

Study of Coagulase-Negative Staphylococci (CNS) Isolated from Hospital Personnel and Hospital Environment

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Coagulase Negative Staphylococcus (CNS) strains were isolated from hospital personnel and hospital environment. Total 320 samples were taken from skin, anterior nares, nails, finger tips, stethoscope, floor, door handle and catheter. Samples were spread on Mannitol Salt agar Medium and one *Staphylococcus* culture from each sample was select for further study. Isolated CNS strains were characterized according to Bergey's manual of determinative bacteriology. Results suggested that five types of CNS strains-*Staphylococcus saprophyticus*, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus sp.* were isolated from hospital personnel and hospital environment and 24.68% strains were Coagulase negative staphylococcus (CNS). Maximum (CNS) strains were present in anterior nares and it was 42.5%. CNS was also present in skin (22.5%), nails (30%), finger tip (27.5), stethoscope (27.5), floor (15%), door handle (20%) and catheter (12.5).

Key words: CNS, *S. saprophyticus*, *S. epidermidis*, *S. hominis*, *S. haemolyticus*.

Coagulase-negative staphylococci (CNS) are a group of adaptable and opportunistic pathogens whose ability to persist and multiply in a variety of environments and causes a wide spectrum of diseases in both humans and animals (Pilipèincová *et al.*, 2010; Akinkunmi and Lamikanra, 2010). These CNS are a part of the normal microflora in human (Agvald-Öhman *et al.*, 2004). Eastick *et al.*, (1996) reported that large, relatively stable reservoirs were identified in the faeces,

around the ear, and in the axilla and nares.

There are reports detailing staphylococcal transmission between people in hospitals and people at home. These cases may relate to outbreak situations involving health-care workers or patients newly transferred from elsewhere (Blok *et al.*, 2003). Other reports document transmission from health-care workers to family members (Calfee *et al.*, 2003; Eveillard *et al.*, 2004) spread between patients in the community (Borer *et al.*, 2002). Boyce *et al.*, (1997) showed that just fewer than half of the nurses entering the rooms of staphylococcal infected patients acquired the patient's strain on gloved hands and aprons. Another study showed that about 12 (17%) of 70 contacts between a health-care worker and staphylococcal colonised patient resulted in

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transmission of staphylococcal infect from the patient to the gloves of the health-care worker (McBryde *et al.*, 2004).

Some other report showed that *Staphylococcus* can be found throughout the general environment, it is hardly surprising that it is also found on more tangible objects within clinical areas. Objects such as computer keyboards, door handles, tourniquets, pens, television sets, stethoscopes, telephones, beds and bedside tables, equipment packaging, paper and patient's notes, and toys are just a few examples (Oie *et al.*, 2002; Panhotra *et al.*, 2005; Ciragil *et al.*, 2006).

Further, these Coagulase-negative staphylococci (CNS) are commonly caused the diseases such as urinary tract infection (Kolawole *et al.*, 2009), intraocular infection (Bannerman *et al.*, 1997), bacteraemia (Singh *et al.*, 2009), prosthetic valve endocarditis (Revilla *et al.*, 2005), endophthalmitis (Ramadan and Menkabo, 2007).

So main aim of present study that isolation, characterization of Coagulase-negative staphylococci (CNS) from hospital personnel and hospital environment. Samples were taken from skin, anterior nares, nails, finger tips, stethoscope, floor, door handle and catheter.

MATERIALS AND METHODS

Isolation of *Staphylococcus* from different specimens

For isolation of *Staphylococcus* from different samples were taken by sterile cotton swab. Such sterile cotton wool swabs, moistened with sterile normal saline were used to collect the specimen. This swab was spread in specific site of sample. Then swab spreaded on Mannitol salt agar plates and incubated at $37 \pm 1^\circ\text{C}$ for 24hr.

Staphylococcus strains grow on mannitol salt agar medium while the growth of most other bacteria will be inhibited due to high salt concentration (7.5%). Strains produce luxuriant growth with yellow zones around colony. Coagulase negative staphylococci will produce small red colonies with no color change to the medium surrounding them. Composition of mannitol salt agar medium is proteose peptone- 10g; Beef extract- 1g; D-Mannitol- 10g; Sodium chloride- 75g; Agar- 15g; Phenol red- 0.025g; Distilled water 1000ml; pH 7.4 ± 0.2

Characterization of Coagulase-negative staphylococci (CNS): Isolated strains were characterized on the basis of gram staining and bio-chemicals tests. Such tests were done according to Bergey's manual of Determinative Bacteriology (Holt *et al.*, 1994).

