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ORIGINAL ARTICLE

A Ten-Year Study of the Epidemiology and Antimicrobial Resistance of Bacterial Pathogens Associated with Otitis in Crete, Greece

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SUMMARY

Background: **Otitis is a very common infection, mainly affecting children, and the main indication for prescribing antibiotics. This study investigated the epidemiology and antibiotic resistance profile of bacterial pathogens associated with otitis in patients of all ages in Crete, Greece.**
Methods: **From January 2013 through December 2022, patients diagnosed with otitis, based on clinical signs and symptoms and otoscopy findings, were enrolled in the study. Ear discharge samples were collected by tympanocentesis or by using sterile swabs and were promptly transported to the microbiology laboratory for further processing. Cultures for bacterial pathogens were performed according to laboratory protocols. Bacteria were identified by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry and antimicrobial susceptibility testing by Vitek 2 system.**
Results: **Out of the 939 samples examined, 600 (63.9%) were positive for bacterial pathogens. *P. aeruginosa* was the most prevalent microorganism detected (29.5%), followed by *S. aureus* (18.9%) and *Enterobacterales* (12.8%). The isolation rate of *P. aeruginosa* significantly increased during the study period (p = 0.0001). *P. aeruginosa* resistance rates to ticarcillin/clavulanate, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem and meropenem were 6.4%, 3.4%, 3.4%, 3.8%, 3.3%, 2.1%, and 2.1%, respectively. Ceftazidime resistance remained around the same levels during the study period, while resistance to piperacillin/ tazobactam, cefepime, imipenem, and meropenem doubled over the years 2018 - 2022 (p = 0.48, p = 0.49, p = 0.65, and p = 0.65, respectively). *S. aureus* penicillin-resistance was extremely high (87.4%). Resistance rates to methicillin, erythromycin, and clindamycin were 21.2%, 14.6%, and 11.9%, respectively. Forty-one percent of the methicillin-resistant strains were multidrug-resistant (MDR). All isolates were susceptible to gentamicin, rifampicin, linezolid, daptomycin, tigecycline, teicoplanin, and vancomycin. Among *Enterobacterales*, high rates of resistance were observed for ampicillin, amoxicillin/ clavulanate, colistin, tigecycline, and tetracycline, ranging from 34.1% to 82.5%.**
Conclusions: ***P. aeruginosa, S. aureus,*** **and the *Enterobacterales* are the predominant bacterial pathogens causing otitis in our area. Regarding Gram-negatives, although resistance rates to commonly-used antibiotics are low, they are increasing over time. Continued surveillance of the prevalence and antimicrobial susceptibility of bacterial pathogens causing otitis is necessary to reassess updated antimicrobial policies and develop appropriate guidelines.**
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INTRODUCTION

Otitis is an upper respiratory tract infection that is more common in children than in adults. There are different types of ear infections, depending on which part of the ear is infected. Two common types are middle ear infection called otitis media (OM) and the external auditory canal infection, otitis externa (OE), called swimmer's ear [1]. Types of OM include acute otitis media (AOM), otitis media with effusion (OME), and chronic suppurative otitis media (CSOM). AOM is a very common infection and a leading cause of health care visits. The total annual number of new AOM episodes is estimated at 709 million, with 51% occurring in children < 5 years of age [2]. AOM represents the most common indication for antibiotic prescription. In the USA, 8.7 million diagnoses of AOM are made every year, and AOM is one of the most common reasons for pediatric outpatient consultation, accounting for an estimated 15 million antibiotic prescriptions every year [3]. By 3 years of age, 60% of children have had experienced at least one episode of AOM, and one third have had three or more episodes [4]. Risk factors include early childhood, daycare attendance, previous history of AOM, upper respiratory tract infections, passive smoker, the presence of atopy, adenoidal hypertrophy, ciliary dysfunction, immune-compromising conditions, and craniofacial anomalies [5].

WHO estimated that 28 thousand deaths annually, with the highest mortality rates in children < 5 years of age, are attributable to complications of OM, such as meningitis and brain abscess [6].

