Indian J Med Res 146, September 2017, pp 420-425
DOI: 10.4103/ijmr.IJMR_1362_15

Quick Response Code:

Staphylococcus hominis subsp. *novobiosepticus*, an emerging multidrug-resistant bacterium, as a causative agent of septicaemia in cancer patients

Nishat Hussain Ahmed[1], Frincy K. Baruah[1] & Rajesh K. Grover[2]

[1]Department of Laboratory Medicine, Division of Microbiology & [2]Department of Clinical Oncology, Delhi State Cancer Institute, New Delhi, India

Received August 28, 2015

***Staphylococcus hominis* subsp. *novobiosepticus* is a new sub-species of *S. hominis*, thus dividing *S. hominis* into subsp. *hominis* and *novobiosepticus*. This study was designed to identify subsp. *novobiosepticus* isolates amongst the *S. hominis* isolated from blood samples of patients with malignancy and septicaemia and to study their resistance profile. The identification was performed by using three simple tests which differentiated between the two sub-species. It was found that 22.8 per cent of *S. hominis* isolates belonged to subsp. *novobiosepticus*.**

Key words D-trehalose - N-acetyl-D-glucosamine - novobiocin - septicaemia - *Staphylococcus hominis* subsp. *hominis* - *Staphylococcus hominis* subsp. *novobiosepticus*

Staphylococcus hominis is one of the three most frequently identified isolates recovered from bloodstream infections (BSIs)[1]. In 1998, characterization of subsp. *novobiosepticus* divided *S. hominis* into two sub-species namely *S. hominis* subsp. *hominis* (SHH) and *S. hominis* subsp. *novobiosepticus* (SHN)[2]. SHN is reported to be exhibiting multidrug resistance, thus leaving narrow therapeutic options[3]. SHN has been reported from the USA, Spain and Brazil as a causative agent of invasive infections and outbreaks[2-5]. This study was undertaken in the Microbiology division of Laboratory Medicine department in Delhi State Cancer Institute, a tertiary care cancer centre in New Delhi, India, over a period of 10 months (September 2013- July 2014) to look for the presence of subsp. *novobiosepticus* in the *S. hominis* isolates from patients with septicaemia and also to characterize their resistance profile to various antimicrobial agents. The study was approved by the institutional ethics committee and written informed consent was obtained from all participants.

Blood samples were collected from all inpatients suspected of having BSI. As far as possible, blood was collected from two different sites at the same time. From adults, 10-20 ml of blood, and from paediatric patients, 5-10 ml of blood was collected and added to BacT/ALERT® FA Plus Blood Culture Bottles and incubated in BacT/ALERT® 3D system (BioMe'rieux, Durham, North Carolina, USA)[6].

Sub-cultures were performed from the culture bottles flagged as positive. The colony characteristics of sub-cultured organisms were examined and Gram staining was done. Gram-positive cocci were identified in VITEK® 2 Compact System (BioMe'rieux, Durham, North Carolina, USA) using Gram-positive GP REF 21342 identification card. The pathogenicity of all coagulase-negative staphylococci (CoNS) isolates was confirmed. They were considered to be pathogenic if (*i*) the same isolate was obtained from blood drawn from two or more peripheral sites, and/or (*ii*) the clinical presentation correlated with the isolate and condition improved on administration of specific antimicrobial therapy. In cases where a single blood specimen was received, or organism was isolated in one out of two samples received, a repeat blood specimen was obtained and similarly cultured. The patients were examined and their records were studied for clinical correlation. Those pathogenic isolates which were identified as *S. hominis* were preserved in nutrient agar slopes at 4°C for later confirmation of sub-species[6]. The pathogenic isolates of CoNS which were not identified as *S. hominis* and those isolates of *S. hominis* which were not considered to be pathogens were excluded from the study. Antimicrobial susceptibility testing was done using VITEK® 2 AST card P-628 (BioMe'rieux). Antibiogram results were expressed as susceptible, intermediate or resistant according to the criteria of the Clinical Laboratory Standards Institute (CLSI)[7].

