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ANTIMICROBIAL SUSCEPTIBILITY OF VIRIDANS GROUP *STREPTOCOCCI ISOLATED* FROM BLOOD OF HOSPITALIZED PATIENTS

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ABSTRACT
The present paper describes the results of blood samples were collected and cultured from 100 patients hospitalized during the period of March 2009 to May 2009, in the regional hospitals at Allahabad (UP). Fifty nine blood culture samples were positive for *Streptococcus* sp., of which 21 VGS strains were isolated. The isolates were identified on the basis of cultural, morphological characteristics and biochemical tests were performed for antibiotic susceptibility. The 21 VGS strains isolated from the blood culture included, *S. mitis* (37%), *S. intermedius* (23.08%), *S. sanguis* (21%), *S. salivarius* (14.28%) and *S. mutans* (11%). Among the different risk factors evaluated *viz.* age and sex, socio-economic status, duration of hospitalization and fever, sex of patients showed a significant association ($p < 0.05$) with the incidence of VGS strains causing infection in the blood of hospitalized patients. The antibiotic sensitivity test was done by disc diffusion method. All VGS strains isolated showed resistance towards Penicillin and Vancomycin but were sensitive towards Erythromycin, Meropenem, Tetracycline, Azithromycin, Gatifloxacin and Ciprofloxacin. *S. mitis* and *S. sanguis* demonstrated resistance towards Ampicillin, however, *S. mutans*, *S. salivarius* and *S. intermedius* showed intermediate sensitivity to Ampicillin. There has been a drift in susceptibility patterns, with resistance issues seen in the general population of hospitalized patients now emerging in febrile neutropenic patients.

INTRODUCTION

Streptococci are gram positive cocci arranged in chains or pairs. They are important human pathogens, causing pyogenic infections with a characteristic tendency to spread as opposed to staphylococcal lesions which are typically localized. Viridans Streptococci are α- hemolytic, normal flora of the oral respiratory tract and gastrointestinal mucosa. They are major cause of bacterial endocarditis in people with damaged heart valves. They enter the blood stream after dental procedures (Azavedo *et al*., 1999). Viridans Streptococci represent a group of 24 currently described *Streptococcus* species that are nutritionally fastidious and mainly α-hemolytic on sheep blood agar (Ruoff, 1991). These viridans group Streptococci (VGS) comprise a significant proportion of the normal flora of the oropharyngeal tract (McWhiney *et al*., 1991); form a highly heterogeneous group of organisms (Francioli, 1995; Whiley and Beighton, 1998). Despite of the overall low virulence, they may cause infective endocarditis, contribute to polymorphic abscess, and invade the blood stream during the state of neutropenia. VGS are associated with endocarditis (Facklam, 1977; Parker and Ball, 1976), and infections of the central nervous, respiratory and musculo-skeletal system. They cause severe infections; they are responsible for up to 39% of the cases of septicemia in neutropenic patients with hematological diseases. The blood stream infection usually occurs in hospitalized patients with mucositis and neutropenia due to anti-neoplastic chemotherapy related toxicity. A blood culture is done when a person has symptoms of blood infection, also called bacteremia. Bacterial infections represent life threatening complications in patients with neutropenia, as has been observed in clinical trials evaluating febrile episodes in this patient group (Carratala *et al.,* 2000). During the past two decades, a trend towards an increasing number of gram positive infections, in particular those caused by Streptococci, has been observed worldwide (Maschmeyer, 1999). Viridans Streptococci are among the most common organisms isolated from cultures of bacteremia samples (Beighton *et al*., 1994; Johnson *et al.,* 2005).

