Nusantara Medical Science Journal
Volume 7 Issue 1, January - June 2022
P-ISSN: 2460-9757, E-ISSN: 2597-7288
Nationally Accredited Journal, Decree No. 36/E/KPT/2019.

Original Article

Microbial Pattern and Sensitivity Analysis of Otitis Externa Patients in Makassar, Indonesia

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ARTICLE INFO

Keywords:
Otitis externa; bacteria pattern; antimicrobial sensitivity;

How to cite:
Tobing JFL., Djamin R., Rahardjo SP. (2022). Microbial Pattern and Sensitivity Analysis of Otitis Externa Patients in Makassar, Indonesia. Nusantara Medical Science Journal, 7(1), 61-69

DOI:
10.20956/nmsj.v7i1.18910

ABSTRACT

Introduction: *Globally, the cases of otitis externa were increased, followed by the incidence of antimicrobial resistance. Several factors, such as untrained medical health personnel and unprescribed antibiotics, are thought to play a major role in this phenomenon. This study aims to determine the bacterial patterns and antimicrobial sensitivity in otitis externa patients in Makassar, South Sulawesi, Indonesia.* ***Methods:*** *This cross-sectional study was conducted from November 2020 to February 2021 at Dr. Wahidin Sudirohusodo and Health Laboratory Center, Makassar, South Sulawesi, Indonesia. There were in total 33 subjects with otitis externa participating in this study. The secretion from the outer ear canal was taken and cultured using Mac Conkey agar for bacteriological examination and Vitek 2 for antimicrobial sensitivity. This study assessed age, sex, and type of otitis externa. All data analyses used SPSS version 26.00 (IBM Corp., Armonk, New York). Bacteria patterns and otitis externa were analyzed using the Fisher test.* ***Results:*** *Fourteen cases (42.4%) were caused by Pseudomonas Aeruginosa. Most Gram-negative bacteria were sensitive to ciprofloxacin, gentamicin, amikacin, and meropenem. Staphylococcus haemolyticus, Staphylococcus capitis, and Staphylococcus epidermidis were the most resistant Gram-positive bacteria. Aerobic bacteria in otitis externa were sensitive to antimicrobials (p<0.023).* ***Conclusion:***

Gram-negative aerobic bacteria were common causes of otitis externa, with Pseudomonas aeruginosa being the most common isolated bacteria. Ciprofloxacin, gentamicin, amikacin, and meropenem were sensitive antimicrobials for otitis externa.

1. INTRODUCTION

Otitis externa is a common infection of the outer ear canal with bacteria as the primary causative agent. It is clinically characterized by pain, pruritus, and edema of the outer ear canal.[1] Factors such as swimming, humid climate, diabetes, or the presence of immunocompromised conditions predispose to the development of otitis externa.[2] First-line treatments include topical antibiotics with or without topical corticosteroids.[3]

It is estimated that 40-62% of antibiotics are prescribed without proper indications. Providing drugs to untrained personnel and buying unprescribed antibiotics are major factors that contribute to this phenomenon.[4-7] Irrational antibiotic use and the high incidence of otitis externa led to an increased incidence of complications from otitis externa. Thus, it is necessary to analyze the antimicrobial resistance patterns of the most frequently isolated microorganisms to help select the effective empirical therapy and develop a rational antimicrobial prescribing policy.[8] Currently, studies examining the relationship between bacterial patterns and otitis externa in Indonesia are still scarce. This study aimed to assess the microbial pattern and sensitivity analysis of otitis externa patients in Makassar, South Sulawesi, Indonesia.

2. METHODS

Subjects included in this cross-sectional study were patients with otitis externa with or without secretions who had never received local or systemic treatment in the ear, nose, throat, and head and neck surgery (ENT-HNS) outpatient clinic of Dr. Wahidin Sudirohusodo and its network hospitals in Makassar, South Sulawesi, Indonesia from November 2020 to February 2021. Subjects were recruited using consecutive sampling. Exclusion criteria were previous history of otitis media, bullous myringitis, otomycosis, acute suppurative otitis media, chronic suppurative otitis media, serous otitis media/effusion, and congenital cholesteatoma and primary acquisition. Otitis media with effusion (OME) history was one of the exclusion criteria as the pathogenic microorganisms involved in OME are different from those found in otitis externa. Thus, it is one of the confounding factors that need to be excluded. The diagnosis of diffuse, circumscribed, and malignant otitis externa was established clinically based on the United Kingdom ENT Trainee Research Network consensus.[9]

This research was conducted based on the ethical approval by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. History taking and sample collection of ear secretions from the outer ear canal with a sterile swab were conducted by the authors, who were experienced otolaryngologists. The sample was inserted into a sterile tube containing brain heart infusion broth (BHIB) transport medium and was closed to prevent contamination. Sample collection was done by the authors. Gram staining and culture

using Mac Conkey agar were performed in each sample. The bacteria were then isolated for less than 24 hours using Vitek 2 (AST GN 93 card) and antimicrobial sensitivity test was performed in Health Laboratory Center for Makassar (*Balai Besar Laboratorium Kesehatan Makassar*), Makassar, South Sulawesi, Indonesia. In addition, the age, sex, and type of otitis externa of all participants were also recorded and assessed in this study.