Viral pathogens, bacterial pathogens, and fungi have been associated with otitis. The most common bacterial pathogens causing otitis are *Streptococcus pneumoniae*, *Hemophilus influenzae, Moraxella catarrhalis*, *Streptococcus pyogenes*, *Stapylococcus aureus*, *Pseudomonas aeruginosa,* and *Enterobacterales* [7].

Antibiotic treatment plays an important role in the management of otitis. AOM is the most frequent cause of antibiotic prescriptions in children below 3 years, accounting for 14% of all antibiotic prescriptions in the UK [8,9]. The rationale for antibiotic prescription mainly includes the prevention of serious but rare complications, such as mastoiditis and meningitis [10]. Therefore, knowledge of bacteriology and the antimicrobial susceptibility profile of causative pathogens is very important. Inappropriate use of antibiotics results in treatment failure and increases the risk of antibiotic resistance. The increasing prevalence of antimicrobial resistance among these pathogens and the spread of multidrug-resistant (MDR) strains remains a global health problem [11].

The frequency and antimicrobial susceptibility patterns of pathogens causing otitis vary significantly by geography, time, age, socioeconomic factors and local antimicrobial prescribing practices. Antimicrobial therapies rely on local patterns of antimicrobial resistance, that should be continuously monitored in order to update treatment guidelines, and contribute to tackling antibiotic resistance.

In Greece, there are limited data regarding bacterial pathogens and their resistance to antimicrobials; it is mainly focused on childhood AOM [12,13].

This study was undertaken to investigate the prevalence of bacterial pathogens associated with otitis and their antimicrobial resistance trends over a 10-year period in Crete, Greece.

MATERIALS AND METHODS

Study setting and design

This retrospective study was conducted from January 2013 through December 2022 at the University Hospital of Heraklion. This hospital is a 771-bed, tertiary care academic institution, serving a population of approximately 750,000 inhabitants. All ear discharge samples that were included in this study were obtained from patients of all ages that were diagnosed with otitis, based on clinical signs and symptoms and otoscopy findings. Immunocompromised patients and those with craniofacial abnormalities were excluded. To avoid selection bias, repeated samples from the same patient were not included. The samples were analyzed by the Hospital Clinical Microbiology Laboratory for the presence of bacterial pathogens. All hospital departments were involved, including emergency, inpatient, and outpatient departments. This study was approved by the Ethical Committee of the University Hospital of Heraklion and met the guidelines of the Declaration of Helsinki (protocol code 87431/24-03-2022).

Microbiology

Cultures and identification

Ear discharge samples were collected under aseptic conditions either by tympanocentesis or by using sterile cotton swabs. Swabs were immediately placed into Amies transport media (bioMérieux SA, Marcy L'étoile, France). The samples were promptly transported to the Microbiology Laboratory for further processing. Cultures were performed in chocolate agar, sheep blood agar, MacConkey and Sabouraud agar plates (BioMérieux). The plates were incubated for 24 - 48 hours at 36°C. Bacterial identification was performed by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry (BioMérieux).

Antimicrobial susceptibility testing

The antimicrobial susceptibility testing (AST) was performed by Vitek 2 system (BioMérieux). *P. aeruginosa* isolates were tested for susceptibility to ticarcillin-clavulanate, piperacillin-tazobactam, ceftazidime, cefepime,

aztreonam, imipenem, amikacin, gentamicin, tobramycin, and ciprofloxacin, using AST-N222 cards. For *Enterobacterales,* both AST- XN01 and AST-N233 cards were used, which contain the following antibiotics: ampicillin, amoxicillin/clavulanate, piperacillin/tazobactam, cefotaxime, ceftriaxone, ceftazidime, cefepime, aztreonam, imipenem, meropenem, amikacin, gentamicin, tobramycin, ciprofloxacin, tetracycline, tigecycline, colistin, and trimethoprim/sulfamethoxazole (TMP/SMX). Additionally, colistin MICs were determined by broth microdilution (BMD) ComASP Colistin (Liofilchem, srl). BMD is recommended by both CLSI and EUCAST as the reference methodology for colistin MIC determination according to the ISO standard 20776-1 [14]. The antibiotics tested against *S. aureus* included penicillin, oxacillin, erythromycin, clindamycin, linezolid, daptomycin, teicoplanin, vancomycin, tetracycline, tigecycline, rifampicin, levofloxacin, and TMP/SMX.

