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Identification and Prevalence of Coagulase-Negative Staphylococci Species from Various Clinical Specimens at a Tertiary Care Hospital in the North-West Region of Rajasthan

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Abstract

Coagulase-negative staphylococci (CoNS) are increasingly recognized as significant opportunistic pathogens, particularly in hospital settings. This prospective study aimed to determine the prevalence and identify CoNS species from various clinical specimens at a tertiary care hospital in North-West Rajasthan, India. A total of 600 clinical specimens were processed, and 105 CoNS isolates were identified using biochemical tests. The prevalence of CoNS was 17.50%. Blood samples had the highest positivity rate (38.19%), followed by urine (25.71%). S. epidermidis was the predominant species (27.61%), followed by S. hemolyticus (20%). CoNS are significant opportunistic pathogens, and accurate identification is crucial for effective patient management.

Keywords: Coagulase-negative staphylococci, Prevalence, Clinical specimens, S. epidermidis.

Introduction

Coagulase-negative staphylococci (CoNS) have long been considered harmless commensals, primarily colonizing human skin and mucous membranes. However, over the past few decades, their role has shifted dramatically from mere contaminants to significant opportunistic pathogens, particularly in hospital settings. This change in perception is driven by their increasing prevalence in various clinical infections and their growing resistance to a wide range of antimicrobial agents [1-3].

Historically, the pathogenic potential of CoNS was largely overlooked. Early reports noted their association with specific conditions like urinary tract infections and endocarditis, but these were considered rare occurrences. The paradigm truly shifted with the rise of modern medicine and the increased use of invasive procedures and indwelling medical devices. Surgical procedures, such as ventriculocaval shunts for hydrocephalus, created new niches for CoNS to colonize, leading to serious bloodstream infections and device-related complications. Staphylococcus epidermidis, in particular, has emerged as the leading cause of such infections due to its unique ability to form biofilms on medical implants, making it

highly resistant to both host immune defenses and antibiotics [4,5]

The identification of CoNS species beyond basic coagulase testing has become critical for both clinical management and epidemiological surveillance. Advances in microbiology have allowed for more precise classification based on biochemical and genetic properties[2]. This is crucial because different CoNS species vary significantly in their virulence and antimicrobial susceptibility profiles [6,7].

In India, especially in the context of tertiary care hospitals, the challenge posed by CoNS is particularly significant. A high rate of methicillin-resistant Staphylococcus aureus (MRSA) and other multidrug-resistant organisms has been well documented, suggesting a similar trend for CoNS. Therefore, understanding the local prevalence of CoNS species and their specific[1,8].

Antimicrobial susceptibility patterns is essential. This data is critical for guiding empirical antibiotic therapy, implementing effective infection control measures, and combating the silent but significant threat of antimicrobial resistance[4,7].

The clinical importance of CoNS is now widely acknowledged. They are a leading cause of hospital-acquired infections, particularly in immunocompromised patients and those with indwelling medical devices. The rise in antibiotic resistance among these species, including methicillin resistance, further complicates treatment[9,10]. Therefore, accurate identification and antimicrobial susceptibility testing of CoNS isolates are critical for effective patient management [10,11].

This study aims to address this gap by comprehensively identifying the prevalent CoNS species from various clinical specimens at a tertiary care hospital in the North-West region of Rajasthan. The findings will provide valuable local data to aid clinicians and microbiologists in making informed decisions for patient care and in developing effective strategies to control the spread of these increasingly important pathogens.

Materials and Methods

Study Design and Setting-This prospective study was conducted at the Department of Clinical Microbiology & Immunology, Sardar Patel Medical College, Bikaner, Rajasthan, from February 2021 to November 2021. The aim was to determine the prevalence of Coagulase-Negative Staphylococci (CoNS) species and find out the risk factors from associated with various CoNS species from various clinical specimens collected at PBM Hospital and its associated facilities.

Sample Collection and Criteria-Clinical specimens, including blood, urine, pus, and other body fluids, were collected using standard aseptic techniques[12].

