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A STUDY ON METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATED FROM CLINICAL SAMPLES

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Introduction

Staphylococci are Gram positive bacteria of the micrococcaceae family they are primarily found on the skin and mucous membranes of the human and other warm blooded animalsand aggregate into small grape like clumps. Staphylococcui related infections are one of the most common causes of nosocomial (Hospital acquired) infections. Yet they are increasingly difficult to treat due to the rate at which the bacteria acquire antibiotic resistance.

The spectrum of staphylococcus aureus ranges from minor skin infections to folliculitis to a life threating illness such as endocarditis, pneumonia and septicemia. Pyogenic infections of the skin are the commonest clinical problems in dermatological practice.

The staphylococcus genus is divided into two groups: Coagulase positive staphylococci and coagulase Negative staphylococci. These two types are distinguished from each other traditionally by their coagulase activity.(they maintain the ability to clot blood plasma).

Staphylococcus can cause skin,heart valve, blood and bone infections, which can lead to septic shock and death. These infections are primarily caused by the toxins which staphylococci produce. For Example, the enterotoxins produced by staphylococci aureus are significant cause of food poisoning and the superantigens can cause toxic shock syndrome if present in the blood stream. In their potent forms, the toxins are responsible for damaging host tissues, inhibiting phagocytosis (where by the host neutralizes the staphylococci toxins and eliminates the bacteria) and causing disease symptoms.

The study of MRSA gaining importance because it is pathogenic and encountered as common causative agent of hospital acquired infection has limited treatment options as only few strains are susceptible to fluroquinolones, trimethprin/sulphomethoxale and gentamicin (or) rifampin, vancomycin often is the only drug of choice.

Limited treatment options are vancomycin often Is the only drug of choice for treatment of severe MRSA infections, although some strains remain susceptible to fluoroquinolone trimethprin/sulphomethoxale and Gentamicin (or) rifampicin.

Who is colonized, general 40% population,50-90% of health care practitioners.

Aims and Objectives

To isolate and characterize coagulase positive staphylococci in various clinical samples from various clinical samples from all age groups.

- To evaluate the antibiotic suceptibilty pattern and drug resistance pattern.
- To isolate MRSA strains on selective media.
- To know the incidence of MRSA in the area.
- Biotyping for MRSA strains.

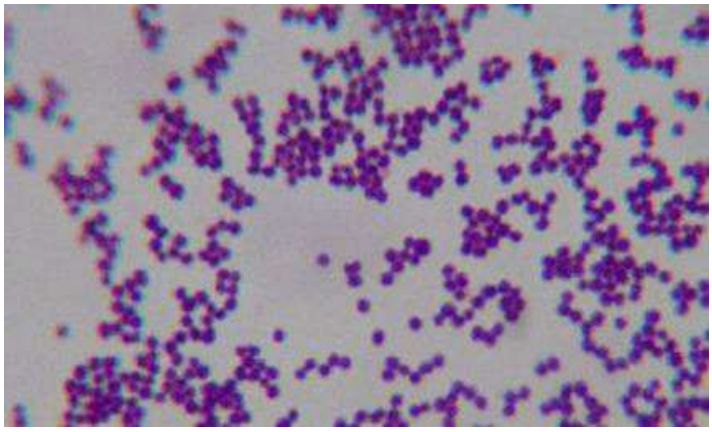
Review of Literature

Staphylococcus aureus belongs to micrococcaceae family. Staphylococci are gram positive cocci arranged in grape like clusters.

Staphylococcus was first observed in human pyogenic lesions by vonrecklinghausen in 1871.Pasteur in 1880 obtained liquid cultures of the cocci from pus and produced abscess by inoculating them into rabbits. A Scottish surgeon, Sir Alexander Ostgon established the causative role of the coccus in absecess and other supurative lesions in 1880.He gave it the name staphylococcus (Staphyle, in greek meaning bunch of grapes “Kokkos” meaning berry).He also noticed that the non virulent staphylococci were also often present on skin surfaces.

Rosenbach(1884) named them staphylococcus aureus due to the production of golden yellow color. Staphylococci are ubiquitous in nature. Staphyloccocci are the normal flora of human anterior nares, nasopharynx, peripheral area and mucosal surfaces. The common source of infection being human patients and carriers. Staphylococci may follow endogenous or exogenous infection. Spread of patients endogenous strains to normally sterile site by traumatic introduction. Also may be transmitted to person by fomites air or unwashed hands of hospital workers especially in the nosocomial setting.

Hospital infections of staphylococci are significant as their frequency and resistance to various antibiotics is ever increasing. Staphylococci are the commonest cause of infection by virtue of their ability to elaborate non-toxic metabolites and some toxins.



Gram positive cocci

STAPHYLOCOCCUS AUREUS PRODUCES ENZYMES AND TOXINS:

Staphylococcus aureus virulence factors (Pathological markers Coagulase positive, Mannitol fermentation,β-haemolysis,Golden yellow pigment, Liquefy gelatin,Produce phosphatase, Reduces potassium tellurite to tellurium, Lipolytic activity and Urease production).

Staphylococcus aureus produces β-lactamases hospital strains are resistant to multiple antibiotics and belonging to phage type 80/81 spread globally in 1950 and 1960 colonising hospital and causing nocosomial infection with such frequency that came to be called hospital staphylococci.The original phage types have since been replaced by other belonging to group 383A but staphylococcus continue to be very common agents in hospital infections.

The majority of hospital cross infection must be treated with an appropriated antibiotic and for this invitro sensitivity testing is required. Resistance strains vary from place and time to time depending on the variety of antibiotics used and also duration of their use.

In 1929, Alexander flemming first discovered pencillin, but it was not until 1940 that it was fully integrated into infectious disease treatment strategies

In the interveining decade, the medical world saw the discovery of a variety of antibiotic agents. Among the most successful were the suphonamides, prodrugs that arrested by inhibiting Nucleic acid biosynthesis.

Sulphonamides are “Prodrugs” these compounds are made active once ingested. The sulphonamides inhibitNucleic acid synthesis by preventing the synthesis of purine bases.

With the successful use of pencillin and sulfonamides, the 1940 were dubbed the antibiotic age. Suddenly, there were a wealth of way to combat infectious diseases.

Pencilin was seen as the “cure-all”.