All results were presented in tables and graphs. Data analysis was done using the chi-square test and a p-value of 0.05 was considered significant. A fisher's exact test was used if the criteria were not met. Statistical analysis was done using SPSS 26.00 (IBM Corp., Armonk, New York).

3. RESULTS

Demographic and Characteristics

A total of 33 patients with otitis externa participated in this study. Otitis externa was most commonly found at a young age, with the largest distribution found in 21-30 years (12 subjects, 37%), followed by 31-40 years, 10-20 years, 41-50 years, and >50 years (Figure 1) age group. Females were at a higher risk of developing otitis externa (20 subjects, 60.6%). The most common type was the diffuse type (19 subjects, 57.6%), followed by circumscribed type (8 subjects, 24.4%) and malignant type (6 subjects, 18,2%). All cases only involved one ear, with the right ear being more commonly affected (19 subjects, 67.6%).

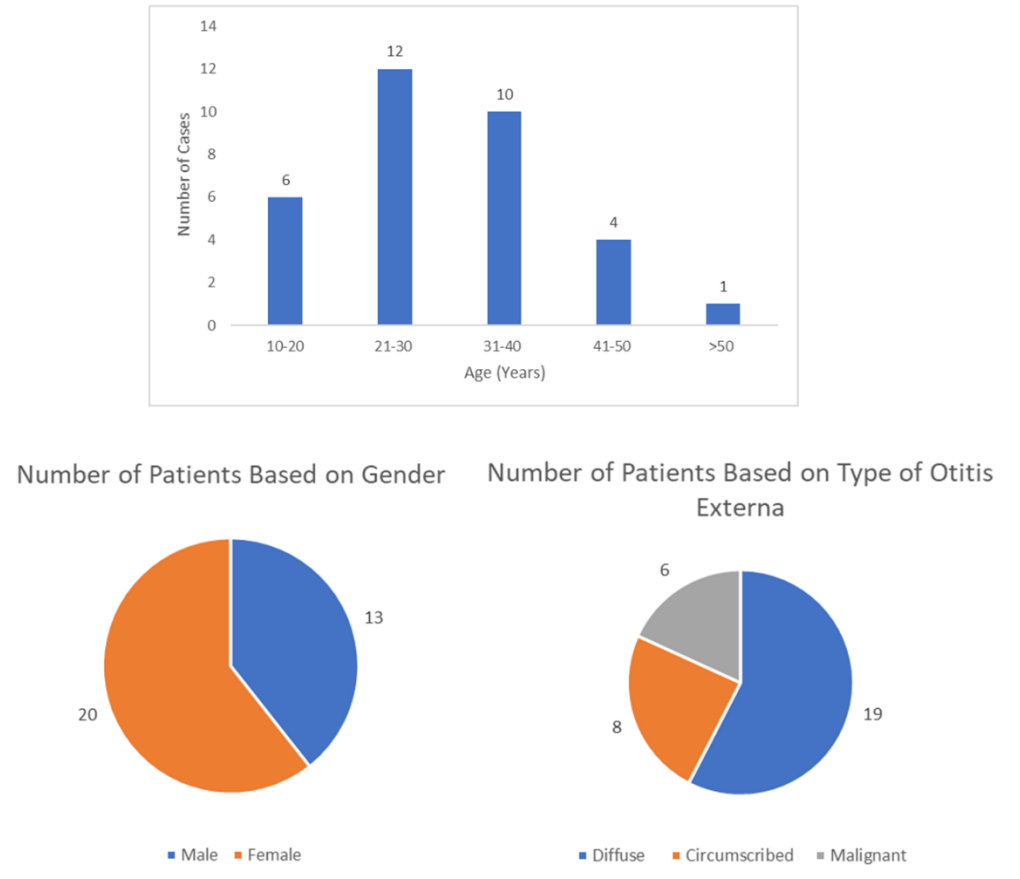

Figure 1. Demographic data

Culture Result

Table 1 shows that Gram-negative bacteria were the more commonly found bacteria in patients with otitis externa regardless of clinical types (26 subjects, 78.8%). In contrast, Gram-positive bacteria were found in only 7 cases (21.2%). *Pseudomonas aeruginosa* was the most common cause of otitis externa in this study and infected 14 out of 33 subjects (42.4%). It accounted for the most frequent causative agent both in the diffuse (9 subjects, 27.3%) and circumscribed (4 subjects, 12.1%) types. However, in the malignant type, *P. aeruginosa* came second (3%) to *Sphingomonas paucimobilis*. *Staphylococcus haemolyticus* and *Staphylococcus epidermidis* were the most commonly isolated Gram-positive bacteria, found in 2 cases (6.1%). Almost all cases (5 out of 7 cases) caused by Gram-positive bacteria were of diffuse type.

