ABSTRACT

BACKGROUND: Immune compromised HIV/AIDS infected children have consistently shown a higher prevalence of chronic suppurative otitis media than their immune competent counterparts. This study aimed to compare the microbial isolates from ear discharges in HIV infected and non-infected children.

METHOD: This was a hospital-based prospective cohort study designed to determine the relative prevalence of the common organisms present in chronically discharging ears of HIV infected children and their antimicrobial sensitivities and compare that of age and sex matched non HIV infected children. Fifty HIV infected children being followed up at the paediatric HIV clinic of a tertiary health facility, had ear swabs obtained for culture and antimicrobial sensitivity. The same was done for another cohort of fifty age and sex matched non HIV infected children.

RESULTS: The mean age of the study population and controls were 6 years 11 months (SD 0.96) and 7 years 11 months (SD 1.00), respectively. Male to female ratio for each group was 1:1. Ear discharge was bilateral in 27 (54%) HIV infected children and in 35 (70%) of the controls. The most prevalent bacterial isolate in both groups was Pseudomonas aeruginosa though it was more prevalent in HIV infected children (P=0.005). Fungal isolates were commoner in non HIV infected children (P=0.001). Ninety percent sensitivity to the 4 Quinolones was recorded by isolates in HIV infected children while sensitivity to the Aminoglycosides was greater in non HIV infected children.

CONCLUSION: Pseudomonas aeruginosa is the predominant organism isolated from HIV infected children with chronic otitis media. Fungal isolates are less frequently encountered in ear discharges of HIV infected children. The 4 Quinolones are the drugs of choice in treatment of Chronic suppurative otitis media in HIV infected children.

KEYWORDS: Chronic suppurative otitis media; Bacteriology; HIV infection; Children
We aimed to study the microbial organisms in CSOM, as well as their antibiotic sensitivities among HIV infected children, and to compare the microbial isolates from ear discharges of HIV infected children and non-infected children.

**MATERIALS AND METHOD**

Fifty consecutive HIV infected children in the study group who were previously diagnosed with HIV infection at the paediatric HIV clinic of a tertiary institution were prospectively recruited during a 1-year period ending October 2014. The control consists of 50 discharging ears of age and sex matched HIV negative children recruited from the ENT clinics of the same tertiary institution. All ears recruited for the study had been discharging for three months or more and had varying degrees of tympanic membrane perforations on otoscopy. Those excluded were not having active ear discharge, or had otitis externa, or did not give consent. Also excluded were children who had used topical or systemic antibiotics in the last 72 hrs before presentation. The study compared the discharging ears of children recruited for the study.

Approval for the study was given by the Institutional Ethical Review Committee. Informed consent was obtained from each subject who was 8 years and above or parent(s) or legal guardian for children below 8 years, following which his/her eligibility for inclusion was further evaluated.

On arrival at the clinic, potential subjects were interviewed and data concerning the age, duration of HIV status, otorrhoea and history of antibiotic usage were taken. Only patients who had not received systemic or topical antibiotics, in the last 72 hrs, were included. Otoscopy was carried out for each patient and the states of the ear canals and ear drums recorded. Exudates in the ear canals were characterized.

Two separate ear swab samples were collected by the author using single-use minitip culture standard sterile swab sticks before cleaning the ear canals of any debris/exudates. Much care was taken to avoid surface contamination. The samples were encased in air tight plastic tubing and then transported to the microbiology laboratory. One of the swabs was sent for bacteriological study. The bacterial swabs were placed on MacConkey agar plates and incubated for 48 hours after which bacterial isolates were identified using standard microbiological methods as outlined below.

Antimicrobial sensitivities were also carried out for the pathogenic bacterial isolates using the Kirby Bauer disc diffusion technique on Muller-Hinton agar and commercial antibiotic discs (Ovoid UK) were used for microbial testing. The second swab sample was sent for fungal studies, after staining with potassium hydroxide.

