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Study and Isolation of Skin Microflora and their Effects on Human Health

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Abstract:- *Staphylococcus species* are Gram positive, cocci shaped bacteria found especially in the wounds of a person. Skin micro flora is the major source of this species and present in the upper epidermis of it. Skin samples were collected to isolate the *Staphylococcus* bacteria and studied their resistance towards antibiotics. In this thesis, samples of different communities were taken by sterilized swap sticks and inoculate. Mannitol Salt Agar Media specialized for *Staphylococcus Species* was used to culture the incubated broth tubes to study the morphology. Biochemical test and Antibiotic Sensitivity Tests were performed to analyze the enzyme activity and Sensitivity Resistance towards antibiotics, respectively. This study revealed the two species of *Staphylococcus- aureus* and epidermidis and their morphology and biochemical characteristics along with resistance towards some antibiotics.

INTRODUCTION

The skin serves as a natural protective barrier against invasion by most microorganisms because of the acidity of the skin, the presence of indigenous flora, the temperature of the skin sebum secreted by oil glands and creation of hypertonic environment by the salts in perspiration. However perspiration and sebum serve as a source of nutrition for certain micro organism and help in their establishment as normal flora of the skin most encountered bacteria on the skin are gram positive cocci including micrococcus and *staphylococcus*. Micrococcus is generally considered to be a saprophyte while *staphylococci* which include 40 species, most of them are harmless and reside normally on the skin and mucous membranes of humans and other organisms. *Staphylococcus aureus* which is a member of the normal flora of the skin is an important and frequently encountered human pathogen. Sebum secreted from oil glands helps the survival of *propioniobacterium acnes*, an anaerobic gram-positive rod in hair follicles. The so-called normal flora (or biota) of the skin vary according to the skin region and the environmental factors. Most pathogenic organisms do not penetrate the unbroken skin but as soon as epidermis is destroyed infection easily occurs. Staphylococci and certain fungi are able to penetrate the hair follicles and cause disease in the deeper tissues. Although human skin harbours many different genera, this exercise deals with the isolation and identification of catalase-positive, salt tolerant members of gram-positive cocci belonging to the family *Micrococcaceae*(*Actinobacteria*) and *Staphylococcaceae (Fimicutes).*

METHODOLOGY

Chemical; Nutrient Broth - HIMEDIA M001- 500 Gms
Mannitol Salt Agar- Microgen – DM1118.
Mueller Hinton Agar: - Microgen – DM 1173.
BIO- CHEMICALS MEDIA:-Coagulase Mannitol Agar Base- HIMEDIA- M272- 100gm.
ANTIBIOTICS:-HEXA G- PLUS 1- HIMEDIA- HX001- 1PK

Antibiotics	Concentration
Penicillin G	10 mcg
Oxacilliin	1mcg
Cephalothin	30mcg
Clindamycin	2mcg
Erythromycin	15mcg
Amoxcyclav	30mcg

TABLE:-1

Antibiotics	Symbol	Concentration
Tetracycline	TE	30mcg
Co- Trimoxazole	COT	25mcg
Cloxacillin	COX	1mcg
Lincomycin	L	2mcg
Cefuroxime	CXM	30mcg
Cefotaxime	CTX	30mcg

TABLE:-2

HEXA G- PLUS 5- HIMEDIA- HX005- 1PK.

Sample collection - From skin of different communities -From railways station sample, Slum area. The study was undertaken in Sadhu Vaswani College, Botany & Biotechnology Department between Feb to May, 2014. (32) Clinical specimens taken by swab from various aged individuals from different communities such as -- with different locations of skin under sterile conditions. The staphylococcus species procedure is determining the identity of Staphylococcus species as below:

Composition of Ingredients-

Ingredients	Gms / Litre
Protease peptone	10 gm
Sodium chloride	75gm
Beef extract	10gm
D-Mannitol	1.5gm
Phenol red	0.025gm
Agar	15gm
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Composition of Ingredients-

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.0 gm
Sodium chloride	5.0gm
Beef extract	1.5gm
Yeast extract	1.5gm
Agar	15gm
Final pH (at 25°C)	7.4±0.2

**Formula adjusted, standardized to suit performance parameters

Growth in Liquid media:

Nutrient Broth is used for the general cultivation of less fastidious microorganisms, can be enriched with blood or other biological fluids.