Table 1. Microbial Distribution based on the type of otitis externa

Bacteria	Type of Otitis Externa			Total (%)
	Diffuse	Circumscribed	Malignant	
GRAM-NEGATIVE				
Pseudomonas aeuroginosa	9 (27.3)	4 (12.1)	1 (3.0)	**14 (42.4)**
Klebsiella pneumoniae	1 (3.0)	1 (3.0)	0 (0)	**2 (6.1)**
Klebsiella oxytoca	0 (0)	1 (3.0)	0 (0)	**1 (3.0)**
Achromobacter xylosoxidans	0 (0)	0 (0)	1 (3.0)	**1 (3.0)**
Enterobacter aerogenese	2 (6.1)	0 (0)	0 (0)	**2 (6.1)**
Enterobacter cloacae	1 (3.0)	1 (3.0)	0 (0)	**2 (6.1)**
Citrobacter karoseri	1 (3.0)	0 (0)	0 (0)	**1 (3.0)**
Sphingomonas paucimobilis	0 (0)	0 (0)	2 (6.1)	**2 (6.1)**
Proteus mirabilis	0 (0)	1 (3.0)	0 (0)	**1 (3.0)**
Total	**14 (42.4)**	**8 (24.2)**	**4 (12.1)**	**26 (78.8)**
GRAM-POSITIVE				
Enterococcus faecalis	1 (3.0)	0 (0)	0 (0)	**1 (3.0)**
Staphylococcus haemolyticus	0 (0)	0 (0)	2 (6.1)	**2 (6.1)**
Staphylococcus capitis	1 (3.0)	0 (0)	0 (0)	**1 (3.0)**
Staphylococcus aureus	1 (3.0)	0 (0)	0 (0)	**1 (3.0)**
Staphylococcus epidermidis	2 (6.1)	0 (0)	0 (0)	**2 (6.1)**
Total	**5 (15.2)**	**0 (0)**	**2 (6.1)**	**7 (21.2)**

Antimicrobial Sensitivity Test

Almost all antimicrobials were sensitive to *P. aeruginosa* (Table 2). Meropenem, amikacin, and ciprofloxacin were found to be sensitive in 14 subjects, followed by cefepime (13 subjects), ceftazidime (12 subjects), and tazobactam (11 subjects). However, *P. aeruginosa* showed high resistance to cefazoline (14 of 33 subjects). Klebsiella pneumoniae and Enterobacter cloacae showed the highest resistance frequency, with each showing resistance to 8 out of the 14 tested antibiotics. *Citrobacter koseri* came second and showed resistance to 7 out of 14 tested antibiotics. All samples of *Staphylococcus haemolyticus* showed complete resistance to 11 out of 14 antibiotics, and only cotrimoxazole showed full efficacy to this species (Table 3).

