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Slime production and antibiotic sensitivity pattern of coagulase negative staphylococci isolated from clinical specimens

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Abstract

Background
Coagulase negative staphylococci (CONS) have become a common cause of nosocomial infections, particularly bloodstream infections and infections related to the indwelling foreign devices like prosthesis. These organisms when attached to the surfaces of foreign bodies may produce an extracellular slime allowing for the persistence of coagulase negative staphylococci on the catheters. Slime production plays an important role in the pathogenesis and drug resistance of CONS infections.

Objective
This study was therefore undertaken to know the incidence of CONS in human infections, role of slime production in its pathogenicity and to study antibiogram of the isolates.

Materials and Methods
100 strains of CONS isolated from different clinical specimens were taken, identified on the basis of cultural characteristics and were classified into biotypes. Further slime test was done to study production of slime to determine its role in the pathogenicity of CONS. All the isolated strains of CONS were subjected to drug sensitivity by disc diffusion method.

Results
Maximum age incidence of 35% was in the age group of 21-30 years and minimum incidence of 5% was in the age group of 51-60 years. Out of 100 cases,46 were males and 54 were females with a male: female ratio of approximately 1:1.17. Out of 71 *Staph. epidermidis* , in 20 cases slime test was positive. Out of 29 cases of *Staph. saprophyticus*, 2 cases gave slime test positive and in 27 cases, slime test was negative. Slime producing strains of CONS were more resistant to antibiotics like gentamicin and kanamycin in comparison to non slime producing strains.

Conclusion
Multiple drug resistance has been shown by the slime producing strains of CONS as compared to non slime producing strains thus proving slime production as a marker of pathogenicity in CONS.

Keywords: Slime production, Coagulase- negative staphylococci, nosocomial infections

Introduction

Previously considered solely as commensal bacteria of the skin, Coagulase negative staphylococci (CONS) are now recognised as major cause of nosocomial and opportunistic infections[1] CONS have become the 3rd cause of nosocomial bloodstream infections as a result of the combination of increased use of intravascular devices and an increased number of hospitalised immunocompromised patients.[2] According to Baird-Parker Classification, CONS have been divided into *Staphylococcus epidermidis* biotypes 1-4 and *Staphylococcus saprophyticus* biotypes1-4. [3]

Among the CoNS species, *S. epidermidis* is the most common organism, amounting from 50 to 80% of isolates causing surgical site infections, prosthetic device associated infections, peritoneal dialysis related infections, cerebrospinal fluid shunt and ophthalmic infections. *S. saprophyticus* is the second most common cause of urinary tract infections accounting for 11-32% of UTI in female outpatient.[4]

These organisms when attached to the surfaces of foreign bodies may produce an extracellular slime which solidifies attachment, prevents the access of effective antimicrobial agents to the cell surface thereby allowing for the persistence of coagulase negative staphylococci on the catheters[5] and aids resistance against cellular host defenses. [6] Pathogenicity was much more likely to be associated with slime producing strains than with slime negative organisms. Both slime production and the species of the organism appeared to be important factors in the determination of pathogenicity.[7]

It has been found that the strains isolated from hospital patients and from hospital environment are commonly resistant to many antibiotics. .[8] These infections are difficult to treat because of the risk factors and multi drug resistant nature of the organisms. [9]In view of multiple drug resistance of these organisms, prevention and control of such infections cannot be over emphasised. Control is only possible by strict aseptic techniques, proper antibiotic policy and good epidemiological study. [8]

The present work was therefore undertaken to know the incidence of Coagulase Negative Staphylococci (CONS) in human infections, to study slime producing activity of CONS to determine if slime production could be used as a pathogenic marker and to study antibiogram of the isolates.

