized water were added to compound to fullfil volume. AP-PCR reaction was performed in automatic PCR device (Techne,USA) under circumstances like:following denaturation for 5 minutes at 94°C, denaturation for 1 minute at 94°C making 40 cycles, adhesion for 2 minutes at 72°C and following last elongation for 6 minutes at 72°C, PCR products were kept at +4°C until extrapolation. Obtained PCR products were conducted in % 12 polyacrilamid gel electrophoresis (PAGE) (20 cm x 20 cm) (Owl Scientific, USA), 120 volts, 80 mA and 10 watts. PAG was painted by silver painting technic later on.

Distributions of 51 *P. aeruginosa* isolates utilized in study as per the clinical samples are shown in Table 1.

Table 1. The distribution of isolators obtained from patient materials with respect to clinical samples

Clinical samples	*P. aeruginosa*
Blood	15
Sputum	6
Aspirate	10
Urine	12
Wound	8
Total	51

Sensitivities of *P. aeruginosa* isolates to different antibiotics by the method of disc diffusion are shown in Table 2.

Table 2. Sensitivity proportions of isolated *P.aeruginosa* to tested antibiotics with disc diffusion methods

ANTIBIOTICS	SENSITIVE	(%)	MEDIAL SENSITIVE	(%)	RESISTANT	(%)
Amicasin	28	55	11	21	12	24
Aztreonam	22	43	9	18	20	39
Netilmycin	38	75	-	-	13	25
Sefepim	35	68	5	10	11	22
Ceftazidime	37	73	-	-	14	27
Ciprofloxacin	39	76	4	8	8	16
Gentamicin	19	37	14	28	18	35
Meropenem	43	84	1	2	7	14
Piperasillin/tazobaktam	39	76	3	6	9	18
Tobramycin	24	47	8	16	19	37
Ýmipenem	43	84	3	6	5	10

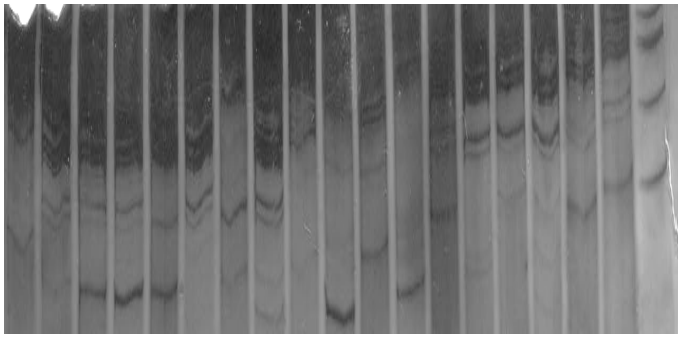
In study, 43 of 51 *P. aeruginosa* isolate were found out sensitive to carbapenems. Sensitivity to imipenem and meropenem from these antibiotics was determined as % 84. Distributions of isolates as per the samples are shown in Table 3. Molecular typing profiles of 43. *P. aeruginosa* which is sensitive to meropenem and imipenem antibiotics with AP 1 primary were determined in AP-PCR (picture 1a and picture 1b). In study, genotypic proximity was determined in number 3,4 and 5 *P. aeruginosa* isolates (picture 1a). In study, no common genotypic profile was found out among left 40 *P. aeruginosa* isolates sensitive to meropenem and imipenem (picture 1a and picture 1b). Besides, a dominant genotypic pattern was not determined among sensitive isolates.

Table 3. 43 *P. aeruginosa* isolates sensitive proportions of meropenem and imipenem antibiotics

Clinical samples	Imipenem			Meropenem		
	D	AD	R	D	AD	R
Blood	13	1	2	12	1	3
Sputum	5	-	-	5	-	-
Aspirate	9	1	-	10	-	-
Urine	10	1	1	10	-	2
Wound	6	-	2	6	-	2
TOTAL	43	3	5	43	1	8

Figure 1a. *P. aeruginosa* isolates sensitive to meropenem and imipenem with AP1 primer were determined in AP-PCR

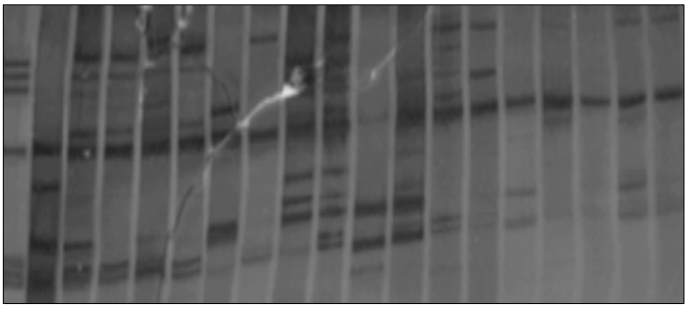
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 M



M: molecular size indicator [ÖX174 *HaeIII* cut (Fermentas, Lituanya) genotypic proximity was determined in number 3,4 and 5 *P. aeruginosa* isolates

Figure 1b. *P. aeruginosa* isolates sensitive to meropenem and imipenem with AP1 primer were determined in AP-PCR

M 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36



M: molecular size indicator [ÖX174 *HaeIII* cut (Fermentas, Lituanya)]

DISCUSSION

More than one antibiotic are used in treatment of infections coused by *P. aeruginosa* Especially in treatment, aminoglycosides, cephalosporins, carbapenems and betalactam/betalactamases inhibitored antipseudomonal penicillin combinations are preferred for the reason of ease-of-use (14). For this reason, in accordance with antibiotic sensitivity results, 43 *P. aeruginosa* isolate determined as sensitive to carbapenems was chosen in research. Sensitivities to imipenem and meropenem from carbapenem antibiotics are determined as % 84. As a result of AP-PCR molecular typing study of this 43 *P. aeruginosa* isolate, no correlation was found out between antibiotic sensitivities and molecular types. In the result of the study, imipenem and meropenem usage can be recommended in *P.aeruginosa* infection treatment. Because, carbapenems used in treatment are separated than other betalactam antibiotics with their wider influence spectrum and strong antibacterial influences. Furthermore, carbapenems are more stabile to plasmid and also chromosomal beta lactamases.

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IN VITRO PHENOTYPIC ANTIBIOTIC SUSCEPTIBILITY PROFILES OF FOOD INDICATOR BACTERIA ISOLATED FROM HOME-MADE ORAL REHYDRATION SOLUTIONS IN NIGERIA

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ABSTRACT

One thousand and ten bacterial isolates from ORS constituents characterised as *Bacillus cereus* var. *mycoides*, *Bacillus subtilis*, *Citrobacter* sp., *Clostridium perfringes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar* Typhi, *Salmonella enterica serovar* Typhimurium, *Shigella dysentariae*, *Staphylococcus aureus* and *Vibrio cholerae* were screened for their *in vitro* antibiotic susceptibility profiles using the agar discs and agar well-diffusion methods. The Gram-negative bacteria from granulated sugar samples had 7.69% phenotypic resistance profiles while the Gram-negative bacteria from table salt samples had between 13.3% and 20.0% resistance profiles. The resistance profiles of Gram-positive bacteria from granulated sugar samples was between 8.0% and 19.0% while the Gram-positive bacteria from table salt samples had between 11.0% and 27.9 % resistance profiles towards the test antibiotic (discs). The bacterial isolates from granulated sugar exhibited resistance of between 36.4% in ampicillin + cloxacillin and 64.9% in metronidazole. while the bacterial isolates from table salt gave an overall resistance of 41.0% - 64.7% towards the twenty-eight test oral paediatric antibiotic suspensions All the bacterial isolates from the table salt and granulated sugar samples displayed multiple resistance to the test paediatric antibiotics, except *Ps. aeruginosa* SA12, *Shigella dysenteriae* SA16C, SA16D, *E. aerogenes* SA18A, SA18AE and *E. coli* SA22A which recorded no (0.0%) resistance to all the test paediatric antibiotics.

KEYWORDS*: antibiotics,* **in vitro***, ORS, paediatric, resistance, susceptibility,*

INTRODUCTION

In the living conditions prevalent in the less developed world, characterised by a lack of potable water, sanitation, and refrigeration, the bacteria and other pathogens that cause diarrhoeal diseases are easily transmitted to young children by contaminated water, hands, and food. As a result, infants in less developed countries suffer on the average six to eight separate episodes of diarrhoeal disease per child (1). In an extremely conservative estimate, investigators calculated that there are at least 750 million to 1 billion episodes of diarrhoea and 4.6 million deaths each year due to diarrhoea in children less than 5 years of age in Africa, Asia (excluding the People's Republic of China), and Latin America (2).

Dehydration is the most important complication of infantile diarrhoea, and if untreated it can lead to death (3). Irrespective of the specific infectious agent causing diarrhoea, the treatment of diarrhoeal dehydration is the same, and involves the replacement of body water and electrolytes (salts in solution). A simple, efficacious, technologically appropriate alternative to intravenous dehydration has become available namely, oral dehydration therapy (ORT) using sugar/ electrolyte solutions. An application of ORT involves its use early in the

course of infant diarrhoea to prevent dehydration (4). It is not possible economically or logistically however to provide a packet of balanced sugar/electrolyte powder to treat every episode of diarrhoea in all young children in developing countries, for that reason, some observers have advocated the use of simple dehydration solutions of table salt and sugar, prepared and administered in the home (5). Several methods have been devised for the preparation of simple sugar/salt solutions that are safe and can be prepared in the home, while the ingredients used in oral dehydration solutions are widely available and easily transported (6)(7). The primary focus of this study therefore, is on the clinical significance of antibiotics as discs and oral paediatric suspensions on bacterial isolates obtained from home-made ORS constituents.

MATERIALS AND METHODS

Samples' collection: Five hundred granulated sugar and three hundred and seventy table salt samples obtained from the Federal capital territory, Abuja; five southwestern states- Lagos, Ogun, Oyo, Osun, Ekiti and Kogi state (a middle belt state) of Nigeria between February, 2001 and December, 2004 were microbially analysed in the laboratory to determine their microbial contents.

Isolation of the microbial flora of the samples: The overnight broth culture (1 ml) of each table salt and granulated sugar samples in alkaline peptone water (pH 8.6) was transferred into sterile plates by plating decimal dilutions of each sample in triplicates, and molten (45^oC) nutrient agar (NA; LAB M), thiosulphate citrate bile sucrose (TCBS; Oxoid) agar, pH 8.2; mannitol salt agar (MSA; LAB M), MacConkey agar (Oxoid), (LAB M) at pH 7.4, cystein lactose electrolyte deficient (CLED; LAB M) and Sabouraud dextrose agar (SDA; LAB M) were aseptically added to the plates and incubated between 24-48 hours at 35^oC for bacterial isolation and at 25^0C for fungal isolation (8). The population, in colony-forming units (CFU), and the characteristics of the colonies were recorded for each medium Purification and preservations of the isolates: Representatives of each different bacterial colony types were randomly picked from the primary plates of each sample and sub-cultured onto sterile plates by the streaking method. The isolates were then sub-cultured by repeated streaking to obtain pure cultures. All the bacterial isolates were kept at 4^0C in triplicates, on Brain Heart Infusion (BHI) agar slants as working and stock cultures.

Characterisation and identification of the isolates: Taxonomic studies were carried out on the purified isolates from the differently analysed samples on the basis of their cultural, morphological, biochemical and physiological characteristics, Tentative identification of the bacterial species was based on the conventional standard phenotypic taxonomic identification characteristics of the strains while the general key used for the identification was by reference to Kloos & Schleifer (9) and Bergey's Manual of Systematic Bacteriology (10).

