

## **Antibiotic Susptipility Profile and Virulence Factors Profile of Staphylococcus Aureus Isolated from Otitis Media**

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### **Abstract**

The current study aimed to find Antibiotic susptipility profile and virulence factors profile of staphylococcus aureus isolated from otitis media, for this purpose 200 ear swabs collected from patients suffering from otitis media whom attending to the ENT consulting clinic at Tikrit Teaching Hospital and the private outpatient clinic in Tikrit city. Bacterial isolation and identification were done , virulence factors profile detected by classical and genetic methods, and Antibiotic susptipility were done.

The result shows that out of 200 ear sample collected from otitis media, staphylococcus aureus isolated in rate of 26.1% (46 out of 200), virulence factor detection by classical methods shows that he results of the current study showed that Staphylococcus aureus possessed protease, lecithinase, and urease by 95.6%, 89.1%, and 89.1%, respectively, while the enzymes denase and hemolysin appeared by 45.6% and 71.7%, respectively. When using genetic methods, the detection of virulence genes FnbA, Etb and Sea were 80%,70% and 23% respectively. The results of the current study showed that the highest rate of resistance of staphylococci towards antibiotics was towards amoxicillin, reaching 67.3%, and the lowest towards vancomycin and azithromycin which are 9.5%

### **Introduction**

Otitis media is defined as inflammation of the membrane lining the middle ear in whole or in part due to various types of microorganisms such as bacteria, fungi or viruses that reach the middle ear through the outer ear or after infection of the respiratory system through the nose or throat through a tube (Ackerman, 2021). There are three types of otitis media which are acute otitis media (lasts less than six weeks), chronic otitis media( usually accompanied by a hole in the eardrum and ear secretions are thrown through the perforated membrane for more than six weeks and the third type, it is called Otitis Media with Effusion which characterize by an inflammation of the middle ear with serous, mucous, or purulent secretions (Poloan et al., 2021).

Staphylococcus is a spherical Gram-positive bacterium with a diameter of 0.5-1.5 micrometers. Arranged singly, in pairs, short chains, or in the form of grape clusters (Murray et. al, 2002). Staphylococcus aureus have many virulence factors such as adhesion factors that interfere with the host's body cells, including Viteronectin, Fibronectin, Laminin, bonesiabprotein, Fibrinogen, Collogen, ealstin, and thrombospondin. (Dego et al.,2002). And many enzyme such as Coagulase, catalase, Hyaluronidase, DNase , lipase , Phosphatase , hemolysin, Leucocidins, and Pyrogenic exotoxins, these factors play important role in pathogenesis of bacteria (Kinney et al.,2022).

### **Materials and methods :**

- 200 ear swabs were collected from patients suffering from otitis media, the Patients attending the ENT consulting clinic at Tikrit Teaching Hospital and the private outpatient clinic in Tikrit city in period from September 2021 until the end of April 2022.
- Bacterial isolation and identification: each swab put in tube contain nutrient broth, then transport to bacteriology laboratory, then sub culturing on nutrient agar, mannitol salt agar and blood agar and cultivation on 37C for 48hours, then sub culturing in same agar and cultivation condition. Gram stain and group of biochemical tests were done as to (Quinn et al.,1998).
- Detection of virulence factors by classical methods which inculde

- a- Protease production : conducted according to (Cruickshank et al.,1975)
- b- Lecithinase production: : conducted according to (Cruickshank et al.,1975)
- c- DNAs: conducted according to (Cruickshank et al.,1975)
- d- Hemolysin production: conducted according to (Cruickshank et al.,1975)
- e- Urease production: conducted according to (Cruickshank et al.,1975)
- Detection of virulence factors by PCR test:
- a- DNA extraction: by using a ready-made kit (G-spin™ Total DNA Extraction) and according to manufacturer instructions.
- b- Primer: primers used in current study as in table (1)

