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IDENTIFICATION OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CHRONIC SUPPURATIVE OTITIS MEDIA

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ABSTRACT

Introduction: Staphylococcus aureus isolated from prolonged suppurative otitis media was the focus on the current investigation. Aims: The goal of this study is the identification of methicillin resistant Staphylococcus aureus isolated from chronic suppurative otitis media. Material and methods: The research population is made up of 100 medical samples were taken from individual with chronic otitis media infection who visited Alfurat Al-Awsat Hospital and AL-Zahra Teaching Hospital for Children in the province of Al-Najaf between January 1, 2023, and March 20, 2023. Results: The samples were injected onto mannitol salt agar (MSA) and then subcultured on blood agar after being incubated for (24 to 48 hours at 37°C). Characterization of colonies, Gram's staining, and conventional biochemical testing. A genomic investigation of MRSA isolates was accompanied using PCR affecting the mecA gene. Staphylococcus aureus bacteria were created in 34.5% of specimens. Genotyping complete polymerase chain reaction and biochemical examination presented 58% correctness in recognizing detaches. 20 bacterial specimens were found to encompass the mecA gene, representing a joining among chronic suppurative otitis media and MRSA resounding the mecA gene. Conclusion: this study shows Staphylococcus aureus is more causative agent for suppurative otitis media and resistant for some antibiotic because resistant to mecA gene.

INTRODUCTION

The description of CSOM is prolonged swelling of the middle ear and mastoid mucosa in which the tympanic tissue is not whole (tear or tympanostomy tube) and liberation (otorrhea) is current [1]. Continuing suppurative otitis media (CSOM) leftovers one of the greatest public infantile chronic communicable illnesses universal, touching varied ethnic and cultural collections both in emerging and manufacturing states. It includes significant illness and can reason extra- and intracranial difficulties. The different meanings of chronic suppurative otitis media and the enclosure of cases with cholesteatoma in stated pervasiveness of prolonged suppurative otitis

media, prevent an precise approximation of the positive commonness and frequency of chronic suppurative otitis media [2].

[3] obligate recognized a past of severe and persistent otitis media, paternal antiquity of prolonged otitis media, and packed circumstances (i.e. great relatives with numerous relations, great diurnal care centres) as important risk influences for CSOM. They could not establish an association between prolonged suppurative otitis media and dislike, persistent better breathing contagions, breastfeeding, gender, paternal age, or inactive smoking. In CSOM, bacteria can reach the middle ear either from the nasopharynx through the Eustachian tube or since the outside ear channel finished a non-intact tympanic tissue [4]. The aerobic microorganisms greatest regularly isolated in prolonged suppurative otitis media are *P. aeruginosa* (18-67%), *S. aureus* (14-33%), Gram-negative bacteria, such as *Proteus spp*., *Klebsiella spp*., and *Escherichia spp*. (4 -43%), and *Haemophilus influenzae* (1-11%) [5].

MRSA is a majorcause of clinic- developed contagions that are flattering progressively problematic to battle since of evolving confrontation to allcurrent antibiotic courses. The evolutionary backgrounds of *methicillin resistant S. aureus* are sick unspoken, no lucid terminology occurs, and there is no agreement on the quantity of main *methicillin resistant S. aureus* replicas or the understanding of duplicates defined from diverse countries [6].

The methicillin resistance gene (mecA) codes for protein binding that methicillin resistance penicillin that ist current in liable straining and is supposed to have remained developed from a vaguely associated classes [7]. MecA is approved moveable hereditary component, the SCCmec, of which 4 methods have been defined that change in scope and hereditary arrangement [8]. Numerous methicillin resistance *Staph spp*. isolates are increase resilient and are predisposed individual to glycopeptide antibiogram such as vancomycin and new drugs. Methicillin resistance *Staph spp*. isolates separates that have reduced defenselessness to glycopeptides antibiogram such as vancomycin and investigational drugs. Methicillin resistance *Staph Spp* isolates that have decreased glycopeptide intermediately susceptible *S. aureus* [9].

The details of advent of methicillin resistance *Staph spp.* are Multifariously and can be credited to crowd issues, contagion regulator performs and antibigram burdens. The entrance of bacteria suspitability phenotypes has been related to the medical usage of antibigram managers to which the bacterial rapid resistant [10].

The aim of this study is the identification of methicillin resistant *Staphylococcus aureus* isolated from chronic suppurative otitis media.

