AL-QADISIYA UNIVERSITY
COLLAGE OF PHARMACY

Isolation and Identification of *staphylococcus aureus* in different clinical cases

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بسم الله الرحمن الرحيم

{ ألم تَرَ أنَّ الله أنزلَ من السَّماء ماءً فأخرجنا به ثمراتٍ مُخْتلفاً ألوانُها ومن الجبالِ جُدَدٌ بيضٌ وحمرٌ مختلفٌ ألوانُها وغرابيبُ سُودٌ ومن النَّاسِ والدَّوابِّ والأنعامِ مُختلفٌ ألوانُهُ كذلك إنَّما يَخشى الله من عبادِهِ العلماءُ إنَّ الله عزيزٌ غفور }

صدق الله العظيم (35 فاطر أية 27-28)

الاهداء

الى من قال في علاه ولا اله سواه

(وقل رب زدني علما) صدق الله العظيم (طه : 114)

الى شفيعنا يوم الدين محمد صلى الله عليه و على اله واصحابه و من اتبعهم بأحسان الى يوم الميعاد..

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الى كل قطرة دم سقطت من اجلك يا عراق .. بلد الحضارات و الامجاد و خير الاوطان ..

الى من جمع بيت سعادتنا و حزننا

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الى من نتمنى ان تبقى صورهم ...في عيوننا

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Abstract :

This study was done to assess the bacterial profile and antibiotic susceptibility pattern of different clinical cases pathogens.

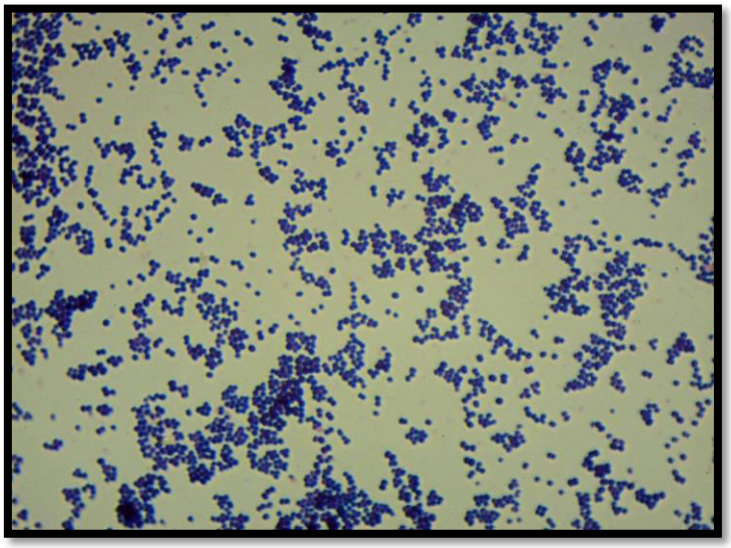
For proper identification of causative microbial, Where 40 samples are collect from different patients with different clinical cases, cultured and subjected to appropriate biochemical tests.

These samples were collected from Teaching, Laboratories Center in AL Qadisiyah during the study period (1st July 2016-1st September 2016) The antimicrobial sensitivity test was carried out by disc diffusion technique using Muller-Hinton agar ,where the results of the study showed that 15 samples gave a positive result for staph.aureus, while 25 sample gave a negative result due to the presence of fungal pathogens or viral or bacterial.

The majority of staph. aureus were sensitive to the amikacin (100 %) , chloramphenicol ؛vancomycin (86.6%) , gentamicin ; gatifloxacin (73.3%) rifampin ; ciprofloxacin (60) , ofloxacin ;levofloxacin (46.6%) and oxacillin (0%)
While resistance to the staph. aureus are oxacillin (100%), ofloxacin;levofloxacin (53.4%) , rifampin; ciprofloxacin (40%), gentamicin;gatifloxacin (26.6%), chloramphenicol (13.4%), vancomycin; amikacin (0%)

Introduction:-

Staphylococcus aureus is a gram-positive coccal bacterium that is a member of the Firmicutes, and is frequently found in the nose, respiratory tract, and on the skin. It is often positive for catalase and nitrate reduction. Although *Staph. Aureus* is not always pathogenic, it is a common cause of skin infections such as abscesses, respiratory infections such assinusitis, and food poisoning. Pathogenic strains often promote infections by producing potent protein toxins, and expressing cell-surface proteins that bind and inactivate antibodies. The emergence of antibiotic-resistant strains of *Staph. aureus* such as methicillin-resistant *Staph.aureus* (MRSA) is a worldwide problem in clinical medicine.



