Asian Pacific Journal of Tropical Disease

Microbiological research https://doi.org/10.12980/apjtd.7.2017D6-242 ©2017 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Bacterial etiologies, antibiotic susceptibility patterns and risk factors among patients with ear discharge at the University of Gondar Hospital, Northwest Ethiopia

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ARTICLE INFO

Objective: To determine the etiologic agent, antibiotic susceptibility patterns and possible risk factors among patients who had ear infection.

Methods: A cross-sectional study was conducted between February 2014 and June 2014 at the University of Gondar Hospital, Northwest Ethiopia among patients with ear discharge. Data were collected by using a semi-structured questionnaire. Ear discharge was inoculated on blood agar, chocolate agar and MacConkey agar plates. Standard procedures were used for identification of etiologic agents. Antibiotic susceptibility tests were performed on Mueller-Hinton agar. Data were entered and analyzed by using SPSS version 20 and \( P < 0.05 \) was considered statistically significant.

Results: Out of 167 patients, 97 (58.1%) were males. The mean age of the study participants was 23.3 years with the age ranging from 4 months to 78 years. Among the 167 study participants, 104 (62.4%) were from patients with chronic ear infection. The prevalence of Gram-negative bacteria was high. Proteus species and Pseudomonas species were the most common isolate. Age and sex had statically significant association with ear infection \( (P = 0.013) \). Multidrug resistances were observed in 100% and 88.4% Gram-positive and Gram-negative bacterial isolates, respectively. The most commonly isolated Gram-positive bacterium was Staphylococcus aureus [43 (25.1%)]. Among the Gram-negative isolates, Proteus species [43 (25.1%)] were the most common isolate. Age and sex had statically significant association with ear infection \( (P = 0.013) \). Multidrug resistances were observed in 100% and 88.4% Gram-positive and Gram-negative bacterial isolates, respectively.

Conclusions: The prevalence of Gram-negative bacteria was high. Staphylococcus aureus, Proteus and Pseudomonas species were the most predominant. Alarming high rates of multiple drug resistance to majority of the commonly used antimicrobial agents were found. Therefore, treatment of ear infection should be based on culture and susceptibility test.

1. Introduction

Otitis media (OM), a kind of ear infection being the most common and occurring mostly in children, is an inflammatory disease of the mucosal lining of the middle ear caused by pathogenic microorganisms[1]. In developing countries, the natural course of the disease is different, leading further complications due to the low socio-economic status, overcrowding, poor hygiene and inadequate health care, and recurrent upper respiratory tract infection[2] increases the complications of OM[3]. Global burden of chronic suppurative otitis media (CSOM) ranges from 65–330 million individuals and 60% of them had hearing impairment. The World Health Organization categorizes countries into low (1%–2%) and high (3%-6%) prevalence of CSOM. Ethiopia belongs to the last group[4]. OM is more prevalent among children than adults because the children’s Eustachian tube is more horizontal and shorter with a
more flaccid cartilage which can easily impair its opening[5,6].

The etiologies of acute otitis media (AOM) are multi-factorial. Both viruses and bacteria are concerned with the ordinary respiratory bacteria often preceded by a viral infection[7]. These are respiratory pathogens that may have been insufflated from the nasopharynx into the middle ear through the Eustachian tube during upper respiratory tract infections[8]. In CSOM, the commonest organisms isolated include Pseudomonas aeruginosa, Escherichia coli (E. coli), Staphylococcus aureus (S. aureus), Streptococcus pyogenes (S. pyogenes), Proteus mirabilis (P. mirabilis) and Klebsiella species[9].

Various antibiotics, including tetracycline, chloramphenicol and penicillin are effective in treating OM. However, the current appearance of Streptococcus pneumoniae (S. pneumoniae) resistant for penicillin and the rising frequency of beta-lactamase producing strains of Haemophilus influenzae (H. influenzae) and Moraxella catarrhalis are concern regarding the use of amoxicillin as the traditional first line treatment for younger children with OM[10]. The high prevalence of OM and the increasing incidence of bacterial resistance to antibiotics necessitating continuous reevaluation of bacterial susceptibility to commonly used antibiotics is necessary[11]. The morbidity due to ear infection in Ethiopia is high and much attention is required in this regard.

Different epidemiological data from Ethiopia and elsewhere showed that the load due to ear infection in terms of physician visits, medications, absences from work and school was very high, indicating that for this problem it needs more attention. Therefore, this study was undertaken to analyze the prevalence of bacterial etiologic agents, antimicrobial susceptibility patterns and risk factors among patients with ear infections visiting ear, nose, and throat (ENT) clinics of University of Gondar Hospital, Northwest Ethiopia.

