VIRIDANS STREPTOCOCCAL BACTERAEMIA IN PAEDIATRIC IMMUNOCOMPROMISED PATIENTS WITH MALIGNANT DISEASE

by

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A Thesis submitted to the Faculty of Medicine, University of Glasgow,

for the degree of Doctor of Philosophy

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August 2001

To Iain, Louise and Amanda

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Dr Joanne Michie for agreeing to my request to pursue the topic presented in this thesis as a PhD research project.

I would like to thank my supervisors: Professor Curtis Gemmell, Dr Joanne Michie and Dr Marcello Riggio for their advice, guidance and encouragement. Many thanks are also due to Professor Jeremy Bagg for making me feel so welcome at the Dental School and for expert advice and inspiration.

Special thanks are due to Dr Donald Morrison for training me in the 'art' of pulsed-field gel electrophoresis and to Peter Sweeney for superb technical assistance.

I am very grateful to my colleagues and friends who comprise 'The Team' of the Schiehallion Ward, RHSC, for their assistance in various aspects of this work. A special mention is due to Dr Brenda Gibson for expert advice, useful discussion and support, and to Nurse Practitioner Nan McIntosh for providing specialist knowledge and inspiring me with her positive attitude.

The staff of the Medical Microbiology Department at Yorkhill and the Oral Microbiology Department at Glasgow Dental Hospital and School helped with discussion and friendship.

I am particularly grateful to the Yorkhill NHS Trust Scholarship Committee for the award of funding to cover my fees throughout the period of this research project and to Aventis Pharma, BioMerieux U.K. Limited, Eli Lilly and Company Limited, Glaxo Wellcome, Pharmacia Limited and Wyeth for providing financial assistance or materials towards this project.

My family provided continued support and encouragement, without which this work would never have been completed. Most of all, I would like to thank my husband, Iain for advice on all things, especially in the fields of molecular biology and computing and for endless support in all aspects of my life.

Schiehallion

Schiehallion is a Munro on Rannoch Moor. The name was chosen for the Haematology/Oncology ward of the Royal Hospital for Sick Children, Glasgow to represent the uphill and relentless struggle faced by families with critically ill children. Schiehallion is famous for its blind summits which represent the many setbacks, disappointments and relapses with which the children and their families have to cope.

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PUBLICATIONS

Kennedy, H.F., Morrison, D., Kaufmann, M.E., Jackson, M.S., Bagg, J., Gibson, B.E.S., Gemmell, C.G., Michie, J.R. (2000). Origins of *Staphylococcus epidermidis* and *Streptococcus oralis* causing bacteraemia in a bone marrow transplant patient. *Journal of Medical Microbiology* **49**, 367-370.

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ABBREVIATIONS

Units

°C: degrees Celsius

g: gram

L: litre

M: Molar

m: metre

m^2: metre squared

mm: millimetre

µm: micrometre

mg: milligram

ml: millilitre

µl: microlitre

v/v: volume per volume

w/v: weight per volume

pH: Hydrogen ion concentration

r.p.m: revolutions per minute

Chemicals and Reagents

EDTA: Ethylenediaminetetraacetic acid

NaCl: Sodium chloride

Tris: (hydroxymethyl)amino-methane

Aesculin: 6-β-D-glucosyl derivative of 6,7-dihydroxycoumarin

SDS: Sodium dodecyl sulphate

Sma I: A restriction endonuclease from *Serratia marcescens*

Miscellaneous

P: Probability

sp: Species (singular)

spp: Species (plural)

ALL: Acute lymphoblastic leukaemia

AML: Acute myeloid leukaemia

ARDS: Acute respiratory distress syndrome

BMT: Bone marrow transplantation

CRP: C-reactive protein

CSF: Cerebrospinal fluid

DNA: Deoxyribonucleic acid

ITU: Intensive therapy unit

MIC: Minimum inhibitory concentration

MUD: Matched unrelated donor

NHL: Non-Hodgkin's lymphoma

PAGE: Polyacrylamide gel electrophoresis

PCR: Polymerase chain reaction

PFGE: Pulsed-field gel electrophoresis

RNA: Ribonucleic acid

RNAase: Ribonuclease

BSAC: British Society for Antimicrobial Chemotherapy

NCCLS: National Committee for Clinical Laboratory Standards

NCTC: National Collection of Type Cultures

PHLS: Public Health Laboratory Service

IATCG/EORTC: International Antimicrobial Therapy Cooperative Group of the European Organization for Research and Treatment of Cancer

SUMMARY

The earliest reports of viridans streptococcal bacteraemia in immunocompromised patients with malignant disease appeared in 1978, and throughout the following decade, several centres reported increased incidence of this infection. The clinical course of this infection was variable, but included a severe form with septic shock and/or acute respiratory distress. The development of such acute symptoms seemed inconsistent with the limited pathogenic properties of these oral commensals.

Amongst the paediatric haematology/oncology patients attending the Royal Hospital for Sick Children, Glasgow, episodes of viridans streptococcal bacteraemia increased from 12% of all microbiologically documented bacteraemias (*i.e.*10/81) in 1993 to 22% (18/83) in 1994. During the first year of this project (which started in December 1994), ITU support was required following the development of viridans streptococcal bacteraemia on 6 occasions, and of these, there were two fatalities.

The overall aim of this study was to improve the management of this infection and to explore preventative startegies. Three different approaches were adopted:

(1) An extensive epidemiological analysis was undertaken – to include all episodes of viridans streptococcal bacteraemia from December 1994 to December 2000.

(2) Phenotypic, followed by genotypic analyses of isolates of viridans streptococci from mouth swabs and blood cultures were carried out to determine whether the mouth was in fact the source of organisms responsible for this infection.

(3) Extensive antibiotic susceptibility studies were performed on all isolates of viridans streptococci from blood culture.

In total, 69 episodes of viridans streptococcal bacteraemia occurred in 54 children. The infection was more often associated with patients with haematological

malignancy (particularly AML), than those with solid tumours, and in the majority (84%) of episodes, the patients suffered from chemotherapy-induced mucositis or other forms of oral compromise. Forty-eight episodes of infection (70% of total) responded well to antimicrobial therapy with no evidence of additional clinical complications. However, in 21 cases (30% of total), pulmonary complications developed, with 8 of these requiring mechanical ventilation and supplemental oxygen. Five of these 8 cases also developed septic shock.

