Original Article

Bacteriological Study and Antibiotic Susceptibility of Some Bacterial Isolates among Otitis Media Patients in Al-Muthanna Province, Iraq

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Abstract

Background: Otitis media is one of the diseases spread globally, especially in Iraq. **Objectives:** The research aims to diagnose the bacteria that cause this disease and find out the extent of its resistance to antibiotics, and thus reach the best antibiotics that eliminate these bacteria. **Materials and Methods:** Overall 100 samples including ear discharge were collected from different ages of patients suffering from complaints of ear infection in Al-Hussein Teaching Hospital in Al-Muthanna City, Iraq through the dates between January 2020 and May 2021. The isolates were detected by the usual cultural techniques, Gram stain, biochemical tests, and VITEK Compact System (bioMérieux, France) for bacterium detection. Antibiotic susceptibility testing was achieved for each isolate using the disc diffusion method by Kirby-Bauer. **Results:** The results were read according to the Clinical & Laboratory Standards Institute (CLSI) criteria, and were divided into three reads (sensitive, intermediate, and resistant). The most types of bacterial isolates identified from positive ear swabs were: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella* spp., *Staphylococcus hemolyticus*, *Staph epidermidis*, *Escherichia coli*, *Serratia* spp., and *Basterulla* spp. The result also showed the highest infection rate of 32 (32%) isolates among the age group (11–20) years old compared with other age groups. The antibiogram result showed that most isolates were resistant to more than four drugs. In addition, 4 (4%) of the isolates were susceptible to every antibiotic examined. **Conclusions:** We conclude the best treatments that were effective against isolates diagnosed with otitis media were Amikacin and Gentamycin.

Keywords: Antibiotic resistance pattern, bacterial isolation, otitis media

Introduction

Ear discharge is one of the most typical signs of ear infection, which is an inflammation of the ear.[1] The three main forms of ear infections are otitis externa, otitis media, and inner ear infections. An external auditory canal infection is known as otitis externa,[2] and otitis media is an inflammation of the mastoid process and middle ear. Children frequently have otitis media.[3,4] Moreover, it encompasses chronic supportive otitis media, otitis media with effusion, and acute otitis media. Although closely related, ear infections have different presentations, associated consequences, and treatments.[5]

In developing nations, ear infections pose a public health risk since they are associated with frequent antibiotic prescriptions, hearing loss, severe disability, and even death.[4] Ear infections can be either acute or persistently purulent.[6] In total, 65–330 million people experience ear infections annually, and 60% of those persons have severe hearing loss.[7] Despite being primarily a condition that affects babies and young children, ear infections can affect adults.[8] The disorder may begin in childhood, appear as a complication of untreated or incorrectly treated

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acute suppurative otitis media, or appear immediately as a chronic condition, Since children's eustachian tubes are anatomically shorter, more horizontal, and have more flaccid cartilage, which can easily hinder their opening, ear infections are a serious health concern for them, particularly in those with low socioeconomic position.[9,10] varied geographic regions have varied ear infection etiologies and prevalence rates.[11] According to a World Health Organization survey, nations can be grouped into those with low ear infections when the prevalence rate of ear infections in children is between 1% and 2% and high when it is between 3% and 6%, and Ethiopia is in the latter group.[12] Although viruses and fungi infections can also cause ear infections, bacteria that are found in the skin of the external ear and enter the middle ear through a chronic perforation, such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus mirabilis*, *Klebsiella pneumonia*, and *Escherichia coli*, are the main culprits.[13]

Additionally, the antimicrobial resistance profile of bacteria differs by community due to geographic differences, regional antimicrobial prescribing policies, and the presence of resistant bacterial strains in a particular area.[14]

Material and Methods

Samples collection

The cross-sectional study involved the exploration of the described cases of ear infection. Overall 100 samples including ear discharges were collected from different ages patients suffering from complaints of ear infection in Al-Hussein Teaching Hospital and outpatient clinic in Al-Muthanna City, Iraq through the dates between January 2020 and May 2021.