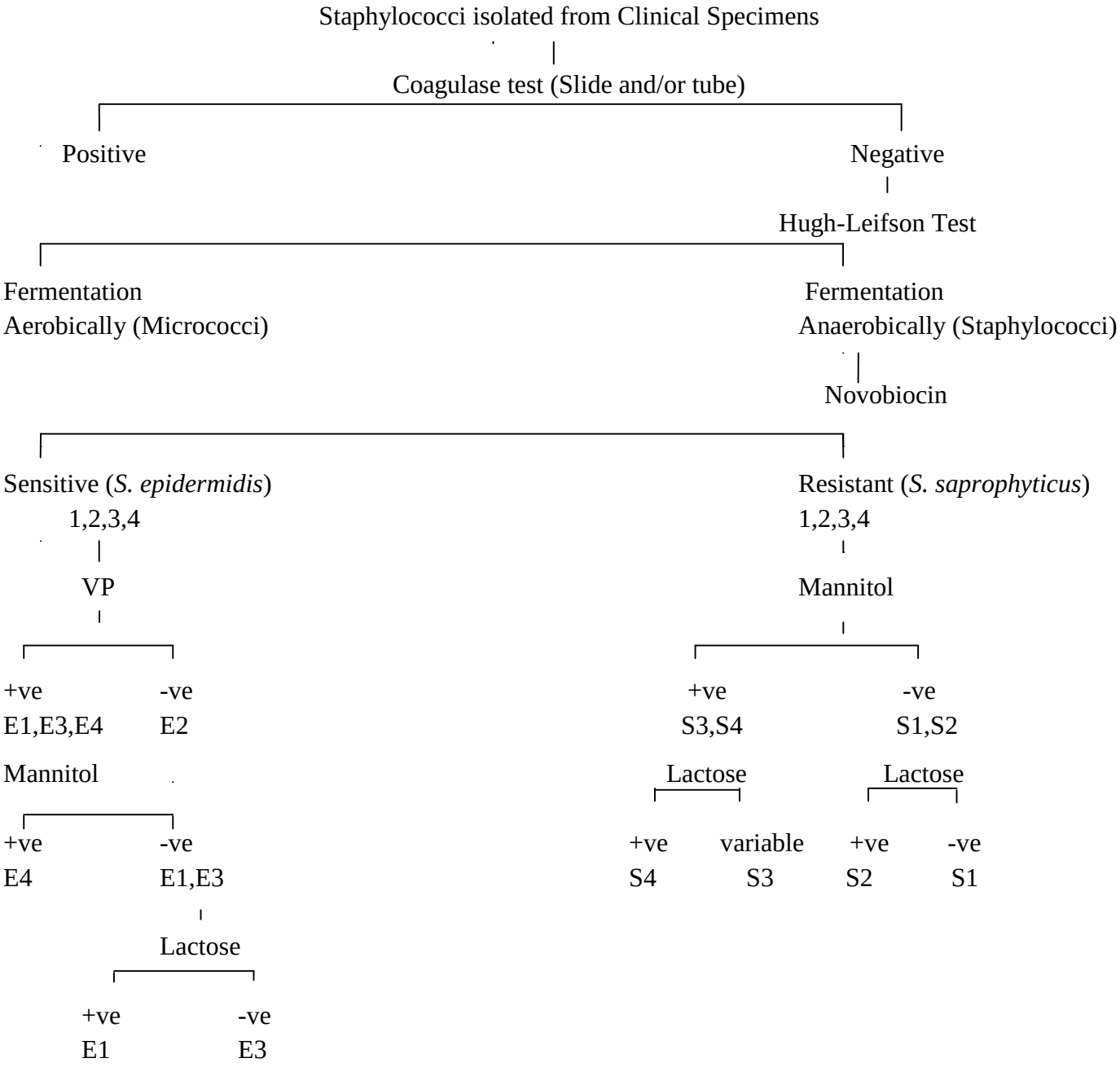
Materials and Methods

One hundred strains of coagulase negative staphylococci isolated from different clinical specimens like wound swab, urine, vaginal swab, ear swab, pus, sputum, C.S.F. and blood etc. were collected from outdoor and indoor patients of Rajindra Hospital, Patiala, belonging to both sexes of different age groups.

The present study was done under following three steps:

1. Biotyping (Baird- Parker) of CONS
2. Slime production
3. Antibiotic sensitivity

For the identification of CONS, cultural characters were studied on Blood agar plate containing sheep blood, McConkey bile salt lactose agar plate, nutrient agar and milk agar. Then coagulase negative staphylococci were classified into biotypes by Baird- Parker classification as shown in the flow chart.



