Microbiology Research Journal International

26(1): 1-8, 2018; Article no.MRJI.45565
ISSN: 2456-7043
(Past name: British Microbiology Research Journal, Past ISSN: 2231-0886, NLM ID: 101608140)

Evaluation of *Mec*A Gene Occurrence of Gram Positive Bacteria Isolated from Patients with Otitis

Ömer Akgül[1*], Yunus Çetin[2], Gülhan Bora[1], Yaser Çetin[3] and Müzeyyen Kaya[4]

[1]Pharmaceutical Microbiology, Faculty of Pharmacy, Yüzüncü Yıl University, Van, Turkey.
[2]Department of Internal Medicine, Van Training and Research Hospital, University of Health Sciences, Van, Turkey.
[3]Department of Otolaryngology, Van Training and Research Hospital, University of Health Sciences, Van, Turkey.
[4]Department of Otolaryngology, Midyat State Hospital, Mardin, Turkey.

Authors' contributions

This work was carried out in collaboration between all authors. Author ÖA designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors ÖA, YaÇ and MK managed the analyses of the study. Authors ÖA, YuÇ and GB managed the literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2018/45565
Editor(s):
(1) Dr. Giuseppe Blaiotta, Professor, Department of Agriculture, Division of "Grape and Wine Sciences", University of Naples Federico II, Via Universita' 100 – Palazzo Mascabruno 80055 Portici, Italy.
Reviewers:
(1) Ilham Zahir, Sultan Moulay Slimane University, Morocco.
(2) Henrique Furlan Pauna, University of Campinas – UNICAMP, Brazil.
(3) Peekate Lekiah Pedro, Rivers State University, Nigeria.
(4) Maria Antonietta Toscano, University of Studies of Catania, Italy.
Complete Peer review History: http://www.sciencedomain.org/review-history/28089

Original Research Article

Received 20 September 2018
Accepted 12 December 2018
Published 03 January 2019

ABSTRACT

Otitis externa (OE) and Otitis media (OM) are two important diseases that affects a significant portion of the communinities in developing countries. The prevalance of otitis media and externa in Easthern Turkey, the proportion of the implicated bacteria that are Gram positive, and their antibiotics resistance profiles was investigated in this study. Ear swab samples were collected from 2000 patients (1000 women and 1000 men) at the Van Training and Research Hospital Department of Otorhinolaryngology, Turkey. The Patients were between the ages of 0 to 80 years. Microbiological analysis was carried out on the samples in the Pharmaceutical Microbiology laboratory, Faculty of Pharmacy, Van Yüzüncü Yıl University, Turkey. The presence of Gram

**Corresponding author: E-mail: o.akgul@yyu.edu.tr;*

positive bacteria was observed in 1,225 ear swab 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 to Cefoxitin, while those isolated from female patients with otitis showed the highest resistance to Erythromycin. Oxacilin resistance was determined for of the *S. aureus* isolates, of which 22 were from the male patients and 14 from the female patients. According to the results of Polymerase Chain Reaction (PCR) the 36 isolates 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 the Easthern Region of Turkey.

Keywords: Otitis; Mec A gene; Staphylococcus spp.; antibiotic resistance.

1. INTRODUCTION

Otitis externa (OE) is defined as infection or inflammation of the external ear canal [1]. It can be limited to external ear. But out side 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 [2-4]. Chronic otitis externa (COE) can continue for three months or more. Patients suffering from COE experiences ear itching, clean or mucoid otorrhea, aural fullnes and ear pain. Chronic otitis externa usually occurs due to a non-infectious and allergic cause [5]. Chronic otitis externa 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 ear drum. As a result of the examination, inflammation of the middle ear can be seen. Children in their early years of life are predisposed to CSOM. But it can also be seen in adults. The disease affects 65-330 million people worldwide[8]. Each year, 31 million people are affected by the disease. 22,6% of them were 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* [4,9]. It has been reported that *Escherchia coli* and *Klebsiella* species are less common etiological agents [10,11]. The annual incidence of OE in people varies between 1:100 and 1:250 [10]. *P. aeruginosa,* followed by *S. aureus* and *Proteus mirabilis* are the most common etiological agent for MOE [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* are reported to be less frequent agents [7]. Age, gender, flora and geographic conditions 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, otorrhea 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 [12,13]. 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 [14,15].