For confirmation of sub-species, all *S. hominis* isolates were subjected to testing for novobiocin susceptibility and the production of acid aerobically from D-trehalose and N-acetyl-D-glucosamine (NAG)[8]. Novobiocin susceptibility was tested by Kirby-Bauer disc diffusion method[8] using 5 µg novobiocin disc (HiMedia® Laboratories, Mumbai) in Mueller-Hinton agar. A zone size of <16 mm after incubation at 35°C for 24 h was considered indicative of resistance. The quality of novobiocin disc was checked using laboratory strains of *Staphylococcus saprophyticus* (resistant) and *S. epidermidis* (sensitive)[8]. Aerobic fermentation of D-trehalose and NAG was tested using peptone water fermentation medium with Andrade's indicator (HiMedia® Laboratories). After overnight incubation of the inoculated medium at 37°C, a change in colour to dark pink was interpreted as positive and to light pink was considered as weak positive. Positive controls (*Klebsiella pneumoniae* for D-trehalose and *Lactobacillus* for NAG) and un-inoculated controls were used with each run[9].

Independently, the bio-profile and antimicrobial susceptibility pattern given by VITEK® 2 compact of all the isolates was re-analyzed and the findings were noted.

A total of 815 blood samples were received in 10 months duration from patients suspected of having BSI. A total of 249 were found positive by BacT/ALERT® 3D system and 172 grew clinically relevant pathogenic bacteria. In all, 102 staphylococci isolates were obtained, of which 92 were CoNS. The most commonly isolated CoNS were *S. hominis* (57/92 isolates, 61.9%). These 57 isolates identified as *S. hominis* by VITEK® 2 compact were further processed for sub-species identification.

Of the 57 isolates, 11 were resistant to novobiocin. Acid production from D-trehalose was positive in 31 isolates, weakly positive in six and negative in 20 isolates. Acid production from NAG was positive in 32 isolates, weakly positive in seven and negative in 18 isolates. Those isolates which were oxacillin (based on MIC given by VITEK® 2 Compact AST P-628 card) and novobiocin resistant were interpreted as belonging to sub-species *novobiosepticus*[7]. All the isolates thus identified as SHN failed to produce acid aerobically from D-trehalose, all except one isolates failed to produce acid aerobically from NAG also (Table I). On re-analysis of VITEK® 2 Compact bio-profile and MIC, it was found that there were five isolates which were both oxacillin resistant (based on MIC given by VITEK® 2 Compact AST P-628 card) and novobiocin resistant (based on the VITEK® 2 Compact bio-pattern). They were identified as *S. hominis* with low discrimination by VITEK® 2 Compact and the contraindicating biotype was shown to be SHN. These five isolates based on VITEK® 2 Compact bio-patterns were also interpreted as SHN. On comparing the two independent results, it was found that there were three isolates which were identified as SHN both by manual methods and with re-analysis of VITEK® 2 Compact bio-profile. Table I shows the demographic, clinical and laboratory characteristics of the patients identified to be having SHN bacteraemia.

All the isolates identified as SHN (by both methods) were uniformly resistant to penicillin, oxacillin and ciprofloxacin. All isolates except one (intermediate resistant) were resistant to erythromycin (92.3% resistance). Resistance to trimethoprim/sulphamethoxazole was 61.5 per cent (eight out of 13 isolates being resistant) and that to

Table I. Demographic, clinical and laboratory characteristics of the patients identified to be having *Staphylococcus hominis* subspecies *novobiosepticus* septicaemia