S. oralis was the species most commonly isolated from blood culture (63% of total isolates of viridans streptococci), and *S. mitis* represented 25% of total isolates. Polymicrobial bloodstream infection occurred in 23% of episodes.

In agreement with the findings of earlier workers, the incidence of viridans streptococcal bacteraemia was highest amongst those patients who had recently received chemotherapeutic regimens containing high-doses of the antimetabolite, cytosine arabinoside, in particular, the CLASP regimen (cytosine arabinoside 3g/m^2 twice daily with sequential asparaginase). Therapy with high-dose cytosine arabinoside is associated with profound neutropenia and severe mucositis. Pulmonary toxicity, also associated with the use of this agent, had previously been proposed by others as a factor related to the development of the acute respiratory distress syndrome (ARDS) in patients with viridans streptococcal bacteraemia. However the present study demonstrated additional contributory factors. Concomitant infection with respiratory viruses or *Pneumocystis carinii* was associated with 6 episodes of viridans streptococcal bacteraemia and 5 of these exhibited respiratory complications with 3 progressing to ARDS.

In phase 2 of the study, phenotypic analysis using a commercial identification system (BioMerieux Rapid ID 32 Strep) plus antibiograms, followed by genotypic analysis

using the technique of pulsed-field gel electrophoresis, demonstrated that in two selected cases, viridans streptococci from the oral cavity were indistinguishable from those isolated from blood cultures. Both mucositis and gingivitis were shown to be probable portals of entry. The same techniques were also used to demonstrate that isolates of *Streptococcus oralis* with *Staphylococcus epidermidis* causing poly-microbial bacteraemia, originated in the oral cavity of a patient with severe mucositis.

Genotypic methods also demonstrated that one strain of *S. oralis* which persistently colonized the mouth of one of the above patients, and which had caused bacteraemia, was also capable, within a short time interval, of colonizing the patient's toothbrush. This demonstrated that a tool used for mouth care could potentially become a reservoir of organisms capable of causing bacteraemia and underscored the importance of regular replacement of toothbrushes used by neutropenic patients.

Early results from the antimicrobial susceptibility study revealed a high rate of resistance to certain β-lactam antibiotics amongst viridans streptococci isolated from blood culture. Of particular concern was resistance to the third generation cephalosporin, ceftazidime, which in combination with amikacin comprised first line empirical antibiotic therapy for episodes of febrile neutropenia at the beginning of this study. Although all isolates were sensitive to vancomycin, this agent's potential for causing nephrotoxicity rendered it unsuitable for first line empirical therapy. Early results of this study showed that, against viridans streptococci, piperacillin/ tazobactam demonstrated superior *in vitro* activity to that of ceftazidime. The author and ward staff had experience of the former's good activity against Gram-negative organisms and good safety profile from participation in IATCG/EORTC Trial IX during 1991-92, therefore, after discussion, in summer 1996, piperacillin/tazobactam replaced ceftazidime as the β-lactam component of first line empirical therapy. This

intervention later provided the opportunity to conduct a retrospective sequential study to compare antimicrobial susceptibility of viridans streptococci from blood cultures prior to this change with those following it.

While ceftazidime was used as empirical therapy, the geometric mean MIC of this agent against viridans streptococci from blood cultures (31 isolates, throughout 19 months (period 1)) was 9.6 mg/L compared with 3.3 mg/L for isolates cultured from blood after empirical therapy was changed to piperacillin/tazobactam (45 isolates over a period of 54 months (period 2)) ($P < 0.05$). Of note, the geometric mean MICs for other β-lactam antibiotics tested (piperacillin/tazobactam, cefaclor, penicillin, cefpirome and meropenem) against these isolates also decreased from period 1 to period 2 (from 2.8 - fold for piperacillin/tazobactam to 1.4 - fold for meropenem)

Prior treatment with first line empirical antibiotics was associated with 93% of episodes of viridans streptococcal bacteraemia during the first interval of time, compared with 85% during the second ($P > 0.25$). The majority of patients who developed viridans streptococcal bacteraemia were receiving cotrimoxazole prophylaxis at the time (93% of episodes during period 1 and 88% during period 2), with resistance rates of 74% and 73% respectively - therefore this was a constant factor throughout. No other form of antibiotic prophylaxis was used. Comparing the patients with viridans streptococcal bacteraemia during period 1 with those of period 2, there were no statistically significant differences between the proportion with haematological malignancy versus solid tumours, the presence of mucosal lesions or the presence of neutropenia or between the number of episodes associated with treatment with high or intermediate-high dose cytosine arabinoside or allogeneic bone marrow transplantation, *i.e.* the therapies most associated with mucositis. The mouth care protocol remained essentially unchanged throughout the two time intervals.

During period 1, viridans streptococcal bacteraemia was associated with 4.6% of all febrile episodes compared with 2.2% during period 2 ($P < 0.005$). This corresponded to 17% of all microbiologically documented episodes during period 1 compared with 8% during period 2 ($P < 0.001$).

While there was little variation in the actual chemotherapy regimens used throughout the two study periods, the proportions of the various regimens used inevitably differed. It was not possible to obtain information on every course of chemotherapy administered throughout the entire study period. However, it was possible to perform an analysis of the total number of patients in the haematology/oncology unit who received CLASP chemotherapy and the development of viridans streptococcal bacteraemia during period 1 compared with period 2. All of these patients had previously received multiple courses of empirical antibiotic therapy. During period 1, eleven courses of CLASP were administered and 7 episodes of viridans streptococcal bacteraemia occurred, compared with 23 courses of CLASP and 4 episodes of viridans streptococcal bacteraemia during period 2 ($P < 0.01$). Therefore, in spite of the continued use of intensive chemotherapeutic regimens, fewer cases of viridans streptococcal bacteraemia occurred after the change in empirical therapy. Loss of mucosal integrity would generally be required to provide a portal of entry. However, amongst patients who have previously received empirical therapy, viridans streptococcal bacteraemia may be less likely to occur if the agent used has a low propensity to select for overgrowth of resistant strains.