Composition of Ingredients-

Ingredients	Gms / Litre
Peptic digest of animal tissue	5.0 gm
Sodium chloride	5.0gm
Beef extract	1.5gm
Yeast extract	1.5gm

**Formula adjusted, standardized to suit performance parameters.

*Composition***

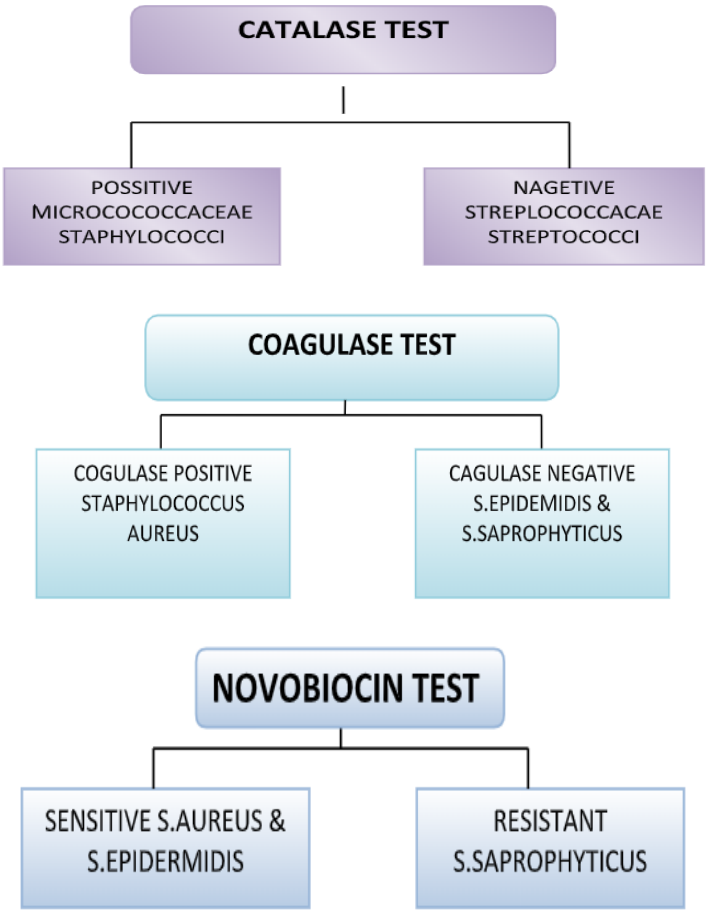
Ingredients	Gms / Litre
Peptic digest of animal tissue	10.000
Sodium chloride	5.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Study of External Morphology by staining procedure:

Gram staining Ingredients	Composition	
Grams Crystal Violet (S012)	Ingredients	Grams/Litre
	Crystal violet	2.0 gm
	Ammonium oxalate	0.8 gm
	Ethyl alcohol 95%	20.0 ml
	Distilled water	80.0 ml
Grams Iodine (S013)	Iodine	1.0 gm
	Potassium iodide	2.0 gm
	Distilled water	300.0 ml
Grams Decolourizer (S032)	Ethyl alcohol (95%)	50 ml
	Acetone	50ml
Safranin (S027), 0.5% w/v (Gram's Counter stain)	Safranin	0.05gm
	Ethyl alcohol 95%	100ml

Biochemical tests:



Antibiotic Sensitivity Test

To test the susceptibility of *Staphylococcus Species* towards antibiotics.

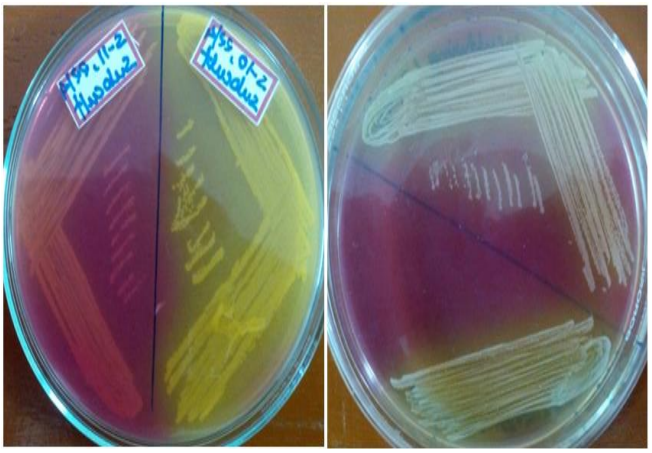
1. Mueller Hinton Agar media was prepared for different bacterial cultures.
2. Prepared discs of Hexa G plus 1 and Hexa G plus 5 were used.
3. Mueller Hinton Plates were inoculated with different bacterial culture and prepared discs were placed on the plates and incubated for 24 hours.
4. Zone of inhibition were observed and studied.