Detection of bacteria

Colonies identification

Ear swabs were collected from patients using a sterile swab. The swabs were transported to the laboratory in Al-Hussein Teaching Hospital immediately by Amies transport medium. Then the swab was cultured on many media including MacConkey agar, Mannitol Salt agar, and Blood agar (Thermo Fisher Scientific, USA), and incubated at 37°C aerobically and anaerobically. The isolates were detected by the usual cultural techniques, Gram stain, biochemical tests, and VITEK Compact System for bacterium detection.[15,16]

Antibiotic susceptibility testing

Antibiotic susceptibility testing was achieved for each isolate using the disc diffusion method by Kirby-Bauer.[8] Mueller–Hinton agar was used with following antibiotic discs (Ampicillin (AMP) 10 µg, Amikacin (AK) 30 µg, Ceftriaxone (CTR) 30 µg, Ciprofloxacin (CIP) 5 µg, Chloramphenicol (C) 30 µg, Amoxicillin 30 µg, Gentamicin (CN) 10 µg, Nalidixic acid (NA) 30 µg, Penicillin G 30 µg, Oxacillin (OX) 1 µg, Tetracycline (TE) 30 µg, Vancomycin (VA) 30 µg, and Augmentin 15 µg, Cephalothin 15 µg). After the bacterial isolates were incubated with antibiotic discs for 24 h at 37°C, the sensitivity was read. The results were read according to the Clinical & Laboratory Standards Institute (CLSI) criteria and were divided into three reads (sensitive, intermediate, and resistant).[17]

Statistical analysis

The statistical analysis was done with the SPSS 23 (IBM) software package. Both the Pearson's chi-square test and the analysis of variance (ANOVA) test were used to determine the statistical differences between the groups. Statistical significance was assigned to the probability of ($P \leq 0.01$).[18]

Ethical approval

The study was conducted following the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patient's verbal and analytical approval before the sample was taken. The study protocol, the subject information, and the consent form were reviewed and approved by a local ethics committee according to document number 1988 (on 12/3/2021) to get this approval.

Results

From January 2020 to May 2021, ear swab samples were collected from patients at Al-Hussein Teaching Hospital and an outpatient clinic who had Otitis media. The present study revealed that 100 ear swabs had bacterial cultures, Among these; 38 (38%) were male and 62 (62%) female, the most type of bacterial isolates identified from positive ear swabs were: *S. aureus* (31%), *P. aeruginosa* 18 (18%), *Klebsiella* spp. 16 (16%), *Staphylococcus hemolyticus 11(11%)*, *Staphylococcus epidermidis* 9(9%), *E. coli* 7(7%), *Serratia* spp. 4(4%), and *Basterulla* spp. 4(4%) as shown in Table 1, $P \leq 0.005$ indicates a significant outcome. In addition, there was statistically significant difference between acute and chronic infections 59 (59%) and 41 (41%), respectively, with $P \leq 0.005$ as shown in Table 2.

The current result also shows the highest infection rate of 32 (32%) isolates among the age group (11–20) years old than the other age groups and the lowest infection rate of 4 (4%) among the age group (more than 40) years old as shown in Table 3.

In the current study, 14 antibiotics were tested against different bacterial isolates diagnosed according to the sensitivity and resistance profile of the antibacterial agent. The best treatments that were effective against isolates diagnosed with otitis media were Amikacin (94.0%), Gentamycin (91.8), Ciprofloxacin (53.7%) had a mediocre antibacterial impact, whereas it had a high level

Table 1: Bacterial species distribution among study participants at Al-Hussein Teaching Hospital according to gender

Strains of bacteria	Total	Male	Female	Total
Staphylococcus aureus	31	10(26.32%)	21 (33.87%)	$P \leq 0.005$
Pseudomonas aeruginosa	18	6(15.79%)	12 (19.35%)	
Klebsiella spp	16	8(21.5%)	8(12.90%)	
Staph hemolyticus	11	4(10.53%)	7(11.29%)	
Staph epidermidis	9	4(10.53%)	5 (8.06%)	
Escherichia coli	7	2(5.26%)	5 (8.06%)	
Serratia spp.	4	2(5.26%)	2(3.23%)	
Basterulla spp.	4	2(5.26%)	2(3.23%)	
Total	100	38 (38%)	62 (62%)	