Table (1): primers used in current study

Primer	Sequence	Size of DNA product	Annealing temp	Annealing time (second)
fnbA	GTGAAGTTTTAGAAAGGTGGAAAGATTAG GCTCTTGTAAAGACCATTTTCTTCAC	643	57	40
etb	CAGATAAAGAGCTTTATACACACATTAC AGTGAACCTATCTTTCTATTGAAAAACACTC	612	56	45
sea	GGTTATCAATGTGCGGGTGG GCGGCACTTTTTCTCTTCGG	102	57	120

- c- Components of the PCR master mix for one sample as in table (2)

Table (2): mixture compound and amount

Compound	Amount (microliter )
DNA Template	2.5
2X Master Mix	12.5
Forward primer	1
Reverse primer	1
DNAse free water	8
Total	25

- d- Thermocycler program: as in table (3).

Table (3): thermocycler program

Step	Temperature	Time (mints)	Cycles
First Denaturation step	94	4	1
Denaturation step	94	1	30
Primer-annealing step:	According to primers type		
DNA extension step	72	1	
Final DNA extension step	72	10	1
End Temperature	4	-----	-----

Antibiotic susceptibility test: conducted by disc diffusion methods and according to (Atlas et al.,1995).

## Results and discussion

Out of 200 ear sample collected from otitis media *Staphylococcus aureus* isolated in rate of 26.1% (46 out of 200)

*Staphylococcus aureus* appear as yellow colony MSA agar (figure 1), and give positive catalase tests and coagulase

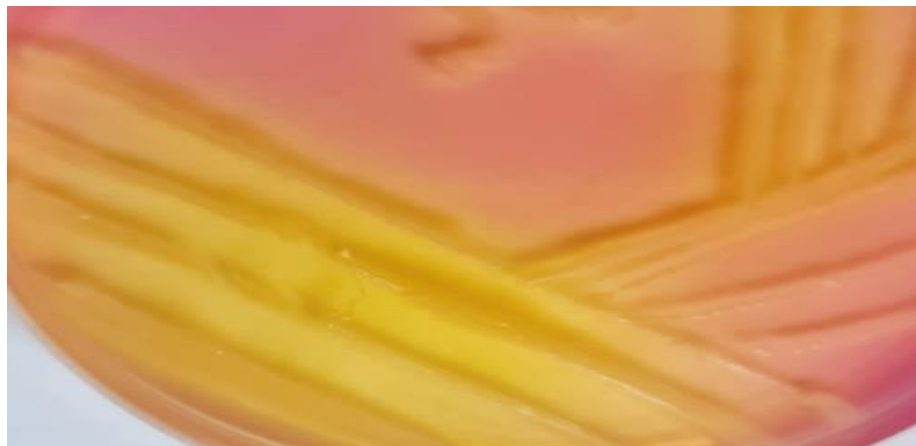


Figure (1): *Staphylococcus aureus* grown in M.S.A

The isolation rate recorded in current study is close to results recorded by( Hateet et al.,2022) which is 30%. Ikeh (2000) refer to The dominance of *Staphylococcus aureus* as a cause of otitis media

virulence factor detection by classical methods shows that he results of the current study showed that *Staphylococcus aureus* possessed protease, lecithinase, and urease by 95.6%, 89.1%, and 89.1%, respectively, while the enzymes denase and hemolysin appeared by 45.6% and 71.7%, respectively.

as from table (4)

Table (4): virulence factor detected by classical methods

Virulence factors	Number of positive isolates	Rate
Protease	44	%95.6
Lecithinase	41	%89.1
DNase	21	%45.6
Heamolysin	33	%71.7
Urease	41	%89.1

When using genetic methods, the detection of virulence genes *FnbA*, *Etb* and *Sea* were 80%,70% and 23% respectively as in table (5) and figure (2,3,4).

