Comparison of Germ Patterns between Cholesteatom Tissue and Tympanic Cavum Mucosa Tissue in COSM with Cholesteatom at Dr. Mohammad Hoesin Hospital, Palembang

Resti Ramdani^{1*}, Abla Ghanie¹, Fiona Widyasari¹, Ahmad Hifni¹, Irfanuddin²

¹Departement of Otorhinolaryngology Head and Neck Surgery, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

²Department of Physiology, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia E-mail: restiramdani@rocketmail.com

Abstract

Chronic Suppurative Otitis Media (CSOM) is middle ear inflammation due to infection characterized by otorrhea through perforation of the tympanic membrane that occurs for more than three months. Identifying bacterial patterns in CSOM is essential for managing CSOM patients with cholesteatoma. Proper administration of antibiotics is crucial to overcome infection and prevent widespread resistance. To compare the germ patterns between cholesteatoma tissue and tympanic cavum mucosal tissue in CSOM patients with cholesteatoma. This observational study has a cross-sectional design, and data collection was carried out using primary data taken from 27 CSOM patients with cholesteatoma tissue swabs and tympanic cavum mucosal tissue from August to October 2023. From the reliability coefficient between the two tissues using the Cohen's Kappa technique, there is a significant concordance in the germ pattern between the tympanic cavum mucosa tissue and the tympanic cavum mucosa of 0.580 (Moderate) p<0.005, with Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumoniae with a significance value of 0.656 (Good) p<0.001. There is a significant concordance in the germ pattern between the toxes with cholesteatoma. The most common bacteria in both tissues are Gram-negative, namely Pseudomonas aeruginosa, proteus mirabilis, and Klebsiella pneumoniae.

Keywords: CSOM with Cholesteatoma, Germ Pattern, Bacterial Culture

1. Introduction

Chronic Suppurative Otitis Media (CSOM) is a common chronic infectious disease worldwide, especially in developing countries. WHO estimates that by 2018, around 486 million people worldwide will be deaf, including 34 million children. A study in Indonesia in 2007 reported 4.2% of hearing loss and deafness cases, with an incidence of hearing loss due to CSO of 3.6%. Research in Palembang in 2021 showed that out of 102 subjects with CSOM, 85.3% had Unilateral CSOM and 14.7% had bilateral CSOM.¹⁻⁵

CSOM is divided into with and without cholesteatoma based on the inflammatory process, perforation of the tympanic membrane, and cholesteatoma. Cholesteatomes are epithelial cysts that can damage the bone. Factors such as the anatomy of the Eustachian tube, the presence of cholesteatoma, environment, immune system, and genetics influence the occurrence of CSOM. Symptoms and complications of CSOM are related to the type of bacteria.⁶⁻¹⁰ This study aims to compare the germ pattern between cholesteatoma tissue and tympanic cavum mucosa tissue in patients with chronic suppurative otitis media.

2. Methods

2.1. Research Design, Population, and Sample

An observational study was conducted at Dr Mohammad Hoesin Hospital Palembang. Sampling was done by consecutive sampling from August to October 2023.

The study involved 52 patients with cholesteatoma who underwent mastoidectomy at Dr. Mohammad Hoesin General Hospital Palembang, fulfilling the inclusion criteria as patients with cholesteatoma who underwent mastoidectomy at Dr. Mohammad Hoesin General Hospital Palembang during August-October 2023 and willing to participate and sign informed consent.

2.2. How it works

Demographic interviews were conducted before obtaining informed consent from patients for intraoperative sample collection at the Central Surgical Installation of Dr Mohammad Hoesin Hospital Palembang. Tissue samples were sent to the Central Laboratory of Dr Mohammad Hoesin Hospital Palembang for germ pattern analysis. Data that were incomplete or did not fulfill the exclusion criteria were excluded.

2.3. Data Collection

Data were obtained from patients who underwent mastoidectomy surgery at the Central Surgical Installation of Dr Moh. Hoesin Hospital Palembang and culture samples were obtained from the Central Laboratory Installatio and the study samples met the inclusion and exclusion criteria. The data obtained were qualitative and quantitative data. Data will be recorded in the research form.

