Detection of virulence factors for methicillin-resistant Staphylococcus aureus isolated from medical staff in Samarra General Hospital

Faten Latif Moza
Dr. Bashar Sadiq Numee

Abstract---S. aureus bacteria is one of the most important types of resistance to antibiotics, including beta-lactam antibiotics, which cause infections acquired in hospitals, which is one of the biggest continuing medical problems and challenges in the world and due to the lack of accurate information and previous studies on Methicillin-resistance Staphylococcus aureus (MRSA) and its true prevalence rates in institutions. The study was conducted on Staphaureus bacteria in (Samarra General Hospital) for the time period from the beginning of the month of November to the end of January. Obtaining (22) isolates out of (100) by phenotypic method. The study showed that the highest prevalence of Staphaureus bacteria was within the laboratory staff and constituted (30%), followed by the nursing staff (10%), then the medical staff (20%), followed by the pharmacy staff at (0%). The study showed that for the age group (21-29) year by (72%), for the age group (30-40) it was (18%), and for the age group over (40 years) it was (9%). The study also showed that the percentage of its presence in males, as it was in females (36%), while the percentage of its presence in males was (63%), and the percentage of Staph.aureus bacteria was higher among those who had repeated seasonal allergies and fever (36%), (36%), followed by those who have a recurrence of tonsillitis (18%), then those who have a recurrence of sinusitis (9%). The study also showed that the percentage of its presence among the health staff according to years of service was from (1-5) years at a rate of (45%), and from (6-10) years it was (27%), and from (11-15) years it was (18%), and more than (16) years (9%). The study also showed that the highest percentage of resistance to bacteria S. Aureus were for the antibiotic Ampicillin, Doxycyclin, Azthromycin and Methicillin, and there was no resistance to the antibiotic Vancomycin, and the percentages were as follows (100%), (100%), (77%) and (50%). While it showed sensitivity to Ciprofloxin (95.4%), Gentamycin (86%), Vancomycin (45.4%), Methicillin (50%) and Azthromycin (22.7%), while it was sensitive to Doxycillin (0%) and Ampicillin (0%). %), the study also showed that the percentage of the number of isolates that showed virulence factors was as follows: The virulence factor was
Lipase (100%), Protease (100%), Urease (100%), Dnase (85.7%) and hemolycin (88.7%).

**Keywords**— virulence, medical staff, methicillin-resistant.

**Introduction**

The World Health Organization (WHO) has recognized that Respiratory System Infection occupies 6% of the total global burden of disease, so this percentage is considered high compared to the rates of Diarrhoea, Malaria and Cancer. Respiratory System Infections cause Respiratory System Infection. With more than 12 million children hospitalized annually (Tazinya et al., 2018), and despite the fever provided by the nasopharynx through mucociliary filtration and immune cells, some invasive pathogens such as Viruses and Bacteria may be able to destroy these defense mechanisms, therefore, work to cause injury, which later develops into serious and fatal injuries when such pathogens reach the lower respiratory tract. In addition, genetic diseases and congenital malformations in children are an important reason for causing Pneumonia infections.

**A brief overview of Staphylococcus aureus**

They are gram-positive spherical shapes arranged in clusters and characterized when they grow on the middle of the culture medium by producing dyes in various colors such as white such as Staphylococcus epidermidis to light yellow to orange S. aureus possesses plasmids, cassette genes, jumpers, and extrachromosomal DNA sequences, which are additional transmissible genetic elements that have a role in genetic evolution (Kuroda et al., 2001).

**S. aureas virulence factors:**

A – virulence factors in the wall of staphylococcus:

**Protein A:**

It is one of the most important components that enter into the composition of the cell wall of S. aureas, as its function is to inhibit the phagocytosis process through the immunoglobulin IgG binding to the FC region, which leads to the removal of the effector function. Aureus, so protein A was recently proposed as a vaccine antigen for it (Brignoli et al., 2019)).

**Capsule:**

It is one of the most important layers that surround bacteria, which is Capsular Polysaccharides, and it has 11 types of capsule that have been identified, and there are strains that have type (CP5)5 or (CP8). (Visansirkul et al., 2020)

**Fibronectin-binding protein**

Adhesion is considered as a first step for the formation of cell membranes that invade the host cells, which enables the germ to escape and cause infection.
**Toxins of Staphylococcus aureus**

*S.aureus* has the ability to produce many toxins that help it cause infection (Oliveire et al., 2018). These toxins were divided into three types (Kim, 2019).

Super antigens (Sag)-1: This is a powerful immune-stimulating exotoxin that causes toxic shock syndrome (TSS) or staphylococcal food poisoning (SFP).