(Microscope image for *staph.aureus*)

(Cole et.al.,2001)

Staphylococcus was first identified in 1880 in Aberdeen, Scotland, by the surgeon Sir Alexander Ogston in pus from a surgical abscess in a knee joint (Ogston , 1984) . This name was later appended to *Staph. Aureus*byFriedrich JuliusRosenbach, who was credited by the official system of nomenclature at the time. An estimated 20% of the human population are long-term carriers of *staph. aureus* (Kluytmans et.al., 1997) which can be found as part of the normal skin flora and in the nostrils (Cole et.al.,2001). *Staph. aureus* is a normal inhabitant of the healthy lower reproductive tract of women (Hoffman and Barbara , 2012). *Staph. aureus* can cause a range of illnesses, from minor skin infections, such as pimples,(Senok et.al., 2009) impetigo, boils, cellulitis, folliculitis, carbuncles, scalded skin syndrome, andabscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and

sepsis. It is still one of the five most common causes of hospital-acquired infections and is often the cause of postsurgical wound infections. Each year, around 500,000 patients in hospitals of the United States contract a staphylococcal infection, chiefly by *Staph. aureus*. (Bowersox and John ,1999).

virulence factors:-

1-Enzymes

Staph. aureus produces various enzymes such as coagulase (bound and free coagulases) which clots plasma and coats the bacterial cell, probably to prevent phagocytosis. Hyaluronidase (also known as spreading factor) breaks down hyaluronic acid and helps in spreading it. *Staph. aureus* also produces deoxyribo, nucleasewhich breaks down the DNA, lipase to digest lipids, staphylokinase to dissolve fibrin and aid in spread, and beta-lactamase for drug resistance (Collee et.al ., 1996)

2-Toxins

Depending on the strain, staph. aureus is capable of secreting several exotoxins, which can be categorized into three groups. Many of these toxins are associated with specific diseases (Dinges et.al.,*2000)*

3-Superantigens

Pyrogenic toxic superantigen have superantigen activities that induce toxic shock syndrome (TSS). This group includes the toxin TSST-1, enterotoxin type B, which causes TSS associated with tampon use. This is characterized by fever, erythematous rash, hypotension, shock, multiple organ failure, and skin desquamation. Lack of antibody to TSST-1 plays a part in the pathogenesis of TSS. Other strains of *staph. aureus*can produce anenterotoxin that is the causative agent of *staph. aureus* gastroenteritis. This gastroenteritis is self-limiting, characterized by vomiting and diarrhea one to six hours after ingestion of the toxin, with recovery in eight to 24 hours. Symptoms include nausea, vomiting, diarrhea, and major abdominal pain (*Becker* et.al.,2003).

4-Exfoliative toxins

EF toxins are implicated in the disease staphylococcal scalded-skin syndrome (SSSS), which occurs most commonly in infants and young children. It also may occur as epidemics in hospital nurseries. The protease activity of the exfoliative toxins causes peeling of the skin observed with SSSS (*Deurenberg* and *Stobberingh* ,2008)*

Other toxins :-

Staphylococcal toxins that act on cell membranes include alpha toxin, beta toxin, delta toxin, and several bicomponent toxins. The bicomponent toxin Panton-Valentine leukocidin (PVL) is associated with severe necrotizing pneumonia in children. The genes encoding the components of PVL are encoded on a bacteriophage found in community-associated MRSA strains.(Jarraud et.al.,2001)

Other immunoevasive strategies:-

Protein A

Protein A is anchored to staphylococcal peptidoglycan pentaglycine bridges (chains of five glycine residues) by the transpeptidasesortase A. Protein A, an IgG-binding protein, binds to the Fc region of an antibody. In fact, studies involving mutation of genes coding for protein A resulted in a lowered virulence of *staph.aureus* as measured by survivaln in blood, which has led to speculation that protein A-contributed virulence requires binding of antibody Fc regions.

Protein A in various recombinant forms has been used for decades to bind and purify a wide range of antibodies by immunoaffinity chromatography. Transpeptidases, such as the sortases responsible for anchoring factors like protein A to the staphylococcal peptidoglycan, are being studied in hopes of developing new antibiotics to target MRSA infections (Tong et.al.,2015)

Disease that caused by staph.aureus and it' streatment :-

1-Bacteremia :

Treatment for bacteremia or blood infection with *staph. aureus* or infection from a medical device – the medical device or the foci of the infection needs to be removed after identification. Of antibiotics β-lactams, oxacillin, nafcillin, cefazolin are preferred . For MRSA vancomycin, daptomycin, linezolid ,Quinupristin/dalfopristin, Cotrimoxazole, Ceftaroline, Telavancin are chosen.

