EVALUATION OF THE *mec*A GENE INVASION RATE OF THE GRAM POSITIVE BACTERIA ISOLATED FROM THE PATIENTS WITH OTITIS

ABSTRACT:

Otitis externa (OE) and Otitis media (OM) diseases are two important diseases that affecting a significant portion of the community. This diseases mostly affect the developing world communities. In this study, we aimed to reveal the prevalence of otitis media in the East of Turkey, the proportion of gram positive bacteria and antibiotic resistance profiles. 2000 ear swab sample (1000 women and 1000 men) were collected from 0 to 80 years old patients, in Van Training and Research Hospital Department of Otolaryngology, Turkey. The microbiological analysis of 2000 ear swab samples was done in Pharmaceutical Microbiology Department, Faculty of Pharmacy, Van Yüzüncü Yıl University, Turkey. Gram positive bacteria reproduction observed in 1,225 ear swaps samples. *Staphylococcus aureus* was found to be the most common isolated bacterium in male and female patients with otitis. *Streptococcus pyogenes* and *Streptococcus pneumoniae* were the second most common isolated bacteria, respectively, in male and female patients with otitis. Gram positive bacteria isolated from male patients with otitis showed the highest resistance rate to Cefoxitin. Gram positive bacteria isolated from female patients with otitis showed the highest resistance rate to Erythromycin. In 36 *S. aureus*, 22 of male and 14 of female patients, Oxacilin resistance were determined. According to the results of Polymerase Chain Reaction (PCR) 36 isolates of *S. aureus* were positive for *mec*A gene. It is thought that the data obtained from this study will contribute to the elaboration of OE and OM diseases in our region and will contribute to the new planned studies.

Keywords; Otitis, mecA, antibiotic resistance

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1. INTRODUCTION

OE is defined as infection or inflammation of the external ear canal [1]. It can be limited to external ear. But outside the tragus and auricle (pinna) might be affected. External ear tract infections are presented by acute and chronic course. External ear tract infection lasting less than six weeks is referred to as “acute otitis externa (AOE)". AOE is also known as ”swimmer's ear". The most important causes of this disease include excessive moisture in the external ear canal, loss of protective serum and prolonged irritation. Fourteen per thousand of the population in the United States has been reported to be affected by AOE disease each year [2, 3, 4]. Chronic otitis externa can continue for three months or more. Chronic otitis externa usually occurs due to a non-infectious and allergic cause [5]. COE, affects 3-5% of the population. OE has a persistent infection type described as "malignant otitis externa (MOE)”. The outer ear canal and surrounding structures are affected in MOE. The disease appears to be an important risk in elderly patients with diabetes [6]. Otitis Media (OM) is the disease that occurs when infectious agents or inflammatory causes affect the middle ear. OM may cause chronic and acute course. Acute Otitis Media (AOM) is characterized by rapid

onset of inflammation. Especially the tympanic membrane perforation and swelling, fullness of sensation, erythema, ear pain, irritation and fever are important clinical findings [7]. Chronic Supurative Otitis Media (CSOM) is a result of AOM that couldn't be treated despite appropriate antibiotics. CSOM is characterized by continuous drainage to the middle ear with perforation of the eardrum. As a result of the examination, inflamed inflammation of the middle ear can be seen. CSOM generally evolves in the early years of life. But it can also be seen in adults. The disease affects 65-330 million people worldwide. Each year, 31 million people caught by the disease. 22,6% of them have found to be under the age of 5. Hearing loss, is one of the most important symptoms of CSOM. CSOM is one of the leading chronic infectious diseases that cause hearing loss and affect children. Hearing loss has a negative effect on children's speech development, education and habits. CSOM can cause death due to different complications. Death in CSOM patients has been shown to occur as a result of intracranial complications such as brain abscesses and meningitis [8].

Bacterial agents are the most common etiological agents of the infections of the external ear canal. It may occur less frequently due to fungal agents or non-infectious causes. The most common etiological agents of the disease are *Staphylococcus aureus* and *Pseudomonas aeruginosa* . It has been reported that *Escherchia coli* and *Klebsiella* species are less common etiological agents. The annual incidence of OE in people varies between 1:100 and 1:250. *P. aeruginosa* is the most common etiological agent for MOE. *S. aureus* and *Proteus mirabilis* are the most common bacterial agents [6]. *Streptococcus pneumoniae*, *Haemophilus influenza* and *Moraxella catarrhalis* are the leading etiological agents of AOM. However, *P. aeruginosa* and *S. aureus* isolated from the patients are the important aerobic microbial agents. *Proteus vulgaris* and *Klebsiella pneumoniae* and reported to be less frequent agents [7]. Age, gender, flora and geographic condition of the region they live in are important factors in disease formation in people [1].

