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ORIGINAL ARTICLE

Molecular Detection of Hospital-Acquired and Community-Acquired Staphylococcus Aureus by PCR Amplification of NUC Gene

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ABSTRACT

A total of 44 Staphylococcus aureus isolates have been reactivated and diagnosed by using bacteriological and biochemical methods and confirmed by VITEK 2 compact system. The specimens were obtained from several causes included vagina, urine, nose wounds and burns. The results of virulence factors investigation showed that 70.5% of S. aureus isolates were beta hemolytic, 56.8% have Dnase activity, 45.5% were gelatinase positive and 91% were biofilm producer. The results showed a great prevalence of MDR strains of S. aureus with significant p value (<0.001) which were 77.2% while the XDR, PDR and sensitive isolates were 14.6%, 2.2% and 6.8% respectively. Hospital acquired infections caused by S. aureus isolates were the majority in the current study which was 72.72% while the community acquired infections were 27.27% with a significant p value (<0.05). Genetic detection results showed mecA was found in 19 of 24 isolates, whereas the nuc gene was found in 20 of 24 isolates.

Keywords: S. aureus, MRSA, nuc, mecA.

INTRODUCTION

The bacteria Staphylococcus aureus can be discovered on the body's skin and upper respiratory tract. Despite being a normal flora, S. aureus has developed into an opportunistic pathogen that leads to a variety of illnesses that seem to be challenging to treat because to the existence of virulence genes (Ahmed and Ahmed, 2022). S. aureus ranks among the most prevalent widespread causes of illness and death owing to an infectious pathogen. This bacterium may lead to a diverse range of illnesses, from minor skin lesions to potentially fatal pneumonia and sepsis, there has recently been a steady and rising attention in the exceptionally large quantity of toxins and also other virulence agents generated by S. aureus, as well as the way they impact illness. (Cheung et al., 2021). Methicillin-resistant MRSA (Methicillin-resistant Staphylococcus aureus) recently developed as a prevalent cause of communal-based or healthcare infections. MRSA now causes 10 fold the amount of illnesses produced by all MDR Gram-negative pathogens collectively. MRSA was been recognized as (1) of (12) urgent illnesses posing a risk to human health by the World Health Organization (WHO) (Craft et al., 2019). S. aureus consider among the most frequent cause of hospital - acquired infections, and hospital workers, as transporters of S. aureus, play a significant role in its spreading through patients. (Karimzadeh and Ghassab, 2022). The S. aureus contains many potential virulence factors including factors that inhibit phagocytosis, such as the capsule and immunoglobulin binding protein A, other virulence agents comprise exterior proteins that encourage host tissue colonization and toxins that can induce host tissue destruction and so generate illness indications. (Hansen, 2019). S. aureus has a variety of virulence factors. They are classified as bacterial cell components implicated in illness and substances discharged into environment by the bacteria. Capsules, protein A, and teichoic acid are bacterial cell elements implicated in pathogenicity. Exo-proteins: nucleases, hyaluronidase, lipases, proteases, and collagenase, as well as exotoxins:.-hemolysis activity , production of leucocidin, and Panton Valentine leucocidin which practically all strains of S. aureus can be a producer for them (Bien et al., 2011). The detection of the mecA gene by using PCR technique is currently regarded the global method for identifying methicillin resistance in S. aureus. (Pillai et al., 2012), Methicillin resistance is generated in the Staphylococcus cassette chromosomal genomic zone by the mecA gene, which originates in Staphylococcus chromosomes. (Gittens-St et al., 2020).

Keywords: S. aureus, Dnase, nuc, mecA, Gelatinase.

MATERIALS AND METHODS

Sample collection: Specimens were obtained from patients hospitalized to Baqubaa teaching hospital with a variety of clinical conditions (wounds, burns, nose, urine, and vaginal swabs). All specimens were cultured on blood agar for hemolysis and mannitol salt agar for mannose fermentation, bio-chemical checks such as Gram stain, catalase check and coagulase check, were utilized to identify the clinical specimens (Jead and Mohammed, 2020).

Detection of virulence factors:

Hemolysis of blood: Inoculate blood agar with pure bacterial culture and incubated in 37 C to 24 hours, the emergence of transparent regions around bacterial colonies developing indicates the susceptibility of bacteria to secretion hemolysin (Dulczak and Kirk, 2005).

