Research Article

ANTIMICROBIAL RESISTANCE PATTERN AND BACTERIAL PROFILE FROM EAR CULTURES

Mehwish Sajjad Mehmood, Wajiha Iffat, Zona Irfan, Ali Hasnain Taqi, Kapil Dev Bhojwani, Shahzaib Ahmed, Ambreen Fatima
Daignostic and Research Laborastory, Dow University of Health Sciences, Karachi, Pakistan
Pakistan

Correspondence:
Mehwish Sajjad Mehmood, Diagnostic and Research Laboratory, Dow University of Medical and Health Sciences, Karachi, Pakistan.
Email:
mehwish.sajjad@duhs.edu.pk

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ABSTRACT

Ear infections cause hearing loss, which is a hallmark of many diseases. Ear infections are more common in children than in adults. This study was conducted to isolate different pathogens from ear culture and to evaluate the antibiotic susceptibility pattern of these organisms. This was a retrospective study conducted in Diagnostic and Research Laboratory, Dow University of Health Sciences, Karachi, Pakistan. All case histories including respiratory culture and antibiotic susceptibility results were analyzed. Culture and antibiotic susceptibility reports of patient were retrieved from record files of the department from 1st January 2021 to 31st December 2021. A total of 691 ear pus samples were identified, most frequent bacterial isolate was Pseudomonas aeruginosa (n=249, 36%) followed by Staphylococcus aureus (n=140, 20%), CONS (n= 77, 11%) and Proteus mirabilis (n=58, 8%). Penicillin had 100% and 98% resistance against Staphylococcus aureus and Coagulase negative staphylococcus, respectively, while there was 100% susceptibility against vancomycin. Colistin has 100% susceptibility against gram negative rods in our study. Growing bacterial resistance is of great concern as it alters the clinical prognosis of ear infection. Further-more this study help in choosing the correct empirical therapy for ear infections.

Key words: Ear Infections; Antibiotic Sensitivity; Anti-microbial Resistance; Karachi, Pakistan

INTRODUCTION

Ear infections are one of the most common causes of hearing impairment. It is estimated that 65-330 million people worldwide are with disable hearing impairment is due to ear infections (1). Over 60% of these cases are preventable, with infections contributing to up to 40% of preventable hearing loss (2, 3). Ear infections are more prevalent in children as compared to adults. Most of the kids affected from otitis media in their first year of life. Ear infections are of three types according to their location Otitis externa, otitis media and Otitis interna (4). Otitis externa is an external auditory canal infection with life time prevalence of 10% (5). It affects more commonly the adults than children, most commonly associated with swimming (6). Otitis media is the middle ear infection causing death of more than 20,000 people globally especially in poor countries. Otitis media leads to perforation of tympanic membrane, otitis externa and mastoiditis which affect motor hearing and balance disorder, intellectual problems and language development (7). Otitis Interna usually presents with loss of balance and sensory neural hearing impairment (1).

Causative organisms for these ear infections can be viruses, bacteria or fungi. More than 90% cases of otitis externa are due to bacteria, most commonly reported are Pseudomonas aeruginosa, Staphylococcus aureus, E.coli, Proteus mirabilis and Streptococcus viridans (8). Common Bacterial isolates that cause otitis media are Streptococcus pneumonia, Hemophillus influenzae, Morexella catarrillis and Streptococcus pyogenes (9). Otitis interna is mostly associated with the progression of middle ear infections and its expension to internal ear (10, 11). Ear cultures were used for bacterial isolation of these ear infections.

Therapeutic challenges posed by antimicrobial-resistant bacteria are a major global concern (12). Many experts view antimicrobial resistance (AMR) as a serious threat, with projections suggesting it could cause 10 million deaths annually by 2050. To address the global AMR crisis, the 68th World Health Assembly adopted five strategic objectives from the Global Action Plan in May 2015(13). The second strategic objective emphasizes research and surveillance, which are crucial for developing targeted intervention policies. To curb the spread of AMR requires continuous monitoring of resistance pattern through development of various national and international surveillance programs. World Health Organization (WHO) advocate for the use of traditional antimicrobial susceptibility tests (AST) in AMR surveillance efforts (14). Hence, this study was designed to identify the bacteriological profile and antibiotic resistance pattern in different samples of ear discharge.

