

***Staphylococcus Aureus* Isolated from Iraqi Pediatrics with Acute Otitis Media: Etiology and Antibiotic Resistance**

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التهاب الأذن الوسطى (OM) هو عدوى شائعة في الأذن غالبًا ما يتم تجاهلها في البلدان النامية. الرضع والأطفال هم الأكثر تأثرًا بـ OM. ومع ذلك ، لا يتم استثناء البالغين. يكلف علاج التهاب الأذن الوسطى مليارات الدولارات سنويًا ويمكن أن يسبب المرض أو الوفاة إذا ترك دون علاج. تكمن المشكلة في معظم البلدان النامية في عدم وجود مبادئ توجيهية لعلاج التهاب الأذن الوسطى. يمكن للمرضى شراء الأدوية مباشرة من الصيدلية بدون وصفة طبية من الطبيب. من بين 203 مسحة أذن ، 161 مسحة كانت إيجابية لمسببات الأمراض. تم العثور على 62 عزلة فقط من المكورات العنقودية الذهبية ، 42 منها كانت حساسة للميثيسيلين (67.8%) و 20 عزلة من المكورات العنقودية الذهبية المقاومة للميثيسيلين (32.2%). أظهرت الدراسة حساسية 59.4% للسيبروفلوكساسين والريفامبيسين والفانكوميسين. لم يظهر البنسلين أي فعالية لأن أكثر من 90% من السلالات الـ 62 كانت مقاومة ، و 77.4% كانت مقاومة للإريثروميسين. يهدف هذا البحث إلى التعرف على البكتيريا المسببة لالتهاب الأذن الوسطى الحاد وتحديد مدى حساسية المكورات العنقودية الذهبية للمضادات الحيوية في طب الأطفال العراقي المعالج في مستشفى الأطفال في بغداد.

Abstract

Otitis media (OM) is a common ear infection often overlooked in developing countries. Infants and children are most affected by O.M, However, adults are not exempted. Otitis media treatment costs billions of dollars annually and can cause morbidity or mortality if left untreated. The problem with most developing countries is that they do not have guidelines for treating otitis media. Patients can buy medications directly from the pharmacy without a doctor's prescription.

Among the 203 ear swabs, 161 were positive for pathogens. Only 62 were found to be *S. aureus* isolates, 42 of which were methicillin-sensitive (67.8%) and 20 were methicillin-resistant *Staphylococcus aureus* (32.2%). The study showed 59.4% sensitivity to ciprofloxacin, rifampicin, and vancomycin. Penicillin showed no effectiveness since more than 90% of the 62 strains were resistant, and 77.4% were erythromycin-resistant.

This research aims to identify the causative bacteria for acute otitis media and determine the susceptibility of *S. aureus* to antibiotics in Iraqi pediatrics treated in a pediatric hospital in Baghdad.

Introduction

Eighty percent of children under the age of three suffer from acute otitis media (AOM), a frequent bacterial infection of the middle ear [1]. According to the National Institutes of Health, nearly 75% of children have acute otitis media at some point [2]. Antibiotic-resistant antibiotics are children's most common cause of antibiotic use [3]. Understanding the epidemiology and etiology of acute otitis media is crucial for choosing suitable antibiotics for therapy, as the overuse of antibiotics has become a public health concern in Iraq [4]. The geographic variation is one of the main reasons for the differences in AOM incidence. There are many pathogens causing otitis media, such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Moraxella* [5]. However, for the last two decades, *Staphylococcus aureus* has been reported to be the main reason for the hospitalization rate of pediatric patients [6, 7]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been linked to pneumonia, skin and soft tissue infections, and bacteremia in pediatrics.

Materials and methods

Collection of samples

The time frame for this retrospective research was from January 1, 2019, to December 1, 2021, and it took place in a private ENT clinic. Patients older than three months but less than three years were considered. As with the 9th revision of the International Classification of Diseases, Clinical Modification Codes have been used as the basis for AOM diagnostic criteria (ICD-9-CM) [3]. An otolaryngologist was used to take ear swabs, which were then cultured for further diagnosis.

Sample culturing

Samples were transported to the microbiology lab and plated on blood, chocolate, and McConkey agar. All agars were incubated at 35 to 37°C for a maximum of 72 hours, and VITEK-2 was used to identify the suspected bacteria.

Antibiotic susceptibility test

Antibiotic sensitivity testing of *S. aureus* was achieved using antibiotic discs including Penicillin, Gentamicin, Erythromycin, Tetracycline, Ciprofloxacin, Sulfamethoxazole-Trimethoprim, Rifampicin, and

Vancomycin. CLSI (Clinical and Laboratory Standards Institute) criteria were used to recognize the inhibition zone [8].

