ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Anti-microbial Susceptibility of *Staphylococcus aureus* Isolated from Different Clinical Infection

Ameer Sadeq Al-Ethari, Enas Jalil Baqer Almayali*

Department of Medical Laboratory Technique, Faculty of Medical and Health Technique, University of Alkafeel. Najaf, Iraq.

***Corresponding Author: Enas Jalil Baqer Almayali**

Abstract

This study investigated *staphylococcus aureus* in many of the infected clinical samples and their resistance to antibiotics .The study included two mainaxes, the first axis is diagnosis of bacterial isolates according to approved diagnostic systems, the second axis is determine the resistance and sensitivity of the antibiotic. A total of 250 clinical samples were collected from several places of the body from Al-Hakim General Hospital and Al-Sadr city medical in Al-Najaf city during the period from November 2018-Febray 2019. Samples were under study (ear, eye, wound, urine, tonsils, diabetic foot and aspiration fluid) for both sexes and different age group. The results of the bacterial diagnosis showed that sample was infected with *staphylococcus aureus* (20 isolated from diabetic foot, 10 isolated from ear ,10 isolated from urine , 15 isolated from wound, 15 isolated from aspiration fluid),depending on characters of culture media, Gram stain, biochemical test and Vitek-2 for identification . The study also examined the sensitive test by testing disk diffusion depending on the locations of the disease. Isolated from ear, wound, diabetic foot, aspiration fluid were resistance to penicillin family. While isolated from urine were variable to it family. All isolated were sensitive to vancomycin, teicoplanin, impenem, meropenam. All isolated were sensitive to amikacin, refampine, except isolates from diabetic foot were resistant to it. Cefidinr more kind of cephalosporin gives sensitivity to study isolated. Quinolones, macrolides, aminoglycoside were variable depending on location of the disease.

Keywords: *S. aureus, Sensitivity*

Introduction

Staphylococcus aureus are Gram positive bacteria, cocci, grape like clusters, non-motile, aerobic or facultatively anaerobic, and catalase positive and grow in a medium containing 10% sodium chloride and at a temperature ranging from 18°C to 40°C. Relatively simple biochemical tests can be used to differentiate *S. aureus* and the other *staphylococci. S. aureus* has positive reactions for coagulase, heat-stable nuclease, alkaline phosphatase and mannitol fermentation. Differentiation of the coagulase-negative *staphylococci* is more complex, however, and is not routinely done in many clinical laboratories unless the isolates are demonstrated to be clinically significant [1]. *Staphylococci* grow readily on most bacteriological media under aerobic or microaerophilic conditions. They grow most rapidly at 37°C but form pigment best at room temperature (20-25°C) [2]. The most important staphylococcal species is *S. aureus*, which is named for its yellow-pigmented colonies (aureus-golden) as the result of the carotenoid pigments that form during their growth. Colonies on solid media are round, smooth, raised, and glistening. *S. aureus* usually forms grey to deep golden yellow colonies [3].*Staphylococcus aureus* are Gram-positive cocci anging from 0.5 to 1.5 µm in diameter, which may or may not contain a polysaccharide capsule. They are non-motile, non-spore forming facultative anaerobes that produce catalase and coagulase enzymes yearly, microbial contamination of marine waters is predicted to be responsible for millions of gastrointestinal and acute respiratory infections (ARIs), in addition to several skin infections Although *S. aureus* is typically a commensal organism, it has been known to be opportunistic. Invasive infections due to wound invasion can lead to numerous

diseases, including scalded skin syndrome, abscesses, septicaemia, pneumonia, food poisoning, and toxic shock syndrome [4].*S. aureus* is a potentially harmful human pathogen associated with both nosocomial and community-acquired infections, and it is increasingly becoming resistant to most antibiotics. Previous studies of *S. aureus* in marine environments have linked swimmers to the dissemination of *S. aureus* in marine water, via the shedding of the bacterium from their nose, skin, and respiratory tract. On recreational beaches, *S. aureus* has occasionally been found in high abundance in both water and sand, which can be directly associated with bather density and human activities around the beach The human skin is directly exposed to infectious agents during swimming and this exposure can lead to the colonization of *S. aureus* with the potential to invade the immune system and cause infections. There is a relationship between seawater exposure and *S. aureus* infection rates which suggests that recreational waters are potential sources of community-acquired *S. aureus* infections. There is also a positive correlation between the concentrations of *S. aureus* and total *staphylococci* to skin, eye, and ear infections among bathers [5].