Multiple antibiotic resistance in methicillin-resistant *S.aureus* (MRSA) strains started to emerge in the 1970s. Nowadays, MRSA has become a very serious problem, leading to an outbreak of resistance, as well as hospital outbreaks and community-acquired infections [16,17]. The multiple antibiotic resistance in MRSA is due to the mecA gene of the bacterium. *mec*A encodes a different penicillin binding proteins (PBP) called PBP 2a, which has low affinity for methicillin and other beta-lactam drugs. Antibiotics other than the beta-lactam group are prominent in the treatment of infections caused by methicillin-resistant *S.aureus* strains. However, it is important to determine the effectiveness of these antibiotics through susceptibility testing before using them for treatment of infections caused by MRSA [17,18].

In this study, we seek 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 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. For microbiological evaluation, swabs were inoculated on 5% sheep blood agar, Chocolate Agar, McConkey agar, Eosin Methylen Blue agar and Mannitol Salt Agar and incubated for 5 days at 37°C. Cultures having the suspected characteristics were evaluated for colony morphologies, Gram staining, catalase and oxidase enzymes. 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 [19]. 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°C; 30 sec in 94°C; 60 sec in 55°C; 60 sec in 72°C; 420 sec in 72°C). Separation of the amplicons were carried out using 1.5% agarose gel electrophoresis containing ethidium bromide at 100 volts 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 were considered as 5% and MINITAB for windows (: 14) statistical program was used for all statistical computations.

3. RESULTS

805 ear swab samples from the 1,000 male patients and 420 from the 1,000 female patients, totaling to 1,225 ear swab samples were positive for the presence of Gram positive bacteria. Gram-positive bacteria were found to be most common in male (0-18 years) patients with otitis externa. The lowest number of positive results 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 to Cefoxitin. While, Gram positive bacteria isolated from female patients with otitis, they showed the highest resistance to Erythromycin. It

was observed that Gram positive bacteria from male and female patients with otitis, showed the highest sensitivity to Vancomycin (Table 3).

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

	MALE (n = 1000)		FEMALE (n = 1000)		p_E	p_M
Age	**Otitis Externa**	**Otitis Media**	**Otitis Externa**	**Otitis Media**		
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**		

pE: Analysis value of OtitisExterna, pM: Analysis value of Otitis Media

Table 2. Gram positive bacteria strains isolated from patients with 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**		

pE: Analysis value of OtitisExterna, pM: Analysis value of Otitis Media

Table 3. Antibiogram results of the isolated 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

pR: Analysis value of antibiotic resistance, pI: Analysis value of antibiotic intermediate, pQ: Analysis value of antibiotic susceptibility

In 36 patients, including 22 male and 14 female, *S. aureus* showed only resistance to Oxacilin. Results obtained from the PCR revealed that 36 *S.aureus* isolates carried the *mec*A gene.

The statistic alanalysis of the rates of the isolates obtained from patients with otitis and the antibiogram are given in Table 1, Table 2 and Table 3. It was observed that gram-positive isolation rates were significant in 0-18 years-old male and female patients with otitis. Gram positive bacteria isolated from the patients with otitis externa at the age of 18-45 were found to be significant of statistical value. The statistical value was not significant in patients with otitis at 45 years of age. P_R and P_I values were found to be significant in the evaluation of resistance status of gram positive bacteria isolated from patients with otitis. The significance of P_S values was found to be different.

