AJMAS
https://journal.utripoli.edu.ly/index.php/Alqalam/index eISSN 2707-7179

Original article

Isolation and Identification of the Bacteria that Causes Otitis Media in Medical Center Hospitals Tripoli, Libya

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ARTICLE INFO

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Received*: 10-08-2023*
Accepted*: 28-10-2023*
Published*: 30-10-2023*

Keywords*. Otitis Media, Staphylococcus Aureus, Streptococcus Pneumoniae, Antibiotic Susceptibilities Test.*

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ABSTRACT

Background and aims. *People of all ages are susceptible to otitis media, a common ear infection, with a higher incidence in developing nations like Sub-Saharan Africa and South Asia, otitis media is the second most prevalent cause of hearing loss and the fifth most common disease worldwide. As a result, the objective of this study is to identify and isolate the bacteria that cause otitis media to use susceptibility testing in Tripoli, Libya, to find appropriate medications.* ***Methods****. Ear discharge samples from 100 patients with chronic ear discharge who had not had antibiotics in the previous five days were taken and cultured. Swab samples were taken from each patient and sent to the microbiology department for examination. The materials were grown, and the isolates were identified using standard microbiological methods. The Kirby-Bauer disc diffusion method was used to test the isolates' susceptibilities to various antibiotics.* ***Results****. Ninety-five out of a hundred ear discharge and swab samples were chosen for further analysis. The results demonstrated that 36 (38%) of the pathogens identified were Staphylococcus aureus, 5 (5%) were Streptococcus pneumonia, 3 (3%) were Staphylococcus epidermidis, 30 (32%) were Pseudomonas aeruginosa, 8 (8%) were Klebsiella Pneumonia, 1 (1%) were Haemophilus influenza, 7 (7%) were Enterococcus Faecalis, 2 (2%) were Acinetobacter pneumanni, 1 (1%) were Citrobacter koseri, and 2 (2%) were Morganella Morgani whereas, 5 samples did not grow.* ***Conclusion****. Adult males are more likely than adult females to have otitis media, according to our study. The data also showed that Staphylococcus aureus was the most typical isolate and the most typical cause of otitis media, with Ciprofloxacin being the most efficient drug.*

Cite this article. *Elyounsi N, Said A, Abuhelala H, Alsharif H, Elkammoshi A. Isolation and Identification of the Bacteria that Causes Otitis Media in Medical Center Hospitals Tripoli, Libya. Alq J Med App Sci. 2023;6(2):666-671.*
https://doi.org/10.5281/zenodo.10051799

INTRODUCTION

Otitis media (OM) is a common condition that affects both industrialized and developing nations, according to research; in fact, it is a major global health problem [1]. It could be chronic, acute, recurrent, suppurative, or nonsuppurative in nature. A common pediatric illness called acute suppurative otitis media (ASOM) has the potential to develop into chronic supportive otitis media (CSO) [2]. A condition affecting the middle ear cleft (tympanic cavity, eustachian tube,

and mastoid air cells) is known as chronic suppurative otitis media (CSOM). It is defined as a continuous perforation of the tympanic membrane that manifests as a mucoid or muco-purulent discharge that lasts for around eight weeks. [3]. A low recovery rate has been noted for the chronic form of otitis media, known as CSOM, which affects people of various ages. For certain populations, especially young children and the elderly, this condition can cause severe and life-threatening issues such as brain abscesses, hearing loss, or meningitis [4]. According to World Health Organization (WHO) estimates from 2015, 32 million children and 328 million adults worldwide (or over 5% of the population) suffer from debilitating hearing loss. The regions of Sub-Saharan Africa, Asia-Pacific, and South Asia have the highest percentages. A significant portion of hearing loss instances can be cured, and the remainder can be prevented with extensive preventative measures. Untreated ear infections that typically result in ear discharge, especially in middle- and low-income countries, are a major factor in hearing loss in children [5].