- **Inclusion Criteria[19,22]:** We included all CoNS isolates from pure cultures. For blood cultures, significance was defined as the same strain isolated from two or more samples within five days. Urine samples with high colony counts (>10^5 CFU/ml) and isolates from patients with clear clinical signs of infection were also included.
- **Exclusion Criteria:** We excluded mixed growths, non-Staphylococcus species, and isolates from patients already on antibiotic therapy.

Laboratory Procedures and Speciation-All samples were processed immediately. Initial identification of Staphylococci was based on colony morphology, Gram staining, and a negative slide and tube coagulase test. We excluded Micrococci using tests like the modified oxidase test [1,4,11].

CoNS isolates were speciated using the comprehensive biochemical scheme developed by Kloos and Schleifer, as modified by Bannerman. This method involved a

series of tests to determine sugar fermentation, nitrate reduction, urease, and novobiocin susceptibility[8,9,10].

Results

The present study was conducted in Department of Microbiology and immunology, Sardar Patel Medical College, for identification and to detect prevalence of cogulase-negative staphylococci and its species in this zone, in various clinical specimens such as blood, urine, pus, CSF, joint fluid, vaginal swab, stitch line, pleural fluid, broncho-alveolar lavage, bronchial-aspirate samples.

Total of 600 samples were collected from pentiens attending different outdoors & indoors of P.B.M. and associated group of hostpitals. Total of 105 strains of CoNS isolated in pure form.

Following various observations were made during the study.

Out of 600 samples, 105 (17.50%) were isolated as Coagulase-negative Staphylococcus. So the isolation rate was 17.50%. The highest number of strains were isolates from the age group 0-15 years 40 (38.09%), followed by the age group 15-30 years 14 (13.33%), 30-45 years 12 (11.42%), 45-60 years 19 (18.09%) and 20 (19.04%) cases belonged to >60 years of age group and out of these 105 positive cases, 63 (60%) were male and 42 (40%) were female. Thus showing the male predominance.

Table 1: CoNS isolated from different specimens

Sn.	Name of sample	No. of sample	Cases positive for CoNS	
			No.	Percentage (%)
1.	Blood	132	38	38.19%
2.	Urine	260	27	25.71%
3.	Stitch line	66	06	5.71%
4.	Pus	42	09	8.57%
5.	Joint fluid	21	04	3.80%
6.	Urinary catheter tip	30	09	8.57%
7.	Body fluid	12	04	3.80%
8.	CSF	21	01	0.95%
9.	Other sample	16	07	6.66%
Total		600	105	100%

Maximum number of Coagulase-negative Staphylococci were isolated from blood 38 (38.19%), followed by urine 27 (25.71%), pus and urinary catheter tip 09 (8.57%), Stitch line 06 (5.71%), body fluid 04 (3.80%), CSF 01(0.95%) and other samples 07 (6.66%). (Table 1).