The final part of the antimicrobial susceptibility study revealed that three newer antibiotics – cefpirome, quinupristin/dalfopristin and linezolid exhibited significant *in vitro* activity against isolates of viridans streptococci, demonstrating that even within the time span of this study, the potential range of antibacterial agents against multiply-resistant Gram-positive bacteria had increased.

INTRODUCTION

CHAPTER 1

VIRIDANS STREPTOCOCCI

VIRIDANS STREPTOCOCCI

1.1 Microbiology

Viridans streptococci, members of the genus *Streptococcus,* are spherical or ovoid bacteria, < 2 μm in diameter, arranged in chains or pairs. They are Gram-positive, facultatively anaerobic, non-motile and catalase-negative.

The term *viridans* is derived from the Latin word *viridis*: green. Many viridans streptococci produce a greenish discolouration (α-haemolysis) when cultured on blood agar due to the partial clearing of erythrocytes. However, some strains produce complete (β) haemolysis while others may be non - (δ) haemolytic.

Viridans streptococci are fastidious with respect to their growth requirements; enriched agars and broths are recommended for optimal recovery from primary cultures. The presence of carbon dioxide generally enhances growth while some strains require anaerobic conditions. Appearance of the colonies varies depending on the composition of the medium and the atmosphere of incubation.

Viridans streptococci are distinct from the pyogenic group of streptococci and can generally be distinguished from *Streptococcus pneumoniae*, (which also produces α-haemolysis on blood agar) by resistance to optochin and lack of bile solubility. Some isolates of viridans streptococci react with Lancefield grouping antisera, but the species do not correspond to specific serogroups and many isolates are non-groupable (Whiley & Beighton, 1998). In addition to the traditional antigenic, biochemical and physiological tests used to identify different species of streptococci in the past, more modern schemes utilizing molecular biological techniques are now available to increase accuracy in this field.

Viridans streptococci can be isolated from all sites of the mouth and comprise a major part of the resident microflora. As a result, the term 'viridans streptococci' has often been used interchangeably with that of 'oral streptococci'. However, in common with the former term, 'oral streptococci' is not a completely accurate description of these organisms as they may also be isolated, to a lesser extent, from other sites, such as the upper respiratory tract and gastrointestinal and genitourinary tracts and are sometimes isolated from the skin.

1.2 Classification and identification of viridans streptococci

Microbial classification is a dynamic area with existing species being re-classified due to the application of more sophisticated techniques and more stringent tests, together with the recognition of genuinely newly-discovered species. Taxonomy and identification are important in making associations between particular species and specific patterns of disease.

1.2.1 Taxonomic history of viridans streptococci

Classification systems for the genus *Streptococcus*, which included streptococci from saliva and the throat, have been proposed since the early twentieth century (Gordon, 1905). However it was not until the 1960s and 1970s that significant advances in this field were achieved. Around this time, Colman and Williams, using chemotaxonomic and genetic approaches, together with biochemical characterization, established the basis for the classification and identification schemes subsequently adopted by many laboratories in the U.K. and the rest of Europe. This system recognized five species of viridans streptococci (*Streptococcus mutans*, *Streptococcus milleri*, *Streptococcus sanguis*, *Streptococcus salivarius* and *Streptococcus mitior*) (Colman & Williams, 1972). In the 1970s in the USA, the system of Facklam (1977) was more widely used. This system assigned strains classified by Colman and Williams as *S. sanguis* to *S.*

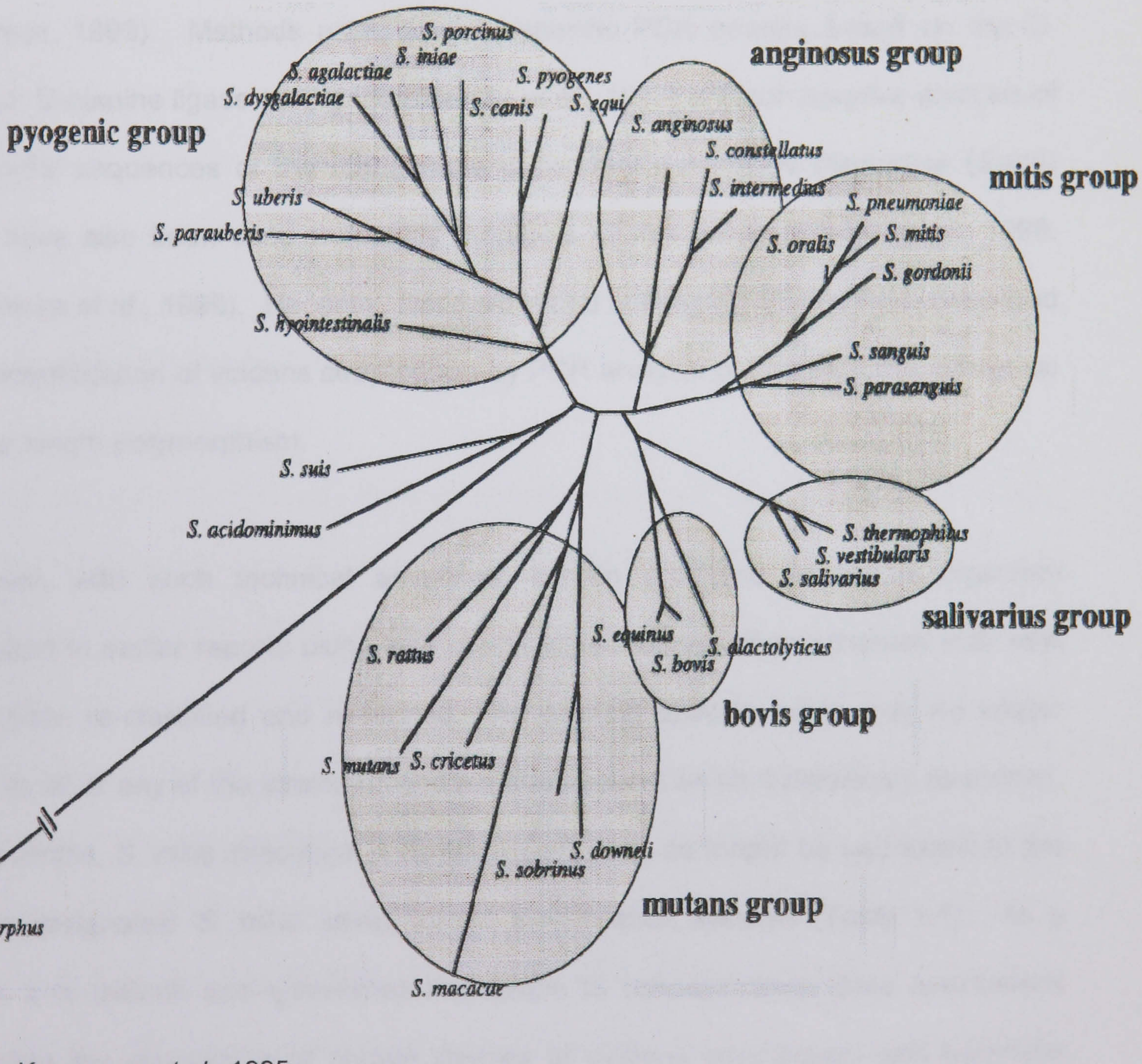
sanguis biotype I and *S. mitior* to *S. sanguis* biotype II or *S. mitis*. It also divided strains of *S. milleri* into *S. anginosus-constellatus* and *S. MG-intermedius*. Inevitably, these differences in classification and nomenclature resulted in considerable confusion in the literature (Facklam, 1984). In the 1980s, by which time molecular biological techniques were becoming established in the field of bacterial taxonomy, Kilian and co-workers devised an identification system based on phenotypic tests which correlated with the advances in genotypic identification (Kilian, Mikkelsen & Henrichsen, 1989). This scheme utilized chromogenic substrates for the detection of glycosidic hydrolases, peptidases, phosphatases, esterases and lipases, together with tests for the detection of IgA protease and neuraminidase activity, as well as conventional tests for carbohydrate fermentation. These authors proposed a scheme for the identification of *S. gordonii*, *S. mitis*, *S. oralis*, *S. sanguis*, *S. salivarius*, *S. mutans*, and *S anginosus (= 'S. milleri* group'). However several of the more recently described species - *S. vestibularis, S. parasanguis* and the three distinct species within the '*S. milleri* group' (*S. anginosus, S. constellatus* and *S. intermedius*) were not included.

