Etiologic agents of otitis media in Benin city, Nigeria

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Abstract
Background: Otitis Media continues to be a major presentation in the ear, nose and throat clinic. Aim: This study aimed to isolate, characterize and identify the bacteriological and mycological etiologic agents of otitis media in Benin city. Patients and Methods: Ear discharge from 569 (299 males and 270 females) patients diagnosed clinically of otitis media between August 2009 and August 2010 were processed to recover the bacterial and fungal etiologic agents. Susceptibility test was performed on all bacterial isolate. Result: Pseudomonas aeruginosa (28.3%) was the predominant bacteria isolate causing otitis media followed by Staphylococcus aureus (21.0%), Klebsiella sp (8.9%), Proteus sp (8.2%), Alkaligenes spp (4.3%), Streptococcus pneumoniae (3.9%), Escherichia coli (3.0%) and Citrobacter freundii (1.7%). Fungi isolated were Aspergillus niger (9.2%), Candida albicans (5.4%), Candida tropicalis (3.0%), Aspergillus flavus (2.1%) and Candida parasilopsis (1.5%). 413 had a single organism isolated from the middle ear culture while twenty (3.51%) patient had mixed organisms isolated. Infection was highest among 0 - 5 years, and lowest among aged 18 - 23. All bacterial isolates were poorly susceptible to the antibacterial agents. Conclusion: The study uncovers a high frequency of bacteria associated otitis media with the finding of fungi too as a significant etiologic agent.

Keywords: Otitis media, etiologic agents, prevalence, Benin city.

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Introduction
Otitis Media (OM) is highly prevalent worldwide [1]. Otitis media is inflammation of the middle ear drum and the inner ear, including a duct known as the Eustachian tube [2]. Otitis media is very common in children [3]. Children below the age of seven years are much more susceptible to otitis media since the Eustachian tube is shorter and at more of a horizontal angle than in the adult and this is also because they have not developed the same resistance to bacteria, fungi and viruses as found in adults [4]. Exposure to smoke crowded living conditions and low socio-economic class are among the risk factor of otitis media [5].

Over 50 percent of the cases of otitis media are caused by bacteria [6]. Occasionally, otitis media may be caused by fungi, viruses, Mycoplasma pneumoniae and Chlamydia trachomatis [7]. A number of studies have reported on the prevalence of otitis media [5, 8]. However, majority of these studies have focused on clinical diagnosis with little or no report on the bacteriological and mycological etiology of otitis media. There exists a dearth of knowledge on a study carried out in this area on the prevalence of otitis media incorporating bacteriological and mycological examination. Against this background, this study aimed to isolate, characterize and identify the bacteriological and mycological etiologic agents of otitis media in Benin City.

Patients and Methods
Benin City ‘The Cradle of Black civilization’ as it is popularly known, is an urban area and capital city of Edo state, southern Nigeria. The city is approximately 25miles north of the Benin River and situated 200miles by road.
east of Lagos. It is home to about 1,147,188 million individuals [9], who are predominantly ‘Edo’s’ with smaller population of other tribal groups from neighboring states. This study was carried out during the period August 2009-August 2010 in Benin City at the Ear, Nose and Throat Clinic in University of Benin Teaching Hospital, Benin City, Nigeria (UBTH). UBTH is a tertiary hospital with a referral status and serves the many private hospitals and government owned specialist hospitals in the state and neighboring states. Five hundred and sixty nine patients with clinical manifestations suggestive of Otitis Media (Discharging ears) (299 males and 270 females) were investigated. The age range of patients was (1 day - 70 years).

Collection of specimens
The samples were collected with sterile swab sticks which were properly labeled for each patient. The swab sticks were taken to the Medical Microbiology Laboratory, University of Benin Teaching Hospital for analysis, within 4 – 6 hours after collection.