Graph 1: Frequencies of CoNS in different specimens

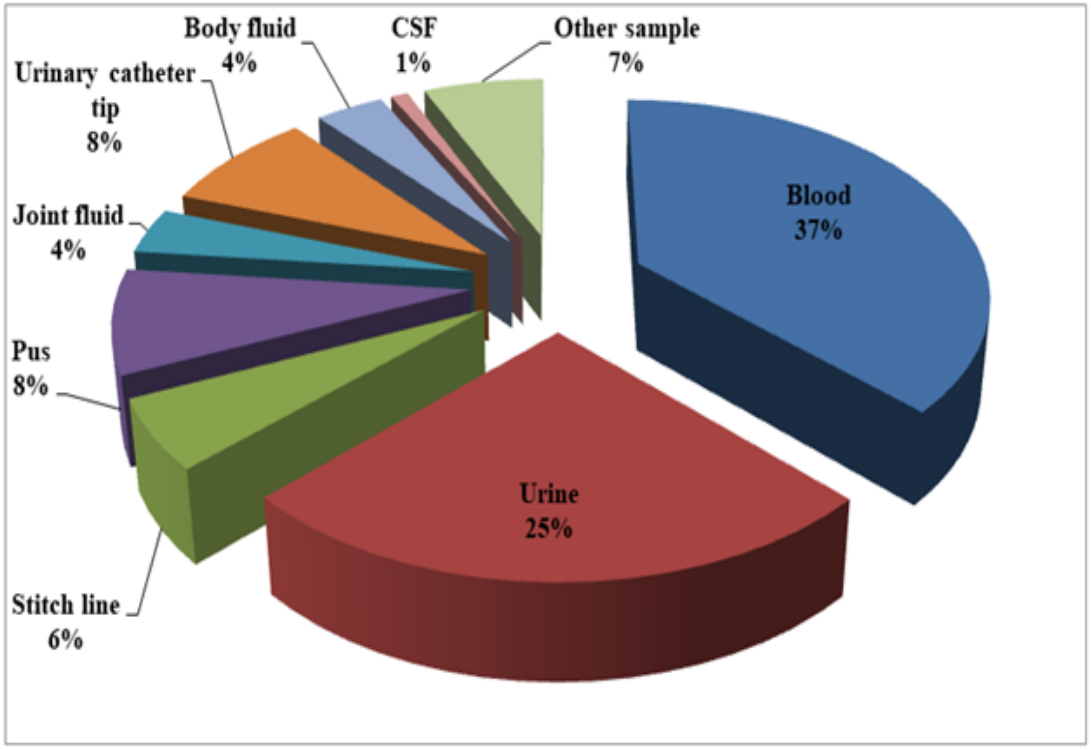


Table 2: Distribution of CoNS among various infections

Various infections	Total CoNS
Surgical wound infections	15 (14.28%)
Septicemia/Blood stream infection	26 (24.76%)
Osteomyleitis (prosthetic implant)	2 (1.90%)
Diabetic wound infection	12 (11.42%)
Burns	19 (18.09%)
Cellulites, Furuncle	7 (6.66%)
Urinary tract infections	18 (17.14%)
Prosthetic wound infection	4 (3.80%)
Deep seated abscesses	2 (1.90%)

Maximum number of CoNS were isolated from patients of Septicemia/Blood stream infection 26 (24.76%) followed by Burns 19 (18.09%), urinary tract infection 18 (17.14%), Surgical wound infections 15 (14.28%), Diabetic wound infection 12 (11.42%), Cellulitis, Furuncle 7 (6.66%), Prosthetic wound infection 4 (3.80%) and least from Deep-seated abscesses and Osteomyleitis 2 (1.90%).(Table 2).

