Bacteriological Study of Carbapenem and Aminoglycoside Resistance in *Staphylococcus Aureus* Recovered from Patient in Baghdad Province

Hajir Adil Anwar[1], Maliha Masaoud[2], Ahmed Salim[3]

[1]MSC Student, Bachelor of Medical Laboratory Technologies, [2]Assist. Prof/ Public Health Sciences/Biostatistucs / College of Health & Medical Technology/Baghdad; [3]Assist. Prof/ Molecular Microbiology / College of Health & Medical Technology/Baghdad

Abstract

Background: In population- and hospital-acquired infections, Staphylococcus aureus is one of the most powerful causative agents. Aminoglycosides are active bactericidal drugs and are frequently used to treat staphylococcal infections in conjunction with beta-lactams or glycopeptides. Staphylococcus aureus is immune to carbapenems and other beta-lactams A different mechanism that can lead to carbapenem resistance is the active expulsion of carbapenems from the periplasmic space after their entry, due to the high distribution of these bacteria that are now immune to other antibiotics, it has become more difficult to treat these drug-resistant strains. **Materials and Methods:** A total of 130 *staph.aureus* isolates were isolated from different clinical samples in some public & private hospitals in Baghdad city during the period from November to December 2019. Bacterial identification was done using conventional cultural & chemical methods & and VITEk 2 cards for identification (GP), while the minimum inhibitory concentration (MIC) testing was performed using disk diffusion & (AST-GP30) cards in VITEK 2 automated system. **Results:** By using disk duffusion test out of 130 of *Staph.aureus* isolate, 76(58.4%) were resistant to aminoglycosids antibiotics (gentamicin) while 29(22.3%) were resistant to carbapenem antibiotics (imipenem) The MIC of different antibiotics was performed on 26 isolates using (VITEK2AST-GP30) showed that 18(69.2%) were resistant to aminoglycosids antibiotics (gentamicin) while 26(100%) were resistant to carbapenem antibiotics (imipenem). **Conclusion:** The MIC of different antibiotics By using disk duffusion *the Staph.aureus* isolate resistant to gentamicin 76(58.4%) and 29(22.3%) were resistant to imipenem, while, by VITEK2AST-GP30 showed 18(69.2%) were resistant to gentamicin , while 26(100% were resistant to imipenem.

***Key words**: Staph.aureu, carbapenems, aminoglycosids*

Introduction

Staphylococcus aureus is cocci-shaped Gram-positive bacteria (stain purple by Gram stain) that appear to be organized in clusters defined as "grape-like"[(1)] After Gram staining when observed under a light microscope. The word 'Staphylococcus' was derived from Greek, meaning bunch of grapes (staphyle) and berry (kokkos). The scanning electron microscopic observation shows approximately spherical cells with a smooth surface. The cell diameter varies from 0.5 to 1.0 μM Thick cell walls, distinctive cytoplasmic membranes and amorphous cytoplasms are seen by transmission electron microscopy of cells[(2)], Under aerobic or microaerophilic conditions, staphylococci grow easily on most bacteriological media. Colonies on solid media are round, smooth, elevated, and glistening On Mannitol Salt Agar circular, 2-3 mm in diameter, with a smooth, glossy surface, S. aureus typically forms gray to deep golden yellow colonies; colonies appear opaque and are frequently pigmented, golden yellow[(3)] Antibiotics have dramatically decreased infectious disease deaths since their introduction into medicine to treat millions of people and can account for the biggest medical breakthrough of the 20th century[(4)] . Imipenem is a beta-lactam antibiotic belonging to the carbapenem subgroup, active against Gram positive as well as Gram negative aerobic