Materials and Methods

Specimen's Collection

From November 2018 to February 2019, two hundred and fifty clinical specimens were collected from patients suffering from infection from Al-Sader medical city, and Al-Hakim General Hospital in Al –Najaf province. Those specimens were collected from patients (male and female, adults and children) suffering from infections. By taken swab from infection area (ear, wound infection, tonsils infection, eye infection, diabetic foot, urine and aspiration fluid).

Specimens Culture and Biochemical Test

The collected specimens were inoculated on three types from culture media which included mannitol salt agar, blood agar, and MacConkey agar. Which considered as predominant enrich media, selective and differential media for the isolation, purification and identification of many types from bacteria [6].The plates were incubated at 37˚C for 24 hours then a single pure isolated colony was transferred to trypticase soya agar for the preservation and to carry out other biochemical tests IMVIC test, catalase test, coagulase and oxidase test that confirmed the identification of isolates [7].

Vitek-2 for Identification

GP identification card has been used for documentation gram positive bacteria. The bacterial suspension was familiar to McFarland standard of 0.5 in 2.5ml of a 0.45% sodium chloride solution with a Vitek -2 instrument (bioMérieux, France). The time between preparation of the inoculum and the card big was continually less than 30 min. The GP identification card is a fully closed system to which no reagents have to be added. The card was put on the cassette designed for use with the Vitek-2 system, located in the instrument, routinely filled in a vacuum chamber, sealed, incubated at 35.5˚C, and automatically subjected to colorimetric measurement (with a new reading head) every 15 min for a maximum gestation period of 8 hours. Data were analyzed using Vitek-2 database, which agrees organism identification in a kinetic mode beginning 180 min after the start of incubation [8].

Sensitivity Test

McFarland Standard Solution

The microbial inoculums was standardized at 0.5 McFarland. In microbiology, McFarland standards were used as a reference to adjust the turbidity of bacterial suspensions so that the number of bacteria will be within a given range [9].

Muller-Hinton Agar

It was prepared according to manufacturer's instructions (Oxoid/ UK), by adding 38 gm in 1000 ml D.W. It was used to determine of antimicrobial susceptibility test (AST) [6].

Result and Discussion

Isolation of Pathogenic Bacteria and Sensitivity Test

During the study period from November 2018 to February 2019, two hundred and fifty clinical specimens were collected from patients. By taken swab from infection area (ear, wound infection, tonsils infection, eye infection, diabetic foot, urine and aspiration fluid) .Were(70) specimen *staphylococcus aureus* as in table one, shows the location of the infection and the number of isolates , it grown on mannitol agar as *staphylococcus* spp and give catalase positive. And they are

resistance to bacitracin; Bacitracin is an antibiotic interfering with the synthesis of peptidoglycan, a major component of bacterial cell walls.

Different types of bacteria have different degrees of susceptibility to bacitracin. This test determines whether the bacterium is either sensitive (susceptible) to bacitracin or resistant to the drug. Knowledge about bacitracin susceptibility is valuable in identification of Gram positive cocci, some of which are susceptible and others of which are resistant [10]. From it give coagulase positive as *S. aureus*, because only *S. aureus* produce coagulase enzyme, Coagulase test is used to differentiate *S. aureus* (positive) from Coagulase Negative *Staphylococcus* (CONS). Coagulase is an enzyme produced by *S. aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin [11].