Gelatinase production: It was conducted to assess capability of the bacteria to yield gelatinases. Gelatin liquefaction medium was inoculated with colonies of S. aureus and 24 hours at 37°C incubation. The culture was kept in the freezer at 4°C for 30 minutes after incubation. Gelatinase is produced by cultures that remain liquefied and show the hydrolysis of gelatin was referred to as a positive result (Cappuccino and Welsh, 2020).

Dnase activity: Dnase test agar with toluidine blue is used to detect deoxy-ribonuclease activity of bacteria and fungi and particularly for identification of Staphylococci, the Dnase activity resulting in the formation of a clear zone around the S. aureus growth (Himedia).

Biofilm formation: A microtiter plate test was employed to check for biofilm growth, according to the procedure. (Almeida et al., 2013). The micro-organisms were implanted on a nutrient broth media at 37°C for 24 h. Next that, 200 µl of micro-organism had been suspended in every 3 wells of a ninety six well of flat-bottom poly-styrene plate and cultured for 24 hours at 37°C by means of the same media as the diluent. Each well was then cleansed by means of distilled water for 3 whiles and forcefully shook previously being completely dehydrated. Fixation of the adherent bacterial cell was conducted by adding of 200 µl of absolute methanol. Then, for 15 min, each well was stained with 200 µl of 0.5% crystal violet. Based on Tang et al., (2011), the quantity of crystal violet eradicated by 95% ethanol in every well and it was calculated by assessing the OD 630 nm by means of an ELISA reader.

Genetic detection

Primers and their sequencing: Table 1 showed the primer sequencing utilized for detecting. The Macrogen Company provided the primers which were lyophilized and suspended in nuclease-free water to a last volume of 100pmol/l as a stock solution. To produce a 10 pmol/µl working primer solution, blend 10 µl of primer standard solution (kept at -20 C) with 90 µl of nuclease-free water.

DNA Extraction: The DNA of the bacterial isolates was obtained utilizing ABIO, pure extraction methodology, DNA was extracted as mentioned by Jasim and Alzubaidy in (2022).

Table 1: primers of coa and mecA genes

Primer	Seq.	Annealing Temp. (°C)	Product Size (bp)
nuc-F	5'CCTGAAGCAAGTGCATTTA CGA-3'	56	166
nuc-R	5'CTTTAGCCAAGCCTTGACG AACT-3'		
mecA-F	5'-TGGCTATCGTGTCACAATCG-3'		310
mecA-R	5'-CTGGAACTTGTTGAGCAGAG-3'		

(Jasim and Alzubaidy, 2022), (Niu et al., 2018)

Estimation of DNA: A Quantus Fluorimeter was used to measure the amount of extracted DNA in order to assess the integrity of extraction for downstream applications.

Agarose Gel Electro-phoresis and DNA Packing: The agarose was prepared according to the manufactured company. The agarose was poured into the gel box and was left to set at room temperature for a half an hour. The gel was placed in the gel box after gently discarding the comb. 1^X of TAE electro-phoresis buffer was loaded into the box till it got 3-5 mm above the edge of the gel. Two micro-liters of loading dye were inserted carefully in separate wells of each 5 µl DNA sample. The PCR yields were loaded immediately. Each well received 5 µl of PCR product immediately. For sixty minutes, electrical energy was switched on at 100v/mAmp.

Statistical analyses: Medcalc program was used. P-values of below than 0.05 were deemed statistically significant. The following is the P-value: 0.01 is the P-value.

RESULTS

Forty four S. aureus isolates were reactivated and diagnosed which were obtained from diiferent clinical sources: vagina, wounds, burns, urine and nasal which were 50 specimens for each source. The specimens were collected from Baaquba Teaching Hospital in the province of Diyala during the period from October 2021 until February 2022. The result of the current study showed that the positive growth for S. aureus was 17.6%. The specimens were cultured on blood agar at first; isolates were primary identified as S. aureus. All isolates were diagnosed by morphological features on blood agar, Gram stain, biochemical test, mannitol salt agar to detect mannitol fermentation which consider a specific feature for S. aureus and finally confirmed by VITEK 2 system. The percentage of S. aureus isolates were distributed as 5 (10%) isolates from vagina, 13 (26%) isolates from wounds, 12 (24%) isolates from burns, 2 (4%) isolates from urine and 12 (24%) isolates from nose as showed in figure 1.

