






Microorganisms isolated from external ear cultures and their antimicrobial susceptibility in patients with chronic suppurative otitis media: A six years experience

 Tuba Muderris¹,  Mustafa Koray Balci²,  Rahim Ozdemir³,  Sureyya Gul Yurtsever¹,  Selcuk Kaya¹

¹Department of Microbiology, Izmir Katip Celebi University, Izmir, Turkey

²Department of Otolaryngology, Ataturk Education and Research Hospital, Izmir, Turkey

³Department of Microbiology, Ataturk Education and Research Hospital, Izmir, Turkey

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Abstract

Aim: Chronic suppurative otitis media (CSOM) continues to be a major health issue in developing countries. The goal of this study was to establish the microorganisms causing CSOM and their susceptibility to antimicrobials.

Materials and Methods: External ear tract swab that came to our laboratory between 2014-2019 were evaluated retrospectively. The data were obtained from the hospital medical records. Data was collected using an electronic database.

Results: The analysis included 185 ear swab samples. These patients 55.1% male and 44.9% female, and average age was 44.29 ± 1.6 (2-86) years. Forty (21.6%) of the patients included in the study were diagnosed with cholesteatoma as having chronic suppurative otitis media. The first three bacteria species isolated from the samples included in the study; *Pseudomonas aeruginosa* (31.9%), *Staphylococcus aureus* (11.9%) and *Escherichia coli* (9.7%). In addition, in 10.8% of the samples *Candida spp.* and in 5.4% of the samples *Aspergillus spp.* were isolated. The first three species isolated from the patients with cholesteatoma; *P. aeruginosa* (6.5%), *S. aureus* (4.3%) and *Candida spp.* (3.2%), the first three species isolated in patients without cholesteatoma; *P. aeruginosa* (25.4%), *E. coli* (8.1%), *S. aureus* and *Candida spp.* (7.6%) was detected. Ciprofloxacin (68.8%) and gentamicin (46.7%) in *P. aeruginosa* isolates, amoxicillin-clavulonic acid (66.7%) and trimethoprim-sulfamethoxazole (42.9%) in *E. coli* isolates were found to be the most resistant antimicrobials.

Conclusion: In conclusion, antimicrobial therapy should not be initiated in CSOM patients without waiting for the culture result. However, in cases where empirical antimicrobial therapy is mandatory, the first choice has been determined as amikacin. Good awareness of microorganisms' antimicrobial sensitivity can lead to appropriate antibiotic usage and treatment success for CSOM. We think that multicenter prospective studies are needed to organize effective treatments of these infections.

Keywords: Antimicrobial susceptibility; chronic suppurative otitis media; microorganisms

INTRODUCTION

Chronic suppurative otitis media (CSOM) is the middle ear and mastoid cavity recurrent inflammation and infection (1) and usually occur with mixed bacterial flora (2). This condition is characterised by ear discharge (otorrhoea) through a perforated tympanic membrane and hearing loss (1). Ear discharge may be transient or continuous (3). Chronic suppurative otitis media is the most important reason of hearing loss in almost every age group (2). Chronic suppurative otitis media can cause serious complications such as mastoid abscess, postauricular fistula and facial palsy, otitic meningitis, lateral sinus thrombosis and cerebellar abscess in rare cases (4,5).

Chronic suppurative otitis media, may develop as a result of repeated and/or poorly treated acute suppurative otitis media, rhinosinusitis and upper respiratory tract infection, especially in childhood (2). Typical pathogens reach the middle ear following insufflations of respiratory pathogens through the eustachian tubes from the nasopharynx or from the external ear canal inwards through a non-intact tympanic membrane (6,7). Usually aerobic and anaerobic bacteria coexist in CSOM, hence the treatment is difficult (2). Studies of microbiological CSOM diagnoses vary in terms of patients age, geographic circumstances, and occurrence of complications such as cholesteatomas, and these differences are likely to affect the variability in recorded pathogens (6). Knowledge of

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Corresponding Author: Tuba Muderris, Department of Microbiology, Izmir Katip Celebi University, Izmir, Turkey

E-mail: tubamuderris@yahoo.com

the true frequency of polymicrobial infection is limited by the differences in collection and culture techniques (8). Traditional collection of swab specimens was associated with contamination with natural skin flora such as *Staphylococcus epidermidis*, diptheroids and anaerobic organisms, like *Propionibacterium acnes* (9).

All possible pathogens in CSOM include aerobes, anaerobes, and fungi. Understanding of the microbiology of chronic otitis media is critical for successful and efficient treatment, and prevention of complications and antibiotic resistance. The aim of this study was to determine microorganisms causing CSOM and their antimicrobial susceptibility in the Ataturk Education and Research Hospital, the largest hospital in the region.

