

## Original Article

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# Study on Prevalence of commensals as pathogens in diabetic patients

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### Abstract:

**Introduction:** The aim of this study is to evaluate the incidence of skin commensals (Coagulase negative staphylococci) as pathogens in diabetic patients. Coagulase negative staphylococcus species is usually considered as normal skin commensals in healthy individual. It can be considered only as opportunistic pathogen and can be reported as pathogen only under clinically significant conditions.

**Materials and methods:** A total of 579 samples were received from various departments and the Samples received were cultured on various different agars and incubated overnight at 37°C. Culture Smear and Gram staining technique was performed. The smear which showed Gram positive Cocci in clusters were taken and subjected for biochemical reactions by Catalase test, slide coagulase (bound coagulase) and tube coagulase (free coagulase) test. The isolates which are catalase positive, tube coagulase negative is subjected for antibiotic sensitivity test. The diabetic status of these patients were evaluated. Fasting blood sugar of more than 126mg/dl and postprandial blood sugar of more than 200mg/dl were considered for further evaluation.

**Results:** From a total of 579 samples 466 organisms were isolated among which 232 (49.7%) organisms were isolated from diabetic patients. Among Coagulase negative staphylococcus species isolated in diabetic patients maximum resistant shown to Penicillin group of drugs (84.6%) and Cefoxitin (84.6%, Oxacillin resistant) Clindamycin (76.9%), Erythromycin (73.0%), Rifampin (65.3%), Gentamycin (34.6%).

**Conclusion:** Coagulase negative staphylococcus species is usually considered as normal skin commensals in apparently healthy individual. It can be considered only as opportunistic pathogen. The present study suggests that we can start patients on antibiotics, whenever it is absolutely necessary to avoid emergence of multidrug resistant organisms.

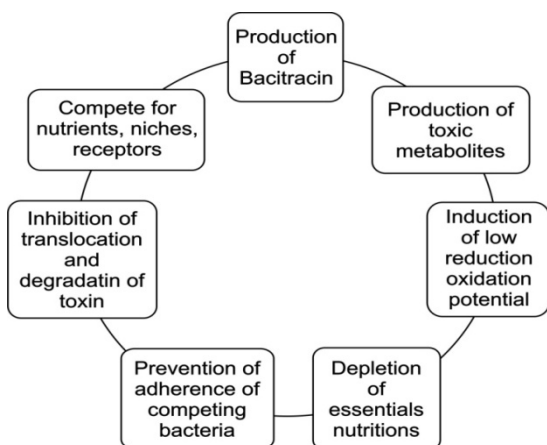
**Keywords:** *Commensals, opportunistic pathogen, immunocompromised, multidrug resistant, CoNS (Coagulase negative staphylococci species)*

**Introduction:**

Human body is made of more than 100 trillion symbiotic microorganisms, known as commensals<sup>1</sup>. They are present on body surfaces covered by epithelial cells and are exposed to the external environment (gastrointestinal and respiratory tract, vagina, skin, etc.)<sup>14</sup>. The host will be colonized by commensals shortly after birth. These commensals gradually becomes home for highly diverse ecosystem during host growth [1, 2, 3]. The composition and function of commensals differ according to different locations, age, sex, race, and diet of the host<sup>[1]</sup>.

**The Skin As A Milieu For Bacterial Growth:**

The first line of defense for human being against foreign invaders is skin <sup>[4]</sup>. Trillions of microbes including bacteria, fungi, viruses, archaea, and small arthropods colonize the skin surface which collectively comprises the skin microbiome<sup>1, 4, 5</sup> (Table 1). The number of bacteria that is colonizing in mucosal and skin surfaces exceeds the number of cells forming human body<sup>14</sup>. Skin plays very important role in protecting human from foreign pathogen<sup>1, 2, 3</sup> by the following mechanisms (Fig 1):



**Fig 1:** Mechanism by which microbial commensals acts against foreign agents invading host (Skin Microflora and Bacterial Infections of the Skin, Katarina Chiller et al).

**Table 1:** Common skin commensals, their location and skin pathology [2,3,4,5].

| S.No | Organism                                   | Location   | Skin Pathology                   |
|------|--|--|----------------------------------|
|      | Gram Positive Organism.                    |  |                                  |
| 1.   | Coagulase negative staphylococcus species. | Upper trunk, Glabrous skin, Head, Forehead, Antecubital, Perineum. |                                  |
| 2.   | Micrococcus species.                       | Cold temperature. Children   |                                  |
| 3.   | Corynebacterium species                    | Intertriginous. Conjunctiva.                                       | Trichomy cosis. Conjuncti vitis. |
| 4.   | Rhodococcus.                               |  |                                  |
| 5.   | Propionibacterium species                  | Sebaceous gland Axilla   | Acne.                            |
|      | Gram Negative Organism.                    |  |                                  |
| 1.   | Acinetobacter                              | Dry areas.   | Burn wounds.                     |

**Condition When Commensals Called As Pathogen:** [1, 2, 3, 5, 8, 15, 16].

- Extremes of age.
- Person with multimorbid condition.
- Immunocompromised (diabetic, chemotherapy etc..)
- Inserted / implanted foreign body.
- Hospital acquired infection.
- Long standing open wound infection and post-operative wound infection.

