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Enumeration of the Antimicrobial Susceptibility Patterns of Different Bacterial Isolates from ENT Patients with Ear Infections

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ABSTRACT

Children are more susceptible to ear infection than adults worldwide especially in developing countries. In Bangladesh like other developing countries antibiotics is a common choice of treatment in an ear infection. This study was sought to determine causative agents both in right and left ear infection and their antibacterial susceptibility pattern from patients with an ear infection. Specimens of ear discharge were collected aseptically using a sterile swab and cultured on MacConkey's agar, blood agar and chocolate agar. After inoculating on appropriate culture media bacterial isolates were identified by their colony morphology, culture characteristics, Gram reaction and biochemical tests. In this study, a total of 70 positive cases of patients were included. Among these 27 (38.6%) were male, and 43(61.4%) were female. The prevalence of ear infection was significantly high in females (61.4%) than males (38.6%). The predominant bacterial isolates from positive culture were *Staphylococcus aureus* 43(61.4%), followed by *Pseudomonas spp.,* 13 (18.6%), *Streptococcus spp.,* 8(11.4%), *Proteus spp.,* 5(7.2%), and *Klebsiella spp*., 1(1.4%). The antibacterial agent like Amikacin and Gentamicin showed high level of antibacterial effect on all identified bacterial isolates. On the other hand, 98.6% of isolates were found highly resistant to Co-Trimoxazole and Flucoxacillin. Moreover, *Streptococcus spp., Proteus spp*., *Klebsiella spp*., and *Pseudomonas spp.,* were highly resistant to multiple drugs (more than 4). A high degree of antibiotic resistance was observed among most of the drugs used in this study. However, Amikacin and Gentamicin were highly effective against the isolated bacterial species. Therefore, culture and susceptibility tests are vital for the appropriate treatment of ear infection.

Keywords: Ear infections, Bacterial isolates, Antibiotic sensitivity, and Resistant patterns.

1. INTRODUCTION:

Otitis media or ear infection is usually an infection and/or inflammation of the middle ear that affects the tympanic membrane and ear discharge is the common symptoms (Bhargava *et al.,* 2005). It is the most common childhood condition for which antibiotic is

prescribed. One of the reasons why children are more likely than adults to get ear infections is that their Eustachian tubes are smaller and more horizontal than the tubes in most adults (Bluestone *et al*., 2001). About 65-330 million people suffer from ear infection worldwide and 60% of them had significant hearing impairment (Woodfield *et al*., 2008). Clinically, otitis media may be classified as acute, sub acute, and chronic suppurative otitis media (CSOM). Acute suppurative otitis media is a pyogenic bacterial infection of the middle air. It is a common disorder occurring at all ages and particularly in children. Following influenza or a common cold infection travels from the nose and nasopharynx to the middle ear usually by the Eustachian tube. The viral infection causes damage to the mucous membrane and then bacteria invade as secondary organisms. The most common organisms include *Haemophilus influenzae, Pneumococcus* (particularly in infants, diabetics, and in the aged) *Moraxella catarrhalis,* beta haemolytic *Streptococci, Staphlycoccus aureus,* non hemolytic *Streptococcus* etc (Moqbul, 2007).

Development and spread of resistant bacteria due to the indiscriminate and over use of antibiotics are global public health problem (Spellberg *et al*., 2008). In addition, antimicrobial resistance profile of bacteria varies among population because of the difference in geography, local antimicrobial prescribing practices and prevalence of resistant bacterial strains in a given area. So there should be up to date information on microbial resistance pattern at national and local levels to lead the sensible use of the existing antimicrobial drugs.

The bacteriological profiles of ear infection are well documented in developed world. However, so far a couple of studies have been conducted on bacteriological profile and antimicrobial sensitivity pattern in most developing countries. It is very essential to study the bacteriological profiles and their antimicrobial resistance pattern for appropriate management of patients with middle ear infection. World Health Organization registers antibiotic resistance as a increasing worldwide problem among the major threats to human health (WHO, 2014).