Isolate number	Age (yr)	Sex	Underlying malignancy	Total leucocyte count (per µl)	BacT/alert (time of positivity in h)	Manual methods			VITEK® 2 compact analysis	
						Acid production from D-trehalose	Acid production from NAG	Novobiocin susceptibility	Confidence level	Contradicting typical bio-pattern
1*	60	Male	Carcinoma prostate	19,430	22	Negative	Negative	Resistant	Very good identification	None
2*	30	Female	Chronic myeloid leukaemia	Not available	45	Negative	Negative	Resistant	Excellent identification	None
3*	25	Female	Hodgkin's lymphoma	3150	20	Negative	Negative	Resistant	Excellent identification	None
4*	19	Male	Ewing sarcoma	1750	23	Negative	Positive	Resistant	Excellent identification	None
5*	25	Male	Carcinoma of gastro-oesophageal junction	13,260	28	Negative	Negative	Resistant	Excellent identification	None
6*	65	Female	Carcinoma gall bladder	20,770	22	Negative	Negative	Resistant	Excellent identification	None
7*	55	Female	Non-Hodgkin's lymphoma	11,070	48	Negative	Negative	Resistant	Low discrimination	*Leuconostoc mesenteroides*
8*	50	Male	Chronic lymphocytic leukaemia	3510	21	Negative	Negative	Resistant	Excellent identification	None
9*,#	69	Male	Carcinoma prostate	16,100	25	Negative	Negative	Resistant	Low discrimination	SHN
10*,#	42	Male	B-cell acute lymphocytic leukaemia	1490	24	Negative	Negative	Resistant	Low discrimination	SHN
11*,#	20	Male	Acute leukaemia	4830	22	Negative	Negative	Resistant	Low discrimination	SHN
12#	18	Male	Malignant round cell tumour of left forearm	10,330	24	Positive	Positive	Susceptible	Low discrimination	SHN
13#	12	Male	Acute myeloid leukaemia	7220	49	Weak positive	Positive	Susceptible	Low discrimination	SHN

*The isolates which were identified as SHN using manual methods; #The isolates which were identified as SHN after re-analyzing bio-profile given by VITEK® 2 compact system. *#The isolates which were identified as SHN by both the methods. SHN, *Staphylococcus hominis* subspecies *novobiosepticus*; NAG, N-acetyl-D-glucosamine

clindamycin was 23.1 per cent (three out of 13 isolates being resistant). All isolates except one were susceptible to gentamicin (7.7% resistance). Susceptibility was maintained in all isolates against vancomycin, linezolid, daptomycin and tigecycline. The minimum inhibitory concentration of vancomycin (as shown by VITEK® 2 Compact AST report) was ≤0.5 µg/ml in two isolates and 1 µg/ml in the remaining 11 isolates (Table II). The difference in resistance patterns of SHH and SHN was not found to be significant using Pearson's Chi square test, except for ciprofloxacin, against which SHN was significantly more resistant than SHH (P<0.05).

In view of CoNS being one of the most important nosocomial pathogens related to BSIs and substantial increase in the frequency of methicillin resistance among CoNS isolates, accurate detection of pathogenic methicillin-resistant CoNS isolates by clinical microbiology laboratories is of crucial importance in guiding therapy and promoting the correct use of glycopeptides[3,10]. Manual identification of CoNS is labour-intensive and requires long incubation (24-72 h). VITEK® 2 automated detection system allows identification in lesser time (6-24 h) and provides high discrimination between species. However, the system identifies only the known pathogens which are fed into the software, and in case the bio-pattern obtained is not matching any organism, it gives a probable identification. Hence, if there is a doubt of a novel pathogen, the bio-pattern needs to be analyzed and interpreted by microbiologists, and also, the confirmation needs to be done by standard manual methods[11].

The most frequently isolated CoNS in our set-up were *S. hominis*, of which 22.8 per cent were SHN. After first described by Kloos *et al*[2] in 1998, SHN has been reported by authors from Europe, Spain and Brazil (Table III)[3-5,12]. The highest number of isolates (67) has been reported by Balejova[12] from different clinical specimens. Palazzo *et al*[5] have reported six isolates from BSI patients, of which one identified as SHN by pulsed-field gel electrophoresis was found to be oxacillin susceptible. They have suggested that novobiocin resistance is intrinsic to SHN and oxacillin resistance has been acquired later on. SHN isolates causing BSIs are up till now described as vancomycin susceptible. However, increased MIC of SHN strains has been reported in an outbreak of BSI from the Intensive Care Unit in Brazil[4]. There are no reports of SHN from India, however, we have earlier reported probable presence of SHN in septicaemia cases, based on retrospective analysis of VITEK® 2 bio-patterns[13].