**Diabetes:**

Diabetes mellitus belongs to a group of metabolic disorders exhibiting high blood sugar level and giving rise to risk of microvascular damage (retinopathy, nephropathy and neuropathy) macrovascular

damage (ischemic heart disease, stroke, peripheral vascular disease). It is associated with high mortality and morbidity level<sup>6</sup>. According to World Health Organization report, an estimated number of people with diabetes are projected to rise from 171 million in 2000 to 366 million in 2030. Several literatures suggest a strong positive correlation between hyperglycemia and skin and soft tissue infection. Recent studies on diabetic patients have observed an increased incidence (ranging 20-50%) of skin and soft tissue infections. Sensory neuropathy, atherosclerotic vascular disease and hyperglycemia all predispose patients with diabetes to skin and soft tissue infections<sup>6</sup>. The aim of our study is to evaluate the incidence of skin commensals (Coagulase negative staphylococci) as pathogens in diabetic patients.

## Materials and Methods:

### *Sampling:*

The study was carried out in Microbiology department at Sree Balaji Medical College and Hospital, Chennai over duration of six months from January 2019 to July 2019. A total of 579 samples (pus and wound swabs) were received from various departments (general medicine, general surgery, dermatology, and orthopedics).

### *Isolation of coagulase negative staphylococci:*

Samples received were routinely cultured on nutrient agar, blood agar, MacConkey agar, Chocolate agar and was incubated overnight at 37°C. Culture which showed significant pure growth with distinct well-separated and creamy white colonies considered for further evaluation.

### *Gram staining and biochemical characterization:*

Culture Smear Gram staining was done. The smear which showed Gram positive Cocci in clusters (fig 3) were taken and subjected for biochemical reactions by Catalase test, slide coagulase (bound coagulase) and tube coagulase (free coagulase) test (fig 4). The isolates which are catalase positive, tube coagulase negative is subjected for antibiotic sensitivity test.

### *Antibiotic Susceptibility Test:*

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method in Muller -Hinton agar plate. The plates were incubated at 37°C for 24 hours. The interpretation of the zones of inhibition was done using the chart adapted from Clinical and Laboratory Standards Institute (CLSI 2017). The isolates were tested for their susceptibility to 10 following antibiotics (Table 2):

**Table 2:** List of antibiotics used and their concentrations

| S.No | Antibiotics Used               | Concentration |
|------|--------------------------------|---------------|
|      | <b>First Line Drugs</b>        |               |
| 1.   | Penicillin                     | 10 Units      |
| 2.   | Cefoxitin                      | 30µg          |
| 3.   | Erythromycin                   | 15µg          |
| 4.   | Clindamycin                    | 2µg           |
| 5.   | Trimethoprim-sulphamethaxazole | 1.25/23.75µg  |
| 6.   | Tetracyclin                    | 30µg          |
| 7.   | Linezolid                      | 30µg          |
| 8.   | Gentamycin                     | 10µg          |
| 9.   | Ciprofloxacin                  | 5µg           |
|      | <b>Second Line Drugs</b>       |               |
| 10.  | Vancomycin(MIC)                | -             |
| 11.  | Rifampin                       | 5µg           |
| 12.  | Chloramphenicol                | 30µg          |

**Diabetic Status:**

The diabetic status of these patients was evaluated. Fasting blood sugar of more than 126mg/dl and postprandial blood sugar of more than 200mg/dl were considered for further evaluation.

**Results:**

579 samples were received, a total of 466 organisms isolated from it. Among which, 303 (65.02%) were Gram negative bacilli, 132 (28.3%) were Gram positive cocci (Fig 2), 30 were (6.43%) Gram positive bacilli, 1 was (0.21%) *Candida albicans*.

Out of 466 organisms isolated 232(49.7%) organisms were from diabetic patients. 156 (67.2%) isolates were Gram negative bacilli (fig 5), 59 (25.4%) isolates were Gram positive cocci (Fig 5), 17(7.32%) were Gram positive bacilli.

Of 59 Gram positive cocci isolated in diabetic patients, 28 isolates are *Staphylococcus aureus* (47.45% ), 26 isolates are Coagulase negative staphylococcus species (44.06%), 3 Enterococcus species (5.08%), 2 streptococcus species (3.38%). Table 3 shows that elderly persons are most commonly affected (57.68% in > 60years age).