2. Materials and methods

2.1. Study design, area and period

A hospital-based cross-sectional study was conducted from February 2014 to June 2014. University of Gondar Hospital served for over six million people. The hospital served as a teaching as well as patient-care providing center for the region. It is one of the largest hospitals in the region. It has an intensive care unit with 18 beds and 13 wards with 518 beds including one ENT clinic. The ENT clinic gives service for about 2640 patients per year.

2.2. Study population

The study populations were all patients with ear discharge attending at the ENT clinic of University of Gondar Hospital during the study period.

2.3. Data collection and laboratory processing

2.3.1. Socio-demographic and clinical data

Socio-demographic data such as age, sex, residence and possible risk factors such as history of previous ear infections, upper respiratory tract infections, hearing loss, recurrent tonsillitis, and introduction of foreign bodies were collected from each study participant by using structured questionnaire conducted by the attending trained nurse.

2.3.2. Culture and identification

Ear discharge was obtained from each patient and kept in Amies transport media to maintain the viability of the bacteria until the specimen was processed. The samples were transported within 30 min to the bacteriology laboratory in the biomedical complex at the School of Biomedical and Laboratory Sciences. Specimens were inoculated directly on to blood, chocolate and MacConkey agar (Oxoid Limited). The blood and MacConkey agar plates were incubated in aerobic and chocolate agar in microaerophilic atmosphere using a candle atmosphere using a candle jar at 37 °C for 24–48 h.

Then, culture media were inspected for the growth of bacteria. Organisms were identified by their characteristic appearance on their respective media, Gram staining reaction and biochemical reactions using the standard method[12]. Members of the family Enterobacteriaceae were identified by using a series of biochemical tests such as carbohydrate fermentation, gas production, H₂S production, citrate utilization, urease production and lysine decarboxylation. For Gram-positive catalase, coagulase, bacitracin and optochin susceptibility test were used.

2.3.3. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was performed for all isolates according to criteria of the National Committee for Clinical Laboratory Standards by the disk diffusion method. A set of antibiotic discs (Oxoid, England) were then delivered on the surface of Muller-Hinton plate. The antimicrobials agents tested were in the following concentrations: ampicillin[11], amoxicillin-clavulanic acid (30 µg), ceftiraxone (30 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), erythromycin (15 µg), gentamicin (10 µg), kanamycin (30 µg), oxacillin (5 µg), penicillin (10 IU), streptomycin (10 µg), trimethoprim-sulphamethoxazole (25 µg) and vancomycin (30 µg). Penicillin, vancomycin, erythromycin and oxacillin were tested only for Gram-positive bacteria[13].

2.4. Quality control

Reference strains of E. coli (ATCC25922) and S. aureus (ATCC25923) were used to check the performance of culture media and antimicrobial susceptibility discs[12].

2.5. Statistical analysis

Data were checked, sorted, categorized and coded manually. The data were entered to SPSS version 20 statistical software for analysis purpose. Data cleaning was done before analysis. Frequencies and cross tabulations were used to summarize categorical variables. Both bivariate and multiple logistic regressions were employed to assess the association between outcome and explanatory variables. P < 0.05 at 95% confidence interval (CI) was considered statistically significant.
2.6. Ethical considerations

Ethical clearance was obtained from the Ethical Review Committee of School of Biomedical and Laboratory Sciences, College of Medicine and Health Sciences, University of Gondar. Permission letter was obtained from University of Gondar Hospital. Consent was secured from each study participant and their family or guardian for children.

3. Results

3.1. Socio-demographic information

Out of the 167 patients, 97 (58.1%) were males resulting in an overall male to female ratio of 1.3:1. The mean age of the patients was 23.3 year. The ages of participants ranged from 4 months to 78 years. Nearly a third, 60 (35.9%), of the patients were younger than 15 years. The majority of the study participants, 126 (75.4%) lived in urban area (Table 1).

| Socio-demographic characteristics | Number | Percentage |
|----------------------------------|--------|------------|
| Sex Male                         | 97     | 58.1       |
| Female                           | 70     | 41.9       |
| Age (year) < 15                  | 60     | 35.9       |
| 15–40                            | 81     | 48.5       |
| > 40                             | 26     | 15.6       |
| Residence Urban                  | 126    | 75.4       |
| Rural                            | 41     | 24.6       |