A study showed that the rate of Viridans Streptococci bacteremia increased 1 per 10,000 to 47 per 10,000 (Elting *et al.,* 1992). Blood stream infection is an important and most frequent condition or cause of morbidity and death in hospitalized patients undergoing respective treatment .The principal species or species groups comprising these Streptococci are *S. mutans, S. salivarius, S. mitis, S. mille*ri (including *S. anginosus, S. constellatus, S. intermedius), S. sanguis, S. oralis* and *S. parasanguis* (Ruoff, 1995). Fatal outcomes, however, have occurred from sustained bloodstream infection with *S. mitis* in neutropenic cancer patients, neonates, and as an associated complication of adult respiratory distress syndrome (Tambekar *et al.,* 2007). Illness is associated with bacteremia which ranges from self limiting infection to life threatening sepsis (Rezende *et al.,* 2002). It has been reported that the antimicrobial susceptibility patterns

of Viridans group Streptococci vary according to geography (Reacher, 2000). In the past VGS, were nearly uniformly susceptible to β-lactam antimicrobial agents, amino glycosides, tetracycline and macrolides. However, their growing resistance to penicillin and other beta-lactam antimicrobial agent is increasingly being recognized as a matter of concern. The prompt initiation of empirical antimicrobial therapy is, therefore, a cornerstone in the management of patients with neutropenic fever today (Bochud *et al*., 1997). The VGS is the cause of bacteremia in blood of most of the hospitalized patients and the emergence of resistance to antimicrobial agents that has led to compromise with currently used prophylactic and therapeutic antibiotic regimens. Therefore, the present study was conducted to find out the most common cause of bacteremia and emergence of, strains of Viridans Streptococci, resistant to multiple antibiotics.

MATERIALS AND METHODS

The present study was conducted in the Department of Microbiology and Microbial Technology in the College of Biotechnology and Allied Sciences in Allahabad Agriculture Institute-Deemed University, Allahabad. Hundred blood samples of hospitalized patients were taken in different culture tubes from the hospitals selected in the study. 2mL of blood was drawn from patient, based on physician's decision on knowledge of infection and the person's clinical condition and medical history. A proforma was prepared based on the age, sex, state of disease, fever, endocarditis, skin infection, lower respiratory tract infection, mucositis, nausea, vomiting, diarrhea, gastro-intestinal tract infection, history of medication, duration of illness. Blood was withdrawn and immediately inoculated under strict aseptic conditions in broth media or brought to the laboratory and stored at 4ºC to be processed later on. The blood sample was cultured in the blood culture bottles containing Brain Heart infusion broth. The bottle without any disturbance was placed in the incubator, at 37ºC for 5-7 days and observed daily for signs of growth, *viz*. cloudiness or a color change in the broth, formation of gas bubbles, or clumps of bacteria. Sub-cultures was done over selective and enriched media, from day 2, with the positive culture bottle and incubated aerobically/anaerobically at 35ºC in 5% CO_2. VGS recovered from the positive blood cultures were identified by cultural, morphological and biochemical methods on the basis of characteristics given in Bergey's Manual of Systematic Bacteriology (Holt *et al*., 1984). They were identified by standard methods, including colony morphology and production of acid from Sorbitol, Lactose, Maltose, Mannitol, Raffinose and Inulin. These isolates were additionally tested for reactions such as optochin test, bile solubility-esculin hydrolysis test, growth in 6.5% NaCl, ammonia production from arginine, acetoin production, hydrolysis of starch and production of glucans and fructans.

The Antimicrobial agents tested were Vancomycin, Norfloxacin, Erythromycin, Ceftriaxone, Meropenem, Tetracycline, Azithromycin, Penicillin, Gatifloxacin, Ampicillin, Ciprofloxacin. Susceptibility testing was performed for each isolate over Muellar Hinton agar, by standard disc diffusion method, following the Clinical and Laboratory Standard Institute (CLSI) guidelines (Wayne, 2006), where isolates were classified as sensitive, resistant or intermediate resistant.

The data obtained during the study was subjected to statistical analysis using χ^2 test and t-test at 5% probability level (Panse and Sukhatna, 1967).

RESULTS AND DISCUSSION

Of the 100 blood samples collected from hospitalized patients, 59 were positive for Streptococcal strains, showing an incidence rate of 35.59% for Viridans Group Streptococci (VGS). On the basis of cultural, morphological characteristics and biochemical tests, the VGS strains were identified as *S. mutans* (11%), *S. mitis* (37%), *S. salivarius* (14.28%), *S. intermedius* (23.80%) and *S. sanguis* (21%) (Fig. 1). Incidence varying from as high as 71.8%-87% and as low as 3%-18% has been reported in studies conducted on VGS (Razonable *et al*., 2002; Edmond *et al*., 2003; Han *et al*., 2006; Huang *et al*., 2007). Viridans Group Streptococcal bacteremia is usually considered to derive from patient's own gastro-intestinal flora. The oral mucosa was the portal entry for Viridans Group Streptococci causing bacteremia in the neutropenic patients. They have been implicated in serious pyogenic infections (Piscitelli *et al*., 1992; Whiley *et al*., 1992). The variation in the recovery rate of VGS in different studies could be due to differences in geographical site, culturing system, use of different culture techniques and patient's selection criteria (Razonable *et al*., 2002; Tunkel *et al*., 2002). Enhancement in VGS infection was observed in hospitalized patients belonging to the age groups of 50yr and above (37.5%) and a