2.4. Statistical Analysis

Univariate analysis was conducted for the characteristics of the study subjects, with numerical data presented as mean and standard deviation, as well as categorical data in graphs and distribution tables. Bivariate analysis used Cohen's Kappa Inter-rater Reliability test on CSOM patients. The analysis results are presented in tables, charts, and narratives with data processing using SPSS for Windows version 26.0.

3. Results

3.1. Characteristics of Research Subjects

Of the initial 34 patients, 6 dropped out because no bacteria grew and 1 only grew mould. The remaining 27 patients were aged 19-60 years (70.4%), with more males (60.7%) than females (39.3%). The most common cholesteatoma stage was stage 4, with 15 patients (55.6%) (table 1).

Table 1. Characteristics of research subjects (N=28)

Characteristics	Total (%)				
Ages					
0-18 years old	5 (18,5)				
19-60 years old	19 (70,4)				
Above 60 years old	3 (11,1)				
Gender					
Man	16 (60,7)				
Woman	11 (39,3)				
Cholesteatoma stage					
Stage 2	2 (7,4)				
Stage 3	3 (11,1)				
Stage 4	15 (55,6)				
Stage 5	7 (25,9)				

3.2. Germ Pattern Characteristics in CSOM with Cholesteatoma

The germ pattern consisted of 27 types of bacteria in 54 samples, with 11 Grampositive species and 16 Gram-negative species. Gram-negative bacteria were found in 66.7% of tympanic cavum mucosa samples and 85.2% of cholesteatome samples. A total of 74.1% of samples showed the same culture at both sites, and 81.5% showed concordant culture results between the two sites (tabel 2).

3.3. Germ Pattern Distribution in CSOM with Cholesteatomes According to Tissue Predilection

Pseudomonas aeruginosa was most prevalent in cholesteatomes (59.3%), followed by Proteus mirabilis (11.1%) and Staphylococcus warneri (7.4%). In the tympanic cavum mucosa, Pseudomonas aeruginosa predominated (37.0%), followed by Staphylococcus aureus (14.8%) and Proteus mirabilis (11.1%) (table 3).

Table 2. Compatibility of culture results with germpredilection

Compatibility	Total (%)						
By Species							
Appropriate	20 (74,1)						
Not Appropriate	7 (25,9)						
Based on grouping (Gram							
negative and Gram positive)							
Appropriate	22 (81,5)						
Not Appropriate	5 (18,5)						

3.4. Group Conformity of Cultured Species in Both Tissues

Of the 27 patients, 22 (81.5%) had a concordant germ pattern between the cholesteatoma tissue and tympanic cavity Gram-negative mucosa. germs were predominant in 66.7% of patients, while 14.8% showed Gram-positive germs at both sites. The Cohen's Kappa test showed a germ pattern concordance between the two tissues of 0.580 (Moderate) with a significance of p < p0.05. where Gram-negative germs predominated (table 4).

3.5. Suitability of Cultured Species in Both Tissues

The results of the reliability coefficient between the two tissues using the Cohen's Kappa technique showed that there was a strong agreement (0.675) with a significance level of p<0.01 between the culture results in the tympanic cavum mucosa tissue and the cholesteatoma tissue. The dominant germ pattern was Pseudomonas aeruginosa.

4. Discussion

This study involved 34 subjects with 27 bacteria growing. Of the 27 samples tested, 11 types of bacteria grew on the mucosal tissue of the tympanic cavum and 9 types of bacteria grew on the cholesteatom tissue. The mean age of the patients was 16.17 ± 44.77 years, with 70.4% aged 19-60 years. There were more males than females, with a ratio of 1.5:1.