Antibiotic susceptibility determination-

Agar disc diffusion method: Seeded Mueller-Hinton agar plates were left for about 15 minutes before aseptically placing the antibiotic discs [amoxicillin (25µg), augmentin (30µg), cotrimoxazole (25µg), nitrofurantoin (300µg), gentamicin (10µg), nalidixic acid (30µg) and tetracycline (30µg) for the Gram-negative bacteria; and ampicillin (10µg), chloramphenicol (30µg), cloxacillin (5µg), erythromycin (5µg), gentamicin (10µg), penicillin (15µg), streptomycin (10mg) and tetracycline (10µg)] on the agar surfaces and incubating the plates aerobically at 37^0 C for 18-24hr. Zones of inhibition and the diameter of the

zones were measured and recorded in millimeter diameter.

Agar well-diffusion method: Antibiotic susceptibility determination of various paediatric antibiotic suspensions (ampicillin/ampicillin-cloxacillin, cotrimoxazole, metronidazole, chloramphenicol, cephalexin and erythromycin) was carried out on the bacterial isolates using the modified agar disc and agar well-diffusion methods. Seeded Mueller-Hinton agar plates for bacterial isolates were prepared by transferring 500µl culture broth of each bacterium unto the agar plates followed by surface streaking of the entire agar surface with sterile wire loop. The seeded agar plates were then left for about 15 minutes before aseptically placing the antibiotic discs onto the agar surfaces and incubating the plates at 35^0C for 18-24 h. Zones of inhibition were measured and recorded in millimeter diameter according to the methods of NCCLS (11) and the modified method of Ogunshe (12) in which sterile semi-solid agar was added to the paediatric antibiotic suspensions to avoid spillage on the agar surface during the agar well-diffusion method.

RESULTS

One thousand and ten bacterial isolates characterised as *Bacillus cereus* var. *mycoides*, *Bacillus subtilis*, *Citrobacter* sp., *Clostridium perfringes*, *Enterobacter aerogenes*, *Escherichia coli*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *P. vulgaris*, *Pseudomonas aeruginosa*, *Salmonella enterica serovar* Typhi, *Salmonella enterica serovar* Typhimurium, *Shigella dysentariae*, *Staphylococcus aureus* and *Vibrio cholerae* obtained from retailed table salt and granulated sugar samples were screened *in vitro* for their phenotypic antibiotic susceptibility/resistance profiles.

Apart from *Vibrio cholerae* SA12A which displayed 100.0% resistance to all the test antibiotics, a very high phenotypic susceptibility profiles to the test antibiotics were recorded among the Gram-negative bacteria from retailed table salt samples. However, out of the 8 test antibiotics (discs) assayed for in this study, antibiotic resistance of 7.69 % was displayed by all the Gram-negative bacteria isolated from retailed sugar samples towards all the test antibiotics while antibiotic resistance of 13.3 % was exhibited by the Gram-negative bacteria against augmentin, cotrimoxazole, nalidixic acid, nitrofurantoin, ofloxacillin, tetracycline and 20.0 % against amoxicillin and gentamicin (Table 1).

The antibiotic resistance recorded among the Gram-positive bacteria from retailed granulated sugar samples were gentamicin (8.0 %), chloramphenicol (10.0 %), erythromycin (13.6 %), streptomycin (15.4 %), ampicillin, cloxacillin, penicillin (16.7 %) and tetracycline (19.0 %). The Gram-positive bacteria from retailed table salt samples had resistance profiles of tetracycline (11.0 %), chloramphenicol (12.6 %), streptomycin (17.0 %), ampicillin (18.7 %), cloxacillin (20.7 %), erythromycin (23.2 %), penicillin (25.7 %) and gentamicin (27.9 %) respectively (Table 2).

The *in vitro* results of the antibiotic susceptibility patterns of the bacterial isolates from the table salt samples against the twenty-eight oral paediatric antibiotic suspensions gave resistance profiles of 36.4 % in ampicillin+ cloxacillin, 38.3 % in erythromycin + ethylsuccinate, chloramphenicol (40.2 %), cotrimoxazole (42.8 %), sulfamethoxazole + trimethoprim (42.9 %), cephalexin (44.4 %), erythromycin (49.5 %), ampicillin (51.9 %), amoxicillin (54.2 %) and 64.9% in metronidazole.. All the bacterial isolates displayed multiple resistance to the test antibiotics as shown in Table 2.

The *in vitro* antibiotic susceptibility patterns of the bacterial isolates from the granulated sugar samples exhibited mono and multi resistance to the twenty eight oral paediatric antibiotic suspensions (Tables 2 & 3).

The overall antibiotic resistance exhibited by the Gram-positive bacterial isolates from granulated sugar samples exhibited resistance profiles of 49.7 % in ampicillin, 50.6 % in cotrimoxazole, 52.1 % antibiotics. in cephalexin, 54.3 % in sulfamethoxazole + trimethoprim, 63.5 % in metronidazole and 64.7 % in chloramphenicol. All the bacterial isolates from the table salt samples exhibited mono and multiple antibiotic resistance (MAR) to all the test paediatric antibiotics, except *Ps. aeruginosa* SA12, *Shigella dysenteriae* SA16C, SA16D, *E. aerogenes* SA18A, SA18AE and *E. coli* SA22A which recorded 100.0 % susceptibility to all the test paediatric

Table 1: The antibiotic resistance profiles (antibiotic discs) of Gram-negative bacterial isolates from retailed granulated sugar and table salt samples

		Antibiotic resistance	
Antibiotic	Conc.	Sugar	Salt
Amoxicillin	25µg	7.69 %	20.0 %
Augmentin	30µg	7.69 %	13.3 %
Cotrimoxazole	25µg	7.69 %	13.3 %
Gentamicin	10µg	7.69 %	20.0 %
Nalidixic acid	30µg	7.69 %	13.3 %
Nitrofurantoin	30µg	7.69 %	13.3 %
Ofloxacillin		7.69 %	13.3 %
Tetracycline	30µg	7.69 %	13.3 %

Table 2: The antibiotic resistance profiles (antibiotic discs) of Gram- positive bacterial isolates from retailed granulated sugar and table salt samples

		Antibiotic resistance	
Antibiotic	Conc.	Sugar	Salt
Chloramphenicol 30µg	10.0 %	12.6 %	
Erythromycin	5µg	13.6 %	23.2 %
Gentamicin	10µg	8.0 %	27.9 %
Streptomycin	10mg	15.4 %	17.0 %
Tetracycline	10µg	19.0 %	11.0 %
Ampicillin	10µg	16.7 %	18.7 %
Cloxacillin	5µg	16.7 %	20.7 %
Penicillin	15µg	16.7 %	25.7 %

Table 3: The antibiotic resistance profiles (Oral paediatric suspensions) of Gram-negative bacterial isolates from retailed granulated sugar and table salt samples

Antibiotics	Antibiotic resistance	
	Sugar	Salt
Ampicillin + cloxacillin	36.4 %	32.8 %
Erythromycin + ethylsuccinate	38.3 %	41.3 %
Chloramphenicol	40.2 %	64.7 %
Cotrimoxazole	42.8 %	50.6 %
Sulfamethoxazole + trimethoprim	42.9 %	54.3 %
Cephalexin	44.4 %	52.1 %
Erythromycin	49.5 %	43.2 %
Ampicillin	51.9 %	49.7 %
Amoxicillin	54.2 %	41.0 %
Metronidazole	64.9 %	63.5 %

DISCUSSION

Fluid replacement therapy that is widely used in medicine in prevention or treatment of dehydration, or as an intravenous therapy to prevent hypovolemic shock, and this may be the reason for table salt being one of the compositions of home-made ORS in addition to sucrose sugar. The significance of ORS is to alleviate morbidity and mortality through fluid loss during gastroenteritis/diarrhoea (13), however, the finding of Ogunshe *et al*. (14) indicates that home-made ORS may serve as means of transmitting gastroenteritis/diarrhoeal and other infectious microbial agents due to the high recovery rates of the indicator bacteria from retailed table salt and granulated sugar samples, more especially from home-made ORS solutions. These bacteria have however, been previously implicated in clinical cases (15)(16)(17)(18).

Though the magnitude of the problem may vary from place to place, the problem of antibiotic resistance is probably amplified in tropical developing countries where infectious conditions account for a substantial percentage of hospital consultations. Several workers in the country and elsewhere have highlighted the problem of antibiotic resistance (19)(20). The bacterial pathogens in this study were assayed for their susceptibility to eight (µg/disc) commonly used antibiotics incorporated in multi-discs, and it was noted that the phenotypic antibiotic resistance profiles of the bacterial isolates used in this study were relatively low except among the Gram-positive bacterial isolates. The results obtained in this study indicated that the Gram-negative bacterial isolates were highly susceptible to the test antibiotics (discs), which is in conformity with the reports of some earlier workers such as Dax (21)(22), but contrary to the previous findings of Ryan *et al*. (23), Chopra *et al*. (24) and Hlavka *et al*. (25) who had all earlier reported a high resistance to same antibiotics by certain clinical bacterial isolates. The high prevalence of susceptibility of the bacterial isolates to nalidixic acid in this study may be attributable to similar finding which stated that nalidixic acid displays good activity against certain Gram-negative pathogens but that a wide variety of bacteria that are resistant to quinolones have been selected from laboratory strains or have been obtained from clinical isolates (22). It could therefore be inferred that the high level susceptibility to nalidixic acid by the bacterial isolates from the ORS constituents (table salt and sucrose sugar) in this study was because the pathogens were non-clinical isolates. Koneman *et al*. (26) reported that aminoglycoside antibiotics,

such as streptomycin and gentamicin, are bactericidal and tend to be most active against Gram-negative pathogens. This may account for the low resistance among the Gram-negative bacterial isolates but very high resistance observed among the gram-positive bacterial isolates in this study.

Examples of penicillins according to Dax (22) are ampicillin; amoxicillin etc., while amoxyillin with clavulanic acid is termed augmentin. The fact that a lower antibiotic resistance was recorded in ampicillin in this study however confirms the earlier findings of Neu (27) that amplicillin is the most recognized of the aminopenicillins and remains a valuable and widely prescribed chemotherapeutic agent as well as being markedly more active against a host of Gram-negative bacilli. Amoxicillin has been claimed by Dax (21) to be essentially comparable to ampicillin in terms of *in vitro* potency. The results obtained in this present study, in which low antibiotic resistance was displayed against amoxicillin by the Gram-positive and Gram-negative bacterial isolates confirms the earlier report of Dax (21).

Schwan & Ebetino (28) earlier reported that the nitrofurans such as nitrofurantoin are medium-spectrum antibacterial agents, which are potent against a variety of Gram-positive and Gram-negative bacteria, assuming that sufficient concentrations are achieved at the site of infection and that resistance is not a problem with the use of these agents. Similarly, Brooks *et al*. (29) supported the potency of gentamicin in a large percentage of bacterial isolates. Having recorded very low resistance to nitrofurantoin in this study supports the earlier findings of Schwan & Ebetino (28) and Brooks *et al*. (29), however, a very high resistance to gentamicin by the Gram-positive bacterial isolates in this study disagrees with the earlier findings of Schwan & Ebetino (28) and Brooks *et al*. (29).