2-Endocarditis :

Treatment of infection of the heart or its valves (Endocarditis) – the foci is removed when possible. Choice of antibiotics includes oxacillin, cefazolin, nafcillin, gentamycin etc. for Methicillin sensitive strains

(MSSA). Others include Ciprofloxacin, rifampin, vancomycin, daptomycin etc. (*Shafie* iet al.,2011)

3-Skin Infections :

Infections of soft tissues and skin – the primary treatment is removal of foci of infection by drainage of pus from abscesses, cellulitis etc. Choice of antibiotics for MSSA include Cephalexin, Dicloxacillin, Clindamycin, Amoxicillin/clavulanate etc. For MRSA antibiotics like Cotrimoxazole, Clindamycin, tetracyclines, Doxycycline, Minocycline, Linezolid etc. may be used. For skin infections local application of antibiotics like Mupirocin 2% ointment are also prescribed.

4-Pneumonia :

Lung infections or pneumonia – for MRSA cases Linezolid, Vancomycin, Clindamycin may be used (*Neely and Maley ,2000*)

5-Bone and joint infections :

For MSSAoxacillin, cefazolin, nafcillin, gentamycin etc. may be used. For MRSA cases Linezolid, Vancomycin, Clindamycin, Daptomycin, Coptrimoxazole may be used.

6-Meningitis :

Brain and meninges infection (meningitis) - for MSSA oxacillin, cefazolin, nafcillin, gentamycin etc. may be used. For MRSA cases Linezolid, Vancomycin, Clindamycin, Daptomycin, Cotrimoxazole etc. may be used.

7-Toxic Shock Syndrome :

Toxic shock syndrome is a rare but serious medical condition caused by a bacterial infection. It is caused when the bacterium *Staphylococcus aureus* gets into the bloodstream and produces toxins.It can cause major organ damage or death if left untreated.Treatment for MSSA are oxacillin, nafcillin, clindamycin and for MRSA cases Linezolid, Vancomycin, Clindamycin may be used. (*Chang* et al .,2003)

Carriage of Staph. aureus :-

About one-third of the U.S. population are carriers of *staph. aureus*.The carriage of *staph. aureus* is an important source of hospital-acquired infection (also called nosocomial) and community-acquired MRSA. Although *staph. aureus* can be present on the skin of the host, a large proportion of its carriage is through the anterior nares of the nasal

passages* and can further be present in the ears.* The ability of the nasal passages to harbour *staph. aureus* results from a combination of a weakened or defective host immunity and the bacterium's ability to evade host innate immunity.Nasal carriage is also implicated in the occurrence of staph infections.*(*Wertheim* et.al.,2005)

Materials and Methods:-

1- Collection of the sample& culture characteristics :

I attended a glass slide with a drop of the colonies from the brine and it covered by a glass slide and examined the optical microscope as colored dye gram to note external appearance of the colonies , containing the shape , size, color ,diameter and height of the colony . (Macfaddin,2000)(Forbes *et al*,2007)

2- Growth on culture media :

A-Growth on blood agar:

1. Prepare the blood agar base as instructed by the manufacturer. Sterilize by autoclaving at 121°C for 15 minutes.
2. Transfer thus prepared blood agar base to a 50°C water bath.
3. When the agar base is cooled to 50°C, add sterile blood agar aseptically and mix well gently. Avoid formation of air bubbles. You must have warmed the blood to room temperature at the time of dispensing to molten agar base. Dispense 15 ml amounts to sterile petri plates aseptically
4. Label the medium with the date of preparation and give it a batch number
5. Store the plates at 2-8°C, preferably in sealed plastic bags to prevent loss of moisture. The shelf life of thus prepared blood agar is up to four weeks. (Atlas , 2004)

(Blood Agar Test)

(Forbes *et al*,2007)

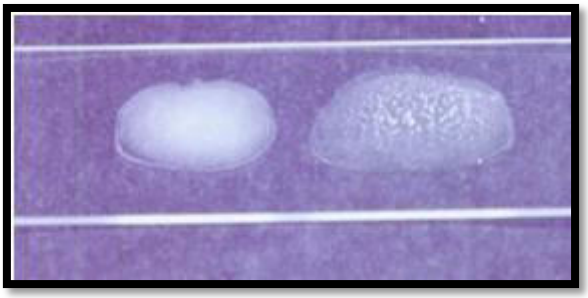
B-Coagulase Test:

Coagulase test is used to differentiate *Staphylococcus aureus* (positive) from Coagulase Negative Staphylococcus (CONS). Coagulase is an enzyme produced by *staph. aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin. *Staphylococcus aureus* produces two forms of coagulase, bound and free.