Graph 2: Distribution of CoNS among various infections

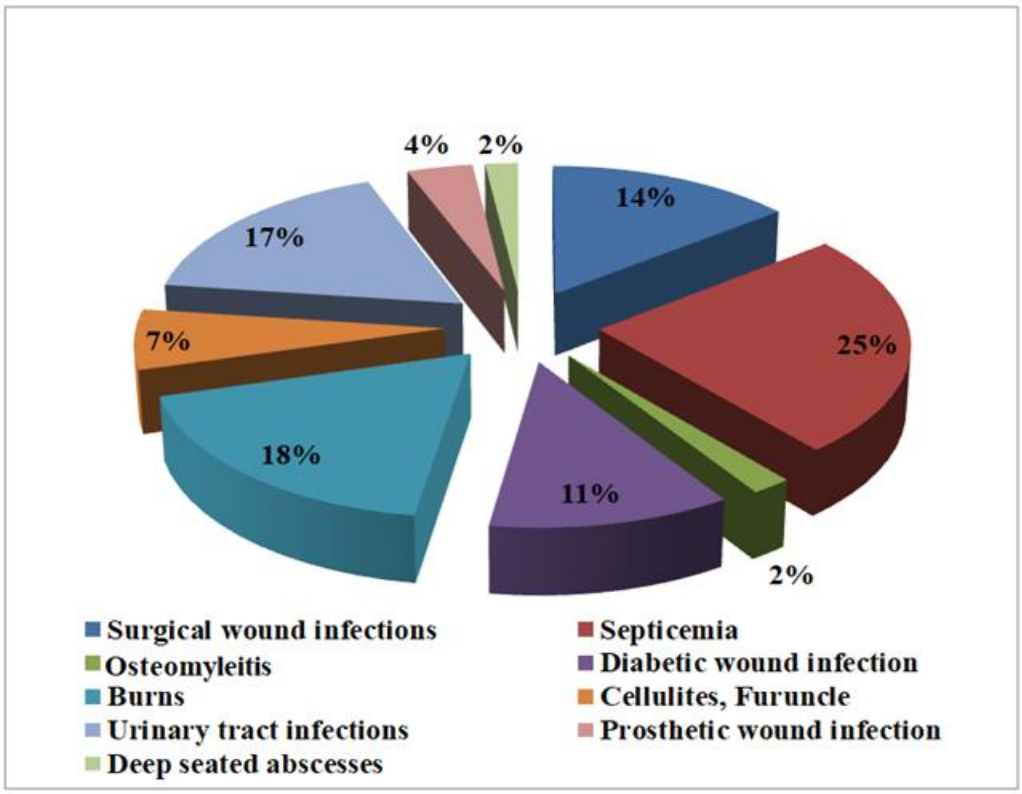
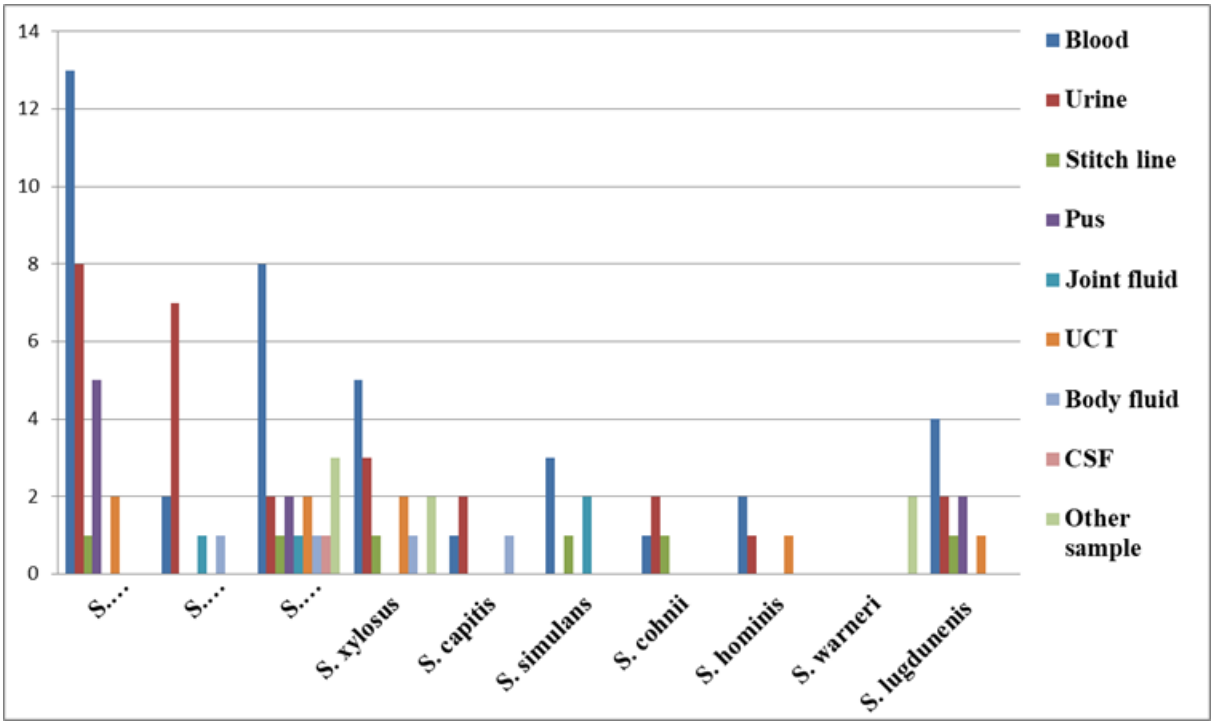