Emergence of pencillinase producing staphylococci in the 1950 led to the development of semi-synthetic pencillins possessing an acyl side chain.whichserically inhibits the action of pencillinase enzymes. Preserving the integrity of the β-lactum ring. These pencillinase resistant pencillins, include Methicillin the first developed, Naficillin and Isoxazyl pencillins, they have excellent activity against methicillin sensitive staphylococci and remain the agents of choice for these infections.

One plasmid (30Kb)encodes heavy metal resistance and β lactamases, the other of 4.4Kb provides tetracycline resistance.In early 1980 MRSA prevalence started to increase again due to new variant designated Epidemic MRSA(EMRSA).Most resistant genes located on chromosomes occurs via three mechanisms.

- Mutation(eg: Rifampin)
- Plasmids(eg:pencillin as in classic MRSA)
- Transposons

RESISTANCE MECHANISM OF MRSA:

There are three known resistantmechanisms in MRSA.

Alteration of penicillin binding proteins(PBPs) termed intrinsicresistance is most common,found in the bacterial cell wall,PBPs are the structures to which βlactams and there byinhibiting cell wall synthesis.

Hyper production of pencillinase, first described in 1984 confers border line resistance.

Organisms which have normal PBPS is a decreased affinity for lactams are classed as modified resistant strains.

Staphylococcus aureus was responsible for 18% of all nosocomial infections causing 1/3 of post operative and skin infections, the proportion of staphylococcial infections due to MRSA is necessary. Reviewrs of public health data reported that MRSA reprented <1% isolate in 1960 and <5% in 1970.In 1995 upto 60% of all staphylococcus aureus infections in intensive care units may be due to MRSA.

Kawashima T.et al(1992)-In a study of nasal carriage of Methicillin resistant Staphylococcus aureus reported 61% pencillin resistance in staphylococcus aureus infections.

Okubu T.,Okamoto et al(1994)-Proposed that most of the methicillin resistant strains are phage type group –III or mixed.Surviving ability of Methicillin resistant strains are longer when compared to sensitive strains.

Rosdahl V.T.,Braveny I.et al(1994)-In a study of MRSA in Europe reported 12.8% were Methicillin resistant staphylococcal strains.The proportion of MRSA in the various European countries ranged from <1% in Scaandinavia to >30% in spain,France and Italy.Rates of resistance were lowest for Rifampin and highest for ciprofloxacin.76% of the isolates belonged to phage group III.

Okamoto R.Yomoda S.et al(1994)-In a study reported the activities of antimicrobial agents against staphylococcal aureus strains isolated in 1990.Strains resistant to pencillin were isolated at the lightest frequency 93.6%,Kanamycin 51.5%,Erythromycin 49.0%,Fluoroquinolone 33.4% and Minicycline 12.3%.The isolation frequencies of MRSA strains were 18.9% in 1981,44.8% in 1990.Most of the drug resistant strains of staphylococcus aureus were also resistant to Methicillin.76% of the MRSA strains were phage typed into groups III or mixed.60% of the MRSA strains were typed into these two groups.

Gasper C.,Jim enez j.et al(1994)-In a study between 1989-92 reported an outbreak of MRSA.Surgical wound, urinary tract and skin infections accounted for 58% of the infections. Carriers 98% death 13%, colonization with MRSA was found in 42%.

Namura S.,Kawai S.(1994)-In astudy showed the frequency of isolated MRSA were resistant to many kinds of antibiotics, therefore MRSA infection was incurable. In skin disorders, it was very difficult toeradicate MRsa because of the sever damage or defect of the skin.

Kawai S.,Asada Y.et al(1995)-In a study of Mrsa isolated from skin infections reported that MRSA resistant to fluroquinolones had gradually increased in recent years. Over half of the strains were resistant to Norfloxacin, Ofloxacin, Ciprofloxacin and Lomefloxacin.

Nishijima S.et al(1995)-n a study on staphylococcal cutaneous infections reported methicillin resistance in 20-40% of staphylococcal strains.

O'Swyer G.,Shafi M.S.(1996)-Showed that the incidence rate for MRSA in elderly was 2.9%.Nostrils were significantly associated with carriage and skin break isolates were significant in the prevalence.

Lund B.,Skov R.L.et al(1997)-Descibed the outbreak of MRSA onvolving 8 patients within a time span of 6 weeks.Bacteriophage typing revealed that 3 different strains of MRSA were involved in an outbreak. This emphasises the importance of typing Mrsa in order to clean up the spread in an outbreak. Specific guidelines for hospital hygiene are necessary to prevent the spread of such strains.

Borremans A.,Reybrouck G.(1997)-In astudy they reported about 5.1% of patients carried MRSA on admissions, mostly without clinical symptoms, the highest percentage 11.6% being in geriatric patients. Hospital acquisition of MRSA occurred in 1.7% of patients and was high in intensive care unit 5.2%.The MRSA strains imported by geriatric patients were only a minor source of nosocomial infection.

Mitchell K.,Wise R.et al(1997)-In a study showed that the prevalence of MRSA in nursing homes in Birmingham was high and that strains may have originated in hospitals.

Olesen J.,Jensen I.(1998)-In a study that increased hygienic precautions,isolation of infected patients,staff and management efforts and a close contact with the microbiologists prevented MRSA from spreading to other hospital wards.

Tan N.H.,Tay Y.K.(1998)-In a study showed that staphylococcus aureus was the commonest organism isolated from both primary and secondary pyrodermas. The MRSA strains were common in secondary pyrodermas.The staphylococcus aureus had a high rate of resistance 89.5% to pencillin and was very sensitive 93% to cloxacillin.

Swanton W.H.(1999)-Showed that the prevalence rate of MRSA was 4.6% during 1995-96.But all strains were sensitive to vancomycin.

Sugahara Y.,Imai C.et al(1999)-In astudy they isolated 125 MRSa strains during the period of January 1990 and December 1994.No vancomycin strains was isolated.

Dr.Kaur G.,Dr.Verenkar M.P.et al(2000)-In a study reported that out of 154 strains had MIC of >=8 gm/ml and only 4 strains showed MIC between 4-8 gm/ml.All strains were sensitive to vancomycin.

Pandit Dakshayani P.et al(2000)-In a study isolated 56.62%MRSA strains from burn wounds infections. But all were sensitive to vancomycin.

Brig V.C.Ohri(2000)-In a study showed that 7% MRSA isolated during the period of 1985-1989,33.6% MRSA isolates during 1988-89 and 34.2% MRSA isolates during 1992.