100% of CONS were reported as pathogen in diabetic patients and 37% of CONS were reported as pathogen in non diabetic patients (Table 4).

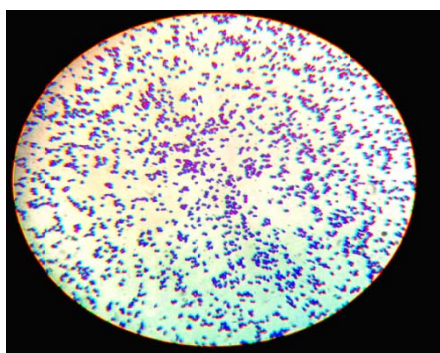


Fig 2: Gram staining shows gram positive cocci in clusters.

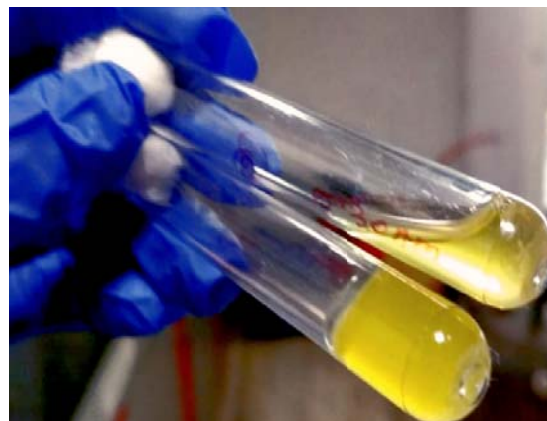


Fig 3: Tube coagulase test (free coagulase) with Quality control in left and test isolate in right. Quality Control in left shows clot formation, test isolate in right doesn't show clot formation.

Table 3: Distribution of diabetic patients by age from whom Coagulase Negative Staphylococcus Species (CONS) has been isolated

| S.No | Age Group | No. of. Patients | Percentage |
|------|-----------|------------------|------------|
| 1.   | 31-40     | -                | -          |
| 2.   | 41-50     | 4                | 15.38%     |
| 3.   | 51-60     | 7                | 26.9%      |
| 4.   | 61-70     | 13               | 50%        |
| 5.   | 71-80     | 1                | 3.84%      |
| 6.   | 81-90     | 1                | 3.84%      |

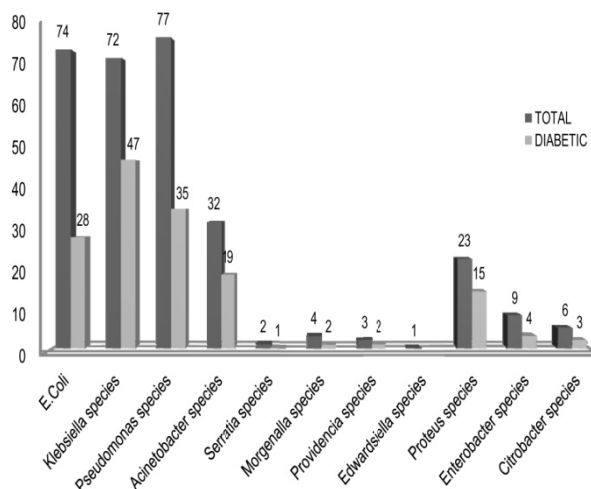


Fig 4: Number of gram negative bacilli isolated from diabetic patients (compared with total isolates).

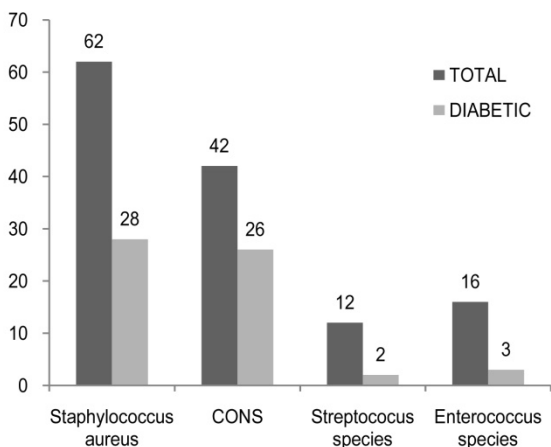


Fig 5: Number of gram positive cocci isolated from diabetic patients (compared with total isolates).

