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Viridans and bovis group streptococci that cause infective endocarditis in two regions with contrasting epidemiology

Venkatesan Naveen Kumar[a], Mark van der Linden[b], Thangam Menon[a], D. Patric Nitsche-Schmitz[c,*]

[a] Department of Microbiology, PG Institute of Basic Medical Sciences, University of Madras, Chennai 600113, Tamil Nadu, India
[b] German National Reference Center for Streptococci, Department of Medical Microbiology, University Hospital RWTH Aachen, D-52074 Aachen, Germany
[c] Department of Medical Microbiology, Helmholtz Centre for Infection Research, D-38124 Braunschweig, Germany

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ABSTRACT

Viridans group (VGS) or bovis group streptococci (BGS) are the major causes for streptococcal infective endocarditis (IE). However, the causative isolates are not sufficiently characterized. Using multilocus sequence analysis we have examined VGS and BGS (VGS/BGS) isolates that caused IE in southern India and Germany, two distant geographic regions with a contrasting IE epidemiology. Other than in Germany, the majority of patients (68%) in Chennai, southern India had an underlying rheumatic heart disease (RHD). In accord with the high prevalence of RHD in the younger population and with the expansive age structure of India, the median age (24 years) of the VGS/BGS endocarditis patients was lower than in Germany (63 years), where RHD is rare and the age structure is contractive. Both in Germany and in southern India, the majority of cases were caused by mitis group streptococci, however, with considerable differences in the spectra of causative (sub)species. BGS endocarditis was more frequent in Germany. The spectrum of VGS/BGS that cause IE differs considerably between distant geographic regions in which different predisposing conditions prevail. Therefore, improved microbiological diagnosis in IE may facilitate determination of the optimal therapy.

Introduction

Infective endocarditis (IE) is a life-threatening disease that affects between 0.3 and 1.5 million people every year worldwide. In-hospital mortality rates of IE range between 11% and 26% (Moreillon and Que, 2004). Delay in treatment results in valvular obstruction, dissemination of septic thrombi to other organs, sepsis, multi organ failure and death of the patient. The infecting organisms can be difficult to eradicate and surgical management is often required in such cases. Most frequently, IE is caused by streptococci, enterococci and staphylococci (Moreillon and Que, 2004; Murdoch et al., 2009). Rheumatic heart disease (RHD) is an important predisposing condition in IE (Yew and Murdoch, 2012). RHD is caused by rheumatic fever, an autoimmune sequela of *Streptococcus pyogenes* infection (Marijon et al., 2012).

The genus *Streptococcus* comprises important human pathogens such as *S. pyogenes* and *S. pneumonia.* However, the most frequently isolated streptococci in IE belong to the viridans group streptococci (VGS) (Douglas et al., 1993; Moreillon and Que, 2004; Murdoch et al., 2009). VGS are a genetically diverse variety of species that are subdivided into the mitis, salivarius, anginosus and mutans group. They are non-β-hemolytic, except for some strains of the anginosus group (Facklam, 2002; Herzberg, 2000). Other non-β-hemolytic streptococci that are frequent in IE belong to the bovis group streptococci (BGS). Most of the VGS and BGS are found in the normal oral, urogenital or intestinal flora of humans. *S. gallolyticus* subsp. *gallolyticus* for instance, is a BGS that resides in the gastrointestinal tract of 2.5–15% of the healthy human population (Hinse et al., 2011). Species determination of VGS based on phenotypic tests alone remains inaccurate (Ikryannikova et al., 2011; Summanen et al., 2009; Teles et al., 2011) but nucleic acid based analyses have been developed to improve the identification of these opportunistic pathogens (Bishop et al., 2009; Garnier et al., 1997; Hoshino et al., 2005; Poyart et al., 1998; Summanen et al., 2009; Teles et al., 2011).