Pruritis, pain and erythema are common in patients with otitis externa. However, edema, autorrhea and permanent hearing loss may develop if the disease persists. The severity of inflammation varies between patients. Approximately 50% of patients have inflammation. Less than 0.5% of patients have life-threatening temporal bone infections [9, 10]. Chronic OE patients are characterized by pruritus, a mild discomfort and erythematous external ear canal. In the treatment of otitis externa, results can be obtained by regulating the causative factors and topical treatment. However, 25% of patients should be treated with systemic antibiotics. [11, 12].

In this study, we aimed to reveal the presence and prevalance of gram-positive bacteria in otitis cases in people living in the East of Turkey, their antibiotic resistance profiles and carriage rate of methicillin resistance.

2. MATERIALS AND METHODS

Patients who were admitted to the otolaryngology department between January 2017 and October 2018 were evaluated. As a result of the examinations of the patients, 2,000 ear swab samples were taken from 1000 male and 1000 female patients with OE and OM findings. For microbiological evaluation, swaps were dosed in 5% sheep blood agar,

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Chocolate Agar, McConkey agar, Eozin Methylen Blue agar and Mannitol Salt Agar and incubated for 5 days at 37°C. Cultures with positive reproduction were evaluated for colony morphologies, gram staining, catalase and oxidase properties. Identification and antibiogram results of isolated bacteria were performed in Vitek II device. The bacteria were stored at -20°C.

*mec*A gene carrier ratio of *S.aureus* strains were determined by PCR method using the mecA F-5'- ACTGCTATCCACCCTCAAAC -3'; R-5'- CTGGTGAAGTTGTAATCTGG-3' oligonucleotide primer series. The genomic DNA extraction of *S.aureus* bacteria produced in TSA agar was performed by using Qiagen DNA mini kit based on the spin column system. DNA extraction products were amplified using DNA amplification kit (VWR, PCR Kit with Taq Polymeras, UK). 40 cycles of amplification were performed by adjusting the PCR conditions (120 sec in 94^{0}C; 30 sec in 94^{0}C; 60 sec in 55^{0}C; 60 sec in 72^{0}C; 420 sec in 72^{0}C). The amplicons were carried out in a 1.5% agarose gel electrophoresis containing ethidium bromide for 100 voltage for 2 hours. The bands were visualized on the BIO-PRINT-ST4 device.

Descriptive statistics for the studied variables (characteristics) were presented as count and percent. Proportions styled outfits were compared with Z test for two proportions styled outfits. Statistical significance levels was considered as 5% and MINITAB for windows (: 14) statistical program was used for all statistical computations.

3. RESULTS

2,000 swab samples from patients with OE and OM were evaluated. 805 of 1,000 male patients and 420 of 1,000 female patients, totally of the 1,225 ear swab samples, were positive for gram positive bacteria reproduction. Gram-positive bacteria was found to be most common in male (0-18 years) patients with otitis externa. The lowest positivity rate was found in women (45+) patients with otitis media (Table 1).

S. aureus was found to be the most common bacterium isolated from male and female patients with otitis. *S. pyogenes* and *S. pneumoniae* were found to be the second most frequent bacteria isolated from male and female patients with otitis. *S. agalactiae,* was isolated from a male patient with OE. Also, *Enterococcus faecalis* was isolated from a female patient with OE. The ratios of the isolated and identified gram positive bacteria are given in Table 2.

Gram positive bacteria isolated from male patients with otitis showed the highest resistance rate to Cefoxitin. In female patients with otitis, showed the highest resistance rate to Erythromycin. It has been revealed that, in male and female patients with otitis, showed the highest rate of sensitivity to Vancomycin. Antibiotic sensitivities of gram positive bacteria were given in Table 3.

In 36 patients, including 22 male and 14 female, gram positive bacteria showed rasistance to Oxacilin. Analysis of PCR revealed that 36 *S.aureus* isolates carried the *mec*A gene.

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The statistical analyzes of the rates of the isolates obtained from patients with otitis and the sensitivities in antibiogram are given in Table 1, Table 2 and Table 3.