1. Slide coagulase test is done to detect bound coagulase or clumping factor.
2. Tube coagulase test is done to detect free coagulase.

Slide coagulase test procedure :

1. Divide the slide into two sections with grease pencil. One should be labeled as “test” and the other as “control
2. 2Place a small drop of distilled water on each area
3. Emulsify one or two colonies of Staphylococcus on blood agar plate on each drop to make a smooth suspension
4. The test suspension is treated with a drop of citrated plasma and mixed well with a needle
5. Do not put anything in the other drop that serves as control. The control suspension serves to rule out false positivity due to auto agglutination
6. Clumping of cocci within 5-10 seconds is taken as positive.(Todar,2002).



(Coagulase test)
.(Todar,2002).

C- Mannitol test:

1. Samples Collection (streak the samples as soon as possible after collection)
2. Allow the plates to warm to room temperature and the agar surface to dry before inoculating.
3. Streak for isolation with a sterile loop.

4. Incubate plates aerobically at 35-37ºC for 24-48 hours.
5. Examine colonial morphology. (Alexander,2004)

(Mannitol test)

.(Todar,2002).

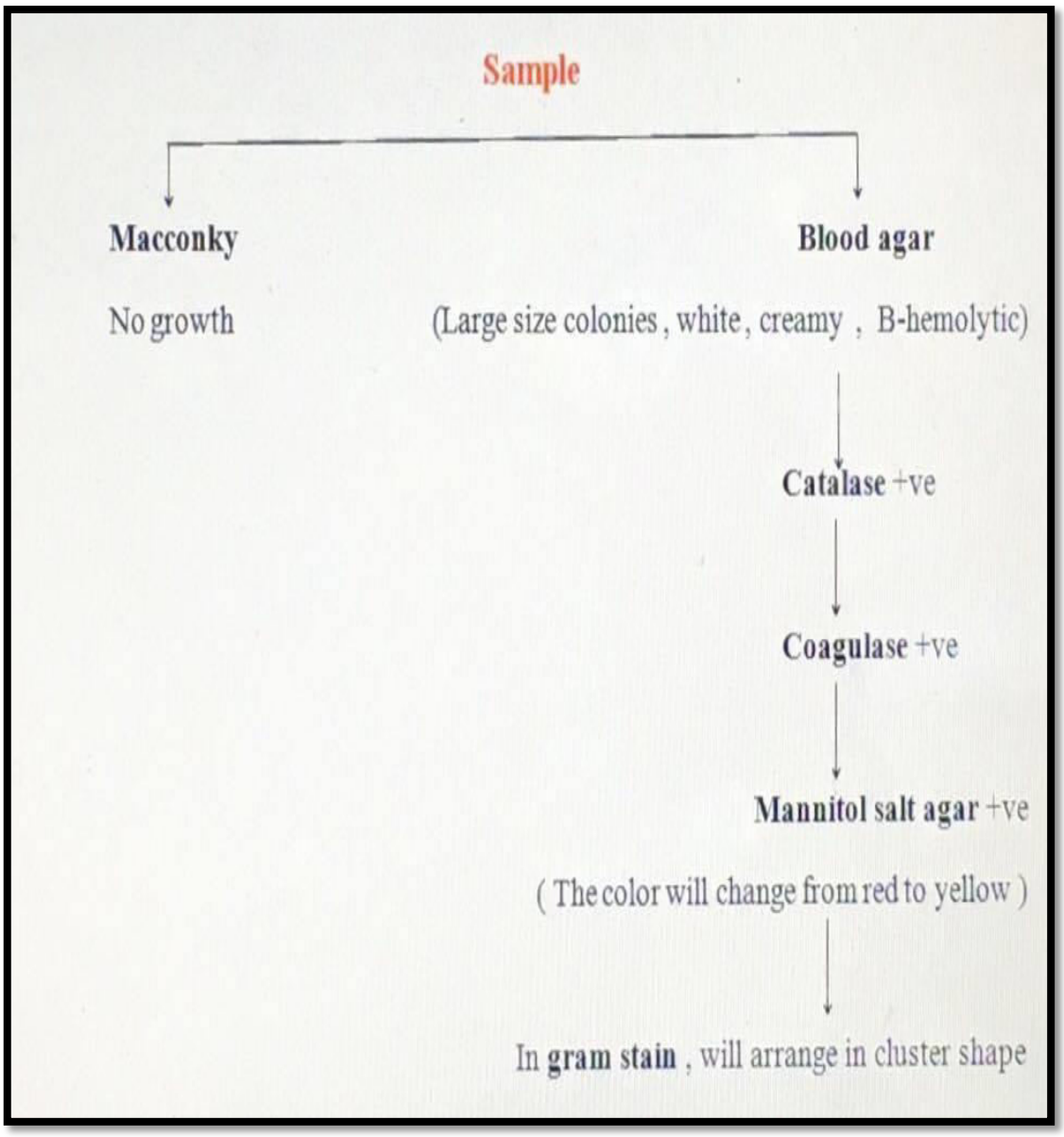


Table : the diagnosis of *staph.aureus* .

Antibiotic sensitivity test of staph.aureus :-

The agar diffusion testis a test of the antibiotic sensitivity of bacteria. It uses antibiotic-impregnated wafers to test the extent to which bacteria are affected by those antibiotics. In this test, wafers containing antibiotics are placed on an agar plate where bacteria have been placed, and the plate is left to incubate. If an antibiotic stops the bacteria from growing or kills the bacteria, there will be an area around the wafer where the bacteria have not grown enough to be visible. This is called a zone of inhibition.

Procedure :

1. Using an aseptic technique, place a sterile swab into the broth culture of a specific organism and then gently remove the excess liquid by gently pressing or rotating the swab against the inside of the tube.
2. Using the swab, streak the Mueller-Hinton agar plate .
    a. To obtain uniform growth, streak the plate with the swab in one direction, rotate the plate 90° and streak the plate again in that direction.
    b. Repeat this rotation 3 times.