Pus(94.3%) was the most common source of MRSA isolates. Betalactamase production was 100% with MRSA.most MRSA isolates were multidrug resistant to most antibiotics, including Gentamicin reaching 80-90%.All MRSA were sensitive to Vancomycin, Clindamycin and Fusidic acid. Bacteriophage typing showed most of the isolates were belonging to lytic groups III and II.Phage types 54/75/96 and 96 were the most prevalent phage types encountered.

D.Majumder et al(2001)-In astudy showed 23.2% MRSA isolates during the period of 2001.

Mahalaxmi et al(2001)-In astudy showed 38% MRSA isolates in skin infections.

Anupurba et al(2003)-In astudy showed 54.85% were found to be MRSA.

THE STAHPHYLOCOCCUS STRAINS ARE IDENTIFIED BY:

- ❖ Coagulase Test
- ❖ Pigmentation
- ❖ Potassium Tellurite Medium
- ❖ Mannitol Fermentation Test
- ❖ Gelatin Liquefaction Test
- ❖ Phosphatase Test
- ❖ Urease Test
- ❖ DNAse Test

Biotyping:

MRSA strains usually exhibit the following characteristics.

- Coagulase test
- Greater biochemical activity,ferment mannite.
- Produce clear hemolysis on blood agar.
- Liquefy gelatin.
- Produce phosphatase.
- In a medium containing potassium tellurite to form black colonies.
- Produce thermostable nucleases which can be demonstrated by theability of boiled cultures to degrade DNA in an agar diffusion test.

❖ **Coagulase Test**

Principle: This test is used to differentiate staphylococci.

Staphylococcus aureus produces two forms of coagulase bound and free Bound coagulase or clumping factor is bound to the bacterial cell wall and reacts directly with fibrinogen. This results in alteration of fibrinogen so that it precipitates on the staphylococcal cell causing the cells to clump when a bacterial suspension is mixed with plasma,

the presence of bound coagulase correlates well with free coagulase an extracellular protein enzyme that causes the formation of a clot. When staphylococcus aureus colonies are incubated with plasma the clotting mechanism involve activation of plasma coagulase reacting factor(CRF) which is a modified derived thrombin molecule to form a coagulase CRF complex.This complex in turn reacts with fibrinogen to produce the fibrin clot.

- **SLIDE COAGULASE TEST:** Divide the slide into two sections with grease pencil. place a drop of normal saline on to each area emulsify a small amount of colony or two colonies from an agar plate of the test strain in each of the two drops to make a smooth suspension. Add a drop of undiluted human or rabbit plasma to mone of the drops and stir gently with a wire. Coagulase positive strains of staphylococci are clumped within 15 sec. Because fibrin is precipitated on the cell surfaces causing them to stick together. The factor causing this is the "Clumpinf factor" or "Bound" coagulase, which is attached to the cell and acts directly on the fibrinogen(Duthie 1954) the tube test measures "free coagulase" wich requires an accessory factor present in the plasma. The second drop is a control to show spontaneous granularity of the strain which if it occurs invalidates the test.
- **TUBE COAGULASE TEST:** Tube coagulase test is a reliable test for coagulase production. citrated, oxalated or heparinised human or rabbit plasma is diluted 1 in 6 with istonic saline. Place 0.5ml of diluted plasma in each of 2 small tubes.to one tube add 5 drops of an overnight broth cultures. Incubate both tubes in water bath and examine after one hour and at intervals upto 24 hrs.

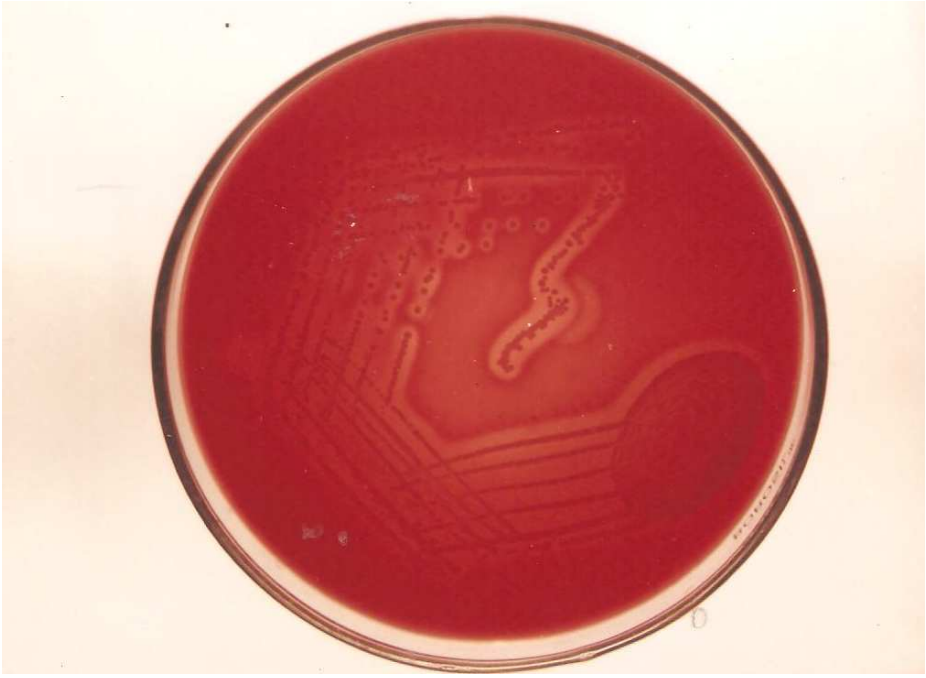
- **PIGMENTATION:** pigment production an the characters of the pigment produced by staphylococci was also noted. Pigment production was best seen on selective media like milk agar.
- **POTASSIUM TELLURITE MEDIUM:** Staphylococcus aureus reduces tellurite to metallic tellurium, which incorporated in the colonies giving them black color.

TEST: Staphylococcus from broth culture were seeded on potassium tellurite media and it was incubated at 37°c fro 24 to 48 hrs. Black pigmented growth was observed.

