ISSN 0975 - 6299 Vol 2 / Issue 3 / Jul - Sept 2011

International Journal of Pharma and Bio Sciences

RESEARCH ARTICLE MICROBIOLOGY

ANTIBIOGRAM OF BACTERIA AND FUNGAL ISOLATES ASSOCIATED WITH OTITIS MEDIA AMONGST CHILDREN IN BAUCHI STATE, NIGERIA

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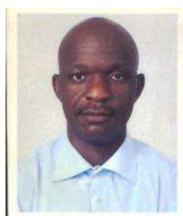

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ABSTRACT

A total of 400 ear swabs were collected from infant and children within the ages of 0-12years. All samples were cultured on MCA, BA, CA, and SDA plates which yielded the following *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa Streptococcus species, Klebssiella spp, and Candida albicans.* These bacteria isolates were tested against selected antibiotics such as Tetracycline, Gentamycin, Ampiclox, Chloramphenicol, Augmentin, Enthromycin, Cotrimoxazole and Ciprfloxacin, using Disc diffusion method. Ciprofloxcin was the most sensitive antibiotic with Tetracycline and Chloramphenicol being the least. The isolate antibiotic susceptibility was statistically significant by ANOVA ($P<0.05$) which on further analysis with LSD and DMRT revealed that Ciprfloxacin isolate sensitivity was significantly different to all the antibiotics while Gentamycin was statistically significant over Tetracycline, Chloramphenicol and Cotrimoxazole. Furthermore, an overall assessment of 75.43% sensitivity and 24.57% resistance respectively was observed which was statistically significant by Chi square ($P< 0.05$) analysis. Isolates of *C. albicans* were tested against selected orally administer antifungal agents such as Fluconazole, Ketoconazole, Griseofulvin and Terbinafine using Microdilution tube method. Terbinafine was the most sensitive and Fluconazole was the least. This study concludes that Ciprofloxcin and Terbinafine are the most effective drugs of choice in first line treatment of Otitis media in children. Hence, for effective management of cases a preceding mycology investigation should supplement bacteriological diagnostic procedures.

KEYWORDS

Antibiogram, Bacteria, Yeast, Otitis media, Children

INTRODUCTION

The ear is an important sensory organ used for hearing and maintenance of body balance[1]. Otitis Media (OM) is the inflammation of the middle ear, a common childhood infection referred to as 'ear infection' which usually occurs as Acute Otitis Media (AOM), Chronic Otitis Media (COM) and Chronic Suppurative Otitis Media (CSOM) in nature[2]. Acute infection is characterized by moderate pain, presence of fluid (typically pus) and possibly fever, chronic cases are characterized by more severe symptoms with or without the presence of effusion while resistant acute cases occur repeatedly due to failure of antibiotics therapy[3]. Resistant and untreated cases, may lead to intracranial or intra temporal complication or both[4]. Although the anatomy of man contributes significantly to the occurrence of OM, especially in children due to the horizontal nature of the Eustachian tube which easily allows the entrance of food particles into the middle ear, hence the buildup of infective agents such as Bacteria, Viruses, Fungi and Yeasts which are the major etiologic agents of OM[2,5]. Bacteria organisms commonly associated with OM include *Staphylococcus aureus, Proteus mirabilis, Streptococcus pneumoniae, Pseudomonas aeroginosa, Heamophilus, influenza* [6,7] and rarely *Morexella catarrhalis* [8] which varies with location and other geographic factors[9]. Reports of continuous emergence of resistant pathogen, as well as the changing pattern of antibiotics effectiveness when used in treatment of OM necessitated this study. This study was aimed at providing update information on the different organisms associated with OM in children with their corresponding antibiotic susceptibility tests within the study area.

MATERIALS AND METHODS

Study Area

This study was carried out at the Specialist Hospital, Bauchi. It is the major health facility of the state located in its capital, Bauchi local Government Area (LGA). Bauchi is a first- order administrative division located in the northern part of Nigeria with a population size of 4,706,909 as reported during the 2006 Census. It is located on latitude N l0^0 30' 0'' and longitude E 10^0 0' 0'' bordered by seven states, Kano and Jigawa to the North, Taraba and Plateau to the South, Gombe and Yobe to the east and Kaduna to the west. The state occupies a total landmass of 549,260 square kilometers with two distinctive vegetations (Sudan and Sahel Savannah) representing about 5.3% of the country's total landmass. It is made of 20 LGA's with 55 different tribes and the principal ethnic groups include Fulani, Hausa, Gerawa, Sayawa, Jarawa, Bolewa, Karekare, Kanuri, Fa'awa, Butuwa, Warjawa, Zulawa and Badawa[10].