Several antimicrobial agents have become available for use in newborns and children with suspected or proven bacterial infections, but the most commonly employed method for antibiotic susceptibility screening is usually the agar disc-diffusion method, using antibiotic discs. Clinically, infantile antibiotic prescriptions are generally made based on the antibiotic (discs) reports; meanwhile, paediatric antibiotic suspensions are usually administered on infants and children. No documented reports on the antibiotic susceptibility patterns of bacterial isolates using paediatric suspensions was obtained except for those of Ogunshe (12) and the results obtained in this study are in accordance with their earlier findings which indicated that infantile gastroenteritic and non-gastroenteritic bacterial isolates were more resistant to paediatric oral suspensions, especially amoxicillin, ampicillin, co-trimoxazole, chloramphenicol, sulfamethazole + trimethroprim and metronidazole. In this study, the *in vitro* results of the antibiotic susceptibility of the bacterial isolates to various paediatric oral suspensions gave a relatively higher percentage resistance than those of antibiotic discs. These findings indicate that the bacterial isolates were more resistant to the paediatric oral suspensions. Recording as high as 49.5% - 64.9% resistance in paediatric antibiotics such as amoxicillin, ampicillin, chloramphenicol, co-trimoxazole, erythromycin, metronidazole and sulfamethazole+trimethroprim shows the health implications that these commonly prescribed and consumed antibiotics may pose in paediatric infectious conditions, especially since it has already been reported that antibiotic resistance is a world-wide problem that is also prevalent in Nigeria (30)(31)(32). Although percentage resistance of other classes of paediatric antibiotics

such as ampicillin and erythromycin+ethylsuccinate suspensions were relatively lower, the percentage resistance is still higher than expected in paediatric chemotherapy having found widespread use particularly as children suspensions.

All the isolated bacteria species isolated from retailed table salts and granulated sugar samples in this study exhibited mono and multi resistance to all the test antibiotic discs and paediatric antibiotic suspensions which indicate the unwholesomeness and clinical implication of the samples especially as constituents of ORS in infantile therapy. This study has shown a worsening trend in the antibiotic resistance profiles of the bacterial isolates. From the overall results of the antibiotic resistance profiles obtained in this study, it is therefore an established fact that the onset of drug resistance threatens virtually all classes of antibacterial agents as well as confirming the predictions of a worsening antibiotic resistance situation especially in paediatric chemotherapy which has become an accepted medical practice. The emergence of antimicrobial-resistant bacterial pathogens has become a major public health concern thus; the use of antimicrobials in any area including disease treatment can potentially lead to widespread dissemination of antimicrobial-resistant bacteria **(33)([34)(35)(36)(37)(38), more especially if such antimicrobial-resistant bacteria are from food sources such as granulated sugar and table salt samples**

All the bacteria species isolated from the table salt samples were mono- or multi-resistant to the test antibiotics and the danger in this phenomenon is that the multi-resistance determinants can be transferred to new bacterial hosts. The situation is made more difficult in developing countries such as Nigeria where antimicrobial drugs are readily available to consumers across the counter with or without prescriptions from medical practitioners. Such a practice can lead to misuse of the antimicrobial drugs with the associated high prevalence of drug resistance among the implicated bacterial isolates.

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RUBELLA IgG ANTIBODY IN WOMEN OF CHILD-BEARING AGE IN OYO STATE.

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ABSTRACT

230 females of childbearing age from four different towns in Oyo State were screened for rubella specific IgG antibody using a sandwich ELISA test kit. Rubella IgG was detected in 215 (93.5%) of the females. 96% of the non-pregnant, 87.5% of the primigravida and 76% of the multigravida screened positive to the antibody. Ogbomoso, Ibadan, Oyo and Iseyin respectively had 96.1%, 94.2%, 90.5% and 88.2% of their samples positive for the rubella IgG antibody in them. It is imperative for the government to ensure that rubella vaccine is made available and routine so as to avert the risk of congenital rubella syndrome.

INTRODUCTION

Rubella is a universally endemic mild febrile disease difficult to diagnose clinically because of its mild symptoms and similarity to other diseases such as measles, scarlatina, infectious erythema, infectious mononucleosis and drug rash. Though of a worldwide distribution, clinically recognized disease occurs less frequently in tropical regions than in temperate zones (*1)*. Humans are the only host of the virus and infection is by contact with nasopharyngeal secretions of infected persons, droplets spread or direct contact with patients.

Gregg (2) discovered the teratogenic nature and the possibility of vertical transmission of rubella. When infection is in the first trimester, it is potent to produce a range of permanent congenital defects like deafness, mental retardation, cardiac abnormalities, and congenital cataracts (3). These conditions are collectively known as congenital rubella syndrome (CRS). Diabetes, thyroid disease and progressive rubella panencephalitis have been reported as late effects of CRS (4).

Incidence rates of rubella was reportedly high in children 5-9 years of age in 1969 (5) while in recent times, it has shifted to young adults and adolescents (6). Rubella surveillance based on clinical reports is not specific because of its mild nature and relatedness to some other viral infections (7). So, it is better done during rubella epidemic. Studies have been tending towards using other body fluids apart from sera for the screening. Laboratory diagnosis using saliva (*7*,), urine (*8*) and low avidity IgG and IgA tests (9) have been found to be reliable for the diagnosis of rubella infection.

MATERIALS AND METHOD

(a) Study Population

Two hundred and thirty females of childbearing age consisting of 169 married and 61 non-married women from Ibadan, Oyo, Iseyin and Ogbomoso in Oyo State had their sera screened for rubella IgG antibody. 2ml venous blood samples were collected from each of the woman after ethical approval and

informed consents were obtained. The sera were frozen at -20^0 C until analyzed.

(b) Method

Rubella Specific IgG antibody in serum was detected with an enzyme linked immunosorbent assay (ELISA) kit (Human Gasellschaft for Biochemica and Diagnosdtica, mbh, Germany). The kit is a sandwich enzyme immunoassay designed to be used on serum samples of 100µl added to microtiter strip wells as a solid phase coated with purified rubella virus antigen (RVAg). Microplate incubation was at 37^0 C for 30 minutes, followed by washing, incubation for an additional 30 minutes with 100µl anti IgG conjugate, washing and development with substrate tetramethyl benzidine (TMB) for 15 minutes. The reaction was then stopped and the results read within 30 minutes.

RESULTS

215 (95.5%) of the females screened had detectable rubella specific IgG while 15 (6.5%) did not. In terms of age distribution, all the 11 females of ages 40-45 years screened positive to the rubella IgG. This is closely followed by ages 35 – 39 years where 28 (91.3%) of the 69 samples from females of ages 25 – 29 years were seropositive to the rubella IgG (Table 1). Ibadan, Ogbomoso, Oyo and Iseyin were used for the study. Table 2 shows the prevalence of rubella in the towns of study.

Of significance to this study are the 3 (4.9%) out of 61 spinsters screened, 9 (6.8%) out of 133 pregnant women and 4 (4.12%) out of 97 non- pregnant women who had no detectable rubella IgG in them.

Table 1: Age Distribution of rubella IgG antibody

Age (Years)	Total Sample	Number –ve (%)	Number +ve (%)
15-19	13	1 (7.7)	12 (92.3)
20-24	48	2 (4.2)	46 (95.8)
25-29	69	6 (8.7)	63 (91.3)
30-34	60	5 (8.3)	55 (91.7)
35-39	29	1 (3.4)	28 (96.6)
40-45	11	0 (0)	11 (100)
Total	230		

Table 2: Rubella IgG antibody Distribution in the study Towns

Towns	Total Sample	Number –ve (%)	Number +ve (%)
Ibadan	120	7(5.8)	113 (94.2)
Ogbomoso	51	2 (3.9)	49 (96.1)
Oyo	42	4 (9.5)	38 (90.5)
Iseyin	17	2(11.8)	15 (88.2)

DISCUSSION

The role of antibody prevalence surveys in immunization programme development and refinement is now being generally appreciated. Such surveys are important for identifying target age groups for measles vaccination (10), providing data on the burden of disease from congenital rubella syndrome (11) and monitoring their respective control programmes. It has been a source of motivation and challenge for both the government and biomedical scientists to work towards prevention rather than curing certain viral infections.

The absolute risk of CRS among children born to mothers infected during pregnancy varies widely in different studies. Miller *et al* (12) found the risk of congenital infection to be 81% and that of malformation 69% after confirmed maternal rubella in the first trimester.

The seropositivity of 93.5% of the female subjects screened is more likely to be due to their exposure to the virus and reinfection rather than rubella vaccination as all the women screened knew nothing about rubella vaccine probably because it is not included in the routine immunization programme in Nigeria. There is an increase in the percentage of women with detectable rubella IgG antibody when the results of this study are compared with what Odelola (13) obtained where 70% of the women screened were seropositive for the rubella IgG antibody. This is likely due to increase in population and overcrowding.

If the risk of congenital rubella infection is 81% and that of malformation is 69% (*10*), it implies that there is a high risk of CRS among the 4.9% negative spinsters, 6.8% pregnant women and 4.12% non pregnant women. The same is true of the multigravids who are still in their active reproductive stages with their ages ranging from 25 – 34 years if they are infected in the first trimester of the next pregnancy. Also, the pregnant seronegative women who are mostly in their second trimester stand a 20% chance of infecting their fetuses with rubella if infected. The increasing detection of rubella IgG antibody in married women suggests that rubella virus is still in circulation in Oyo state. Considering the potential dangers of CRS, it is important that rubella vaccine be included in the routine immunization programme of the country as it confers a lifelong protection against the virus in women and children who are the ones really affected.

As at 2002 when this work was done, the available data about the prevalence of rubella IgG in Nigeria were (13, 14, 15) all revealed that the virus was circulating then but now with this study, there is an increased seropositivity. This may be due to increasing population, poor hygiene, poverty and overcrowding. It is hoped that the virus will not mutate some day to become more virulent than it is today. If this happens, children and females will suffer for it. To avoid this, it is necessary to concentrate on sparsely populated areas where the dwellers are more seronegative than in densely populated areas and schoolgirls for routine rubella vaccination. The vaccine should also be made available and mandatory for all pregnant women in their antenatal clinics. Coupling strengthened immunization programme with regular surveillance and sensitization of the women about the virus will go a long way to help curtail the risk of CRS.

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THE PATTERN OF THE FREQUENCY OF HBSAG, HBEAG, ANTI-HCV AND ANTI-HBE IN PATIENTS WITH HAEMOGLOBIN GENOTYPE HBSS AND HBSC IN A RURAL COMMUNITY.

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ABSTRACT

Sixty HbSS sickle cell anaemic patients aged 17.45 ±10.1years (Female=30, Male=30) and sixty HbSC sickle cell disease patients aged 20.6±11.0years(Female=30,Male=30) were recruited for the investigation. Haemoglobin genotype of each of the patient was determined by electrophoresis. HepatitisB's' antigen, HBeAg,anti-HBe, and anti-HCV in patients' plasma were determined by Enzyme Immunoassay.

The frequencies of HBsAg, anti-HBe, HBeAg +HBsAg, HBsAg + antiHBe, in HbSS(6.7% , 20%,13.3%, and 20% respectively) were higher than those of HbSC(5% ,8.3%, 5% , and 3.3% respectively). The frequency of anti-HCV + anti-HBe in HbSC was higher compared with that of HbSS patients (3.3% Vs 0%).The frequency of HBeAg in female HbSS and HbSC patients was higher than their male counterparts.(HbSS:16.7%Vs 10%;HbSC:6.7% Vs 3.3%).Higher frequency of HBsAg was found in HbSS male patients than the females (26.7% Vs 13.3%).The frequency of anti-HBe in HbSS male patients and HbSC female patients was higher than those of HbSS female patients and HbSC male patients respectively(HbSS:10% Vs 3.3%; HbSC: 10% Vs 6.7%).The frequency of HBeAg+ HBsAg obtained in HbSS male patients and HbSC female patients was higher than the results obtained from HbSS female patients and HbSC male patients (HbSS: 16.7% Vs 10%; HbSC:6.7% Vs 3.3%).The frequency of HBsAg + anti-HBe in HbSS female patients was higher than in HbSS male patients.(23.3% Vs 16.7%).None of the patients plasma was found to contain both HBeAg + anti-HBe.