Table 3: Species distribution of CONS among different clinical samples

Species	Blood	Urine	stitch line	Pus	Joint fluid	UCT	Body fluid	CSF	Other sample	Total
S. epidermidis	13	8	1	5	0	2	0	0	0	29
S. saprophyticus	2	7	0	0	1	0	1	0	0	11
S. hemolyticus	8	3	1	2	1	2	1	1	2	21
S. xylosus	5	3	1	0	0	2	1	0	2	14
S. capitis	1	2	0	0	0	0	1	0	0	4
S. simulans	3	0	1	0	2	0	0	0	0	6
S. cohnii	1	2	1	0	0	0	0	0	0	4
S. hominis	2	1	0	0	0	1	0	0	0	4
S. warneri	0	0	0	0	0	0	0	0	2	2
S. lugdunenis	4	2	1	2	0	1	0	0	0	10
Total	38	27	06	09	04	09	04	01	07	105

S. epidermidis, S. hemolyticus, S. lugdunesis, and S. xylosus were more common in a blood culture respectively. S. saprophyticus was commonly isolated in urine and S. epidermidis, S. hemolyticus, and S. lugdunesis were also found in pus and other cilinical specimens. (Table 3)

Graph 3: Species distribution of CONS among different clinical samples



Discussion

The main problem in reporting of CoNS isolated from any clinical sample is to rule out either colonization or contamination and report it as a true pathogen. In circumstances where false positive CoNS reports are given, it leads to not only unnecessary treatment, increase in cost but also further increases antimicrobial resistance. Repeated isolation of CoNS from clinical specimen of same pateint are useful in predicting true infections.[10]

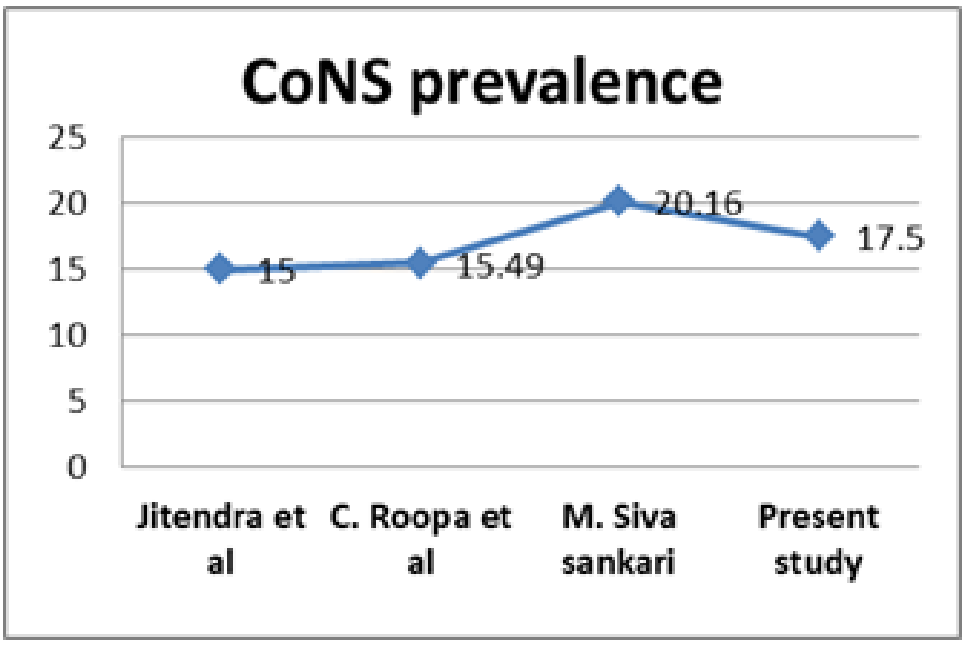
This study was undertaken to find out the prevalence of coagulase-negative Staphylococcus species in the various clinical specimens. Total 600 clinical specimens of all ages and both sexes were studied. Among these, 105 (17.50%) were found to be positive for coagulase-negative Staphylococci.

In the present study, out of 600 patients specimens 105 (17.50%) were found positive for CoNS infection. similar results were seen in other studies such as Jitendra et al (2017)[13] (15%), C. Roopa et al (2015)[14] (15.49%) and M. Siva sankari (2020)[15] (20.16%).

