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Full Length Research Paper

In vitro activity of antibacterial drugs upon microorganisms from patients with chronic otitis media

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This study was conducted to generate microorganisms isolated from cultures of ears taken from the patients diagnosed to have chronic otitis media and determine and compare the in vitro effects of different antibiotics on these microorganisms. Between June 2009 and October 2010, 127 ear cultures were taken from 100 patients who applied to the Department of Otolaryngology of Ataturk University Faculty of Medicine Hospital diagnosed to have chronic otitis media in the otolaryngological investigation. Antimicrobial susceptibility of isolated strains was determined by the disc diffusion method according to the CLSI criteria. From the cultures investigated, 277 (6 ferment and 16 fungus) microorganisms were generated. Among the isolated pathogen microorganisms, *pseudomonas* spp. (24,91%), *staphylococcus spp* (13,00%), *proteus* spp. (5,05%) were the three most frequently isolated pathogen agents. 4 *Peptococcus* spp. (1,44%) and 3 *Peptostreptecoccus* spp. (1,08%) multiplied and it was observed that there was 2,52% anaerobic reproduction among all microorganisms. It was also seen that *Pseudomonas* spp. strains were more susceptible to gentamicin (97,1%), imipenem (94,2%), amikacin (84,1%) and ciprofloxacine (78,3%). In the study, vancomycine, teicoplanin, telithromycin and linozolidin were found to be the most effective antibiotics for all *staphylococcus*. It was seen that p*roteus* spp. strains were more susceptible to gentamicin (92, 9%), ciprofloxacin (%92, 9) and piperacillin (92, 9%). In the antimicrobial treatment required to reach effective outcomes in the treatment of patients with chronic otitis media, it is important to monitor the frequency of effective microorganisms and their rates of resistance to antimicrobial agents.

Keywords: Chronic otitis, antibiogram, microorganism, in vitro, antibacterial.

INRODUCTION

Otitis media (OM) is the infection and inflammation of the mucosa lining the Eustachian tube and the air-filled spaces in the middle ear and temporal bone. OM is classified as acute, subacute or chronic, depending on the type of onset and duration of the disease. If perforation and infection-inflammation findings last more than 3 months after an OM attack, this is referred to as chronic otitis media (COM). An acute OM attack with suppurative discharge lasting more than 6 weeks and not responding to medical treatment is regarded as chronic suppurative otitis media (CSOM) (1). CSOM cases may be prolonged, due to patients disregarding ear discharge and to incorrect and inadequate treatment. That is in turn due to treatment not being based on investigation of culture and antibiotic sensitivity.

Uninformed use of antibiotics may lead to delays in diagnosis and surgical treatment, since it suppresses the symptoms and findings of COM complications. If not treated, the disease may lead to serious, life-threatening problems. Complications are investigated under two

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classifications, extracranial and intracranial. Extracranial complications include mastoiditis, subperiosteal abscesses (postauricular abscess, gland abscesses, zygomatic abscesses) fistulized mastoiditis, facial paralysis, labyrinthitis, labyrinthine fistula and petrositis. Intracranial complications may be very serious and lead to meningitis, extradural abscess, subdural empyema, brain abscesses, lateral sinus thrombosis and otitic hydrocephaly (3,4). Sequelae resulting from the natural course of COM, such as sensorineural hearing loss, tympanic membrane perforation, erosion of the ossicular chain, tympanosclerosis and adhesive OM, also represent serious health problems (4).

The aim of this study was to compare the in vitro effectiveness of drugs with various antibacterial properties on active micro-organisms obtained from ear cultures taken from COM patients.

MATERIALS and METHODS

One hundred patients diagnosed with COM through anamnesis and examination at the Erzurum Atatürk University Faculty of Medicine Research and Training Hospital ENT Clinic, Turkey, between June 2009 and October 2010 were included. Patients with known systemic disease at anamnesis and with a diagnosis requiring constant drug use were excluded. Once informed consent had been received, patients' outer ears were cleaned with alcohol.