Studies conducted in the United Kingdom, the United States, India, Ghana, Japan, Sierra Leone, and Nigeria have discovered certain groups of organisms recorded in CSOM. Ear infections are attributed to viral, bacterial, or fungal causes. Nonetheless, the most common causes of ear infection (Pepto streptococcus, Propionibacterium) are bacterial isolates such as aerobic (e.g., *Escherichia coli, Pseudomonas aeruginosa, Streptococcus pyogenes, Staphylococcus aureus, Klebsiella species, Proteusmirabilis*) or anaerobic (e.g., *Bacteroides species*) [6-8].

Furthermore, it can be challenging for the clinician to investigate the precise origin of the sickness because the earache symptoms and indicators sometimes confound the infection etiology. As a result, the practitioner might recommend antibiotics in addition to the disease's cause. Viruses or fungi could be at blame for the ear infection. Patients would experience stress, there would be financial loss, and most importantly, there would be antibiotic resistance [9]. To properly treat patients with ear infections, it is essential to look at the microbiological profiles of ear infections and gauge the level of antibiotic resistance. Antibiotic resistance is also a growing global issue that poses serious risks to human health, according to the World Health Organization [10]. The current study aims to specify the causative microorganisms of otitis media (OM) and to determine their antibiotic agent susceptibility.

METHODS

Patients

A total of 100 outpatients with otitis media (55 male and 40 females) visited outpatient clinics in Tripoli Medical Centre during the period of March to September 2013 were enrolled in this study. Samples were collected in sterile swab sticks; gender, age, and whether they had taken any antibiotics for at least a week before the swab were recorded for each patient; the swabs were each properly labeled. The medical microbiology laboratory received the samples as soon as they were collected for analysis.

Sample processing

The culture mediums Blood Agar, MacConkey Agar, and Chocolate Agar were used to inoculate all specimens. At 37°C, petri dishes underwent a 24-hour incubation period. Colonies were initially identified based on their morphological and cultural characteristics. All isolated bacterial species underwent antimicrobial susceptibility testing using the Kirby-Bauer disc diffusion method in accordance with Clinical and Laboratory Standard Institute guidelines on plated Mueller Hinton agar (Oxoid, UK), where they were incubated for 24 hours at 37°C using 16 antibiotic discs, which were Gentamicin GN (10µg), Ciprofloxacin CIP (5µg), Amoxicillin AMC (30µg), Imipenem IMP (10µg), Tobramycin TOB (10µg), Piperacillin PRL (10µg) ,Trimethoprim SXT (25µg ,(Ampicillin AMP (100µg) Amikacin AK (30µg), Cefoxitin FOX (30µg), Ceftriaxone CRO (30µg), Tetracycline TE (30µg), Erythromycin E (15µg), Penicillin P (10U), Ceftazidime CAZ (30µg), and Vancomycin VA (30µg).

Statistical analysis

Chi square (X2) test was used to analyze the data, and it was presented as percentage and numbers. P value of less than the 0.05 was considered significant.

RESULT

One hundred patients with otitis media were chosen at random from an otorhinolaryngology ward, regardless of age or gender. The bacteria were found in 95 ear swabs taken from otitis media patients. During the study, 100 specimen isolates were collected. About 55(58%) of the infected were males, 40 (42%) were females, and 5 samples showed no growth (Table 1).

Table 1. The numbers and percentage of bacterial strains isolated from patients infected with otitis media in Tripoli, Libya

Age Groups	Female N=40		Male n= 55		Total (95)	
		%	n	%	n	%
01 - 20 years	15	37.5	8	14.5	23	32
21 - 40 years	5	12.5	7	12.5	12	14
41 - 65 years	20	50	40	72	60	54
(Growth & No growth)	40+3=43		55+2=57		95	100

Patients with otitis media were divided into three age groups: those aged 1 to 20 years (23 bacteria isolates 24%), those aged 21 to 40 years (12 bacteria isolates 12.5%), and those aged 41 to 65 years (60 bacteria isolates 63%). Males had a much higher incidence than females in general, particularly in the age group 41-65, as seen in (Table 2).