Escherichia coli ATCC 25922, *Klebsiella pneumoniae* ATCC 700603, and *S. aureus* ATCC 29213 were used as quality control strains. The Clinical and Laboratory Standards Institute (CLSI) M100-Ed32 guideline was used to interpret results for all other antimicrobials studied, except for tigecycline for which the U.S. Food and Drug Administration (FDA)-recommended MIC breakpoints were applied [15]. For the purpose of the MDR classification, isolates displaying intermediate susceptibility to antimicrobials were considered as "non-susceptible".

Additionally, the modified CLSI ESBL confirmatory test was performed for detection of extended-spectrum beta-lactamases (ESBLs) employing cefotaxime (30 µg) and ceftazidime (30 µg) disks alone and in combination with clavulanate (10 µg), on which both boronic acid (BA) and EDTA were dispensed [16].

Multidrug-resistant (MDR) bacteria were defined as isolates non-susceptible to at least one agent in ≥ 3 antimicrobial categories [17].

Statistical analysis

Statistical analysis was conducted by chi-squared and Fisher´s exact test, as appropriate. A p-value of < 0.05 was regarded as indicative of statistical significance. The selected cutoff p-value was two-tailed. All statistical analyses were performed with Graphpad Prism version 4.0 software (GraphPad Software Inc, CA, USA).

RESULTS

Demographic characteristics

During the study period, 939 specimens were examined, one from each patient. Processed specimens highly increased in the second 5-year period. Pathogens were isolated from 600 patients (63.9%). Out of those, 331 (55.2%) were male and 269 (44.8%) were female. The mean patient age was 28 ± 8.7 years (range, 10 days - 96 years), with approximately one-third of them being ≤ 4 years (Table 1).

Table 1. Distribution of the pathogen by age and gender.

Age (years)	n (%)
0 - 4	203 (33.8)
5 - 9	66 (11)
10 - 19	76 (12.7)
20 - 29	30 (5)
30 - 39	46 (7.7)
40 - 49	41 (6.8)
50 - 59	40 (6.7)
60 - 69	43 (7.2)
≥ 70	55 (9.2)
Gender	**n (%)**
Male	331 (55.2)
Female	269 (44.8)

More than half of the samples (56.6%) were obtained from patients admitted to the Emergency Department (ED), 20.7% were from outpatient clinics, and 14.1% were from Pediatrics. During the study period, the proportion of positive samples ranged from 49.2% to 76.7%, with the higher rate observed in 2013.

Distribution and prevalence of bacterial pathogens

During the ten-year period, a total of 800 strains were isolated from 600 ear exudate samples. Out of these pathogens, 435 (54.4%) were Gram-negative bacteria, 301 (37.6%) were Gram-positive bacteria, and 64 (8%) were fungi. *P. aeruginosa*, *S. aureus,* and *Enterobacterales* accounted for 58.2% of the total isolates. *P. aeruginosa* was the most prevalent microorganism (n = 236; 29.5%), followed by *S. aureus* (n = 151; 18.9%) and *Enterobacterales* (n = 101; 12.8%). Among the 101 *Enterobacterales* identified, *Proteus mirabilis* was the most commonly isolated bacteria (19.8%), followed by *K. pneumoniae* (16.8%) and *Enterobacter cloaceae* (13.9%). *S. pyogenes* (7%), *H. influenzae* (5.1%), and *S. pneumoniae* (4.6%) were less frequently isolated. *P. aeruginosa* and *S. aureus* were most frequently isolated from all age groups, with higher frequencies in the 30 - 39-years age group. *H. influenzae* and *S. pneumoniae* predominated in the 0 - 9 age group, while *Enterobacterales* were more common in the over 65 age group. The isolation rate of *P. aeruginosa* significantly increased during the study period ($p = 0.0001$), while there were lower increases in the isolation rates of *S. aureus* and the *Enterobacterales* ($p = 0.16$ and $p = 0.77$, respectively). Monomicrobial growth was found in 429 (71.5%) cases, while in 171 (28.5%) cases polymicrobial growth was observed. *P. aeruginosa* and *S. aureus* co-existed in 22.8% of polymicrobial cultures.