In the study area, there is no such type of recent data that shows the magnitude of the problem. Therefore the aim of this study was to determine the bacterial isolates and their drug susceptibility patterns from patients suspected of having a middle ear infection those were referred to Lab Zone and Hormone Center located in Tangail, Bangladesh.

2. MATERIALS AND METHODS:

A total of 70 specimens with clinically diagnosed case of acute and chronic suppurative otitis media by ENT specialist were included in present study.

2.1. Isolation and Identification - The ear discharge samples were collected aseptically using sterile cotton swab techniques from patients of the hospital and transported to the microbiology laboratory immediately for processing by Ameis transport media (Oxoid company). Upon receipt of the samples, they were inoculated on MacConkey, (5-10%) sheep blood agar plates, Chocolate agar plates and incubated aerobically at 37^0C for 24 hours. Discrete colonies of isolates were sub cultured on Nutrient agar to obtain pure culture for characterization and swarming feature of *Proteus spp*.

Pure isolates of bacterial pathogens were characterized by gram reaction, colony characteristics, and biochemical tests. Biochemical tests were carried out included: coagulase, catalase, urease, oxidase, carbohydrate fermentation, motility, gas production, citrate utilization and indole test. Bacterial species were identified as per the standard microbiological methods (Cheesbourgh, 2006; Abedin *et al*., 2020).

2.2. Antimicrobial Susceptibility Testing - Antimicrobial susceptibility testing was performed for all bacterial isolates on Muller Hinton agar by disc diffusion method (Abedin *et al.,*2020). Antimicrobial susceptibility of the both gram positive and gram negative bacterial isolates to commonly used antimicrobials was tested.

The pattern of drug sensitivity was elucidated as per Clinical and Laboratory Standards Institute (CLSI, 2012). *E. coli* ATCC 25922 and *S. aureus* ATCC 25923 were used as reference strain for quality control of antimicrobial susceptibility tests.

3. RESULTS:

In this study, a total of 70 specimens were included. Among theses 38.6% were male and 61.4% were female with majority under age 15 (**Table 1**). Among the positive cultures, 100 % had single bacterial infections. The majority of the species were Gram positive bacteria which accounted for 51(72.8%). The type of bacteria identified from positive ear swabs were *Staphylococcus aureus* 43 (61.4%), following *Pseudomonas spp.,* 13 (18.6%), *Streptococcus spp.,* 8 (11.4%), *Proteus spp.,* 5 (7.2%), and *Klebsiella spp.,* 1(1.4%). The most frequent bacterial species in both left and right ear infection was *Staphylococcus aureus* with the frequency of 30 (69.8%) and 13 (48.2%) respectively (**Table 2**).

Table 1: Age and sex specific distribution of pathogenic bacterial species among study subjects at Lab zone.

Bacterial species	**Sex**			**Age-group (Years)**				
	Male	Female	Total	0-12	13-24	25-36	>37	Total
Staphylococcus aureus	16(37.2)	27(62.8)	43(61.4)	16(37.2)	10(23.3)	9(20.9)	8(18.6)	43(61.4)
Streptococcus spp.	4(50.0)	4(50)	8(11.4)	3(37.5)	3(37.5)	1(12.5)	1(12.5)	8(11.4)
Pseudomonas spp.	5(38.5)	8(61.5)	13(18.6)	3(23.1)	2(15.4)	2(15.4)	6(46.1)	13(18.6)
Proteus spp.	1(20.0)	4(80)	5(7.2)	5(100)	0	0	0	5(7.2)
Klebsiella spp.	1(100)	0	1(1.4)	0	1(100)	0	0	1(1.4)
Total	**27(38.6)**	**43(61.4)**	**70(100%)**	**27(38.6%)**	**16(22.9%)**	**12(17.1%)**	**15(21.4%)**	**70(100%)**

Table 2: Infection site specific distribution of pathogenic bacterial species among study subjects.