The results revealed that ninety-five of the hundred specimens, 36 (38%) of the pathogens were identified with *Staphylococcus aureus*, 5 (5%) with *Streptococcus pneumoniae*, 3(3%) with *Staphylococcus epidermidis*, 30 (32%) with *Pseudomonas aeruginosa*, 8 (8%) with *Klebsiella Pneumonia*, 1 (1%) with *Haemophiles influenzae*, 7 (7%) with *Enterococcus Faecalis*, 2 (2%) with *Acinetobacter pneumanni*, 1 (1%) with *Citrobacter koseri*, 2 (2%) with *Morganella Morgani* whereas, five samples did not grow as illustrated in the Table (1).

Gram stain was used to determine the type of bacteria that had been isolated. According to the findings, 51 (53%) of bacteria pathogens were Gram-positive, whereas 44 (46%) were Gram-negative (Table 3). Biochemical tests such as Catalase, Coagulase, and Oxidase were also used.

Table 2. The numbers and percentages of bacteria isolated from patients infected with otitis media according to their gender and age groups in Tripoli, Libya.

Isolated Bacteria	Female (40)		Male (55)		Total (95)	
	No	**%**	**NO**	**%**	**NO**	**%**
Staphylococcus aureus	22	55%	14	25%	36	38%
Streptococcus	1	3%	4	7%	5	5%
Staphylococcus epidermidis	2	5%	1	2%	3	3%
Pseudomonas aeruginosa	9	23%	21	38%	30	32%
Klebsiella Pmeumonia	2	5%	6	11%	8	8%
Haemophilus influenzae	0	0%	1	2%	1	1%
Enterococcus Faecalis	1	3%	6	11%	7	7%
Acinetobacter pneumanni	1	3%	1	2%	2	2%
citrobacter koseri	0	0%	1	2%	1	1%
Morganella Morgani	2	5%	0	0%	2	2%
No growth	3	60%	2	40%	5	100%

Table 3. The pattern of Gram-positive and Gram-negative pathogens in bacterial otitis media.

Isolated	Number	%
Gram-positive	51	54%
Gram-negative	44	46%
Total	95	100%

The antibiotic susceptibility of the organisms was evaluated and the results for the ten most common strains are shown in Table 1. Of the sixteen antibiotics that are more commonly available and that are used to treat the ear infection, Ciprofloxacin has the highest susceptibility rate (52%) of all isolates evaluated, followed by Tobramycin and Gentamicin (40%). Furthermore, the susceptibility rates to Imipeneme, Piperacillin, Amikacin, Ceftazidime Vancomycin, percentage were (36%, 28%, 27%, 26%, and 22% respectively). In contrast, Amoxycillin, Trimeyhprim, Cefoxitin, Tetracycline, and Erythromycin were showed fewer effects on bacteria isolated (18%, 14%, 16%, 12%, and 17% respectively). Finally, Penicillin and Ampicillin were shown the lowest susceptible to these strains (3% and 6% respectively) Table 4.

Table 4. Sensitive pattern of bacterial isolates from patients infected with otitis media in Tripoli, Libya