Table 2. Comparison of antimicrobial resistance rates in *P. aeruginosa* isolates over the 2 study periods (2013 - 2017 and 2018 - 2022).

	2013 - 2017 (94 isolates)			2018 - 2022 (142 isolates)			p-value
Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	
Ticarcillin/ Clavulanate	88/94 (93.6)	2/94 (2.1)	4/94 (4.3)	130/142 (91.6)	1/142 (0.7)	11/142 (7.7)	0.36
Piperacillin/ Tazobactam	92/94 (97.9)	-	2/94 (2.1)	136/142 (95.8)	-	6/142 (4.2)	0.48
Ceftazidime	91/94 (96.8)	-	3/94 (3.2)	137/142 (96.5)	-	5/142 (3.5)	1.00
Cefepime	92/94 (97.9)	-	2/94 (2.1)	135/142 (95.1)	-	7/142 (4.9)	0.49
Aztreonam	82/94 (87.2)	9/94 (9.6)	3/94 (3.2)	96/118 (81.4)	18/118 (15.3)	4/118 (3.4)	0.46
Imipenem	93/94 (98.9)	-	1/94 (1.1)	138/142 (97.2)	-	4/142 (2.8)	0.65
Meropenem	93/94 (98.9)	-	1/94 (1.1)	138/142 (97.2)	-	4/142 (2.8)	0.65
Amikacin	93/94 (98.9)	-	1/94 (1.1)	139/142 (97.9)	-	3/142 (2.1)	1.00
Gentamicin	93/94 (98.9)	-	1/94 (1.1)	136/142 (95.8)	-	6/142 (4.2)	0.25
Tobramycin	93/94 (98.9)	-	1/94 (1.1)	140/142 (98.6)	-	2/142 (1.4)	1.00
Colistin	94/94 (100)	-	-	142/142 (100)	-	-	NA
Ciprofloxacin	89/94 (94.7)	1/94 (1.1)	4/94 (4.2)	100/105 (95.2)	1/105 (1.0)	4/105 (3.8)	0.98

NA not applicable.

Table 3. Comparison of antimicrobial resistance rates in *S. aureus* isolated over the 2 study periods (2013-2017 and 2018 - 2022).

	2013 - 2017 (69 isolates)			2018 - 2022 (82 isolates)			p-value
Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	
Penicillin	9/69 (13)	-	60/69 (87)	10/82 (12.2)	-	72/82 (87.8)	1.00
Oxacillin	55/69 (79.7)	-	14/69 (20.3)	64/82 (78.0)	-	18/82 (22.0)	0.84
Gentamicin	69/69 (100)	-	-	82/82 (100)	-	-	NA
Levofloxacin	67/69 (97.1)	-	2/69 (2.9)	71/77 (92.2)	-	6/77 (7.8)	0.28
Erythromycin	56/69 (81.2)	2/69 (2.9)	11/69 (15.9)	32/82 (39.0)	39/82 (47.6)	11/82 (13.4)	< 0.00001
Clindamycin	59/69 (85.5)	-	10/69 (14.5)	74/82 (90.2)	-	8/82 (9.8)	0.46
Linezolid	69/69 (100)	-	-	82/82 (100)	-	-	NA
Daptomycin	69/69 (100)	-	-	82/82 (100)	-	-	NA
Teicoplanin	69/69 (100)	-	-	82/82 (100)	-	-	NA
Vancomycin	69/69 (100)	-	-	82/82 (100)	-	-	NA
Tetracycline	63/69 (91.3)	-	6/69 (8.7)	77/82 (93.9)	-	5/82 (6.1)	0.75
Tigecycline	69/69 (100)	-	-	82/82 (100)	-	-	NA
Fusidic acid	46/69 (66.7)	19/69 (27.5)	4/69 (5.8)	51/82 (62.2)	3/82 (3.7)	28/82 (34.1)	< 0.00001
Rifampicin	69/69 (100)	-	-	82/82 (100)	-	-	NA
TMP/SMX	67/69 (97.1)	-	2/69 (2.9)	76/82 (92.7)	-	6/82 (7.3)	0.29

TMP/SMX trimethoprim/sulfamethoxazole, NA not applicable.