Specimen collection

Samples were collected randomly from patients (Infants and Children within the ages of 0-12years) attending the Ear, Nose and Throat (ENT) Clinic of the Specialist Hospital Bauchi. These ear swabs were collected before the commencement of antibiotics therapy using sterile swab sticks and immediately taken to the laboratory for both Bacteriological and Mycological investigation.

Isolation of Pathogens

Bacteriology of specimens: Collected sample were inoculation onto MacConkey Agar (MCA),

Blood Agar (BA) and Chocolate Agar (CA) plates[11]. MCA and BA plates were incubated aerobically at 37 ^oC for 24 hour while CA plates were incubated at 37 ^oC for 24 hour in presence of 2-5% Carbon dioxide. Post incubation, colonial and cultural morphology were read and documented. Pure isolates were obtained by repeated sub culturing of these organisms onto Nutrient Agar (NA) plates, and then maintaining on NA agar slants at 4 ^oC until required.

Identification and biochemical characterization of Bacterial Isolates: The cultural and physiological characteristic of isolates was observed and noted 24 hours post incubation. Motility test and other biochemical reactions such as Catalase, Coagulase, Indole, Urease, Oxidase and Citrate test were further conducted[11]. Results obtained were compared with the Identification and Biochemical reaction tables[12].

Mycology of specimens: Collected sample were inoculated on to Saboraud Dextrose Agar (SDA) [13]. All plates were inoculated under aseptic conditions and incubated aerobically at 25 ^oC for up to 72hours. Plates were observed macroscopically at intervals to check for yeast cell. Plates that yielded creamy, mucoid and musty colonial appearance were suspected to be yeast cells and investigated further.

Identification and characterization of Yeast isolates: These suspected yeast isolates were gram stained and observed for the thick walled, round and budding clamydospores. This was accompanied by Serum tube test (physiological reactions) to observe for Germ tubes production as well as acid and gas production from sugar reactions[12].

Bacterial Inoculums: Bacterial inoculums were prepared using the McFarland turbidity standard which gives turbidity scale of approximate bacterial density of 1.2 x 10^9 CFU/ml[12].

Yeast inoculums: Yeast inoculums were prepared using Colorimetric method. This gives an approximate inoculums density range of about 2-4 x 10^6 CFU/ml which was adjusted to 5ml using YPD broth to yield a final concentration of 2-4 x 10^4 CFU/ml[14].

Quantification of prepared microbial inoculums: The cell number in the prepared microbial inoculum suspension was determined using Heamatocytometer method. A 1:50 dilution was prepared by introducing (0.1) ml of prepared inoculums into 5ml of distilled water. Then, one (1ml) ml of new dilution was charged into the heamatocytometer and covered with cover slip. All cells were enumerated using a manual counter at x10 objective[15].

Drugs preparations

Antifungal Drugs were dissolved in sterile distilled water and 100% Dimethylsulphoxide (DMSO) [15,16,17]. Stock solutions of 1000ug/ml were prepared for all drugs followed by the preparation of a ten 2-fold serial dilutions of varying concentrations and dilution factors. Ketoconazole (KET) and Fluconazole (FLU) were dissolved in 20ml of sterile distilled water while Griseofulvin (GRI) and Terbinafine (TER) were dissolved in 50ml DMSO to yield the initial 1000ug/ml concentration. A ten 2-fold serial dilution were prepared using a dilution factor of 1:20, 1:40, 1:80, and 1:160 to yield a range of various concentrations. Using pasture pipette, 0.1ml of each stock solutions were dispensed into 2ml of sterile distilled water and DMSO to yield the initial dilution factor of 1:20, then 9 sterile test tubes each were assembled for each drug type containing 1ml of the sterile distilled water and DMSO each. Then, 1ml of the 1:20 preparation was serially diluted into the 9 sterile test tubes to yield a dilution range of 1:40 – 1:10240 and a concentration of 50ug/ml-0.10ug/ml.

Antibacterial sensitivity testing: The antibacterial susceptibility testing was carried out using Disc diffusion assay[11,12]. Pure isolates of bacteria were tested against selected antibiotics using multidisc of Erythromycin (ERY), Gentamycin (GEN), Chlorophenicol (CHL), Ampiclox (AMP), Cotrimaxozole (COT), Tetracycline (TET), Augumentin (AUG) and Ciprofloxacin (CIP).

Prepared inoculums were streaked onto Diagnostic Sensitivity Test Agar (DSTA) under aseptic condition and commercially obtained antibiotics multidisc were carefully placed onto the surface of the streaked plates using a sterile forceps and incubated at 37°C for 24 hours. All plates were prepared in duplicate, after 24 hours the zones of inhibition were measured. Obtained results were compared with the zones-size interpretation chart[12] and the frequency of sensitive or resistance antibiotics were recorded.