E1 = *S. epidermidis* biotype 1
E2 = *S. epidermidis* biotype 2
E3 = *S. epidermidis* biotype 3
E4 = *S. epidermidis* biotype 4

S1 = *S. saprophyticus* biotype 1
S2 = *S. saprophyticus* biotype 2
S3 = *S. saprophyticus* biotype 3
S4 = *S. saprophyticus* biotype 4

Further slime test[10] was done to study production of slime to determine its role in the pathogenicity of CONS. The presence of slime was detected on glass tube surfaces by staining with safranin and grading was done as follows:

Negative : no staining of walls

1+ : very slight colour on sides of the tube
2+ : light staining along walls of the tube
3+ : heavy staining along walls of the tube
Grades negative and 1+ : reported as slime test negative
Grade 2+ and grade 3+ : reported as slime test positive

All the isolated strains of CONS were subjected to drug sensitivity by disc diffusion method. The commercial antibiotic discs (Span diagnostics limited) were used on nutrient agar to know the sensitivity and kept in incubator overnight. The recording of the results was done on the next day.

The antibiotics used were: penicillin ,ampicillin, erythromycin, kanamycin, tetracycline, gentamicin, ciprofloxacin, clindamycin, cefotaxime, vancomycin, nitrofurantoin and nalidixic acid (nitrofurantoin and nalidixic acid were used for UTI only). Zone of inhibition equal to or more than mentioned in the zone interpretation chart (Span diagnostics limited) was taken as sensitive, otherwise resistant.

Results

Maximum age incidence of 35% was in the age group of 21-30 years and minimum incidence of 5% was in the age group of 51-60 years. Above 60 years, only 8% suffered from CONS infections. Mean age was 31.3.Out of 100 cases,46 were males and 54 were females with a male: female ratio of approximately 1:1.17.

Out of 100 isolates of CONS, 63 strains were isolated from patients admitted in the different wards of Rajindra Hospital, Patiala while 37 strains were isolated from outdoor patients.

Among the clinical materials listed in table 1, urine specimen received were 36% followed by pus 22% , vaginal swab 13%, wound swab 11%, blood 7%, aural swab 6%, sputum 3% and CSF 2%. Using Baird-Parker classification system, 71 out of 100 CONS strains were typed as *Staph. epidermidis* and 29 as *Staph. saprophyticus*.

Table 1 Distribution of clinical material

Name of clinical material	No. of specimens	Percentage
Urine	36	36 %
Pus	22	22%
Vaginal swab	13	13%
Wound swab	11	11%
Blood	7	7%
Aural swab	6	6%
Sputum	3	3%
CSF	2	2%
Total	100	100

Out of 71 *Staph. epidermidis* isolated, 34 (47.8%) belonged to biotype E_1, followed by biotype E_2, 23 (32.4%) and biotype E_4, 14(19.8%) respectively. Out of 29 *Staph. saprophyticus* isolated, 5(17.3%) belonged to biotype S_2 and 24 (82.7%) belonged to biotype S_3 respectively. (Table 2)

Table 2 Different biotypes of CONS (n=100) using Baird-Parker classification

Different types of CONS	Biotypes	No. isolated	Percentage
Staph. epidermidis (n=71)	E_1 E_2 E_4	34 23 14	47.8% 32.4% 19.8%
Staph. saprophyticus (n=29)	S_2 S_4	5 24	17.3% 82.7%

Out of 71 *Staph. epidermidis*, in 20 cases slime test was positive. Out of 29 cases of *Staph. saprophyticus*, 2 cases gave slime test positive and in 27 cases, slime test was negative.(Table 3)

Table 3 Slime production in CONS (n=100)

Staph. epidermidis (71)		*Staph. saprophyticus* (29)	
Positive	Negative	Positive	Negative
20	51	2	27
(28.2%)	(71.8%)	(6.9%)	(93.1%)

The antibiotic sensitivity pattern of slime producers has been listed in table 4 while that of non slime producers has been listed in table 5.