MATERIALS and METHODS

External ear tract swab samples taken from patients with CSOM that came to our laboratory between January 2014–December 2019 were evaluated retrospectively. The data were collected from hospital medical records using an electronic database.

Patients with otorrhoea for twelve weeks or more were considered as CSOM. Presence of choleastatoma were diagnosed on the basis of otoscopic findings, temporal bone imaging (CT and MRI), and the presence or absence of cholesteatoma during surgery.

Until collecting samples the patients had not been treated with antimicrobial agents. External ear canal samples were taken with a cotton swab using a sterile otoscope to prevent contamination. The samples were sent to the microbiology laboratory in Stuart transport medium.

External ear tract swab samples were planted in 5% sheep blood agar and Eosin Methylene Blue media and incubated at 37°C for 18–24 hours for bacteriological examination. For fungal examination, samples were sown in two separate Sabouraud dextrose agar (SDA). One of the SDAs was incubated at room temperature and the other at 37°C for 1–3 weeks, the reproduction was checked daily during the incubation period.

Strains found to be bacteria in Gram staining were evaluated by colony morphology and biochemical tests (oxidase, coagulase and catalase test, three sugar iron test, urea hydrolysis test, Indole, Methyl Red, citrate test). Identification and antimicrobial susceptibilities of isolated bacteria were done using automated system (Phoenix, BD, USA). Antimicrobial susceptibility tests were evaluated according to the Clinical and Laboratory Standards Institute for the first two years and European Committee on Antimicrobial Susceptibility Testing criteria for the last four years (10,11).

Strains found to be yeast in gram staining were identified by traditional methods (germ tube test, colony morphology on corn-meal Tween 80 agar and urease test) and automated system (Phoenix™ 100-yeast ID, BD, USA) for the samples that revealed yeast. Identification in mold-growing samples was done according to the

microscopic appearance of the hif and spore structures in the preparations prepared with lactophenol cotton blue and the colony appearance of mold.

The study is approved by institutional review board (2020-GOKAE-0291).

RESULTS

Between the years 2014 and 2019, 320 ear swab samples came to our laboratory and growth was detected in 189 (59.1%) of these samples. Repetitive growths were excluded from the study. Eventually, 185 ear swab samples were included to the study. Single microorganism growth was detected in all samples. Of these patients, 55.1% were male and 44.9% were female, and the mean age was 44.29 ± 1.6 (2–86) years. Forty (21.6%) of the patients had the diagnosis of chronic suppurative otitis media with cholesteatoma. The distribution of samples by years were as follows; 21.1% in 2019, 12.4% in 2018, 34.6% in 2017, 6.5% in 2016, 13% in 2015, and 12.4% in 2014. The most common three bacteria species isolated from the samples were; *Pseudomonas aeruginosa* (31.9%), *Staphylococcus aureus* (11.9%) and *Escherichia coli* (9.7%). In addition, *Candida spp.* were isolated in 10.8% and *Aspergillus spp.* were isolated in 5.4% of the samples. While the most common three species isolated from the patients with cholesteatoma were *P. aeruginosa* (6.5%), *S. aureus* (4.3%) and *Candida spp.* (3.2%), the most common three species isolated in patients without cholesteatoma (noncholesteatoma) were; *P. aeruginosa* (25.4%), *E. coli* (8.1%), *S. aureus* and *Candida spp.* (7.6%). The distribution of the isolates is shown in Table 1.

Table 1. Distribution of the isolates in patients with noncholesteatoma and cholesteatoma

Isolated organism	Noncholesteatoma n (%)	Cholesteatoma n (%)	Total n (%)
Gram-positive bacteria			
<i>S. aureus</i>	14 (7.6)	8 (4.3)	22 (11.9)
CNS	10 (5.4)	2 (1.1)	12 (6.5)
Gram-negative bacteria			
<i>P. aeruginosa</i>	47 (25.4)	12 (6.5)	59 (31.9)
<i>E. coli</i>	15 (8.1)	3 (1.6)	18 (9.7)
<i>Achromobacter spp.</i>	10 (5.4)	2 (1.1)	12 (6.5)
<i>Klebsiella spp.</i>	7 (3.8)	0	7 (3.8)
<i>Candida spp.</i>	14 (7.6)	6 (3.2)	20 (10.8)
<i>Aspergillus spp.</i>	8 (4.3)	2 (1.1)	10 (5.4)
Others	20 (10.8)	5 (2.7)	25 (13.5)
Total	145 (78.4)	40 (21.6)	185 (100)

Enterococcus spp., *Proteus spp.*, *Serratia spp.*, *Acinetobacter spp.*, *Alcaligenes spp.*, *Streptococcus spp.*, *Citrobacter spp.*, *Enterobacter spp.*, *Morganella spp.*
CNS: Coagulase Negative Staphylococcus