METHODOLOGY

The current examination appropriated residence in the Bacteriology and Molecular Laboratories of the College of Health and Medical Technologies at Al-Furat Al-Awsat Technical University in Iraq, between January 1 and March 20, 2023. The research population consisted of 100 clinical specimens from patients with chronic otitis media who visited Al-Zahra Teaching Hospital for Children and Alfurat Al-Awsat Hospital in Al-Najaf region. Every research participant exhibited an active purulent discharge from a ruptured tympanic membrane.

Clinical specimens are promptly directed transfer to the lab after collection. Ear swabs were placed on blood agar and incubated for 24 to 48 hours at 37°C on Mannitol agar plates in the lab. Initial colonial morphology on various media, hemolysis on blood agar, pigment formation, and Gram's staining of the culture were used to diagnose the isolates.

The biochemical test of *Staph aureus* is accomplished due to [11].

Antibiotic Susceptibility Test of *Staph aureus* is accomplished due to [12].

Total DNA Extract: After an overnight incubation period, transfer one colony of cultured bacteria to two milliliters of sterile nutritional broth and let it incubate for eighteen to twenty-four hours at 37 ◦C. Utilizing the Genomic DNA Mini plasmid kit (Favorgen/Taiwan) in agreement through the producer's directions, the DNA was extracted and purified. Genes VatA and mecA were found using the whole DNA.

PCR Amplification and Gel Electrophoresis: PCR was performed on the DNA of each isolate to find specific genes, like the mecA gene. MecA-F5: 5′-TGCTCAATATAAAATTAAAACAAACTACG-3′ and mecA-R5-L: 5′-GAAGTATGACGCTATGATCCCAATCTAACTTC-3′ are the precise primer pairs that were utilized [13], the primer was initially denaturated at 95ºC for three minutes, and the annealing temperature was set at 58 °C, producing a PCR product of 108 bp. thirty-three cycles divided into four parts: four minutes of final extension at 72 oC, 30 sec of annealing at 54 ºC, 30 sec of extension at 72 ºC, and thirty seconds of denaturation at 95ºC. An estimation of the amplified products' size was made for PCR using 2% agarose gel electrophoresis. After staining the gel with 4 µL of 10 mg/mL ethidium bromide (Sigma, USA), it was operated at 80 volts for 90 minutes. A solitary band was visible at the proper place using an ultraviolet light transillumintor (Cleaver, UK). Next, a gel documentation system (Cleaver, UK) was used to take pictures of the band. A 100 bp ladder (Bioneer, Korea) was used to assess the molecular weights of enlarged products [14].

RESULTS AND DISCUSSION

Culturing identification: According to the morphological analysis of the bacteria, 58/100 (or 58%) of the isolates of *Staphylococcus aureus* on mannitol salt agar that showed as yellow colonies due to mannitol commotion, which was identified as a possible source of the germs. In the current investigation, 58 isolates grew on agar plates and showed typical Staphylococcus aureus characteristics. According to the results of the biochemical tests, 40/58 (68.9%) of the isolates were *Staphylococcus aureus*, as shown (table 1).

Table (1): The biochemical tests for *Staphylococcus aureus*

Test / **Bacteria**	Coagulase	Mannitol fermentation	Catalase	Urease	Motility	Kliglar iron agar H2S	Indole
Staph. aureus	+	+	+	+	-	-	-

According to [15], MRSA is most prevalent in infants, the elderly, and people with chronic illnesses such injury stayers, recipients for transplant organ, cancer patients undergoing diabetics, steroid users, chemotherapy, intravenous drug users, and HIV patients. Higher levels of contamination in the human body are caused by the accumulation of bacteria. In addition to the vagina, it can occur in the skin, perineum, throat, and gastrointestinal tract [11].

The most important pathogen for humans and animals among the Staphylococci species is Staphylococcus aureus. The creation of golden pigment also sets it apart. The simplest method used in laboratories to differentiate coagulase-negative staphylococci from S. aureus is the coagulase test. The methods utilized in this work for gram staining, conventional biochemical tests, and colony characterisation are similar to the S. aureus phenotypic features reported by [16]. Based on the pathogenic bacteria's ability to hemolyze red blood cells, blood agar is typically employed to separate them [17]. S. aureus may be distinguished by its colony shape as well as by

the results of all biochemical tests, including DNase, coagulase, catalase, urease, and Gram's staining [18].

