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ORIGINAL ARTICLE

Fate of airborne coagulase-negative staphylococci

Suchithra Sudharsanam[1], Shanthi Mathias[1], Ramya Barani[1], Madhan Sugumar[2], Jeshina Janardhanan[2], Ravi Annamalai[1], Sandhya Swaminathan[3], Padma Srikanth[1]

1. Sri Ramachandra Medical College and Research Institute, Sri Ramachandra University, Porur, Chennai, Tamil Nadu, India
2. Christian Medical College and Hospital, Department of Medicine Unit I & Infectious Diseases, Vellore, Tamil Nadu, India
3. National Environmental Engineering Research Institute, CSIR-complex, Taramani, Chennai, Tamil Nadu, India

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Abstract

Hospital indoor air can be a source for transmitting nosocomial infections in resource limited settings. This study was undertaken over two months period (February – March 2010) in Chennai, India, to 1) characterise bacteria isolated from indoor air of healthcare facility; and 2) establish whether environmental and clinical isolates are similar by molecular typing methods. Daily visits were made to microbiology laboratory to determine clustering of cases of nosocomial infections. Patients with illnesses related to respiratory tract and skin and soft tissues were included. Clinical strains (from laboratory) with similar antibiogram patterns were systematically stocked. Indoor air samples were collected from such locations by exposed plate method for 30 minutes. Growth was identified by standard microbiological procedures. Phenotypically similar strains were further subjected to genotyping by polymerase chain reaction and pulsed field gel electrophoresis (PFGE) to confirm similarity. Coagulase-negative staphylococci (CNS) were only isolated from both environmental and clinical samples during the study period. Totally, 15 clinical and six environmental strains of CNS were isolated over two months. One airborne and one clinical strain, isolated subsequently from a patient in the same location, had similar phenotypic (biochemical and antibiogram) characteristics. Isolates were identified

Corresponding Author

Ms. Suchithra Sudharsanam
Sri Ramachandra Medical College & Research Institute,
Sri Ramachandra University, Porur, Chennai 600116, India.
Email: suchisanam@gmail.com

as *Staphylococcus haemolyticus* using API STAPH system (bioMérieux), and confirmed to be meticillin resistant CNS using appropriate primers. When subjected to typing by PFGE, they were found to be similar (F=0.51). This is the first study documenting the similarity of CNS of environmental and clinical origin, thus establishing the possibility of air as a source for transmitting nosocomial infections.

Keywords: Nosocomial infections; *Staphylococcus haemolyticus*; Air microbiology.

Introduction

Air can be a source for transmitting nosocomial infections.[1] Airborne nosocomial infections are transmitted directly or indirectly through air and may cause respiratory (primarily pneumonia) and surgical-site infections.[2] Earlier studies have shown increasing evidences of airborne transmission in nosocomial outbreaks of meticillin resistant *Staphylococcus aureus* (MRSA),[3,4] *Acinetobacter* spp.[4,5] and *Pseudomonas* spp.[6]

Studies carried out in healthcare facilities in different parts of India have documented the presence of high bacterial loads in operating rooms, including *Staphylococcus aureus*, coagulase-negative *Staphylococcus* spp., *Acinetobacter* spp. and *Klebsiella* spp.[7,8,9] There are concerns among clinicians if the presence of such nosocomially significant microorganisms in indoor air of healthcare facilities can pose a threat in resource limited settings, especially in the context of a developing country.

This study was undertaken at a tertiary healthcare facility in Chennai, India, to 1) characterise bacteria isolated from indoor air of healthcare facility; and 2) establish whether environmental and clinical isolates are similar by molecular typing methods.

Methods

Sample collection

The study was conducted over a period of two months (February – March 2010) in a tertiary healthcare facility situated in West Chennai, India. Daily visits were made to the clinical microbiology laboratory to determine any clustering of cases of nosocomial infections among clinical samples received from patients with illnesses related to respiratory tract and skin and soft tissues. Cases (after 48 h of admission) from ward/ intensive care unit (ICU) were screened for growth of same species with similar antibiogram patterns. Such clinical strains from the laboratory were systematically stocked; air samples were collected in parallel from locations where cases with similar growth were admitted. Indoor air samples (in duplicates) were collected using exposed plate method,[10] by exposing the plates for 30 minutes.[11] Media used for sampling included blood agar and MacConkey agar. Plates were incubated aerobically at 37°C for 24-48 hours. Growth was identified by Gram staining, colony morphology and appropriate biochemical tests.[12]

Phenotypic identification

Blood agar plates were observed for haemolysis. The haemolytic colonies were taken and Gram staining was done. Gram-positive cocci (GPC) were further subjected to catalase and coagulase tests. If they were GPC in clusters and catalase positive but coagulase-negative, they were identified as coagulase-negative *Staphylococcus* spp. (CNS). They were further speciated using the API STAPH system (bioMérieux).