In the early 1990s, Beighton and co-workers in the U.K. developed a more comprehensive system (Beighton, Hardie & Whiley, 1991). They collected strains, the taxonomic position of which had been established by DNA-DNA hybridization or by SDS-PAGE studies (Whiley, 1987). Phenotypic tests were then performed. These included the ability to produce acid from nine carbohydrates, to hydrolyse aesculin and arginine and to hydrolyse ten fluorogenic (4-methylumbelliferone-linked) glycosidase substrates. This battery of tests allowed representatives of all reported human viridans streptococci at that time, to be distinguished. Subsequent to this work, molecular taxonomic analyses identified the species *Streptococcus crista* (Handley *et al.*, 1991), *Streptococcus peroris* and *Streptococcus infantis* (Kawamura *et al.*, 1998), all members of the mitis group.

1.2.2 The current classification

Figure 1.1 shows a recent representation of the genus *Streptococcus* comprising the pyogenic group, the bovis group and the four species groups of viridans streptococci: the mitis, salivarius, anginosus and mutans groups (Kawamura *et al.*, 1995).

Figure 1.1 Phylogenetic relationships of members of the genus *Streptococcus* by rRNA gene sequence analysis



Source: Kawamura *et al.*, 1995.

(N.B: Three more species of viridans streptococci are now recognized as members of the mitis group: *S. crista* (Handley *et al.*, 1991), *S. peroris* and *S. infantis* (Kawamura *et al.*, 1998)).

With the advent of molecular biological techniques the classification of viridans streptococci has been improved and clarified. Techniques used include whole genomic DNA-DNA hybridization (Adnan *et al.*, 1993; Whiley *et al.*, 1990; Whiley & Beighton, 1991; Kikuchi *et al.*, 1995, Kawamura *et al.*, 1998), DNA-ribosomal RNA hybridization (Rudney & Larson, 1993, Rudney & Larson, 1994), and rRNA gene sequencing (Whiley *et al.*, 1990; Bentley, Leigh & Collins, 1991; Kawamura *et al.*, 1995; Kawamura *et al.*, 1998). Arbitrarily primed-PCR protocols have also been developed to facilitate identification of viridans streptococci of the mitis group (Rudney & Larson, 1999). Methods using species specific PCR primers based on the D-alanine: D-alanine ligase (*ddl)* gene (Garnier *et al.*, 1997) and comparative analysis of the partial sequences of the manganese dependent superoxide dismutase (*SodA*) gene have also been used to identify members of this group (Poyart *et al.*, 1998, Kawamura *et al.*, 1999). Recently, DeGheldre and colleagues (1999) have described rapid identification of viridans streptococci by PCR analysis of transfer DNA intergenic spacer length polymorphism.

However, with such technical advances, certain problems arise; an organism described in earlier reports using less sophisticated identification schemes may now have been re-classified and re-named. An existing species name may no longer apply to all or any of the strains of viridans streptococci which it previously described. For example, *S. mitis* described in earlier reports may no longer be equivalent to the isolate designated *'S. mitis'* using current identification systems (Table 1.1). As a result, it is difficult and sometimes impossible to retrospectively draw conclusions regarding the association of certain species of viridans streptococci with particular disease presentations.

Table 1.1 Present, compared with previous nomenclature of selected species of viridans streptococci belonging to the mitis group

Present nomenclature	**Previous nomenclature**
S. sanguis *S. gordonii*	*S. sanguis* I
S. oralis *S. mitis*	*S. sanguis* II / *S. mitior* / *S. mitis*

1.2.3 Commercial systems for identifying viridans streptococci

Conventional identification of viridans streptococci may be a lengthy process, requiring special biochemical tests and sera not generally used in the clinical microbiology laboratory. Commercial identification kits provide a convenient, quicker alternative and also a standardized means of testing, whereby the results from different laboratories can be compared. However, it has been demonstrated that for certain species of viridans streptococci, commercial systems may not provide results of comparable accuracy with those obtained from conventional tests or DNA-DNA hybridization (Hinnebusch, Nikolai & Bruckner, 1991, Beighton, Carr & Oppenheim, 1994) and as taxonomy of viridans streptococci advances, it takes considerable time to incorporate recent revisions into commercial systems.