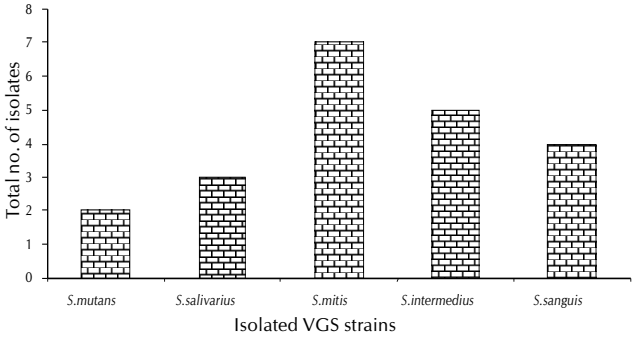

Figure1: Distribution of Viridans Group Streptococcal strains isolated from blood of hospitalized patients

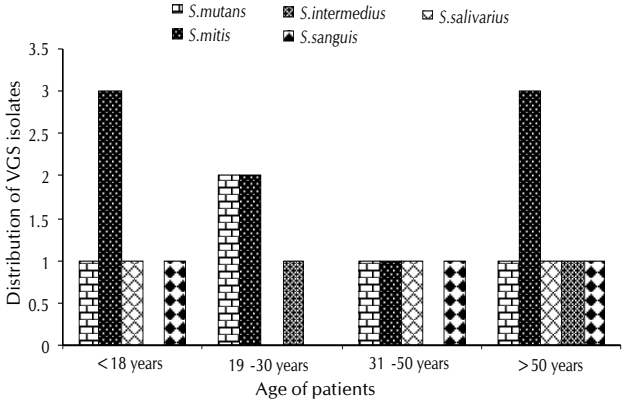

Figure 2: Distribution of VGS strains isolated from blood of hospitalized patients with respect to age

Table 1: Incidence of VGS spp. isolated from blood of hospitalized patients with respect to sex

Sex	Total no. of samples	Samples positive for VGS	Distribution of VGS isolates				
			S. mutans	*S. mitis*	*S. sanguis*	*S. salivarius*	*S. intermedius*
Male	67	15(22.38%)	2(13.33%)	5(33.33%)	3(20%)	2(13.33%)	3(20%)
Female	33	6(18.18%)	-	2(33.33%)	1(16.66%)	1(16.66%)	2(16.66%)

For Sex: $t_{cal.}(3) > t_{table}(2.231)$; S = Significant

comparatively lower incidence in age groups below 18yr and between 31-50yr (Anthony *et al.*, 2001; Cherif *et al.*, 2003; Lyytikainen *et al.*, 2004; Chulamokha *et al.*, 2006). On analyzing the data statistically, the difference was found to be non significant ($p > 0.05$). Further as in the present work, study carried out by Richardson *et al.* (2004) suggested no significant association of age with the VGS colonization in the blood of hospitalized patients (Fig. 2). A high incidence of Viridans group Streptococcal infection in blood was observed in male patients (22.35%) as compared to the female population (18.18%). Statistically, difference was found significant, thereby showing the influence of sex *viz*. male and female, over the incidence of Viridans Streptococci (VS) causing infection ($p < 0.05$) (Table 1). The present study was found in line with the observations of some other workers, showing a significant association of sex with the incidence of VG Streptococcal infection in the blood of hospitalized patients (Carratala *et al.*, 2000; Rezende *et al.*, 2002; Cherif *et al.*, 2003; Lyytikainen *et al.*, 2004; Chulamokha *et al.*, 2006; Huang *et al.*, 2007).