This research work has therefore been used to examine the pattern of HBeAg, HBsAg, anti-HCV, and anti-HBe in the plasma of patients with haemoglobin genotype HbSS and HbSC in rural community.

KEYWORDS: Pattern, Frequency, HepatitisB, Hepatitis C, Antibody, Surface ('s') and Envelope ('e') antigens.

INTRODUCTION

Clinically significant abnormal hemoglobin that can be found in tropical countries include HbS, HbC, HbD and HbE (1, 2, 3)

Haemoglobin S is formed when valine replaces glutamic acid in the β globin chain (6th amino acid osition) of haemoglobin. In deoxygenated state HbS has poor solubility forming polymers in red cells. Polymerization leads to changes in the red cell membrane and metabolism, causing the cells to become rigid and distorted with a sickle cell shape. (1, 3). The sickle cells adhere to vascular endothelium and to one another blocking small blood vessels. They become trapped in the cell spleen and haemolyse easily (3).

The term sickle cell disease is used to describe sickle cell anaemia (HbSS) and the range of HbS related disorders of similar clinical severity e.g. when HbS is inherited with HbC, HbD or HbO. Sickle cell can also occur when HbS is inherited with β^o thalassemia gene.

Haemoglobin C is found in West Africa. It is formed when glutamic acid is replaced by lysine in the β globin chain. Homozygous inheritance causes mild haemolytic anemia and splenomegaly. (3).

When HbC is inherited with HbS (HbS+C), the condition can cause symptoms similar to, but less severe than sickle cell anaemia (4).

Splenomegaly is more common in HbSS than HbSC disease, in some patients it causes only mild haemolytic anemia. (3).

The clinical features of sickle cell disease, which include sickle cell anaemia (HbSS) and HbS related disorders such as HbSC are:- haemolytic anaemia, jaundice, fever, painful swelling of hands and feet skeletal changes, painful infarcts, pulmonary complications, kidney damage, leg ulcers, increased risk of salmonella and pneumococcal infection, visual impairment, immune function impairment, gallstones, haemolytic and aplastic anemia. (3).

Hepatitis "e" antigen or HbeAg is known to be intimately associated with Hepatitis B virus - HBV replication and the presence of infectious Dane particles in the blood. It appears soon after HbsAg and persists for a short time, disappearing when recovery being. Persistence of e antigen indicates chronic liver disease. (3, 4).

Antibody to HbeAg (anti-HBe) may be found in the convalescence stage and often in chronic hepatitis and the carrier states (5).

The detection of HbsAg in the serum indicates an infection caused by the hepatitis B virus. It is the first marker to appear and may be observed 2 to 3 weeks before the clinical and biological symptoms of the disease (5, 6). Hepatitis B surface antigen (HbsAg) persisting beyond 6 months in serum denotes chronic hepatitis. (3, 6, 7). Hepatitis (virus of HCV is an envelope RNA virus. Antibodies directed to the major immunodominant determinants of the viral proteins are detected in patients infected with HCV early in the course of infection (8, 9) The diagnosis of hepatitis C infection is usually made serologically by detecting anti-HCV IgG in the serum. (5, 8, 9). Most patients infected with HCV become carriers. Antibody is detectable 6-8weeks after infection (8, 9).

This present research work was therefore designed to examine the serological pattern of anti-HCV, HBsAg, HBeAg and anti-HBe in patients with HbSS and HbSC in rural community.

MATERIALS AND METHODS

Participants: - Sixty HbSS sickle cell anaemic patients (Female == 30, Male = 30) aged 17.45 ± 10.1years and sixty participants with HbSC genotype (female = 30, male = 30) aged 20.6 ± 11.0 years were recruited from three Local Governments; Saki-West, Saki-East and Atisbo) in Oke-Ogun, the Northern part of Oyo State, Nigeria for the study.

MATERIALS

Sample:- Five milliliters of blood was collected from each of the participants and preserved in NaEDTA anticoagulant bottles. The plasma was extracted from each of the blood samples for the senelogical studies while the red cells was used for haemoglobin genotype.

METHODS:-

(a) Haemoglobin genotyping was carried out by electrophoresis described by Cheesbrough[5] (2002).

(b) Hepatitis C antibody detection in plasma/serum was carried out by Enzyme immunoassay for the determination of antibodies to Hepatitis C virus in serum and plasma using reagent kit of DIA. PRO, Diagnostic Bioprobes Srl Via Columella n° 31, 20128 Milano, Italy. E-mail diapro@tin.it

(c) Hepatitis B 'e'(envelope) antigen and antibody in the plasma was detected by Enzyme immunoassay using the reagent kit

of DIA.PRO, Diagnostic Bioprobes Srl via Columella n°31,20128 Milano, Italy. E-mail-diapro@tin.it

(d) Hepatitis B surface ('S') antigen detection in the plasma of the patients was carried out by Enzyme immunoassay Technique using MONOLISA AgHBs Plus reagent kit of BIO-RAD, 3, Bd Raymond Poincare 92430 MARNES LA COQUETlE.

RESULTS

TABLES 1A & 1B

The two tables present the serological pattern of HBeAg, HBsAg, anti-HCV and anti-HBe in the sera of HbSS and HbSC patients. The frequency of anti-HCV and anti-HbeAg found in HbSS and HbSC patients was the same. However higher frequency of anti-HBe and HBsAg was found in HbSS than HbSC patients.

None of the HbSS and HbSC patients was found to have both HBeAg and anti-HBe in a serum. Both HBeAg and HBsAg were detected in a single serum of some patients with the frequency of this observation higher in HbSS patients than in HbSC participants. . Two HbSC patients presented with the presence of both anti-HCV and anti-HBe in their respective serum and this was not found in the HbSS patients' sera.

Table 1a: Pattern of HBeAg, HBsAg, anit-HCV, and anti-HBe in the sera of HbSS and HbSC patients.

Hb Genotype	Anti-HCV	Anti-HBe	HBeAg	HBsAg
HbSS n = 60	2 (3.3%)	4 (6.7%)	8 (13.3%)	12(20%)
HbSC n = 60	2 (3.3%)	3 (5%)	8 (13.3%)	5 (8.3%)

Table 1b

Hb Genotype	**HBeAg + HBsAg**	**HBsAg + Anti-HBe**	**HBeAg + anti-HBe**	**Anti-HCV + any of HBsAg, HBeAg, and anti-HBe**
HbSS n= 60	8 (13.3%)	12 (20%)	-	0 (0%)
HbSC n = 60	3 (.5%)	2 (3.3%)	-	Anti-HCV + anti-HBe 2(3.3%)

Table-2a: Gender serological pattern of Anti-HCV, HBeAg HBsAg and anti-HBe in HbSS and HbSC patients.

Gender	Hb Genotype	Anti-HCV	HBeAg	HBsAg	Anti-HBe
Male	HbSS	1 (3.3%)	3 (10%)	8 (26.7%)	3 (10%)
	HbSC	1 (3.3%)	1 (3.3%)	4 (13.3%)	2 (6.7%)
Female	HbSS	1 (3.3%)	5 (16.7%)	4 (13.3%)	1. 3.3%)
	HbSC	1 (3.3%)	2 (6.7%)	4 (13.3%)	3 (10%)

Table -2b: Gender serological pattern of HbeAg + HbsAg, HbsAg + anti-HBe, HBeAg + anti-HBe, and anti-HCV + any of HBsAg, HBeAg, and antiHBe in HbSS and HbSC patients.

Gender	Hb Genotype	HBeAg + HBsAg	HBsAg + anti-HBe	HBeAg + anti- HBe	Anti-HCV + any of HbsAg, HbeAg Anti-HBe
Male n = 30	HbSS HbSC	5 (16.7%) 1 (3.3%)	5 (16.7%) 1 (3.3%)	- -	0 (0%) Anti-HCV + Anti-HBe 1 (3.3%)
Female n = 30	HbSS HbSC	3 (10%) 2 (6.7%)	7 (23.3%) 1(3.3%)	- -	0 (0%) Anti-HCV + Anti-HBe 1(3.3%)

TABLES 2A AND 2B

These tables present the result of serological pattern by gender of anti-HCV, HBeAg, HBsAg and anti-HBe in HbSS and HbSC patients. The frequency of anti-HCV in HbSS and HbSC male and female patients was the same but a higher frequency of HbeAg was found in HbSS and HbSC female patients than their male counterparts. Higher frequency of HBsAg was found in HbSS male patients than HbSS female patients. The same frequency of HBsAg was found in HbSC male and female patients. Male patients with haemoglobin genotype of SS have higher frequency of anti-HBe in their sera than their female counterparts. Higher frequency of anti-HBe was also found in HbSC female patients than their male counter parts.

Higher frequency of the presence of HBeAg and HBsAg in a single serum was found in HbSS males than HbSS females and in HbSC females than HbSC males. The frequency of the presence of both HBsAg and anti-HBe in a serum was higher in HbSS females than HbSS males but the same frequency was observed in male and female HbSC patients. Anti-HCV and antiHBe were found together in a single serum of on each of HbSC male and female.

DISCUSSION

Higher frequency of anti-HBe found in patients with Hbgenotype HbSS than HbSC may be associated with the higher incidence of hepatitis B infection in HbSS than HbSC due to higher frequency of anaemia in HbSS than HbSC a contributive factor to the possibilities of hepatitis B viral infection through transfusion of infected blood. (3, 5, 6, 7). The presence of this antibody indicates past hepatitis B infection and probably chronic, carrier or convalescence state. (3,5,6,7). This supports the findings of this recent study that some patients with HbSS and HbSC has persistence of HbsAg (though more in the former) which indicates chronic hepatitis B infection in these patients as evidenced by the presence of both HbsAg and anti-HBe together in some sera. Sickle cell anaemia is also more severe compared with other haemoglobinopathes including HbSC which accounts for higher frequency of the presence of both HBsAg and anti-HBe together in some sera of HbSS than HbSC (2, 3, and 11).

Above explanations also hold for higher frequency of HBsAg, HBeAg + HBsAg, and HBsAg + anti-

HBe in HbSS than in HbSC patients. This may also be associated with impaired immune function which is more in sickle cell anaemia compared to HbSC and other haemoglobinopathes. (3, 5, 10). This makes them to be more susceptible to infections. The pathological basis for this susceptibility to infections is complex. Defective splenic function is the most important factor .There is also abnormalities of opsonization, alternate complement pathway, antibody production, leucocyte function, and cell-mediated immunity (3, 12, 13, and 14)

Presence of anti-HBe in serum in most cases indicates convalescence from Hepatitis B infection. (3). Presence of anti-HBe (or anti-HBe together with anti-HCV) in some sera of HbSC patients can be associated with this fact with reference to hepatitis B infection. Presence of anti-HCV in these patients indicates hepatitis C infection. (2, 5). None of the HbSS patients was found to possess anti-HBe + anti-HCV (or anti-HBe plus any of HBsAg, HB eAg) in their sera compared to HbSC patients that have frequency of 2%. This may be attributed to the fatal severity of co- infections in HbSS than the HbSC patients. (3). This is also consistent with the fact that HbSC is less severe than HbSS patients and the impairment in immune function as explained earlier. (3, 5, 10).