METHODS

Study Design and Study Population

This retrospective descriptive cross-sectional study aimed to determine the prevalence of various otisis media pathogens and assess their susceptibility profiles. The ear swab specimens of both inpatients and outpatients at the Microbiology Laboratory of the Dow University Hospital in Karachi, Pakistan were collected from January 1, 2021, to 30th June, 2021 after receiving ethical approval from the institutional ethical council under form number IRB-2554/DUHS/EXEMPTION/2022/783. Study population consisted patients who exhibited clinical symptoms of ear infection as diagnosed by physicians and visited the inpatient and outpatient departments (OPDs) of the hospital. All age groups and both genders' ear pus samples were included. Samples that were duplicates or that lacked necessary information were excluded.

Identification and Confirmation of the Isolated Bacteria

Pus samples received subjected to Gram staining followed by inoculation on Chocolate agar, 5% Sheep blood agar, and MacConkey agar plates and incubated at 37 C for 18 to 24 hours. After 24 hours of incubation positive culture plates with bacterial colonies further processed for identification of bacterial isolates followed by antimicrobial susceptibility testing. Identification of isolates was performed according to standard bacteriological technique and distinguished based on gram stain, colony morphology, and biochemical tests such as Catalase, Coagulase, Bile esculin (BE), Optochin (OP) Bacitracin (BC) discs for Gram positive isolates and Oxidase, Urease, Citrate, Indole, Triple sugar iron tests for gram negative isolates. Analytical Profile Index (API 20E) was further used to identify gram negative rods.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed on Muller-Hinton agar by Kirby-Bauer Disk Diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines (15). For Gram positive bacteria, Penicillin (P) (10 units), Erythromycin (E) (15µg), Clindamycin (DA) (2µg), Neomycin (N) (30µg), Tetracycline (TET) (30 µg) and Trimethoprim/Sulphamethoxazole (SXT) (1.25/23.75 µg) were used. For Gram negative bacteria, Ampicillin (AMP) (10µg), Amoxicillin-Clavulanic acid (AMC) (30/10 µg), Piperacillin-Tazobactum (TZP) (100/10µg), Ceftriaxone (CRO) (30 µg), Ceftazidime (CAZ)(30 µg), Cefoperazone/ Sulbactam 2:1 (105 µg), Meropenem (30 µg), Ciprofloxacin (CIP) (5 µg), Levofloxacin (5µg), Gentamicin (CN) (10µg), Tobramycin (10µg), Minocycline (30µg), and Trimethoprim/Sulphamethoxazole (SXT) (1.25/23.75µg). The susceptibility breakpoints were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines (16). Escherichia coli (ATCC25922), Staphylococcus aureus (ATCC25923) and Pseudomonas aeruginosa (ATCC27853) were used as quality control strains for culture and susceptibility testing.

Statistical Analysis

The data was analyzed using Statistical Package for Social Sciences (version 20). Frequencies and percentages were calculated for variables such as gender, microorganism, and antibiotic susceptibility. Age was treated as a continues variable, and the mean and standard deviation were computed for analysis.

RESULTS

A total of 691 samples of ear culture were taken from suspected patients of ear infection. Majority of them were females (n=347, 50.3%) as compared to males (n=344, 49.7%). Majority of patients about 55% were belonged to the pediatric age group table1.

Most frequent bacterial isolate was Pseudomonas aeruginosa (n=249, 36%) followed by Staphylococcus aureus (n=140, 20%), CONS (n=77, 11%) and Proteus mirabilis (n=58, 8%). Others included Morganella moraganii (n=10, 2%), Enterococcus species (n=8, 1%), Enterobacter species (n=7, 1%), Pseudomonas species (n=6, 0.9%), Proteus vulgaris (n=4, 0.7%), Burkholderia species (n=2, 0.2%), Nocardia_(n=1, 0.1%) Klebseilla spp (n=1, 0.1%) and Serratia (n=1, 0.1%). Remaining organisms that were the etiological factor of ear infection are shown in Figure 1.