Table 4 Antibiotic sensitivity pattern of slime producers (CONS) (n=22)

S.no	Name of antibiotic	Disc content	Sensitive		Moderately Sensitive		Resistant	
			No.	% age	No.	% age	No.	% age
1	Penicillin	10 units	1	4.5	-	-	21	95.5
2	Ampicillin	10 mcg	3	13.6	1	4.5	18	81.9
3	Tetracycline	30 mcg	4	18.2	2	9.1	16	72.7
4	Erythromycin	15 mcg	7	31.8	-	-	15	68.2
5	Kanamycin	30 mcg	7	31.8	2	9.1	13	59.1
6	Gentamicin	10 mcg	9	40.9	1	4.5	12	54.6
7	Clindamycin	2 mcg	17	77.3	-	-	5	22.7
8	Ciprofloxacin	5 mcg	19	86.4	-	-	3	13.6
9	Cefotaxime	30 mcg	19	86.4	-	-	3	13.6
10	Vancomycin	25 mcg	22	100	-	-	-	-
11	*Nitrofurantoin	300 mcg	4	66.6	-	-	2	33.7
12	*Nalidixic acid	30 mcg	4	66.6	-	-	2	33.7

*Used for UTI isolates only

All the 22 (100%) slime producers of CONS were sensitive to vancomycin. 19 (86.4%) isolates were sensitive to both cefotaxime as well as ciprofloxacin. 17(77.3%), 9(40.9%) ,7(31.8%) , 7(31.8%) , 4(18.2%), 3(13.6%) and 1(4.5%) isolates were sensitive to clindamycin, gentamicin, erythromycin, kanamycin, tetracycline ,ampicillin and penicillin respectively. 4(66.6%) out of 6 urinary isolates were sensitive to nitrofurantion and nalidixic acid.

Table 5 Antibiotic sensitivity pattern of non slime producers (CONS) (n=78)

S.no	Name of antibiotic	Disc content	Sensitive		Moderately Sensitive		Resistant	
			No.	% age	No.	% age	No.	% age
1	Penicillin	10 units	5	6.4	-	-	73	93.6
2	Ampicillin	10 mcg	16	20.5	4	5.1	58	74.4
3	Tetracycline	30 mcg	29	37.2	3	3.8	46	59
4	Erythromycin	15 mcg	33	42.3	-	-	45	57.7
5	Kanamycin	30 mcg	45	57.7	4	5.1	29	37.2
6	Gentamicin	10 mcg	62	79.5	-	-	16	20.5
7	Clindamycin	2 mcg	64	82.1	-	-	14	17.9
8	Ciprofloxacin	5 mcg	67	85.9	2	2.6	9	11.5
9	Cefotaxime	30 mcg	72	90.2	-	-	6	9.8
10	Vancomycin	25 mcg	78	100	-	-	-	-
11	*Nitrofurantoin	300 mcg	17	56.7	4	13.3	9	30.0
12	*Nalidixic acid	30 mcg	17	56.7	4	13.3	9	30.0

*Used for UTI isolates only

All the 78(100%) isolates of non slime producers were sensitive to vancomycin. 72(90.2%), 67(85.9%), 64(82.1%), 62(79.5%) were sensitive to cefotaxime, ciprofloxacillin, clindamycin and gentamicin respectively. 17(56.7%) were sensitive to nitrofurantion and nalidixic acid.

Discussion

CoNS are resident flora of the human body and are not harmful to ordinary individuals. However, they cause serious infections in compromised hosts, especially patients with prosthetic valves, prosthetic joints, cerebrospinal fluid shunts, or intravascular catheters. Recent progress in medicine has resulted in an increase number of compromised hosts. Because of this, CoNS has become one of the most common causes of nosocomial infections .

In the present study of 100 isolates of CONS, maximum age incidence of 33% was in the age group of 21-30 years and minimum of 5% was in the age group of 51-60 years though it affects all the age groups. Similar observations were made in studies by Alex AM et al[11] in which age group of 21-30 yr showed highest isolation of CONS (23.2%) followed by 51-60yr (13.4%) and <1 yr(13.4%) and by Rani JN et al[12]in which incidence of CONS was high in 21-30 years age group (42.7%) than the other age groups.