Maximum incidence of VGS causing infection was observed in patients belonging to higher (29.68%) class followed by the lower (20%) and medium (16.66%) socio-economic status group; however the difference was statistically non significant (Azavedo *et al.*, 1999). No significant association was observed for the duration of hospitalization with the incidence rate of VGS causing infection. However, patients hospitalized for 2weeks (53.33%) or less (50%), showed higher incidence for VGS, than the patients staying for 15-21 days (28.57%) or more than 21days (38.4%) (Edmond *et al.*, 2003; Chulamokha *et al.*, 2006). Fever was observed in almost all patients included in the study. In the study maximum incidence of VGS causing infection was observed in patients having fever for 17 days or more (46.15%) followed by an average of 10.5 days (20.68%) of persistent fever. Fever may persist for days, even though blood culture results rapidly becomes negative; therefore no significant association of fever was observed with the prevalence of VGS causing infection in blood (Richardson *et al.*, 2004 and Han *et al.*, 2006).

Antibiotic sensitivity testing

The isolated VGS strains were subjected to Antibiotic susceptibility test, using 11different commercially available antibiotic discs. Antibiotic susceptibility pattern of 21 VG Streptococci revealed varying degree of sensitivity. All the strains of VGS were found resistant to Penicillin and Vancomycin but showed sensitivity towards Erythromycin, Meropenem, Tetracycline, Azithromycin, Gatifloxacin and Ciprofloxacin. Intermediate susceptibility towards Ampicillin was observed for *S. mutans*, *S. salivarius* and *S. intermedius* while *S. mitis* and *S. sanguis* were found resistant to the drug. *S. mitis* and *S. intermedius* showed resistance to Ceftriaxone while *S. sanguis* exhibited intermediate susceptibility for Norfloxacin and Ceftriaxone (Table 2). The present finding shared the observations made by several workers (Wishplinghoff *et al.*, 1999; Alcaide *et al.*, 2001; Seppala *et al.*, 2003; Richardson *et al.*, 2004; Tambekar *et al.*, 2007; Huang *et al.*, 2007), determining the prevalence activity of VGS over penicillin, β-lactam antimicrobials and the fluoroquinolones. The study revealed high rates of penicillin and vancomycin resistance among current blood culture isolates of VGS, which was in agreement with the results previously reported from South Africa (Mashmeyer, 1999) and Spain (Burden *et al.*, 1991; Beighton *et al.*, 1994. Erythromycin, Meropenem and Ceftriaxone demonstrated uniform activity while Tetracycline and Ciprofloxacin were also highly active against this collection of VGS bloodstream isolates (Bochud *et al.*, 1994). The variations observed in the susceptibility pattern could be due to the factors like activity of strains, inoculum size, nature and time of inoculation, methods used, composition and nature of culture media and other experimental conditions. Also, it may be due to the unavailability for analysis of patients information pertaining to individual blood culture isolates such as specific disease associations or patient's antibiotic histories. Other factors like local environmental characteristics that affect the nosocomial micro

Table 2: Antibiotic susceptibility pattern for VGS isolates

Antibiotics	Concentration	Distribution of VGS Isolates				
		S.mutans	*S.salivarius*	*S.mitis*	*S.intermedius*	*S.sanguis*
Vancomycin	30μg	–	–	–	–	–
Norfloxacin	10μg	+ +	+ +	+ +	+ +	+
Erythromycin	15μg	+ +	+ +	+ +	+ +	+ +
Ceftriaxone	30μg	+ +	+ +	–	–	+
Meropenem	10μg	+ +	+ +	+ +	+ +	+ +
Tetracycline	30μg	+ +	+ +	+ +	+ +	+ +
Azithromycin	15μg	+ +	+ +	+ +	+ +	+ +
Penicillin	10 units	–	–	–	–	–
Gatifloxacin	5μg	+ +	+ +	+ +	+ +	+ +
Ampicillin	10μg	+	+	–	+	–
Ciprofloxacin	5μg	+ +	+ +	+ +	+ +	+ +

+ Intermediate; + + Sensitive; – Resistant

flora, its resistance patterns, antibiotic toxicity, cost of care and patient related factors should also be taken into account (Bronzwaer *et al*., 2002).

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