The antibiotic discs used were Ofloxacin (30mcg), Ceftriaxone (20mcg), Ciprofloxacin (5mcg), Cefazidime (30mcg), Cefixime (30mcg), Cefuroxime (30mcg), Augmentin (30mcg), Cotrimoxazole (1.25/23.75mcg), Amoxycillin (10mcg), Tetracycline (30mcg), Gentamycin (10mcg), Erythromycin (10mcg) and Cloxacillin (5mcg).

The antibiotic disc impregnated culture plates were incubated overnight at 37°C. The diameter zone of inhibition was measured and recorded as resistant or susceptible according to the national Committee for Clinical Laboratory Standards interpretation criteria.

Data collected was outlined in tabular form as shown in Tables 1 to 4 and analyzed using epi info version 3.5.1 in which P values less than 0.05 were considered significant at 95% confidence limit.

**RESULTS**

During the study period, a total of 100 children (200 ears) ranging between 3 months and 15 years were evaluated for organisms present in their chronically discharging ears. 50 of these (100 ears) were confirmed HIV positive children while the other 50 (100 ears) were controls. The mean age for HIV positive children was 6 years 11 months ± 4 years 9 months while that of the control group was 7 years 11 months ± 3 years 11 months (P=6.2). Male to female ratio was 1:1 for both the study and control groups.

The CSOM was bilateral in 27 (54%) of the study group and in 35 (70%) of the control thereby making a total of 162 ear swab cultures available for analysis (77 in study group and 85 in the control).

In table 1 the distributions of identified microorganisms were compared between HIV infected children and non-infected children. The commonest bacteria isolate in both groups was Pseudomonas aeruginosa, but the difference in the frequencies of isolation between the two groups was statistically significant (p =0.005). However there is predominantly more fungal isolates in the HIV negative group than the positive children as shown in table 2 (P <0.001).

The in vitro antibiotic sensitivity pattern of common antibiotics tested against the isolates recovered from ears of 50 HIV infected children was outlined in Table 3.

The group of 4-quinolone antibiotics namely: Ofloxacin (90%) and Ciprofloxacin (88%) demonstrated the highest level of sensitivity. This was followed by Ceftriaxone (71%), Gentamycin (65%) and Cefazidime (50%). The Penicillin based antibiotics demonstrate a
much lower sensitivity with Amoxycillin (10%) being the least sensitive in this category. Tetracycline (11%) and Cotrimoxazole (5%) are the least sensitive antibiotics against isolates of CSOM in HIV infected children.

Table 4 showed the in vitro antibiotic sensitivities of microbes isolated from discharging ears in non HIV infected children. The antibiotic sensitivity patterns in the HIV positive and negative groups as outlined in tables 3 and 4 respectively indicated that overall the bacterial isolates among the HIV positive children showed highest susceptibility to Ofloxacin and Ciprofloxacin, whereas antibiotic susceptibility was highest with Gentamicin among the HIV negative children. The Penicillin was the least effective antibiotics in both groups.

In table 5, a comparison is made between the in vitro antibiotic sensitivities of microbes in discharging ears of the HIV infected and non infected children. With the exception of the 4-quinolone Ciprofloxacin all the other classes of antibiotics tested demonstrated greater sensitivity in discharging ears of non HIV infected children compared to their HIV infected counterparts (p<0.001).

**DISCUSSION**

Chronic Suppurative Otitis Media has been reported to account for as much as 85.7% of discharging ears in HIV infected children. Such a high prevalence rate compared to those in non infected children prompted this study on the organisms fuelling chronicity in HIV infected children to see if they were different from those of non infected children.

In the present study, Pseudomonas aeruginosa was the most common bacterial isolate in HIV infected children with a prevalence of 23.4%. Though Pseudomonas aeruginosa was also the most prevalent in non infected children at 5.9%, the difference in prevalence rates was statistically significant (P=0.005). This suggests that the background immunosuppression may create conditions that favor the chance of isolating Pseudomonas aeruginosa in more ear discharges. The prevalence of Pseudomonas aeruginosa in ear discharges of children infected was also found to be the highest at 24.4% in the ARROW trial in Uganda and Zimbabwe. In contrast to our findings, Ugochukwu et al reported that Proteus spp (25%), Staphylococcus aureus(25%) and Klebsiella spp (25%) were the most frequently isolated organisms, with Pseudomonas spp constituting only 17.9% of the isolations.