Table (5) Virulence factors of *Pseudomonas aeruginosa* bacteria detected by genetic methods

Gene	Rate of positive isolates
<i>FnbA</i>	%80
<i>Etb</i>	%70
<i>Sea</i>	%23.3

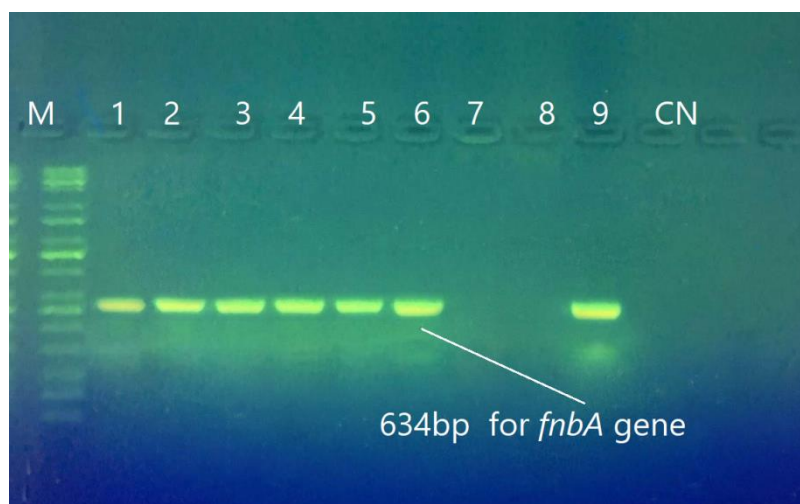


Figure (2) Results of electrophoresis of *Staphylococcus aureus* for the detection of *fnbA* gene. M : DNA marker. pore 1-6 indicate positive results. It gave DNA regions of 634 pb

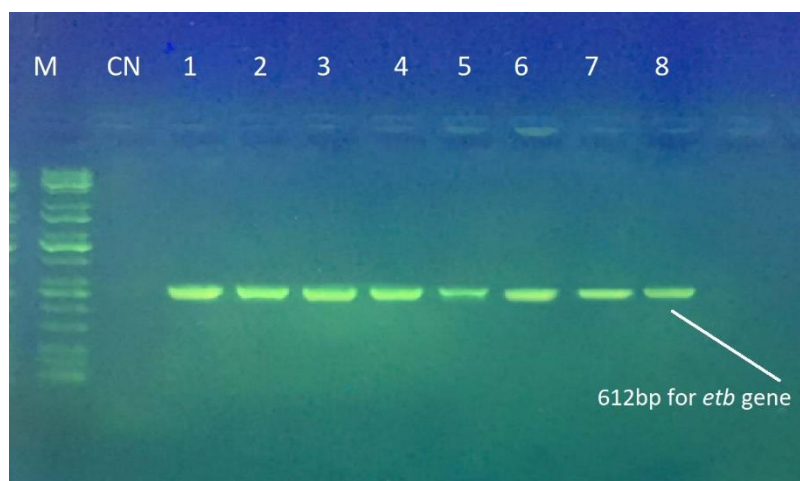


Figure (3) Results of electrophoresis of *Staphylococcus aureus* for the detection of *etb* gene. M : DNA marker. pore 1-8 indicate positive results. It gave DNA regions of 612 pb

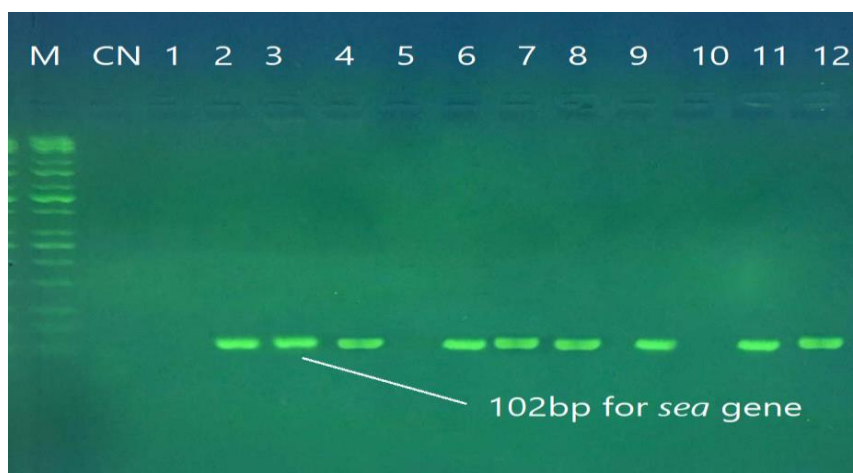


Figure (4) Results of electrophoresis of *Staphylococcus aureus* for the detection of *sea* gene. M : DNA marker. pore 3,4,6,8,10,11,12 indicate positive results. It gave DNA regions of 102 pb