Three specimens of discharge from the middle ear were taken from patients using sterile swabs. The first specimen was added to medium containing 2 ml Brain-Heart-Infusion Broth (BHIB)(DIFCO) for bacterial culture, the second to broth with minced meat for anaerobic culture and the third to Sabouraud Dextrose Agar (SDA) (DIFCO) medium for fungal culture. A total of 127 swabs from the 100 patients, some from the right ear and some from the left, were included in the study. After BHIB passages were incubated for approximately 3 h at 36±1 °C, 5% Sheep Blood Agar (OXOID), Eosin Methylene Blue Agar (EMB) (OXOID) and Chocolate Agar (waxed jar incubation for 5-10% CO_2) (OXOID) passages were prepared, and the new cultures were left to incubate. For fungal isolates, SDA media were seeded twice at 25 ^{O}C and 37 ^{O}C and monitored for fungal growth for one week. Fungal colonies were described in terms of microscopic spore architecture and biochemical characteristics. For bacterial cultures with growth, bacteria were described using conventional methods. For bacteria, colony morphologies, gram staining characteristics, catalase activities, oxidase activities, sugar fermentations, H_2S–gas characteristics, PYR test results and novobiocin, optochin and bacitracin sensitivities were investigated. When necessary, a Vitek 2 Compact automatic bacterial identification device was used for all bacteria determined using conventional methods. Antibiotic sensitivity tests were performed using the "disk diffusion" method in Mueller-Hinton according to CLSI guidelines (2).

Descriptive statistical analysis was performed. The effectiveness of various drugs by species of pathogen was examined using cross tables. Chi square analysis was performed. One-way ANOVA was used to compare numerical variables in multiple groups. Post hoc analysis was performed using Tamhane's test.

RESULTS

A total of 277 micro-organisms were obtained from ear cultures, 255 bacteria, 6 yeast and 16 mold. The distribution of these micro-organisms is shown in Table 1.

Fourteen mixed type growths were identified in the cultures examined, multiple bacteria in 12 and bacteria and fungi together in two. More than one micro-organism grew in 14 of the 127 cultures taken (11.02%). The distribution of these micro-organisms is shown in Table 2.

Gram-negative micro-organisms were determined in 104 cultures (39.03%). Sensitivity levels of Gram-negative micro-organisms to major antibiotics in clinical use are shown in Table 3.

The gram-positive pathogen bacteria in this study were investigated under two headings, coagulase negative staphylococci and *S. aureus*. Both pathogens were classified according to whether or not they were sensitive to methicillin. Sensitivity levels of Gram-positive micro-organisms to major antibiotics in clinical use are shown in Table 4.

DISCUSSION

Inadequate and ineffective use of antibiotics in recent years has led to an increase in resistant strains, and therefore to therapeutic failures. CSOM generally develops as a result of Eustachian dysfunctions arising due to untreated or unhealed AOM, and results in long-term use of antibiotics (5). Antibacterial drugs to be used in treatment should be chosen on the basis of case severity. Topical ear drops are sufficient in mild cases. In more serious cases in which inflammation has progressed to the retroauricular region, systemic antibiotics should be used (6). Accurate identification of micro-organisms and determination of antibiotic sensitivities will contribute to the prevention of possible sequelae and potential new infection attacks (7).

The two micro-organisms most frequently isolated from the outer ear passage are the normal flora elements coagulase negative staphylococci and diphtheroid strains (*Corynebacteria*spp.) (8,9). The two most frequently isolated agents as pathogens are *S. aureus* and *P. aeruginosa* (8,10). *S. aureus* and *P. aeruginosa* were also the two most frequently isolated pathogens in our study. Kılıç et al. (11) reported a *P. aeruginosa* rate of 33%, an *S. aureus* rate of 5% and *Proteus* spp. at 3%.

Table 1. Distribution of micro-organisms grown in all cultures.

Mikroorganism	n	(%)
BACTERIA		
Diphtheroid bacilli	85	30,69
Pseudomonas spp	69	24,91
Coagulase negative methicillin-resistant Staphylococcus	10	3,61
Coagulase negative Methicillin-sensitive Staphylococcus	10	3,61
Methicillin-sensitive S.Aures	10	3,61
Methicillin-resistant S.Aures	6	2,17
Proteus spp.	14	5,05
E. coli	11	3,97
Enterobacter spp.	8	2,89
Neisseria spp.	13	4,69
Group A beta hemoliytic strep.	4	1,44
S.pneumoniae	3	1,08
H. influenzae	3	1,08
Klebsiella spp.	1	0,36
Citrobacter spp.	1	0,36
Peptecoccus spp.	4	1,44
Peptestreptecoccus spp.	3	1,08
YEAST		
Candida albicans	5	1,81
Non-albicans yeast	1	0,36
MOLD		
Aspergillus fumigatus	8	2,89
Aspergillus niger	4	1,44
Penicillium spp.	3	1,08
Mucor	1	0,36

Table 2. Distribution of micro-organisms in mixed-type cultures.