Results and Discussion

Microbiology and Epidemiology

During the study period, 203 children aged 0–3 years with AOM were identified at private ENT clinics. The mean age was 24 months. 52% of the children were under one year old. The female-to-male ratio was 0.55:1, as shown in Table 1.

79.4% (161 of 203) of the samples were positive for pathogenic bacteria, with *Staphylococcus aureus* being the most common (30.5%), followed by *Streptococcus pneumoniae* (26.6%), *Haemophilus influenzae* (4.9%), *Streptococcus pyogenes* (3.4%), *Candida albicans* (2.9%), *Klebsiella pneumoniae* (3.4%), *Escherichia coli* (3.4%), and fungal infection (3.9%). These data are presented in Table 2 and Figures 2 and 3. The percentage of gram-positive isolates was 84%. In contrast, gram-negative bacteria were detected in about 16% of samples, as presented in Figure 1.

Table 1: Children's information with AOM

	AOM		Staphylococcus aureus	
Male	104	65%	38	56%
Female	57	35%	24	44%
Age	No.	%	No.	%
0-1	52	37%	15	47%
1-2	48	34%	10	31%
2-3	41	29%	7	22%

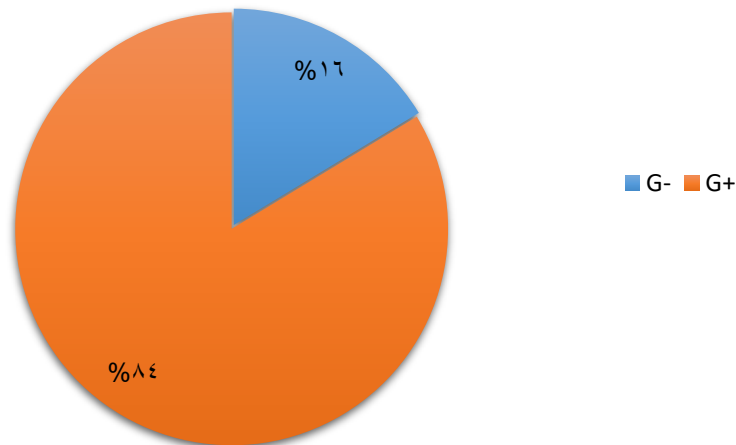


Figure 1: Types of microbial isolates.

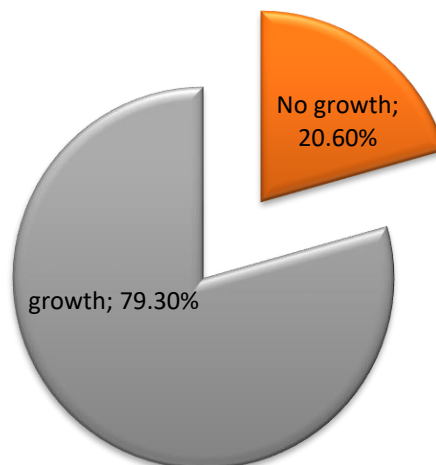


Table 2. Microorganisms growth on swabs taken from the middle ear of children with acute otitis media

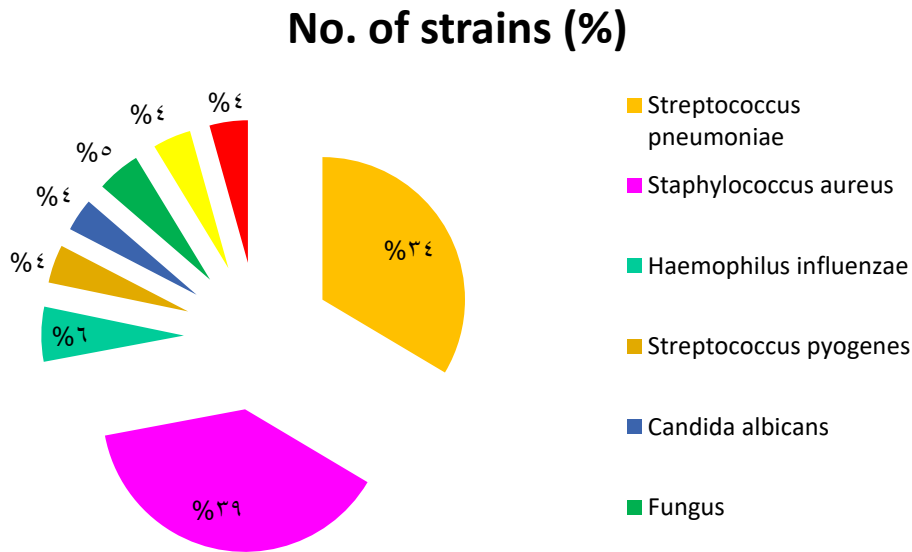


Figure 3 percentages of microbial strains of middle ear swabs.