Various commercial identification kits have been marketed (Hinnebusch, Nikolai & Bruckner, 1991). However those used most commonly in clinical microbiology

laboratories in the U.K. have been the API 20 Strep system, followed more recently by the Rapid ID 32 Strep method (BioMerieux, Basingstoke, U.K.). The former system performs less satisfactorily (West *et al.*, 1998) as it does not contain certain critical tests such as urea hydrolysis and N-acetyl-β-glucosaminidase.

The study of Kikuchi and co-workers (1995) assessed the accuracy of the Rapid ID 32 Strep system in identifying 171 isolates of viridans streptococci. Results were compared with those of DNA-DNA hybridization and conventional physiological tests. Eighty-seven percent of strains were identified correctly. Incorrect identification was obtained for 8% and no identification for 5%. It was difficult to differentiate some strains of *S. mitis* from *S. oralis,* and *S. parasanguis* and *S. crista* could not be identified because neither of these organisms were included in the identification database at that time. The conclusions of these workers were that this system could be used for the differentiation of most species for which phenotypic characteristics had been described if the database were revised according to the more recently reported amended criteria, with the reservation that a few species such as *S. mitis* and *S. oralis* may be problematic. Since then, the APILAB database has been revised to Version 2.0 (from March, 1998) and now includes *S. parasanguis* (BioMerieux, 1998).

Until recently, published studies investigating the usefulness of the Rapid ID 32 Strep system have used the earlier identification database (Version 1.1) (West *et al.* 1998; Jensen, Konradsen & Bruun, 1999), therefore evaluation of the updated version has not been generally available.

1.3 Viridans streptococci and the oral ecosystem

1.3.1 Acquisition of viridans streptococci

The mouth of the newborn baby is usually sterile. However from the first feeding onwards, the mouth is regularly inoculated with micro-organisms and the process of acquisition of resident oral microflora begins. By one month of age, virtually all infants are colonized with at least one species of viridans streptococcus. Colonizing micro-organisms originate in food, milk or water or may come from the saliva of individuals close to the baby (Kononen, 2000). Acquisition of micro-organisms from the birth canal itself may also occur (Carlsson & Gothefors, 1975).

The role of saliva in transmission of micro-organisms has been confirmed conclusively. Bacteriocin-typing of strains has enabled the transfer of *S. mutans* from mother to child via saliva to be followed (Berkowitz & Jordan, 1975). Similarly, comparisons of the DNA of a variety of oral bacteria indicated that the same strain was found within mother-child pairs and within family groups and that different patterns are observed between such groups (Caufield *et al.*, 1988; Caufield & Walker, 1989; Alaluusua *et al.*, 1993).

From birth, the human oral cavity is a highly selective environment which can only be colonized by certain micro-organisms (Kononen, 2000). The predominant 'pioneer species' in the mouth are streptococci. *S. salivarius* usually becomes established within the first 48 hours of life (McCarthy, Snyder & Parker, 1965). More recently, Pearce and colleagues (1995) have also identified *S. mitis* I and *S. oralis* as early colonizers, in a study of streptococcal species isolated from the mouths of neonates at different sampling times - 1-3 days, 2 weeks and 1 month *post partum*. The ability of some pioneer viridans streptococci to produce immunoglobulin A1 protease may influence their ability to survive in this habitat (Cole *et al.*, 1994).

With time, the metabolic activity of the pioneer community can modify its environment. Change in pH or redox potential or provision of new receptors or nutrients allows colonization by new organisms (Marsh & Martin, 1992). Once established, bacterial species tend to persist in the mouths of infants (Carlsson *et al*, 1970; Kononen *et al.*, 1999). However, at a clonal level, there exists a high degree of diversity amongst strains (Fitzsimmons *et al.*, 1996) and the turnover rate of these may be higher in children than in adults. Among more than 600 *S. mitis* biotype I isolates from two families, including two infants, collected four times at 3-monthly intervals, some persisting clones were found in adults but none in infants (Hohwy, 1997). The results from a study by Kononen and co-workers (1994) demonstrated a similar pattern for the oral anaerobe, *Prevotella melaninogenica*.

In the oral cavity, acquisition of viridans streptococci along with many other micro-organisms continues with age. Species which prefer to colonize hard surfaces, appear or increase in number once teeth have erupted. The oral microflora of teenagers differs from that of most 5 year-olds. Eighteen to forty percent of 5 year-olds are colonized with black-pigmented anaerobes, while 90% of teenagers aged 13-16 years harbour these organisms. It has been proposed that the increased prevalence of this bacterial group during puberty might be due to hormones entering the gingival crevice and acting as a nutrient source (Marsh & Martin, 1992). The process of microbial succession continues until a stable situation or 'climax community', comprising highly diverse species of micro-organisms is established.

1.3.2 Distribution of viridans streptococci

Viridans streptococci have species-specific predilections for anatomic areas of the oral cavity and pharynx. For example, in the healthy adult mouth, *S. sanguis* and

S. mitis I are commonly associated with the buccal mucosa, whereas the pharyngeal mucosa is more likely to be colonized with *S. salivarius* (Fransden, Pedrazzoli & Kilian, 1991). On the teeth, mutans streptococci and members of the anginosus and mitis groups are found as these organisms have a high affinity for hard surfaces. On the dorsum of the tongue, the major species found are *S. mitis* II and *S. salivarius* (Fransden, Pedrazzoli & Kilian, 1991).

In contrast to the oral microflora of adults, it has been found that *S. sanguis* is not generally isolated from the buccal mucosa of neonates and *S. mitis* II rarely colonizes the dorsum of the tongue (Pearce *et al.*, 1995, Fransden, Pedrazzoli& Kilian, 1991). *S. oralis* was commonly recovered from the oral mucosa of neonates by Pearce and co-workers (1995), however it was found almost exclusively on the teeth of adults (Fransden, Pedrazzoli & Kilian, 1991). In the adult mouth, on average, streptococci represent 28% of the total cultivable microflora from supragingival dental plaque, 29% from the gingival crevice, 45% from the tongue and 46% from saliva (Marsh & Martin, 1992).