Antibiotics	Bacterial isolates										Total
	S. aureus	Strepto coccus	S. epider midis	P. aeregin osa	H. influen zae	K. Pmeum onia	Entero coccus	Acineto bacter	Citroba cter	M. Morgan i	
Gentamicin (10µg)	(36%) 13	(80 %) 4	(33 %) 1	(50%) 15	(0 %) 00	(38 %) 3	(14%) 1	(0 %) 0	(100%) 1 1	(0%) 0	(40%) 38
Ciprofloxacine (5µg)	(44%) 16	(80 %) 4	(67 %) 2	(57 %) 17	(100%) 1	(63 %) 5	(29 %) 2	(50 %) 1	(100%) 1 1	(0%) 0	(52 %) 49
Amoxycillin (30µg)	(31%) 11	(60 %) 3	(33 %) 1	(0%) 0	(0%) 0	(0%) 0	(29%) 2	(0 %) 0	(0 %) 0	(0%) 0	(18%) 17
Imipenem (10µg)	(22%) 8	(40%) 2	(33%) 1	(47 %) 14	(100 %) 1	(25%) 2	(57%) 4	(50 %) 1	(100 %) 1 1	(0%) 0	(36%) 34
Tobromaycin (10µg)	(36%) 13	(0 %) 0	(33 %) 1	(50 %) 15	(100 %) 1	(38 %) 3	(14 %) 1	(100%) 2	(100 %) 1 1	(50%) 1	(40%) 38
Pipreacillin (10µg)	(3 %) 1	(20%) 1	(33 %) 1	(53 %) 16	(100 %) 1	(25 %) 2	(43%) 3	(0 %) 0	(100 %) 1 1	(50 %) 1	(28%) 27
Trimeyhprim (25µg)	(22%) 8	(20 %) 1	(33 %) 1	(0 %) 0	(0%) 0	(25 %) 2	(0%) 0	(0 %) 0	(100 %) 1 1	(0%) 0	(14%) 13
Ampicillin (100µg)	(6 %) 2	(20 %) 1	(33 %) 1	(0 %) 0	(0%) 0	(0%) 0	(14 %) 1	(50 %) 1	(0%) 0	(0%) 0	(6%) 6
Amikacin (30µg)	(11%) 4	(0 %) 0	(0%) 0	(57 %) 17	(0 %) 0	(38 %) 3	(0 %) 0	(0 %) 0	(100 %) 1 1	(50 %) 1	(27%) 26
Cefoxitin (30µg)	(28%) 10	(0 %) 0	(33 %) 1	(0%) 0	(0 %) 0	(38 %) 3	(0%) 0	(0 %) 0	(100 %) 1 1	(0 %) 0	(16%) 15
Ceftriaxone (30µg)	(17%) 6	(20%) 1	(0%) 0	(3%) 1	(100 %) 1	(38%) 3	(0 %) 0	(0 %) 0	(100 %) 1 1	(0%) 0	(14%) 13
Tetracucline (30µg)	(14 %) 5	(40 %) 2	(0%) 0	(7%) 2	(0 %) 0	(0%) 0	(29 %) 2	(0 %) 0	(0 %) 0	(0 %) 0	(12%) 11
Erythromycin (15µg)	(31 %) 11	(40 %) 2	(33 %) 1	(0 %) 0	(0 %) 0	(13 %) 1	(14 %) 1	(0 %) 0	(0 %) 0	(0 %) 0	(17%) 16
Penicillin (10U)	(3%) 1	(0%) 0	(33 %) 1	(0 %) 0	(0%) 0	(0%) 0	(14%) 1	(0 %) 0	(0 %) 0	(0 %) 0	(3%) 3
Ceftazidim (30µg)	(3%) 1	(0 %) 0	(0 %) 0	(60 %) 18	(100%) 1	(50%) 4	(0%) 0	(0 %) 0	(0 %) 0	(50%) 1	(26%) 25
Vancomycin (30µg)	(39%) 14	(20 %) 1	(33 %) 1	(0%) 0	(0%) 0	(0%) 0	(71%) 5	(0 %) 0	(0 %) 0	(0 %) 0	(22%) 21

DISCUSSION

Overall, bacterial ear infections are a widespread problem worldwide and can lead to preventable hearing loss [11, 12]. Microbes can affect various parts of the ear, including the skin, cartilage, ear canal, and different cavities [13]. There are three types of ear infections: acute suppurative otitis media (ASOM), chronic suppurative otitis media (CSOM), and otitis externa (OE) [14]. CSOM is a chronic disease that affects people of all ages and has a low recovery rate.

The prevalence of ear infections varies among different populations. In this study, it was found that adults (ages between 41 and 65) had a higher incidence of ear infections compared to children. However, in north-central Nigeria, a high prevalence of the disease was found among pediatric patients by about 50% [15].

The high incidence of middle ear infections in men found in this study is consistent with the findings of other studies. In our study, 58% of the male participants were found to be sick, while only 42% of the female participants were contaminated. These results are comparable to those reported by Ethiopian researchers, where 64.8% of the patients were male and 35.2% were female [16]. Another study conducted by Kaur et al [17]. also showed a higher percentage of males (61%) compared to females (39%) with middle ear infections. Mohammad et al reported comparable results [18]. One explanation for this gender disparity could be that women are more hesitant to seek medical attention for hearing loss due to social factors. It is possible that cultural or societal norms discourage women from seeking help for their hearing problems, leading to a lower representation of females in these studies.