4. DISCUSSION AND CONCLUSION

OE and OM are among the most important diseases known in ear infections. Approximately 10% of young and old people are exposed to this disease. [13-14] . Each year, AOE attracts attention as an important disease in which 1 person in 100 people is affected. The average number of OE cases in the community is reported to be 95%. On the other hand, COE cases were below 5%[15] . It has been reported in the world that the prevalence of OM is at different rates. In Ethiopia studies, the prevalence of disease is reported to be 90%. [16.17]. In a study conducted in Nigeria, the disease prevalence was reported to be 81.9% [18]. In a study of acute OM patients in Russia, this rate was reported to be 32% [19]. Canada, Alaska, Greenland, Australia Aborigines and Native Americans have reported that the incidence of CSOM is between 7% and 46%. A low incidence of 1% to 6% is reported in South Pacific islands, Korea, Africa, India and Saudi Arabia [8].

Since 1940, *S. aureus* was reported to be the most common pathogen agent in patients with AOE. The incidence of *S. aureus* has been reported to vary from 8.5% to 29% [20] . Wasihun et al. In a study conducted in 2015 [7] *S. aureus* were reported to be the most isolated agent in OM patients. In a study conducted by Tanon-Anoh et al. [21] in 2006 *S. pneumoniae* was reported to be the leading etiological agent. In a study conducted in Brazil reported that the *S. pneumoniae* and *M. catarrhalis* were found to be the leading etiological agents [22] . A series of studies conducted in CSOM patients worldwide reported that *S. aureus* was the most common pathogen in India, Nepal, Singapore and Nigeria [23, 24]. Other studies indicated that,*S. aureus* was found to be the most common pathogen in Pakistan, Iran and Saudi Arabia [8, 25]. In a study conducted by Shavit et al. [6] in 2016, ear swab samples of 88 patients with OE were evaluated. The culture results of 78 of them were positive. Bacterial agents was found to be *S. aureus* (7, 9%), *Streptococcus* spp. (1, 1.3%) and CNS (1, 1.3%). In a study conducted in Italy between 2012 to 2013, 100 ear swab samples were collected from 58 men and 42 women. 49 bacteria were found to be gram positive. CNS (22, 17.6%) was reported to have the highest isolation rate among Gram-positive bacteria [1]. In a study conducted by Yazdi MMK et al. [13] in 2015, 15 *S. aureus* and 11 CNS were isolated from 186 patients with OE and OM. Noguerira et al. isolated *S. aureus* from 27 patients with OE. *S. aureus* and *P. aeruginosa* were isolated together in 5 patients. In a study conducted in Russia, bacterial positivity were observed in 172 of 541 patients with AOM. It has been reported that *S. pneumoniae* was isolated in 109 of 172 patients [19] . A study conducted by Rolan et al. in 2002 indicated that the highest rates was 9.1% for S.epidermidis and 7.8% for *S.aureus*. In this study, *S. aureus* was found to be the most isolated bacterium in male and female patients with otitis. It has been observed that *S. pyogenes* and *S. pneumoniae* were the second most common isolated bacteria in male and female patients with otitis. *S. agalactiae* were isolated from a male patient with OE. In addition, *Enterococcus faecalis* was isolated from a female patient with OE. It is thought that the differentiation of flora bacteria is an important factor in bacterial isolation in people living under different climate and geographic conditions.

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According to different research results in the world, there are differences between sex and disease. In some studies, men have been reported to be more infected than women. [26.27]. However, one study reported that women were exposed to more infections than men [28]. In a study conducted by Wasihun et al. indicated that there is no significant difference between bacterial agents and sex. In our study, it was found that gram positive bacteria were mostly in male (0-18 years) patients with otitis externa. While the lowest reproduction was found in women with otitis media (45+).

In antimicrobial evaluation, it was reported that isolates were resistant to many different antibiotics. In a study conducted by Roland et al. [20] in 2002 reported that gram positive bacteria shows different resistance rates. They reported that the resistance rates of S. epidermidis isolates were 23% to neomycin, 11% to oxcillin and 12% to ofloxacin. And the resistance rates of *S. aureus* isolates were 6.3% to neomycin, 2.7% to oxcillin and 4.5% to ofloxacin. In Italy, 14 *S. aureus* was isolated from patients with OE. 3 of the *S. aureus* isolates showed resistance to methicillin. These isolates' resistance rates was 66.6% to ciprofloxacin, 33.4% to erythromycin and 66.6% to vankomycin, respectively [1]. *S. aureus* isolates showed low rates of resistance to ciprofloxacin, gentamicin and erythromycin [7]. 4 patients with *S. aureus* have been treated with Ciprofloxacin [29].In a study conducted by Kaushik et al. [30] in 2010 indicated that aminoglycoside and fluoroquinolone antibiotics were efficient for the *S. aureus* isolated patients. In this study, 805 bacteria isolated from male patients developed the lowest resistance against vancomycin, oxacilin and SXT, respectively. 420 bacterium isolated from female patients developed the lowest resistance against Vancomycin, Oxacilin and Rifampicin, respectively. Different rates of resistance to antibiotics were observed and the evaluation of the antibiogram results in the treatment was found to be important.