Isolation of bacteria
Specimens were inoculated onto blood, chocolate and MacConkey agar plates. All plates were incubated for 24 hours aerobically with the exception of chocolate agar that was incubated in a candle jar. Emergent colonies were identified according to standard bacteriological methods [10]. Disc susceptibility test was performed according to National Committee for Clinical Laboratory Standards (NCCLS) [11].

Isolation of fungi
The swab sticks were streaked directly on the well labeled Sabouraud Dextrose Agar (SDA) plates and incubated at room temperature. The growth was identified based on their morphological and cultural characteristics and microscopic examination was done using lactophenol blue staining technique [12]. Germ tube method and clomidospore formation where used to differentiate Candida albicans from other species of Candida. API 20C (Biomerieux France) was also used for the speciation of Candida.

Statistical analysis
Data were analyzed using chi square (X2) test using the statistical software SPSS v 16. $P$ value of less than 0.05 was considered significant.

Results
From the 569 patients enrolled in the study, there were 466 isolates. Four hundred and thirteen patients (72.6%) had a single organism isolated from the middle ear culture, while twenty (3.51%) patients had mixed organisms isolated. Pseudomonas aeruginosa was the most prevalent etiologic agent of otitis media while the least was Candida parasilopsis (Table 1). Patients that were 5 years or younger had significantly higher prevalence of otitits media compared with other children and adults; but there was no significant difference in gender in the prevalence of otitis media (Table 2). The antibiotic susceptibility profile of the bacteria isolates revealed high level resistance (Table 3). Lincomycin, erythromycin, ampicillin and tetracycline were not active against any of the bacterial isolate.

| Organisms Isolated          | Total Number of Isolates (%) |
|----------------------------|------------------------------|
| Staphylococcus aureus       | 98(21.0)                     |
| Streptococcus Pneumoniae    | 18(3.9)                      |
| Klebsiella spp              | 40(8.9)                      |
| Proteus spp                 | 38(8.2)                      |
| Pseudomonas aeruginosa      | 132(28.3)                    |
| Escherichia coli            | 14(3.0)                      |
| Alkaligenes spp             | 20(4.3)                      |
| Citrobacter freundii        | 8(1.7)                       |
| Aspergillus niger           | 43(9.2)                      |
| Candida albicans            | 25(5.4)                      |
| Candida tropicalis          | 14(3.0)                      |
| Candida parasilopsis        | 7(1.5)                       |
| Aspergillus flavus          | 10(2.1)                      |
| Total                      | 466                          |

| Sex         | No. Tested | No. Infected (%) |
|-------------|------------|------------------|
| Males       | 293        | 218(74.4)        |
| Females     | 276        | 213(77.1)        |
| Age (Years) |            |                  |
| 1Day – 5    | 193        | 185(95.9)        |
| 6 – 11      | 97         | 79(81.4)         |
| 12 – 17     | 73         | 60(82.2)         |
| 18 – 23     | 85         | 33(57.7)         |
| 24 – 29     | 40         | 20(50.0)         |
| 30 – 35     | 40         | 31(77.5)         |
| >36         | 41         | 26(63.4)         |

Discussion
Otitis media is frequently encountered in tropical and subtropical areas [13]. Diagnosis of this disease is often based solely on the clinical symptoms. The objectives of this study were to determine the prevalence, bacterial and fungal etiologic agents associated with Otititis media in Benin City, Nigeria. Otitis media was found in (76.1%) of cases of patients presenting with clinical manifestations of otitis media from a total of 569.