Yeast sensitivity testing: The antifungal susceptibility testing was performed following the Yeast broth micro dilution protocol M27 reference method of National Committee for Clinical Laboratory Standard [15,17,18] with slight modification. Pure Isolates of yeast were tested against some selected orally administered antifungal drugs such as Ketoconazole (KET) (Hovid-200mg), Fluconazole (FLU) (Drugfield-50mg), Griseofulvin (GRI) (Hovid-500mg) and Terbinafine (TER) (Norvatis–500mg).

Ten (10) test tubes each were assembled for each antifungal drugs and all procedure was carried out in duplicate. Each tube contained 1ml of the prepared 2-fold drug concentrations ranging from 50ug/ml-0.10ug/ml in the test tubes. Then, 1ml of the prepared inoculums was inoculated into these drugs concentration to yield a new range of 25ug/ml-0.05ug/ml. For each sets of experimental tubes, two drug-free controls were included, one with the Saboraud Dextrose Broth(SDB) and sterile normal saline alone (sterile control) and the other with SDB and prepared inoculums suspension (growth control). All tubes were incubated at 35°C for 48 hours and observed macroscopically by comparing experimental tubes with sterile and growth control tubes.

Determination Minimal Inhibitory Concentration (MICs): MICs were determined according to M27-method of broth micro dilution for yeast[18]. After 24 hours of incubation, growth in experimental tubes were compared with those of sterile and growth control tubes. The concentration with the least inhibition next to the tube without or at least without any significant inhibition was taken as the MIC_{90} and the concentration at which 50% of its initial cell size was inhibited was considered as the MIC_{50} for all antifungal drugs.

Determination of Minimal Fungicidal Concentration (MFC): This was determined according to the method[16]. After the determination of MIC, the total volume from each experimental tubes, stating from the last tube in which growth was observed up to the highest drug concentration were transferred into another sterile test tubes containing 5ml of SDB (PH=6.5) and incubated at 35°C for up to 72 hours. After 72 hours, growth was observed visually after shaking these tubes and MFC was observed. MFC correspond to the lowest concentration in which 99% of yeast cells are inhibited or no viable growth.

Data analysis

All the isolates and isolate sensitivity/inhibition were expressed as frequencies and percentages which were subjected to Chi square, one way-ANOVA, LSD, DMRT and T-test.

RESULTS

Out of the Four hundred (400) ears swabs collected and screened, three hundred and thirty-two specimens (332) yielded microbial growth (83%), 301 (75.25%) of these were bacteria isolates, 31(7.75%) were yeast isolates while 68 (17%) yielded no microbial growth(Table 1). Eight (8) of the 332 yielded both bacteria and yeast isolates and the sensitivity distribution pattern of the Eight (8) antibiotics routinely used in treatment of OM, shows that 218 (75.43%) isolates out of the 289 bacteria were sensitive to all antibiotics while 71 (24.57%) were resistant which was statistically significant by Chi square ($P<0.05$) analysis. Ciprofloxacin (CIP) – 95 (32.87%) was the most sensitive of these antibiotics, followed by Gentamycin (GEN) – 38 (13.15%) and Tetracycline (TET) – 5 (1.73%), Chloramphenicol (CHL) and Cotrimaxazole

(COT) – 11 (3.81%) at the same level of sensitivity were least susceptible as indicated in table 2. These isolate antibiotic susceptibility was statistically significant by ANOVA ($P<0.05$) which on further analysis with LSD and DMRT revealed that CIP isolate sensitivity was significantly different to all the antibiotics while GEN was statistically significant over TET, CHLOR and COT, with no significant difference over ERY, AUG and AMP.
The MICs of four antifungal drugs against 31 clinical isolates of *Candida albicans* as shown in Table 3 indicates different drug concentrations that inhibits 50% and 90% of the Isolates (MIC_{50} and MIC_{90}) respectively after 48 hours of incubation and all these isolates tested were susceptible to the 4 antifungal drugs used with TER being the most effective and FLU been the least. This least activity of fluconazole, MIC_{50} and MIC_{90} were statistically in significant ($P>0.01$) by T-Test. Also the MFC of the four antifungal drugs against the clinical isolates of *C. albicans* shows the different drug concentrations that inhibits 99% of cells or at which no visible yeast cells was observed as shown in table 3.

