TMP UNIVERSAL JOURNAL OF RESEARCH AND REVIEW ARCHIVES

VOLUME 1 | ISSUE 2 | YEAR 2022 | OCT - DEC 2022

RECEIVED	REVISED	ACCEPTED
15/09/2022	20/10/2022	15/11/2022

Article Type: Research Article

Available online: www.tmp.twistingmemoirs.com

ISSN: N/A

CHARACTERIZATION OF MULTIDRUG RESISTANT STAPHYLOCOCCUSAUREUS ISOLATED FROM VARIOUS CLINICAL SAMPLES.

***[1]Farah B.Abd, [1]Mohsen Hashem Risan, [1]Dhafar N. Al-ugaili**

**[1]Department of Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq*

ABSTRACT

Staphylococcus aureusis a major hospital and community pathogen that is attributed to a wide variety of infections in humans and bio film production is one of the most important virulence factors of S. aureus that contributes to its multiple drug resistance. Therefore, searching for a valuable alternative to the used antibiotics is considered an important goal for study. For this reason one hundred and fifty different clinical samples were collected from various clinical sources and healthcare workers in Al-Imame in Al-Kadhimae in Medical City,Al-Numan Teaching Hospital, Medical City/Teaching laboratories and Central Child Teaching Hospital during the period from1/10/2020to 1/2/2021 in Baghdad City. Isolates were identified by conventional methods (cultural, microscopic and biochemical tests) in addition to the identification by the VITEK® 2Compact, and fifty isolates were recorded as Staphylococcus aureus.

Keywords: Resistant, Staphylococcus, aureus, VITEK® 2, clinical samples.

CORRESPONDING AUTHOR

Name: Farah B.Abd

Affiliation: Department of Biotechnology, College of Biotechnology, Al-Nahrain University, Baghdad, Iraq

Email: m_risan@yahoo.com

INTRODUCTION

The Staphylococci group is considered as one of the most important human pathogenic organisms in the skin, nasal mucosa and oral mucosa; it is recognized as a group of opportunistic pathogens

that is responsible worldwide infections in hospitals. Staphylococci are positive for catalase, which is (considered)a defining feature that distinguishes Staphylococci from Strep to coccus and they were oxidase negative. Susceptibility to lysostaph in is considered to be another feature of Staphylococci because it comprises many residues of glycine in the cross-bridge between peptidogly can layers (Plata etal. 2009).

Staphylococcus aureus(S. aureus) is a Gram positive bacteria non-motile, catalase and coagulase positive, non-spore-forming and as facultative anaerobic, cocci, blue-violet as single, pairs or irregular as grape-like clusters, about (0.4-1.2μm) in diameter, during their growth have a yellow colonies named(aureus; means golden)on nutrient rich media(Yan et al.,2016).Staphylococcus aureus shows high degree of tolerance that enable them to grow under low humidity, high pressure, and high salt concentration (15%NaCl); also, the bacteria can tolerate pH range (4.2-9.3); It could grows at temperature between (15oC-45oC), and multiply to produce toxins that lead to disease development(AlkhafajiandAlsaimary,2020).Staphylococcus aureus is
The main pathogenic bacterium responsible for nosocomial and public acquired infections, often (25-50%) of the human are colonized with S. aureus and is one of the common human pathogenic bacteria that cause diverse infections in both male and female (Suhailiet al.,2018). The bacteria found to localize the mucous membranes, nose, mouth, upper respiratory tracts, intestinal, groin, mammary glands, perinea area in males, hair, and genitourinary of human also its interpreted in the production of abscesses, pus, fatal sepsisandsepsis.

MATERIALS AND METHODS

Sample collection of Staphylococcusaureus

A total of 150 samples were collected from patients (Skin swab, Nasal swab, Ear swab), health care workers (Skin swab and Nasal swab),and operation theater (various places of operation theater before and after sterilization), hospital words. Samples were collected in the period between1/10/ 2020 and 2/1/2021, from several locations in Baghdad; Al-ImameinAl-Kadhimaein Medical City, Al-Numan Teaching Hospital, Medical City/Teaching laboratories and Central Child Teaching Hospital.