This bacteria has unique ability of growing on a high salt (7.5% sodium chloride), which was found in mannitol agar. The high salt concentration inhibits the growth of most bacteria other than *Staphylococcus* [12]. On mannitol salt agar, pathogenic *S. aureus* produces small colonies and color of medium was changed from pink to yellow due to fermentation the mannitol sugar and producing acid which, in turn, changes the indicator from pink to yellow [13]. The final identification was performed with the automated vitek-2 compact system using GP, ID cards which contained 64 biochemical tests and one negative control [8].

Table 1: Showing the location of the disease with *staphylococcus* aureus and number of isolates

Location of disease	Number of isolates
Diabetic foot	20
Wound	15
Aspiration fluid	15
Urine	10
Ear	10
Total	**70**

The results of the sensitivity test for isolates were determined according to the location of the disease depends on WHO international standards for antibiotics, as clearly in the tables 2,3,4,5,6.

Table 2: Showing Sensitivity test to *staphylococcus aureus* isolated from diabetic foot

Types of antibiotics	S . Test	Types of antibiotics	S . Test
Amoxiclave	R	Ciprofloxacin	R
Ampicillin/cloxacillin	R	Levofloxacin	R
Pipracillin/tazobactam	Variable	Moxifloxacin	R
Ticarcillin	R	Norfloxacin	R
Carbencillin	R	Nalidixicacid	R
Vancomycin	H.S	Refampine	R
Teicoplanin	Variable	Trimethoprime	R
Imipenem	H.S	Trimethoprime / sulfonamide	R
Meropeneam	H.S	Azithromycin	R
Cefotaxime	R	Gentamycin	R
Ceftriaxone	R	Amikacin	R
Cefixime	R	Tobramycin	R
Ceftrazidine	R	Tetracycline	R
Cefidiner	R	Deoxycyclin	R
Cefpodoxime	R	Cefepime	R
Cefodizime	R	Cefoxitin	R

R = Resistance, H.S = High sensitive, M.S = Moderate sensitive

Table 3: Showing Sensitivity test *staphylococcus aureus* isolated from wound swab

Types of antibiotics	S . Test	Types of antibiotics	S . Test
Amoxiclave	Variable	Ciprofloxacin	Variable
Ampicillin/cloxacillin	R	Levofloxacin	H.S
Pipracillin/ tazobactam	M .S	Moxifloxacin	Variable
Ticarcillin	R	Norfloxacin	H.S
Carbencillin	R	Nalidixicacid	variable
Vancomycin	H.S	Refampine	H.S
Teicoplanin	H.S	Trimethoprime	R
Imipenem	H.S	Trimethoprime / sulfonamide	H.S
Meropeneam	H.S	Azithromycin	H.S
Cefotaxime	H.S	Gentamycin	variable
Ceftriaxone	Variable	Amikacin	variable
Cefixime	R	Tobramycin	H.S
Ceftrazidine	R	Tetracycline	R
Cefidiner	H.S	Deoxycyclin	R

Cefpodoxime	R	Cefepime	R
Cefodizime	R	Cefoxitin	M.S

R = Resistance, H.S = High sensitive, M.S = Moderate sensitive

Table 4: Showing Sensitivity test to *staphylococcus aureus* isolated from aspiration fluid

Types of antibiotics	S . Test	Types of antibiotics	S . Test
Amoxiclave	R	Ciprofloxacin	H.S
Ampicillin/cloxacillin	R	Levofloxacin	H.S
Pipracillin/ tazobactam	R	Moxifloxacin	H.S
Ticarcillin	R	Norfloxacin	H.S
Carbencillin	R	Nalidixicacid	R
Vancomycin	H.S	Refampine	H.S
Teicoplanin	H.S	Trimethoprime	R
Imipenem	H.S	Trimethoprime / sulfonamide	H.S
Meropeneam	H.S	Azithromycin	H.S
Cefotaxime	R	Gentamycin	H.S
Ceftriaxone	R	Amikacin	H.S
Cefixime	R	Tobramycin	R
Ceftrazidine	R	Tetracycline	M.S
Cefidiner	H.S	Deoxycyclin	R
Cefpodoxime	R	Cefepime	R
Cefodizime	R	Cefoxitin	R