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. [20,21]. 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 otherhand, COE cases were below 5% [22]. 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%. [9, 23]. In a study conducted in Nigeria, the disease prevalence was reported to be 81.9% [24]. In a study of acute OM patients in Russia, this rate was reported to be 32% [25]. 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% [26] . In a study conducted in 2015 [7] *S. aureus* was reported to be the most isolated agent in OM patients. In a study conducted by Tanon-Anoh et al. [27] *S. pneumoniae* was reported to be the leading etiological agent. In a study conducted in Brazil, it was reported that *S. pneumoniae* was the leading etiological agent [28]. A series of studies conducted in CSOM patients world wide showed that *S. aureus* was the most common pathogen in India, Nepal, Singapore and Nigeria [29,30]. Other studies indicated that, *S. aureus* was found to be the most common pathogen in Pakistan, Iran and Saudi Arabia [8,31]. In a study conducted by Shavit et al. [6] ear swab samples of 88 patients with OE were evaluated. Bacterial agents were found to be *S. aureus* (7, 9%), *Streptococcus* spp. (1, 1.3%) and Coagulase Negative *Staphylococcus* (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 highestisolation rate among Gram-positive bacteria [1]. In a study conducted by Yazdi MMK et al. [20], 15 *S. aureus* and 11 CNS were isolated from 186 patients with OE and OM. Noguerira et al. [21] 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 presence were observed in 172 of 541 patients with AOM. It has been reported that *S. pneumoniae* was isolated in 109 of 172 patients [25]. A study conducted by Roland et al. [26] indicated that the occurrence of Gram positive bacteria 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 52.2% of male and %35.6 of female patients with otitis. It was observed that *S. pyogenes* (16%) and *S. pneumoniae* (3.4%) were the second most common isolated bacteria in male and female patients with otitis. *S. agalactiae* (0.1%) was isolated from a male patient with OE. In addition, *Enterococcus faecalis* (0.1%) was isolated from a female patient with OE. It is thought that the differentiation of flora bacteria is an important factor in bacterial isolation from people living under different climate and geographic conditions.

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 [32, 33]. However, one study reported that women were exposed to more infections than men [34]. A study conducted by Wasihun et al. [7] indicated that there is no significant difference between the type of bacterial agents isolated from both 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 occurance was 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. [26] it was reported that gram positive bacteria shows different resistance level. They reported that the resistance of *S. epidermidis* isolates were 23% to Neomycin, 11% to Oxcillin and 12% to Ofloxacin. The resistance 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. The resistance of these isolates was 66.6% to Ciprofloxacin, 33.4% to Erythromycin and 66.6% to Vankomycin, respectively [1]. *S. aureus* isolates showed low resistance to Ciprofloxacin, Gentamicin and Erythromycin [7]. 4 patients with *S. aureus* have been treated with Ciprofloxacin [35]. In a study conducted by Kaushik et al. [36] it was indicated that aminoglycoside and fluoroquinolone antibiotics were efficient for the *S. aureus* isolated from patients. In this study, 805 bacteria isolated from male patients showed little or no resistance to Vancomycin, Oxacilin and Trimethoprim/ sulfamethoxazole. 420 bacterium isolated from female patients showed little or no resistance to Vancomycin, Oxacilin and Rifampicin. Different level of resistance to antibiotics were observed and the evaluation of the antibiogram results in the treatment was found to be important.

There are limited reports of methicillin-resistant *S. aureus* isolated from patients with otitis in studies available to the researchers. In a study on otologic infections, three pediatric patients were diagnosed with MRSA. The researchers reported that MRSA in children may be available from the community and may cause otitis externa and otitis media [37]. In Italy, 3 of the *S. aureus* isolates in patients with OE showed resistance to methicillin [1]. In this study, Oxacilin resistance was determined for the *S. aureus* isolates, of which 22 were from the male patients and 14 from the female patients. According to the results of the Polymerase Chain Reaction (PCR) the 36 isolates were positive for *mec*A gene. It was evaluated that MRSA may affect patients in different parts of the world. The correct evaluation of the presence of MRSA is important in patients with otitis.