3.2. Magnitude of bacterial etiologic agents and clinical feature of participants

Out of the 167 study participants with ear discharge, 154 (92.2%) were showed bacterial growth. More than half of the isolates, 100 (58.5%) were Gram-negative bacteria, the rest, 71 (41.5%) were Gram-positive. A total of 171 bacterial pathogens were recovered from 167 discharges inoculated. Seventeen (10.2%) of the discharge showed mixed growth, while 137 (82.0%) showed single bacterial growth. The rest 13 (7.8%) had no bacterial growth (Table 2).

| Bacterial isolate | AOM | COM | Total |
|-------------------|-----|-----|-------|
| S. aureus         | 43  | 25.1|
| P. mirabilis      | 10  | 5.8 |
| Staphylococcus epidermidis (S. epidermidis) | 11 | 6.4 |
| S. pneumoniae     | 4   | 2.3 |
| S. pyogenes       | 3   | 1.8 |
| P. mirabilis      | 24  | 14.0|
| Pseudomonas spp.  | 23  | 13.5|
| Proteus vulgaris  | 16  | 9.4 |
| Klebsiella pneumoniae (K. pneumoniae) | 12 | 7.0 |
| Providencia rettgeri (P. rettgeri) | 3 | 1.8 |
| Klebsiella ozaenae (K. ozaenae) | 9 | 5.3 |
| Klebsiella oxytoca (K. oxytoca) | 4 | 2.3 |
| Citrobacter spp.  | 5   | 2.9 |
| E. coli           | 2   | 1.2 |
| Enterobacter cloacae (E. cloacae) | 1 | 0.6 |
| H. influenzae     | 1   | 0.6 |
| Total             | 171 | 100.0|

3.3. Possible risk factors of ear discharge

Both bivariate and multivariate logistic regression analyses were done to assess the possible risk factors of infections among ENT patients. Statistical significant associations were observed between sex and age. Males were 21 times more likely to have bacterial ear infection as compared to females [adjusted odds ratios = 21.200 (1.910–23.570)]. Those who had ages under 15 years were 4.3 times more likely to expose and those aged between 15 and 40 years were reduced the risk of ear infection by 99% as compared to those who have age over 40 years [(adjusted odds ratios = 4.300 (0.084–222.000) and 0.010 (0.000–0.220)], respectively. However, residence, chronic tonsillitis, upper respiratory tract infection, history of previous ear infection, long-term usage of antibiotic and the
introduction of foreign bodies were not statistically significant with ear discharge (Table 4).

3.4. Antimicrobial resistance patterns of bacterial isolates

The antimicrobial resistance patterns of the Gram-positive (n = 71) and Gram-negative bacterial isolates (n = 100) from ear infection against 13 and 9 antimicrobial agents were presented in Tables 5 and 6, respectively. The predominant isolate, S. aureus revealed high level of resistant to penicillin (38 (88.4%) and for ampicillin (41 (95.3%). All Gram-positive isolates showed 100.0% multiple drug resistance (resistance to two or more drugs). All of the isolates of S. aureus (100%) were sensitive to ciprofloxacin and one isolate of S. aureus was resistant to chloramphenicol.

The second predominant isolate, Protues spp. (n = 40) showed resistance to ampicillin (31 (77.5%), amoxicillin (20 (50.0%), chloramphenicol (19 (47.5%), kanamycin (7 (17.5%), streptomycin (21 (52.5%) and trimethoprim-sulfamethoxazole (3 (7.5%). The Gram-positive isolates were 100.0%, 97.5% and 86.5% susceptible to ceftriaxone, ciprofloxacin and gentamicin, respectively. Out of the 100 Gram-negative isolates, 80.0% strains were also identified as multiple drug resistant. In general, ceftriaxone, ciprofloxacin and gentamicin were the most effective drugs against Gram-positive and Gram-negative bacteria (Tables 5 and 6).

3.5. Multidrug resistance

Multidrug resistance to or more antibiotics was observed in 71 (100%) and 80 (80%) of Gram-positive and Gram-negative bacteria, respectively (Tables 7 and 8). The overall prevalence of multidrug resistance in both groups was 151 (88.3%).