**Exfoliative** toxins-2: They break down and degrade the skin, causing Staphylococcal scalded skin syndrome (SSS).

**Cytotoxins**-3: They degrade aureus cells on the surface proteins (the fibronectin-binding protein (Joss et al., 2017).)

**Clumping factor**-4: This *S.aureas* surfactant binds to the fibrinogen protein via poorly understood interactions, promoting infection (Herman – Bausier et al., 2018)).

**Leukocidin**-5

It is a toxin that destroys polymorph nuclear leukocidin cells, inhibiting phagocytosis, and degrades these cells through holes in the plasma membrane (Gordon and Lowy, 2008), causing cytoplasmic granules to break out, causing a condition called degranulation (Francis et al., 2005).

**Hemolytic toxins**

It has importance in increasing the pathogenicity of *S.aureus* bacteria by analyzing red blood cells (erythrocytes) and destroying platelets, and it represents three important types of exotoxins (Jawetz et al., 2019).

**Resistance of S.aureus to beta-lactam antibiotics**

**Resistance Staphylococcus aureus to β – Lactams**

These antibiotics from the group of β-lactams, penicillins and cephalosporins, inhibit the final stage in the synthesis of the peptidoglycan layer in the cell wall, which consists of two amino sugar units (NAG) N-acetylglucos and N-acetylmuramic acid (NAM) (Le Minor and Veron, 1989)

Connected to these two units are four short peptide chains, where cross-linking occurs between these chains by new peptide bridges with the help of Penicillin Binding Proteins, to form a cohesive and solid layer that protects bacteria from external osmotic stresses) 1991 (Tortora et al. ,

**S. aureus bacteria resistance to the antibiotic Methicillin:**

Several studies have confirmed that the main factor in the resistance of *S.aureus* bacteria to the antibiotic methicillin and other types of antibiotics is due to its possession of the mec A gene. This gene originates on a large mobile genetic element on the chromosome. Genetic mobile element called Staphylo coccus-chromosomal cassette mec (Scc mec) (Weese et al. At least eight types of this gene have been identified and named as Type 1, Type 2) and so on until we get Type 8 (Type 8)) Van et al., 2009).
Genetic Origin of Antibiotic Resistance

Most bacteria resistant to antibiotic have the ability to genetic change and this change may be on the basis of the chromosome or within genetic factors outside chromosomal called plasmids (Baron, et al., 1998), and it can be transmitted from one gene to another after it.

Global spread of MRSA-resistant bacteria:

Since the discovery and diagnosis of the first MRSA isolate in the United Kingdom in 1961, it has become the main and primary cause of infections associated with health workers in all parts of Europe, Asia, Australia and America (Boyce et al., 2006). It should be noted that the environment and presence of S. aureus bacteria is at the front of the nostrils and that reliable and reliable studies have shown that the nostrils are the most suitable place for the isolation of this bacteria (Williams, 1963).

Methods of screening for MRSA

There are two main methods for diagnosing MRSA bacteria. The first method is to investigate it by phenotypic methods such as the use of the method of diffusion using cefoxitin and the use of the method of diffusion of the antibiotic oxacillin screen agar testing (Velasco et al., 2005). The second method for diagnosing MRSA bacteria is to investigate it using genotypic methods, such as using the polymerase chain reaction (PCR), which is more accurate than using phenotypic methods for diagnosis (Zheng et al., 1999), and this is mainly due to its possession of many Variable factors to a large extent affect the extent to which the resistance gene is expressed by phenotypic methods, as some isolates do not show the resistance gene for it mec A unless antibiotic treatment is used to create a selective pressure that highlights this gene (Martineau et al., 2000), and some strains fall Within the resistant strains possessing the mec A resistance gene called Borderline strains, which may contain Altered Pencillin Binding proteins or may have the tendency to overproduction of Pencillinase, and this causes difficulty in interpreting the results and in reconciling and diagnosing them with strains carrying the mec A resistance gene by familiar phenotypic methods (Brown, 2006).

Since partial methods are not available to all health institutions, the National Committee for Clinical Laboratory Standards (NCCLS) has recommended the use of the phenetipc method, which is the method of spreading the antibiotic cefoxitin tablet at a concentration of 30 micrograms on a standard culture medium inoculated with growth Standard bacteria A standard incubation period of 18-24 hours at a temperature of 35-37 degrees Celsius for the diagnosis of MRSA bacteria and the routine identification of the mec A resistance gene and its prevalence rates in medical laboratories (Skov et al., 2005).