In the present study, no significant difference was seen in resistance pattern of SHH and SHN isolates (except in ciprofloxacin). All SHN isolates had

Table II. Antimicrobial susceptibility results of the *Staphylococcus hominis* subspecies *novobiosepticus* isolates

Isolate number	Group A					Group B			Group C		TIG	Vancoycin MIC (µg/ml)
	P	Ox	E	CL	TMX	VA	LZD	DAP	G	CIP		
1	R	R	R	S	R	S	S	S	S	R	S	1
2	R	R	R	R	S	S	S	S	R	R	S	1
3	R	R	R	S	S	S	S	S	S	R	S	1
4	R	R	R	R	R	S	S	S	S	R	S	1
5	R	R	R	S	S	S	S	S	S	R	S	1
6	R	R	R	S	R	S	S	S	S	R	S	1
7	R	R	R	S	R	S	S	S	S	R	S	1
8	R	R	I	S	R	S	S	S	S	R	S	1
9	R	R	R	R	R	S	S	S	S	R	S	1
10	R	R	R	S	S	S	S	S	S	R	S	≤0.5
11	R	R	R	S	R	S	S	S	S	R	S	1
12	R	R	R	S	R	S	S	S	S	R	S	1
13	R	R	R	S	S	S	S	S	S	R	S	≤0.5

[#]The grouping of antibiotics is according to Ref. 7. P, penicillin; OX, oxacillin; E, erythromycin; CL, clindamycin; TMX, trimethoprim/sulphamethoxazole; VA, vancomycin; LZD, linezolid; DAP, daptomycin; G, gentamicin; CIP, ciprofloxacin; TIG, tigecycline; S, sensitive; R, resistant; I, intermediate; MIC, minimum inhibitory concentration; CLSI, Clinical and Laboratory Standards Institute

Table III. Studies from all over the world reporting *Staphylococcus hominis* subspecies *novobiosepticus* as causative agents of invasive infections

Yr	References	Place of reporting	Key features of study	Methods of identifying SHN	Number of SHN isolates reported	Remarks
1998	Kloos *et al*[2]	USA	New subspecies SHN formed	Phenotypic characterization, DNA-DNA hybridization, PFGE, Plasmid profile analysis, DNA base composition, *MecA* gene detection	26	Phenotypic and genotypic distinguishing characteristics defined. Mechanism of combined resistance to novobiocin and methicillin hypothesized.
2004	Balejova[12]	Europe	Identification of SHN isolated from blood and other clinical specimens	Biochemical tests kit, Novobiocin resistance, Molecular confirmation at NRL	67	Demonstration of biofilm formation by SHN.
2005	Chaves *et al*[3]	Spain	Identification and determining clinical relevance of SHN isolates	Novobiocin resistance, Failure to produce acid aerobically from D-trehalose and NAG, PFGE	32	SHN important nosocomial pathogen in neonates.
2008	Palazzo *et al*[5]	Brazil	Outbreak of SHN BSI in ICU	Novobiocin resistance, Failure to produce acid aerobically from D-trehalose and NAG, PFGE	6	One strain identified as SHN found to be methicillin sensitive.
2008	d'Azevedo *et al*[4]	Brazil	Outbreak of SHN BSI in ICU	Automated bio-identification and PFGE *MecA* gene detection	3	Resistance to teicoplanin and increased MIC to VA.
2014	Roy *et al*[13]	India	Communication of cases of BSI indicated to be caused by SHN	Retrospective analysis and interpretation of bio-profile of automated identification	7	First case series from India pointing towards SHN as a pathogen of BSI in cancer patients.
2014	Authors, present study	India	Prospective study to identify SHN as a cause of BSI in cancer patients	Novobiocin resistance, Failure to produce acid aerobically from D-trehalose and NAG	13	First study from India indicating presence of SHN as a causative agent of BSI in cancer patients.

SHN, *Staphylococcus hominis* subspecies *novobiosepticus*; BSI, bloodstream infection; ICU, Intensive Care Unit; PFGE, pulsed-field gel electrophoresis; NAG, N-acetyl-D-glucosamine; VA, vancomycin; MIC, minimum inhibitory concentration; NRL, National Reference Laboratory

vancomycin MICs ≤1 µg/ml; four SHH isolates had vancomycin MICs of 2 µg/ml. However, the MICs against vancomycin need to be confirmed using the gold standard microbroth dilution method to know the actual trend towards increasing MIC.

Small sample size was the major limitation of our study. Studies with large sample size and those involving molecular tests are required to know the actual load, susceptibility pattern and clinical prognosis of this multidrug-resistant CoNS.