Pigmentation test

Potassium Tellurite medium

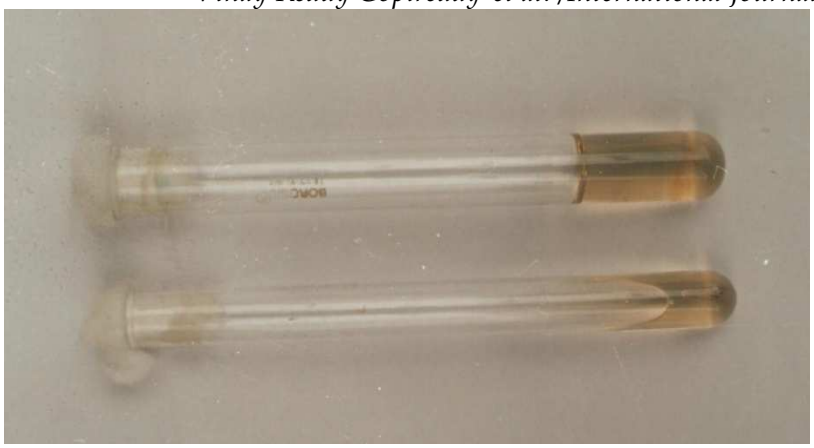
❖ **β HAEMOLYSIS ON 5% SHEEP BLOOD AGAR**: Stahylococcus broth culture was seeded on 5% sheep blood agar and incubated at 37°c for 24 hrs.T he plates were examined for the presence of haemolysis. Then the plates were kept in refrigerator for a period of 24 hrs and examined again. The haemolysis appearing after refrigeration was taken as β haemolysis.



❖ **GELATIN LIQUEFACTION:**

PRINCIPLE:This test is used to determine the ability of an organism to produce proteolytic enzymes (Gelatinases) that liquefy gelatin.

TEST: The liquefaction was tested by stab inoculation of the medium with overnight culture of staphylococci. After stabbing the tubes wereincubated at 37°c ans tested periodically for liquefaction till 14 days. The liquefaction was tested by cooling the tubes in refrigerator. At the end of 14 days if there was no liquefaction the such strains were noted as negative for gelatinase activity.



- **MANNITOL FERMENTATION:** Mannitol fermentation was tested aerobically with mannitol broth.A 0.5% concentration of mannitol was used with phenol as the indicator.Seitz filtered solution of mannitol was added to the sterilized broth and tubed in 5 ml amounts.Tubes were inoculated with fresh broth culture of staphylococci and incubated at 37°c.The tubes were examined for acid production at 24 hrs and 48 hrs.

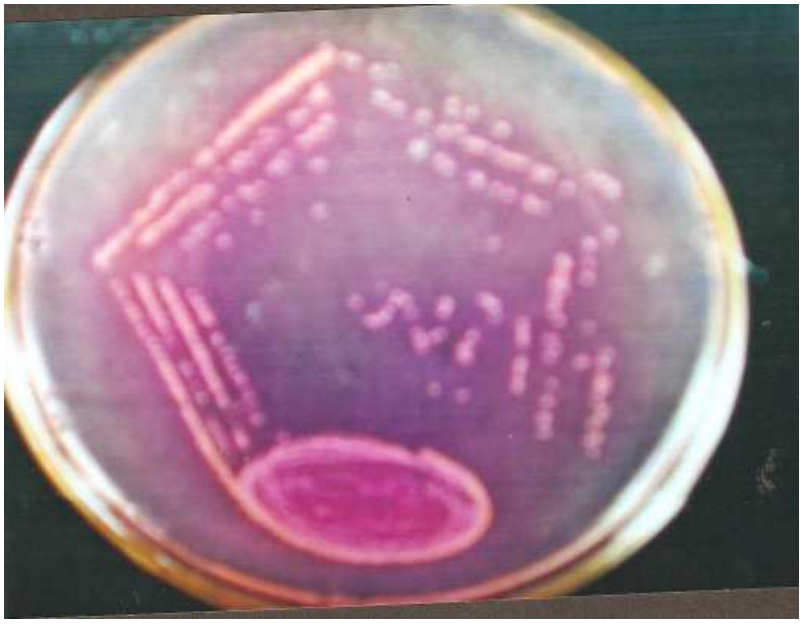
Urease test,Phosphatase test

- **PHOSPHATASE TEST:** most strains of staphylococcus aureus produce enzyme phosphatase, for detection of this enzyme staphylococcus aureus is grown on Nutrient agar containing Phenolphthalein diphosphate. Enzyme phosphatase acts on Phenolphthalein salt to release free Phenolphthalein. The colonies turn pink when exposed to ammonia vapours due the presence of free Phenolphthalein.

❖ **UREASE TEST:**

PRINCIPLE: This test detects the ability of an organism to produce urease enzyme. The organism is inoculated on the entire slope of christensen's medium which contains urea and phenol red as indicator in addition to other constituents including agar. The presence of water converts urea into ammonia and carbondioxide. Ammonia makes the medium alkaline and phenol red indicator changes to purple pink in color.

TEST: The entire slant surface of cristensen's urease agar was heavily inoculated and incubated at 37°c.the slant was examined after 4 hrs and after overnight incubation. Urease positive culture changes the color of the indicator to purple pink.



PHOSPHATASE TEST

❖ **DNAse:**

PRINCIPLE: This is used to determine the ability of an organism to hydrolyze DNA. The medium is plae green complex.If the organism is growing on the medium it hydrolyses DNA. The colour fades and the colony is surrounded by colorless zone.

TEST: Inoculate the DNAse agar with organism to be tested and streak for isolation. Incubate aerobically at 35°c for 18 to 24 hr

Materials and methods:

The material for present study was collected from patients A total 100 -130 samples are collected such as pus, sputum/throatswab, urine, blood from all patients belonging to all age groups. All the samples were aseptically handled and processed.

Collection of samples:

The wound lesion was cleaned with sterile normal saline with cotton woolswabs. The crusts were removed with sterile needle. The pus was collected by sterile swabs and processed.

Blood was collected through veinpuncture under aseptic conditions in a blood culture media set. Skin was disinfected with 70% alcohol before collecting the blood.

Sputum was collected in a sterile screw cap container.