Also, a new culture medium was manufactured by the manufacturers called CHROM agar, as the color of the MRSA bacteria colonies on this medium is violet.
after incubation for 24 hours at a temperature of 37 °C, while other isolates sensitive to MSSA are blue, white or beige

**Samples collection**

100 samples were obtained from the medical staff of Samarra General Hospital and these samples were distributed to the hospital departments. The samples were taken using a swab from the nose Nasal Swab. This swab was inserted into the nostril and moved in a circular motion and then taken out to be planted on the mannitol saline medium where the dishes were incubated. at 35°C for 24 hours to see growth in dishes Askarian et al., 2009.

**Results and Discussion**

The spread of aureus in the medical staff of Samarra General Hospital The current study showed that the percentage of carriers of S.aureus bacteria in general among the medical staff included in the research that was conducted in (Samarra General Hospital) in Salah al-Din Governorate was (22%), as these percentages were the highest that was reached by Askaria and his group (2009) 5.3% in Namazi Hospital in Iran and less than what was found by researcher AL-Shekhli (2003) (64%) in Baghdad. The current study also showed that the percentage of carriers of S.aureus bacteria among the medical staff was (30.2%) among the laboratory staff (10.1%) for the nursing staff and (20.4%) for the physicians in Samarra General Hospital, as these percentages seem to be higher Much of what was found by Mathanraj and his group (2009) (1.8%) in India as well as less than what Masano and his group found (30%) in Britain.

**Percentages of the distribution of S.aureus bacteria on the age groups of the medical staff included in the research**

The medical personnel included in the research were divided according to age groups into three groups (21-29) (30-40) (more than 40). The study showed that the highest percentage of carriers of S.aureus bacteria is the first age group (72%), followed by the age group The second age group (18%), then the third age group was (9%), as in the table. These results were in agreement with the findings of ((Osama, et al., 2016), as it was the highest percentage in the first age group.

**Numbers and Percentages of S.aureus Distribution by Sex**

The current studies showed that the horizontal pregnancy rate of S.aureus bacteria in males in general from the medical staff covered in the study in Samarra General Hospital in Salah al-Din Governorate (63%) 22:14, while the percentage in females was (36.4%) 22:8 of Carriers of S.aureus bacteria. These results are much less than what was reached (AL-Dahbi and Al-Mathkhury, 2013) and were close to what was stated (Osama et al., 2016).
**Percentage distribution of S.aureus bacteria according to the job of the medical staff**

The current studies showed that the percentage of carriers of S.aureus bacteria within the laboratory staff included in the study in Samarra General Hospital was (30%), and the rest of the percentages were (10%) nursing staff (ZERO), pharmacist staff (20%) medical staff. These results were close to what was stated by (Osama et al., 2016). The highest percentage of laboratory staff was (25%) and the rest of the percentages were (22.6%), (17.1%), (8.5%) distributed among the nursing, pharmacy and physicians’ cadres, and they vary. From what was stated by (Fadeyi et al., 2010), the highest percentage of doctors’ staff was (65.2), followed by nursing (64.4) and then laboratory (25%).

The percentages that the highest percentage of S.aureus isolates was in the laboratory staff among the medical staff may be due to the fact that the laboratory staff deals with pathological samples that may carry pathogenic factors and may be transmitted to it easily, in addition to the pollution caused by these factors to the yeast environment and equipment Laboratory and workbench surfaces and may remain a source of contamination from several weeks to several months Al-Khafaji (2010). It has been observed that the process of cleaning laboratory tops in the hospitals under study is repeated once a day and in some cases simple detergents are used that do not rise to the level of disinfection and sterilization, in addition to that. The method and method of cleaning is usually wrong, and the place where some meals are eaten inside the laboratory sections on a daily basis may be one of the reasons for the high rate of S.aureus in the staff, and among the other reasons is the lack of attention of the laboratory staff to the place of sterilization, which is caused by the lack of health awareness and lack of sufficient knowledge Occupational and biological safety position.

As for the nursing staff, the percentages of direct contact with patients and patients or those who are sleeping are periodically continuous and for the length of their stay in the hospital wards with patients, they are susceptible to acquiring pathological factors from the hospital environment or from inpatient patients (Osama et al., 2016; Fadeyi et al., 2010).

As for the pharmacy staff, the percentage of isolates for them is less or no than that of the laboratory staff and the nursing staff, and this is an expected result due to the lack of contact with inpatient and reviewing patients, and the pharmacy departments in hospitals are isolated from disease halls, and there is no with samples or pathological factors Osama, et al.(2016; Shibabow et al., 2013).

As for the lowest percentage of S.aureus isolates, it was among the doctors’ cadre, and the reason was due to the coincidence with the issue of sterilization after examination and examination of patients, and adherence to occupational and biological safety standards, as well as their wearing of masks, paws and suits before entering for operations.
**Staphylococcus aureus isolate rates, distributed according to years of service**

It was found that the percentage of isolates from S.aureus according to the years of service was 45% of those covered by the initial examination, and it was found that the percentage of isolates from S.aureus according to the number of years of service was (27%) of those covered by the second examination, and the percentage of the third examination was (18%). As for the percentage of the fourth examination, it was (9%) of all those covered by the examination.