Isolation and identification of *Staphylococcusaureus*

The isolate was identified according to the standard laboratory test. S.aureus identified and confirmed by the following: Specimenswere cultured in mannitol salt agar (MSA) and blood agar base (BA), in cubatedat 37°C for 24 hours. All the primary screened isolates were then subjected to various morphological and biochemical tests to ensure their identity. [1-10]

Morphological Examination

Staphylococcusaureus growth on Mannitol Salt Agar (MSA)

Staphylococcus can grow on mannitol salt agar medium with a highs alt concentration (7.5%) NaCl, these media inhibits the growth of other than the *staphylococci* bacteria. Mannitol-fermenting *staphylococci* changes color of MSA from the alka line(red)to the acidic(yellow),while the rest of the Staphylococcus will grow without produce a color change of the medium(Gillet*et al*.,2002).

Blood Hemolysis

Bacterial culture was streaked on blood agar(BA),and the nincubated for 24 h at 37°C. A Green zone appeared around the *S. aureus*colonies, denoting α- hemolysis; while a clear zone indicates β- hemolysis. [11-20]

Microscopic Examinations

All bacterial isolates were subjected to Gram stain to check the irresponsive to the stain,

arrangement and the irshapes (the shape of the bacteria was observed as blue cocci, arranged in grapes like irregular clusters). A small portion of the suspected colony of the positive culture was placed and fixed on a clean microscopic glass slide. Gram staining technique was followed and all slides were examined under oil emersion.

Bio chemical characteristics

Catalase test
From each clinical isolates a single colony was smeared on a slide and drops of (3%) H_2O_2 were added. The appearance of bubbles indicated positive results.

Coagulase test
Two types of the coagulase tests were used to detect the presence of coagulaseenzymein *S.aureus*:

1. Coagulase slide test: Suspended *S. aureus* colony with a saline drop on a clean glass-slide, mixed with a drop of human plasma, the result appears within ten seconds, as a coarse coagulate that can be seen by the naked eye indicates a positive result.
2. Coagulase tube test:Anisolated colony is taken from the petri dish and is solved in 1 ml of diluted plasma, the tube was incubated at 37^0C and achieve d after1-4h,positive results will clot.

Oxidase Test
A few drops of the oxidase reagent (1%N, N, N, N-tetramethylep-phenylenediaminedihydro chloride), were placed on a filter paper, then an isolated bacterial colony was added a paper by wooden stick. The colony's color changes to dark purple within10-15 seconds when the result is positive.

Bacterial Identification using VITEK2 System:

Bacterial isolates identification was carried out by VITEK2 system which is an automated microbiology system employed growth-based technique. From clinical samples, a single colony of bacterial culture was suspended in with 3ml of normal saline. The turbidity was checked to equal (0.5) McFarland via turbidity meter for determining inoculums density of Gram positive bacterial isolates. [21-24]

RESULT

Isolation and Identification of Staphylococcus aureus
One hundred and fifty different clinical samples that were collected from various clinical sources and healthcare workers in Al-Imame in Al-Kadhimae in Medical City, Al-Numan Teaching Hospital, Medical City/Teaching laboratories and Central Child Teaching Hospital. During the period from 1/10/2020 to 1/2/2021 in Baghdad, Iraq. Fifty isolates (33%) were characterized as *S.aureus* depending on the conventional cultural, biochemical and microscopic examination in addition to a confirmatory test by the VITEK® 2Compact system. The rest of the clinical samples which represent (67%) were found to be related to different genus of pathogenic bacteria. That the number and percentage of isolates according to the sources in table (1)

Table:-1. Number of samples according to the sources of collection

Source of Samples	No. of Samples	No. Isolates	Percentage fromisolates (%)
Earswap	21	7	14
Hospitalwards	24	8	16
Noseswap	31	13	26
OperationRoom	20	4	8
HealthCareWorker	31	11	22
skinswap	23	7	14
Total	150	50	100

CULTURAL IDENTIFICATION

All the collected clinical specimens were streaked on MSA media, which is considered a selective and differential medium containing high concentration of sodium chloride (7.5%) to inhibit the growth of other than Staphylococci. The S.aureuson this medium appear edas form yellow golden(Figure1)due to fermenting the mannitol salt changing the phenol red to golden, smooth, raised, mucoid and glistening colonies while S. epidermidis tends to form colonies with pink zones (Carroll et al., 2016). On the other hand; the same specimens were found to produce hemolysis when cultured on blood agar media with smooth colony shape (Gillespie and Hawkey, 2006). Finally, the positively selected isolates were maintaining Ed for further steps in our work using Brain Heart Infusion (BHI) broth and agar medium.