After the samples were processed in the laboratory without delay after collection. Direct smear was done from samples and stained by Grams stain and samples wre inoculated on to Nutrient broth, Nutrient agar,5%sheep blood agar, MacConkey's agar medium, and paltes wre incubated at 37°c for overnight incubation and colony characters were studied and smears wre made from such colonies, stained by Grams staining and examined under oil immersion lens morphological identification, violet colored spherical shaped organisms arranged in clustered were identified as staphylococci. Afterconfirmation by smear examination, strains were further tested for the production of bound coagulase by slide test andfree cagulase enzyme using tube coagulase test(Mackie & Mccartney:Practical Medical Microbiology 14^{th} ed) All the confirmed staphylococcus aureus strains were subsequently tested for methicillin resistance based on Kirby bauer disc diffusion method using oxacillin disc(1micro gram)obtained from Hi-media laboratories private limited. The isolates were considered methicillin resistant if zone of inhibition was 10mm or less. Further the antibiotic susceptibility of methicillin resistant staphylococcus aureus strains was determined on the day of their isolation by the modified Kirby bauer disc diffusion method on Mueller Hinton agar using the criteria of standard zone sizes of inhibition to define sensitivity or resistance to different antimicrobials.

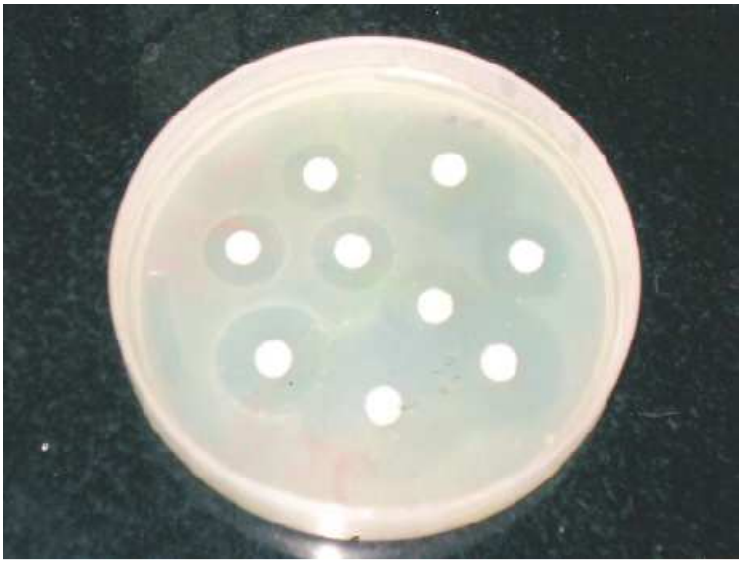
The antibiotics used were ciprofloxacin(5µg),gentamicin(10µg),amikacin(30µg),

cefotoxime(30µg),amoxyclav(30µg),ceftazidine(30µg)gatifloxacin(5µg),cefoperazone(75µg)

ceftrazidine(30µg0,oxacillin(1µg),penicillin(10INU).Obtained from Hi-Media Lab Pvt Ltd.

Finally the data were recorded and analyzed at the completion of the study as per recommendation of the NCCLS.

After confirmation by smear examination strains of staphylococcus aureus were subcultured on to nutrient agar slants at 4°c in the refrigerator, before testing for any activity the stock strains were first subcultured into broth and from broth cultures, further sub cultures made on to the appropriate media which were required for that particular test.



Results:

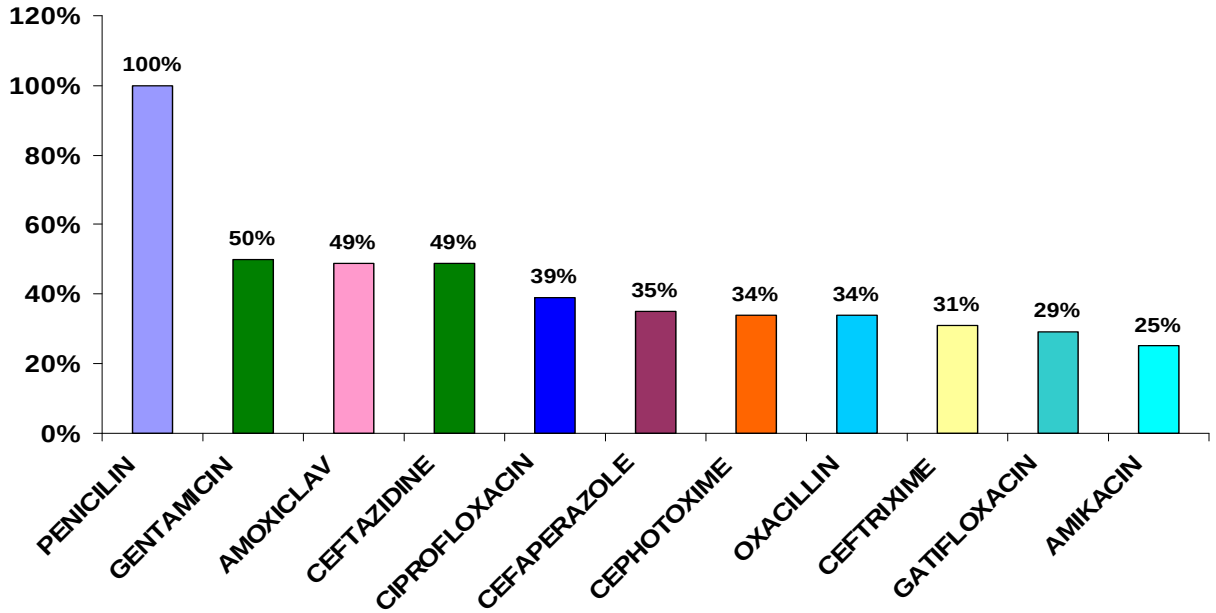
- The present study included 130 strains of staphylococci isolated from various clinical samples from all age groups of patients. Comprising of Blood, sputum, pus, urine.
- MRSA percentage was 34%
- Incidence in males were found to be more than females
- MRSA was isolated more in age group 21-30 yrs (23).Age distribution showing 7 in 1-10yrs,23 in 21-30 yrs,7 in 31-40 yrs,17 in 41-50 yrs,12 each in 51-60 yrs and 61-70 yrs,3 in 71-80 yrs & 2 cases in81-90 yrs in age group.

- Out of 130 samples 100 wre coagulase positive staphylococci and 30 were coagulase negative staphylococci. By biotyping 100 strains of coagulase positive staphylococci showed phosphatase production 93%, mannitol fermentation 90%, pigment production 96%, urease production 76%, and gelatinLiquefaction 66%.
- The strains of 34 isolates of MRSA when incubated on Mueller Hinton agar at 30°c,for 24 hrs showed highest percentage of resistance to Oxacillin(100%), followed by gentamycin(61.7%),ceftazidine(52.9%),amoxyclav(50%),ciprofloxacin(47%),cefaperazole(47%),Ceftrixime(41.7%),gatifloxacin(41.7%),cephotoxime(41.7%),amikacin(35.2%).