Higher frequency of HBeAg was found in HbSS and HbSC females than their male counterparts. Presence of HBeAg indicates viral replication and infectivity, also in most cases absence of antibody to 'e' antigen to stop viral replication and infectivity (6, 7). This fact and findings can also be associated with the result of this study, that there was a higher frequency of anti-HBe in HbSS male than the females .Antibody to 'e' antigen in HbSC females was also higher than in the males this may be associated with the report of Walter and Engler(1995) [14]that there is a difference between women and men in antibody response from immunization to hepatitis B virus.Lockshin and Volcker(1999) [12] that sex hormone-control of some measures of adaptive and innate immunity, clinical inflammation, infection handling and healing rates differ between men and women. They further explained that not all autoimmune diseases are female predominant. Some thyroid, rheumatic and hepatic diseases have very high female / male ratios but the female/ male ratio is 1 for many autoimmune diseases and <1 for some. Female / male ratios measure incidence commonly cited sex discrepant autoimmune diseases do not differ in severity between the sexes. (12).

The frequency of HbsAg was found to be higher in HbSS male patients than in HbSS females is not consistent with the report of Walter and Engler [14] that women in military may have an increased hepatitis B infection.

The frequency of HbeAg + HbsAg in HbSS males was higher than the HbSS females, and higher frequency of HbeAg + HbsAg was also found in HbSC females than HbSC males. These may be attributed to the fact that there is a difference between women and men in antibody response from immunization to hepatitis B. (14). Infection handling and healing rates differ little between men and women (12). Antigens's' and 'e' to hepatitis B are cleared on the production of antibodies directed to the two antigens.(6,7,10)

Higher frequency of HBsAg + Anti-HBe was found in HbSS females than HbSS males. This may indicate carrier state and that the virus (Dane particle) is not replicating and infective because of the presence of anti-HBe.(6,7,10). It may also indicate chronic infection because of the persistence

of HBsAg in their sera. (5, 6, 7, 10).The gender difference can be attributed to the reports of Lockshin and Volcker (1999) [12] and Walter and Engler (1995) [14]

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SUSCEPTIBILITY PROFILE OF YEAST-LIKE ORGANISMS ISOLATED FROM HIV/AIDS PATIENTS; USING NCCLS MACRODILTION METHOD COMPARED WITH AGAR DIFFUSION TECHNIQUE.

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ABSTRACT

Yeast like opportunistic fungal infection has been reported globally amongst HIV/AIDS patients, particularly as the etiologic agent of oral thrush. Fluconazole antibiotic has been most popularly employed in treating cases of oral thrush in HIV/AIDS patients. Recent reports have recorded antifungal drug resistance amongst immunocompromised subjects. This constitutes a big problem in the management of opportunistic candidiasis. The NCCLS micro/macrodilusion sensitivity testing procedure is expensive, cumbersome and requires a level of sophistication. This study was designed to compare NCCLS M-27-A macrodilution method (expensive) with agar diffusion technique (cheap and simple), to provide a reliable rapid alternative to the new pressing need for antifungal routine sensitivity testing. Sputum specimens from 213(108 females and 105 males) HIV positive patients were plated onto SDA. The isolates were identified by morphotyping, microscopy and speciated using germ tube test, and battery of biochemical sugar fermentation; and assimilation tests. Fluconazole agar diffusion susceptibility testing was carried out on each isolate, compared with the NCCLS macrodilution sensitivity assay standard.

Of the 74 isolates tested for fluconazole sensitivity, 59(79.7%) were sensitive (zone diameter $\geq$ 19mm, mean diameter 28mm), 6(8.1%) were Sensitive Dose Dependent (S-DD) (zone diameter 13-18mm, mean diameter 16mm), while 9(12.2%) were resistant (zone diameter $\leq$ 12mm) using agar diffusion method, matched with 58(78.4%) sensitive MIC $\leq$ 8µg/ml, 9 (12.2%) S-DD MIC 16-32µg/ml and 7(9.5%) resistant MIC >64µg/ml profile, using the NCCLS macrodilution assay. The differences between the test method (Agar diffusion) and the control standard method (NCCLS-M 27-A broth Macrodilution MICS) were not statistically significant using t-test (two tail) (t = 4.302656, P=1.0). Among the *C. albicans* isolates, 26(86.7%) were sensitive to fluconazole. The rank of susceptibility was *C. albicans* > *C. tropicalis* > *C. krusei*.

It is concluded that broncho-oro-pharyngeal Candida and other yeast-like species existed in about one third of the HIV and AIDS patients studied; in which *C. albicans* was the most prevalent, while about ten percent of all the Candida isolates were resistant to fluconazole. The reliability of germ tube production as a confirmatory test for *Candida albicans* in HIV infection was as high as 96.7% and is therefore, recommended for continued use. Agar diffusion compared favourably with the NCCLS macrodilution technique, hence it is recommended for routine antifungal sensitivity test on all isolates of yeast-like cells from HIV and AIDS subjects.

KEY WORDS: HIV/AIDS, oral thrush, yeast-like cells, fluconazole resistance, NCCLS vs agar diffusion technique.

INTRODUCTION

Fungal infections have been reported from the early days of HIV|AIDS epidemic (Hody-son and Rachanis, 2002). In USA death due too mycoses increased from 10th most common infectious disease in 1980 to the 7th. in 1997 (Mc-Neil, et al 2001).

The rates of mortality for different mycoses varied markedly according to the HIV status, but were consistently higher among males, blacks and age group $\geq$ 65 years of age. (Mc-Neil, et al 2001; Lamagni, et al 2001).

Mucosal candidiasis is prevalent in HIV infection and occurs in almost all the patients at sometime during the course of the HIV disease (Odds, 1994). Oropharyngeal Candidiasis (OPC) occurs in about 84% of the cases (Neil, 1996). Esophageal

candidiasis (EC) and OPC remain the most common opportunistic infection of those infected with HIV and are considered 'AIDS Defining Illness (ADI) (Macher 1988; Jabrarisk, et al 2004).

Candida albicans (Maenza et al, 1996; Dobosz and Marczynska 2004) is the most implicated. Yeast-like cells are found globally, some occur as normal flora (*C. albicans*) in wet areas (cavity, genitalia, large intestine and skin) of about 20% of humans.

Other possible etiologic agents of oral thrush include *C. krusei, C. tropicalis, C. parapsilosis, and C. glabrata* (Hauman, et al 1993; Klein, et al 1984; Odds, 1994).

Azole, particularly fluconazole antfungal agent has been the most effective drug in the treatment of yeast-like infections (White, et al 2002).

Antifungal drug resistance is fast becoming a major problem, particularly the fluconazole amongst the immunocompromised population (Jabra Rizk, et al 2004). According to Maenza, et al (1996), the major risk factors for fluconazole resistance were advanced immunosuppression and prolonged exposure to azoles (Andrew 2003). Again, Jones, et al (1994) and Vargas, et al (2000) confirmed switching on oral candida isolates from HIV|AIDS patients at frequency between one and two orders of magnitude higher than those of healthy control.

Lynch and Sobel (1994) reported several fold increase in MICs to azoles tested, 0.31-40.0µg|ml for fluconazole. Carrillo-Munoze, et al reported mean MIC of 5.53µg|ml in 1999 on various yeast-like cells.

The newer classes of antifungal drugs – the echinocardins, pnemocardins and others are quite expensive and are not available in Nigeria.

The increased reports of anticandidal resistance and the expanding drug therapy options prompted the need for clinically relevant antifungal susceptibility testing (Lewis, et al 1998).

The NCCLS method is expensive, time consuming and somehow sophisticated as stated before. Other method like the E-test strip is easier but marred by high discrepancies, having only about 74.5% reproducibility.

Currently, very few studies have been carried out on the effectiveness of the new trend in the management of EC, OPC and other mucosal fungal infections including chemotherapy and drug sensitivity pattern of yeast-like isolates from immunocompromised (HIV|AIDS) population in Nigeria in particular and Africa in general.

This study was therefore designed to compare the NCCLS M 27 A macrodilusion sensitivity testing method with agar diffusion technique for easy and routine use in HIV/AIDS opportunistic infections diagnostic laboratories.

MATERIALS AND METHOD

ORGANISMS

A total of 74 isolates were recovered from 213 adult patients presented with oral disorder attending ARV clinic for the first time between October, 2004 to December, 2005.

Isolates were identified by grams reaction, germ tube and battery of biochemical sugar assimilation and fermentation tests (Rohde and Hartmann et al 1980; Claderone, 2002). Isolates were stored as water suspensions until used (Pfaller, 1999). Prior to sensitivity testing, each isolate was passaged onto 2% glucose Sabouraud Dextrose Agar (SDA) (OXOID), supplemented with chloroamphenicol (50mg|L) (Rejane, et al, 2002) to ensure purity and viability. Cell suspension adjusted to the turbidity of

a 0.5 McFarland standard, as described by NCCLS for Candida species were used.

SUSCEPTIBILITY TESTING

Reference antifungal powder marked 'Physicians sample' was obtained from Pfizer pharmaceuticals Lagos, Nigeria. Macrodilution susceptibility was performed according to NCCLS M-27-A Standard. The MIC end points were read visually following 72 hours incubation. The interpretative criteria for fluconazole were those published by NCCLS (2002) and are: susceptible, MIC ≤ 8µg|ml, susceptible dose dependent (SDD), MIC 16-32µg|ml and resistance ≥ 64µg|ml.

For agar diffusion, 150mm diameter plates containing Mueller-Hinton agar (Difco Laboratories) supplemented with 2% glucose and methylene blue (0.5µg|ml) at a depth of 4mm were used. The solid agar surfaces were inoculated from the test organism suspension employed in the corresponding macrodilution procedure, before the holes were made. In the holes 0.1ml of the various concentrations of the fluconazoles were dispensed. The plates were incubated in the air at 35 °C, read first at 24 hours and subsequently at 72 hours.

The interpretative criteria for fluconazole agar test were those published by Pfaller, et al (1999): Sensitive, zone diameter of ≥ 19mm, SDD, zone diameter of 15-18mm, and resistant, zone diameter of ≤ 14mm.

QUALITY CONTROL

Quality control (QC) was performed in accordance with, the NCCLS recommendations, however, in place of *C. parapsilosis* ATTC 22019 (22-33mm) recommended, *C. parapsilosis* previously isolated in Lagos from an AIDS patient that clinically responded to a single dose of fluconazole without a repeat episode in 6 months was used; whose zones of inhibitions were consistently 20-40mm for all MICs 0.625-64µg/ml**.**This was included in all the assays to check the accuracy of the drug dilution and the reproducibility of the results.

RESULTS

Seventy three (34.7%) out of the 213 patients gave positive culture results for yeast-like species. One patient out of the culture positive ones harbored double yeast-like cells which were identified separately, bringing the total number of isolates to 74. Only 70(94.6%) of the isolates could be adequately speciated. *Candida albicans* 30 (40.5%), was the most frequently isolated species, the rest were non-*Candida albicans* Candida (NCAC) species, Table 4.

The gender distribution, prevalence, species distribution and Frequency of the isolates have been published in a different report.

Out of the 74 isolates tested for fluconazole sensitivity, 59(79.7%) were sensitive (zone diameter ≥ 19mm [mean diameter 28mm]) 6(8.1%) were susceptible dose dependant (S-DD) (zone diameter 13-18mm [mean diameter 16mm]} , while 9(12.2%) were resistant (Zone diameter ≤ 12mm} using agar diffusion technique table 1, compared with 58(78.4%} sensitive MIC ≤ 8µg|ml, 9(12.2%) SDD MIC 16-32µg|ml and 7(9.5%) resistant MIC > 64µg|ml using NCCLS macrodilution assay method Table 2. Table 3 shows the comparative analysis of both methods. The discrepancies between the two approaches were not statistically Significant using t-test (two tail) (t=4.302656, p=1.0).