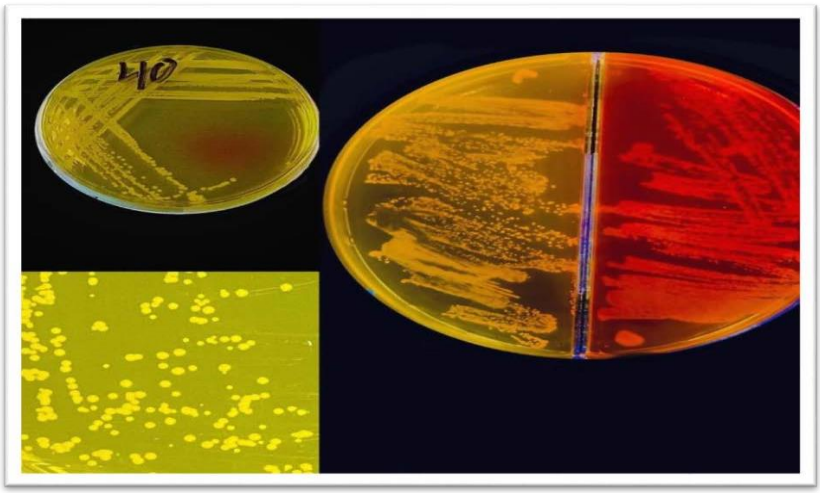

Figure:-1 Growing of *Staphylococcus aureus*colonies on MSA (Mannitol salt agar) after 24 hours of incubationon37°C.

Microscopic Characterization

Microscopic examination showed Gram positive cocci that arranged inpaired or grape like clusters but usually non- spore forming as mentioned by (Carroll etal.,2016).

Biochemical Characterizations

The basic biochemical tests of the all S. aureus isolates were showed a positive reaction for catalase and coagulase tests which confirm the ability of the test ed isolates to synthesize the se enzymes that act as a virulence factors enable the bacteria to develop infection. While negative

results were obtain for oxidase test which refer to the inability of the isolates to produce this enzyme. All the tested bacterial isolates gave positive results for the catalase enzyme through the formation of bubbles (figure 2).which refer to the release of O2from hydrogen peroxide H2O2. Most isolates were able to yield the coagulase enzyme. This enzyme has an important role in S. aureus pathogen city. As it enable the bacteria to form protective barriers of fibrin around themselves, making them highly resistant to phagocytes is and some other anti microbial agents (Aryal, 2018).

S. aureus secreted coagulase enzyme that converted the plasma to clot as shown in figure (2)by effective prothromb into form thromb in which converts fibrinogen to fibrin. S. aureus can produce fibrinolys in, which can lyseclots within4h.These strains would have misdiagnosed as coagulase negative staphylococci if the tube coagulase test had been incubated overnight (Bello and Qahtani, 2005).

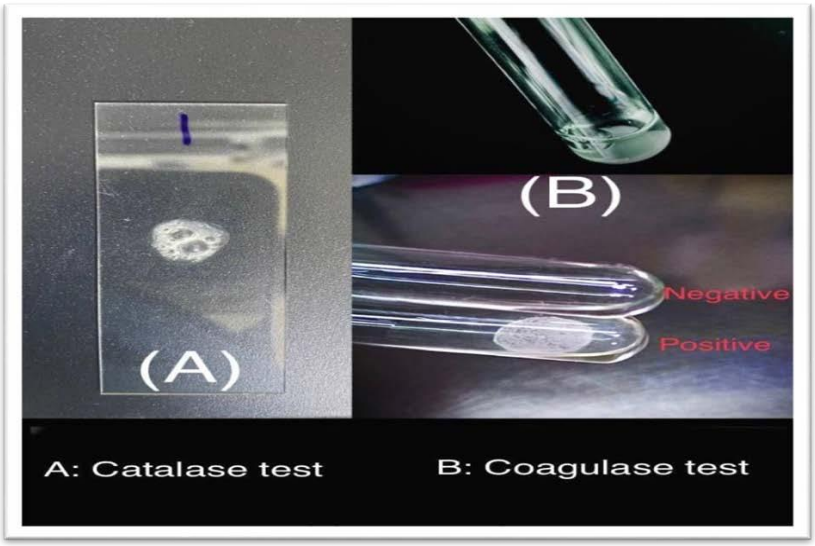

Figure:-2 Detection of *Staphylococcus aureus* by Catalase and coagulase test. Identifying with VITEK2 system.

Further confirmatory identification of the bacteria to the species level was carried out using the vitek2 system that included a number of the biochemical test in addition to the antibiotic susceptibility profile that specific for each bacterial species therefore it can give information to the species level.