In our study, out of 100 isolates,54 were found in females and 46 in males , male- female ratio being 1:1.17. A study by Rani JN et al[12] also reported more CONS isolation among female patients (61.3%) than male patients (38.7%).However , studies by Usha MG et al[13] and James J et al[14] showed preponderance of CONS infections in males- 59% and 68% respectively more than the females.

In the present study of 100 isolates of CONS, 63 belonged to patients admitted in hospital wards and 37 from the outdoor clinics. This shows that CONS infection occurs mostly in hospitalised patients.

In this study, isolation of CONS from the various clinical samples was: urine 36% followed by pus 22% , vaginal swab 13%, wound swab 11%, blood 7%, aural swab 6%, sputum 3% and CSF 2%. This finding is in concordance with another study by Kumar S et al[15] who obtained maximum isolates of CONS from urine samples (41.75%) followed by blood (16.67%).Similarly ,Mohan U et al[16] isolated 48.4%CONS from urine,17.7% from pus,14.5% from catheter tips, 4.7% from blood and 2.1% from skin and conjunctival swabs. However ,studies by Sharma V et al[17] and Mane PM et al[18] have reported majority of CONS infections from blood samples - 46.33% and 42.7% respectively.

In the present study, all the CONS strains were isolated in pure culture from various infections. Similar results were reported by Vijayalaxmi et al [19], Bhalla P et al[20], Joshi et al [21], Pal N et al [22], Phatak et al [23]. This strongly implicates its aetiological role in causation of diseases in man.

Out of 100 CONS strains isolated, 71 strains belonged to *Staph. epidermidis* and 29 strains to *Staph. saprophyticus* using Baird-Parker classification system .This result is comparable with a study by Mohan U et al[16] who showed that *Staph. epidermidis* was the most common species isolated (82.9%) followed by Staph. saprophyticus (15.62%).

In the present study, among the *Staph. epidermidis* strains, biotype E_1 was the commonest organism isolated- 34 (47.8%) the and thus correlates with studies of Vijayalaxmi et al [19] - 46.6% and Nayak et al[24] - 40.7%. The prevalence of biotype E_2 in present study was 23 (32.4%).

We did not get any biotype E_3. Vijayalaxmi et al[19], Karchmer et al [25] also did not find any biotype E_3 strains in their study. Joshi et al [21] found 2 (2.86%) strains to be of biotype E_3.

We found 14 (19.8%) strains to be of biotype E_4, in the present study. This correlates with the studies of Vijayalaxmi et al [19] and Phatak J et al [23]who found biotype E_4 to be 20 (26.67%) and 18 (21.9%) respectively.

In the present study, out of 71 *Staph. epidermidis* strains, 22(28.2%) showed slime production, while 51 (71.8%) were negative for the test. Christensen et al [10] , Mohan U et al[16], Nayak et al[24] and Valli KP[26] reported 44%,48.7%, 65.8% and 72.6% cases to be slime positive.

In our study, only 2 (6.9%) of the 29 strains of *Staph. saprophyticus* showed slime production. Similarly, Phatak J et al[23] reported only 1 (4.13%) out of 24 strains to be slime positive. However, Mohan U et al[16] reported slime positivity in 26.6% strains of *Staph. saprophyticus*.

In our study, vancomycin was 100% effective against all the strains of CONS whether slime producing or non slime producing which is consistent with the findings of Deighton et al [27], Javadpour S et al[28], Jayakumar R et al[29] and Pal N et al[20]. However, slime producing strains showed only 45.4% sensitivity to gentamicin while non slime producers showed 79.5% sensitivity to gentamicin .Similarly, slime producers were only 40.9% sensitive to kanamycin against non slime producers who showed 62.8% sensitivity towards kanamycin. Thus, It is evident that slime producing strains of CONS are more resistant to antibiotics in comparison to non slime producing strains as studied by Deighton et al[26] , Phatak J et al[21] and Mohan U et al. [16]

Conclusion

CONS are emerging as potential pathogens in hospital settings. Slime production has a role in pathogenesis and drug resistance of CONS infections. . In view of multiple drug resistance of these organisms, control is only possible by strict aseptic techniques, proper antibiotic policy and good epidemiological study.

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