Table 4. Comparison of antimicrobial resistance rates in *Enterobacterales* isolated over the 2 study periods (2013 - 2017 and 2018 - 2022).

	2013 - 2017 (49 isolates)			2018 - 2022 (52 isolates)			p-value
Antibiotic	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	Susceptible n (%)	Intermediate n (%)	Resistant n (%)	
Ampicillin	6/49 (12.2)	-	43/49 (87.8)	11/48 (22.9)	-	37/48 (77.1)	0.19
Amoxicillin/ Clavulanate	21/49 (42.9)	6/49 (12.2)	22/49 (44.9)	25/51 (49.0)	1/51 (2.0)	25/51 (49.0)	0.13
Piperacillin/ Tazobactam	47/48 (40.5)	1/48 (6.9)	-	39/42 (92.9)	3/42 (7.1)	-	0.33
Cefotaxime	48/49 (98.0)	-	1/49 (2.0)	49/52 (94.2)	-	3/52 (5.8)	0.61
Ceftriaxone	48/49 (98.0)	-	1/49 (2.0)	49/52 (94.2)	-	3/52 (5.8)	0.61
Ceftazidime	48/49 (98.0)	-	1/49 (2.0)	49/52 (94.2)	-	3/52 (5.8)	0.61
Cefepime	48/49 (98.0)	-	1/49 (2.0)	50/51 (98.0)	-	1/51 (2.0)	1.00
Aztreonam	48/49 (98.0)	-	1/49 (2.0)	49/51 (96.1)	-	2/51 (3.9)	1.00
Imipenem	48/48 (100)	-	-	52/52 (100)	-	-	NA
Meropenem	49/49 (100)	-	-	52/52 (100)	-	-	NA
Amikacin	45/49 (91.8)	-	4/49 (8.2)	44/52 (84.6)	-	8/52 (15.4)	0.35
Gentamicin	43/49 (87.8)	-	6/49 (12.2)	47/52 (90.4)	-	5/52 (9.6)	0.75
Tobramycin	39/48 (81.3)	-	9/48 (18.7)	36/45 (80.0)	-	9/45 (20.0)	1.00
Colistin	28/49 (57.1)	-	21/49 (42.9)	29/52 (55.8)	-	23/52 (44.2)	1.00
Ciprofloxacin	45/49 (91.8)	-	4/49 (8.2)	39/42 (92.9)	-	3/42 (7.1)	1.00
Tetracycline	34/49 (63.4)	-	15/49 (30.6)	23/48 (47.9)	-	25/48 (52.1)	0.04
Tigecycline	31/49 (63.3)	-	18/49 (36.7)	29/42 (69.0)	-	13/42 (31.0)	0.65
TMP/SMX	42/49 (85.7)	-	7/49 (12.3)	40/41 (97.6)	-	1/41 (2.4)	0.06

TMP/SMX trimethoprim/sulfamethoxazole, NA not applicable.

Antimicrobial susceptibility testing

P. aeruginosa was the most frequently isolated pathogen from positive samples. During the ten-year study period, *P. aeruginosa* showed the lowest rate of resistance for tobramycin (1.3%). Among β-lactams, resistance rates to ticarcillin/clavulanate, piperacillin/tazobactam, ceftazidime, cefepime, aztreonam, imipenem, and meropenem were 6.4%, 3.4%, 3.4%, 3.8%, 3.3%, 2.1%, and 2.1%, respectively. Ceftazidime resistance remained around the same levels during the two periods. Resistance to piperacillin/tazobactam, cefepime, imipenem, and meropenem doubled over the years 2018 - 2022 ($p = 0.48$, $p = 0.49$, $p = 0.65$, and $p = 0.65$, respectively) (Table 2). Similarly, rates of resistance to aminoglycosides, amikacin and gentamicin, increased from 1.1% and 1.1%, respectively, over the years 2013 - 2017, to 2.1% and 4.2%, respectively, over the period 2018 - 2022 ($p = 1.00$ and $p = 0.25$, respectively) (Table 2). On the other hand, resistance to ciprofloxacin decreased over the two five-year periods ($p = 0.98$) (Table 2).