All isolates of Gram-negative bacilli (GNB) were presumptively identified by conventional methods including haemolysis on sheep blood agar and growth on MacConkey agar for lactose fermentation. Lactose fermenting and non lactose fermenting organisms were characterised to the genus level, based on the basic biochemical methods like catalase, oxidase, motility, indole, triple sugar iron test, urease, citrate and H_2S production.[12] Appropriate standard ATCC control strains were included for biochemical tests.

Antimicrobial susceptibility testing

Susceptibilities of the isolates were determined by disk diffusion method. The following antimicrobial agents (Hi-Media Company Limited, India) were tested: ampicillin (A) (10 µg), gentamycin (G) (10µg), linezolid (Lz) (30 µg), vancomycin (Va) (30 µg), bacitracin (B), cloxacillin (Cx) (1 µg), cephalexin (Cp)

(30 µg), cefotaxime (Ce) (30 µg) and ciprofloxacin (Cf) (5 µg). *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as controls. Results were expressed as susceptible or resistant according to criteria recommended by the Clinical and Laboratory Standards Institute (2009).[13]

Genotypic characterisation

Phenotypically (biochemical and antibiogram pattern) similar strains were further subjected to genotyping by polymerase chain reaction and pulsed field gel electrophoresis (PFGE) to confirm the similarity.

Polymerase chain reaction (PCR)

Bacterial DNA was extracted from pure cultures of *Staphylococcus* isolates recovered from clinical specimen and indoor air, as per the protocol deduced by Goldenberger *et al.* with modifications.[14] Fresh overnight bacterial cultures on nutrient agar were suspended overnight in 500µl of normal saline and centrifuged at 14,500 rpm for 10 minutes. Bacterial pellets were re-suspended in 0.2ml of Triton X-100 buffer and incubated for 30 minutes at 95°C with agitation. The tubes were then cooled to 4°C and centrifuged for 10 minutes at 14,500 rpm and 5µl of the supernatant was used directly for amplification. Amplification was carried out in a PTC-200 thermal cycler (MJ Research Inc., Watertown, Mass.). Amplification was done using commercially available kit (Red dye master mix kit), custom synthesized primers and reagents procured from Bangalore Genei Pvt. Ltd (Bangalore, India). Primers were used at 20 pmol concentrations; 0.2µl of the primer was used for CNS amplification. Details of the primers and the cycling conditions used in the study that were chosen based on the published

Table I. Primers used and cycling conditions

Organism	Primer used	Primer sequence	Target site	Cycling conditions	Amplicon size (bp)	Reference
Staphylococcus spp.	TStaG422 TStaG765	5 -GGC CGT GTT GAA CGT GGT CAA ATC A -3 5 -TIA CCA TTT CAG TAC CTT CTG GTA A -3	tuf gene	thermal cycling (3 min at 96°C and then 40 cycles of 1 s at 95°C for the denaturation step and 30 s at 55°C for the annealing-extension step)	370	Martineau *et al.*[15]
	16Sup	5- GTG CCA GCA GCC GCG GTA A -3 5- AGA CCC GGG AAC GTA TTC AC -3	16S rDNA	denaturation for 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s at 55°C and 30 s at 72°C, and final elongation for 2 min at 72°C	886	Poulsen *et al.*[16]
	mecup	5- GGG ATC ATA GCG TCA TTA TTC -3 5- AAC GAT TGT GAC ACG ATA GCC -3	*mecA*		527	
	nucPCR	5-TCA GCA AAT GCA TCA CAA ACA G -3 5-CGT AAA TGC ACT TGC TTC AGG -3	*Nuc*		255	

literature[15,16] are summarised in Table I. A strain of meticillin resistant *Staphylococcus haemolyticus* that was confirmed biochemically and tested repeatedly positive at the time of standardisation of multiplex PCR was used as positive control for *mecA* and 16S rDNA genes. *Staphylococcus aureus* ATCC 25923 was used as positive control for *tuf* gene in conventional PCR and for *nuc* gene in multiplex PCR, while water was included as negative control.