Table 4: Comparison between reporting of CONS species in diabetic and nondiabetic.

| Diabetic status | Total Cons Isolated | Reported as Cons | Percentage | Reported as Commensals | Percentage |
|-----------------|---------------------|------------------|------------|------------------------|------------|
| Diabetic        | 26                  | 26               | 100%       | -                      | -          |
| Non Diabetic    | 16                  | 6                | 37.5%      | 10                     | 62.5%      |

Table 5: Percentage of Susceptibility and resistance of CONS isolated from Diabetic patients

| S.No | Antibiotics     | Cons Susceptibility |            | Cons Resistance |            |
|------|-----------------|---------------------|------------|-----------------|------------|
|      |                 | Number              | Percentage | Number          | Percentage |
| 1.   | Penicillin      | 4                   | 15.3%      | 22              | 84.6%      |
| 2.   | Cefoxitin       | 4                   | 15.3%      | 22              | 84.6%      |
| 3.   | Erythromycin    | 7                   | 26.9%      | 19              | 73.0%      |
| 4.   | Clindamycin     | 6                   | 23.7%      | 20              | 76.9%      |
| 5.   | Cotrimaxazole   | 14                  | 53.8%      | 12              | 46.1%      |
| 6.   | Tetracyclin     | 14                  | 53.8%      | 12              | 46.1%      |
| 7.   | Linezolid       | 16                  | 61.5%      | 10              | 38.4%      |
| 8.   | Gentamycin      | 17                  | 65.3%      | 9               | 34.6%      |
| 9.   | Ciprofloxacin   | 14                  | 53.8%      | 12              | 46.1%      |
| 10.  | Rifampin        | 9                   | 34.6%      | 17              | 65.3%      |
| 11.  | Chloramphenicol | 16                  | 61.5%      | 10              | 38.4%      |
| 12.  | Vancomycin      | 16                  | 61.5%      | 10              | 38.4%      |

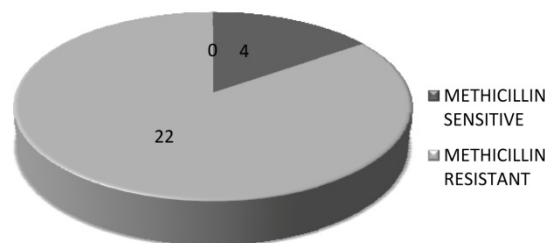


Fig 6: Number of methicillin sensitive and resistant strains isolated in diabetic patients.

Among Coagulase negative *staphylococcus* species isolated in diabetic patients maximum resistant was shown to Penicillin group of drugs (84.6%) and Cefoxitin (84.6%, Oxacillin resistant) Clindamycin (76.9%), Erythromycin (73.0%), Rifampin (65.3%), and Gentamycin (34.6%) (Table 4 and Fig 6).

Maximum sensitivity was shown to Gentamycin (65.3%), chloramphenicol (61.5%), Vancomycin (61.5%), Linezolid (61.5%), cotrimaxazole (53.8%), Tetracyclin (53.8%), ciprofloxacin (53.8%).

Among 22 isolates that is resistant to Penicillin group, 5 isolates are Vancomycin resistant (vancomycin is the drug of choice for methicillin resistant strains).

2 isolates are Multidrug resistant (resistant to all first line drug, sensitive only to Rifampin and Vancomycin).

### Discussion:

Coagulase negative *staphylococcus* species is usually considered as normal skin commensals in apparently healthy individual [9, 18]. It can be considered only as opportunistic pathogen. In our study we have evaluated the incidence of commensals (CoNS) as pathogen in diabetic patients.

Of 26 strains isolated in diabetic patients, most of the strains (84.6%) are resistant to Penicillin and Cefoxitin (oxacillin). This result

is comparable with studies of Nagaraju Vanaparathi et al (2017) and J.Baba et al (2015). In our study the isolates were 65.3% susceptible to gentamicin, which is *corroborating* with the results of J.Baba et al., 2015. Chloramphenicol and ciprofloxacin (53.8%) were also effective against CoNS which is correlating with the results of J.Baba et al., (2015).

In the present study 2 isolates were Multidrug resistant (Sensitive only to Vancomycin and rifampin) and among 22 isolates which were resistant to penicillin group, 5 isolates were Vancomycin resistant (Vancomycin is the drug of choice for MRCoNS) (Table 5 and fig 7).

### Conclusion:

Coagulase negative staphylococcus species is usually considered as normal skin commensals in apparently healthy individual. It can be considered only as opportunistic pathogen and can be reported as pathogen only under clinically significant conditions in which patient needs prompt antibiotic treatment to improve patient's health conditions.

In present study, CoNS showed maximum sensitivity to Gentamycin and maximum resistant to Penicillin and methicillin (Oxacillin resistant), 10 isolates were Vancomycin resistant (5 MRCoNS), 2 isolates were Multidrug resistant.

The present study suggests that, we can start patients on antibiotics, whenever it is absolutely necessary to avoid emergence of multidrug resistant organisms. So, when CoNS are isolated from elderly, multimorbid, immunocompromised patients and patients with inserted/implanted foreign body, Hospital acquired infections, post-operative wound should be considered seriously and

antibiotic susceptibility test should be done on these isolates for prompt diagnosis and treatment.

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