and anaerobic bacteria. It exerts bactericidal effects by disrupting the synthesis of the cell wall[5] , By mutation-derived modifications of their PBPs, Gram-positive bacteria become immune to carbapenems and other beta-lactams[6] . Aminoglycosides are active, wide-spectrum antibiotics that function by protein synthesis inhibition[7] . Inactivation of antibiotics by aminoglycoside modifying enzymes (AMEs) encoded by genetic elements is the key mechanism of resistance to aminoglycosides[8]. Aminoglycosides bind to the bacterium's 30s ribosomal subunit and disrupt the translation of RNA, leading to bacterial death. This class of antibiotics is also used to treat infections, such as bacterial endocarditis caused by staphylococci and enterococci, in conjunction with beta-lactams and glycopeptides The three mechanisms of aminoglycoside resistance are changes in the location of the drug's ribosomal binding site, decreased drug permeability, and drug inactivation by enzymes. An significant mechanism of resistance in staphylococcal species is enzymatic inactivation by aminoglycoside-modifying enzymes (AMEs) Based on the modifying effects, these enzymes are categorized into three categories: aminoglycoside acetyl transferases (AACs), aminoglycoside phosphotransferases (APHs) and aminoglycoside nucleotidyltransferases (ANTs) Three enzymes, AAC (6')/APH (2 '), APH (3)-III, and ANT (4), are encoded respectively by the genes aac (6')-Ie/aph (2'), aph (3)-IIIa, and ant (4)-Ia. Among Staphylococcus species, these are the most common modifying enzymes and are clinically important[9]

Materials and Methods

A total of 130 *Staphylococcus aureus* isolates were collected from different clinical specimens in Baghdad/ Iraq during the period from November to December 2019. These specimens were collected from 130 specimens obtained from inpatients suffering from different infections by taking swabs from Burn, wound, nasal, Skin ulcer,Ear or from blood, urine, from patients who attending to different medical centers in Baghdad province.

Table1:-Types and the numbers of clinical samples collected during the present study.

Specimen types	Numbers
Nasal swab	33
Blood	18
Wound swab	25
Urine	15
Burn	35
Otitis media	1
Knee bursitis	1
Skin Ulcer	1
C.S.F	1
Total number	130

Clinical samples were collected from Teaching laboratories of Medical City, Al-Waseti hospital, Ibn-Anafes hospital, Princesses hospital, Peadiatric hospital ect… in Baghdad. Bacteria were cultured on Manitol salt agar and blood agar in aerobic condition at 42 C for 24-48 h. Then identified by conventional biochemical tests and by using of VITEK 2 Automated system using (GP) cards.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed as recommended by the Clinical & Laboratory Standards Institute (CLSIs) using disks containing containing ceftazidime (CAZ:30 µg), ceftriaxone (CRO:30 µg), cefotaxime (CTX:30 µg), gentamicin(GM:10 µg), imipenem (IPM:10 µg), ciprofloxacin (CIP:5 µg), Naldixic acid(NA:30 µg), Tetracycline(T:30 µg) Isolates were considered to be gentamicin resistant when the zone around gentamicin was = 12 mm, intermediate 13-14 mm and sensitive =15 mm,VITEK 2 system using (AST- GP30) was used & the MIC for these antibiotics was obtain.

Results

By using disk diffusion test out of 130 of *Staph.aureus* isolate, 76(58.4%) were resistant to aminoglycosids antibiotics (gentamicin) while 29(22.3%) were resistant to carbapenem antibiotics (imipenem) The MIC of different antibiotics was performed on 26 isolates using (VITEK2AST-GP30) showed that 18(69.2%) were resistant to aminoglycosids antibiotics (gentamicin) while 26(100%) were resistant to carbapenem antibiotics (imipenem)

Disk Diffusion Test (DDT)

The antibiotic disc diffusion test was done using 8 different clinically important antibiotics This study showed that most of *staphylococcus aureus* isolates were highly resistant against the antibiotics used shown in Table2

Table 2: Distribution of resistant isolates against tested antibiotics using Disc diffusion test

Antibiotic discs	No.&% of resistant isolates
Imipenem	29(22.3%)
Ceftriaxone	121(93%)
Cefoxitin	120(92.3%)
Ceftazidime	113(86.9%)
Gentamicin	76(58.4%)
Ciprofloxacin	91(70%)
Tetracycline	107(82.3%)
Naldixic acid	123(94.6%)

The conformational identification of *staphylococcus aureus* was performed using VITEK2 system (VITEK-2 GP Kit). show in table(3)

Table (3). Antibiotic susceptibility of Carbapenem and gentamicin resistant *Staph.aureus* isolates