In this study, it has been observed that the otitis disease was common in our region. Uncontrolled use of antibiotics and having wrong preferences in empirical treatments lead to increased resistance problem. Antibiotic selection should be done according to susceptibility tests. It is anticipated that this study will contribute to advanced studies.

COMPETİNG İNTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Pane G, Cacciola G, Giacco E, Mariottini GL, et al. Assessment of the antimicrobial activity of algae extracts on bacteria responsible of external otitis. Marine Drug. 2015;13(10):6440-6452.
2. Nussinovitch M, Rimon A, Volovitz B, et al. Cotton-tip applicators as a leading cause of otitis externa. Int J Pediatr Otorhinolaryngol. 2004;68(4):433-435.
3. Sander R. Otitis externa: a practical guide to treatment and prevention. Am Fam Phys. 2000;63(5):927-36.
4. Brook I. Treatment of otitis externa in children. Pediatr Drug. 1999;1(4):283-289.
5. Wipperman J. Otitis externa. Prim Care: Clin Office Pract. 2014;41(1):1-9.
6. Shavit SS, Soudry E, Hamzany Y, et al. Malignant external otitis: factors predicting patient outcomes. Am J Otolaryngol. 2016; 37(5):425-430.
7. Wasihun AG, Zemene Y. Bacterial profile and antimicrobial susceptibility patterns of otitis media in Ayder Teaching and Referral Hospital, Mekelle University, Northern Ethiopia. Springer Plus. 2015; 4(1):701.
8. Mittal R, Lisi CV, Gerring R, et al. Current concepts in the pathogenesis and treatment of chronic suppurative otitis media. J Med Microbiol. 2015;64(10): 1103-1116.
9. Olina M, Cametti M, Guglielmetti C, et al. External otitis. Recenti Prog Med. 2002; 93(2):104-107.
10. Rosenfeld RM, Brown L, Cannon CR, et al. Clinical practice guideline: Acute otitis externa. Otolaryngol Head Neck Surg. 2006;134(4):4-23.
11. Jayakar R, Sanders J, Jones E. A study of acute otitis externa at Wellington Hospital, 2007–2011. Australas Med J. 2014;7(10): 392-399.
12. Osguthorpe JD, Nielsen DR. Otitis externa: Review and clinical update. Am Fam Phys. 2006;74(9):1510-1516.