1.3.3 Microbial diversity in the oral cavity

In addition to viridans streptococci, the mouth supports the growth of a wide variety of other micro-organisms. These include diverse bacterial species with yeasts, viruses and, on occasions, protozoa. Many of the bacteria are fastidious in their nutritional requirements and are difficult to culture and identify in the laboratory; many are obligate anaerobes (Table 1.2).

Table 1.2 Bacterial genera found in the oral cavity

	Gram-positive	**Gram-negative**
Cocci	*Enterococcus* *Peptostreptococcus* *Streptococcus* *Stomatococcus*	*Moraxella* *Neisseria* *Veillonella*
Bacilli	*Actinomyces* *Bifidobacterium* *Corynebacterium* *Eubacterium* *Lactobacillus* *Propionibacterium* *Rothia*	*Actinobacillus* *Campylobacter* *Capnocytophaga* *Centipeda* *Eikenella* *Fusobacterium* *Haemophilus* *Leptotricha* *Mitsuokella* *Porphyromonas* *Prevotella* *Selenomonas* *Simonsiella* *Treponema* *Wolinella*

Adapted from: Marsh and Martin, 1992.

The diverse habitats unique to the oral cavity together with a variety of nutrients can support this mixed population. In addition, in dental plaque, gradients develop in parameters of ecological significance, such as oxygen tension and pH, providing

conditions suitable for the growth and survival of micro-organisms with a wide spectrum of requirements. The distribution of micro-organisms is also related to their ability to adhere at a site, as well as to the need for their nutritional and environmental requirements to be satisfied. Many species of bacteria have been shown to adhere by specific molecular interactions between adhesins located on their cell surface and ligands on the host. These ligands are derived mainly from the acquired pellicle and mucus coat on enamel and mucosal surfaces respectively. Under the conditions found in the healthy mouth, no one bacterial population has a particular advantage and numerous species can co-exist.

1.3.4 Persistence of the oral microflora and 'colonization resistance'

The persistence of the resident oral microflora is dependent on the ability of these organisms to obtain nutrients and multiply in the mouth. Nutrients are derived mainly from the metabolism of endogenous substrates present in saliva and gingival crevicular fluid. Superimposed on these components are exogenous nutrients, which are supplied intermittently via the diet; the most significant of these are carbohydrates and casein. The concentration of nutrients will affect the growth rate and physiology of the microflora, as will any changes in pH resulting from microbial metabolism. The fluctuating conditions of nutrient supply and environmental change require the microflora to possess biochemical flexibility.

In the healthy mouth, members of the resident microflora, including viridans streptococci, prevent colonization by more pathogenic micro-organisms; a phenomenon called 'colonization resistance'. This can be achieved by various means including competition for mucosal adherence sites and the production of bacteriocins. One such bacteriocin is hydrogen peroxide, produced by certain members of the mitis group, which exerts an inhibitory effect on the growth of competing bacteria (Garcia-

Mendoza, 1993). The indigenous species may also be more efficient in utilizing natural substrates in the mouth so that invading organisms cannot flourish. The metabolism of the resident microflora, producing changes in pH or redox potential, can also make conditions unsuitable for colonization by other organisms. Host factors also play a role in colonization resistance. Immune and innate host defences also help exclude invading organisms (Marsh & Martin, 1992).

1.3.5 Disruption of the oral ecosystem

Colonization resistance may be impaired by factors which compromise the integrity of the host defences or perturb the resident microflora (Bagg, 1990; Sixou *et al.*, 1996; Lucas *et al.*, 1997; Sixou *et al.*, 1998). Classical examples are the use of broad-spectrum antibiotics or cytotoxic chemotherapy but other more subtle mechanisms can apply. Fibronectin has been shown to prevent adhesion of *Pseudomonas aeruginosa* to buccal epithelial cells (Woods *et al.*, 1981; Woods *et al.*, 1983). Levels of fibronectin in seriously ill adults and in infants are lower than those in healthy adults and may account for the higher rates of colonization by Gram-negative bacilli in these subjects.

The ecology of the oral cavity also varies with diet. The regular intake of dietary carbohydrates can lead to the enrichment of aciduric and cariogenic organisms such as mutans streptococci (Grindefjord *et al.*, 1991), while poor oral intake in debilitated cancer patients may also influence the microbial composition.

The very diverse resident oral microflora with its characteristic composition, exists, for the most part, in harmony with the host. However, components of this microflora can act as opportunistic pathogens when the habitat is disturbed or when micro-organisms are found at sites not normally accessible to them.

1.4 Infections associated with viridans streptococci

1.4.1 Introduction

In the past, the isolation of viridans streptococci from blood culture or cerebrospinal fluid has often been regarded as contamination. Viridans streptococci are frequently considered to be commensal organisms of low virulence. Their major disease associations were formerly limited to dental caries (Bagg *et al.*, 1999) and endocarditis (Douglas *et al.*, 1993). Over the last twenty years however, these bacteria have emerged as significant pathogens in certain patient populations, particularly the immunocompromised (Cohen *et al.*, 1983; Sotiropolous *et al.*, 1989; Classen *et al.*, 1990; Guiot *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Villablanca *et al.*, 1990; Weisman *et al.*, 1990; McWhinney *et al.*, 1991; Burden *et al.*, 1991; Elting, Bodey & Keefe, 1992; Awada *et al.*, 1992; Steiner *et al.*, 1993; Donnelly *et al.*, 1993; Bochud *et al.*, 1994; Richard *et al.*, 1995; Wisplinghoff *et al.*, 1999; Bilgrami & Feingold, 2000; Marron *et al.*, 2000).

The following section provides background information on the major disease associations of viridans streptococci. Infection in the immunocompromised host will be discussed in Chapter 3. It should again be emphasized, that due to the history of numerous taxonomic changes, it is often difficult to definitively associate any particular species responsible for infection in earlier studies with species identified using recent systems.