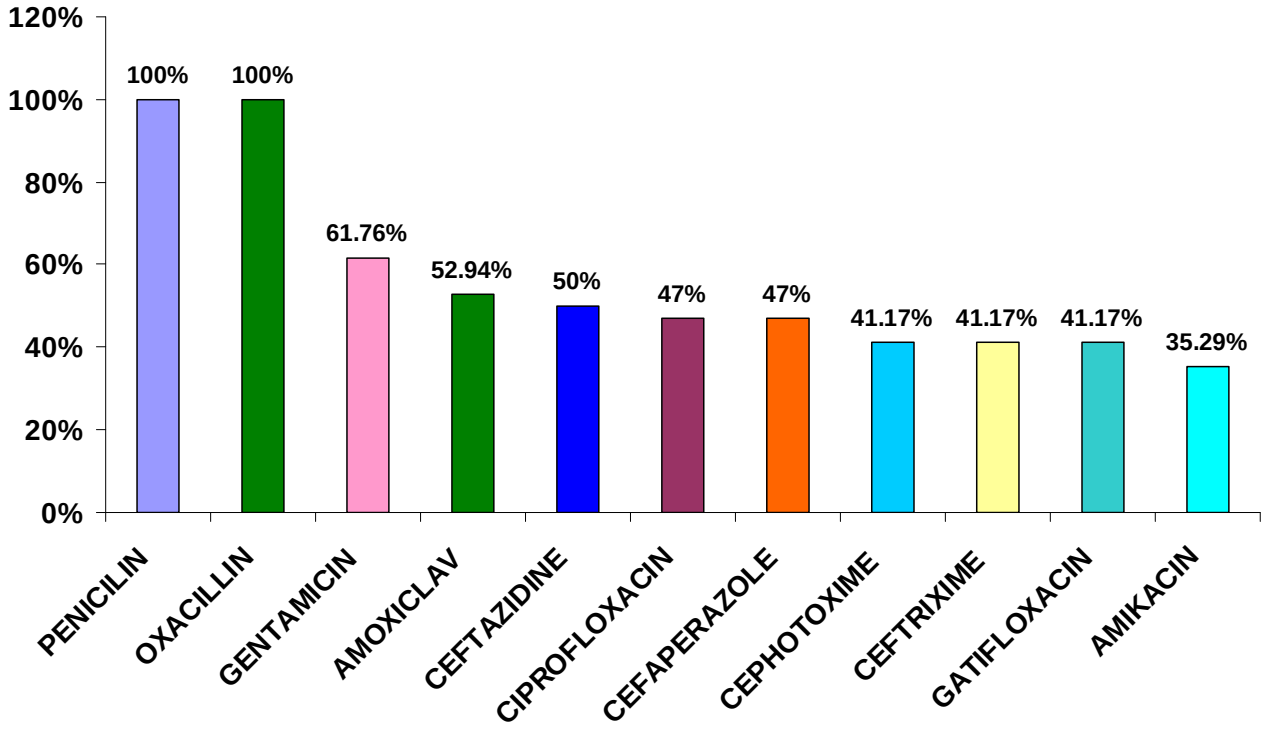
BACTERIOLOGICAL ANALYSIS

Organism	No of cases	%
Coagulase Positive Staphylococcus	100	77%
Coagulase Negative Staphylococcus	30	23%

RESISTANCE PATTERN OF 100 ISOLATES OF STAPHYLOCOCCUS AUREUS



RESISTANCE PATTERN OF 34 ISOLATES OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS

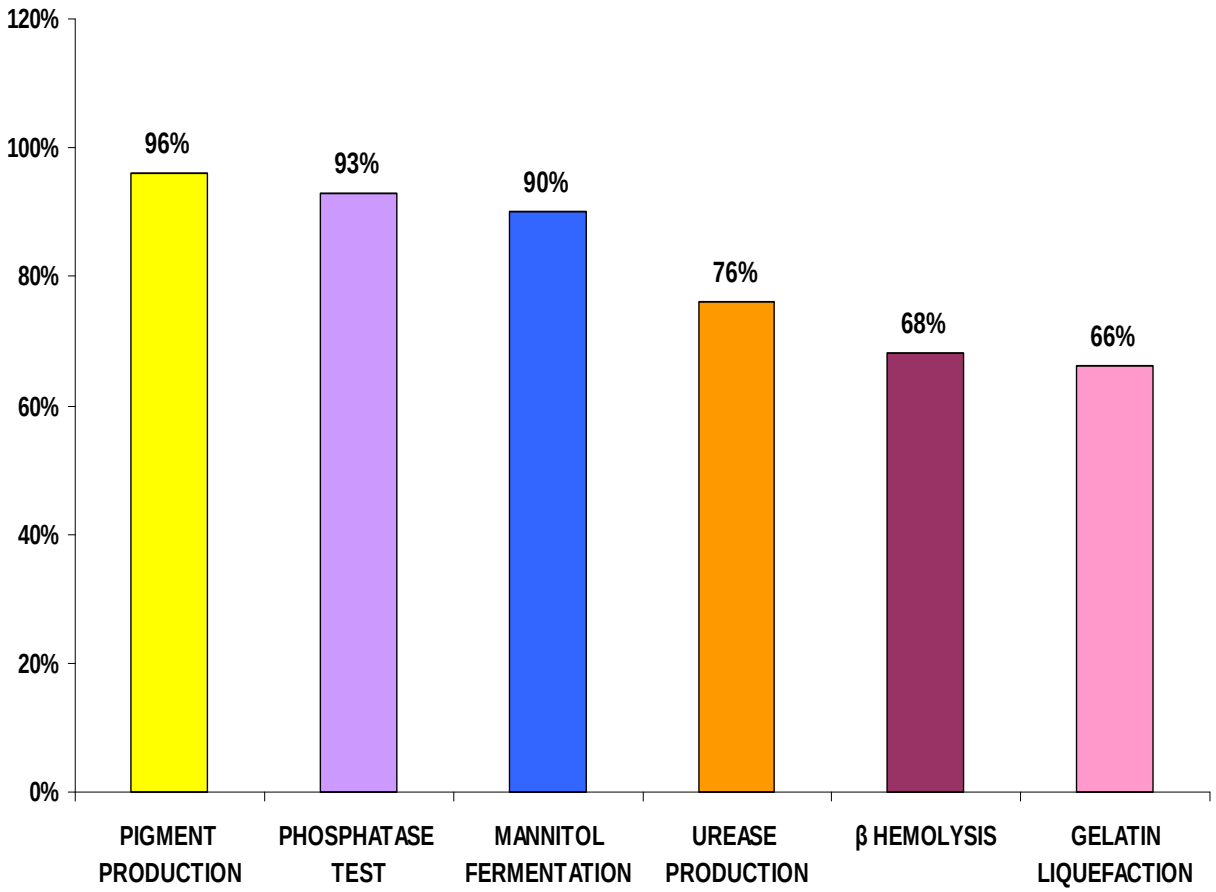


ANTIBIOGRAM OF 34 ISOLATES OF STAPHYLOCOCCUS AUREUS

S.No.	PENICILLIN	AMIKACIN	GENTAMICIN	CEFTAZIDIME	CEPHOTAXIME	CEFTRIAXONE	CIPROFLOXACIN	CEFOPERAZOLE	GATIFLOXACIN	AMOXICLAV	OXACILLIN
1	R	R	R	R	R	R	R	R	S	S	R
2	R	S	S	S	S	S	S	S	S	S	R
3	R	S	S	S	S	S	S	S	S	S	R
4	R	S	S	S	S	S	S	S	S	S	R
5	R	R	R	R	R	R	S	R	S	R	R
6	R	S	S	S	S	S	S	S	S	S	R
7	R	S	S	S	S	S	S	S	S	S	R
8	R	S	S	S	S	S	S	S	S	S	R
9	R	S	R	S	S	S	S	S	S	S	R
10	R	S	S	S	S	S	R	S	R	S	R
11	R	S	R	R	R	R	R	R	R	R	R
12	R	S	R	R	R	R	R	R	R	R	R
13	R	R	R	R	S	S	R	S	S	R	R
14	R	R	R	R	S	S	S	S	S	R	R
15	R	R	R	R	R	R	R	R	R	R	R
16	R	S	S	S	S	S	S	S	S	S	R
17	R	R	R	R	R	R	R	R	R	R	R

18	R	S	S	S	S	S	S	S	S	S	R
19	R	S	R	R	S	R	S	S	S	S	R
20	R	R	R	R	R	S	R	R	R	R	R
21	R	S	S	S	S	S	S	S	S	S	R
22	R	S	S	S	S	S	S	S	S	S	R
23	R	S	S	S	S	S	S	S	S	S	R
24	R	S	R	S	S	S	R	S	S	S	R
25	R	S	R	S	S	S	S	S	S	S	R
26	R	R	R	R	R	R	R	R	S	R	R
27	R	R	R	R	R	R	R	R	R	R	R
28	R	R	R	R	R	R	R	R	R	R	R
29	R	S	R	R	R	R	R	R	R	R	R
30	R	R	R	S	S	S	R	R	R	R	R
31	R	S	R	R	R	R	R	R	R	R	R
32	R	R	R	R	R	R	R	R	R	R	R
33	R	S	R	R	S	S	S	R	R	R	R
34	R	S	S	R	R	R	S	R	R	R	R

BIOTYPING OF OVERALL RANGE OF COAGULASE POSITIVE STAPHYLOCOCCUS AUREUS

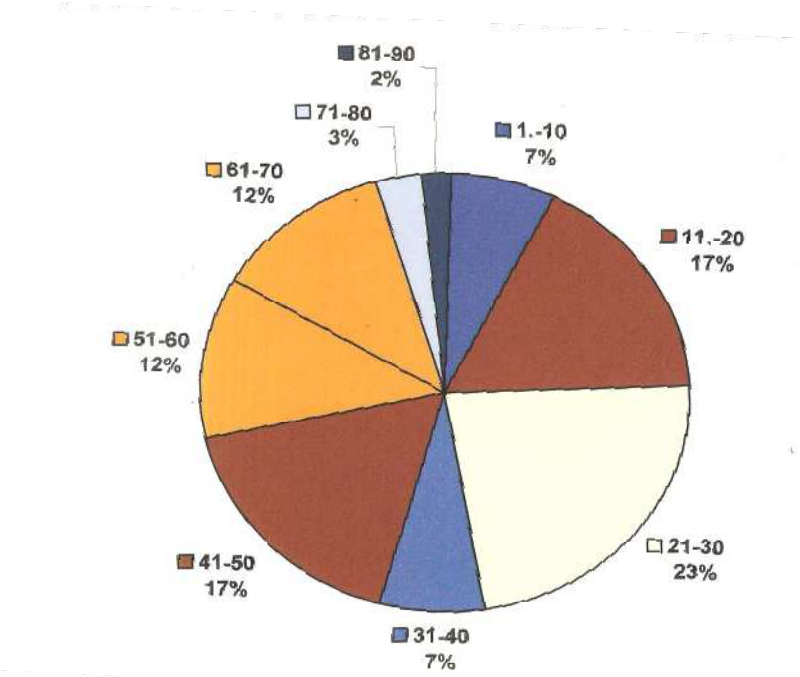


OVERALL RANGE OF ACTIVITY OF COAGULASE POSITIVE STAPHYLOCOCCUS AUREUS ISOLATED

TEST	POSITIVE STRAINS	NEGATIVE STRAINS	% OF POSITIVE STRAINS
Phosphatase Production	93	7	93%
Mannitol Fermentation	90	10	90%
Pigment Procuction	96	4	96%
Urease Production	76	24	76%
Gelatin Liquefaction	66	32	66%
β.Haemolysis	68	32	68%

Age Distribution

AGE	NO: OF CASES
01-10	07 Cases
11-20	17 Cases
21-30	23 Cases
31-40	07 Cases
41-50	17 Cases
51-60	12 Cases
61-70	12 Cases
71-80	03 Cases
81-90	02 Cases



Discussion:

MRSA are often found in the skin and in nose of healthy individuals who are carriers, Invasion of skin by MRSA occurs and some times get in to the blood stream to cause more svere infections in the internal organs.

MRSA strains are not only resistant to methicillin but also to many other types of antibiotics. Urinary catheters and tubes going into veins or parts of the body are contaminated by MRSA and lead to urinary infection and septicemia.