Table 2. Antimicrobial Sensitivity of Gram-Negative Bacteria

Gram-negative Bacteria																										
Antibiotic	*Pseudomonas aeruginosa*			*Klebsiella pneumoniae*			*Klebsiella oxytoca*			*Achromobacter xylosoxidans*			*Enterobacter aerogenes*			*Enterobacter cloacae*			*Citrobacter koseri*		*Sphingomonas paucimobilis*			*Proteus mirabilis*		
	S	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**S**	**R**	**I**	**S**	**R**	**I**
Amoxicillin	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-	-	2	-	-	1	-	-	-	1	-	-
Ampicillin	-	-	-	-	2	-	-	1	-	-	-	-	-	2	-	-	2	-	-	1	-	-	-	1	-	-
Ceftriaxone	-	-	-	1	1	-	1	-	-	-	1	-	2	-	-	1	1	-	-	1	1	1	-	1	-	-
Cefotaxime	-	-	-	1	1	-	1	-	-	-	-	-	1	-	-	1	1	-	-	-	-	-	-	1	-	-
Ceftazidime	12	1	1	2	-	-	1	-	-	1	-	-	2	-	-	1	-	1	1	-	1	-	1	1	-	-
Cefepime	13	-	1	2	-	-	1	-	-	-	1	-	2	-	-	2	-	-	1	-	2	-	-	1	-	-
Gentamicin	14	-	-	1	1	-	1	-	-	-	1	-	2	-	-	2	-	-	1	-	1	-	1	1	-	-
Cotrimoxazole	-	-	-	1	-	-	1	-	-	1	-	-	2	-	-	1	1	-	-	1	2	-	-	-	1	-
Cefazoline	-	14	-	-	1	1	-	-	1	-	1	-	-	2	-	-	2	-	-	1	1	1	-	-	-	1
Meropenem	14	-	-	2	-	-	1	-	-	1	-	-	2	-	-	2	-	-	1	-	2	-		1	-	-
Amikacin	14	-	-	2	-	-	1	-	-	-	1	-	2	-	-	2	-	-	1	-	1	1	-	1	-	-
Ciprofloxacin	14	-	-	1	1	-	1	-	-	-	-	1	2	-	-	2	-	-	1	-	-	1	1	1	-	-
Nitrofurantoin	-	-	-	-	-	2	1	-	-	-	-	-	-	-	2	1	1	-	-	1	-	-	-	-	1	-
Tazobactam	11	2	1	1	-	1	1	-	-	1	-	-	2	-	-	1	1	-	-	1	1	-	1	1	-	-

S= Sensitive . R=Resistant. I=Intermediate

Table 3. Antimicrobial Sensitivity of Gram-Positive Bacteria

Gram-positive Bacteria															
Antibiotic	***Enterococcus faecalis***			***Staphylococcus haemolyticus***			***Staphylococcus capitis***			***Staphylococcus aureus***			***Staphylococcus epidermidis***		
	S	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**	**S**	**R**	**I**
Amoxicillin	1	-	-	-	2	-	-	1	-	-	-	-	-	1	-
Ampicillin	1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Cefotaxime	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-
Ceftazidime	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-
Cefepime	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-
Gentamicin	-	-	-	1	-	1	1	-	-	-	-	-	1	-	-
Cotrimoxazole	-	-	-	2	-	-	1	-	-	1	-	-	2	-	-
Cefazoline	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-
Meropenem	-	-	-	-	2	-	-	1	-	-	-	-	-	1	-
Ciprofloxacin	-	1	-	-	2	-	1	-	-	1	-	-	2	-	-
Clindamycin	-	-	-	1	1	-	1	-	-	1	-	-	1	1	-
Erythromycin	-	1	-	1	1	-	1	-	-	1	-	-	1	1	-
Azithromycin	-	-	-	1	1	-	1	-	-	1	-	-	1	1	-
Nitrofurantoin	1	-	-	1	-	-	1	-	-	-	-	-	-	-	-
Tazobactam	1	-	-	-	2	-	-	1	-	-	-	-	-	1	-

S= Sensitive . R=Resistant. I=Intermediate

4. DISCUSSION

This study showed that otitis externa was more prevalent in the 10-20 years age group which was different from a study by Kiakojuri et al., which revealed that middle-aged individuals were at a higher risk of developing otitis externa [10] and by Rowlands et al. who did not find any particular age group to be at a higher risk of acquiring otitis externa [11]. This discrepancy might be caused by children's higher activities carried out outside the room, which exposed them to excessive heat, humidity, and dust. In addition, ear cleaning activities such as using cotton buds might also play a role.[12]

Females were found to be more commonly affected (20 subjects, 81.8%), which was in line with a previous study which showed that the incidence of otitis externa was

higher in women (63.6%) compared to men (36.4%). This might be caused by the habit of frequently cleaning the ear in women, which can be a predisposing factor for otitis externa. However, these results differ from another study where men (55%) were more frequently affected by otitis externa (45%). This discrepancy may be due to differences in the distribution of research characteristics. The correlation between sex and the incidence of otitis externa still has to be further proven with larger sample size.[12]

Based on the type of otitis externa, diffuse otitis externa was the most encountered type (19 subjects, 57.6%), followed by circumscribed otitis externa (9 subjects, 24.2%) and malignant otitis externa (6 subjects, 18.2%). In line with a previous study that showed diffuse otitis externa patients (78.9%) were found more frequently.[13]