Table 2. Distribution of the isolates in patients with noncholesteatoma and cholesteatoma

%	CN	TZP	CAZ	FEP	AK	IMP	MEM	CP	AMC	TG	ST	LZ	VA
P. aeruginosa													
Noncholesteatoma	25	30	20.5	28.9	0	20	20	44.4	-	-	-	-	-
Cholesteatoma	62.5	50	60	54.5	33.3	50	40	87.5	-	-	-	-	-
Total	46.7	36.7	28.3	35.4	18.8	36.4	27.3	68.8	-	-	-	-	-
E. coli													
	-	37.5	25	38.9	-	12.5	14.3	-	66.7	12.5	42.9	-	-
S. aureus													
	-	-	-	-	-	-	-	12.5	7.1	-	0	0	0

CN: Gentamicin, TZP: Piperacillin-tazobactam, CAZ: Ceftazidime, FEP: Cefepime, AK: Amikacin, IMP: Imipenem, MEM: Meropenem, CP: Ciprofloxacin, AMC: Amoxicillin-clavulonic acid, TG: Tigecycline, ST: Trimethoprim-sulfamethoxazole, LZ: Linezolid, VA: Vancomycin.

Noncholesteatoma: Antimicrobial resistance distribution of *P. aeruginosa* isolates isolated from patients without cholesteatoma

Cholesteatoma: Antimicrobial resistance distribution of *P. aeruginosa* isolates isolated from patients with cholesteatoma

When the antimicrobial resistances were evaluated, *P. aeruginosa* isolates found to be commonly resistant to ciprofloxacin (68.8%) and gentamicin (46.7%) and *E. coli* isolates found to be resistant to amoxicillin-clavulonic acid (66.7%) and trimethoprim-sulfamethoxazole (42.9%). In addition, most resistant antimicrobials in *P. aeruginosa* isolated from the patients with cholesteatoma were ciprofloxacin (87.5%) and gentamicin (62.5%), most resistant antimicrobials in *P. aeruginosa* isolated from the patients without cholesteatoma were ciprofloxacin (44.4%) and trimethoprim-sulfamethoxazole (30%). While *P. aeruginosa* isolates were sensitive to amikacin (18.8%) and meropenem (27.3%), and *E. coli* isolates were sensitive to imipenem (12.5%) and meropenem (14.3%). In addition, *P. aeruginosa* isolates in patients with and without cholesteatoma were found to be least resistant to amikacin (0%), carbapenems (20%), amikacin (33.3%) and meropenem (40%) respectively. Antimicrobial resistance distribution is shown in Table 2.

DISCUSSION

Treatment options for CSOM include topical antibiotics with or without steroids, systemic antibiotics, topical antiseptics and ear cleaning, all of which can be used on their own or in various combinations (1). The increasing prevalence of resistant bacteria, as well as altering the susceptibility to antibiotics, indicates that continuous and periodic bacteriological surveys is needed (12). Bacteriological studies of CSOM patients have been used as recommendations for the selection of antibiotics (12). The causative microorganisms in CSOM patients may vary depending on the conditions such as the geographical and climatic conditions, the age of the patient, whether sterile conditions can be met while taking the culture and the presence of otitis complications such as cholesteatoma (13).

Pseudomonas aeruginosa and *S. aureus* are the most frequently isolated bacteria in the cultures taken from both external ear canals and middle ears in CSOM cases (14-16).

In previous studies conducted among patients with CSOM, the first two most frequently isolated bacteria in the external ear canal swab samples were reported as *P. aeruginosa* and *S. aureus* (2,12,13,17,18). In these studies, *P. aeruginosa* was reported between 17.1% and 42.4%, and *S. aureus* was reported between 10.6% and 40.6% (2,12,13,17,18). In our study, the most common bacteria detected in cultures taken from the external ear canal in CSOM cases were *P. aeruginosa* (31.9%) and *S. aureus* (11.9%), in accordance with the literature. As we mentioned earlier, the difference between the rates may be attributed to the specimen collection and culture techniques, factors associated with patient like age and geographical conditions, and the presence of complications.

Chronic otitis media is categorized as noncholesteatomatous or cholesteatomatous based on the presence or absence of cholesteatoma (12). Cholesteatoma of the middle ear is an accumulation of desquamated keratin in the tympanic cavity from a multilayered ectopic epithelial mass (19). In chronic otitis media with cholesteatoma, *P. aeruginosa* and *S. aureus* are the most commonly reported isolates (19-21). Kim et al. reported that while the most common microorganism in patients with cholesteatoma was *S. aureus* (26%), the second microorganism was *P. aeruginosa* (14%). Also in that study, it was stated that while the rate of *S. aureus* decreased over the years, the rate of *P. aeruginosa* increased (22). Compatible with the literature, in our study, the most frequently isolated microorganisms in patients with cholesteatoma were *P. aeruginosa* (6.5%) and *S. aureus* (4.3%).