R = Resistance, H.S = High sensitive, M.S = Moderate sensitive

Table 5: Showing Sensitivity test to *staphylococcus aureus* isolated from urine

Types of antibiotics	S . Test	Types of antibiotics	S . Test
Amoxiclave	Variable	Ciprofloxacin	variable
Ampicillin/cloxacillin	Variable	Levofloxacin	variable
Pipracillin/ tazobactam	Variable	Moxifloxacin	variable
Ticarcillin	R	Norfloxacin	variable
Carbencillin	Variable	Nalidixicacid	R
Vancomycin	H.S	Refampine	Variable
Teicoplanin	H.S	Trimethoprime	R
Imipenem	H.S	Trimethoprime / sulfonamide	R
Meropeneam	H.S	Azithromycin	R
Cefotaxime	M.S	Gentamycin	R
Ceftriaxone	R	Amikacin	H.S
Cefixime	Variable	Tobramycin	Variable
Ceftrazidine	R	Tetracycline	R
Cefidiner	H.S	Deoxycyclin	R
Cefpodoxime	R	Cefepime	R
Cefodizime	R	Cefoxitin	M.S

R = Resistance, H.S = High sensitive, M.S = Moderate sensitive

Table 6: Showing Sensitivity test to *staphylococcus aureus* isolated from ear swab

Types of antibiotics	S . Test	Types of antibiotics	S . Test
Amoxiclave	Variable	Ciprofloxacin	R
Ampicillin/cloxacillin	R	Levofloxacin	R
Pipracillin/ tazobactam	R	Moxifloxacin	R
Ticarcillin	R	Norfloxacin	R
Carbencillin	R	Nalidixicacid	R
Vancomycin	H.S	Refampine	H.S
Teicoplanin	H.S	Trimethoprime	R
Imipenem	H.S	Trimethoprime / sulfonamide	R
Meropeneam	H.S	Azithromycin	R
Cefotaxime	R	Gentamycin	R
Ceftriaxone	Variable	Amikacin	H.S
Cefixime	R	Tobramycin	R
Ceftrazidine	Variable	Tetracycline	R
Cefidiner	Variable	Deoxycyclin	R

Cefpodoxime	R	Cefepime	R
Cefodizime	R	Cefoxitin	Variable

R = Resistance, H.S = High sensitive, M.S = Moderate sensitive

The major targets for antibiotics in *staphylococci* are (i) the cell envelope, (ii) the ribosome and (iii) nucleic acids. Several novel targets emerged from recent targeted drug discovery programmed including the ClpP protease and FtsZ from the cell division machinery. Resistance can either develop by horizontal transfer of resistance determinants encoded by mobile genetic elements via plasmids, transposons and the *staphylococcal* cassette chromosome or by mutations in chromosomal genes. Horizontally acquired resistance can occur by one of the following mechanisms: (i) enzymatic drug modification and inactivation, (ii) enzymatic modification of the drug binding site, (iii) drug efflux, (iv)bypass mechanisms involving acquisition of a novel drug-resistant target, (v) displacement of the drug to protect the target.

Acquisition of resistance by mutation can result from (i) alteration of the drug target that prevents the inhibitor from binding, (ii) depression of chromosomally encoded multidrug resistance efflux pumps and (iii) multiple stepwise mutations that alter the structure and composition of the cell wall and/or membrane to reduce drug access to its target. This review focuses on development of resistance to currently used antibiotics and examines future prospects for new antibiotics and informed use of drug combinations [14]. The major inhibitory target for ß-lactam antibiotics in *S. aureus* is the bi functional transglycolylase-transpeptidase PBP2.