The prevalence of Proteus mirabilis (14.3%) and Staphylococcus aureus (16.9%) also agreed with the findings in the ARROW study which as well noted that Staphylococcus aureus was the least sensitive in this category. Tetracycline (11%) and Cotrimoxazole (5%) are the least sensitive antibiotics against isolates of CSOM in HIV infected children.

A similar trend of greater prevalence in the ear discharges of HIV infected children was evident for Proteus mirabilis and Staphylococcus aureus. Proteus mirabilis was also significantly more likely isolated in HIV infected children than in non infected children (P=0.1) and for Staphylococcus aureus, P=0.05. There was no statistically significant difference in the prevalence of Klebsiella sp, Streptococcus pneumonia and Coliform bacilli between the ear discharges in HIV infected and non infected children. This could be as a result of Streptococcus pneumonia mediated URTI in children generally with the great propensity of ascending infection to the middle ear. Therefore it may not be a surprise to find it in equal frequency irrespective of the immune status of the child. For Klebsiella sp and Coliform bacilli, it may be tempting to assume that immunocompromise does not favor the infectivity of these organisms any more than it does in the immunocompetent.

The fungal isolate A. fumigatus has a greater chance of being isolated from ear discharges of non infected children where the prevalence is as much as 29.4%. This compared to a prevalence of 6.5% was statistically significant (P=0.001). A. fumigatus is a well known contaminant of chronic otorrhoea that causes only superficial mycoses. Based on the foregoing observation, it is possible that immunosuppression such as conferred by HIV does not encourage increased prevalence of the superficial mycoses but may rather encourage the causative organisms to be more virulent though this bears further research as there is dearth of literature on this subject matter. However, it is also possible that prior exposure to antibiotics in these children had altered the composition of the middle ear flora with elaboration of opportunistic fungal infections since virtually all subjects had used one form of antibiotics or the other before presentation.

There was no statistical difference in the prevalence of Yeast Spp and Candida albicans between the HIV infected and non infected children. Overall, Bacteria isolates were more likely encountered in ear discharges of HIV infected children than in non infected children who showed more significant tendency towards fungal isolates.

Bacterial isolates in CSOM demonstrate a high sensitivity to the quinolone group of antibiotics. Despite concerns regarding their use in paediatric practice, trials in treatment of otitis media have been carried out. Findings in the current study also showed that in both HIV infected and the non infected
Indeed, all the other groups of antibiotics tested such as the Penicillins, the Macrolides and the Sulphonamides showed that the bacterial isolates were less sensitive to them in CSOM in HIV infected children than they were in their non HIV infected counterparts (P=0.001).

CONCLUSION

Our study indicated that ear discharges of HIV infected children most commonly grow Pseudomonas aeruginosa followed by Proteus mirabilis and Staphylococcus aureus. Contrary to expectation, fungal isolates were less frequently encountered from the immunocompromised HIV infected children and the most prevalent fungal isolate was Aspergillus flavus.

The quinolone group of antibiotics remain the drug of choice in the treatment of CSOM in children irrespective of the presence of immunodeficiency.

Table 1: Distribution of Ear Discharge Microbial Isolates Between HIV Infected and Non infected Children

| Micro-organisms         | HIV Infected Children | Non-infected Children | (P-value) |
|-------------------------|-----------------------|-----------------------|-----------|
|                         | Number (%)            | Number (%)            |           |
| Pseudomonas aeruginosa  | 18(23.4)              | 5(5.9)                | (0.005)   |
| Proteus Mirabilis       | 11(14.3)              | 5(5.9)                | (0.1)     |
| Staphylococcus aureus   | 13(16.9)              | 5(5.9)                | (0.05)    |
| Klebsiella spp          | 7(9.1)                | 6(7.1)                | (0.75)    |
| Streptococcus pneumonia | 5(6.5)                | 6(7.1)                | (0.90)    |
| Coliform bacilli        | 5(6.5)                | 6(7.1)                | (0.90)    |
| Aspergillus flavus      | 5(6.5)                | 25(29.4)              | (0.001)   |
| Yeast spp               | 5(6.5)                | 11(12.9)              | (0.25)    |
| Candida albicans        | 0(0.0)                | 11(12.9)              | -         |
| No Isolate              | 8(10.4)               | 5(5.9)                | (0.5)     |

