STAPHYLOCOCCUS NEPALENSIS: A NEW SPECIES FROM NEPAL

Kripa Ghimire,[1] Rajeshwar Reddy Kasarla[2]

ABSTRACT

The Genus *Staphylococcus* belong to the family Staphylococcaceae which comprise of 53 valid species. These are the normal flora of mucocutaneous membranes of mammals and usually of felines. *Staphylococcus nepalensis* is one of the member of genus *Staphylococcus* which show close resemblance to *Staphylococcus cohnii, Staphylococcus gallinarum* and *Staphylococcus succinus*. Thus, phenotypic tests are not sufficient to characterize them sufficiently, molecular intervention is required for their correct identification. In recent times, the cases associated with *S. nepalensis* is surging and this may create problems as they are seen to be highly resistant towards some of the antibiotics like cefoxitin and penicillin. Their potential to transfer resistant genes to *Staphylococcus aureus* isolated from human clinical samples via plasmid and their ability to form biofilm are threatening as they may lead to increase in burden of antimicrobial resistance. There may be more cases of infection with *S.nepalensis* but may have remained hidden due to limited number of research. Thus, the knowledge and scientific information on *Staphylococcus nepalensis* is still unknown or relatively limited; hence this comprehensive review of *S.nepalensis* is undertaken to highlight its importance and research.

KEYWORDS

DNA sequencing, New species, *Staphylococcus nepalensis*

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INTRODUCTION

Coagulase-negative staphylococci (CoNS) frequently inhabits mucocutaneous sites of humans, mammals and birds and also isolated from the environment.[1] Though there are evidences of CoNS producing infections in humans and animals, most of the laboratories do not speciate CoNS.[2-4] However, increasing cases of infection with CoNS along with their resistance towards methicillin is quite alarming. *Staphylococcus nepalensis* is a coagulase-negative *Staphylococcus* found in food products, such as dry-cured ham and fish sauce.[1,5,6]

Historical Background

S.nepalensis, in early 2000s, was isolated from the respiratory tract of goats having pneumonia from the Himalayan region of Nepal. In the study conducted by Spergser et al, the researchers isolated four unidentified strains of staphylococci recovered from three nasal swabs and were named PM34, RW78, CW1T respectively and one strain isolated from lung sample was named MM3. These isolates were considered as a single species as they share almost similar biochemical and physiological properties, protein profiles obtained after SDS-PAGE (sodium dodecyl-sulfate polyacrylamide gel electrophoresis) and identical genomic fingerprints generated after enterobacterial repetitive intergenic consensus-polymerase chain reaction (ERIC)-PCR. Strain CW1(T) revealed highest similarities in16S rDNA sequence to *Staphylococcus cohnii* subsp. urealyticus ATCC 49330(T), *Staphylococcus saprophyticus* subsp. saprophyticus ATCC 15305(T), *S. cohnii* subsp. cohnii ATCC 29974(T), *Staphylococcus arlettae* ATCC 43957(T), *Staphylococcus gallinarum* ATCC 35539(T), *Staphylococcus succinus* ATCC 700337(T) and *Staphylococcus xylosus* ATCC 29971(T) (99.0, 98.8, 98.8, 98.4, 98.2, 98.1 and 98.1%, respectively), hence, it was assigned to a single genus *Staphylococcus*. While classifying these four strains in the genus *Staphylococcus*, the polar lipid composition, fatty acid profiles, quinone systems and diagnostic cell-wall diamino acid were tested and these properties complied with the Genus *Staphylococcus*.[7-9]

Bacteriological Properties

These are Gram positive cocci measuring in 1.1-1.6 um in diameter, non-capsulated and non-motile. These grow on P agar, after incubation of 48 hours, show colonies that are circular, low-convex, smooth, glossy, opaque white and 2–6 mm in diameter. Growth is optimum at body temperature and cannot grow at 45ºC and 15˚C. On biochemical evaluation, these are catalase positive, oxidase negative and coagulase negative. Urea hydrolysis test is positive, hydrolyses Tween-80 and aesculin. It shows resistance towards novobiocin, bacitracin, vibriostatic agent O/129, lysozyme, metronidazole and optochin. Produces acid from D-glucose, D-fructose, D-mannose, maltose, lactose, trehalose, mannitol, sucrose, L-arabinose, N-acetylglucosamine, galactose, glycerol, D-xylose, and salicin.[10]