Table 3. Predilection characteristics of breeding results

Etiology	Total (%)
From the Mucosa of the Tympa	nic
Cavum	
Pseudomonas aeruginosa	10 (37,0)
Staphylococcus aureus	4 (14.8)
Proteus mirabilis	3 (11,1)
Klebsiella pneumoniae	2 (7.4)
Staphylococcus epidermidis	2 (7.4)
Staphylococcus warneri	1 (3.7)
Citrobacter koseri	1 (3.7)
Staphylococcus haemolyticus	1 (3.7)
Staphylococcus paucimobilis	1 (3.7)
Acinetobacter lwoffii	1 (3.7)
Achromobacter xylosoxidans	1 (3.7)
From Cholesteatoma	
Pseudomonas aeruginosa	16 (59,3)
Proteus mirabilis	3 (11,1)
Staphylococcus warneri	2 (7.4)
Klebsiella pneumoniae	1 (3.7)
Citrobacter koseri	1 (3.7)
Staphylococcus haemolyticus	1 (3.7)
Staphylococcus paucimobilis	1 (3.7)
Achromobacter xylosoxidans	1 (3.7)
Staphylococcus epidermidis	1 (3.7)

Table 4. Species group conformity of second tissue culture results

	(_	_						
	Gram- negativ e		Gram- positive		Total		alue	asym p	
	n	%	n	%	n	%			
Gram-	18	66,	0	0	1	66,			
negati		7			8	7			
ve	5		4	14,			0,58	0,15	
Gram-		18,		8	9	33,	0	8	
positiv		5				3			
е									

This study is slightly different from previous studies that showed more female patients, with a percentage of 57.2% and an age of 10-56 years.¹¹⁻¹⁵ Chronic Suppurative Otitis Media (CSM) can occur in all age groups, including children, due to upper respiratory tract infections and different tubal structures. Smoking habit in males may be a risk factor for recurrent upper respiratory tract

infections.4,16-17

This study shows the distribution of cholesteatoma degree with the most stage groups in stage 4 (55.6%), followed by stage 2 (7.4%), stage 3 (11.1%), and stage 5 (25.9%). Previous studies have shown that the degree of invasion of cholesteatoma is mostly in stage 2 (28.57%) and stage 3 (71.43%). The analysis showed the relationship between the degree of cholesteatome invasion and auditory bone damage was 75.2%. Delay in treatment, incorrect use of ear drops, and lack of education on CSOM with cholesteatoma can complications. The cause success of mastoidectomy surgery is influenced by operator skill, surgical technique, postsurgical care, and counselling to the patient or family.¹⁸⁻²² Good post-surgery care is essential to ensure that the healing process goes smoothly, the ear cleaning mechanism works properly, and to prevent infection.^{16,23-24}

The study found a higher occurrence of single bacterial growth (78%) compared to polymicrobial infections (22%), in line with previous research showing a predominance of isolates. monomicrobial Pseudomonas aeruginosa, a Gram-negative bacterium, was the most prevalent pathogen in both tympanic cavity mucosal tissue (35.7%) and cholesteatoma tissue (57.1%). Additionally, Staphylococcus aureus, Proteus mirabilis, and Klebsiella pneumoniae were notable bacterial species. Analysis of cholesteatoma microbiota revealed the presence of Staphylococcus, Actinobacteria (including Corynebacterium), Brevibacterium, and Cutibacterium. The higher prevalence of Gram-negative bacteria in cholesteatoma tissue may contribute to tissue damage through the secretion of toxins and destructive enzymes.^{4,25-26}

Table 5. Species suitability of second tissue culture res	ults
---	------

		Cholesteatoma								
		Ps. aeruginosa	•	Proteus mirabilis		Staph. haemolyticus	Sphmon. paucimobilis	K.pneum.pneumoniae	Achro. xylosoxidans	Staph. epidermidis
	Ps.aeruginosa	10	0	0	0	0	0	0	0	0
	Staph.warneri	0	1	0	0	0	0	0	0	0
	Staph.aureus	3	0	1	0	0	0	0	0	0
	Proteus mirabilis	1	0	2	0	0	0	0	0	0
	CITIO.KOSEII	0	0	0	1	0	0	0	0	0
	² Staph.haemolyticus	0	0	0	0	1	0	0	0	0
	Sphmon.paucimobilis	5 0	0	0	0	0	1	0	0	0
	K.pneum.pneumonia	e 0	0	0	0	0	0	2	0	0
	Aci.lwoffii	1	0	0	0	0	0	0	0	0
	Achro.xylosoxidans	0	0	0	0	0	0	0	1	0
	Staph.epidermidis	1	0	0	0	0	0	0	0	1