Microorganism 1	Microorganism 2	n
Pseudomonas spp.	*E. coli*	5
Pseudomonas spp.	*S. aureus*	1
Pseudomonas spp.	*Enterobacter spp.*	1
Pseudomonas spp.	*Aspergillus spp.*	1
Pseudomonas spp.	*Candida spp.*	1
Pseudomonas spp.	*Coagulase negative methicillin-resistant Staphylococcus*	2
E. coli	*Coagulase negative Staphylococcus Methicillin-* ***sensitive***	1
Enterobacter spp.	*S. aureus*	1
Citrobacter spp	*Pseudomonas spp.*	1

Another study in Turkey involving 75 patients reported *P. aeruginosa*, *S. aureus* and *Proteus* spp. rates of 43.5%, 20.5% and 8.9%, respectively (12). İmamoğlu et al. (13) identified 13 kinds of bacteria in 100 ear cultures. A single bacterium was isolated in 85 (87.62%) cases and more than one bacterium in 11 (11.34%), while bacteria and aspergillus fungus together in one (1.03) case. There was no growth in 3 (3.09%) cases. The most frequently growing bacteria in cultures are *Pseudomonas* spp*., S. aureus* and *Proteus* spp. Brook (14) cited levels in a 50-case series of 72% *Pseudomonas* spp., 14% *Proteus* and 14% *S. epidermidis*. Ural and Elçi(15) reported 40% staphylococci, 25% *Proteus* spp., 14% *Pseudomonas* spp., 7% *E. coli*, 3% hemolytic streptococci and 3% Candida in their 100 cases. In another study performed in our region, 800 bacteria, 22 yeast and 20 mold grew from outer ear culture specimens. The two most frequently isolated pathogens among the micro-organisms identified were *S. aureus* in 124 (17.8%) specimens and *P. aeruginosa* in 119 (17.1%). Other Gram-negative micro-

Table 3. The most frequently isolated Gram-negative strains and their antibiotic sensitivity levels (%).

n (102)	*Pseudomonas spp. (69),*	*Proteus* spp.(14)	*E. coli* (11)	*Enterobacter* spp.(8)	*Chi-Square*	*P*
Amikacin	84,1	85,7	90,9	87,5	0,39	0,94
Gentamicin	97,1	92,9	81,8	75	8,02	0,04
Tobramycin	-	78,6	81,8	100	1,93	0,38
Cefazolin	-	71,4	45,5	37,5	2,91	0,23
Cefuroxime	-	85,7	72,7	62,5	4,23	0,37
Ceftriaxone	46,4	-	-	-	-	-
Cefoperazone	-	78,6	81,8	100	1,93	0,38
Ceftazidime	47,8	-	-	-	-	-
Cefoxitin		85,7	81,8	50	3,86	0,14
Cefepime	44,9	-	72	63	-	-
Ampicillin	-	64,3	36,4	25	3,71	0,15
Ampicillin / sulbactam	65,2	-	-	-	-	-
Piperacillin	23,2	92,9	81,8	100	42,00	<0,001
Piperacillin / tazobactam	37,7	-	-	-	-	-
Ticarcillin / clavulonic acid	39,1	-	-	-	-	-
Amoxicillin / clavulonic acid	-	-	-	50,4	-	-
Trimethoprim / sulphamethoxazole	-	71,4	72,7	87,5	0,79	0,67
Imipenem	94,2	-	-	-	-	-
Ciprofloxacin	78,3	92,9	63,6	100	5,48	0,13

Table 4. The most frequently isolated Gram positive bacteria and their antibiotic sensitivity levels (%).

Mıcroorganism (n)	Coagulase negative methicillin sensitive Staphylococcus (10)	Coagulase negative Methicillin-resistant Staphylococcus (10)	Methicillin sensitive S.Aures (10)	Methicillin resistant S.Aures (6)	Chi-Square	P
Cefoxitin	100	0	100	0	36,00	<0,001
Penicillin	80	0	70	0	20,77	<0,001
Ampicillin	30	0	40	0	7,27	0,06
Amoxicillin/clavulonic acid	90	0	100	0	32,38	<0,001
Ampicillin/sulbactam	90	0	100	0	32,38	<0,001
Vancomycin	100	100	100	100	-	-
Teicoplanin	100	100	100	100	-	-
Gentamycin	80	70	100	50	5,91	0,11
Telithromycin	100	100	100	100	-	-
Clindamicin	70	40	100	50	9,00	0,02
Erythromycin	50	20	100	50	13,32	0,004
Trimetoprim/sulphamethoxazole	90	70	100	100	5,62	0,13
Ciprofloxacin	60	50	100	66,7	6,62	0,08
Moxifloxacin	100	80	100	83,3	4,14	0,24
Rifampin	80	70	100	66,7	3,86	0,27
Linozolid	100	100	100	100	-	-