RESULTS AND DISCUSSION

In the result, two species of *Staphylococcus Species* were isolated. They are-

- *Staphylococcus aureus and*
- *Staphylococcus epidermidis*

Study on their enzyme activity through biochemical test and susceptibility towards different antibiotics were also studied over *Staphylococcus Species.*



Subculturing plates of *staphylococcus species.*
pure culture of *staphylococcous epidermidis.*

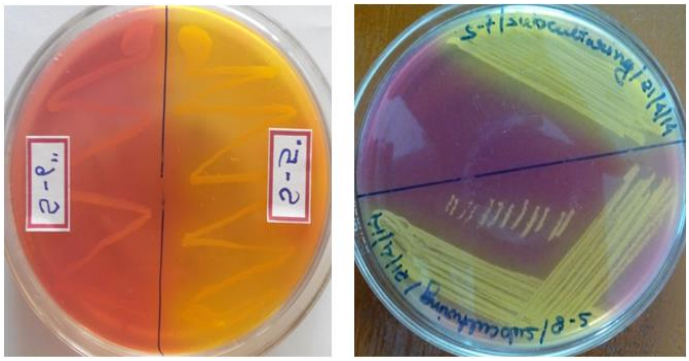
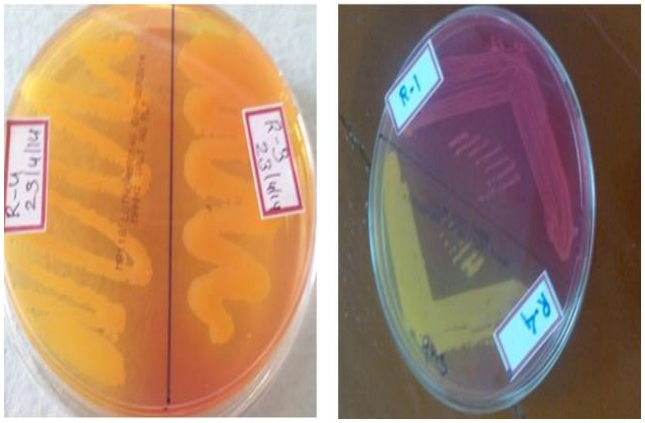


Plate showing cultures of two different species
pure culture of *staphylococcus aureus* of *staphylococcus* from slum area in mannitol salt agar.



Culture plate of *Staphylococcus Epidermidis*
pure cultures of *Staphylococcus Aureus and*
From railways station sample
Staphylococcus Epidermidis

Pure culture plate of *Staphylococcus species*
NAM media showing *Staphylococcus aureus &*
Staphylococcus epidrmidis.

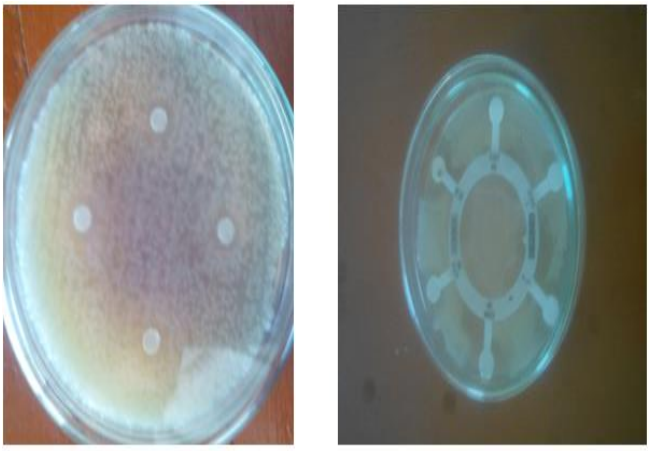


Plate showing antibiotic sensitivity of Staphylococcus.

DISCUSSION

S. Aureus and *S. Epidermidis* have emerged as an important cause of infections in patients with indwelling medical devices. Slime as a virulence factor of *S. Epidermidis* in medical implant/device related infections was documented in the past. It was also shown that comparatively higher numbers of slime producing isolates of S. epidermidis from the keratitic lesions were adherent to artificial surfaces as compared to the commensally isolates from the eye. Breakage of the skin barrier may lead to the transformation of *S. aureus* from a commensally colonizer to an invading pathogen, and its multitude of virulence factors enables it to adhere to and survive on and in the host cells. In most categories of hospitalized patients, S. aureus nasal carriage has been identified as a major risk factor for developing subsequent infections. Asserting that *S. aureus* infections are of endogenous origin is supported by studies revealing that isolates from nosocomial S. Aureu s infections were identical to nasal carrier isolates in 80% or more of the patients. In non-surgical patients who were *S. ureus* nasal carriers, the risk of acquiring a nosocomial *S. aureus* bacteraemia was three times higher than in no carriers. To gain an understanding of *S. aureus* nasal carriage and the connection with subsequent infection, the *S. aureus* population structure needs to be defined. In our studies, we investigated the population structure of *S. aureus* in adult nasal carriers from a general population and among healthcare workers.

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