**Detection of virulence factors**

**Hemolysin Production**

Hemolysin enzyme is one of the virulence factors possessed by some bacteria. This enzyme has the ability to degrade the cell membrane of red blood cells (Arthur et al., 2002), and the results of the study currently showed that 20 out of 100 isolates, or about (90.9%) of S.aureus isolates, are capable of producing this enzyme, and these results agreed. With the results of Al-Naimi (2002), who obtained a percentage of (87.5%) of the isolates of this bacterium, and the ability to analyze the blood by this enzyme produced by different bacterial species depends on many factors, including blood type, as well as the presence of cholesterol and serum in the used blood helps to inhibit the decomposition process (Abdulwahid et al., 2001).

**Dnase production**

This enzyme has the ability to affect the structure of DNA, and this enzyme is one of the advantages of pathogenic bacteria. This bacteria produces two types of this enzyme, the first is heat-sensitive Dnase, which determines the pathogenicity of S.aureas bacteria, and the second is heat-resistant and is symbolized by Tnase, whose production is the most important. The advantages of this bacteria. (Rosato et al., 2009).

The results of the detection of DNase enzyme by Staphylococcus isolates showed that members of the species S.aureus were able to produce this enzyme by (86.3%), 19 out of 100 isolates where colonies were surrounded by a transparent halo in Dnase medium. This study agreed with Abdullah (2008). Which obtained 60 out of 67 isolates of S.aureus bacteria.

**Urease production**

The results of the detection of urease enzyme showed that all S.aureus isolates produced this enzyme by 100%, and the importance of urease enzyme lies in its ability to decompose urea into ammonia and carbon dioxide gas. Which converts the medium from acidic to basic (Forbes, et al., 2007) as in the picture, and the results of this detection were close with (Abdullah, 2008).
**Lecithinase and Lipase production**

Twenty-two out of 100 isolates of S.aureus that produced lecithins were obtained (100%), and this result is in contrast to Khalaf (2008) who obtained 51% of isolates that were producing this enzyme, and the importance of this enzyme lies in the fact that it works to break down phosphorylated lipids in the cell membranes, and then degrades and leads to tissue death, as well as this enzyme works to separate the complex lipoproteins in the medium containing the egg yolk, and this interaction is called Lecitho Vitellin. (Kumar et al., 1997)

As for the lipase enzyme, the results showed that 100% of S.aureus isolates produced this enzyme. 22 out of 100 did not agree with the result of Khalaf (2008). The importance of this lipase enzyme lies in the fact that it works on the analysis of fats and thus facilitates the penetration of skin tissues.

**Proteases Production**

The current study showed that 22 isolates and (100%) of S.aureus isolates produced proteinase and did not agree with Mahmoud (2006) (50%) study. And, consequently, the immune system. (Karisson et al., 2001)

**Antibiotic Susceptibility Test**

Seven types of antibiotics were used, and showed a clear discrepancy in terms of resistance and sensitivity, as the isolated bacteria showed high resistance against Doxycyclin, %, 100%, Ampicillin, followed by, %,methicillin 50%, 4.1% Ciproflox Giusti and colleagues (1999) indicated that the resistance against cephalosporins increased in staphylococci carrying the Mec A gene responsible for the production of penicillinase, in addition to the fact that some isolates varied in their sensitivity to antibiotics, and this is due to the bacteria having several mechanisms of antibiotic resistance, such as neutralizing the activity of the antibiotic by The production of certain enzymes that paralyze the action of the antigen, the transformation of the place on which the antigen works, and a change in the permeability of bacterial cells to prevent the entry of the antigen or through their possession of the Efflux System (Levinson and Jawetz, 2004).As for the beta-lactam antagonists, it showed a resistance of (50.4%) to anti-methacillin, where the type S.aureus resisted this anti-methacin by (50.3%), while it agreed with (Peck et al., 2009), which obtained (15.4%) anti-methicillins.

Also, Fuda et al., (2004) confirmed that the resistance of S.aureus bacteria to penicillin compounds does not include only penicillinase and beta-lactamase enzymes, but goes beyond that to the production of (PBPS) present in the cytoplasmic membrane that binds to the cell wall, and these proteins may be the target of all antigens of the penicillins and cephalosporins groups, they are It works by changing the target site for beta-lactam antigens, thus producing bacterial resistance to it. The amount of penicillin-binding proteins is not entirely related to the resistance to beta-lactam antigens.
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