The study found unique bacteria like Staphylococcus aureus and Acinetobacter Iwoffii in the tympanic cavity mucosa. Gramnegative bacteria, including Pseudomonas aeruginosa, Proteus mirabilis, and Klebsiella pneumoniae, were prevalent in both cholesteatoma tissue and mucosa. There was a significant link between bacterial profiles in cholesteatoma tissue and middle ear mucosa. Understanding these germ patterns could aid in managing patients with chronic suppurative otitis media and cholesteatoma, especially concerning Gram-negative bacteria. Study limitations may have influenced results, and environmental factors like air temperature variations could impact bacterial growth outcomes.²⁷⁻²⁸

5. Conclusion

Pseudomonas aeruginosa was

predominant in tympanic tissue and cholesteatoma. Antibiotics were recommended according to the cholesteatom culture results. Need departmental cooperation for postoperative antibiotics policy.

6. Acknowledgements

The researchers also acknowledge the support and cooperation from the ENT department, microbiology laboratory, pharmacy department, and hospital management at RSUP Dr. Mohammad Hoesin Palembang.

References

- Mittal R, Lisi C V, Gerring R, Mittal J, Mathee K, Narasimhan G, et al. Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. J Med Microbiol. 2015 Oct;64(10):1103–16.
- Chole RA, Nason R. Chronic Otitis Media and Cholesteatoma. In: Wackym A, Snow JB, editors. Ballenger's Otorhinolaryngology Head and Neck Surgery. 18th ed. Connecticut: People's Medical Publishing House; 2016. p. 245– 56.
- Chole RA, Sharon JD. Chronic Otitis Media, Matoiditis, and Petrositis. In: Flint PW, Francis HW, Haughey BH, Lesprance MM, Lund VJ, Robbins T, editors. Cummings Otolaryngology Head and Neck Surgery. 7th ed. Philadelphia: Elsevier; 2021.
- 4. Ghanie A, Hasni R, Hifni A, Widyasari F, Bahar E. Comparison of Germ Patterns and Antimicrobial Susceptibility in Chronic Suppurative Otitis Media with Cholesteatoma and Without Cholesteatoma in Dr. Mohammad Hoedin Hospital. Bioscientia Medicina: Journal of Biomedicine and Translational Research. 2021.

- Anggraeni R, Carosone-Link P, Djelantik B, Setiawan EP, Hartanto WW, Ghanie A, et al. Otitis media-related hearing loss in Indonesian school children. Int J Pediatr Otorhinolaryngol. 2019 Oct;125:44–50.
- Adriztina I, Adenin LI, Lubis YM. Efficacy of Boric Acid as a Treatment of Choice for Chronic Suppurative Otitis Media and Its Ototoxicity. Korean J Fam Med. 2018;39(1):2.
- Uddén F, Filipe M, Reimer A, Paul M, Matuschek E, Thegerström J, et al. Aerobic bacteria associated with chronic suppurative otitis media in Angola. Infect Dis Poverty. 2018 May 3;7(1):42.
- Luers JC, Hüttenbrink KB. Surgical anatomy and pathology of the middle ear. J Anat. 2016 Feb;228(2):338–53.
- Francis HW. Anatomy of the Temporal Bone, External Ear, and Middle Ear. In: Flint PW, Francis HW, Haughey BH, Lesprance MM, Lund VJ, Robbins T, editors. Cummings Otolaryngology Head and Neck Surgery. 7th ed. Philadelphia: Elsevier; 2021.
- Gacek RR. Anatomy of the Auditory and Vestibular Sistem. In: Wackym A, Snow JB, editors. Ballenger's Otorhinolaryngology Head and Neck Surgery. 18th ed. Connecticut: People's Medical Publishing House; 2016.
- Massa HM, Lim DJ, Kurono Y, Cripps AW. Middle Ear and Tuba EustachiusMucosal Immunology. Mucosal Immunol. 2015;1923–42.
- Khattak SF, Sheikh NA, Aleem A, Farooq M, Nadeem K. Microbiological Profile From Middle Ear And Nasopharynx In Patients Suffering From Chronic Active Mucosal Otitis Media. J Ayub Med Coll Abbottabad. 2017;29(4):610–3.
- Anggraeni R, Hartanto WW, Djelantik B, Ghanie A, Utama DS, Setiawan EP, et al. Otitis Media in Indonesian Urban and Rural School Children. Pediatric

Infectious Disease Journal. 2014 Oct;33(10):1010–5.