The rank of susceptibility has been published in another eport.

TABLE 1: 25 µg/ml AVERAGE ZONE OF INHIBITION PROFILE

SENSITIVE DIAMETER (≥ 19mm)				SENSITIVE DOSE DEPENDENT DIAMETER (13-18mm)		RESISTANCE DIAMETER ≤ 12mm	
P.CODE	**DIAMETER**	**P.CODE**	**DIAMETER**	**P.CODE**	**DIAMETER**	**P.CODE**	**DIAMETER**
MR/10/01	39.0	MR/1/44	38.0	LU/11/21	14.5	MR/10/02	
MR/10/03	29.0	ID/1/45	31.5	MR/1/42	17.5	LU/10/08	
MR/10/04	38.5	ID/1/46	29.0	MR/1/43	13.0	LU/11/17	
MR/10/01	38.5	ID/1/47	27.5	ID/1/50	18.0	LU/11/20	
MR/10/06	24.0	ID/1/48	34.0	LU/1/54	17.5	MR/12/23	
LU/10/07	3.8	ID/1/49	32.5	MR/2/59	13.0	MR/12/26	
LU/10/09	18.5	ID/1/51	18.5			MR/12/33	
LU/10/10	41.0	ID/1/52	21.0			MR/2/58	
MR/11/11	30.0	ID/1/53	22.0			LU/2/66	
MR/11/12	29.5	LU/1/55	22.5				
MR/11/13	20.5	LU/1/56	29.5				
MR/11/14	24.0	LU/1/57	38.0				
MR/11/15	23.5	MR/2/60	18.5				
MR/11/16	32.5	MR/2/61	24.0				
MR/11/18	25.5	MR/2/62	21.0				
MR/11/19	41.0	MR/2/63	26.0				
MR/11/22	31.5	LU/2/64	22.0				
MR/12/24	32.5	LU/2/65	29.5				
MR/12/25	21.5	LU/2/67	36.5				
MR/12/27	19.0	ID/2/68	24.0				
MR/12/28	23.5	ID/2/69	24.0				
MR/12/29	19.0	ID/2/70a	29.0				
MR/12/30	27.5	ID/2/70b	25.0				
MR/12/31	19.5	ID/2/71	24.5				
MR/12/32	20.0	ID/2/72	27.5				
LU/12/34	29.0	ID/2/73	36.0				
LU/12/35	29.0						
LU/12/36	18.5						
LU/12/37	24.0						
MR/1/38	46.0						
MR/1/39	31.5						
MR/1/40	23.5						
MR/1/41	29.0						
Total			**n = 59**	**n = 6**		**n = 9**	

Key: P CODE= Patients'hospital code number, MR= Medical Research, ID= Infectious Disease hospital and LU= LUTH hospital.

TABLE 2
THE MICs OF MACRODILUTION PROCEDURE CONDUCTED

SENSITIVE MIC ≤ 8µg/ml				S-DD MIC › 8 -32µg/ml		RESISTANT MIC ≥ 64µg/ml	
P-CODE	MIC	P-CODE	MIC	P-CODE	MIC	P-CODE	MIC
MR/10/01	0.625	MR/1/43	8.0	MR/10/02	32	LU/10/08	>64
MR/10/03	0.1	ID/1/45	0.25	MR/10/06	>8	LU/11/17	-
MR/10/04	0.25	ID/1/46	1.0	MR/12/27	>8	LU/11/20	>64
MR/10/05	0.25	ID/1/47	1.0	MR/11/33	32	MR/12/23	-
MR/10/07	1.0	ID/1/48	0.25	LU/12/36	32	MR/12/26	-
LU/10/09	4.0	ID/1/49	1.0	MR/11/44	32	MR/2/58	-
LU/10/10	1.0	ID/1/50	8.0	ID/1/51	>8	LU/12/66	-
MR/11/11	0.25	ID/1/52	0.25	MR/2/59	32		
MR/11/12	0.625	ID/1/53	1.0	MR/2/60	32		
MR/11/13	4.0	LU/1/54	8.0				
MR/11/14	1.0	LU/1/55	8.0				
MR/11/15	4.0	LU/1/56	0.25				
MR/11/16	1.0	LU/1/57	0.625				
LU/11/18	1.0	MR/2/61	4.0				
LU/11/19	0.25	MR/2/62	1.0				
LU/11/21	4.0	LU/2/63	1.0				
LU/11/22	0.25	LU/2/64	4.0				
MR/12/24	1.0	LU/2/65	1.0				
MR/12/25	0.25	ID/2/67	0.25				
MR/12/28	4.0	ID/2/68	1.0				
MR/12/29	1.0	ID/2/69	4.0				
MR/12/30	4.0	ID/2/70a	0.25				
MR/12/31	8.0	ID/2/70b	1.0				
MR/12/32	4.0	ID/2/71	0.25				
LU/12/34	0.25	ID/2/72	4.0				
LU/12/35	4.0	ID/2/73	0.25				
LU/12/37	1.0						
MR/1/38	0.25						
MR/1/39	4.0						
MR/1/40	4.0						
MR/1/41	0.25						
MR/1/42	8.0						
Total		**n = 58**		**n = 9**		**n = 7**	

Key: P.CODE= Patients' hospital code number, MR= Medical Research, ID= Infectious Disease hospital and LU= LUTH hospital.

TABLE 3: The COMPARATIVE ANALYSIS OF NCCLS MACRODILUTION AND AGAR DIFFUSION METHODS FOR FLUCONAZOLE SENSITIVITY PROFILE.

METHOD	SENSITIVE n(%)	S-DD n (%)	RESISTANT n (%)	TOTAL n(%)
NCCLS MACRO DILUTION	58 (78.4)	9 (12.2)	7(9.4)	74(100)
AGAR ZONE DIAMETER	59(79.7)	6 (8.1)	9(12.2)	74(100)

T-test (two tail) t = 4.3027, P = 1.0

S-DD = Sensitive Dose Dependent.

TABLE 4: THE NCCLS MACRODILUTION FLUCONAZOLE SUSCEPTIBILITY PROFILES OF VARIOUS YEAST-LIKE SPECIES ISOLATED

CANDIA SPECIES	NO ISOLATED	SENS. NO	(%)	SENS. DD NO	(%)	RESISTANT NO	(%)
C .albicans	30	26	86.7	1	3.3	3	10
C. tropicalis	13	11	84.6	1	7.7	1	7.7
C. krusei	5	2	40.0	1	20.0	2	40
C. glabrata	4	4	100		-		-
C.pseudotropicalis	3	1	33.3	2	66.7	-	
C.parapsilosis	3	1	100	2	66.7	-	
C. famata	3	3	100		-		-
C. kefyre	2	2	100		-		-
C.gulliermondii	1	1	100		-		-
R. rubra	1	1	100		-		-
T. cutaneum	1	-			-	1	100
C. dubliniensis	1	-	1	100	-	-	
C. neoformance	4	3	75		1	25	-
Indeterminate	3	3	100		-		-
Total NO:	**74**	**58**		**9**	**7**		

DISCUSSION

Oral thrush caused by yeast-like organisms having been reported as a common opportunistic infection amongst immunocompromised HIV and AIDS patients requires a closer marking.

That *Candida albicans* (40.5%) was the most implicated has equally been reported by Ehrahim, et al (2002) and Rejane, et al (2002); 52.4% and 57.4% from Bahraim and Brazil respectively.

Essentially, it has been reported that all species of candida isolates from HIV and AIDS patients in America are potentially pathogenic (Jabria-Rizik, et al (2004); Colman, (1998). However, the knowledge of the particular species involved in any episode is imperative, since some species of yeasts are known to be intrinsically resistant to some antifungals eg *C. krusei* to fluconazole (Regane, et al 2002). Azole(Fluconazole in particular) is considered the drug of choice for the treatment of oral candidiasis associated with HIV and AIDS patients (White, et al 2002).

Recent reports have indicated development of yeast-like cells resistance to fluconazole antifungal (Gabriel, 1991; Maenza, et al 1996; White, et al 2002; Sangeorzan, et al 1994 and Jabra-Rizk, et al 2004). Factors associated with the development of yeast-like cells resistance have been severally reported to include include: Secondary (after previous drug exposure) resistance, prolonged clinical treatment, low dosage and advanced immunosuppression (Million, et al 1994; White, et al 2002). These factors are gradually indicated in Nigerian ARV clinics.

From the result of this study, 9.5% of clinical isolates of oral thrush yeast-like organisms showed invitro resistance to Fluconazole in Nigeria. This report is in agreement with Million et al who reported 5-10% in 1994 from Europe ,but lower than 6- 36% reported by Priscila de Laet Sant' Ana, et al from Brazil, in 2002. This could be explained in concord with the peer notion that there exist intrinsic and geographical differences amongst yeast-like organisms (Jabra-Rizk et al 2000), or that resistant strains spread with each passing time.

The sensitive *C. albicans* isolates had a mean MIC of 2.2µg/ml, (standard ≤ 8µg/ml) for macro and microdilution methods. Lynch and Sobel (1994) reported MIC of Fluconazole tested as 0.31 – 40.0µg/ml, Pfaller; et al (1999) reported 1.25 - 2.5µg/ml for *C. albicans.* And 5.0 – 50.0µg/ml for *C. glabrata*; and Carrillo-Munoze et al (1999) reported mean MIC of 5.53µg/ml. This relatively lower MIC reported here may be attributed to less abuse of the antifungal agent in the area studied, probably because it is not common unlike common antibacterial agents and the drug is somewhat expensive.

Having established the need for fluconazole susceptibility tests on clinical isolates of yeast cells, this report put the resource poor setting into perspective, comparing the NCCLS drug sensitivity testing standard (macrodilution) with agar diffusion method.

From the result of this study there are: 59 versus 58 Sensitive (S) group, 6 versus 9 Sensitive Dose Dependant (S-DD) and 9 versus 7 Resistant group (R). The discrepancies between the standard method (The NCCLS M27-A broth macrodilution method) and the agar diffusion method were essentially minor using t-test (two tailed), t = 4.302656, p = 1.0. This report agrees with the work of Drussel, et al (1998) who reported complete agreement in percentage of the comparative study of disc diffusion method and NCCLS microdilution method.

Since there are reports of intrinsic and geographical differences between different populations of yeast cells and with the report of extensive variety in yeast species recovered from Nigeria by Jabra-Rizk et al in 2000, this report recommends intermittent if not routine susceptibility testing of all-yeast-like isolates, particularly from immunocompromised (HIV/AIDs) subjects using less cumbersome and cheaper agar diffusion technique.

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ONCHOCERCIASIS AMONGST CHILDREN OF AN ENDEMIC COMMUNITY IN EDO STATE, NIGERIA

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ABSTRACT

Onchocerciasis among 278 children (0-15yrs) of Ekpan village, a hyperendemic community in Uhunmwode Local Government Area of Edo State, Nigeria was investigated using the prevalence of nodules as index. The overall prevalence of palpable nodules was 26.3%. Nodule prevalence increased with age and the association was found to be statistically significant (P<0.001). More males than females presented with onchocercal nodules; and the difference was also found to be statistically significant (P<0.05). Majority of the nodules found were located on the head, followed by the abdomen. Of the 186 children eligible to take ivermectin, only 124 (66.7%) actually took the drug. The efficacy of ivermectin against onchocerciasis is demonstrated in the observation that those who took the drug presented with fewer nodules. Therefore, a timely introduction of eligible children (>5yrs) to the treatment programme is advocated.