Table 4:

Name	Place	Year	CoNS prevalence
C. Roopa et al	Karnataka	2015	15.49%
Jitendra et al	Jaipur	2017	15%
M. Siva sankari	Chennai	2020	20.16%
Present study	Bikaner	2021	17.50%

Graph 4:



Sample wise distribution

In the present study coagulase-negative Staphylococcus were isolated most frequently from blood samples (38.19%) followed by urine (25.71%), pus & UCT (8.57%), stitch line (5.71%), body fluid (3.80%) and other specimens (6.66%). Similar observation was also reported by Priya Datta et al (2018)[16] who isolated CoNS mostly from blood (56.66%) and urine (43.33%), Maj Puneet Bhatt et al (2014)[17] isolated mostly from blood (34.7%) and catheter tip (28.7%), Y. Kavitha et al (2014)[18] isolated mostly from blood (31.65%) and urine (30.8%), Dimple Raina et al (2020)[19] isolated mostly from blood (45%) and pus (21.6%), M M Ehsan et al (2013)[20] isolated mostly from blood (45.9%) and pus (36.7%), Xiao Xue Ma et al (2011)[21] isolated mostly from blood (47.3%) and wound (10.5%).

Age wise Distribution

In the present study (table 3), out of 105 cases maximum numbers of positive cases were found in the age group of 0-15 years that was (38.09%) followed by age group >60 (19.04%), this is than followed by age group 46-60 (18.09%), age group 16-30 (13.33%) and while only (11.42%) cases were belong to age group 31-45 years.

In other study by C. Roopa et al (2015)[14], maximum numbers of positive cases were from age group between 61-70 years i.e. (20.53%) while minimum from <10 years of age i.e. (7.14%) and by Jitendra et al (2017)[13], maximum numbers of positive cases were from age group between 21-30 years i.e. (25%) while minimum from <10 years of age i.e. (7.25%).

So most of the patients were children and older age group i.e. 0-15 years and age >60 years. This could be explained as due to decreased immunity, prolonged hospitalization (nosocomial infections by opportunistic microorganism) and other associated co-morbidities in these age groups.

Sex wise distribution

In the present study (table 4) number of positive cases for CoNS was found to be higher in males 63 (60%) as compared to females 42 (40%). Other studies also reported a higher incidence of CoNS in males (52.33%) than females (46.67%) as Sby Jitendra et al (2017)[13], CoNS in males were (48%) and females were

(36%), as by Mariraj et al (2017)[22], CoNS in males (59%) and females (41%), as by Usha et al (2013)[23], and CoNS in males (64.9%) and females (36.1%), as by Golia et al (2015)[24].

More incidence in males during the present study may attribute to the fact that the majority of the males are more exposed to infection as compared to females due to their outdoor activities.

Distribution of various infections

In present study (table 5) maximum number of CoNS isolated from septicemia/blood stream infection 26 (24.76%) followed by Burns 19 (18.09%), urinary tract infection 18 (17.14%), surgical wound infections 15 (14.28%), diabetic wound infection 12 (11.42%), cellulites, furuncle 7 (6.66%), prosthetic wound infection 4 (3.80%) and least from deep seated abscesses and Osteomyleitis 2 (1.90%).

In other study by M. Siva sankari (2020)[15], maximum number of CONS were isolated from urinary tract infection (23.3%) followed by diabetic wound infection (18.3%) and burns wound infection (15%).

Distribution of CoNS species

In the present (table 6) study, out of total 600 cases, 105 (17.50%) were positive for CoNS infections, out of these 29 (27.61%) yielded S. epidermidis, 21 (20%) yielded S. hemolyticus, 14 (13.33%) S. xylosus, 11 (10.47%) S. saprophyticus and 10 (9.52%) S. lugdunensis, 06 (5.71%) S. simulans, 04 (3.80%) S. capitis, S. hominis and S. cohnii and least 02 (1.09%) S. warneri.

Similar result was seen as S. epidermidis (38.33%), S. saprophyticus (35%) and S. hemolyticus (15%), by Jitendra et al (2017)[13], S. epidermidis (56.60%) and S. haemolyticus (21.60%) by Priya Datta et al (2018)[16], and S. epidermidis (29.7%) and S. haemolyticus (20.2%) by Mariraj et al (2017)[22].