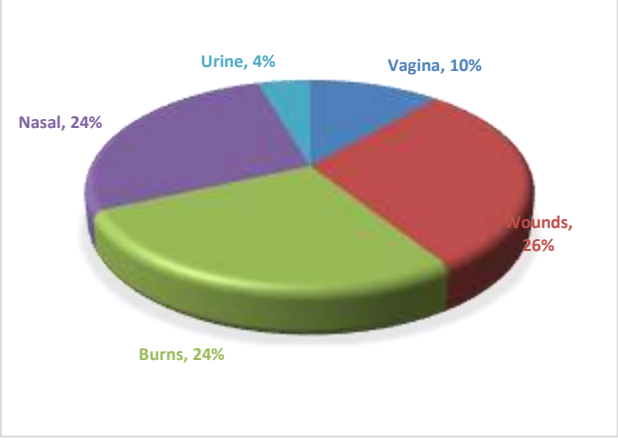

Figure 1: Percentage of Staphylococcus aureus

The outcomes of the present study was in agreement with El-Tawab et al. in 2022, who reported that the frequency of S. aureus among different clinical sources was 11.5%. The current finding indicate a low incidence of S. aureus in urine and it was disagree with Ibisanmi et al., in 2022 who reported that S. aureus was most frequently found in urine (32%). Our outcomes were analogous to the work conducted by Shahi, et al. in 2018 who has an incidence of 14.4%. S. aureus is a natural skin flora that can be transmitted through fissures, abrasions, wounds, medical surgeries, burns, and parenteral catheters, causing pyogenic illnesses. (Khanal et al., 2018).

MRSA nosocomial infections pose a significant concern to burn sufferers. S. aureus is a common pathogen across the world and one of the most common causes of nosocomial infections in high-risk units like the burn intensive care unit (ICU). Because of the excessive use of broad-spectrum antibiotics throughout the years, methicillin resistant S. aureus (MRSA) has become one of the most common bacteria in burn units, causing invasive infections in burn patients worldwide, with infection rates of more than 50% recorded. (Norbury et al., 2016). With observed MRSA epidemics across many hospitals, which ultimately led in risks such as pneumonia, sepsis, and bacteremia in burn patients, burn ICU has evolved into a main reservoir for MRSA in the healthcare setting. Burn patients are much more exposed to bacterial infestation because of physical deprivation of the barrier function of the skin and decrease in cell-mediated immunity. (Khan et al., 2018).

The findings confirmed that 44 (100%) of the strains were (CoPS), as determined by the tube or slide technique. S. aureus can cause serious infections and must be distinguished from opportunistic coagulase negative staphylococci. The coagulase test distinguishes S. aureus from those other staphylococci. Furthermore, not even all S. aureus are coagulase producer, and not all coagulase + staphylococci are S. aureus. Other assays, in addition to the coagulase test, should be done to enhance the diagnosis of S. aureus (UK Standards for Microbiology Investigations, 2022).

In mannitol salt agar medium, all isolates caused fermentation of mannitol and changed the color of colonies to yellow as a result of acids generation that give to reducing the medium's pH and transforming the color of phenol red to yellow, S. aureus might ferment the mannose and generate yellow zones in the reddish agar., this test differentiates between S. aureus and S. epidermidis, which has the capability to procedure colonies with red zones on mannitol salt agar (Bobai et al., 2022).

Detection of virulence factors: Some virulence factors were conducted for S. aureus strains in the present work which are discussed below

Hemolysin: The result of the present work displayed that 70.5% of S. aureus isolates have the ability to produce Beta hemolytic activity on blood agar with significant difference (< 0.01) while 29.5% of S. aureus produced gamma hemolysis (Table 2).

Table 2: Virulence factors

Virulence factor	No. of positive	percentage	p Value
Hemolysin	31	70.5%	0.007
Dnase	25	56.8%	0.366
Gelatinase	20	45.5%	0.546

This result was in agreement with a local study in Diyala by Jasim and Alzubaidy in (2022) and in the province of Baghdad by Al Ani and Al Meani (2018) who reported that 82% and 65.6% of S. aureus give beta hemolysis respectively, while it wasn't compatible with Al-Taey in (2021) who reported that 100% of S. aureus isolated exhibit beta hemolysis.