Site of bacterial infections	**Sex**			**Number of bacterial isolates (%)**					
	Male	**Female**	**Total**	***Staphylococcus aureus***	***Streptococcus spp.***	***Pseudomonas spp.***	***Proteus spp.***	***Klebsiella spp.***	**Total**
Left Ear infections (Ear -LT)	18(41.9)	25(58.1)	43(100)	30(69.8)	3(7.0)	8(18.6)	2(4.6)	0	43(100)
Right ear infections (Ear-RT)	11(40.7)	16(59.3)	27(100)	13(48.2)	5(18.5)	5(18.5)	3(11.1)	1(3.7)	27(100)

Table 3: Overall resistance pattern of antimicrobial agents tested for bacterial species of ear infection.

Antibacterial agent	**Resistance pattern of bacterial isolates, n (%)**				
	S. aureus (n=43)	*Pseudomonas spp.* (n=13)	*Streptococcus spp.* (n=8)	*Proteus spp.* (n=5)	*Klebsiella spp,* (n=1)
Amikacin	1(2.3)	0(0)	0(0)	0(0)	0(0)
Gentamicin	3(7.0)	1(7.7)	1(12.5)	0(0)	0(0)
Ceftriaxone	7(16.3)	6(46.1)	0(0)	3(60)	1(100)
Tetracycline	21(48.8)	10(76.9)	8(100)	5(100)	1(100)
Cephalexin	19(44.2)	5(38.5)	8(100)	5(100)	1(100)
Cefixime	34(79.1)	13(100)	3(37.5)	3(60)	0(0)

Ciprofloxacin	23(53.5)	3(23.1)	3(37.1)	1(20)	1(100)
Clindamycin	18(41.9)	8(61.5)	8(100)	5(100)	1(100)
Cephradine	17(39.5)	6(46.1)	7(87.5)	5(100)	1(100)
Azithromycin	21(48.8)	2(15.4)	4(50.0)	2(40)	1(100)
Amoxiclave	30(69.8)	11(84.6)	4(50)	2(40)	1(100)
Co-Trimoxazole	43(100)	13(100)	8(100)	5(100)	0(0)
Cephuroxime	12(27.9)	10(76.9)	1(12.5)	2(40)	1(100)
Trimethoprime/ Sulphamethoxazole	23(53.5)	13(100)	4 (50)	2(40)	1(100)
Flucloxacilin	43(100)	13(100)	8(100)	5(100)	1(100)

A total of 15 commonly prescribed antibiotics in the study area were tested against the identified bacterial isolates for detecting antimicrobial resistance pattern. About 97.7% of identified bacterial isolates showed remarkable susceptibility to Amikacin and 72.8% to Gentamicin. On the other hand, 98.6% isolated bacterial species showed high degree of resistance to Co-Trimoxazole and Flucoxacillin (**Table 3**).

Staphylococcus aureus, showed high degree of susceptibility to Amikacin (97.7%) and Gentamicin (93.0%). However, 100% of these organisms were resistance to Co-Trimoxazole and Flucoxacillin. *Pseudomonas spp.,* also showed sensitivity to Amikacin (100%) and Gentamicin (92.3%) whereas 100% of these showed resistance to Cefixime, Co-Trimoxazole, Trimethoprime and Flucloxacilin. Similarly, *Streptococcus spp.,* showed high degree of sensitivity to Amikacin, Ceftriaxone (100%) and Gentamicin (87.5%). On the other hand, 100% of these showed resistance to Tetracycline, Cephalexin, Clindamycin, Co-Trimoxazole and Flucoxacillin. 100% of *Proteus spp.,* showed sensitivity to Amikacin and Gentamicin and also showed resistance to Tetracycline, Cephalexin, Clindamycin, Cephradine, Co-Trimazole and Flucloxacilin. Similarly *Klebsiella spp.,* (100%) showed sensitivity to Amikacin, Gentamicin, Cefixime and Co-Trimoxazole and showed resistant to Ceftriaxone, Tetracycline, Cephalexin, Ciprofloxacin, Clindamycin, Cephradine, Azithromycin, Amoxiclave, Cephuroxime, Trimethoprime, Flucloxacilin.