Pulsed Field Gel Electrophoresis (PFGE)

PFGE was the method of choice to determine the association between the clinical and environmental strains as it is a very reproducible, and highly discriminatory sub-typing method capable of identifying the transmission source of bacterial pathogens. Genomic DNA was extracted from the study isolates of CNS and digested with *Sma*I, and was then subjected to PFGE as previously described by McDougal *et al*. with modifications.[17] *Staphylococcus aureus* ATCC 25923 was used as control. Gel was stained with ethidium bromide and captured. Patterns (pulsotypes) were compared visually and considered indistinguishable if all bands were shared.

16S rDNA gene amplification and sequencing

DNA sequencing was carried out with an Applied Biosystems ABI model 3700 sequencer and the protocols of the manufacturer (PE Applied Biosystems, Foster City, California) by Sanger method, using the BigDye Terminator cycle sequencing ready reaction kit.

Results

Retrospective analysis of the laboratory reports of the in-patients revealed the occurrence of staphylococcal wound infections in an orthopaedic ward of the healthcare facility. Based on this, a systematic walk-through was conducted in the ward to determine local environmental conditions. Air samples were collected by exposed plate method. Samples were processed and microorganisms were characterised as per standard procedures. CNS were found to be the predominant isolate. Daily visits were made to the laboratory and samples from in-patients of the ward were screened for occurrence of any nosocomial staphylococcal wound infections. Strains recovered from air and clinical samples were stocked.

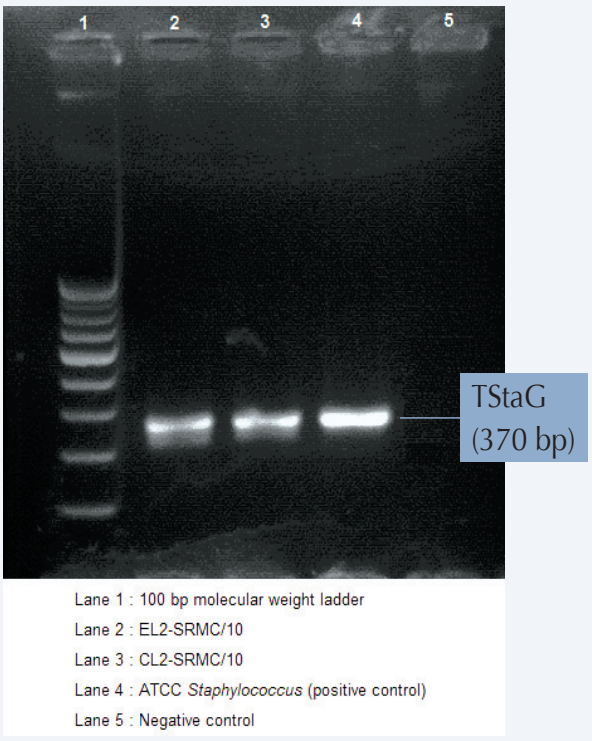
Indoor air from different hospital locations was found to harbour CNS and *Pseudomonas* spp. as predominant bacterial isolates. Of these, only CNS were isolated simultaneously from both environmental and clinical samples during the study period.

A total of 15 clinical and six environmental strains of CNS were isolated over a period of two months. Both the environmental and clinical strains of CNS obtained during the same time period were subjected to phenotypic and genotypic characterisation to determine the similarity. Of these, one clinical and one environmental strain of CNS collected in a week's time-period were found to have similar phenotypic characteristics with identical antibiotic susceptibility pattern, being resistant to meticillin and sensitive to vancomycin and linezolid (Table II). The isolates were identified to be *Staphylococcus haemolyticus* using the API STAPH system (bioMérieux), and confirmed to be meticillin resistant CNS using appropriate primers (Table II; Figure 1). The environmental strain was first isolated from the indoor air of the orthopaedic ward and the clinical strain was isolated with an interval of eight days from the patient admitted subsequently in the same ward. Laboratory reports revealed the recovery of CNS from a tissue sample. The sample was taken from a patient admitted to the orthopaedic ward. From the medical records, it was found that the patient had diabetes mellitus, and was admitted with an open wound in the lower extremity. Initial culture of the swab sample from the open wound did not show any significant growth. The wound became infected following hospitalisation. The tissue sample was sent for culture to the laboratory following wound debridement procedure.

Macrorestriction with *Sma*I separated the chromosomal DNA into 10 to 16 well-resolved fragments ranging in size from 100 to 390 kb. The two study isolates produced between 14 and 12 bands, yielding a diverse array of DNA profiles. A total of two distinct PFGE patterns EL2 and CL2, differing by ≤ 3 bands was observed. The PFGE pattern of EL2 and CL2 were found to be closely related based on Tenover's criteria[18] (Figure 2). The study isolates were submitted to GenBank and have been allotted the GenBank accession numbers JF306643-JF306644.