From the total of 62 isolates, 42 were methicillin-susceptible *Staphylococcus aureus* (MSSA), whereas 20 (32.2%) were methicillin-resistant *S. aureus* (MRSA). Ciprofloxacin, rifampicin, and vancomycin were sensitive against 59.4 percent of the isolates. Table 3 and Figure 4 show that 58 strains (93.5%) were resistant to penicillin and 77.4% were resistant to erythromycin.

Table 3: Antibacterial susceptibility of *Staphylococcus aureus* isolated from AOM children

Antibiotics	Rate of susceptibilities					
	Overall (n = 62)		MSSA (n = 42)		MRSA (n = 20)	
	sensitiv e	resistan ce	sensitiv e	resistan ce	sensitiv e	resistan ce
Penicillin	4	58	7	35	0	20
Gentamicin	35	27	26	16	13	7
Erythromycin	14	48	14	28	3	17
Tetracycline	23	39	21	21	5	15
Ciprofloxacin	37	25	21	21	13	7
Sulfamethoxazole-trimethoprim	36	26	29	13	15	5
Rifampicin	37	25	28	14	16	4
Vancomycin	37	25	29	13	11	9

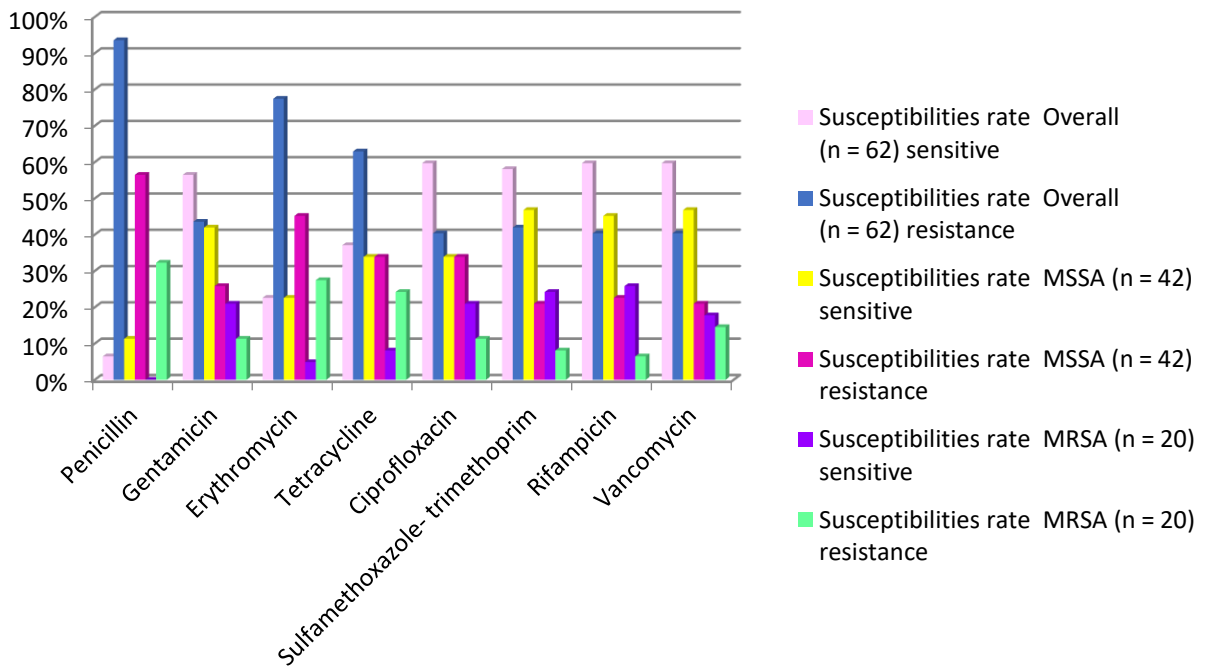


Figure 4. Percentages of antimicrobial susceptibility of Staph. Aureus isolates.