Table 4

| Risk factors                          | Bacterial infection | Total | Crude odds ratio (95% CI) | AOR (95% CI) |
|--------------------------------------|---------------------|-------|---------------------------|--------------|
|                                      | Yes                 | No    |                            |              |
| Age < 15                             | 59                  | 1     | 4.200 (0.426–56.800)      | 4.300 (0.084–222.000) |
| 15–40                                | 70                  | 11    | 0.530 (0.110–2.560)       | 0.010 (0.000–0.220) |
| > 40                                 | 24                  | 2     | 2.710 (0.870–8.490)       | 21.200 (1.910–23.570) |
| Sex Male                             | 92                  | 5     |                            |              |
| Female                               | 61                  | 9     |                            |              |
| Residence Urban                      | 117                 | 9     | 0.550 (0.170–1.750)       | 2.410 (0.320–17.930) |
| Rural                                | 36                  | 5     |                            |              |
| Previous ear infection               | Yes                 | 110   | 0.217 (0.070–0.680)       | 0.720 (0.013–38.850) |
|                                      | No                  | 43    |                            |              |
| Upper respiratory tract infection    | Yes                 | 22    | 3.300 (1.010–10.790)      | 0.730 (0.059–8.890) |
|                                      | No                  | 131   |                            |              |
| Recurrent tonsillitis                | Yes                 | 6     | 13.600 (3.470–53.260)     | 0.340 (0.001–1.510) |
|                                      | No                  | 147   |                            |              |
| Introduction of foreign body to the ear | Yes              | 22    | 4.460 (1.410–4.110)      | 0.180 (0.010–3.380) |
|                                      | No                  | 131   |                            |              |
| Long-term antibiotic use             | Yes                 | 106   | 0.333 (0.109–1.012)       | 3.143 (0.379–26.070) |
|                                      | No                  | 47    |                            |              |
| Duration of infection                | 2 weeks             | 43    |                            |              |
|                                      | > 2 weeks           | 110   | 4.605 (1.460–14.520)      | 4.901 (0.160–147.870) |
| Hearing problem                      | Yes                 | 90    | 0.389 (0.124–12.160)      | 2.129 (0.290–15.570) |
|                                      | No                  | 63    |                            |              |

*: The presence of significance association.

Table 5

Antimicrobial resistance patterns of Gram-positive bacteria isolates among patients with ear discharge attending at Gondar University Hospital, Gondar, Northwest Ethiopia from February 2014 to June 2014.

| Antibiotics tested | Number of resistance isolate [n (%)] | Total [n (%)] |
|--------------------|--------------------------------------|---------------|
|                    | S. aureus (n = 43) | Entrocuccus spp. (n = 11) | S. epidermidis (n = 10) | S. pneumoniae (n = 4) | S. pyogenes (n = 3) |
| Penicillin         | 38 (88.4)           | 10 (90.9)       | 9 (90.0)                | 2 (50.0)              | 2 (66.7)          | 61 (85.9) |
| Ampicillin         | 41 (95.3)           | 6 (54.5)        | 2 (20.0)                | 2 (50.0)              | 2 (66.7)          | 61 (85.9) |
| Amoxicillin        | 30 (69.8)           | 3 (30.0)        | 2 (50.0)                | 2 (50.0)              | 0 (0.0)           | 37 (52.1) |
| Ceftriaxone        | 13 (30.2)           | 0 (0.0)         | 1 (10.0)                | 4 (100.0)             | 3 (100.0)         | 21 (29.5) |
| Ciprofloxacin      | 0 (0.0)             | 6 (54.5)        | 5 (50.0)                | 0 (0.0)               | 0 (0.0)           | 4 (14.3)  |
| Chloramphenic      | 1 (2.3)             | 0 (0.0)         | 0 (0.0)                 | 0 (0.0)               | 0 (0.0)           | 1 (1.4)   |
| Erythromycin       | 38 (88.4)           | 0 (0.0)         | 9 (90.0)                | 3 (75.0)              | 2 (66.7)          | 47 (66.2) |
| Gentamicin         | 19 (44.1)           | 0 (0.0)         | 4 (40.0)                | 3 (75.0)              | 0 (0.0)           | 13 (18.3) |
| Kanamycin          | 15 (34.9)           | 11 (100.0)      | 3 (30.0)                | 1 (25.0)              | 2 (66.7)          | 32 (45.1) |
| Oxacillin          | 18 (41.9)           | 11 (100.0)      | 4 (40.0)                | 4 (100.0)             | 0 (0.0)           | 37 (52.2) |
| Streptomycin       | 28 (65.2)           | 11 (100.0)      | 3 (30.0)                | 4 (100.0)             | 3 (100.0)         | 49 (69.0) |
| Cotrimoxazole      | 9 (20.9)            | 1 (9.1)         | 7 (70.0)                | 4 (100.0)             | 3 (100.0)         | 24 (33.8) |
| Vancomycin         | 19 (44.1)           | 1 (9.1)         | 7 (70.0)                | 1 (25.0)              | 3 (100.0)         | 31 (43.7) |
Many studies indicate that OM is an important public health problem especially in children and it is the most common indication for antibiotics prescribing[6]. The rate of bacterial isolation in discharging ear in our study was 91.6%. Similar results also reported in Addis Ababa (96.6%)[14] and Nigeria (76.1%)[15].