Acknowledgment

Authors thank Ms Nishi Sharma, Technologist, Delhi State Cancer Institute, for her technical help.

***Conflicts of Interest*:** None.

References

1. Burnie JP, Naderi-Nasab M, Loudon KW, Matthews RC. An epidemiological study of blood culture isolates of coagulase-negative staphylococci demonstrating hospital-acquired infection. *J Clin Microbiol* 1997; *35* : 1746-50.
2. Kloos WE, George CG, Olgiate JS, Van Pelt L, McKinnon ML, Zimmer BL, *et al*. *Staphylococcus hominis* subsp. *novobiosepticus* subsp. Nov. a novel trehalose- and N-acetyl-D-glucosamine-negative, novobiocin- and multiple-antibiotic-resistant subspecies isolated from human blood cultures. *Int J Syst Bacteriol* 1998; *48* (Pt 3) : 799-812.
3. Chaves F, García-Alvarez M, Sanz F, Alba C, Otero JR. Nosocomial spread of a *Staphylococcus hominis* subsp. *novobiosepticus* strain causing sepsis in a neonatal Intensive Care Unit. *J Clin Microbiol* 2005; *43* : 4877-9.
4. d'Azevedo PA, Trancesi R, Sales T, Monteiro J, Gales AC, Pignatari AC, *et al*. Outbreak of *Staphylococcus hominis* subsp. *novobiosepticus* bloodstream infections in São Paulo City, Brazil. *J Med Microbiol* 2008; *57* : 256-7.
5. Palazzo IC, d'Azevedo PA, Secchi C, Pignatari AC, Darini AL. *Staphylococcus hominis* subsp. *novobiosepticus* strains causing nosocomial bloodstream infection in Brazil. *J Antimicrob Chemother* 2008; *62* : 1222-6.
6. Collee JG, Marr W. Culture of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie and McCartney practical medical microbiology*. 14th ed. New York: Churchill Livingstone; 1996. p. 121-4.
7. Clinical and Laboratory Standards Institute. *Performance Standards For Antimicrobial Susceptibility Testing; Twenty Third Informational Supplement*. *M100-S23*. Vol. 33. Wayne, PA: CLSI; 2013.
8. Winn W Jr., Allen S, Janda W, Koneman E, Procop G, Schreckenberger P, *et al*. *Koneman's color atlas and textbook of diagnostic microbiology*. 6th ed. Philadelphia: Lippincott Williams and Wilkins; 2006.
9. Collee JG, Miles RS, Watt B. Tests for the identification of bacteria. In: Collee JG, Fraser AG, Marmion BP, Simmons A, editors. *Mackie and McCartney practical medical microbiology*. 14th ed. New York: Churchill Livingstone; 1996. p. 133-4.
10. Hussain Z, Stoakes L, Lannigan R, Longo S, Nancekivell B. Evaluation of screening and commercial methods for detection of methicillin resistance in coagulase-negative staphylococci. *J Clin Microbiol* 1998; *36* : 273-4.
11. Spanu T, Sanguinetti M, Ciccaglione D, D'Inzeo T, Romano L, Leone F, *et al*. Use of the VITEK 2 system for rapid identification of clinical isolates of staphylococci from bloodstream infections. *J Clin Microbiol* 2003; *41* : 4259-63.
12. Balejova M. *Staphylococcus hominis* subsp. *novobiosepticus* - New pathogen in nosocomial infections. 14th European Congress of Clinical Microbiology and Infectious Diseases; European Society for Clinical Microbiology and Infectious Diseases, Prague, Czech Republic, May 1-4, 2004. Available from: *https://www.ncbi.nlm.nih.gov/pubmed/15113312*, accessed on March 13, 2015. [Abstract number: 902_p1354].
13. Roy P, Ahmed NH, Biswal I, Grover RK. Multidrug-resistant *Staphylococcus hominis* subsp. *novobiosepticus* causing septicemia in patients with malignancy. *Indian J Pathol Microbiol* 2014; *57* : 275-7.

Reprint requests: Dr Nishat Hussain Ahmed, Ocular Microbiology Section, Dr. RP Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi 110 029, India
e-mail: drnishathussain@rediffmail.com