Table 2: Bacterial species distribution among study participants at Al-Hussein Teaching Hospital according to type of infection

Strains of bacteria	Total	Acute infection	Chronic infection	*P* value
Staphylococcus aureus	31	13 (22.03%)	18 (43.90%)	$P \leq 0.05$
Pseudomonas aeruginosa	18	8 (13.56%)	10 (24.39)	
Klebsiella spp.	16	10 (16.95%)	6 (14.63%)	
Staphylococcus hemolyticus	11	8 (13.56%)	3 (7.32%)	
Staphylococcusepidermidis	9	5 (8.47%)	4 (9.76%)	
Escherichia coli	7	7 (11.86%)	0 (0.0%)	
Serratia spp.	4	4 (6.78%)	0 (0.0%)	
Basterulla spp.	4	4 (6.78%)	0 (0.0%)	
	100	59 (59%)	41 (41%)	

Table 3: Age groups-distribution of bacterial species among study participants at Al-Hussein Teaching Hospital according to type of infection

Bacterial isolates	Total	0–10 (years)	11–20 (years)	21–30 (years)	31–40 (years)	More than 40 (years)
Staphylococcus aureus	31	9	11	8	2	1
Pseudomonas aeruginosa	18	6	6	3	2	1
Klebsiella spp	16	4	4	4	3	1
Staphylococcus hemolyticus	11	3	4	2	2	--
Staphylococcus epidermidis	9	2	3	1	3	--
Escherichia coli	7	2	2	1	1	1
Serratia spp.	4	1	1	1	1	--
Basterulla spp.	4	1	1	1	1	--
$P \leq 0.005$	100	28	32	21	15	4

of antibacterial activity against all identified bacterial species. Additionally, Ampicillin (81.8%), Amoxicillin (65.0%), Ceftriaxone (72.2%), Cephalothin (78.2%), and Penicillin G (81.5%) were all highly resistant to all identified bacterial species [Table 4].

Regarding to antibiogram, the current study showed that most isolates 77 (77%) were resistant to more than four drugs. In addition, 4 (4%) of the isolates were susceptible to every antibiotic examined. This high rate of multidrug resistance may be a result of drug misuse.

Discussion

Ear infections are a problem in all age groups, which have begun to increase dramatically in Iraq in recent years. We note in this study when the age was greater, the fewer ear infections, as children are more susceptible to infection compared with adults, and this is consistent with several studies, including Ethiopia [13,19] and in Benin.[20] This is due to several reasons, including the weak immune status of children, as well as the anatomical position of the Eustachian tube in childhood, as it is short and of a horizontal nature, which increases the possibility of inflammation. Additionally, children's increased susceptibility to upper respiratory infections and malnutrition, all these reasons contribute to the development of otitis media.[21]

The present study showed *S. aureus* was the most prevalent isolates among patients *31 (31%)*, followed by *Pseudomonas aeruginosa* 18 (18%) and *Klebsiella* 16 (16%).

Table 4: Antibiotic sensitivity and resistance profiles overall for bacterial otitis media

Antibiotic agent (*n*)	Total	Antibiotic susceptibility pattern *n* (%)		
		Sensitive	Intermediate	Resistant
Vancomycin	53	22 (41.5%)	0 (0.0)	31 (58.4)
Ciprofloxacin	54	29 (53.7)	3 (5.5)	24 (44.4)
Gentamycin	49	45 (91.8)	1 (2)	3 (6.1)
Amoxicillin	20	5 (25)	2 (10)	13 (65)
Amikacin	50	47 (94)	0 (0.0)	3 (6)
Ceftriaxone	54	9 (16.6)	6 (11.11)	39 (72.2)
Cephalothin	46	10 (21.7)	0 (0.0)	36 (78.2)
Ampicillin	55	10 (18.2)	0 (0.0)	45 (81.8)
Naldixic acid	50	12 (24)	0 (0.0)	38 (76)
Penicillin G	54	11 (20.3)	0 (0.0)	44 (81.5)
Oxacillin	40	4 (10)	5 (12.5)	31 (77.5)
Augmentin	25	5 (20)	0 (0.0)	20 (80)
Chloramphenicol	27	13 (48.1)	1 (3.7)	13 (48.1)
Tetracycline	26	9 (34.6)	0 (0.0)	17 (65.4)