The statement suggests that in the current study, *S. aureus* was found to be the most significant pathogen associated with otitis media. This finding contrasts with studies conducted in northeastern Ethiopia, where Proteus was found to cause inflammation after *S. aureus*. Other published data from Africa and other parts of the world have reported bacteria of the genus Pseudomonas, particularly *Pseudomonas aeruginosa*, being associated with otitis media [19-21].

The statement also proposes that the difference in findings could be attributed to climatic and geographical variations between Libya and other countries. It implies that environmental factors may play a role in determining the prevalence of different pathogens causing otitis media in different regions.

It appears that both ciprofloxacin and tobramycin have shown high susceptibility rates against all bacterial pathogens isolated from middle ear secretions in the research being referred to. In another study conducted in northern Ethiopia, gentamicin and ciprofloxacin were found to be effective against over 90% of isolates. Additionally, chloramphenicol and co-trimoxazole were effective against some isolates. Erythromycin has also been shown to be effective specifically against *S. aureus* in previous studies [9,16].

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عزل وتشخيص البكتيريا المسببة لالتهاب الأذن الوسطى في مستشفيات المركز الطبي طرابلس، ليبيا

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المستخلص

الخلفية والأهداف. الأشخاص من جميع الأعمار معرضون للإصابة بالتهاب الأذن الوسطى، وهو عدوى شائعة في الأذن، مع ارتفاع معدل الإصابة به في الدول النامية مثل أفريقيا جنوب الصحراء الكبرى وجنوب آسيا، يعد التهاب الأذن الوسطى ثاني أكثر الأسباب شيوعًا لفقدان السمع وخامس أكثر الأمراض شيوعًا في جميع أنحاء العالم. ونتيجة لذلك، فإن الهدف من هذه الدراسة هو تحديد وعزل البكتيريا المسببة لالتهاب الأذن الوسطى لاستخدام اختبار الحساسية في طرابلس، ليبيا، للعثور على الأدوية المناسبة. **طُرق الدراسة**. تم أخذ عينات من إفرازات الأذن من 100 مريض يعانون من إفرازات الأذن المزمنة والذين لم يتناولوا المضادات الحيوية في الأيام الخمسة السابقة وزرعوها. تم أخذ عينات مسحة من كل مريض وإرسالها إلى قسم الأحياء الدقيقة لفحصها. تمت زراعة المواد وتم تشخيص العزلات باستخدام الطرق الميكروبيولوجية القياسية. تم استخدام طريقة الانتشار القرصي Kirby-Bauer لاختبار حساسية العزلات للمضادات الحيوية المختلفة. **النتائج**. تم اختيار خمسة وتسعين من أصل مائة عينة من مفرزات ومسحات الأذن لمزيد من التحليل. أظهرت النتائج أن 36 (38٪) من مسببات الأمراض التي تم تحديدها كانت المكورات العنقودية الذهبية، 5 (5٪) كانت العقدية الرئوية، 3 (3٪) كانت المكورات العنقودية الجلدية، 30 (32٪) كانت الزائفة الزنجارية، 8 (8٪) كانت Klebsiella Pneumonia، 1 (1%) المستدمية النزلية، 7 (7%) المكورات المعوية البرازية، 2 (2%) الراكدة الرئوية، 1 (1%) Citrobacter koseri، و2 (2%) مورجانيلا مورجاني، بينما، 5 عينات لم تنمو. **الخاتمة**. وفقًا لدراستنا، فإن الذكور البالغين أكثر عرضة للإصابة بالتهاب الأذن الوسطى من الإناث البالغات. وأظهرت البيانات أيضًا أن المكورات العنقودية الذهبية كانت المعزولة الأكثر شيوعًا والسبب الأكثر شيوعًا لالتهاب الأذن الوسطى، وكان دواء سيبروفلوكساسين هو الدواء الأكثر فعالية.

الكلمات الدالة. التهاب الأذن الوسطى، المكورات العنقودية الذهبية، المكورات العقدية الرئوية، اختبار الحساسية للمضادات الحيوية.