Table 1
Microbial growth distribution of specimen collected

Growth	Frequency	Percentage (%)
Bacterial isolates	301	75.25
Fungal isolates	31	7.75
No growth	68	17.0
Total	**400**	**100**

Table 2
Sensitivity pattern of isolates to selected antibiotics in treatment of Otitis Media

Isolates	Antibiotics								Total	
	ERY	GEN	CHL	COT	TET	AMP	AUG	CIP	RES	SEN
S. aureus	4	9	-	-	5	12	7	46	33	83
P. mirabilis	2	26	-	5	-	4	6	21	18	64
E.coli	3	-	9	-	-	1	-	15	5	28
Streptococcus Sp	3	2	-	4	-	3	3	5	7	20
Ps aeroginosa	2	-	-	-	-	3	2	5	7	12
Klesiella spp	-	-	1	2	2	-	2	1	3	11
Total	**14**	**38**	**11**	**11**	**5**	**25**	**19**	**95**	**71**	**218**
Frequency %	4.84	13.15	3.81	3.81	1.73	8.65	6.57	32.87	24.57	75.43

10mm or less-Resistant and 11mm or more-sensitive *n = 289*

Key:
ERY-Erythromycin
GEN-Gentamycin
CHL-Chloramphenicol
COT-Clotrimazole
TET-Tetracycline
AMP-Ampiclox
AUG-Auguementin
CIP-Ciprofloxacin

Table 3

MICs and MFCs of four antifungal drugs against of C. albican Isolates (ug/ml) Antifungal Drug Arithmentic mean of MIC (ug/ml)

		MIC_{50}	MIC_{90}	MFC
FLU		12.50	25.0	50.00
KET		1.56	3.13	6.25
GRI		0.39	0.78	1.56
TER		0.10	0.10	0.20
T test: P ≥0.01	=	H_o Accepted	Range = 50- 0.10ug/ml	

Key:
FLU= Fluconazole
KET= Ketoconazole
GRI= Griseofulvin
TER=Terbinafine

DISCUSSION

The 301/400 positive bacterial isolates were *Staphylococcus aureus, Proteus mirabilis, Escherichia coli, Pseudomonas aeruginosa Streptococcus species,* and *Klebssiella spp.* While 31 fungal isolates were *Candida albicans* as shown in Table 1. This is similar to the findings which reported *S. aureus* as the most predominant organisms with a prevalence rate of 25.0% [7]. The antibiogram of eight (8) commonly used antibiotics in the treatment of OM in Table 2 showed an overall assessment with 75.43% sensitivity and 24.57% resistance. Ciprofloxacin was the most sensitive (32.87%), Gentamycin (13.15%), Ampiclox (8.65%), Augumentin (6.57%) and the least being Chloramphenicol and Cotrimaxozole at 3.81%. This finding was in agreement with the results of previous researchers [6,19,20]. However, contrasts with some other reports [9,21,22] which indicates Amoxicillin, Trimethoprim-Sulphamethoxazole, Ofloxacin and Gentamycin as the most sensitive antibiotics respectively. Reports of a high sensitivity of Ceftazine, Ceftriazone and Gentamycin were as documented[23]. Table 3 shows the MICs and MFCs of 4-antifungal drugs against isolates of *C. albicans* and drug concentration required to inhibit 50% and 90% of the isolates hence MIC_{50} and MIC_{90} respectively, which reveals no resistance with all the antifungal drugs tested against the isolates. These isolates were less susceptible to Fluconazole with MIC_{50} = 12.50µg and MIC_{90} = 25.0 µg, Ketoconazole with MIC_{50} = 1.56 µg and MIC_{90} = 3.13 µg. However, Terbinafine was found as most effective of drugs against all isolates at MIC_{50} and MIC_{90} = 0.10µg with an in–vitro activity order of terbinafine > Griseofulvin > ketoconazole >fluconazole as reported [15,16,17]. This least activity of fluconazole, MIC_{50} and MIC_{90} were statistically in significant at P>0.01 as earlier reported [16]. Minimum Fungicidal Concentration (MFC) of these 4 antifungal drugs indicates the concentration at which ≥ 99% of cells or no viable cells are noticed. FLU had MFC of 50 µg/ml while TER had 0.10 µg/ml. This confirms that TER as most effective [24].

In conclusion, this work provides information that will assist clinician and scientists monitor the trends of microbial susceptibility and influence the choice of effective medication especially for the treatment of yeast oriented OM. Thus, mycological test be carried out alongside other laboratory protocols and drug susceptibility testing before prescription of drugs. Antibiotics that require less frequent dosing (one or twice a day) shorter courses (five days or less) which enhance compliance and reduce the development of resistance is strongly advocated.

ACKNOWLEDGEMENT

The authors' wishes to acknowledge the HOD in charge of the Bauchi Specialist Hospital Laboratory and the staff of the ENT Clinic especially Mr. S Hanakuka for their support and efforts during collection and processing of specimens as well as Pharm. Olatunji A. Olabode of Supreme Pharmaceuticals and Chemicals Ltd for donating reagents, antibiotics discs and anti-mycotic drugs used during the course of this research.

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