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Microbiological Profile and Antibiotic Resistance Patterns of Intraoperative Cholesteatoma Cultures

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Abstract

Objective

We aimed to identify the bacteria and fungi recovered from cholesteatoma tissue collected during surgery at a secondary-care center and to describe the antibiotic susceptibility profiles of the bacterial isolates.

Materials and methods

This retrospective, observational, and descriptive study included a convenience sample of 16 patients with chronic suppurative otitis media and histopathologically confirmed cholesteatoma who underwent surgery at a secondary-care center between March 2023 and March 2025. Patients were included based on the availability of intraoperative tissue-culture results and complete clinical records. Cultures were obtained during routine care without standardized criteria for selecting patients. Tissue samples were collected using sterile microalligator forceps, placed in Stuart's transport medium, and processed at an external microbiology laboratory using blood agar, chocolate agar, MacConkey agar, and Sabouraud agar. Bacterial cultures were incubated at 37°C in an atmosphere enriched with CO_2 for approximately 72 hours, whereas fungal cultures were maintained for up to 21 days. Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK® 2 system (bioMérieux, Marcy-l'Étoile, France). Susceptibility results were recorded as susceptible, intermediate, or resistant according to laboratory reports. Demographic characteristics, primary or revision surgical status, prior antibiotic use, histopathological confirmation, and microbiological findings were reviewed retrospectively. Quantitative variables were summarized using means and standard deviations, whereas categorical variables were expressed as absolute and relative frequencies.

Results

All 16 patients had histopathologically confirmed acquired cholesteatoma and a history of antibiotic use. Eleven patients underwent primary surgery, and five underwent revision surgery. Bacteria were isolated in nine cases (56.3%), fungi in two (12.5%), and no growth was observed in five (31.3%). The most frequently recovered microorganisms were *Proteus mirabilis* and *Turicella otitidis*, with two isolates each. Resistance was observed to fluoroquinolones, erythromycin, clindamycin, selected cephalosporins, and trimethoprim-sulfamethoxazole. All isolates tested for gentamicin were susceptible except for one *T. otitidis* isolate, which was classified as intermediate. Amikacin and carbapenems also showed activity against the isolates for which they were tested.

Conclusions

This study identified a heterogeneous microbiological profile in intraoperative cholesteatoma tissue cultures, with varied antibiotic susceptibility patterns among isolates. These findings should be interpreted cautiously given the small sample size, retrospective design, limitations of conventional culture methods, and differences in the antibiotics tested. Larger studies incorporating anaerobic cultures and molecular techniques are needed to better characterize the cholesteatoma microbiota and its antibiotic resistance patterns.

Categories: Otolaryngology, Infectious Disease
Keywords: antimicrobial resistance, bacterial infections, cholesteatoma, chronic suppurative otitis media, fungal infections

Introduction

Cholesteatoma is a non-neoplastic lesion of the middle ear characterized by the accumulation of keratinizing squamous epithelium that may be either congenital or acquired [1]. The acquired form is more prevalent and clinically significant due to its association with chronic otorrhea, progressive conductive

hearing loss, and the risk of local bone destruction [2].

The pathophysiology of acquired cholesteatoma has been linked to persistent infection and the presence of a polymicrobial environment within the lesion, encompassing Gram-positive cocci, Gram-negative bacilli, anaerobes, and fungi, frequently presenting as mixed infections [2,3]. Increasing evidence indicates that these microorganisms can form biofilms, structured microbial communities embedded in an extracellular matrix that provides protection against host immune responses and antimicrobial agents [4,5]. The virulence of these organisms and their capacity to form biofilms are thought to contribute directly to disease chronicity, therapeutic resistance, and postoperative recurrence [6], highlighting the need for a comprehensive understanding of the microbiota of these lesions. Although the microbiological profile of cholesteatoma has been investigated previously [7-11], further research is needed to better characterize the microorganisms involved and their antimicrobial resistance patterns.

Therefore, we aimed to identify the bacteria and fungi recovered from cholesteatoma tissue collected during surgery at a secondary-care center and to describe the antibiotic susceptibility profiles of the bacterial isolates.