N = number of drugs tested

The presence of *S. aureus* with a high rate of infection is consistent with several previous studies including the study in Ethiopia.[13,19] In addition, a study in Benin by Akinjogunla *et al*.,[20] the variation in climate and geographic changes could be the cause of variance in the rate of isolates, as middle ear infections are more common as the temperature rises due to the increased colonization of bacteria in the ear.[22] Since ear infections are on the increase from time to time, it is necessary to study and diagnose these bacterial infections and test the effectiveness of antibiotics to choose the appropriate treatment for them. Therefore, this study attempts to conduct an antibacterial test for each bacteria to know the treatments that these bacteria resist and distinguish them from the treatments that help us eliminate them according to Table 3 Depending on the rate of sensitive isolates, the most effective antibiotics against studied bacterial isolates caused otitis media were Amikacin (94.0%), Gentamycin (91.8), and Ciprofloxacin (53.7%) had a mediocre antibacterial impact, whereas it had a high level of antibacterial activity against all identified bacterial species. On the other hand, most of the bacterial isolates showed significant resistance to many of the antibiotics studied, as follows, Ampicillin (81.8%), Amoxicillin (65.0%), Ceftriaxone (72.2%), Cephalothin (78.2%), and Penicillin G (81.5%). These results were fully consistent with preceding studies, including Dessie, Ethiopia [13,19] and study in Iraq.[23] The sensitivity of isolates to some antibiotics, such as ciprofloxacin, varies from one isolate to another, and this is due to several reasons, including the emergence of new resistant strains as a result of the misuse of these antibiotics.

While Ceftriaxone, Ciprofloxacin, Amoxacin, and Chloramphenicol were incongruent with the current study. With a sensitivity of 94.1%, *P. aeruginosa* also showed remarkable susceptibility to Amikacin and Gentamycin. However, it was resistant to Nalidixic acid (70.6%), Ceftriaxone (94.1%), and Penicillin (100.0%), this is consistent with.[24] This showed that *P. aeruginosa* has resistance (100%) to most of these antibiotics. This study's data differs from one similar to it that was conducted in Ethiopia by Abera.[13] Antimicrobial resistance profiles of bacteria vary throughout populations due to differences in geography, local antimicrobial prescribing patterns, and the occurrence of resistant bacterial strains, which are the most likely causes of this heterogeneity.[13,22] Antibiogram results [Table 5] from the current study showed that nearly all isolates (98.6%) were drug-resistant to at least one antibiotic Furthermore, 97.1% of isolates were multidrug-resistant against two drugs or more. One isolate proved positive for each tested antibiotic. It is possible that drug abuse is to blame for the high rate of multidrug resistance.[19,25,26]

Conclusions

The present study showed *S. aureus* was the most prevalent isolates among patients followed by *Pseudomonas aeruginosa* and *Klebsiella*. The best antibiotics that were effective against isolates diagnosed with ear infections were Amikacin and Gentamycin. Antibiogram results from the current study showed that nearly all isolates were drug-resistant to at least one antibiotic agent.

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Table 5: Antibiogram to species of bacteria from patients with otitis media

Bacterial species	Total	Multiple antibacterial resistance					
		R_0	R_1	R_2	R_3	R_4	$R_{>4}$
Staphylococcus aureus	31	1	2	4	4	1	19
Pseudomonas aeruginosa	18	0	0	0	0	0	18
Klebsiella spp.	16	0	0	0	0	2	14
Staphylococcushemolyticus	11	2	0	0	0	2	7
Staphylococcus epidermidis	9	1	0	0	0	3	5
Escherichia coli	7	0	0	0	0	0	7
Serratia spp.	4	0	0	0	0	0	4
Basterulla spp.	4	0	0	0	0	1	3
Total	100						

R0 is sensitive to all tested antimicrobials; R1, R2, R3, R4, and R>4 are, respectively, resistant to one, two, three, four, and more than four antibacterials

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Conflicts of interest
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