1.4.2 Dental caries

Dental caries is a plaque-associated infection (Bagg *et al.*, 1999). The development of plaque follows a definite pattern of bacterial colonization. Adhesion by pioneer species (*S. mitis, S. oralis, S. sanguis*) is followed by a gradual increase in the

complexity of the microflora to a climax community of high species diversity, including many filamentous and obligately anaerobic bacteria. A variety of studies have found a strong association between viridans streptococci of the mutans group and the development of dental caries. Evidence has also emerged that microbial succession may occur during the different stages of lesion development, both on enamel and on root surfaces. Mutans streptococci are associated with early demineralization while lactobacilli are implicated more with lesion progression and cavitation. Two features that, if not unique, are certainly distinctive properties of cariogenic bacteria are the ability to rapidly transport sugars, when in competition with other plaque bacteria, and to convert sugars rapidly to acid, even under extreme environmental conditions, such as at a low pH. Few oral bacteria are able to tolerate acidic conditions for prolonged periods, but mutans streptococci and lactobacilli are not only able to remain viable at low pH, but are able to continue to metabolise and multiply (Hamilton & Buckley, 1991). Such conditions would favour the proliferation of these organisms, possibly at the expense of other oral bacteria and thus increase demineralization.

1.4.3 Dental abscesses

A dental abscess is a collection of pus in the pulp or around the root of a tooth, which usually results from necrosis following the progression of dental caries. Viridans streptococci are often recovered from dental abscesses and are believed to be involved in the early pathogenesis of this infection. As the abscess develops there is a progression from a facultative to an obligate-anaerobic state. The presence of different species of both facultatively and obligately anaerobic oral bacteria may enhance abscess formation more efficiently than would be possible in infection by a single species (Lewis, MacFarlane & McGowan, 1990).

Most oral abscesses result in some bacteria accessing the bloodstream. Usually this bacteraemia is transient as host defence systems readily eliminate the organisms. Occasionally, however, septicaemia may develop (Marinella, 1998) and/or metastatic abscesses may form in the brain, liver or kidneys.

1.4.4 Endocarditis

Bacteraemia caused by viridans streptococci is usually of little clinical consequence in healthy individuals. Those with poor dental hygiene may even suffer viridans streptococcal bacteraemia as a result of innocuous activities such as chewing or tooth-brushing (Roberts *et al.*, 1997). Further causes include dental extraction, periodontal surgery, bronchoscopy and certain upper respiratory tract and oesophageal procedures (Daly *et al.*, 1997; Roberts *et al.*, 1997; Dajani *et al.*, 1997; The American Heart Association, 1997). However, as a sequela of viridans streptococcal bacteraemia, patients with cardiac compromise may develop infective endocarditis. Conventional microbiological techniques and ribotyping have confirmed that the mouth may be the source of viridans streptococci causing endocarditis (Fiehn *et al.*, 1995).

In the pre-antibiotic era viridans streptococcal endocarditis was always fatal. Without adequate therapy, progressive damage to the heart valves occurred, resulting in cardiac failure and death. Today, viridans streptococcal endocarditis accounts for about 40% of all cases of infective endocarditis (Working Party of the BSAC, 1998) with a mortality rate of 15-30%. Viridans streptococci of the mitis group, are most commonly involved, representing over 50% of all isolates. *Streptococcus mutans* and *S. salivarius* are the next most frequently isolated species. In patients with prosthetic cardiac valves, viridans streptococci are a common cause of late-onset endocarditis (Stanbridge & Isalska, 1997).

Various studies have suggested that conditions which alter the blood flow in the heart may predispose to endocarditis. When blood flow is slowest, deposition of platelets and fibrin may form a thrombotic vegetation. It is believed that viridans streptococci present in the bloodstream due to the development of bacteraemia, bind to this platelet-fibrin aggregate (Manning *et al.*, 1994). Further deposition of platelets and fibrin together with bacterial multiplication, result in increase in the size of the vegetation thus impairing valvular function.

Previously, the major predisposing factor for the development of viridans streptococcal endocarditis was rheumatic heart disease, the incidence of which has now declined. While this condition must still be considered a predisposing factor, other risk factors include prosthetic cardiac valves, previous bacterial endocarditis, systemic-pulmonary shunts, congenital heart disease and mitral valve prolapse (when associated with a systolic murmur) (Working Party of the BSAC, 1982 & 1990; The American Heart Association, 1997).

1.4.5 Meningitis

Viridans streptococci are an infrequent cause of meningitis, representing less than 5% of all cases of bacterial meningitis (Cabellos *et al.*, 1999). Isolation of α-haemolytic streptococci, other than *S. pneumoniae,* from CSF can also represent contamination. Careful clinical examination and precise interpretation of CSF laboratory results is of paramount importance. Of the viridans streptococci, members of the 'mitis' or 'salivarius' groups are most frequently isolated (Cabellos *et al.*, 1999). However meningitis caused by *S. constellatus* has also been reported (Roca, Romera & Simon, 1998). Viridans streptococcal meningitis may occur in all age groups including neonates of mothers with genital tract colonization with these organisms (Hellwege *et al.*, 1984).

Rare cases of viridans streptococcal meningitis have been reported following lumbar puncture (Schneeberger, Janssen & Voss, 1996). In this setting, the source of the infecting organisms may be the patient's endogenous flora, or contamination of a drug, or may be due to poor infection control by the physician. Two cases of viridans streptococcal meningitis have been described following percutaneous glycerol rhizotomy of the trigeminal ganglion (James, Kibbler & Gillespie, 1995). Meningitis has also been reported in association with viridans streptococcal bacteraemia, following upper gastrointestinal endoscopy and cauterization for gastric bleeding (Carley, 1992), and is a very rare complication of dental treatment (Colville *et al.*, 1993; Shetty, Keyser & Ridgeway, 1998).

Clinical symptoms include fever, headache, meningeal signs and confusion. With antimicrobial therapy, mortality rates are low. The majority of fatalities occur in association with viridans streptococcal bacteraemia in immunocompromised patients, in whom the symptoms and signs of meningitis may be more difficult to detect because of an impaired inflammatory response. Of particular concern are reports of meningitis caused by penicillin-resistant viridans streptococci in paediatric neutropenic patients (Balkundi *et al.*, 1997; Tokuda *et al.*, 2000). Viridans streptococcal meningitis has also been reported in association with endocarditis (Cabellos *et al.*, 1999).

1.4.6 Pneumonia

Pneumonia associated with viridans streptococci has been described in both immunocompromised and immunocompetent individuals (Pratter & Irwin, 1980; Marrie, 1993). It must be stressed however that viridans streptococci are often isolated from cultures of lower respiratory tract specimens as normal flora contaminants. Viridans streptococci grow more readily on culture media than more fastidious organisms such as *S. pneumoniae* - the α-haemolytic streptococcus

associated with classical bacterial pneumonia. Viridans streptococcal infection has become more commonly associated with a different form of pulmonary pathology - acute respiratory distress syndrome in haematology/oncology patients - to be discussed in Chapter 3.