S. aureus was the most frequently isolated pathogen among Gram-positive bacteria. Resistance to penicillin was extremely high (87.4%). Resistance rates to oxacillin, erythromycin, and clindamycin were 21.2%, 14.6%, and 11.9%, respectively. Forty-one percent of the methicillin-resistant (MRSA) strains were MDR. Resistance to clindamycin and tetracycline decreased from 14.5% and 8.7% (2013 - 2017) to 9.8% and 6.1% (2018 - 2022), respectively ($p = 0.46$, $p = 0.75$, respectively) (Table 3). Low rates of resistance were detected for TMP/SMX (5.3%) and levofloxacin (5.5%). All isolates were susceptible to gentamicin, rifampicin, linezolid, daptomycin, tigecycline, teicoplanin, and vancomycin. *Enterobacterales* were the third most frequently isolated pathogens. High rates of resistance were observed for ampicillin, amoxicillin/clavulanate, colistin, tigecycline, and tetracycline, ranging from 34.1% to 82.5% Although resistance rates to cephalosporins, monobactams, and amikacin were low, they increased over time, whereas resistance to ampicillin, gentamicin, ciprofloxacin, tigecycline, and TMP/SMX decreased during the last five years (Table 4). Only 4% of the isolates produced ESBLs, and all of them were susceptible to carbapenems.

DISCUSSION

The present study illustrates the prevalence and antimicrobial resistance profiles of bacterial pathogens isolated from patients of all ages with otitis between 2013 and 2022 in Crete, Greece. In this study, 63.9% of the ear discharge samples were positive for at least one pathogen. Higher rates have been found in developing countries, such as Iran (97.3%), India (91%), and Ethiopia (80.4%) [18-20]. These discrepancies are likely due to differences in methodology, antibiotic treatment before bacteriologic testing, and local hygienic conditions. Consistent with other reports, males were more frequently affected than females, probably due to gender differences in immune responses [20-23]. Although otitis can occur at any age, it is most commonly seen between the ages of 6 to 24 months [2]. In the present study, approximately one-third of the patients were 4 years old or younger, which is similar to what was reported in Ethiopia (31%) [24]. Children between the ages of 6 months and 2 years are more susceptible to ear infections, because their eustachian tubes are shorter, narrower, and more horizontal, which makes them more difficult to drain. Additionally, they are more frequently exposed to upper respiratory tract infections, and their immune system is still developing [2].

Among the 800 pathogens, 54.4% were Gram-negative bacteria, 37.6% were Gram-positive bacteria, and 8% were fungi. The predominance of Gram-negatives over Gram-positives is consistent with observations reported by other investigators [20,22,25]. In our study polymicrobial growth was detected in 28.5% of cases, which is lower than that found by Weckwerth et al. (37.5%) [26], but higher than that observed by others [20,25,27]. The most prevalent bacterial pathogens found in this study were *P. aeruginosa, S. aureus,* and *Enterobacterales,* which is in line with other reports [20,22,23,25,26,28]. However, some studies identified *Proteus* spp. as the predominant microorganism, followed by *S. aureus* and *Psedomonas* spp. [29,30]. The difference in bacterial distribution could be attributed to geographical variations. *P. aeruginosa* can thrive well in the ear environment and is difficult to eradicate. It damages the tissues and inactivates antibiotics by various enzymes and toxins [31]. Otopathogenic *P. aeruginosa* can enter and survive inside macrophage in the context of ear infections. This will lead to evasion of killing, and this lack of pathogen clearance by phagocytes contributes to persistence of infection [32]. *S. aureus* and *P. aeruginosa* were co-isolated in 39 cases. It has been shown that coexistence of these two microorganisms alters growth and metabolism of both species, resulting in a highly virulent and antibiotic-resistant infection that might have a large impact on clinical outcome of the patient [33,34]. Additionally, *S. aureus* and *P. aeruginosa* form biofilms that are resistant to antibiotics, contributing to chronicity and recurrent infections [35].