VGS often lead to detectable bacteremia after dental procedures (e.g. a tooth extraction) and injuries that cause breaches in soft tissues (Daly et al., 1997; Roberts et al., 1997; Westling et al., 2002). Fingerprinting techniques have demonstrated endogenous infection in patients with IE by VGS of their own oral flora (Fiehn et al., 1995). Another cause for endogenous IE is bacteremia with BGS that originates from the gastrointestinal tract. IE and other

* Corresponding author at: Helmholtz Centre for Infection Research, Inhoffenstraße 7, D-38124 Braunschweig, Germany. Tel.: +49 531 6181 4504; fax: +49 531 6181 4499.
E-mail address: Patric.Nitsche@helmholtz-hzi.de (D.P. Nitsche-Schmitz).

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infections with BGS are potential diagnostic indicators of malignancy as they are significantly associated with colonic cancer (Klein et al., 1977; Shanan et al., 2011).

The pathogenesis of VGS endocarditis is partially understood. In predisposed individuals congenital defects and degenerative processes such as inflammation or autoimmune diseases such as RHD produce lesions in heart valves in which subendothelial tissue is exposed. During transient bacteremia VGS adhere to the proteins of the subendothelial extracellular matrix (Christie et al., 2002; Love et al., 1997; McNab et al., 1996; Sommer et al., 1992) or to the thrombus that is formed at the site of endothelial injury. Procoagulatory effects of VGS drive the formation of the so called vegetation that is composed of bacteria and thrombus (Burnette-Curley et al., 1995; Erickson and Herzberg, 1990, 1993; Herzberg, 1996; Herzberg et al., 1992). Less is known about the pathogenesis of BGS endocarditis. BGS share virulence associated properties with VGS. They harbor homologues of genes that have been implicated in the virulence of VGS, but the role of these homologues in the pathogenesis of BGS infections is still elusive (Vollmer et al., 2010). Recently, contribution of a pilus operon to the formation of valvular vegetations by *S. gallolyticus* was shown in an in vivo model of IE (Danne et al., 2011). The pilus operon encodes three pilus proteins. One of them, Acb binds collagen (Danne et al., 2011; Sillanpaa et al., 2009). Ninety percent of *S. gallolyticus* isolates from cases of IE carried this pilus operon (Danne et al., 2011). Pathomechanisms exerted by VGS or BGS during IE may vary between group, species or even strains. Moreover, they are likely to depend on different predisposing conditions in the patient.

Prevalence of certain strains as well as predisposing factors of IE in the local population can differ greatly between distant geographic regions. Together this may crucially influence the spectrum of VGS or BGS that cause IE in different parts of the world. Using multilocus sequence analysis (MLSA) we have uncovered the spectrum of VGS and BGS (sub)species that caused IE in the Chennai region in southern India and in Germany, two distant geographical regions in which IE etiology was highly dissimilar.

Materials and methods

Study isolates

VGS and BGS isolated from IE were collected from 12/2005 to 4/2012 at the University of Madras in Chennai, India (53 isolates) and from 7/2000 to 12/2011 at the National Reference Centre for Streptococci in Aachen, Germany (59 isolates). Bacteria from Indian cases were isolated at the Department of Microbiology, University of Madras. Isolation criterion was diagnosis of IE and growth of the isolate in at least two cultures from three samples of venous blood that were taken consecutively in 1 h intervals at different anatomical sites. For blood cultures 45 ml of Brain Heart Infusion broth supplemented with 0.04% sodium polyanethol sulphonate was inoculated with 5 ml of venous blood and incubated at 37 °C, 5% CO_2. Bacteria from German cases were isolated in different collaborating diagnostic laboratories, before they were sent to the National Reference Centre for Streptococci for central collection. The participating diagnostic laboratories applied their local criteria and standardized methods for isolation. For further analyses the bacteria were cultivated for 24–48 h at 37 °C on tryptose agar with 5% sheep blood or in Todd Hewitt Broth containing 0.5% yeast extract (THY). Streptococcal isolates were examined using phenotypic tests (Facklam, 2002) and/or *sod*A single locus analysis (Bishop et al., 2009; Hoshino et al., 2005) and stored at -80 °C in broth containing 20% glycerol.