It has been reported that children less than 5 years are more prone to otitis media due to shorter and more horizontal Eustachian tube, lower immunity of children compared to adults and the fact that bacteria adhere better to epithelial cells of children that adults [3, 4, 14].This was observed in this study as children 5 years and younger had a higher prevalence of otitis media (95.9%). In this study there was no significant difference between male and female patients in the prevalence of otitis media (74.4%, 77.1%), respectively. This result is in agreement with others [15]. Some studies have reported male gender to be a significant risk factor for acquisition of otitis media [4, 14]. The most common pathogen isolated from the ear canal of patients with otitis media is bacteria [15].
Table 3 Antibiotic susceptibility profile of the bacterial isolates

| Bacterial isolate             | OFX  | CN  | CXM | L   | AMX | E   | AUG | AMP | TE | OXC |
|-------------------------------|------|-----|-----|-----|-----|-----|-----|-----|----|-----|
| *Staphylococcus aureus*       | 53(54.1) | 35(35.7) | 13(13.3) | 0   | 0   | 0   | 21(21.4) | 0   | 0  | 16(16.3) |
| *Streptococcus pneumoniae*    | 9(50.5) | 7(38.9) | 1(5.6) | 0   | 1(5.6) | 0   | 11(61.1) | 0   | 0  | 2(11.1) |
| *Klebsiella spp*              | 16(40.0) | 8(20.0) | 7(17.5) | 0   | 0   | 0   | 7(17.5)  | 0   | 0  | 0    |
| *Proteus spp*                 | 11(28.9) | 6(15.8) | 6(15.8) | 0   | 0   | 0   | 3(7.9)   | 0   | 0  | 0    |
| *Pseudomonas aeruginosa*      | 91(68.9) | 46(34.8) | 0   | 0   | 0   | 0   | 0    | 0   | 0  | 0    |
| *Aspergillus niger*           | 5(35.7) | 2(14.3) | 3(21.4) | 0   | 0   | 0   | 4(28.6)  | 0   | 0  | 0    |
| *Candida albicans*            | 7(35.0) | 5(25.0) | 1(5.0) | 0   | 0   | 0   | 2(10.0)  | 0   | 0  | 0    |

| Citrobacter freundii          | 3(37.5) | 2(25.0) | 0   | 0   | 0   | 0   | 1(12.5) | 0   | 0  | 0    |

OFX: Ofloxacin, CN: Gentamycin, CXM: Cefuroxime, L: Lincomycin, AMX: Amoxicillin, E: Erythromycin, AUG: Augmentin, AMP: Ampicillin, TE: Tetracycline, OXC: Oxacillin.

P. aeruginosa was the predominant isolate causing otitis media generally. This is in agreement with previous studies in Nigeria [14, 16], but differs from studies in developed countries were *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catarrhalis* predominate [17, 18]. Other bacteria isolates recovered in descending order were *Staphylococcus aureus*, *Klebsiella spp*, *Proteus spp*, *Streptococcus pneumoniae*, *Escherichia coli*, *Alkaligenes spp* and *Citrobacter freundii*.

Fungal species with the highest frequency was *Aspergillus niger* while the fungi with the lowest frequency were *Candida albicans*, *Aspergillus flavus*, *Candida tropicalis* and *Candida parasilopsis*. *Aspergillus niger* is an opportunistic filamentous fungi, it has been identified as the cause of bilateral otomycosis [19]. *Aspergillus niger* grows on cerumen, epithelial scales and detritus deep in the external canal. The resulting accumulation of these inflammatory materials along with cerumen and fungal debris result in plug formation, which is extremely significant and usually leads to diminished hearing ability; pruritis, irritation of the surface layer of the external ear itself is a predisposing factor for bacterial colonization. There may be superficial erosion of membranes.

The susceptibility profile of the bacteria isolates recovered revealed high level resistance. Prescriptions of antibiotics without laboratory guidance as well as over the counter sales of antibiotics without prescription is common in the Nigerian setting and have been suggested as possible reasons for increase resistance observed in the country [20, 21].

Conclusion

In conclusion, an overall prevalence of 76.1% of otitis media was observed among patients attending the ear, nose and throat clinic in University of Benin Teaching Hospital, Benin City, Nigeria. *Pseudomonas aeruginosa* and *Aspergillus niger* was the predominant bacterial and fungal isolate causing otitis media. Susceptibility rates to antibiotics were generally poor.

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