Antimicrobial susceptibility pattern of bacterial isolates was evaluated on the panel of antibiotics. Substantial numbers of bacteria isolated from culture were resistant to more than one antibiotic. In this study, penicillin had 100% and 98% resistance against Staphylococcus aureus and Coagulase negative staphylococcus, respectively. Gram positive had 100% susceptibility against vancomycin. Pseudomonas aeruginosa has shown increased resistance against tobramycin (60%) and levofloxacin (42%). E. coli has greater resistance above 50% against Penicillin, cephalosporins, fluroquinolones and Sulfamethoxazole. Colistin has 100% susceptibility against gram negative rods in our study. Klebsiella pneumoniae and Acinetobacter resistance pattern can be seen in Table 2.

Table 1: Demographic characteristics of respondents

Characteristics	Frequency (%)
Gender	
Male	344 (49.7%)
Female	347(50.3%)
Clinical isolates	
Gram-positive	127 (11%)
Gram-negative	986 (89%)
Age group	
0 months- 16 years	382(55%),
>16 years (Adult)	309(45%),

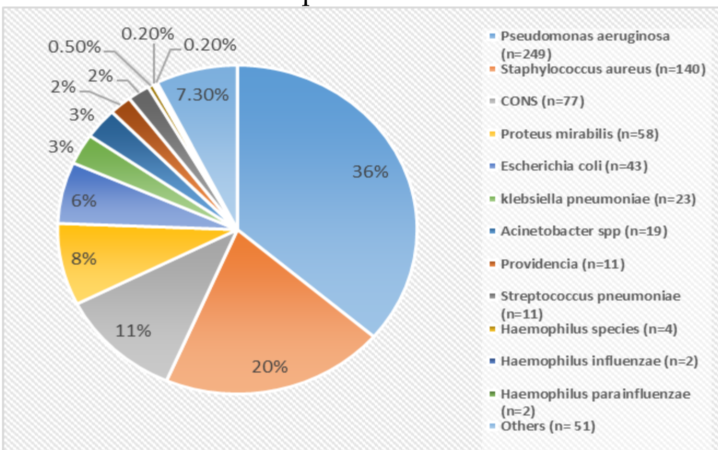

Figure I: Distribution of Pathogen isolated from ear cultures

Table 2. Antibiotic resistance profile (% Resistant) of bacterial isolates in ear culture

Antibiotics	*P.aeruginosa* (n=249)	*S.aureus* (n=140)	*CONS* (n=77)	*P.mirabilis* (n=58)	*E.coli* (n=43)
Penicillin and inhibitors					
Penicillin (P) (10 units)	NT	100% (n=140)	98 100% (n=77)	N	NT
Ampicillin (AMP) (10 µg)	NT	NT	NT	71% (n=41)	87% (n=37)
Amoxicillin clavulanate (AMC) (20/10 µg)	NT	64% (n=89)	54% (n=41)	49% (n=28)	26 43% (n=18)
Piperacillin tazobactam (TZP) (100/10 µg)	5 11% (n=28)	NT	NT	3% (n=2)	4 13% (n=6)
Cephalosporins					
Cefoxitin (FOX) (30µg)	NT	64	54 61% (n=47)	NT	NT
Cefuroxime CXM (30 µg)	NT	NT	NT	54% (n=31)	65% (n=28)
Cefixime (CFM) (5 µg)	NT	NT	NT	51 55% (n=32)	6473% (n=31)
Ceftriaxone (CRO) (30 µg)	NT	NT	NT	30 33% (n=19)	60 65% (n=28)
Ceftazidime	13% (n=32)	NT	NT	NT	NT