3. Allow the plate to dry for approximately 5 minutes.
4. Use an Antibiotic Disc Dispenser to dispense discs containing specific antibiotics onto the plate.
5. Using a flame-sterilized forceps, gently press each disc to the agar to ensure that the disc is attached to the agar.
6. Plates should be incubated overnight at an incubation temperature of 37 °C .
7. The zone of inhibitionmust be measure and compared with a standard table . (Bauer et.al., 1959)

Results and Discussion:-

The samples were planting on the media , the dishes were incubated at a temperature of 37 Celsius and within 24 hours, and has pursued growth until noon, and then has the purification and diagnosis process occur .
Where the results of the study showed that 15 samples gave a positive result for *staph. aureus* , while 25sample gave a negative result, the negative result due to the presence of fungal pathogens or viral or bacterial.

Table:Show different case staphylococcus aureus for Culture

Age	Patient	Specimen	Result of culture
1-10 Years	**P1**	Nipple discharge	Growth of *staph . aureus*
	P2	Ear swab	Growth of *staph . aureus*
	P3	Tonsel swab	Growth of *staph . aureus*
10-20 Years	**P4**	Urine	Growth of *staph . aureus*
	P5	Urine	Growth of *staph . aureus*
20-30 Years	**P6**	Urine	Growth of *staph . aureus*
	P7	Urine	Growth of *staph . aureus*
	P8	Urine	Growth of *staph . aureus*
	P9	Semen	Growth of *staph . aureus*
30-40 Years	**P10**	Urine	Growth of *staph . aureus*
	P11	Nipple	Growth of *staph . aureus*
40-50 Years	**P12**	Urine	Growth of *staph . aureus*
	P13	Pus	Growth of *staph . aureus*
50-60 Years	**P14**	Wound swab	Growth of *staph . aureus*
60-70 Years	**P15**	Wound swab	Growth of *staph . aureus*

Table :Percentage of sensitivity and resistance in our samples

Patient	Gen	gati	Van	Ch	Levo	rif	oflox	amik	cip	oxa	pip	meth
P1	S	S	S	R	R	R	R	S	S	R	R	R
P2	S	S	S	S	R	S	R	S	R	R	R	R
P3	S	R	S	S	S	S	S	S	S	R	R	R
P4	S	R	S	S	R	S	S	S	S	R	R	R
P5	S	S	S	S	S	R	S	S	R	R	R	R
P6	S	S	S	S	S	S	R	S	S	R	R	R
P7	S	S	S	S	S	S	R	S	S	R	R	R
P8	R	S	S	S	R	S	R	S	R	R	R	R
P9	R	S	S	S	R	R	R	S	S	R	R	R
P10	S	R	S	R	R	S	R	S	R	R	R	R
P11	R	S	S	S	S	S	S	S	S	R	R	R
P12	S	S	S	S	R	S	S	S	R	R	R	R
P13	S	S	S	S	S	R	S	S	S	R	R	R
P14	R	S	S	S	R	R	R	S	R	R	R	R
P15	S	R	S	S	S	R	S	S	S	R	R	R
%S	73.3	73.3	100	86.6	46.6	60	46.6	100	60	0	0	0
%R	26.6	26.6	0	13.4	53.4	40	53.4	0	40	100	100	100