The transglycosylase domain of the enzyme is responsible for transferring the disaccharide pentapeptide building block of peptidoglycan from membrane-bound lipid II to growing polysaccharide chains while the transpeptidase (TP) domain cross-links the glycine cross-bridge of the fourth D-alanine of an adjacent chain [15]. Aminoglycosides ,these drugs enter bacterial cells by energy-dependent binding to the cell wall and energy-dependent transport across the cytoplasmic membrane, finally binding to one or more ribosomal sites, thus inhibiting protein synthesis (Maranan,1997). Resistance in *staphylococci* results from any of three events: [1] a chromosomal mutation leading to altered aminoglycoside binding to ribosomes; [2]. Ineffective transport of aminoglycosides into the bacterial cell, producing low-level cross-resistance to most aminoglycosides; and, most commonly, [3] enzymic modification of aminoglycosides [16].

In the last case, resistant strains have the aminoglycoside-modifying genes *acc, aph* and *ant*, which code for aminoglycoside acetyltransferases, phosphotransferases and adenyltransferases, respectively. The acetylated, phosphorylated or adenylated aminoglycosides do not bind to ribosomes, and thus do not inhibit protein synthesis [17].Although fluoroquinolones were introduced in the 1980s for the treatment of Gram-negative bacterial infections, their Gram-positive spectrum of activity meant that they were also used to treat infections caused by *Pneumococci* and *staphylococci* [16]. The primary target of quinolones is bacterial DNA gyrase, without which DNA replication is inhibited [18]. Quinolone resistance emerged rapidly, particularly among methicillin-resistant strains, by the stepwise acquisition of chromosomal mutations.

This involved mutations in the quinolone-resistance-determining region of the enzyme-DNA complex, reducing the affinity of quinolone for its targets (DNA gyrase and topoisomerase IV) [16].Vancomycin has been considered the accepted standard of therapy for methicillin-resistant *Staphylococcus aureus* (MRSA) and is commonly used as an alternative for methicillin-suceptible *Staphylococcus aureus* (MSSA) in cases of allergy or intolerance to other agents. MRSA strains exhibiting elevated vancomycin MICs (minimum inhibitory concentration) within the susceptible range have been associated with increased treatment failure and mortality.

The aim of our study was to evaluate for evidence of elevated vancomycin MICs among S. aureus isolates associated with pneumonia or skin/soft tissue infection (SSTI) in our adult inpatient population, Elevated vancomycin MICs were uncommon in our sample among adult inpatients with pneumonia or SSTI caused by S. *aureus*. Vancomycin appears to remain a viable option for the treatment of these infections in our patient population [19]. Meropenem is a broad-spectrum carbapenem antibiotic with

excellent activity against many aerobic Gram-positive, aerobic Gram-negative, and anaerobic organisms. meropenem is an effective and viable alternative for monotherapy of these challenging infections. The regimen of meropenem 500 mg every 8 hours is appropriate for the treatment of infections caused by most pathogens [20].Tetracycline has been a widely used antibiotic because of its low toxicity and broad spectrum of activity. However, its clinical usefulness has been declining because of the appearance of an increasing number of tetracycline-resistant isolates of clinically important bacteria. Two types of resistance mechanisms predominate: tetracycline efflux and ribosomal protection. A third mechanism of resistance, tetracycline modification, has been identified, but its clinical relevance is still unclear. For some tetracycline resistance genes, expression is regulated. In efflux genes found in gram-negative enteric bacteria, regulation is via a repressor that interacts with tetracycline. Gram-positive efflux genes appear to be regulated by an attenuation mechanism. Recently it was reported that at least one of the ribosome protection genes is regulated by attenuation. Tetracycline resistance genes are often found on transmissible elements. Efflux resistance genes are generally found on plasmids, whereas genes involved in ribosome protection have been found on both plasmids and self-transmissible chromosomal elements (conjugative transposons) [21].

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