P. aeruginosa has many different mechanisms of antimicrobial resistance that may include production of antibiotic-inactivating enzymes, efflux systems, and modification of the permeability of the outer membrane [36]. In the present study, regarding antimicrobial resistance, *P. aeruginosa* exhibited low rates of resistance to clinically relevant antimicrobials with anti-pseudomonal activity, such as piperacillin/tazobactam, ceftazidime, cefepime, carbapenems, aminoglycosides, and ciprofloxacin. In contrast, rates of resistance to these antibiotics were significantly higher in bloodstream infections (BSI) and other invasive infections by *P. aeruginosa* in Greece [37,38]. However, we should take into account the doubling of the rate of resistance to piperacillin/tazobactam, cefepime, and the carbapenems over the period 2018 - 2022.

S. aureus was the second most prevalent pathogen isolated from patients with otitis. In a previous report from Greece, it has been found that *S. aureus* was the causative agent in approximately one-half of children with AOM [13]. It is also a major pathogen found in 30.6% of cases of acute otitis externa (AOE) in the USA [39]. Although penicillin resistance was extremely high, erythromycin and clindamycin resistance rates were reported at 14.6% and 11.9%, respectively, which are lower than that reported in studies from Saudi Arabia and Tanzania [40,41]. Among patients with *S. aureus* otitis, we identified MRSA in 21.2% of cases, similar to that last reported in 2009 [13]. It is noteworthy that 41% of the MRSA strains of the present study were MDR. MRSA represents a significant healthcare burden worldwide, as patients with MRSA have more difficult-to-treat infections due to multi-resistance, which are associated with much higher costs of care [42]. It has been shown that patients who failed treatment with antibiotics to MRSA experienced side effects greater than those who failed treatment with antibiotics to MSSA [43]. Park et al. found that MRSA infections in various OM cases showed different resistance patterns. MRSA infections in patients with chronic otitis media (COM) and chronic cholesteatomatous otitis media (CCOM) were more resistant to antibiotics than those in patients with OME and AOM [44].

However, in the present study all isolates were susceptible to vancomycin, teicoplanin, tigecycline, gentamicin, rifampicin, daptomycin, and linezolid, so they can be used to effectively treat *S. aureus* otitis.

Among *Enterobacterales*, *P. mirabilis* was more prevalent (19.8%), which is in agreement with reports of other investigators [20,26]. Rates of resistance to most antimicrobial agents were low, with increasing trends over time, while rates of ESBL production were also low.

This study has some limitations that should be acknowledged. First, it is a single-center study; thus, the results represent the microbiology and antimicrobial resistance patterns of a specific area. Furthermore, as this study mostly included microbiological data, there are no clinical data on the type of infection, infection severity, comorbidities, treatment outcomes, or antibiotic exposure history, all of which are essential for contextualizing re-

sistance patterns and establishing clinical relevance. Additionally, the study does not stratify data by patient demographics or care setting (e.g. inpatient vs. outpatient), which limits the ability to draw meaningful epidemiological conclusions across clinical contexts. And then, the lack of molecular characterization (e.g. of ESBL, MRSA, or carbapenemase-producing strains) significantly limits the study's contribution to understanding resistance mechanisms or guiding infection control efforts.

P. aeruginosa, S. aureus, and the *Enterobacterales* are the predominant bacterial pathogens causing otitis in our area. Regarding Gram-negatives, although resistance rates to commonly-used antibiotics are low, they are increasing over time. Vancomycin, teicoplanin, linezolid, tigecycline, gentamicin, rifampin, and daptomycin were highly active against *S. aureus.* These data emphasize the need for continued surveillance of the epidemiology and the antimicrobial resistance that will help reassess updated antimicrobial policies and develop appropriate guidelines. The implementation of clinical guidelines that promote accurate diagnosis and judicious use of antibiotics will lead to a decline in the incidence of otitis.

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