(CAZ) (30 µg)					
Carbapenem					
Meropenem (MEM) (10 µg)	4 27% (n=67)	NT	NT	9 2% (n=1)	7 10% (n=4)
Glycopeptides					
Vancomycin (VAN)	NT	0% (n=0)	0% (n=0)	NT	NT
Macrolides					
Erythromycin (E) (15 µg)	NT	69 52% (n=73)	77 80% (n=62)	NT	NT
Lincosamide					
Clindamycin (DA) (2 µg)	NT	30 23% (n=32)	33 21% (n=16)	NT	NT
Oxazolidinones					
Linezolid (LZD) (30 µg)	NT	0% (n=0)	0% (n=0)	NT	NT
Aminoglycosides					
Gentamicin (CN) (10 µg)	27 15% (n=37)	31 21% (n=29)	30 19% (n=15)	23% (n=13)	26 (12)
Tobramycin (TOB) (10 µg)	60 34% (n=84)	NT	NT	16 34% (n=20)	13 43% (18)
Neomycin (30 µg)	NT	74% (n=103)	42% (n=32)	NT	NT
Tetracycline					
Tetracycline (TET) (30 µg)	NT	45 34 (n=47)	34 39% (n=30)	NT	NT
Fluoroquinolones					
Ciprofloxacin (CIP) (5 µg)	33% (n=82)	NT	NT	44% (n=25)	52 60% (n=26)
Levofloxacin (LEV) (5 µg)	42 60% (n=150)	NT	NT	43 38% (n=22)	50 87% (n=38)
Folate pathway antagonist					
Trimethoprim-sulfamethoxazole (SXT) (1.25/23.75 µg)	NT	37% (n=52)	59% (n=45)	82 70% (n=41)	50 65% (n=28)
Lipopeptides (Polymyxins)					
Colistin (CT) (Colistin agar dilution) (2 µg/mL and 4 µg/mL colistin	0% (n=0)	NT	NT	NT	0% (n=0)

NT means Not tested

DISCUSSION

The current study aimed to assess the antimicrobial susceptibility profile of various microorganism (n=691) isolated from infected patients. In the present study, the majority of afflicted with otitis media were children (55%) compared to adult cases. This finding aligns with studies conducted in Ethiopia and Benin that reported otitis media has caused substantial health concern among children in developing countries (17-19). Children are more susceptible because of their shorter eustachian tube, more horizontal, and has more flexible cartilage, making it easier to obstruct the ear opening and leading to ear infections (17, 20). The increased prevalence is attributed further to their immune status, recurrent exposure to upper respiratory tract infections, and malnutrition (21).