Table II. Antibiotic susceptibility pattern and genotypic characteristics of environmental and clinical strains of coagulase-negative staphylococci

STRAIN ID		EL2-SRMC/10	CL2-SRMC/10
ORIGIN OF SAMPLE		Environmental	Clinical
TYPE OF SAMPLE		Air	Tissue
DATE OF ISOLATION		01.03.2010	09.03.2010
SPECIATION OF THE ORGANISM (API STAPH)		*Staphylococcus haemolyticus*	*Staphylococcus haemolyticus*
ANTIBIOTIC SUSCEPTIBILITY	Ampicillin	R	R
	Erythromycin	R	R
	Gentamicin	R	R
	Ciprofloxacin	R	R
	Cephalexin	R	R
	Cephotaxime	R	R
	Cloxacillin	R	R
	Oxacillin	R	R
	Vancomycin	S	S
	Linezolid	S	S
	Tetracycline	S	S
	Rifampicin	S	S
GENOTYPIC CHARACTERISATION (BY POLYMERASE CHAIN REACTION)	TStaG (370 bp)	+	+
	16Sup (886 bp)	+	+
	Mecup (527 bp)	+	+
	nucPCR (255 bp)	-	-



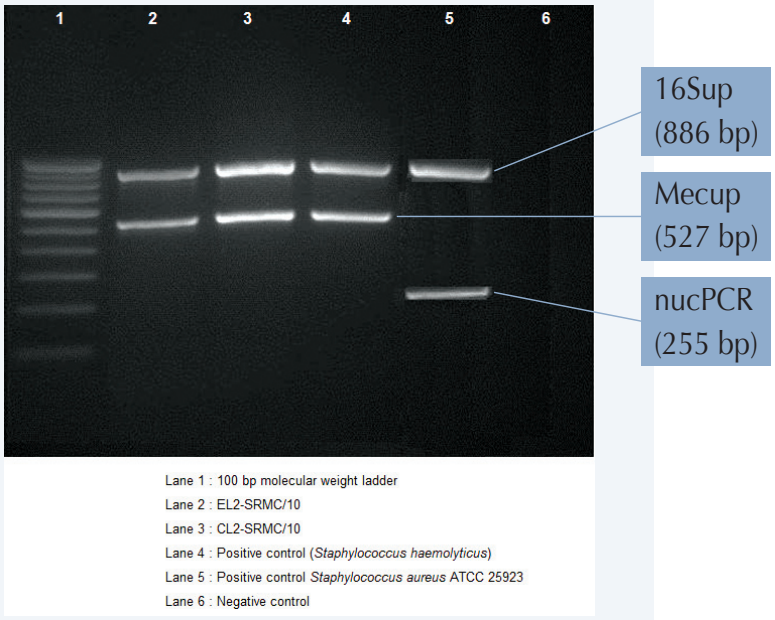


Figure 1. Gel electrophoresis of PCR products amplified from environmental and clinical strains of *Staphylococcus haemolyticus*

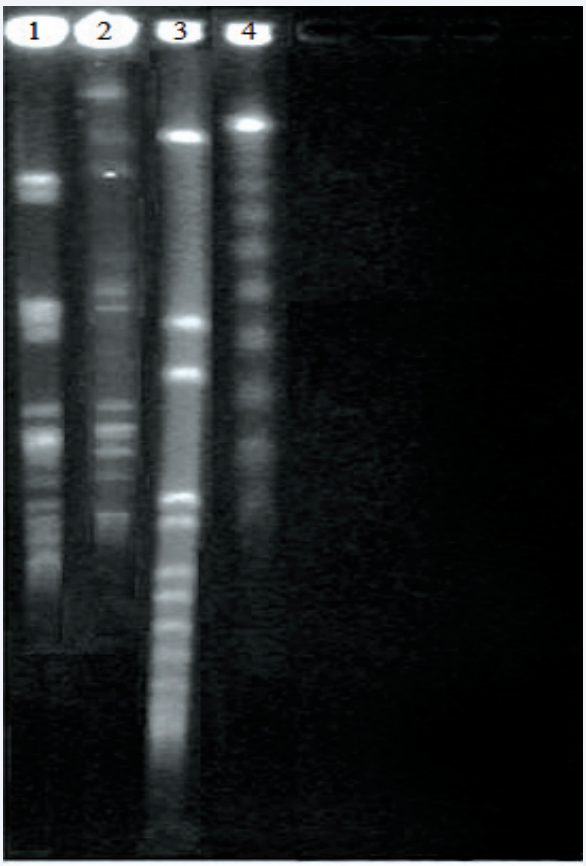


Figure 2. PFGE of *SmaI* digest of chromosomal DNA

Discussion

Nosocomial infections by CNS are on the rise. About 30% of the nosocomial blood-stream infections are caused by CNS, of which bacteraemia related to indwelling devices are predominant. Other infections by CNS include central nervous system shunt infections, endophthalmitis, surgical site infections, peritonitis in patients with continuous ambulatory peritoneal dialysis and foreign body infections. Approximately 55 – 75% of the nosocomial CNS are meticillin resistant. In addition, they also cause endocarditis and urinary tract infections in immunocompetent hosts.[19]