organisms isolated from the outer ear were *Proteus* spp. in 53 (7.6%) specimens, E. *coli* in 50 (7.2%), *Enterobacter* spp. in 45 (6.5%), *Citrobacter* spp. in 13 (1.9%) and *Klebsiella* spp. in 4 (0.6%) (16). A total of 277 micro-organisms grew in the cultures taken in our study, 255 bacteria, 6 yeast and 16 mold. Fourteen mixed-type growths were observed, with more than one bacteria present in 12 of the cultures examined and bacteria and fungi together in 2. The most frequent pathogens were *Pseudomonas* spp. (24.91%), *S. aureus* (5.88%), *Proteus* spp. (5.05%) and *E. coli* (3.97%).

Ural and Elçi (15) reported that gentamycin is 100% effective against staphylococci and *Proteus* and *Pseudomonas* spp. and also against *E. coli*. In another study, the lowest level of resistance in *P. aeruginosa* strains were against imipenem (4%), meropenem (7%) and

and amikacin (8%), while the highest resistance levels were against piperacillin (70%), cefoperazone (60%) and ticarcillin-clavulanic acid (%54). A 25% level of resistance to ciprofloxacin was determined. No resistance to carbapenems and a 2% resistance to ofloxacin and ciprofloxacin were identified in *Proteus* strains. The highest resistance was determined against trimethoprim-sulfamethoxazole (47%) and ampicillin (44%). No resistance to imipenem, meropenem and methymycin was observed in *E. coli* and *Enterobacter* strains, while a low level of resistance to ciprofloxacin was determined (16). The most effective antibiotics against *Pseudomonas* spp. in our study were gentamycin (97.1%) and imipenem (94.2%), the least effective being piperacillin (23.2%). Gentamycin, ciprofloxacin and piperacillin were equally effective (92.9%) against *Proteus* strains. Amikacin was the most effective antibiotic against *E. coli* at 99.9%, while tobramycin, piperacillin and ciprofloxacin were the most effective against *Enterobacter* spp. with 100% sensitivity. *Proteus* spp., *E. coli* and *Enterobacter* spp. strains were resistant to ampicillin. Vancomycin, teicoplanin, telithromycin and linezolid antibiotics were 100% effective against methicillin-sensitive coagulase negative staphylococci and methicillin-resistant coagulase staphylococci strains, while these were resistant to ampicillin. Anaerobic bacteria levels of approximately 30-50% have been reported among the agents in CSOM cases. Ninety percent of these are cases with cholesteatoma (17). Among anaerobic micro-organisms, anaerobic Gram-positive cocci and *Bacteroides* are the most implicated (18). The anaerobic bacteria obtained by Altuntaş et al. (19) in their study were *Peptococcus*, *Peptostreptococcus* and *Bacteroides*. Four Peptococcus spp. (11.44%) and 3 Peptostreptococcus (1.08%) grew in our study, representing 2.52% of all micro-organisms.

Outer ear canal infections are generally bacterial, and some 10% are fungal otitis externa (8,20). *Aspergillus* is reported to be the most commonly encountered agent in cases of otomycosis, followed by *Candida* spp. (8). Other fungal agents encountered are *Mucor, Rhizopus* and *Penicillium*. In one study performed in our region, *A. fumigatus* was isolated in 18.4%, *Candida* spp. in 16.0%, *A. niger* in 10.4%, *Penicillium* in 5.8% and *Mucor* in 1.2% of cases of otomycosis (21). Fungi were isolated at a level of 7.94% in the cultures taken in our study, with 6 yeast (*Candida* spp. 2.17%) and 16 mold (*A. fumigatus* 2.89%, *A. niger*1.44%, *Penicillium* 1.08% and *Mucor* 0.36%).

In conclusion, differences stemming from variation in outcomes due to environmental, cultural, economic and educational factors can be seen in the distribution of otitis agents' sensitivity to antibiotic profiles. Increasing insufficient and ineffective use of antibiotics in recent years has led to an increase in resistant strains and therefore to therapeutic failures. We therefore think that the most correct approach is to provide treatment by isolating the pathogen agent and considering the results of sensitivity to antibiotics tests.

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