Coagulase test: This test is useful in differentiating Coagulase positive and coagulase-negative staphylococci. It is done by two methods-

Slide agglutination test: Dense suspensions of *Staphylococci* from culture are made on two ends of clean glass slide. One should be labeled as "test" and the other as "control". The control suspension serves to rule out false positivity due to autoagglutination. The test suspension is treated with a drop of citrated plasma and mixed well. Agglutination or clumping of cocci within 5-10 seconds is taken as positive.

Tube coagulase test: Three test tubes are taken and labeled "test", "negative control" and "positive control". Each tube is filled with 0.5 ml of 1 in 10 diluted rabbit plasma. To the tube labeled test, 0.1 ml of overnight broth culture of test bacteria is added. To the tube labeled positive control, 0.1 ml of overnight broth culture of known *S. aureus* (coagulase positive) is added and to the tube labeled negative control, 0.1 ml of sterile broth is added. All the tubes are incubated at 37°C and observed up to four hours. Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube. If the test remains negative until four hours at 37°C , the tube is kept at room temperature for overnight incubation.

RESULTS AND DISCUSSION

Total 320 samples were isolated from hospital personnel and hospital environment. Samples were taken from skin, anterior nares, nails, finger tips, stethoscope, floor, door handle and catheter. Growth of *Staphylococcus* strains were observed on Mannitol salt agar medium and one *Staphylococcus* culture from each sample was select for further study. Gram staining and cell arrangement confirmed that all isolated strains were gram positive *Staphylococcus*. Further, biochemical characterization of isolates confirmed that five types of CNS strains- *Staphylococcus*

saprophyticus, *Staphylococcus epidermidis*, *Staphylococcus hominis*, *Staphylococcus haemolyticus*, *Staphylococcus sp.* were isolated from hospital personnel and hospital environment (Table-1). Similar observations have been mentioned in Bergey's manual of determinative bacteriology (Holt *et al.*, 1994) and Cowan and Steel's Manual for the identification of medical bacteria (Barrow and Feltham, 1993).

24.68% strains were Coagulase negative staphylococcus (CNS). Maximum (CNS) strains were present in anterior nares and it was 42.5%. CNS was also present in skin (22.5%), nails (30%), finger tip (27.5), stethoscope (27.5), floor (15%), door handle (20%) and catheter (12.5) (Table-2). Such finding showed that CNS strains were present in personnel and hospital environment. And also suggests that it is possibility that such personnel

and hospital environment may spread the diseases.

Similarly, few reports suggested that *Staphylococcus* strains were present in floor, bed linen, over bed table (Boyce *et al.*, 1997; Sexton *et al.*, 2006). McBryde *et al.*, (2004) reported about spreading of disease by healthcare persons. Other reports document transmission from healthcare workers to family members (Calfee *et al.*, 2003; Eveillard *et al.*, 2004), spread between patients in community (Borer *et al.*, 2002).

Our result and scientific evidences confirmed that isolated CNS strains were present in hospital personals, hospital environment. These CNS strains can spread to person to person, air to person, articles to person etc. Therefore, we should aware about transmission so that we can reduce or control communicable diseases.

Table 1. List of different Coagulase negative staphylococcus (CNS) in various samples from the hospital personnel and environment

S.No	Coagulase negative staphylococcus (CNS)	Number	Percentage
1	<i>Staphylococcus saprophyticus</i>	22	27.84
2	<i>Staphylococcus epidermidis</i>	39	49.36
3	<i>Staphylococcus hominis</i>	10	12.65
4	<i>Staphylococcus haemolyticus</i>	06	07.59
5	<i>Staphylococcus sp.</i>	02	02.53
Total		79	

Table 2. Distribution of Coagulase negative staphylococcus (CNS) in various samples from the hospital personnel and environment

S.No	Specimen	Number of samples	No of CNS	(Percentage)
1	Skin	40	09	22.5
2	Anterior nares	40	17	42.5
3	Nails	40	12	30.0
4	Finger tips	40	11	27.5
5	Stethoscope	40	11	27.5
6	Floor	40	06	15.0
7	Door handle	40	08	20.0
8	Catheter	40	05	12.5
	Total sample	320	79	24.68

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