One hundred clinical specimens were taken after patients with prolonged otitis media, and these were grown on Blood Agar isolated and MSA, a medium that promotes the development of some bacteria while inhibits the growth of others, making it difficult to discriminate between them over time. Contains a high concentration of 7% salt, 1% mannitol sugar, 1% agar (solidifying agent), 7% casein enzyme digest, 7% animal tissue enzyme digest, 7% beef extract, and 1% phenol red indicator. Because most bacteria are inhibited by these components, MSA is selective against G- bacteria and only slightly discerning against G+ bacteria that can withstand in elevation of salt concentrations. Moreover, [11] state that it serves as a differential media for staphylococci that ferment mannitol. It contains phenol red, a pH indicator that can be used to find the acid these bacteria create, as well as the carbohydrate mannitol. By generating an acidic byproduct that changes the phenol red in the agar to yellow, S. aureus creates colonies that are yellow in color. According to [19], it is used to selectively isolate presumed pathogenic (pp) Staphylococcus species, which are subsequently validated by Gram stain and biochemical tests (Catalase, Coagulase, and other assays).

Susceptibility Test: All isolates (40) that yielded positive results for Culturing identification underwent an antibiotic susceptibility test; the results are displayed in Table 3.

The study's findings showed that the *Staph. auerus* isolates had significant rates of antibiotic resistance, with high sensitivity to ofloxacin (95.2%), methicillin (85.7%), minocycline (70), and cefaclor (52.4%). It displayed intermediate resistance to augmentin (59.5%), nalidixic acid (57.1%), erythromycin (43.1%), and dipracillin (25.8%), as well as strong resistance to gentamicin (73.6%) (Figure 1).

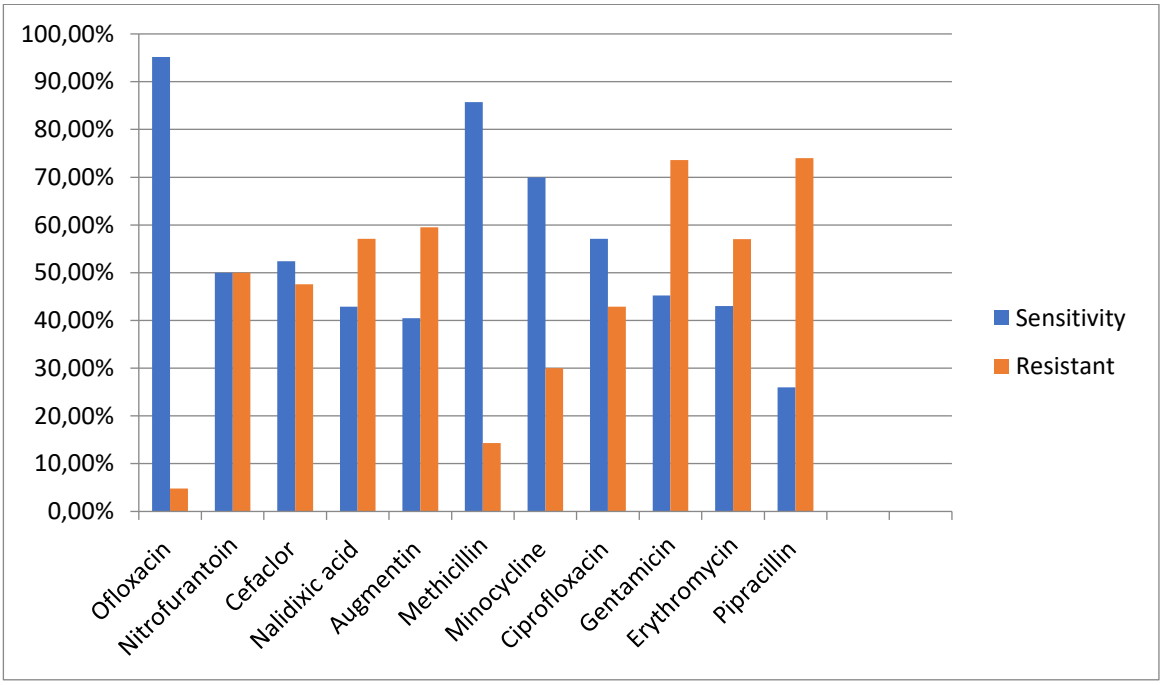

Figure 1: Antibiotics sensitivity test for 20 *Staph. auerus*isolated .

This work identified a susceptibility to antibiotics pattern for S. aureus and MRSA isolate from chronic otitis media, which contrasts with the results of [20], who described that MRSA resistance to cefoxitin was confirmed by PCR. Though [21] reported that penicillin, ampicillin, and cloxacillin found antimicrobial resistance of MRSA at 93.4%, 88.9%, and 83.3%, respectively.According to [22] in Palestine, all MRSA isolates were vancomycin- and gentamicin-sensitive and were identified by cefoxitin disc diffusion. According to a [23] in Italy, 60.58% of

MRSA cases were susceptible to every antibiotic tested, whereas 39.42% of cases showed resistance to at least one antimicrobial.