13. Daneshrad D, Kim JC, Amedee RG. Acute otitis externa. J La State Med Soc. 2002; 154(5):226-228.
14. McCoy SI, Zell ER, Besser RE. Antimicrobial prescribing for otitis externa in children. Pediatr Infect Dis J. 2004; 23(2):181-183.
15. Rowlands S, Devalia H, Smith C, et al. Otitis externa in UK general practice: A survey using the UK General Practice Research Database. Br J Gen Pract. 2001; 51(468):533-538.
16. Wenzel RP, Reagen DR, Bertino JS, Baron EJ, Arias K. Methicillin-resistant Staphylococcus outbreak: A consensus panel's defination and management guidelines. Am J Infect Control. 1998; 26(2):102-10.
17. Eliopoulos G. Antimicrobial agents for treatment of serious infections caused by resistant Staphylococcus aureus and Enterococci. Eur J Clin Microbiol Infect Dis 2005;24(12):826-31.
18. Güler İ, Kılıç H, Atalay MA, el al. In-vitro susceptibility of methicillin-resistant Staphylococcus aureus strains to antibiotics. Dicle Med J. 2011;38(4):466-470.
19. Mamishi S, Mahmoudi S, Sadeghi RH, et al. Genotyping of Staphylococcus aureus strains among healthcare workers and patients in the tertiary referral Children's Medical Hospital in Tehran, Iran. British J Biomed Sci. 2012;69(4):173.
20. Yazdi MMK, Ghalavand Z, Yazdi AK, et al. Antibiotic resistante pattern of Pseudomonas aeruginosa in patients with otitis in Tehran hospitals. Int J Analytic Pharma Biomed Sci. 2015;9:50-6.
21. Nogueira JCR, Margareth de Fátima F, Lima EO, et al. Identification and antimicrobial susceptibility of acute external otitis microorganisms. Braz J Otorhinolaryngol. 2008;74(4):526-530.
22. Seid A, Deribe F, Ali K, et al. Bacterial otitis media in allage group of patients seen at Dessie referral hospital, North East Ethiopia. Egypt J Ear Nose Throat Allied Sci. 2013;14(2):73-78.
23. Diriba M, Solomon G, Hailu N. Isolation and antimicrobial susceptibility pattern of bacterial pathogens causing otitis media in children in Jimma hospital, South Western Ethiopia. Ethiop J Health Sci. 2004;14(2): 89-100.
24. Osazuwa F, Osazuwa E, Osime C, et al. Etiologic agents of otitis media in Benin city, Nigeria. North Am J Med Sci. 2011; 3(2):95.
25. Mayanskiy N, Alyabieva N, Ponomarenko O, et al. Bacterial etiology of acute otitis media and characterization of pneumococcal serotypes and genotypes among children in Moscow, Russia. Pediatr Infect Dis J. 2015;34(3):255-260.
26. Roland PS, Stroman DW. Microbiology of acute otitis externa. Laryngoscope. 2002; 112(7):1166-1177.
27. Tanon-Anoh MJ, Kacou-Ndouba A, Yoda M, et al. Particularities of bacterial ecology of acute otitis media in an African subtropical country (Coted'Ivoire). Int J Pediatr otorhinolaryngol. 2006;70(5):817-822.
28. Pereira M, Pereira MR, Cantarelli V, et al. Prevalence of bacteria in children with otitis media with effusion. J Pediatr (RioJ). 2004;80(1):41-48.
29. Yeo SG, Park DC, Hong SM, et al. Bacteriology of chronic suppurative otitis media–a multicenter study. Acta Otolaryngol. 2007;127(10):1062-1067.
30. Asish J, Amar M, VinayHajare AS, et al. To study the bacteriological and mycological profile of chronic suppurative otitis media patients and their antibiotic sensitivity pattern. Int J Pharma Bio Sci. 2013;4: 186-199.
31. Ahmad S. Antibiotics in chronic suppurative otitis media: A bacteriologic study. Egypt J Ear Nose Throat Allied Sci. 2013;14(3):191-194.
32. Egbe C, Mordi R, Omoregie R, et al. Prevalence of otitis media in Okada community, Edostate, Nigeria. Maced J MedSci. 2010;3(3):299-302.
33. Muluye D, Wondimeneh Y, Ferede G, et al. Bacterial isolates and drug susceptibility patterns of ear discharge from patients with ear infection at Gondar University Hospital, Northwest Ethiopia. BMC Ear Nose and Throat Disord. 2013;13(1):10.
34. Hassan O, Adeyemi RE. A study of bacterial isolates in cases of otitis media in patients attending oauthc, Ile-Ife. Afr J Clin Exper Microbiol. 2007;8(3):130-136.
35. Bhat V, Aziz A, Bhandary SK. Malignant Otitis Externa-A Retrospective Study of 15 Patients Treated in a Tertiary Healthcare

Center. J Int Advan Otol. 2015;11(1):72-76.

36. Kaushik V, Malik T, Saeed SR. Interventions for acute otitis externa. Cochrane Database Syst Rev. 2010;1: Cd004740.

37. Felipe Santos AB, Leila A, Eavey RD. Methicillin-resistant *Staphylococcus aureus* Pediatric Otitis. Arch Otolaryngol Head Neck Surg. 2000;126(11):1383-1385.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history/28089