Materials And Methods

Study design and setting

We conducted a retrospective, observational, and descriptive study involving patients who underwent surgery at a secondary-care center between March 2023 and March 2025. Due to the retrospective design, informed consent was waived.

Study population and eligibility criteria

The study population included patients of either sex diagnosed with cholesteatomatous otitis media, confirmed by histopathological examination, who underwent surgical intervention with intraoperative sampling of cholesteatomatous tissue for microbiological analysis. Patients with incomplete clinical records were excluded. A total of 16 patients met the eligibility criteria and were included in the final analysis.

Intraoperative cultures were obtained during routine clinical care without a standardized protocol or predefined criteria for selecting patients for culture. The study therefore comprised a retrospective convenience sample defined by the availability of intraoperative culture results and the specified eligibility criteria. The total number of cholesteatoma operations performed at the center during the study period was not available for this review. Consequently, the proportion of surgical cases represented by these 16 patients could not be determined.

Sample collection and microbiological analysis

During surgery, cholesteatomatous tissue samples were obtained using sterile microalligator forceps and immediately placed in Stuart's transport medium for laboratory processing. Samples were processed at an external microbiology laboratory using blood agar, chocolate agar, MacConkey agar, and Sabouraud agar. Bacterial cultures were incubated at 37°C in an atmosphere enriched with CO_2 for approximately 72 hours, whereas fungal cultures were maintained for up to 21 days. Culture results were classified according to the presence of bacterial growth, fungal growth, or no microbial growth.

Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK® 2 system (bioMérieux, Marcy-l'Étoile, France), with biochemical identification cards and antimicrobial susceptibility cards. Fungal identifications were recorded as reported by the external laboratory.

Antimicrobial susceptibility results were recorded as susceptible (S), intermediate (I), or resistant (R), according to the laboratory reports. The antimicrobial agents evaluated varied among bacterial isolates. Antimicrobial susceptibility testing was not performed for the isolate identified as coagulase-negative staphylococci (CoNS), because this analysis was not routinely conducted by the microbiology laboratory.

Data collection and statistical analysis

Demographic, clinical, and microbiological data were retrospectively obtained from clinical records and laboratory reports. The variables collected included age, sex, cholesteatoma type (congenital or acquired), surgical status (primary or revision), histopathological confirmation, history of antibiotic use, microbiological culture results, microorganisms isolated, and antibiotic susceptibility patterns. Prior antibiotic exposure was described as a history of use. Individual agents, routes of administration, treatment durations, and the interval between antibiotic treatment and sample collection were not analyzed.

Data were analyzed using descriptive statistics. Age was summarized using the mean, standard deviation (SD), and range. Categorical variables, including sex, cholesteatoma type, surgical status, prior antibiotic use, histopathological confirmation, and microbiological culture results, were expressed as absolute

frequencies and percentages. The distribution of culture outcomes was calculated based on the total number of patients (n = 16), whereas the relative frequency of each microorganism was determined using the total number of positive cultures (n = 11). Antibiotic susceptibility findings were described individually for each bacterial isolate according to the susceptibility categories reported by the laboratory and the antimicrobial agents evaluated.

Results

A total of 16 patients diagnosed with cholesteatoma were included, with a mean age of 41.1 years (SD, 23.0; range, 3-73 years). Eleven patients (68.8%) were male, and five patients (31.3%) were female. Histopathological examination confirmed cholesteatoma in all 16 patients (100%). All cases were acquired; no congenital cholesteatomas were included. Eleven patients (68.8%) underwent primary surgery, and five patients (31.3%) underwent revision surgery. All patients had a history of antibiotic treatment before surgery, although specific treatment regimens and their timing relative to culture collection were not analyzed. Among patients with bacterial growth, six were male and three were female; both patients with fungal growth were female, and all five patients with negative cultures were male.

No microbial growth was obtained in five cultures (31.3%). Bacteria were isolated in nine patients (56.3%) and fungi in two (12.5%). Each positive culture yielded a single organism, for a total of 11 isolates. Of these, *Proteus mirabilis* and *Turicella otitidis* were the most frequently recovered organisms, with two isolates each (18.2%). Coagulase-negative staphylococci (CoNS), *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Sphingomonas paucimobilis*, and *Corynebacterium amycolatum* each accounted for one isolate (9.1%). Fungal isolates comprised *Cladosporium* spp. and *Aspergillus terreus*, with one isolate each (9.1%) (Figure *1*).