The clinical findings of patients with ear infection (predominantly CSOM), the peak-age prevalence (mostly children younger than 15 years of age) in our study (Table 4) are similar to findings of previous studies done in Ethiopia[16]. In developed countries, however, AOM was more commonly seen than CSOM[17]. This disparity partly may be explained by the lack of health awareness in the community, inadequate health infrastructure, and limited access to medical care and lack of specialists in developing countries.

Different studies indicate that OM is an important public health problem affecting all age groups and sexes. It is the most frequent problem affecting all age groups and sexes. It is the most frequent problem especially in children and it is the most common indication for antibiotics prescribing[6]. The rate of bacterial isolation in discharging ear in our study was 91.6%. Similar results also reported in Addis Ababa (96.6%)[14] and Nigeria (76.1%)[15].

The clinical findings of patients with ear infection (predominantly CSOM), the peak-age prevalence (mostly children younger than 15 years of age) in our study (Table 4) are similar to findings of previous studies done in Ethiopia[16]. In developed countries, however, AOM was more commonly seen than CSOM[17]. This disparity partly may be explained by the lack of health awareness in the community, inadequate health infrastructure, and limited access to medical care and lack of specialists in developing countries.

Different studies indicate that OM is an important public health problem affecting all age groups and sexes. It is the most frequent disease for a child to visit a physician[18] and age has been considered a predisposing factor for ear infection, especially in children ($P < 0.0001$)[19]. In the same way in this study, 35.9%
of OM patients were children less than 15 years old which is comparable to 45% and 46% reported in Ethiopia[20] and Nigeria[21], respectively. This indicates that children were more prone to have ear infections. The high prevalence may be related to different factors such as anatomy of the eustachian tubes which are short, broad and straight nature allowing the bacteria into the middle ear. Additionally, the nutritional status of the children and other health problems like upper respiratory tract infections which are important for contribution of middle ear bacterial infection are high in young children because of immature immunity[21].

In the present investigation, significant ear infection was detected in 58.1% vs. 41.9% (P = 0.013) (Table 4) in male and female patients, respectively. Similar findings have been reported in studies conducted in Gondar University Teaching Hospital, Ethiopia (63.7% vs. 36.3%) (P = 0.017)[18] and Nigeria[19]. To the contrary study done in Sudan, females were more affected by ear discharge. This may be due to the difference between ear cleaning habit of the males and females. In some traditions, females use cotton swabs to clean their ears and this may contribute for the introduction of microorganisms from the external surface to the middle ear[22].

However, in some other studies[23], there is no difference on the prevalence of ear infections between males and females.

Different studies showed that the etiologic agents of ear infection accounted for mainly to Gram-negative bacteria[24]. Similarly, the majority of the ear infection in the present study were caused by Gram-negative bacteria (58.4%) which is also similar to previous studies that have been conducted in Gondar (56.9%)[23] and Addis Ababa (60.5%)[14] and Nigeria (224 (65.9%))[15]. In the present study, the majority of the patients 137 (89%) had single bacterial isolates change through time due to differences in climate and geographical variations in different countries.

In conclusion, the prevalence of bacterial isolates accounted for 91.6% of the patients included in the study. Majority of the isolates were Gram-negative from discharging ear. S. aureus, Pseudomonas spp. and Proteus spp. were the most common isolated pathogens. Being male and age less than 15 years were factors associated with ear infection.

Majority of the bacterial isolates had multiple antibiotic resistant patterns. Hence, antibiotics susceptibility test is mandatory
before prescribing any antibiotics. Ceftriaxone, ciprofloxacin and gentamicin were the most effective drugs against the Gram-positive and Gram-negative bacteria.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgments**

We are gratefully to Dr. Legsse Garedew, Dr. Desalgne Mengesha, Mr. Yared Asfaw, Mr. Mastewal Baye and Mr. Semegne Handiebo for providing materials for the research project. We are grateful to Sr. Senayet Eyayou, data collector, for her cooperation during sample collection, Gondar University Hospital ENT clinic staff and study participants. We would like to thank the Department of Medical Microbiology, School of Biomedical and Laboratory Sciences Collage of Medicine and Health Sciences, University of Gondar for giving this opportunity. Last but not the least, we greatly acknowledge Amhara Regional Health Bureau for financial support (Grant No. 11213)

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