In this study, it has been observed that the otitis disease was common in our region. It was concluded that the patients' knowled about the risk factors of the disease increased the prevalence of the disease. This is the first study that demonstrates the prevalence of otitis disease in detail in our region. It is thought that this study will contribute to advanced studies.

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Table 1. Gram positive bacteria rates isolated according to age and gender in patients with otitis

	MALE (n = 1000)		**FEMALE (n = 1000)**			
Age	**Otitis Externa**	**Otitis Media**	**Otitis Externa**	**Otitis Media**	p_E	p_M
0-18	384	57	161	18	0.001	0.004
18-45	105	36	109	25	0.001	0.021
45+	174	49	80	27	0.041	0.492
Sum	**663**	**142**	**350**	**70**		

p_E: Analysis value of Otitis Externa, p_M: Analysis value of Otitis Media

Table 2. Gram positive bacteria strains isolated from patients otitis

	MALE (n = 1000)			FEMALE (n = 1000)			
Bacteria strains	**Otitis Externa**	**Otitis Media**	**Bacteria strains**	**Otitis Externa**	**Otitis Media**	p_E	p_M
S. pneumoniae	85	-	*S. pneumoniae*	24	10	0.001	0.001
S. pyogenes	120	40	*S. aureus*	301	55	0.001	0.001
S. agalactiae	1	-	*Enterococcus faecalis*	1	-	0.001	-
S. aureus	422	100	*S. epidermidis*	19	2	0.001	0.001
S. epidermidis	25	1	*S. haemolyticus*	5	3	0.001	0.001
S. haemolyticus	10	1	-	-	-	0.001	0.498
SUM	**663**	**142**	**SUM**	**350**	**70**		

p_E: Analysis value of Otitis Externa, p_M: Analysis value of Otitis Media

Table 3. Antibiogram results of gram positive bacteria

	MALE (n = 805)			FEMALE (n = 420)					
							p_R	p_I	p_S
Antibiotics	R	I	S	R	I	S	0.001	0.001	0.001
Benzylpenicilline	190	-	615	170	52	198	0.001	0.001	0.001
Ciprofloxacin	154	52	599	121	52	247	0.001	0.001	0.001
Erythromycin	165	72	568	259	61	100	0.001	0.001	0.001
Fosfomycine	152	30	623	152	18	250	0.001	0.001	0.001
Fucidic Acid	153	54	598	251	52	117	0.001	0.001	0.001
Linezolid	111	-	694	60	11	349	0.001	0.001	0.001
Oxacilin	22	-	783	14	-	406	0.001	-	0.939
Ampicilline/Sulbactam	176	-	629	55	-	365	0.001	-	0.081
Daptomycine	161	12	632	78	2	340	0.001	0.001	0.633
Tetracycline	166	2	637	64	7	349	0.001	0.001	0.435
Vancomycin	1	-	804	1	-	419	0.001	-	985
Cloramphenicol	100	37	668	45	17	358	0.001	0.001	0.679
Levofloxacin	244	32	529	116	24	280	0.001	0.001	0.837
Rifampicin	132	19	654	35	44	341	0.001	0.001	0.992
SXT	62	-	743	46	12	362	0.001	0.001	0.269
Cefoxitin	377	92	336	107	77	236	0.001	0.001	0.001
Ceftriaxone	272	71	462	166	15	239	0.001	0.001	0.909
Clindamycin	216	81	508	99	10	311	0.001	0.001	0.008
Gentamicin	152	66	587	89	16	315	0.001	0.001	0.664
Amikacin	133	-	672	155	77	188	0.001	0.001	0.001
Moxifloxacin	212	28	565	122	31	267	0.001	0.001	0.108

p_R: Analysis value of antibiotic resistance, p_I: Analysis value of antibiotic intermediate, p_Q: Analysis value of antibiotic susceptibility

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