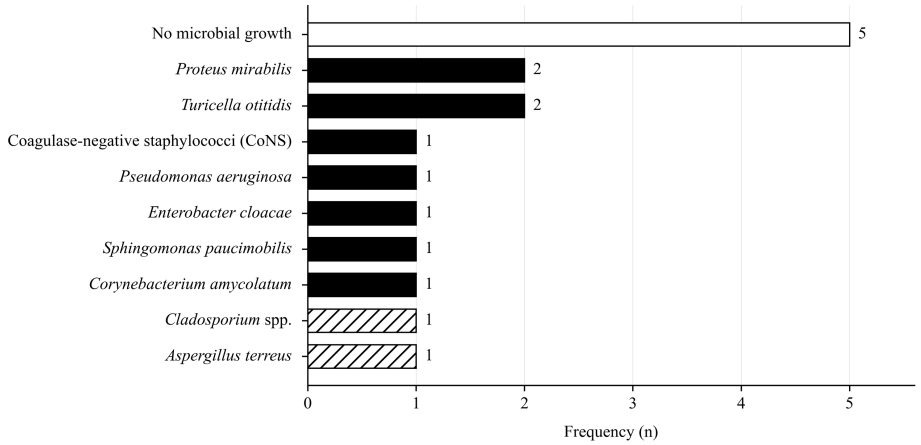


FIGURE 1: Microbiological distribution in intraoperative cholesteatoma cultures

Antibiotic susceptibility results were available for eight of the nine bacterial isolates; the CoNS isolate was not tested (Figure *2*). Both *P. mirabilis* isolates were susceptible to all antibiotics tested except nitrofurantoin. *P. aeruginosa* exhibited resistance to the cephalosporins evaluated while retaining susceptibility to carbapenems, aminoglycosides, and fluoroquinolones. *E. cloacae* was resistant to cephalexin and nitrofurantoin but susceptible to all remaining agents tested. *S. paucimobilis* demonstrated resistance to all fluoroquinolones tested and showed intermediate susceptibility to ceftazidime while remaining susceptible to all other agents tested.

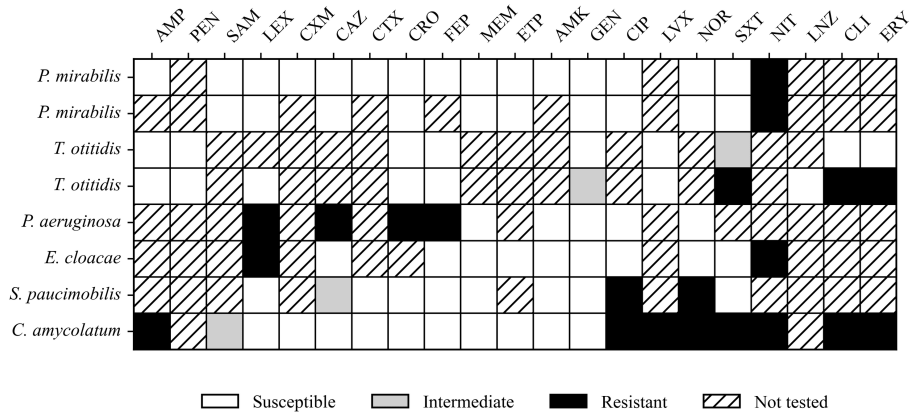


FIGURE 2: Antibiotic susceptibility profiles of bacterial isolates recovered from cholesteatoma tissue

AMP: ampicillin; PEN: penicillin; SAM: ampicillin-sulbactam; LEX: cephalexin; CXM: cefuroxime; CAZ: ceftazidime; CTX: cefotaxime; CRO: ceftriaxone; FEP: cefepime; MEM: meropenem; ETP: ertapenem; AMK: amikacin; GEN: gentamicin; CIP: ciprofloxacin; LVX: levofloxacin; NOR: norfloxacin; SXT: trimethoprim-sulfamethoxazole; NIT: nitrofurantoin; LNZ: linezolid; CLI: clindamycin; ERY: erythromycin.