S. aureus may produce a wide range of protein toxins, which are most likely to blame for the signs that arise during an infection. Several of these proteins generate erythrocyte membrane destruction, resulting in hemolysis. (Bennett et al., 2015; Hansen, 2019). S. aureus is known of its capability to produce four

decomposition types (alpha, beta, delta and gamma) which have different antigenic and chemical effect on red blood cells (Quinn et al., 2011).

Dnase production: The result of the present work displayed that 56.8% of S. aureus strains have the capability to produce Dnase while 43.2% didn't exhibit any Dnase activity with no significant difference (Table 2), this result was in agreement with the outcomes of Khwen et al (2021) who exhibited that (66%) of strains were Dnase producer (Figure 2).

Figure 2: Dnase activity on Dnase test agar with toluidine blue

Gelatinase production: The outcomes of the present work displayed that 45.4% of S. aureus strains have the capability to exhibit gelatinase activity by hydrolyzing gelatin while 54.5% didn't exhibit gelatinase activity with any significant difference (Table 4-3), this result was compatible with Jasim and Alzubaidy, in 2022 who found that 42% of S. aureus isolates produced gelatinase and disagree with Salman and Ali (2017) in province of Diyala who stated a great capability of S. aureus (100%) to create this enzyme.

A connective tissue protein is vital in pathogenicity since it permits bacteria to metabolize gelatin and consume the resulting small peptides for energy. Gelatinase capability can improve their virulence and pathogenicity, particularly in immune-compromised people (Iseppi et al., 2020).

Biofilm formation: Biofilm formation was conducted for the 44 isolates of S. aureus by mico-titer plate method (MTP), the MTP method is a technique used for studying primary bio-film development on abiotic surface and it is a colori-metric approach that use pigments such as crystal violet to label adherent bio-films and estimate them by absorbance measurements by micro-titer plate reader (De Jesus and Dedeles, 2020). Based on the OD of the control, S. aureus strains were having different ability to produce biofilm with significant difference (<0.001). The result of the present work showed that only 9% of S. aureus isolates were non biofilm producer while 91% have the ability to produce biofilm which 61% were moderate biofilm producer and 30% were strong biofilm producer (Table 3).

Table 3: Biofilm formation by Staphylococcus aureus

Biofilm	No. of positive	percentage	p Value
Non-producer	4	9%	< 0.001
Moderate producer	27	61%	
Strong producer	13	30%	

The outcomes of the present work was disagree with Samadi et al. (2018) who found that S. aureus isolates were (strong, 1%), (moderate, 8.2%) and (weak, 54.1%), while (36.7%) of them had no capability to attach, it was also disagree with Sahm (2019) and Yu et al. (2020) who found that 100% of S. aureus isolates have the ability to yield bio-film.

The alterations in bio-film concentration among the strains in the present work perhaps due to numerous reasons. Differences in the capability of isolates to form biofilms or perhaps differences in the first number of cells that effectively adhere to and dissimilarities in the value and amount of quorum-sensing signaling molecules produced from each isolate play important roles (Abdulammer, 2018).

The precision of the microtiter plate approach to quantify the small amounts generated may account for the rapid throughput of biofilm production. Which is more precise, simple, and sensitive in detecting biofilm development. When studying the early stages of biofilm formation, the microtiter plate method can be used because it uses stable conditions and can be used to study many factors necessary for biofilm formation, such as flagellum, pili, and genes that play an important role in the production of exo-polysaccharides (Obaid, 2019).

Multi-drug resistance Hospital acquired and Community acquired S. aureus: High prevalence of multi-drug resistance (MDR, XDR and PDR) isolated were observed in the current study by 93.18%, the MDR isolates were the highest in all sources and it was 77.2% overall while the XDR, PDR and sensitive isolates were 14.6%, 2.2% and 6.8% respectively. These results were closely related to the outcomes of Jasim and Alzubaidy in (2022) who reported that 100% of S. aureus were categorized as multi drug resistance, they also indicated that the incidence of MDR isolates were 82%, XDR isolates 14% and PDR 4%. The outcomes of the present work displayed that the majority of strains were belonging to hospital acquired 72.72% while the community acquired infections caused by S. aureus were 27.27%. Multidrug resistance is a serious public health problem. It may be connected to antimicrobial medications and is recognized as among the most important worldwide public health risks of the twenty-first century. Furthermore, as a result of therapeutic failure and its prevalence in healthcare expenses, this issue has raised both mortality and morbidity. Antibiotics used in human therapeutics in significant quantities led in the selection of harmful microorganisms resistant to several medications (Catalano et al., 2022).