Pseudomonas aeruginosa can grow well in the absence of special nutrients, it proliferates at room temperature, and it is highly resistant to antibiotics, making it difficult to treat (12). In our study, we found that the highest resistance rates in *P. aeruginosa* isolates were against gentamicin (46.7%) and ciprofloxacin (68.8%), which are the two most commonly used local antimicrobials. The most sensitive antimicrobials in these isolates were amikacin (18.8%),

meropenem (27.3%) and ceftazidime (28.3%). Gul et al. reported the resistance rates of gentamicin, ciprofloxacin and amikacin in *P. aeruginosa* isolates were 15%, 8% and 15%, respectively. The bacteria were highly sensitive to imipenem and ceftazidime in mentioned study (2). Malkoc et al. reported the resistance rates of gentamicin, ciprofloxacin, amikacin and ceftazidime in *P. aeruginosa* isolates as 15%, 25%, 8% and 35%, respectively. In addition, the most sensitive antimicrobials in this study were found to be carbapenems and amikacin (17). Tok et al. reported the resistance rates of gentamicin, ciprofloxacin and amikacin in *P. aeruginosa* isolates as 50%, 10% and 80%, respectively. In addition, the most sensitive antimicrobials in that study were found to be imipenem and ceftazidime (18). We think that high gentamicin and ciprofloxacin resistance in *P. aeruginosa* isolates is often associated with the initiation of empirical antimicrobial therapy without waiting for culture and antimicrobial susceptibility and the use of these antimicrobials as the first choice.

In previous studies, *E. coli* isolation rates in the external ear canal swab samples ranged from 7% to 23.7% (12,17,18). In our study, this rate (9.7%) was compatible with the literature. Antimicrobial resistance rates of *E. coli* isolates isolated from the external ear canal differs in the literature (2,17,18). Tok (18), Malkoc (17) and Gul (2) reported that the most resistant antimicrobials in their study were ampicillin and gentamicin, amoxicillin-clavulonate and cefazolin, cefazolin and trimethoprim-sulfamethaxazole, respectively. In addition, Tok et al. (18) reported that cephalosporins, ciprofloxacin, amikacin and imipenem, Gul et al. (2) reported that ciprofloxacin and imipenem and Malkoc et al. (17) reported that amikacin, carbapenems and ciprofloxacin were the most sensitive antimicrobials. In our study, the first two antimicrobials with high resistance were determined as ampicillin, amoxicillin-clavulonate and the first two antimicrobials with high sensitivity were determined as imipenem and meropenem. We think that the variation in resistance rates is due to the difference of antimicrobials used during the treatment between the centers.

Although the most frequently isolated bacteria from ear cultures are *P. aeruginosa*, *S. aureus*, and *E. coli*, many different pathogens can cause CSOM, as demonstrated in our study. We also detected *Achromobacter spp.*, *Klebsiella spp.*, *Proteus spp.*, *Enterobacter spp.*, *Serratia spp.*, *Enterococcus spp.*, *Citrobacter spp.*, *Alcaligenes spp.*, *Morganella spp.* and *Acinetobacter spp.* in the external ear canal cultures which were taken from patients with CSOM in our study. Since bacterial pathogens can be very diverse in suppurative chronic otitis media, it is vital to apply treatment protocols rather than empirical treatments to prevent a failure in treatment. It is reported that anaerobic bacteria are responsible for almost 15% in chronic otitis media cases (23). However, in our study, no anaerobic culture was made, which is the major limitation of our study.

In the classical treatment of chronic otitis media, usually local and/or systemic antimicrobials covering the bacterial factors are given, but fungal factors should not

be ignored in patients living in warm and moist regions, receiving immunosuppressive therapy, taking long-term antibiotics and with a history of diabetes mellitus (24). In their study, Ceviker et al. isolated 3% *Candida spp.* and 10.6% mold fungus (13). Malkoc et al. reported an isolation rate of 3.2% *Candida spp.* and 1.9% *Aspergillus spp.* (17). In our study, 10.8% *Candida spp.* and 5.4% *Aspergillus spp.* were detected among patients with CSOM. We think that our higher rates may be related to the high humidity and temperature rates of the region where our hospital is settled.

CONCLUSION

In conclusion, we think antimicrobial therapy should not be initiated in CSOM patients without waiting the culture result. However, in cases where empirical antimicrobial therapy is mandatory, the first choice should be amikacin. Good awareness of microorganisms' antimicrobial sensitivity can lead to rational antibiotic usage and treatment success for CSOM. We think that multicenter prospective studies are needed to organize effective treatments of these infections.

Conflict of interest: The authors declare that they have no competing interest.

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