Consistent with the results from previous studies [3, 10, 14-16], *P. aeruginosa* was the most frequently isolated bacteria from our cohort (14 subjects, 42.4%). To our knowledge, only one study contradicts this finding, where Kiakojuri et al. showed that most cases of otitis externa were caused by gram-positive bacteria.[10] The frequent finding of *P. aeruginosa* might be attributed to their ubiquitous habitat, including soil and water, which explains the high number of otitis externa in people with frequent aquatic activities, such as swimming.[17] The loss of the protective mechanism of the external canal due to reduced or loss of cerumen due to predisposing factors (such as swimming) leads to a pH change from acid to alkaline and increases the risk of bacterial infection.[18] *Pseudomonas aeruginosa* in otitis externa is hypothesized to be distinct from strains isolated from other infections as the strain in otitis externa was shown to produce a lower level of pyocyanin and less urease with no mucoid-producing strains. One possible explanation is that the *P. aeruginosa* strain found in otitis externa represents those coming from natural habitats as opposed to other strains found in other diseases that might have experience adaptation to the human environment.[19]

Sensitivity analysis found that all 14 isolates (100%) of *P. aeruginosa* were sensitive to quinolones and gentamicin.[15] This finding was supported by data from other studies [14, 15], where both agents were found to be effective in all isolates. Topical quinolones and gentamicin in the form of ear drops are the first-line treatments in uncomplicated otitis externa due to their excellent efficacy and safety profile.[1, 3]

Interestingly, while other studies suggested *S. aureus* as being the second most isolated species [20, 21], the result of our cohort showed that cases caused by *S. aureus* were comparable to those caused by other species such as *Klebsiella pneumoniae, Enterobacter aerogenes, Enterobacter cloacae, Sphingomonas paucimobilis, Staphylococcus haemolyticus,* and *Staphylococcus epidermidis*. We also found some species that had not been identified in other studies, such as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Achromobacter xylosoxidans, Citrobacter koseri,* and *Sphingomonas paucimobilis*. In addition, although otitis externa is often a polymicrobial infection [3], no cases with more than one infectious agent were found.

Third and fourth-generation cephalosporins were also found to be effective in almost all isolates and may serve as an alternative therapeutic option; this favorable effect was supported in a Brazilian study [14]. Surprisingly, all isolates showed resistance to cefazoline, which is one of the most common antibiotics administered in otorhinolaryngology patients in Indonesia. The high frequency of cefazoline administration for prophylaxis in various otorhinolaryngology cases in Indonesia [7],

might have contributed to this high resistance. Clinical practice guidelines for otitis externa published by the American Academy of Otolaryngology-Head and Neck Surgery Foundation did not advise administering systemic cephalosporins as they were indicated to have caused increased disease persistence and recurrence.[3]

Overall, ciprofloxacin, gentamicin, amikacin, and meropenem showed the highest sensitivity against Gram-negative bacteria. This was shown by the low rate of resistance to the four antimicrobials. Only one isolate of *Klebsiella pneumoniae* and *Achromobacter xylosoxidans* were resistant to gentamicin. Among the eight types of Gram-negative bacteria, only *Achromobacter xylosoxidans,* and *Sphingomonas paucimobilis* were resistant to ciprofloxacin with one sample in each type.

Unlike Gram-negative bacteria, almost all antimicrobials were resistant to Gram-positive bacteria isolated in this study. *Staphylococcus haemolyticus*, *Staphylococcus capitis*, and *Staphylococcus epidermidis* were the most resistant Gram-positive bacteria. They showed resistance to most tested antibiotics, such as amoxicillin, cefotaxime, ceftazidime, tazobactam, cefazoline, meropenem, ciprofloxacin, and cefepime. However, these three species were sensitive to gentamicin, cotrimoxazole, and nitrofurantoin. *Enterococcus faecalis* was still sensitive to amoxicillin and ampicillin and resistant to ciprofloxacin and erythromycin. Meanwhile, *Staphylococcus aureus* was sensitive to cotrimoxazole, ciprofloxacin, erythromycin, azithromycin, and clindamycin.

Due to equipment limitations, a few anaerobic microbes, both Gram-positive and Gram-negative, could not be identified. In addition, the kit for oral cephalosporin antibiotics was not available due to material shortage resulting from the COVID-19 pandemic. These issues need to be tackled in upcoming studies.

5. CONCLUSION

Based on the results of this study, it can be concluded that Gram-negative aerobic bacteria are common causes of otitis externa, with *Pseudomonas aeruginosa* being the most common isolated bacteria. Based on our study, quinolones, gentamicin, amikacin, and meropenem can be considered for the management of otitis externa. As the level of antimicrobial resistance is increasing, we recommend carrying out periodic sensitivity and resistance tests.

ACKNOWLEDGMENTS

The authors acknowledge the laboratory technicians who prepared the agar plates and assisted in sample handling.

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Conflict of Interest Statement:

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.