Where :

Gen=gentamicin
gati=gatifloxacin
van =vancomycin
chl=chloramhenicol
levo= levofloxacin
rif =Rifampin
Oflox =ofloxacin
amik =amikacin
cip=ciprofloxacin
oxa=oxacillin
pip=piperacillin
meth= methicillin

Obvious from the table that the staph.aureus show mixed response to antibiotics :-

Aminoglycoside (gentamicin ,amikacin)

S% = (73.3 , 100)% respectively , inhibit the aminoglycosides' action, which occurs via protonated amine and/or hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosomal subunit. (Carter *et al*,.,2000)

R% = (26.6 , 0)% respectively , three main mechanisms of aminoglycoside resistance mechanisms are currently and widely accepted: aminoglycoside modifying enzymes, ribosomal mutations, and active efflux of the drug out of the bacteria.Aminoglycoside-modifying enzymes inactivate the aminoglycoside by covalently attaching either a phosphate, nucleotide, or acetylmoiety to either the amine or the alcohol key functional group (or both groups) of the antibiotic.(*Sakon et al*,.,1993)

Quinolone (fluoroquinolones)

Gatifloxacin **,** ciprofloxacin :
S% = (73.3 , 60)% respectively
R% (26.6 , 40) % respectively
Quinolones exert their antibacterial effect by preventing bacterial DNA from unwinding and duplicating.

Ofloxacin , levofloxacin :
S% = (46.6)%
R% = (53.4)% , mutations at key sites in DNA gyrase or topoisomerase IV can decrease their binding affinity to quinolones, decreasing the drugs' effectiveness.(*Hooper,* 2001)

Penicilllins

oxacillins , pipracillins :

S%=(0)%
R%(100)% , Staphylococcal resistance to penicillin is mediated by penicillinase (a form of β-lactamase) production: an enzyme that

cleaves the β-lactam ring of the penicillin molecule, rendering the antibiotic ineffective. (Lowy ,1998).

Methicillin :
S%=(0)%
R%(100)% , resistance is conferred by the *mecA* gene, which codes for an altered penicillin-binding protein (PBP2a or PBP2') . This allows for resistance to all β-lactam antibiotics, and obviates their clinical use during MRSA infections. (Chambers ,2001)

Glycopeptides (vancomycin)

S% = (100)%
R%=(0)%
Increase in use of vancomycin to treat infections caused by methicillin-resistant staphylococci. In situations where the incidence of MRSA infections is known to be high, the attending physician may choose to use a glycopeptide antibiotic until the identity of the infecting organism is known.(Kirst *et al*, ., 1998)

Chloramphenicol

S%= (86.6)%
Chloramphenicol is a bacteriostatic by inhibiting protein synthesis. It prevents protein chain elongation by inhibiting the peptidyltransferase activity of the bacterial ribosome. It specifically binds to A2451 and A2452 residues in the 23S rRNA of the 50S ribosomal subunit, preventing peptide bond formation. While chloramphenicol and the macrolide class of antibiotics both interact with ribosomes, chloramphenicol is not a macrolide. It directly interferes with substrate binding, whereas macrolides sterically block the progression of the growing peptide.(*Jardetzky, 1963*)

R%=(13.4)% , chloramphenicol is reduced membrane permeability, mutation of the 50S ribosomal subunit, and elaboration of chloramphenicol acetyltransferase.(*Moore . et al,,2013)*

Rifampin

S%= (60)% , Rifampicin inhibits bacterial DNA-dependent RNA synthesis by inhibiting bacterial DNA-dependent RNA polymerase. (Calvori *et al*,.,1965)

R%=(40)% , Resistance to rifampicin arises from mutations that alter residues of the rifampicin binding site on RNA polymerase, resulting in decreased affinity for rifampicin. Resistance mutations map to the *rpoB* gene, encoding the beta subunit of RNA polymerase.(*Feklistov . et al*,,2008).

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