Isolate	Specimen	MIC (µg/ml) of selected antibiotics determined by VITEK 2 system							
		IMI	GM	T	ER	VA	AZ	RF	F
SAU1	Urine	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAB2	Burn	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAB3	Burn	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAW1	Wound	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAB4	Burn	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAB5	Burn	(R)	≥16 (R)	≤1 (S)	≥8 (R)	2 (S)	(R)	1 (S)	≥16 (S)
SAB6	Burn	(R)	≥16 (R)	≤1 (S)	2 (I)	1 (S)	(R)	1 (S)	32 (S)
SAB7	Burn	(R)	≥16 (R)	≥16 (R)	≥8 (R)	1 (S)	(R)	1 (S)	≥16 (S)
SAU2	Urine	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SABL3	Blood	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAN3	Nasal	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAB8	Burn	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SABL2	Blood	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAN2	Nasal	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAU3	Urine	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAW2	Wound	(R)	≤0. 5 (S)	≤1 (S)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAW3	Wound	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	≥32 (R)	≥16 (S)

Cont... **Table (3). Antibiotic susceptibility of Carbapenem and gentamicin resistant *Staph.aureus* isolates**

SAW4	Wound	(R)	≤0. 5 (S)	≥16 (R)	≥8 (R)	≤ 5 (S)	(R)	≥32 (R)	32 (S)
SAB9	Burn	(R)	≤0. 5 (S)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	1 (S)	≥16 (S)
SAB10	Burn	(R)	≤0. 5 (S)	≤1 (S)	≤0.25 (S)	≥32 (R)	(S)	1 (S)	32 (S)
SAB11	Burn	(R)	≤0. 5 (S)	2 (S)	0.5 (S)	≥32 (R)	(S)	≤0.5 (S)	≥16 (S)
SAU4	Urine	(R)	≥16 (R)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	16 (R)	≥16 (S)
SABL1	Blood	(R)	≤0. 5 (S)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	≥32 (R)	32 (S)
SAN1	Nasal	(R)	≤0. 5 (S)	≥16 (R)	≥8 (R)	≥32 (R)	(R)	≤5 (S)	32 (S)
SAB1	Burn	(R)	(R)	≥16 (R)	4 (R)	≥32 (R)	(R)	≥32 (R)	32 (S)
SABL4	Blood	(R)	≤0. 5 (S)	≤1 (S)	≤0.25 (S)	≥32 (R)	(S)	≤0. 5 (S)	32 (S)

Abbreviation IMI; imipenem;T, tetracycline; ER;erythromycin; GM;gentamicin; AZ;azethromycin; RF; rifampicin; F;nitrtofurantion;VA;vancomycin

Discussion

Staphylococcus aureus has a collection of virulence factors and the potential to develop resistance to most antibiotics, a major human pathogen. The constant appearance of new clones, making Staph, further strengthens this ability. The therapeutic use of methicillin led to the emergence of methicillin-resistant S. aureus, a "superbug." Auroraeus (MRSA) [(10)] . In present study; isolates shows resistance to many β-lactam antibiotics, aminoglycosides and quinolones and carbapenem and cephalosporins. Out of 130 isolates 76(58.4%) were resistant to aminoglycosids antibiotics (gentamicin) while 29(22.3%) were resistant to carbapenem antibiotic (imipenem) by disk diffusion test. The MIC of different antibiotics was performed on multi drug resistant (26) isolates using (VITEK2AST-GP30) showed that 18(69.2%) were resistant to aminoglycosids antibiotics (gentamicin) while 26(100%) were resistant to carbapenem antibiotics (imipenem) The resistance to the potent antibiotics carbapenems and aminoglycosides is increased. Resistance is often acquired from outside sources through horizontal transfer to genes, although chromosomal mutation and selection of antibiotics are also important. The capacity of S. aureus to develop resistance to any antibiotic is remarkable[(11)] In this research, the low resistance of Staph.aureus isolates to several antibiotics may be due to the widespread use of antibiotics, leading to a rise in the number of resistant multidrug species, including MRSA[(12)] . A fundamental biological property of *S. aureus* is the ability to asymptomatically colonize normal people. Approximately 30% of humans are asymptomatic nasal carriers of *Staph. aureus*; i.e., *Staph. aureus* is normal flora. *Staph. aureus* carriers are at higher risk of infection and they are presumed to be an important source of spread of *Staphylococcus aureus* strains among individuals. The primary mode of transmission of *Staphylococcus aureus*