- GBD 2019 Hearing Loss Collaborators. Hearing loss prevalence and years lived with disability, 1990-2019: findings from the Global Burden of Disease Study 2019. Lancet. 2021 Mar 13;397(10278):996–1009.
- Nogues JC, Pérez-Losada M, Preciado D. Review of otitis media microbiome studies: What do they tell us? Laryngoscope Investig Otolaryngol. 2020 Oct;5(5):936–40.
- Handoko E, Indrasworo D, Harun A. Hubungan Derajat Kolesteatom dengan Keberhasilan Operasi Mastoidektomi Radikal pada Penderita Otitis Media Supuratif Kronik. 2019;49(2):99-107.
- Kuo CL, Shiao AS, Yung M, Sakagami M, Sudhoff H, Wang CH, et al. Updates and knowledge gaps in cholesteatoma research. Biomed Res Int. 2015;2015:854024.
- Castle JT. Cholesteatoma Pearls: Practical Points and Update. Head Neck Pathology. 2018 Sep;12(3):419–29.
- Kuo CL. Etiopathogenesis of acquired cholesteatoma: prominent theories and recent advances in biomolecular research. Laryngoscope. 2015 Jan;125(1):234–40.
- Yung M, Tono T, Olszewska E, Yamamoto Y, Sudhoff H, Sakagami M, et al. EAONO/JOS Joint Consensus Statements on the Definitions, Classification, and Staging of Middle Ear Cholesteatoma. J Int Adv Otol. 2017 Apr;13(1):1–8.
- 21. Merchant SN, Rosowski JJ, Shelton C. Reconstruction of the Middle Ear. In: Wackym A, Snow JB, editors. Ballenger's Otorhinolaryngology Head and Neck

Surgery. 18th ed. Connecticut: People's Medical Publishing House; 2016.

- Chong LY, Head K, Webster KE, Dew J, Richmond P, Snelling T, et al. Sistemic antibiotiks for chronic suppurative otitis media. Cochrane Database Syst Rev. 2021 Feb 4;2(2):CD013052.
- Widyatama IK, Handoko E, Dian A. Hubungan Kadar Interleukin-6 Kolesteatom dengan Derajat Kerusakan Tulang Pendengaran Pasien Otitis Media Supuratif Kronik. 2014;44(2):83-95
- Kuczkowski J, Sakowicz-Burkiewicz M, Iżycka-Świeszewska E. Expression of the receptor activator for nuclear factor-κB ligand and osteoprotegerin in chronic otitis media. Am J Otolaryngol. 2010;31(6):404- 409.
- Fujikawa T, Tanimoto K, Kawashima Y, Ito T, Honda K, Takeda T, et al. Cholesteatoma has an altered microbiota with a higher abundance ofStaphylococcus species. Laryngoscope Investig Otolaryngol. 2022 Dec 1;7(6):2011–9.
- Mahajan T, Dass A, Gupta N, et al. Bacteriological Profile in Attico-antral type of Chronic Suppurative Otitis Media. Indian J Otolaryngol Head Neck Surgery. 2019;71(2):1412-1221
- Weiss JP, Antonelli PJ, Dirain CO. Microbiome Analysis of Cholesteatoma by Gene Sequencing. Otology & Neurotology. 2019 Oct;40(9):1186–93.
- Malak HA, Abulreesh HH, Organji SR, Elbanna K, Shaaban MR, Samreen, et al. Immune sistem evasion mechanisms in Staphylococcus aureus: current understanding. Vol. 14, Journal of Pure and Applied Microbiology. Journal of Pure and Applied Microbiology; 2020. p. 2219–34.