Among the two *T. otitidis* isolates, a divergent susceptibility pattern was observed. One isolate was susceptible to all tested agents except trimethoprim-sulfamethoxazole (SXT), for which it was classified as intermediate. The second isolate was resistant to clindamycin, erythromycin, and SXT and was classified as intermediate in susceptibility to gentamicin. *C. amycolatum* also exhibited resistance to clindamycin and erythromycin, with additional resistance to fluoroquinolones, nitrofurantoin, SXT, and ampicillin, but it was classified as intermediate in susceptibility to ampicillin-sulbactam.

Overall, all isolates tested for gentamicin susceptibility were susceptible except for one *T. otitidis* isolate, which was classified as intermediate. Amikacin and carbapenems also showed favorable activity in the isolates for which they were tested.

Discussion

This study provides a microbiological characterization of intraoperative cholesteatoma tissue from a secondary-care center. The series comprised patients with acquired, histopathologically confirmed cholesteatoma undergoing either primary or revision surgery, all with a history of antibiotic use. Bacteria were isolated in 56.3% of cases, and fungi in 12.5%, while no growth was observed in 31.3%. Notably, the microbial profile identified in this series diverges from the pattern most consistently reported across culture-based studies, in which *P. aeruginosa* predominates, while *S. aureus* and *P. mirabilis* are also among the most frequently isolated organisms [7-11].

In our series, the most frequent bacterial isolates were *T. otitidis* and *P. mirabilis*, with no recovery of *S. aureus*. The prominence of *T. otitidis* is particularly noteworthy. Although this bacterium has historically been regarded as a commensal otic organism, accumulating evidence supports its role as an emerging pathogen associated with otitis media, otitis externa, and mastoiditis [12]. However, this divergence should be interpreted cautiously given the small sample size and may reflect geographic variation and differences in sampling methodology. The non-standardized selection of patients for culture further limits comparisons with other surgical series.

The 31.3% no-growth rate exceeds the 12.5% reported in a preoperative ear-canal swab series [10]. However, culture negativity does not imply microbial absence. In a recent cholesteatoma tissue study, bacterial DNA was detected in all specimens by droplet digital PCR despite positive cultures in only half of the cases [13]. Notably, conventionally reported genera such as *Pseudomonas* and *Staphylococcus* represented less than 0.001% of total sequencing reads, suggesting that dominant taxa may differ substantially from those identified by culture-based methods. Similarly, other molecular approaches, including 16S rRNA gene sequencing and metagenomic next-generation sequencing, have demonstrated higher detection rates and the ability to identify organisms that are not readily recovered by standard culture, such as anaerobes and low-abundance taxa [2,3,14,15]. Collectively, these findings suggest that the no-growth rate observed in the present series may, at least in part, reflect methodological limitations rather than the true absence of microorganisms. Although all patients had previously received antibiotics, the absence of an analysis of treatment regimens and their timing precludes determining whether antibiotic exposure contributed to negative cultures or the observed susceptibility patterns.

Fungal recovery in 12.5% of cases falls within the broad range reported across prior series [7,8,10,11]. The fungal isolates comprised *Cladosporium* spp. and *Aspergillus terreus*. The recovery of *Aspergillus* is consistent with previous culture-based studies reporting *Aspergillus* and *Candida* among the predominant fungal genera [7,8,10,11]. Additionally, *Aspergillus* was the most commonly detected microbial genus in a recent metagenomic next-generation sequencing study [2]. Interestingly, all fungal isolates were recovered from female patients; however, given the small sample size, no sex-based conclusions can be drawn.

Previous studies have suggested that specific pathogens may play a role in cholesteatoma pathogenesis: CoNS were more abundant in cholesteatomas than in chronic otitis media, while *S. aureus* and *P. aeruginosa* were increased in both diseases compared with the external auditory canal flora; specifically, the abundance of *S. aureus* was positively correlated with cholesteatoma volume [16]. In addition, *Aspergillus* spp. have been linked to severe bone erosion in cholesteatoma [2].