S. epidermidis was more commonly isolated species in other studies also i.e. Usha et al (2013)[23], Rajyalakshmi Gunti et al (2016)[25], Golia et al (2016)[24] and C. Roopa et al (2015)[14] 32%, 42%, 46.3% and 50.8% respectively.

Conclusion

Among the 600 Staphylococcal isolates, 105 were coagulase-negative Staphylococci. Speciation of CoNS was done by using the simplified scheme proposed by Kloos and Schleifer (1975) method, S. epidermidis was found to be the predominant organism followed by S. hemolyticus. This simple and inexpensive methodology will be useful in a routine microbiology laboratory for the presumptive identification of CoNS.

The clinical significance of CoNS is increasing in indwelling device related infections, endocarditis, septicaemia, blood stream infections and urinary tract infections. It is therefore recommended to know the clinically relevant CoNS upto species level and their antibiotic susceptibility during course of infection, which will help in administrating appropriate antibiotics. These in turn will reduce morbidity, mortality and prevent implant removal. Taking into consideration that the etiological importance of CoNS has been often neglected, the present study confirmed their pathogenic role, therefore these microorganisms should not be ignored or classified as mere contaminants.

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Legend Figures:

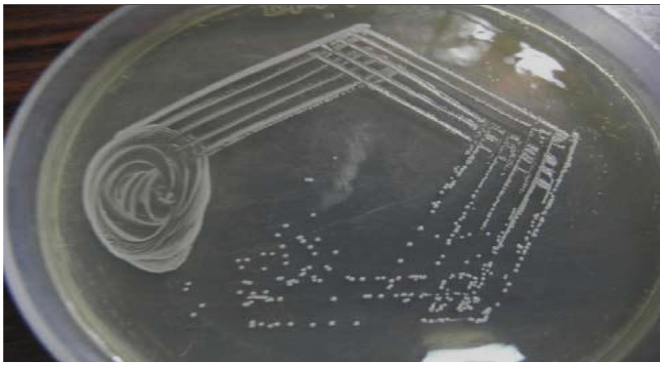


Figure 1: Nutrient Agar Medium-White Opague Colonies

Figure 2: Stphylococcus Species-Mannitol Salt Agar

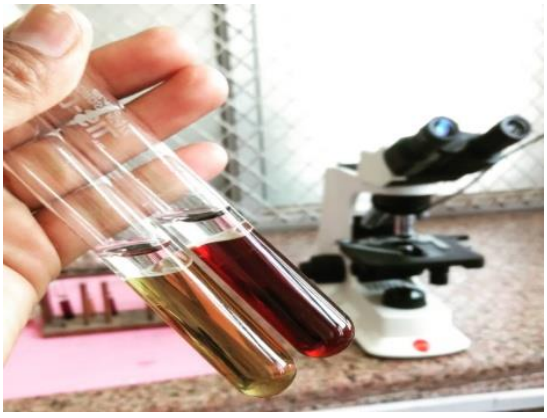
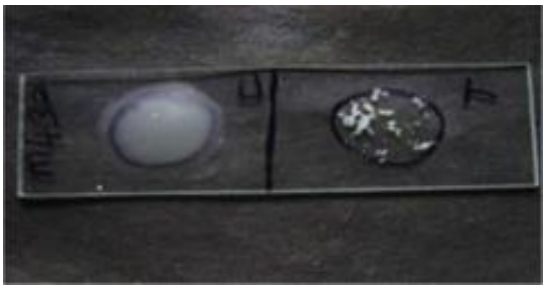


Figure 3: Ornithine Decarboxylase Test



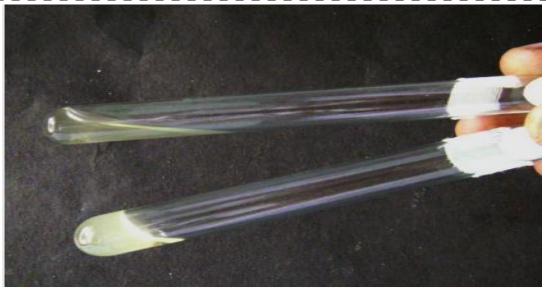


Figure 4: Slide and Tube Coagulase Test