Genetic detection of mecA and nuc genes: A total of 24 isolates were selected to conduct gene detection for mecA gene and nuc gene. 12 isolates were selected as community acquired S. aureus and 12 isolates as hospital acquired S. aureus, the isolates were selected according to resistance classification which were PDR, XDR and MDR. Genetic detection results showed mecA was found in 19 of 24 isolates, whereas the nuc gene was found in 20 of 24 isolates.

PCR detection of the mecA gene is currently regarded the global technique for identifying MRSA (Pillai et al., 2012), mecA gene is originating in Staphylococcus chromosomes and influences S. aureus pathogenicity by generating methicillin resistance in the Staphylococcus cassette chromosome genomic zone (Gittens-St et al., 2020). PBP2a is a mobile extrinsic genetic element transported on a genomic island, is coded by the mecA gene. (Alkharsah et al., 2018). As it blocks the active location from binding B-lactams, PBP-2a has a lower affinity for β-lactams than the usual penicillin-binding protein-2 (PBP2) formed by MSSA (Hussain, et al., 2019). Additional CA-MRSA strains comprise the staphylococcal cassette chromosome mec (SCCmec) element type IV or V, conferring resistance to β-lactam antibiotics (Liao et al., 2021).

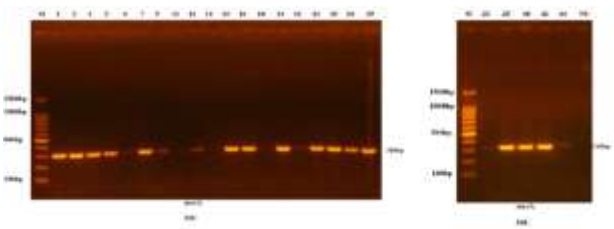

Figure 3: Outcomes of the magnification of mecA primers in Staphylococcus aureus specimens fractionated on 1.5% agarose gel electro-phoresis marked with Eth.Br. M: 100bp ladder marker, NC: negative control.

The differences in mecA gene prevalence among Iraqi regions, or even worldwide, may be attributed to the variation of

geographical distribution, sources of isolates and types and accuracy of techniques used. Variability may also be attributed to healthcare facility factors such as the implementation and oversight of infection prevention programs and the reason for antimicrobial use, which differs between institutions. It is fair to speculate that the group under research may have been affected by similar agent strains at the same area and time.

The result showed that (20 of 24) 83.3% of isolates were having the nuc gene (Figure 4), this result was in agreement with Avila-Novoa et al. in 2018 who reported that 83.3% of S. aureus isolates were having the nuc gene, Karimzadeh and Ghassab in 2022 reported in their article that 100% of S. aureus isolates were positive for nuc gene and disagree with Javid et al. (2018) who reported that 34.2% of S. aureus have nuc gene. According to multiple research, 15%-80% of S. aureus isolates from a variety of sources can generate enterotoxin, the nuc gene acts as an indicator, and the existence of the heat resistant nuclease gene (nuc) is firmly linked to the generation of enterotoxin and can be considered an evidence of infection with enterotoxin producer S. aureus (Lowy, 1998; Brakstad et al., 1992; Karimzadeh and Ghassab, 2022).

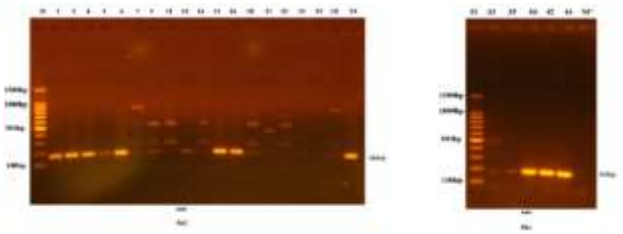

Figure **Error! No text of specified style in document.**: Outcomes of the magnification of nuc primers in Staphylococcus aureus specimens fractionated on 1.5% agarose gel electro-phoresis marked with Eth.Br. M: 100bp ladder marker, NC: negative control.

CONCLUSION

The frequent occurrence of the nuc gene, those codes for poisons, can serve as a warning sign as well as a major hazard to public health. As a result, the foci of risk may be discovered rapidly using the PCR approach, and any infection and contamination can be avoided. It is critical to keep such bacteria under control. It is critical for every society's health-care system to appropriately detect essential and common diseases in the hospital.

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