Majority of children between the ages of 6 months and 3 years were prone to develop otitis media (22, 23). In the present study, the proportion of ear infection was found to be comparable among both females (50.3%) and males (49.7%). Similar findings were observed in other studies (24-26).
In the current study, Pseudomonas aeruginosa (n=249, 36%) were the mostly prevalent followed by S. aureus (n=140, 20%) Coagulase negative Staphylococcus CONS (n=77, 11%) and Proteus mirabilis (n=58, 8%). This is in accordance with the with research conducted worldwide has consistently identified Pseudomonas aeruginosa as the most isolated Gram-negative pathogen from ear discharge (27, 28) along with similar trends in other countries (29-31). The high isolation rate of Pseudomonas aeruginosa may be attributed to its ability to outcompete other organisms and its resistance to antibiotics. Additionally, Pseudomonas aeruginosa uses its pili to adhere to middle ear 's diseased epithelium. Once attached, it produces enzymes such as proteases to evade the body's normal defense mechanisms against infections (25).
Although in many studies Pseudomonas aeruginosa were not the most causative agent causing otitis media. A possible explanation for this discrepancy is that most respondents had otitis media for less than two weeks. Pseudomonas aeruginosa are typically isolated from chronic otitis media, lasting more than two weeks. Additionally, Certain bacterial growth mainly dependent upon specific times or seasons of the year(32). The results of this study indicated that S. aureus was the second most prevalent pathogen, found in 20.0% of children with ear discharge. This finding aligns with previous studies conducted in Ethiopia and in Nepal (21, 23, 33, 34). The likely reason for the high prevalence of S. aureus in middle ear infections is its widespread presence and the outstretched prevalence of resistant strains in the external auditory canal and upper respiratory tract (23).
Pseudomonas aeruginosa has shown increased resistance against tobramycin (60%), levofloxacin (42%). Ceftazidime (13%) Ciprofloxacin (33%), Gentamicin (27%) was effective in treatment of ear infection caused by Pseudomonas aeruginosa. Colistin, Meropenem and Piperacillin tazobactam showed to be highly effective against Pseudomonas aeruginosa. Fewer studies showed a comparable result where Pseudomonas aeruginosa was extremely sensitive to ciprofloxacin and gentamicin (18, 28). In a recent study, meropenem followed by gentamicin and ciprofloxacin was found to be effective against Pseudomonas aeruginosa (35). In the present study, most gram-negative clinical isolates were more sensitive to meropenem, Piperacillin tazobactam and Colistin. The high sensitivity rate of meropenem is likely be attributed to its infrequent prescription in our setting. Similar finding were also observed in a study conducted in Tanzania (31, 35).
Staphylococcus aureus is a predominant bacterial agent resulting in ear infections and poses a significant global challenge due to its resistance to many common treatments (36). The rise of MRSA (methicillin-resistant Staphylococcus aureus) has further complicated the infection management. According to the World Health Organization (WHO), individuals with MRSA infections are 64% more likely to die compared to those with drug-sensitive infections (37). In this study, penicillin had 100% and 98% resistance against Staphylococcus aureus and Coagulase negative staphylococcus, respectively while S. Aureus and Coagulase negative staphylococcus showed 45% and 35% resistance to Tetracyclines. The resistance to tetracyclines is comparatively less to a study conducted in Ethiopia (38) Gram positive bacteria Showed significant resistance to Neomycin, Erythromycin, Amoxicillin clavulanate, Cefoxitin, although Clindamycin and Gentamicin showed to be effective against Staphylococcus aureus and Coagulase negative staphylococcus respectively. The recent study conducted in Tanzania also reported high resistance to amoxicillin/clavulanic acid, ceftazidime, and trimethoprim-sulfamethoxazole (31, 35). Gram positive bacteria showed 100% susceptibility against vancomycin and Linezolid. A comparable results were also reported in a study conducted in Ethiopia (38). Although one of the study reported that Staphylococcus aureus showed high level of susceptibility to Vancomycin (21).
The major concern revealed in the present study is the rise in antimicrobial resistance, that poses a significant greatest global public health challenge. High levels of resistance to these antibiotics could be attributed to underfunded healthcare systems, inappropriate and irrational antibiotic usage, poor hygiene practices, inadequate infection prevention and control measures in hospitals, limited availability of rapid diagnostics, lack of awareness among users, improper prescribing practices inadequate awareness of on antimicrobial resistance among healthcare professionals, improper prescribing practices by physicians, insufficient surveillance of resistance development, self-prescription by patients, limited access to local antibiogram data and patient negligence(12, 39,

40). The current study aligns with the directives set forth by the WHO. However, there is a pressing need to raise awareness and improve prescribing practices, enhance hygiene standards, and increase investment in resources for hospital infection prevention and control. These efforts are essential for mitigating antimicrobial resistance rates.
Recommendation:
A nationwide antimicrobial surveillance program is strongly advocated to ensure the appropriate prescription of antibiotics, coupled with strict adherence to antibiotic usage policies. This essential measure is necessary to mitigate the spread of drug-resistant microbes and the associated complications within the country. Therefore, alongside empiric treatment for ear infections, conducting culture and antimicrobial susceptibility tests should be made as standard and obligatory practices. This approach is crucial for effective management of ear infections, mitigating associated complications at the individual, household, and healthcare system levels, and curbing the emergence of drug resistance in communities. As for the new cases, it is recommended to take a swab before initiating regimen-based treatments, particularly when resistance is already suspected. Further research is imperative to identify strains with high resistance and to characterize resistant strains using molecular techniques.
A limitation of this study is the absence of data on the specific types of ear infections, Consequently, the study could not compare the association between bacterial isolates and the types of infections. Additionally, the study did not gather detailed information on other resistance patterns, such as Extended Spectrum Beta-Lactamase (ESBL).

CONCLUSION

Bacterial ear infection causes a significant health burden that affects all ages especially young children and females. The most prevalent bacteria were P. aeruginosa, S. aureus P. mirabilis and E. coli. Most of the antibiotics showed to be highly resistant to Penicillins, Amoxicillin clavulanate, Erythromycin, cephlosporins, Meropenem, Piperacillin tazobactam and Vancomycin were found to be highly effective in treatment of ear infection.

Conflict of interest:

The authors declare no conflict of interest

Ethical consideration:

The study was approved by local research ethics committee

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