A study conducted to investigate the prevalence of MRSA and MRCoNS found the rate of multidrug resistance to be 69% for MRSA and 72.5% for MRCoNS strains.[20] Another study recorded an increasing trend of MRCoNS in neonatal septicaemia, with the prevalence of MRCoNS during 2008, 2009 and 2010 being 41.57%, 47% and 57.36% respectively.[21]

Chylak *et al.* carried out a study, where meticillin resistant strains of *S. haemolyticus* isolated from patients and from the hospital ward environment were typed.[22] The study findings suggested that a nurse may have been the source of infection because the same genotype of *S. haemolyticus* was isolated from her nasal anterior as from the majority of patients.

Airborne nosocomial staphylococcal infections have been documented. Shiomori *et al.*[23] quantitatively investigated the existence of airborne meticillin-resistant *Staphylococcus aureus* (MRSA) in a hospital environment, and found the isolates from the air and inanimate environments to be identical to the MRSA strains that caused infections or colonisation in in-patients. The study thus indicated that MRSA gets re-circulated among the patients, the air and the inanimate environments, especially when there is movement in the rooms. However, so far, no studies have reported the possibility of airborne CNS in causing nosocomial infections.

In this study, an attempt was made to look for the existence of a correlation between airborne (environmental) and clinical strains of CNS recovered during the study period. Based on the retrospective analysis of the laboratory reports, it was found that there was occurrence of staphylococcal wound infections in an orthopaedic ward that were nosocomial in origin. Air samples were collected from the orthopaedic ward by exposed plate method. Daily visits were made to the laboratory, and samples were screened for the occurrence of any nosocomial staphylococcal wound infections. Strains recovered from air and clinical samples were stocked.

Thus, a total of 15 clinical and six environmental strains of CNS were isolated over a period of two months. When these strains were subjected to screening by phenotypic and genotypic methods for similarity, only two strains (one clinical and one environmental) isolated in a week's time were found to have similar phenotypic characteristics with identical antibiotic susceptibility pattern. Both the strains were found to be meticillin resistant *Staphylococcus haemolyticus*. They were found to be closely related when subjected to typing by PFGE. Since basic infection control practices are adhered to during patient care and no outbreak

was reported during this period, we believe that our study suggests air as a possible pathway for nosocomial pathogens indicating the need for conducting future studies to explore this pathway.

Airborne transmission of *S. aureus* has not been linked to areas other than operating rooms, burn units, and neonatal nurseries.[24] To the best of our knowledge, this is the first study documenting the similarity of clinical and environmental strains of meticillin resistant CNS in an orthopaedic ward. This study indicates that air can be a possible source of nosocomial infection. The patient may have acquired the infection from the air due to particle fall-out.

Although airborne transmission of nosocomial infection by CNS was observed, incidence of only one such case over a period of two months is noteworthy. Though 15 clinical and six environmental strains of CNS were isolated over a period of two months, only one isolate of CNS from clinical specimen was similar to an isolate of CNS recovered from indoor air. This suggests that air may be an infrequent source for these infections.

Swab samples were not collected from patients / health personnel / care takers present in the same location to determine the possible source of these nosocomial agents. In view of the available resources and lack of data on microbial indoor air quality, the study was undertaken to determine the microorganisms in hospital indoor air, look for isolation of any similar organisms from patients with illnesses related to respiratory tract and/ or skin and soft tissues admitted in the same locations, and determine the association between clinical and environmental isolates. Determining the possible sources of airborne CNS is beyond the scope of the study.

The occurrence of only one episode of airborne transmission of nosocomial infection suggests that regular environmental sampling may not be required. These organisms are frequently shed in air by the occupants, and may settle on surfaces including high touch areas like door knobs due to particle fall-out. Hands of health personnel may get colonised through contact with such surfaces.

Targeting measures towards strict adherence to proper hand hygiene practices might be a better option than spending resources on frequent environmental sampling, which may not directly impact outcome of patient care. Air sampling may however be conducted as a part of active surveillance during outbreaks of nosocomial infections.

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