Among the isolates recovered, the two *T. otitidis* isolates displayed divergent susceptibility profiles. One isolate remained broadly susceptible, whereas the second was resistant to clindamycin, erythromycin, and SXT. Resistance to erythromycin and clindamycin has been previously documented [12,17,18], as has resistance to SXT [19]. In contrast, 100% susceptibility to linezolid was reported in a series of adults with active infection and *T. otitidis* isolation [12], while very low minimum inhibitory concentrations have been reported in another study [18]. Regarding *C. amycolatum*, a previous report documented high resistance to ceftriaxone, imipenem, and moxifloxacin in otologic infection [20].

With the exception of nitrofurantoin, both isolates of *P. mirabilis* demonstrated broad susceptibility to all antibiotics tested, including β-lactams, carbapenems, aminoglycosides, fluoroquinolones, and SXT. This pattern is consistent with a previous report [21]. Resistance to nitrofurantoin was consistent with previously reported resistance to nitrofurans in *P. mirabilis* [22]. Nonetheless, some studies have reported variable resistance to fluoroquinolones and aminoglycosides [23,24]. The single *P. aeruginosa* isolate was resistant to the cephalosporins tested but remained susceptible to carbapenems, aminoglycosides, and fluoroquinolones. This partly aligns with studies in which fluoroquinolones, carbapenems, later-generation cephalosporins, and aminoglycosides remain active against this pathogen, although resistance varies for the latter two [24,25]. It differs, however, from other reports highlighting reduced ciprofloxacin susceptibility among *P. aeruginosa* isolates [9,23].

The observed resistance of *E. cloacae* to cephalexin is consistent with its known intrinsic resistance profile [26]. Although its isolation is uncommon in otologic series [24], *E. cloacae* has substantial potential to acquire multidrug resistance [26]. In contrast, *S. paucimobilis* exhibited susceptibility to the other cephalosporins tested, carbapenems, aminoglycosides, nitrofurantoin, and SXT, with resistance to fluoroquinolones and an intermediate result for ceftazidime. While specific resistance data for *S. paucimobilis* in cholesteatoma or otologic infections are limited, reports from other clinical contexts describe common resistance to narrow-spectrum β-lactams, variable resistance to fluoroquinolones, aminoglycosides, and SXT, and relative susceptibility to carbapenems and later-generation cephalosporins [27,28]. Notably, *Sphingomonas* spp. and *Cladosporium herbarum* have been found to have a lower relative abundance in cholesteatomatous tissue compared with controls [29].

Culture and antimicrobial susceptibility testing can help guide antibiotic selection when treatment is indicated. Susceptibility-guided antibiotic therapy has been associated with higher dry-ear rates than empirical treatment in patients with chronic suppurative otitis media and middle-ear cholesteatoma [30].

The primary limitations of the study include the small sample size, retrospective design, absence of anaerobic processing and molecular detection, and variation in the antibiotic panels tested across isolates. The lack of standardized criteria for obtaining cultures introduces potential selection bias. Furthermore, because the total number of cholesteatoma operations during the study period was unavailable, the representativeness of the included cases could not be assessed. Although prior antibiotic use was reported in all patients, specific agents, routes, durations, and timing were not analyzed, limiting interpretation of their potential relationship with culture yield and susceptibility patterns. The findings also describe primary and revision procedures together and do not establish differences between these groups. Additionally, the CoNS isolate was not subjected to antimicrobial susceptibility testing because this analysis was not routinely performed by the microbiology laboratory. These limitations should be considered when interpreting and generalizing the findings.

Conclusions

This study describes a heterogeneous microbiological profile in intraoperative cholesteatoma tissue cultures, with antibiotic susceptibility varying even among isolates of the same species. Susceptibility should therefore be assessed for each isolate rather than inferred from organism identity alone. These findings provide a local reference but have limited generalizability. Larger studies using standardized sampling, anaerobic cultures, and molecular techniques are needed to further characterize the cholesteatoma microbiota and its antibiotic resistance patterns.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Acquisition, analysis, or interpretation of data: Ángel G. Soto-Pardo, Sarah Moreno-Uriarte, Samsara Zazueta-Chavez

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Disclosures

Human subjects: Informed consent for treatment and open access publication was obtained or waived by all participants in this study. **Animal subjects:** All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of interest:** In compliance with the ICMJE uniform disclosure form, all authors declare the following: **Payment/services info:** All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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