4. DISCUSSION:

Ear infection is the common problem to all age groups especially in children in the study area. In our study, majority of the patients were found under age 15 (38.6%). The factors responsible for children to be more susceptible to ear infection include their immune status, the shorter and horizontal nature of Eustachian tubes, frequent exposure to upper respiratory tract infections and malnutrition (Bluestone *et al*., 2001).

Current study showed significant difference on the prevalence of ear infection in genders. Females were more affected group (61.4%) than male (38.6%) similar findings were also reported by Hassan *et al*. (2007) and Akter *et al*. (2015). This may be due to ear cleaning habit of females. In some traditional habit, females use cotton swabs to clean their ear and this may contribute the introduction of microorganisms from the external surfaces in to the middle ear. However, in other studies, there is no difference on the prevalence of ear infections between males and females (Parry *et al*., 2002).

In this study, the predominant bacterial isolates were *Staphylococcus aureus* 43 (61.4%) followed by *Pseudomonas spp.,* 13 (18.6%) which is similar to other studies (Akter *et at*., 2015; Prakash *et al.,* 2013, and Abedin *et al.,*2020). However, in other studies in Ethiopia, predominant bacterial isolates were *Proteus spp.,* followed by *Staphylococcus aureus* (Abera *et al*., 2011). Probable explanation to this difference in isolated bacterial species is due to the effect of climate and geographical variation among the countries (Uddin *et al*., 2017). The other organisms which have been isolated in our study were *Streptococcus spp., Proteus spp., Klebsiella spp*.

The pattern of the bacterial isolates from ear infection and their antimicrobial susceptibility testing could make drug selection in antimicrobial chemotherapy more rationally. Therefore, the present study tried to

address antimicrobial susceptibility and resistance pattern of the commonly isolated bacteria from ear infection. This study revealed that *Staphylococcus aureus* showed sensitivity to Amikacin (97.7%) and Gentamicin (93.0%). Whereas, 100% of these organisms showed resistance to Co-Trimoxazole and Flucloxacilin. *Pseudomonas spp.,* also showed high degree of susceptibility to Amikacin (100%) and Gentamicin (92.3%). However, 100% of these were resistant to Co-Trimoxazole, Flucoxacillin and Trimethoprime.

Streptococcus spp., showed high degree of sensitivity to Amikacin, Ceftriaxone (100%) and Gentamicin (87.5%). On the other hand 100% of it showed resistance to Tetracycline, Cephalexin, Clindamycin, Co-Trimoxazole and Flucloxacilin. *Proteus spp.,* was also susceptible to Amikacin and Gentamicin (100%). However, 100% of it was resistant to Tetracycline, Cephalexin, Clindamycin, Cephradine, Co-Trimoxazole and Flucloxacilin.

Regarding to antibiogram, our study indicated that almost all bacterial isolates shown resistant to two and more commonly prescribed drugs in the study area. Multidrug resistance could be attributed by indiscriminate use of antibiotics (Ferede *et al.,*2001).

5. CONCLUSION:

The current study stated that the majority of the bacterial isolates were gram positive bacteria. *Staphylococcus aureus*, *Streptococcus spp., Pseudomonas spp.*, *Proteus spp*., and *Klebsiella spp.,* were commonly isolated etiology of ear infection both in children and adults. All of the isolates (98.6%) were resistant to two drugs (Co-Trimoxazole and Flucloxacilin) in the study area. Moreover, multidrug resistant isolates against four and more drugs were 52.6 % as well. However, Amikacin and Gentamicin were highly active against majority of the isolated bacteria. Therefore, culture and antibiotic susceptibility test is mandatory for proper treatment of ear infection in the study area.

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7. CONFLICT OF INTEREST:

Authors declare that no competing interest exists to publish the present research work.

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