Detection of Hemolysin in *Staphylococcus* isolates

A total of 49 out from the 50 studied strains (90%) showed haemolysis on blood agar plates, *S. aureus*usually displays a golden yellow pigment as shown in the Figure(3). 42 of the 50 strains (84%) had beta hemolytic phenol type, while five(10%)of the isolates showed alphahaemolytic with dark and greenish color. Only three isolates (6%) were recorded as negative results as it didn't show any hemolysis, table (2).

This result came in accordance with AL-Khzarji (2020) who reported *S.aureus* haemolysis on blood agarplate, 14 %(5/36)were alpha hemolysis and 86%(31/36)gave beta haemolysis. On the other hand Almwafy (2020) showed that (30.95%)of *S.aureus* isolates were alpha haemolysis while (30.95%) had the ability to make beta hemolysis and 38.09% posse the capacity to make gammahemolysis.While,Jahan*etal*.(2015)reportedapositiveβ-hemolyicactivityforall the tested isolates.

Table:-2. Haemolysis of *Staphylococcusaureus* isolates on blood agar

Hemolysis type	Number(No.)	Percentage (%)
Beta Hemolysis	42	84
Alpha Hemolysis	5	10
Gamma Hemolysis	3	6

Figure:-3 Hemolysis is by *Staphylococcus aureus* on blood agar medium

Hemolysins, that cause damage to the red blood cell membrane, is one of the main virulence factors produce by *S. aureus* and play an important role in their pathogenesis as it participates in the bio film (Al Laham*et al*., 2015). And causes β-hemolytic (complete hemolytic); in addition a number of *S. aureus* strains produceα-hemolysin (*S.aureus* in complete hemolytic phenotype)(SIHP) strain have been related with nosocomial infection (Zhang *et al*., 2016).In(2016)denReijer and h is colleagues noticed four toxins(alpha-toxin, gamma-Hemolysin B and leukocidins D and E) in all bio films forming strains. The detection of *S. aureus* toxins, notably alpha toxin, in bio films is well established roles in skin infections (Kobayashi *et al*., 2015). Alpha-toxin causes pore-forming (cytolytic) including skin tissue, obstructs the innate and adaptive immune responses, in addition, it is necessary for bio film growth on mucosal surfaces (Anderson *etal*, 2012).

REFERENCES

1. Alfatemi, S.M.H., Motamedifar, M., Hadi, N. and Saraie, H.S.E. (2014).Analysis of virulence genes among methicillin resistant *Staphylococcusaureus*(MRSA)strains. Jundishapur Journal of Microbiology, 7(6):e10741.
2. Alkhafaji, B.A. and Alsaimary, I.E.(2020).Comparative Molecular Analysis of Mecca, Sea and Seb Genes in Methicillin-Resistant *Staphylococcus aureus* (MRSA).Journal of Clinical & Biomedical Research,2(3):1-8.
3. AL-Khzarji, N. Z. R. (2020). Detection of Bio film formation and qacgenes in Staphy lococcusspp and inhibition of efflux pump using Ananas comosus extract.Thesis,University of Baghdad,IRAQ.
4. Al Laham NA. Species identification of clinical coagulase-negative staphylococci isolated in Al-Shifa hospital Gaza using matrix-assisted laser desorption/ionization-time of flight

