Identification of viridans streptococci associated with bacteraemia in neutropenic cancer patients

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Summary. Twenty-three viridans streptococcal isolates from pyrexial neutropenic patients with various malignant diseases were studied in a comprehensive identification scheme. Fourteen isolates were identified as Streptococcus oralis, five as S. mitis and two as S. salivarius but the remaining two could not be identified reliably. The virulence mechanisms associated with the ability of these species to survive and grow in vivo require further investigation but may involve the production of specific glycosidase and proteolytic enzyme activities.

Introduction

Viridans streptococci were first described as causes of septicemia in children with cancer in 19781 and since then there have been numerous reports of such infections in neutropenic patients with malignancies.2-5 A typical clinical presentation includes shock and respiratory symptoms that may progress to the adult respiratory distress syndrome and some of these episodes have proved fatal.2,3,6 The apparent increase in the incidence of viridans streptococcal infections in compromised patients has been attributed to various factors, including the use of aggressive chemotherapeutic regimens that damage mucosal surfaces5 and the use of selective gut decontamination, particularly with the quinolones.3,9

When viridans streptococci causing infections have been further characterised, the commercial identification kit, API-20 Strep system (bioMérieux, La Balme les Grottes, France) has often been used. This method has identified most isolates as either "Streptococcus mitis" or "S. sanguis".2,6,7,9-11 Recently, however, there have been several significant changes in the taxonomy of the viridans streptococci; emended descriptions of S. sanguis, S. oralis, S. mitis, S. anginosus, S. intermedius and S. constellatus have been reported12,13 and new species S. gordonii,13 S. crista,14 S. vestibularis15 and S. parasanguis16 have been described. These taxonomic changes are not yet incorporated into commercial streptococcal identification kits. Other identification schemes have been described12,17 that incorporate many of these taxonomic changes, and this will result in the improved characterisation of medically important isolates.

We have compared results obtained with the API-20 Strep system with those obtained with a more comprehensive scheme,17 applied to a collection of viridans streptococci isolated from cases of neutropenic bacteraemia in an attempt to improve characterisation of the species responsible for this clinical picture and to obtain further understanding of the virulence mechanisms involved.

Materials and methods

Source of bacteria and strain identification

Blood cultures from pyrexial neutropenic patients treated for various malignant diseases at the Christie Hospital, Manchester, over a 3-year period were processed by either the Bactec radiometric or non-radiometric systems. Isolates were identified as viridans streptococci by standard criteria and were further characterised by the API-20 Strep system. Patient characteristics and identification of some of these strains have been described previously.2 The same strains were then identified according to the scheme of Beighton et al.17 that determines the ability of isolates to produce acid from nine carbohydrates, to hydrolyse aesculin and arginine and to hydrolyse 10 fluorogenic (4-methylumbelliferone-linked) glycosidase substrates; all tests were performed in microtitration trays. On the basis of these 21 different test results, strains of viridans streptococci may be assigned to one of the currently recognised species.

Results

Twenty-three separate isolates of viridans streptococci were available for identification by both schemes.
Carbohydrate fermentation mostly limited to lactose (an unacceptable profile) another as

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Identification by this scheme was not satisfactory.

With the more recent scheme we found that the majority of strains were either S. oralis (14) or S. mitis (5). S. oralis was characterised by the production of sialidase, β-N-acetylglucosaminidase, β-N-acetyl-galactosaminidase and, usually, β-galactosidase, with carbohydrate fermentation mostly limited to lactose and N-acetylglucosamine. S. mitis strains generally produced sialidase and often fermented melibiose, raffinose and N-acetylglucosamine. Two strains were identified as S. salivarius (most tests were variable but the majority of isolates produced arabinosidase), whereas the strains identified by the API-20 Strept system as "S. sanguis I" and S. lactis/S. diacetyl could not be identified.

**Discussion**

Some confusion arises when the API-20 Strept system is used to identify viridans streptococci as it employs taxonomic nomenclature which has now been superseded. Some of this confusion arises because, for example, S. oralis and S. mitis are related but separate from S. sanguis strains, because they have a distinct cell-wall composition, deficient in glycerol teichoic acid and significant amounts of rhamnose but containing a ribitol teichoic acid. On the basis of these cell-wall similarities, Colman and Williams18 grouped these two species into "S. mitior". However, genetic18 and physiological studies26 have clearly demonstrated the separate taxonomic status of these species, for which Kilian *et al.*18 have reported emended descriptions. The development of a new and improved version of the streptococcal identification kit by bioMérieux (Rapid ID 32 Strept system) may improve the reliability of identification but, of 17 strains of S. mitis, 11 required additional tests for identification and one was not identified, and of 22 strains of S. oralis, 14 required additional tests and one was not identified.23 With this kit, consideration should also be given to whether it can produce the correct identification of members of the "S. milleri group"—S. intermedius, S. constellatus and S. anginosus—and the fact that no strains of S. parasanguis or S. crista were included in the validation exercise previously reported.21 Therefore, the identification of these strains may still present difficulties. The API-20Strept system is unsuitable for the identification of these species.

Although many different species of viridans streptococci have been described, only three (S. salivarius, S. oralis and S. mitis) were isolated from blood cultures from pyrexial neutropenic patients. Allowing for the different descriptions of species used in other laboratories, these results are in accord with those of others,1,2,4,7,9,11 who reported the isolation of similar species from blood cultures of patients with similar medical histories.

The reasons for the preponderance of S. oralis and S. mitis strains in this collection of isolates from pyrexial neutropenic patients is unclear. The most likely source of these strains is the oral cavity where they form a significant component of the oral flora22 and from which they may gain access to the circulatory system as a result of dental manipulations, including simple toothbrushing—which often results in transient bacteraemia, even in healthy subjects. There have been many studies of the interactions between viridans streptococci derived from the oral cavity, and salivary and serum glycoproteins that have shown that strains now identified as S. oralis and S. mitis have the greatest affinity for salivary mucin because they possess receptors for the terminal portion of sialic acid-terminating oligosaccharide side-chains.23 Similar studies have demonstrated that strains of these two species also have the greatest affinity for transferrin;24 this would appear to be mediated by the same binding mechanism as for salivary mucin.

These species also have considerable ability to degrade and utilise glycoproteins for growth. Van der Hoven *et al.*25 reported that oral S. oralis strains exhibited the greatest ability to use salivary mucins for growth and we have reported that S. oralis and S. mitis strains are the most proteolytic of the viridans.
These species, compared with representatives of all other species of viridans streptococci, display the greatest proteolytic activity when tested with various synthetic fluorogenic protease substrates, human serum transferrin and bovine serum albumin. These findings indicate that S. oralis and S. mitis are the most effective of all the viridans streptococci in obtaining nutrients from host-derived glycoproteins and this ability might explain the preponderance of these species amongst viridans streptococci isolated from neutropenic patients with septicemia.

These data provide a further insight into the role of viridans streptococci in septicemia that occurs in neutropenic patients and suggest that S. oralis and S. mitis are the species most likely to be isolated. To understand the virulence mechanisms of these species, more detailed information on their ability to replicate in vivo is required.

References