Discussion

Accurate epidemiological data on the cause and burden of AOM are needed to help clinicians choose an effective antibiotic treatment for AOM in children and to help public health policymakers make decisions. In this research, the prevalence among males was 65% higher than that of females, at 35%. These findings are consistent with other studies conducted in Iraq [10], Nigeria [11], Pakistan [12], Ethiopia [13, 14], and Uganda [15]. However, several studies have shown that infection occurs predominantly in women [16–20]. Kvistad et al. (2004) suggested that male dominance may be due to lifestyle choices and environmental influences [21].

No growth was observed in 20.6% of ear swabs, slightly higher than what was reported by Gorems et al. [23], Al-Marzoqi, et al. [17], and Karim [22]. This may be due to the possible presence of chlamydia, mycoplasma, or viruses. These viruses include a respiratory syncytial virus, influenza, and adenovirus. As demonstrated by some literature [24, 25], these act as causative agents of otitis media.

The infectious agents that cause otitis media and their susceptibility to antimicrobial agents change with time, age, and whether the infection is acute or chronic [26]. In this investigation, researchers found that gram-positive bacteria, as opposed to gram-negative bacteria, were the most common isolates from individuals with otitis media. This finding is consistent with previous studies [17, 27, 19, 20, 14, 28]. This finding differs from studies in which gram-negative bacteria were predominant [29, 30, 9]. According to the climatic and geographical differences in the study area, this difference may be associated with the different bacterial spectra of otitis media.

Multiple international investigations [31, 32, and 20] have shown that *Staphylococcus aureus* is the most prevalent bacterial cause of chronic otitis media. *P. aeruginosa*'s high isolation rate in this research may be explained by its biofilm phenotype, making it easier for the bacteria to enter the middle ear through the external auditory canal. The isolation of fecal pathogens such as *Escherichia coli* and *Klebsiella pneumoniae* suggests that these persons may be at risk of infection due to poor sanitation and economic disadvantage.

On the other hand, *Staphylococcus aureus* was the most common cause of acute otitis media (30.5%), consistent with previous studies [33, 34, 14] but different from *Streptococcus aureus* reported by other investigators. *pneumoniae*, *Moraxella influenzae*, and *Haemophilus influenzae* predominated [35, 36]. Ear infections may have several causes related to the immune system, genes, pathogens, and the surrounding environment.

The previous studies have noted different epidemiology and etiology, suggesting the most common pathogens for AOM are *Staphylococcus pneumoniae* and influenza. *Streptococcus pneumoniae* and

Staphylococcus aureus were shown to be the most common causative pathogens of AOM in the current investigation, which is consistent with previous studies from southern China [1, 3, 36].

In the era of generalized immunity against pneumococcal infection, *Haemophilus influenza* became the leading AOM pathogen, which means that with the introduction of protein-conjugated vaccines directed against seven serotypes (PCV7), the dominance of the primary pathogen has been altered [38]. Therefore, *S. aureus* has been considered the leading cause of AOM in children, particularly in Saudi Arabia, since the PCV7 vaccine was included in the standard immunization schedule [39].

In this retrospective analysis, we found *S. pneumoniae* and *S. aureus* to be the most common pathogens, with *S. aureus* detected in 30.5% and 26.6% of AOM samples, respectively. Most *Staphylococcus aureus* bacteria are multidrug-resistant, which means they are resistant to erythromycin, penicillin, and tetracycline. Historically, the most common pathogens for AOM were *Staphylococcus pneumoniae* and *Haemophilus influenzae* [35].

S. aureus's ability to form a biofilm, drug resistance and ubiquitous nature are considered factors attributed to its ability to cause AOM. All the bacterial isolates in the current study had very different antibiotic susceptibility profiles.

Previous studies have shown that ampicillin and amoxicillin-clavulanic acid have little effect on gram-negative bacterial resistance [40]. In our investigation, ciprofloxacin and gentamicin were effective against more than 56 percent of the resistant tested bacterial strains. Ettehad et al. [31] discovered that ciprofloxacin was more effective than other antibiotics for treating otitis media. Otitis media is a common bacterial infection. The research shows that it is efficient against both gram-positive and gram-negative bacteria [41]. The AOM recommends amoxicillin-clavulanic acid, antibiotic medications, ciprofloxacin, and gentamicin for treating these illnesses. In a study conducted by a French research team [42], the multiple gram-negative and gram-positive microbes were shown to be more susceptible to ciprofloxacin and amoxicillin-clavulanic acid. The kind of infection, isolation and antibiotic use determines these changes.

Previous research found that many isolates had high levels of MDR, which is consistent with the study's current results [14; 34; 44; 43]. Experts believe that this may be due to misuse of antibiotics, incorrect treatment methods, or bacterial features of the biofilm of some of the dominant isolates [14, 34].

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