DNA extraction

DNA was extracted from overnight cultures on blood agar plates by alkaline lysis and subsequent neutralization (Hartas et al., 1998). Alternatively, DNA was extracted from the bacteria of a 10 ml overnight culture in THY disrupted with zirconia beads using a FastPrep device (MP Biomedicals, USA) at 4 m/s for 40 s and subsequent use of the DNeasy Blood and Tissue Kit (Qiagen, Germany). Extracted DNA was examined spectrophotometrically and by 1% agarose gel electrophoresis. DNA was stored at −20 °C.

PCR amplification and sequencing of seven house-keeping genes

Seven house-keeping genes *map*, *pfl*, *pyk*, *ppaC*, *rpoB*, *sodA* and *tuf* were amplified by PCR using pairs of primers given earlier (Bishop et al., 2009) with following exceptions. The *sodA*-gene of some BGS isolates was amplified using the alternative forward primer sodA_bfw (WCAYCATGATAAACATCAYGC). Primers map_m_fw (GCWGATTCHTGCTGGGCHT ATGC) and map_m_rv (TTARTAAGTCCYTTCTTCDCCCTG) were used to amplify *map* of *S. mutans* and *S. sanguinis*. The PCR reaction mixture consisted of 5 μl of DNA extract (0.3–1 μg of DNA), 1× PCR Buffer containing 1.5 mM $MgCl_2$, dNTP-mix (0.2 mM per dNTP), 10 pmol of each primer, 1 unit of Taq DNA polymerase filled with PCR grade water to a final volume of 50 μl. PCR was conducted with an initial denaturation at 94 °C for 3 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at temperatures specified below for 1 min and extension at 72 °C for 90 s. Final extension was carried out at 72 °C for 5 min. Genes *map*, *pfl* and *tuf* were amplified at 55 °C, *sodA*, *ppaC*, *pyk* and *rpoB* at 50 °C annealing temperature. Amplicons were sequenced in both directions using the primers described above and the Big Dye Terminator reaction (Applied Biosystems, USA) at Macrogen Inc. (Seoul, Korea) or at the Helmholtz Centre for Infection Research.

Multilocus sequence analysis (MLSA)

Sequences from 427 isolates that were analyzed earlier (Bishop et al., 2009) and from additional type strains ATCC 43143 *S. gallolyticus* subsp. *gallolyticus*, ATCC BAA-2069 *S. gallolyticus* subsp. *gallolyticus*, ACA-DC 198 *S. macedonicus*, ATCC 43144 *S. gallolyticus* subsp. *pasteurinanus*, CJ18 *S. infantarius*, AZ 3a *S. tigurinus* and 1366 *S. tigurinus* were retrieved from the GenBank database. Sequences were aligned, trimmed, edited and concatenated using the software BioEdit 7.0.1 (Isis Pharmaceuticals). Cluster analysis of the concatenated nucleotide sequences (3063 bp) of aforementioned seven house-keeping genes was conducted with MEGA 5.1 using the neighbor-joining method. Robustness of the nodes was tested by bootstrapping with 500 replicates. New sequences were deposited in GenBank (accession numbers KF288146-KF288929)

Results

Regional spectra of VGS or BGS from cases of IE

From a total of 112 cases of VGS or BGS (VGS/BGS) endocarditis that occurred from 2000 to 2011 in the Chennai region in southern India (53 cases) or in Germany (59 cases) the causative isolates were collected together with corresponding but anonymous clinical data (Suppl. Table 1). All Indian cases of IE were monobacterial. Based on the available data, potential co-infections could not be excluded for the German cases. Species of the isolates were assigned based on the multilocus sequence analysis (MLSA) described by Bishop and colleagues (Bishop et al., 2009). The neighbor-joining tree of 427 isolates from the MLSA database (http://www.eMLSA.net), bovis group type strains and the IE isolates from this study is shown in