Total 77(100.0) 85(100.0)

P values < 0.05 were significant at 95% confidence limit
Table 2: Organism Groups From Discharging Ears

| MICROBE    | STUDY          | CONTROL        | (P-value) |
|------------|----------------|----------------|-----------|
|            | n(%)           | n(%)           |           |
| Bacteria   | 59(76.6)       | 33(39.0)       | (0.001)   |
| Fungi      | 10(13.0)       | 47(55.3)       | (0.001)   |
| No Isolate | 8(10.4)        | 5(6.0)         | (0.50)    |
| Total      | 77(100.0)      | 85(100.0)      |           |

P values < 0.05 were significant at 95% confidence limit

Table 3: In vitro Antibiotic Sensitivity Pattern of Microbes Isolated in 50 HIV Infected Children

| Drugs Sensitivity | Total No of isolates tested | No Sensitive | No Resistant | (%)    |
|-------------------|-----------------------------|--------------|--------------|--------|
| Ofloxacin         | 22                          | 19           | 3            | 90.476 |
| Ceftriaxone       | 14                          | 10           | 4            | 71.428 |
| Ciprofloxacin     | 18                          | 16           | 2            | 88.888 |
| Ceftazidime       | 4                           | 2            | 2            | 50.000 |
| Amoxycillin/Clavulanate | 23                  | 6            | 17           | 26.086 |
| Cotrimoxazole     | 18                          | 1            | 17           | 5.555  |
| Amoxycillin       | 10                          | 1            | 9            | 10.000 |
| Tetracycline      | 18                          | 2            | 16           | 11.111 |
| Gentamycin        | 23                          | 15           | 8            | 65.217 |
| Erythromycin      | 7                           | 2            | 5            | 28.571 |
| Cloxacillin       | 8                           | 2            | 6            | 25.000 |
Table 4: In vitro Antibiotic Sensitivity Pattern of Microbes Isolated Ears of in HIV non-Infected Children

| Drugs Sensitivity       | Total No of isolates tested | No Sensitive | No Resistant | (%)   |
|-------------------------|-----------------------------|--------------|--------------|-------|
| Ciprofloxacin           | 18                          | 15           | 3            | 81.5  |
| Ceftazidime             | 10                          | 7            | 3            | 70.6  |
| Amoxycillin/Clavulanate| 14                          | 8            | 6            | 55.8  |
| Cotrimoxazole           | 18                          | 12           | 6            | 64.7  |
| Erythromycin            | 18                          | 13           | 5            | 72.2  |
| Gentamycin              | 18                          | 16           | 2            | 88.3  |
| Amoxycillin             | 15                          | 10           | 5            | 55.5  |

Table 5: Comparison of Sensitivities of Commonly used Antibiotics

| Antibiotic               | % Sensitivity (Non Infected) | % Sensitivity (HIV Infected) | Chi Square (P value) |
|--------------------------|------------------------------|-----------------------------|----------------------|
| Ciprofloxacin            | 81.5                         | 88.9                        | 1.976 (0.25)         |
| Amoxycillin/Clavulanate | 55.8                         | 26.1                        | 18.6 (0.001)         |
| Cotrimoxazole            | 64.7                         | 5.6                         | 76.0 (0.001)         |
| Gentamycin               | 88.3                         | 65.2                        | 14.7 (0.001)         |
| Ceftazidime              | 70.6                         | 50.0                        | 8.33 (0.005)         |
| Amoxycillin              | 55.5                         | 10.0                        | 47.85 (0.001)        |
| Erythromycin             | 72.2                         | 28.0                        | 38.72 (0.001)        |

P values < 0.05 were significant at 95% confidence limit
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