is by direct contact, usually skin-to-skin contact with a colonized or infected individual, although contact with contaminated objects and surfaces or might also play a role. Various host factors, including loss of the normal skin barrier, presence of underlying diseases such as diabetes and acquired immunodeficiency syndrome, or defects in neutrophils function predispose to infection. Infections caused by antibiotic-resistant strains of *S. aureus* have reached epidemic proportions globally. The overall burden of staphylococcal disease, particularly that caused by methicillin *resistant S. aureus* strains (MRSA), is increasing in many countries in both healthcare and community settings[(11)] .

Conclusion

Out of 130 of *Staph.aureus* isolate, 76(58.4%) were resistant to gentamicin, while 29(22.3%) were resistant to imipenem, so The MIC of different antibiotics by using (VITEK2AST-GP30) showed 18(69.2%) were resistant to gentamicin , while 26(100% were resistant to imipenem.

Ethical Clearance: None

Source of Funding: None

Conflict of Interest: None

References

1) Taylor TA, Unakal CG. Staphylococcus Aureus. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK441868/

2) Arumugam Gnanamani, Periasamy Hariharan and Maneesh Paul- Satyaseela .Staphylococcus aureus: Overview of Bacteriology, Clinical Diseases, Epidemiology, Antibiotic Resistance and Therapeutic Approach, Frontiers in Staphylococcus aureus, Shymaa Enany and Laura E. Crotty Alexander, IntechOpen, (2017). DOI: 10.5772/67338.

3) **3-**Sagar A. Cultural and Biochemical characteristics of Staphylococcus aureus October 13, 2017 Microbe Notes

4) Nesme J, Simonet P. The soil resistome: a critical review on antibiotic resistance origins, ecology and dissemination potential in telluric bacteria. *Environ Microbiol*. 2015;17(4):913-930. doi:10.1111/1462-2920.12631

5) https://go.drugbank.com/drugs/DB01598

6) Meletis G. Carbapenem resistance: overview of the problem and future perspectives. *Ther Adv Infect Dis*. 2016;3(1):15-21. doi:10.1177/2049936115621709

7) Krause KM, Serio AW, Kane TR, Connolly LE. Aminoglycosides: An Overview. *Cold Spring Harb Perspect Med*. 2016;6(6):a027029. doi:10.1101/cshperspect.a027029

8) Rahimi F. Characterization of Resistance to Aminoglycosides in Methicillin-Resistant Staphylococcus aureus Strains Isolated From a Tertiary Care Hospital in Tehran, Iran. *Jundishapur J Microbiol*. 2016;9(1):e29237. doi:10.5812/jjm.29237

9) Mahdiyoun SM, Kazemian H, Ahanjan M, Houri H, Goudarzi M. Frequency of Aminoglycoside-Resistance Genes in Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates from Hospitalized Patients. *Jundishapur J Microbiol*. 2016;9(8):e35052. doi:10.5812/jjm.35052

10) Lakhundi S, Zhang K. Methicillin-Resistant Staphylococcus aureus: Molecular Characterization, Evolution, and Epidemiology. Clin Microbiol Rev. 2018 Sep 12;31(4):e00020-18. doi: 10.1128/CMR.00020-18. PMID: 30209034; PMCID: PMC6148192.

11) Chambers HF, Deleo FR. Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nat Rev Microbiol*. 2009;7(9):629-641. doi:10.1038/nrmicro2200.

12) Naimi HM, Rasekh H, Noori AZ, Bahaduri MA. Determination of antimicrobial susceptibility patterns in Staphylococcus aureus strains recovered from patients at two main health facilities in Kabul, Afghanistan. *BMC Infect Dis*. 2017;17(1):737. doi:10.1186/s12879-017-2844-4.