Infections with MRSA can be only treated with antibiotics that are administered intravenously as the choice of antibiotics are limited and organisms are resistant to most of the routine antibiotics. Risk of side effects with these antibiotics are more than the usual antibiotics.

It is observed that the previous studies there was a steady rise in the incidence of MRSA ranging from 0.05% to 56.62%.

An incidence of less than 10% was observed by Barber et al 1961(0.05%), Baired et al1962(0.2%)

Barber and water worth 1962(2.2%),Bulger rj et al 1989(4%),Connawal HNFL Sapico JZ Montogmetric et al 1991(4.5%),Boyce jm M Landry Tr Deetz Nd HI DUpont et al 1981(6%),

Brig VC Ohri 1985-1989(7%) & Barry A,.F Gracia LD Tharup et al 1970(9%).

There is an increased incidence of MRSA ranging from 11 to 35% supported by the studies of BARRET ff RF Maghee &M.Finland et al 1968(11%),Nanner aw,Wmm Kirby,J.C Sherries Nd H TUrck et al1976(11%),Banner and EJ Kauer et al 1079(12%),Rosdahl Vt Bravery I et al 1994(12.8%),Banner and Keyser,Routree and Baired et al 1968(15%),Okamato R Yomoda S et al 1981(18.9%),Nishjima et al 1995 (20-40%),Majumdar et al 2001(23.2%),Brig VC Ohri 1988-1989(33.6%),Brig Vc Ohri 1992(34.2%).

An incidence of more than 35% was observed by Mahalaxmi et al 2001(38%),Okomoto R Yomoda S et al 1990(44.8%),Anupurba et al 2003(54.85%) and Pandit Dakshayani 2000(56.62%)

My study revealed 34% which is agreeing with the study of Brig VC Ohri 1992(34.2%),Nishijima et al 1995(20-40%),Mahalaxmi 2001(38%).

Conclusion:

- The present study included 130 strains of staphylococci isolated from various clinical samples from all age groups.
- 100 were Coagulase Positive Staphylococci and 30 were Coagulase Negative Staphylococci.
- 96%Coagulase Positive Staphylococci were pigment Producing strains.
- Ataphylococcus aureus were isolated more in males.
- Majority of strains were frompus.
- The MRSA Percentage was 34%.
- All MRSA were Resistant to Pencillin and found to be 64.7% sensitive to Amikacin.
- Among Coagulase Positive Staphylococci 24 Strains were sensitive and 9 were resistant to the all antibiotics.
- Majority of the MRSA were sensitive to Cephotoxime(20 strains),Ceftriaxone(20) and Cefaperazone(18).

Appendix:

***CHRISTENSENS UREASE AGAR:-**

COMPOSITION:-

Peptone-1gm

Sodium Chloride-5gm

Dipotassium Hydrogen Phosphate-2gm

Phenol Red-6ml

Agar-20gm

Distilled water-1000ml

Sterilise the Basal Medium by Autoclave

10% glucose-10ml

20% urea solution-100ml

Sterilized by filteration.

Preparation:-The basal medium was sterilized by autoclave and cooled then glucose and urea were added. This broth was dispensed into sterile tubes approximately 4 -5ml per tube to get along slant, short butt this medium was allowed to cool in slanted position and stored in the refrigerator at 4 degrees Celsius.

***GELATIN:-**

COMPOSITION:-

Nutrient Agar-100ml

Gelatin-12gm

Preparation:-To 100ml of nutrient agar, 12gms of gelatin was added and sterilized by steaming at 100 degrees Celsius for 30 minutes the medium was dispensed in tubes and the tubes were stored in refrigerator at 4 degrees Celsius.

***HIGH SALT AGAR:-**

COMPOSITION:-

Nutrient Agar-100ml

Sodium Chloride-8gms

Preparation:-To 100ml of nutrient agar, 8gms of NaCl was added and sterilized by autoclave and then poured into sterile petridishes.

***MANNITOL SALT AGAR:-**

COMPOSITION:-

Nutrient Agar-100ml

Mannitol-1.0%

NaCl-7.55

Phenol Red-0.3%

Preparation:-To 100ml of nutrient agar,1% of mannitol,7.5% NaCl,0.3% Phenol red were added and sterilized by autoclave then the medium was poured into sterile petridishes.

***MUELLER HINTON AGAR:-**

COMPOSITION:-

Beef Infusion-30gms/lit

Caseinhydrolysate-17.5gm/lit

Starch-1.5gm/lit

Agar Agar-17gm/lit

Ph-7.4

Preparation:-Emulsify the starch in asmall amount of cold water, pour into beef infusion and add the casein hydrolysate and the agar, make up the volume to 1liter with distilled water, dissolve the constituents by heating gently at 100 degrees clesius with agitation. Filter if necessary.Adjust the ph 7.4 dispense in screw caped bottles and sterilized by autoclaving at 121 degree Celsius for 20minutesand pour into plates.

***PHENOLPHTHALEIN PHOSPHATE AGAR:-**

COMPOSITION:-

Nutrient Agar-98ml

sodiumPhenolphthalein Diphosphate Agar 0.6% solution-2ml

Preparation:-sodium phenolphthalein diphosphate was taken from afresh batch and dissolved in water to 0.6% concentration sterilized by filteration and 2ml was added to 98ml of melted nutrient agar then the medium was poured into petridishes.

***SHEEP BLOOD AGAR:-**

COMPOSITION:-

Peptone-1gm

Beef Extract-1gm

Nacl-500mg

Agar Agar-2.5gm

Sheep Blood-5-10ml

Distilled water-100ml

Preparation:-To 100ml of distilled water 1gm of peptone, 1gm beef extract and 500mg Nacl were added.ph was adjusted to 7.2 then the agar agar 2.5gm was added. Sterilized by autoclave and cooled to 50 degrees Celsius.5-10ml of sheep blood was added and the flask was shaken well and the medium was poured into sterile petridishes.

***POTASSIUM TELLURITE:-**

COMPOSITION:-

Blood Agar-100ml

Potassium Tellurite-1ml(35gm/lit)

Preparation:-To 100ml of blood agar,1ml of potassium tellurite was added before pouring into plates.

***MILK AGAR:-**

COMPOSITION:-

Nutrient Agar-100ml

Skimmed Milk-50ml

Preparation:-To 100ml of nutrient agar,50ml of sterilized milk was added and then poured into sterile petridishes.

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