Clinical Significance

One of the case study conducted in Japan reported *Leuconostac lactis* and *S. nepalensis* bacteremia in a 71-year-old man who experienced Boerhaave syndrome after a meal. In that study, two sets of blood culture were taken. Blood cultures were positive for Gram-positive cocci, which were further identified by matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry.[11]

In a study conducted by Nevakova et al, five isolates of coagulase-negative staphylococci were obtained from human urine, the gastrointestinal tract of squirrel monkeys, pig skin and from the environment. The biochemical evaluation of these isolates corresponded with *Staphylococcus xylosus* species. On partial 16S rRNA gene sequences of these strains were in close resemblance with *Staphylococcus nepalensis reference strains.*[1]

A recent study of Andrade-Oliveira et al. analysed the ability of feline strains of *S. nepalensis* to transfer antimicrobial resistance genes to *Staphylococcus aureus* isolated from humans through characterization of plasmid.[12]

A recent study suggested that the production of the pro-apoptotic peptide corisin by an *S. nepalensis* strain that may lead to apoptosis of lung epithelial cells. The bacteria shed lung corisin levels significantly resulting in acute exacerbation of idiopathic pulmonary fibrosis.[13]

In a study by Seng R et al., CoNS were isolated from hospital environments and community environments which include surfaces of medical trolleys, intravenous poles, patient beds etc. *S. nepalensis* was detected in 0.3% cases. These strains were highly resistant to cefoxitin and penicillin. Also, these strains showed biofilm production by polymerase chain reaction, Congo red agar and microtitre plate method.[14]

A novel surface protein contributing to cell aggregation, adherence to abiotic surfaces, and biofilm formation named as *S. xylosus* surface protein A (SxsA). Comparative genomic analysis revealed that sxsA is present in the core genome of *Staphylococcus nepalensis* and upon deletion of SxsA, adherence to abiotic surface was completely abolished.[15]

S. nepalensis have recently been isolated from guano of bats indicating that guano accumulated near human dwellings and buildings impose risk for human health as they possess capsule but other virulence factors have not been identified.[16]

Detection

1. By conventional methods

1.1. Culture: Culture media used for the growth of *S.nepalensis* include P agar and trypticase soy agar with or without 5% sheep blood. On incubation for 48 hours, colonies produced are S-type, opaque, white and 2-6mm in size. They are usually facultative anaerobic with an optimum growth temperature of 30ºC.[17]

1.2. Biochemical properties: *S.nepalensis* are catalase positive, produces acid from lactose. D-glucose, sucrose. Mannose, trehalose and galactose. These show positive results for beta glucuronidase and beta galactosidase. These bacteria are negative for oxidase, hyaluronidase, coagulase, clumping factor, indole production and H2S production. *S. nepalensis* are resistant to novobiocin, bacitracin, vibriostatic agent (O/129), lysozyme, metronidazole and optochin but they are sensitive to lysostaphin.[7,10,18]

2. By advanced methods

2.1. DNA sequencing: The 16S rRNA gene sequencing and phylogenetic analysis can be performed.

2.2. Ribotyping: Ribotyping can be carried out by using two restriction endonucleases EcoRI and HindIII and the digoxigenin labelled probe that is complementary to 16S and 23S rRNA . EcoRI or HindIII restriction fragments were transferred to the nylon membrane by vacuum alkalic blotting, and then hybridised with the labelled probe overnight at 56ºC. Digitised ribotype patterns were evaluated by GelCompar II software.[8,9,19]

2.3. MALDI-TOF (Matrix assisted laser desorption/ionization- time of flight) : Identifies bacteria via their protein profiles (mass spectrum).

Industrial Importance

A study by Amryta P, et al explored the biosurfactant potential of a novel strain, Staphylococcus nepalensis (KY024500) isolated from rocks of earthquake-prone area, demonstrating Microbial Enhanced Oil Recovery (MEOR) potential of the biosurfactant as more efficient than commercial ones.[20]

CONCLUSION

CoNS are isolated in various samples in clinical practice in recent times but these bacteria are not studied to their species level. This often leads to missed diagnosis. *S. nepalensis* although are the normal flora of the mammals, but may pose threat to humans as there are few reports of infection caused by these bacteria along with their capacity to transfer drug resistant genes to *S.aureus*. Since studies on *S.nepalensis* are very few, the diseases and their outcome may be challenging in upcoming days. Hence, further research is needed to understand virulence, pathogenicity, control and treatment of *Staphylococcus nepalensis* infections, and its industrial application.

CONFLICT OF INTEREST

None

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