mass spectrometry. *Curr Res Bacteriol.* 2017;10:1–8.
5. Almwafy, A. (2020). Preliminary Characterization and Identification of Gram Positive Hemolysis Bacteria. Al-Azhar Journal of PharmaceuticalSciences, 62(2):96-109.
6. Anderson MJ, Lin YC, Gillman AN, Parks PJ, Schlievert PM, Peterson ML (2012) Alpha-toxin promotes Staphylococcus aureus mucosal bio film formation. *Front Cell Infect Microbiol* 2: 64.
7. Aryal,S.(2018).CatalaseTest-Principles,Uses,Procedure,Result Interpretation with precautions.
8. Bello, C. S. S., and Qahtani, A.(2005). Pitfalls in the routine diagnosis of *Staphylococcus aureus*. African Journal of Biotechnology, 4(1): 83-86.
9. Brown,A.E.;andSmith,H.(2014)Benson'smicrobiologicalapplications:laboratorymanualing eneralmicrobiology.14th.McGrawHillEducation.
10. Carroll, K. C., Morse, S. A., Mietzner, T. and Miller, S. J. (2016).Melnick and Adelberg's Medical Microbiology: (27th ed.). McGraw-HillEducation.US.pp.127,146.
11. den Reijer, P. M., Haisma, E. M., Lemmens-den Toom, N. A., Willemse, J., Koning, R. A., Demmers, J. A., et al. (2016). Detection of alpha-toxin and other virulence factors in biofilms of *Staphylococcus aureus* on polystyrene and a human epidermal model. *PLoS ONE* 11:e0145722. doi: 10.1371/journal.pone.0145722
12. Gao,M.;Sang,R.;Wang,G.andXu,Y.(2019).Association of pvlgene with incomplete hemolytic phenotype in clinical *Staphylococcusaureus*.Infection And DrugResistance,12:1649.
13. Gillespie S and. Hawkey. P. M (2006). Principles and Practice of Clinical Bacteriology. Gillet, Y., Issartel, B., Vanhems, P., Fournet, J. C., Lina, G., Bes, M.,Etienne, J. (2002). Association between *Staphylococcus aureus*strainscarryinggeneforPanton-Valentine leukocidins and highly lethalnecrotising pneumonia in young immuno competent patients.Lancet,359(9308):753–759.
14. Hata, D. J. and Thomson, R. B. (2017). Gram Stain Benchtop Reference Guide:AnIllustrated Guide to Microorganisms and Pathology Encountered in Gramstained Smear.John &WileySons,NY,USA.
15. Jahan, M., Rahman, M., Parvej, M. S., Chowdhury, S. M., Haque, M. E., Talukder, M. A. and Ahmed, S. (2015). Isolation and characterization of *Staphylococcus aureus* from raw cow milk in Bangladesh, Journal of Advanced Veterinary and Animal Research, 2(1): 49-55.
16. Karmakar,A.,Dua,P.,and Ghosh,C.(2016).Biochemical and Molecular Analysis of *Staphylococcusaureus* Clinical Isolates from Hospitalized Patients. The Canadian Journal of Infectious Diseases and Medical Microbiology, 2016: 9041636.
17. Kobayashi,S.D.,Malachowa,N.,andDeLeo,F.R.(2015).PathogenesisofStaphylococcusaureusabscesses.TheAmericanJournalofPathology,185(6):1518–1527.
18. Plata,K.,Rosato,A.E.,andWegrzyn,G.(2009).*Staphylococcusaureus*as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. Acta Biochimica Polonica, 56(4), 597–612.
19. Prescott, L. M., Harley, J. P., and Klein, A. (2007). Microbiology of fresh water.Mc Graw Hill, New-York,(56): 669–682.
20. Suhaili, Z., Rafee, P. '.MatAzis, N.,Yeo, C. C., Nord in, S. A., AbdulRahim,A.R.,Al-Obaidi,M.,andMohdDesa,M.N.(2018).Characterizationofresistancetoselectedantibioticsand Panton-Valentine leukocid in-positive *Staphylococcusaureus* in a healthy student population ata Malaysian University.Germs,8(1): 21–30.
21. Tong, S.Y., Holden, M.T., Nickerson, E.K., Cooper, B.S., Köser, C.U.,Cori, A., Jombart, T., Cauchemez, S., Fraser, C., Wuthiekanun, V. and Thai padungpanit, J. (2015). Genome sequencing defines phylogeny and spread of methicillin-resistant *Staphylococcusaureus* in a high transmission setting.GenomeResearch,25(1):111-118.
22. Zhang H, Zheng Y, Gao H, et al. Identification and characterization of Staphylococcus aureus strains with an incomplete hemolytic phenotype. *Front Cell Infect Microbiol*. 2016;6:146.
23. Wijesundara, W. M. D. A., Rajapakse, R. G. S. C., Jayatilake, J. A. M.A., &Jayatilake, J.

A. M. S. (2019). A preliminary study of mecA gene expression and methicillin resistance in staphylococci isolated from thehuman oral cavity. Sri Lankan Journal of Infectious Diseases, 9(1): 42–48.

24. Yan,X.,Li,Z.,Chlebowicz,M.A.,Tao,X.,Ni,M.,Hu,Y.,Li, Z.,Grundmann,H.,Murray,S.,Pascoe,B.andSheppard,S.K.(2016).Genetic features of livestock-associated *Staphylococcusaureus* ST9 isolates from Chinese pigs that carry the lsa(E) gene for quinupristin/dalfoprist in resistance.International Journal of Medical Microbiology,306(8): 722-729.