From study of Nume & Moza (2022) shows that 88% and 95.6% of *Staphylococcus aureus* produce hemolysin and protease. *Staphylococcus aureus* possesses a hemolytic enzyme, which works to break down red blood cells, liberate hemoglobin, and destroy cell membranes. It is known in several types as type alpha b, and in several types alpha is known as pore forming toxin, which is toxic to many cells, such as blood cells. Platelets and hepatocytes, beta hemolytic, known as sphingomyelinase c, which is toxic to red and white blood cells, gamma hemolytic, which is toxic to macrophage cells and neutrophils, delta hemolytic, which has the ability to analyze the wall of mast cells, and hemolytic abscon (produced from coagulase-negative cocci (Keogh et al., 2019) hemolysin. This enzyme has the ability to influence the structure of DNA, and this enzyme is one of the advantages of pathogenic bacteria. *Staphylococcus aureus* produces two types of this enzyme. Advantages of *Staphylococcus aureus* (Rosato et al., 2017).

In a study conducted by (Raheema, & Abed, 2019) found that *S. aureus* possesses genes, *etb*, and *sea* in rate of 32% and 71%. In a study conducted by Song et al. (2016), found that the *seb*, *tst*, and *eta* genes appeared at a rate of 19.6%, 12.8%, and 11.3%. In a study conducted by Sina et al (2013), it was found that the frequency of *eseb* and *sea* was 44% and 32%. , while in study of Li et al. (2018) showed the frequency of *fnbA*, *eta*, and *sea* genes was 70%, 15%, and 66%, respectively. *fnbA* acts as a gene encoding an adhesion protein, which is the basis for pathogenic events, while the gene *etb* acts as a gene encoding exfoliative toxin proteins, while *sea* encodes for enterotoxins (Peacock et al., 2022)

Table (6) Antibiotic susceptibility test

Antibiotic	Resistant	Intermeted	Sensitive
Amoxicillin	31 (%67.3)	4 (%8.6)	11 (%23.9)
Tetracycline	28 (%60.8)	2 (%4.3)	16 (%34.7)
Chloramphenicol	11 (%23.9)	4 (%8.6)	31 (%67.3)
Gentamicin	11 (%23.9)	1 (%2.1)	34 (%73.9)
Imipenem	9 (%19.5)	3 (%6.5)	34 (%73.9)
Vancomycin	7 (%15.2)	0 (%0)	39 (%84.7)
Erythromycin	11 (%23.9)	1 (%2.1)	34 (%73.9)
Azithromycin	7 (%15.2)	0 (%0)	39 (%84.7)
Ceftazidime	13 (%28.2)	5 (%10.8)	28 (%60.8)
Ciprofloxacin	15 (%32.6)	3 (%6.5)	28 (%60.8)
Trimethoprim	24 (%52.1)	1 (%2.1)	21 (%45.6)
Cefotaxime	15 (%32.6)	3 (%6.5)	28 (%60.8)
Ampicillin	29 (%63.0)	3 (%6.5)	14 (%30.4)





Figure (5): Antibiotic susceptibility test

From table (6) and figure (5,6) showed that the highest rate of resistance of staphylococci towards antibiotics was towards amoxicillin, reaching 67.3%, and the lowest towards vancomycin and azithromycin which are 9.5%. in study conducted by (Abdullah, 2015), he found that the rate of resistance of staphylococci to the antibiotic vancomycin was (10%), while the rate of resistance was (60%) to the antibiotic rifampin, while the rate of resistance was (70%) to the antibiotic chloramphenicol.

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