KEY WORDS: Onchocerciasis, children, Nigeria, nodules, prevalence, ivermectin.

INTRODUCTION

Ochocerciasis is a serious health and socio-economic problem in Nigeria and other West African countries (1-3). It affects mostly the rural population, among who are the farmers who produce the bulk of food and industrial raw materials. Nigeria is estimated to have 30-40% of the world's cases or 60% of the cases in West Africa (4). Studies by Gemade *et al.* (3) showed that the disease was more widely distributed in Nigeria than previously assumed. The study provided accurate estimates of the population living in high risk areas of the disease. In the process, the team also identified communities to be given priority in the treatment with ivermectin.

Mass treatment with ivermectin in endemic communities usually excludes among others children below 5 years of age, although this group may constitute a sizeable proportion of some communities. According to Akogun (5), children under 10 years of age with head nodules have serious and irreversible eye lesions which significantly affect their visual acuity. Moreover, such an untreated population of children along with adults refusing to participate in community treatment programme with ivermectin, could constitute a steady reservoir for the re-infection of the treated population. In view of the little attention that has been given to this segment of the population, we undertook an investigation of onchocerciasis in the children of a hyper-endemic community in Edo State of Nigeria, using the presence of palpable nodules as our index.

SUBJECTS AND METHODS

Study Area

The study was carried out in Ekpan village, located in Uhunmwode Local Government Area of Edo State, Nigeria. The village is located in the rainforest zone of Nigeria between longitude 5°56.660′E and latitude 6°43.210′N at an elevation of 279m ASL as determined using the Garmin III Plus Global Positioning System (GPS). The inhabitants of the community are predominantly farmers with a population of 643 at the time of this study.

Advocacy and mobilization

Advocacy visits were paid to the community head, seeking consent for the survey. Consent to undertake the survey was granted after the objectives of the study were highlighted. Mobilization of the community for the survey was undertaken by the Community Directed Distributor of ivermectin (CDD) on the directives of the village head and elders committee.

Sampling method

There were 643 individuals in Ekpan village, which constituted the entire population. The mean number of the residents per house was 6 while the mean number of children (0-15yrs) per house was 3. A sample size of 264 was derived from a microfilaria prevalence of 77% (6) with an added 10% to improve the response rate to 90%, giving a final sample size of 293. All children 0-15yrs born in the community were eligible to participate in the study. However, only 278/293 consented to participate in the survey, giving a 94.8% response rate. The participants were examined from their head to the feet for the presence of palpable nodules. Number of observed nodules and site of occurrence were noted and recorded. Examination for palpable nodules was undertaken in individual participant's houses under minimal privacy.

Data collection and analysis

Data collected were analyzed using PEPI (7), the computer programme for epidemiological studies and association was established using the Pearson's Chi-square test.

RESULTS

Prevalence of nodules by age and sex

A total of 278 children comprising 133 (47.8%) males and 145 (52.2%) females were studied in Ekpan village (Table 1). The ages of the children ranged from 0-15 years with a majority, 150 (54.0%) in the 5-10 years age group, followed by those in the 0-4 years age with 92 (33.1%). The overall prevalence of palpable onchocercal nodules was 26.3%. The prevalence of palpable nodules among the affected children increased with age as also shown in Table 1 and the association was found to be statistically significant ($P<0.001$). More males than females presented with palpable onchocercal nodules; and the difference was also found to be statistically significant ($P<0.05$).

Distribution of nodules on the affected children

Figure 1 shows the distribution of palpable nodules in different parts of the body of the affected children. Majority 60 (21.6%) of the nodules were located on the head, followed by the abdomen with 44 (15.8%) and the thorax with 38 (13.7%).

Ivermectin intake and nodule prevalence

Table 2 shows that 92 (33.1%) of the children studied were underfives and these were not eligible to take ivermectin. The children eligible for ivermectin were the 186 (66.9%) who were 5 years and above and of this number only 124 (66.7%) of them actually took the drug. Table 3 shows the prevalence of palpable nodules among children who were eligible for ivermectin. Prevalence of

palpable onchocercal nodules was higher among the untreated than in the treated and the difference was found to be statistically significant (P<0.000).

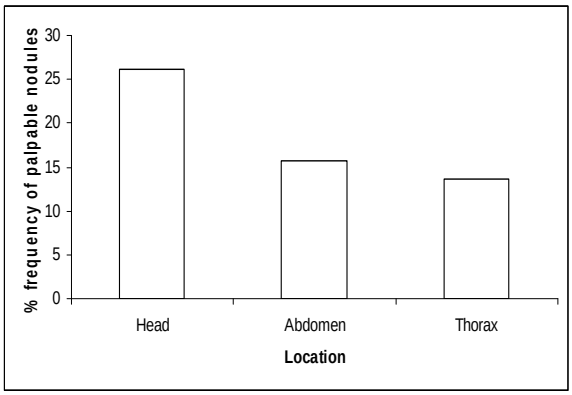

Fig. 1. Distribution of palpable nodules on the body of affected children in Ekpan village

Table 1. Overall prevalence of palpable nodules among the children of Ekpan village by age and sex.

Age group (yrs)	Children with nodules (%)		Children without nodules (%)		Total (%)
0-4	6	(6.5)	86	(93.5)	92 (33.1)
5-10	47	(31.3)	103	(68.7)	150
11-15	20	(55.6)	16	(44.4)	(53.9)
Total	73	(26.3)	205	(73.7)	36 (12.9)
	χ^2=36.6:				278
	df=1;P< 0.001				(100)
Sex					
Male	43	(32.3)	90	(67.7)	
Female	30	(20.7)	115	(79.3)	
Total	73	(26.3)	205	(73.7)	133
	χ^2=4.9;				(47.8)
	df=1;P<0.05				145
					(52.2)
					278
					(100)

Table 2. Age, ivermectin intake and frequency of palpable nodules among the children of Ekpan village

Age (yrs)	No & % examined	No and % frequency of ivermectin ntake Yes	No
<5	92 (33.1)	-	92 (100)
>5	186 (66.9)	124 (66.7)	62(33.3)
Total	278 (100)	124 (44.6)	154 (55.4)

Table 3. Prevalence of palpable nodules among children eligible to receive ivermectin treatment

	No and % frequency of nodules		
Eligible children	**Nodules present**	**Nodules absent**	**Total**
Treated	16(12.9)	108 (87.1)	124 (66.7)
Not treated	51(82.3)	11 (17.7)	62 (33.3)
Total	67 (36.0)	119 (64.0)	186 (100)

χ^2=86.6; df=1;P<

DISCUSSION

The use of palpable onchocercal nodule prevalence as index for determining the level of endemicity in communities has been in practice since its validation in Nigeria (8, 9) except that it was used for adults and the threshold for treatment in endemic communities set at 20% (10). Following its validation in Nigeria, nodule palpation became the method of choice for rapid community diagnosis of onchocerciasis and its validity has since been confirmed by several independent studies (11-13). For our study, the attractiveness of this method lay in its non-invasiveness as against skin-snipping which is resented among the adult population and dreaded by children. Other reasons for which nodule palpation has been recommended include its simplicity, applicability and practicability, acceptability and non-technicality (3,11,13,14,15). Furthermore, this method has been found to reduce blood transmissible infections such as HIV/AIDS and hepatitis B (16,17).

The overall prevalence of nodules among the children studied was 26.3% compared with the 38.7%in the adult population from the same study location (18). This result pattern, which has also been observed elsewhere (19) shows that infection is acquired early in life in this community.

The prevalence of nodules was observed to increase with age. This observation conforms to the pattern of the disease (5,19-26). Consequently, in hyper-endemic communities such as Ekpan, where transmission is already intense at young age, the process of early infection may predispose most individuals to acquiring increasingly higher worm burden as age progresses (12). Differences observed in the prevalence of nodules between male and female children tend to suggest that male children are more exposed to infective bites. In farming communities such as Ekpan it is common practice for young males to accompany their fathers to work in the farm thus exposing them more to *Simulium* bites. This observation is in good agreement with those of Brabin (27) who concluded that any gender-specific differences in symptomatology and microfilarial densities derive from differences in exposure rather than from inherent sex differences.

On the distribution of nodules, it was observed to be more on the head and trunk of the children studied. In Africa where *S. damnosum* and *S. neavvei* complexes bite mainly on the ankles and legs of adults, nodules are most commonly located around the pelvics. In children however, because their head is closer to the ground than those of the adults, it makes the head and trunk the likely sites open to infective bites (28). Moreover, the head of infants may be the only area exposed when on their mother's back or when laid on the ground while their mothers are busy with farming (28).

With the low prevalence of nodules among the underfives (Table 2), it is obvious that this group does not constitute a significant source of infection in the community. This is in good agreement with the observations of Duke and

Moore (20) who in their study of different age groups in a Cameroon forest village concluded that apart from occasional individuals, children under 5 yrs are of little importance as a source of transmission. This cannot be said of children, 5yrs and above, who are infected and very accessible to *Simulium* flies when they engage in farming and other outdoor activities in the community. If this segment of the population is neglected in the ongoing ivermectin treatment programme in the community, they may constitute a good reservoir of infection and re-infection for the treated population. It needs to be stressed that adults in the community need to take the annual treatment programme more seriously as this will effectively reduce the microfilariae available for transmission to the younger members of the community.

From the results presented in Table 3, it is obvious that ivermectin is efficacious in the control of onchocerciasis as children who took the drug presented with fewer nodules. Apart from the microfilaricidal effect of ivermectin, the drug has been shown to reduce fecundity and cause death of as much as 30% of adult *Onchocerca volvulus* (29, 30). From the foregoing, it is obvious that timely introduction of eligible children (5yrs and above) in endemic communities to ivermectin treatment will reduce morbidity and the clinical manifestations of the disease.

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THE PREVALENT BACTERIAL ISOLATES OF DENTAL CARIES IN SCHOOL AGE CHILDREN ATTENDING THE DENTAL CLINIC OF OAUTHC, ILE-IFE

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ABSTRACT

The study was conducted at the dental clinic of Obafemi Awolowo University Teaching Hospital Complex, Ile-Ife. A total of 100 carious samples were collected from children of varying age and sexes. The bacteria isolated were *S. mutans*: 45.6%, *Lactobacillus spp*: 41.2% and *S. aureus*: 13.2%. Out of the 100 samples, 88(5) had mixed growth of bacteria and the common bacteria combinations were *S. mutans* and *Lactobacillus spp.*(43.2%) , *S. mutans* and.*S. aureus* (38.6%) and *Lactocillus spp* and *S. aureus* (18.2%)The distribution pattern of dental caries in relation to gender showed a higher frequency in females than males with the initiator *S. mutans* having 565.8% in female and 44.2% in males. The organisms appear to be more prevalent in children of 6-10 years considering the initiator S. *mutans* being 73.1% while ages 1-5 years were least affected with 5.8%. prevalence. Pefloxacin, Chloramphenicol, Ceftriaxone and Ciprofloxacin are most effective against the caries-inducing organisms with an average susceptibility range of 76.1% to 92.2%.

INTRODUCTION

Dental caries is one of the most common disorders in the world, second only to common cold. It is the most important cause of tooth loss in younger people due to high dependence on dietary sucrose and frequency of eating,[1] Caries is a disintegration of the teeth beginning at the surface and progressing inwards.