Vancomycin, tigecycline, rifampin, daptomycin, and linezolid are among the effective treatments for Staphylococcus species [24,37]. In the current investigation, S. aureus shown resistance to ampicillin at 100%, cefoxitin at 90.9%, erythromycin at 65.9%, and imipenem at 43.2%. ampicillin (100%), clindamycin (63.3%), cephalothin (59.5%), tetracycline (57%), cotrimoxazole and bacitracin (53.2%), and erythromycin (51.9%) were the antibiotics to which *S. aureus* isolates showed the greatest resistance, according to [25]. Additionally, the results we obtained agree with the discoveries of [26] and [27].

Molecular study

The genomic DNA was extracted directly from 58 samples of individuals and was used for PCR to determine antibiotic resistance genes for *Staphylococcus aureus*. Whole DNA extracted directly were subjected to 2% agarose gel electrophoresis.

Figure (2): DNA samples only; 2% Agarose gel electrophoresis at 72 volts for 30 minutes. No. (1–11) Sample

1- detection of the mecA gene: By a polymerase chain reaction technique with confident advancing and reverse primers, all segregates were inspected to discovery the mecA gene, which converts for the enzymes that prompt the tetracycline antibiotics. Created on the results described in Figure 3 of the current examination, mecA gene-tested isolates accounted for 20 (34.5%) of the *S. aureus* isolates.

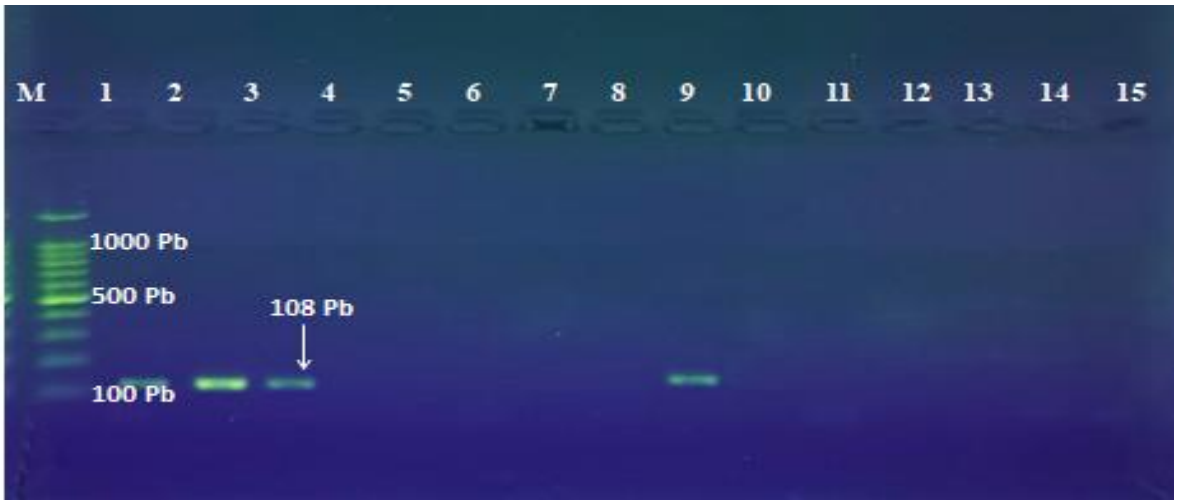

Figure (4): 2 % Agarose gel electrophoresis at 80 volt for 90 minutes for mecA gene tested.

The bacteria are resilient to methicillin and other penicillin antibiotics recognitions to a gene called MecA. In calculation to endorsing bacterial cell wall creation, MecA is also complicated in encoding the PBP2A protein [28]. In the training approved available by [29], the occurrence of this gene was projected at 46.5% amongst 43 MRSA strains. Dependable with preceding discoveries, this gene is also current in *S. aureus* from Pakistan [30], [31]and [32].

Primer intensification of the MecA gene presented that 20 isolates (34.5%) were optimistic for this gene, which encodes the manufacture of adapted penicillin-binding protein (PBP). These results are consistent with the study of [33,34]. The results showed that in India [35] and New Zealand, where MRSA isolates are common (28%), the mecA gene was detected in 38% and 73% of detaches. [36] 24% of the noticed isolates encompass the MecA gene.

CONCLUSION

This study show *Staphylococcus aureus* is more causative agent for suppurative otitis media and resistant for some antibiotic because resistant to mecA gene**.**

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