Caries is not caused by a single organisms, rather it results from the damage caused by complex micro-organisms. There is however a central role for *S. mutans* in the initiation of dental caries. *S. mutans,* Lactobacilli and Antinomycetes have been reported to play a role in the pathogenesis of dental caries.[2] Gabris et al[2] indicated that coronal caries is largely a disease of children with steady increase until 15 years of age and then diminishes in early children. Root caries as earlier reported by Hilson[3] particularly affect the proximal surfaces of the cheek teeth and is primarily a disease of older adult. The pattern of dental caries is similar in members of the same family over several generations. Environmental factors such as diet and oral hygiene habits play a large role in causing dental caries. The development of dental caries also depends on genetic, hormonal, notional and many other factor[4] the clearest singly factor in caries epidemiology still remain sugar. In the recent times, there has been so much discussion and emphasis on the issue of dental caries increase in the developing countries vis a vis the rate of sugar consumption in these countries. The study was therefore undertaken to example the bacteria etiology and their

antigiogram in children as well as the current prevalence rate of the infection for which there is no recent report in this environment.

MATERIALS AND METHODS

STUDY DESIGN

All patients (children) that had visible carious on their teeth attending the Dental clinic of Obafemi Awolowo University Teaching Hospital Complex (OAUTHC). Ile-Ife, Osun State, Nigeria between March 2004 and February 2005 were studied and swabs were obtained. The study included the documentation of age and sex of the subjects.

SAMPLE COLLECTION AND PROCESSING

A total of one hundred samples were obtained from 100 subjects who had not commenced treatment as at the time of c collection of samples. The control samples were collected from apparently normal healthy children with no history of dental carries. Samples were transported immediately to the laboratory for processing. For the bacteriological processing, Chocolate, Cystine Lactose Electrolyte Deficient (CLED) and MacConkey were employed. The Chocolate and CLED agar were incubated at 5-10%. CO_2 at 35-37% C for 24hours and the MaConkey aerobically at 35-37%C for 24hours. All isolates were characterized using the scheme of Chessbrough[5] and Cowan[6]. Susceptibility to antimicrobial agents were done by the disk diffusion method using Diagnostic Sensitivity Testing (DST) agar as described by NCCLS[7].

RESULTS

Bacteria were isolated from 100.0% of the subjects. The bacteria isolated were *Streptococcus mutans. Lactobacillus spp* and *Staphylococcus aureus, S. mutan* had ghest frequency (45.6%), *Lactobacilllus spp* (41.2%) and S. aureus (13.2%) as shown in table 1.

Table 2 revealed that specimens from 88.0% of the subjects had mixed growth of bacteria and the bacteria combinations were *S. mutans* and *Lactobacillus spp* (43.2%).
S. mutans and *S. auareus* (38.6%) and the least combination was *Lactobacillus spp* and *S. aureus* (18.2%).

Table 3 shows the distribution pattern of dental caries in relation to gender and age indicating that it occurs more frequently in females than in males with the initiator *S. mutans* being 55% in female and 44.2% in males followed by *Lactobacillus spp* being 72.3% in females and 27.7% in males . *S. aureus*_ occur more in female (66.7%) than in males (33.3%).
Distribution pattern of dental caries among the subjects in relation to age shows that the S. disease is most prevalent among children of age 6-10years considering the initiator *S. mutans* having 73.1% while ages 1-5years are least affected as recorded in table 4. In table 5 is presented the distribution of the organisms isolated from the control subjects giving *S. albus* (70%) with males having higher frequency (40%) and females (30%). *Klesiella spp* gave a 30% made up of females (20%) and males (10%).

Table 6 shows the antibiotic susceptibility test of the isolates with pefloxacin chloramphenicol, ceftriaxone and Ciprofloxacin being most effective giving average susceptibility of 92.9%, 81.8%, 78.6% and 76.1% respectively. Also shown in table 6 is the susceptibility pattern of the isolates from control sample to some antibiotics.

TABLE I: Frequency of distribution of the organisms implicated

Organisms	Frequency	Percentage Frequency (%)
S. mutans	52	45.6
Lactobacillus spp	47	41.2
S. aureus	15	13.2
TOTAL	114	100. 0

TABLE 2: Frequency of bacterial combination in mixed culture obtained from dental caries

Organisms	Frequency	Percentage Frequency (%)
S. mutans and *Lactobacillus spp*	38	43.2
S. mutans and *S aureus*	34	38.6
Lactobacillus and *S. aureus*	16	18.2

TABLE 3: Distribution according to sex in percentage

	Male	Female
Isolated Organism	N (%)	N (%)
S. mutans	23(44.2)	29(55.8)
Lactobacillus spp	13(27.7)	34(72.3)
S. aureus	5(33.3)	10(66.7)

TABLE 4: Distribution according to age in percentage

Isolated Organism	1-5years	6-10years	11-15years
	N(%)	N(%)	N(%)
S. mutans	3(5.8)	38(73.1)	11(21.1)
Lactobacillus spp	3(6.4)	3(6.4)	41(87.2)0
S. aureus	4 (26.0)	6(41.0)	5 (33.3)

TABLE 5: Distribution according to sex in percentage of the control

Isolated Organism	Male	Female
	N(%)	N(%)
S. albus	4 (40.0)	3 (30.0)
Klebsiella spp	1 (19)	2 (20.0)

TABLE 6: Susceptibility Pattern of the Isolates from dental caries and from control to Some antibiotics

	S. mutans	*Lactobacillus sp*	*S. aureus*	*Klebsiella*	*S. albus*
No. of isolates	52	47	15		
Antibiotics	N (%)	N (10.00)	N (%)		
Augmentin	48(92.30)	5 (10.60)	7 (46.70)	100.00	0.00
Amoxacillin	0 (0.00)	5 (10.60)	2 (13.30)	0.00	0.00
Chloramphenicol	52 (100.00)	47(100.00)	10(66.70)	100.00	100.00
Ceftriaxonne	42 (80.70)	44(93.60)	15(100.00)	100.00	100.00
Co-Trimoxazole	25 (80.10)	0 (0.00)	5 (33.30)	0.00	0.00
Ciprofloxacin	32 (61.50)	30 (63.80)	12 (80.00)	100.00	100.00
Fortum	0 (0.00)	0 (0.00)	0 (0.00)	100.00	100.00
Erythromycin	0 (0.00)	40(84.10)	6 (40.00)	0.00	100.00
Gentamycin	20 (38.50)	0 (0.00)	13 (86.70)	100.00	100.00
Tetracyclin	0 (0.00)	26 (55.30)	6 (40.00)	100.00	100.00
Pefloxacin	52 (100.00)	40 (85.70)	12 (86.70	100.00	100.00

DISCUSSION AND RECOMMENDATIONS

The results obtained showed that children are affected by dental caries as they advanced in age, and this is in line with the reports of Gabris[2] et al emphasizing that the frequency of dental caries increases steadily until 15years or so and then diminishes in early adulthood. *Streptococcus mutans* appear as the most common organism associated with dental caries and closely followed by *Lactobacillus spp* as revealed in table 1. This high percentage of *S. mutan* supports previous reports of some researchers[(8,9)] that the initiation and progression of dental caries is closely associated with *S. mutans*. It can therefore be inferred *S. mutans* play an important role in the aetiology of caries in human.

In the present study, Lactobacillus was not found in non-caries teeth but seen with a lower percentage in carious teeth compared to *S. mutans*. This suggest that Lactobacillus probably plays a role in the initiation of caries and their presence in caries lesions may be an indication of their involvement in the progression of such lesions. This can be buttressed by the highest frequency of mixed culture of *S. mutans* and *Lactobacillus spp* as revealed in table 2.Relatively small number of *S. aureus* were isolated from the carious teeth but not in non-carious teeth. The emergence of *S. aureus* in the caries lesions can be attributed to the facultative habit of *S. aureus*.

In this study Hilsons[3] report that dental caries appear to be more common in girls than boys. The high frequency of *Lactobacillus spp* in females recorded further confirms his work that the early eruption of teeth in females provides the anaerobic state for Lactobacillus to thrive. This result is also in conformity with the work done by Seibert[10] et al while investigating the S. mutans level and caries prevalence in low-income school children 47% of the children with caries had high S. mutan level, females had S. mutans level than males in the 9-13years group. Analysis of variance test indicates that the level for older females (9-13years) were significantly

higher than those observed in males in the same age group. Staphylococcus albus and Klebsiella spp were isolated from the control children. The presence of Klebsiella support the work of Gerald[11] et al that Gram negative rods are isolated from the mouth and that diet has a marked influence on the relative composition of mouth flora.

The antibiograms of the micro-organisms isolated (table 6) indicate a wide range of sensitivity to different antibiotics with Ceftriaxone, Pefloxacin and Chloramphenicol having the highest sensitivity patter. It therefore seems that the use of these drugs may reduce e the incidenc e and severity of caries in individual and communities. However, first line drugs for S. mutans as recommended by Tierny and colleagues[12] include Penic illin and/or Gentamycin. In this work S. mutans has not shown high sensitivity of Gentamycin (38.50%) nor to Amoxicillin which represent the Penic illins. It could be inferred that resistance strains of S. mutans are emerging and this reqire further studies. Further studies are also necessary to access the level of protection against dental caries offered by the use of these antibiotics.

RECOMMENDATIONS

As dental caries is reappearing in many countries as public health crisis, the following steps can be taken to curtail the disease:-

Information:-

The passage information in health system is the basis of health education and therefore efforts should be intensified on oral health education in schools as this will interrupt the currently progressing rate of dental caries among children. The general public should be well informed about the importance of good oral hygiene. Pregnant women and nursing mothers should be educated on the mode of transmission of normal flora from their mouth to their children.

Chewing sticks for tooth cleaning should be encouraged as they have been shown to have inhibitory effect t on microbial flora causing dental caries. Import of foreign method of tooth cleaning should not be overemphasized.

Diet:-

While emphasizing sugar in the case of dental caries development, the role of balanced diet in the maintenance of good health cannot be over looked. The rate of consumption of sugar can be reduced by restricting to the meal times since it is impossible and quite impracticable to totally stop the consumption of sugar. Calcium in diet has the potentials of building stronger teeth.

Fluorides:-

The government at every level should strongly consider fluoridation of public drinking water as a worthwhile project. In addition to the present effort to ensure that bottled and sachet water are portable for drinking, NAFDAC (National Agencies of Food and Drugs Administration and Control) should also insist that such water have adequate content of fluoride ions. Fluoride levels in main food items should be determined and made known to the public. This calls for urgent need for fluoride level research in every country. All tooth pastes lacking fluoride or insufficient fluoride level should be banned from the market.

Fissure sealing:-

Clinical experience has shown that some children are at high risk of caries attack . In such children, prophylactic administration of fissure sealing should be provided as soon as the teeth pit and fissure erupt into the mouth.

Dental check-up:-

A periodic dental check-up at least twice a year for growing children should be encouraged. Dentists should endeavour to visit schools (Nursery, Primary and Secondary) for check-ups on growing children.

Microbial Monitoring:-

As science is dynamic, the periodic monitoring of the oral microbial flora and their drug sensitivity pattern should be carried out and necessary information passed to the public. Dental caries, though a disease of great antiquity is not a disease of the past since it is reappearing in many countries as a public health crisis. It is therefore important that the public health be taught on prevention, early recognition (visible white spot on the tooth with excruciating pain) and reporting to dental clinic for proper prognosis. This underscores the need for laboratory diagnosis, confirmation of dental caries and the antibiograms of incriminating micro-organisms for proper management of patients and to reduce the development of resistant strains or multiple resistance to antibiotics.

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