1.4.7 Infection in neonates

There are several reports describing viridans streptococcal infection in neonates, most commonly associated with obstetric complications such as prolonged rupture of membranes, premature labour or peri-partum fever. Cases of bacteraemia (Broughton, Krafka & Baker, 1981; Moomjian, Sokal & Vijayan, 1984; Spigelblatt *et al.*, 1985) and meningitis (Hellwege *et al.*, 1984) have been described, usually occurring within the first week of life. The mode of transmission is probably vertical as the female genitourinary tract may be colonized with viridans streptococci. Species involved are usually members of the mitis group and, less commonly, *S. salivarius* (Spigelblatt *et al.*, 1985; West *et al.*, 1998). The infection generally responds well to antibiotic therapy. Rare fatal cases of viridans streptococcal bacteraemia in this patient group tend to be associated with extreme prematurity (Moomjian, Sokal & Vijayan, 1984).

1.4.8 Infections associated with the anginosus group of viridans streptococci

The anginosus group of viridans streptococci is associated with endogenous pyogenic infections, which sharply delineates these organisms from the other viridans streptococci (Hardie & Whiley, 1994; Jacobs, 1997). Disruption of mucosal surfaces due to surgical procedures, ulceration and perforation has been associated with infection. Other predisposing factors include diabetes mellitus, malignancy and immunosuppression (Brook & Frazier, 1994; Jacobs, 1997). It should be emphasized,

however, that haematology/oncology patients are generally much more susceptible to infection by viridans streptococci of the mitis rather than the anginosus group.

S. intermedius appears to associated with abscesses of the brain and liver, while *S. anginosus* is more commonly isolated from gastrointestinal and genitourinary infections (Hardie & Whiley, 1994; Jacobs *et al.*, 1995; Yamamoto *et al.*, 1999). *S. constellatus* is recovered from a wide range of sites including the thorax (Jacobs *et al.*, 1995). Infection by viridans streptococci of the anginosus group may involve pathogenic synergism with other bacterial species (Lewis, MacFarlane & McGowan, 1990; Brook & Frazier, 1994; Quinlivan *et al.*, 1996; Nagashima, Takao & Maeda, 1999). Bacteraemia caused by anginosus group streptococci is uncommon (Gossling, 1988).

1.5 Pathogenicity

Historically, viridans streptococci have been regarded as organisms which lack traditional virulence factors. However various subtle mechanisms contribute to pathogenicity in infection by these organisms.

1.5.1 Cariogenic activity

The properties that confer pathogenicity to cariogenic bacteria are related to the ability to rapidly catabolise dietary carbohydrates to acid, and for the organisms to survive and proliferate under the fluctuating conditions of pH in plaque. Mutans streptococci are both acidogenic and aciduric and thus can play an active role in demineralization of enamel (Marsh & Martin, 1992).

1.5.2 Adhesion

In cases of endocarditis, the efficient adherent ability of some species of viridans streptococci, particularly of the mitis group, allows them to attach to vegetations (Manning *et al*, 1994). Evidence from *in vitro* studies suggests that fibronectin may mediate the binding of viridans streptococci to damaged heart valves. The extent to which strains of viridans streptococci can adhere to fibronectin, fibrinogen and platelet-fibrin clots *in vitro* may be related to their ability to produce endocarditis in animal models (Manning *et al.*, 1994). Further animal studies suggest that the production of dextran, a feature of several members of the 'mitis' group, also correlates with the ability to induce endocarditis (Douglas *et al.*, 1993).

1.5.3 Virulence mechanisms of the anginosus group

As mentioned previously, members of the anginosus group are unique amongst the viridans streptococci in their association with purulent infection and have been shown to produce extracellular enzymes which contribute to pathogenicity (Jacobs, 1997). The production of the hydrolytic enzymes, hyaluronidase and chondroitin sulphate depolymerase may facilitate the spread of these organisms through host tissues (Homer *et al.*, 1993). Anginosus group streptococci also produce ribonuclease and deoxyribonuclease, enzymes which may facilitate liquefaction of pus (Homer *et al.*, 1993).

1.5.4 Glycolytic and proteolytic activity

It has been shown that the rate of growth of certain species of viridans streptococci on the teeth of primates is generally unaffected by the availability of the host diet (Beighton & Hayday, 1986). *S. oralis* and *S. mitis* persist in the mouths of neutropenic patients with cancer in spite of reduced or negligible food intake. By virtue of their

glycolytic and proteolytic activity, these species possess the greatest capacity of all viridans streptococci to degrade glycoproteins *in vitro* (Homer, Whiley & Beighton, 1990; Rafay, Homer & Beighton, 1996). Such enzymatic activity may also allow these species to obtain nutrients from host-derived salivary glycoproteins *in vivo* and may be related to their persistence in the mouths of patients with cancer. This finding may, in turn, be associated with the predominance of these particular species in viridans streptococcal bacteraemia in this patient group (Beighton, Carr & Oppenheim, 1994).

1.5.5 Induction of cytokines

It has been demonstrated that cell wall products of viridans streptococci, such as lipoteichoic acid, can induce the proinflammatory mediators, nitric oxide and tumour necrosis factor (English *et al.*, 1996). In addition, a number of investigators have shown that extracellular fractions of viridans streptococci can induce cytokine production (Soto *et al.*, 1998, Matsushita *et al.*, 1995; Takada *et al.*, 1993). Engel and co-workers (1996) showed that interleukin-6 levels in blood of two neutropenic patients with severe viridans streptococcal sepsis, were much higher than those of control patients with uncomplicated bacteraemia caused by Gram-positive organisms. A potential association of cytokine induction by viridans streptococci with development of severe sepsis, exclusively in neutropenic patients with cancer, will be discussed further in Chapter 3.

1.6 Antibiotic susceptibility

Reduced susceptibility of viridans streptococci to penicillin was first described in 1949 (Krumwiede). However around that time little clinical significance was attached to this or other early reports. Resistance to penicillin amongst viridans streptococci, particularly of the mitis group, is now common in many hospitalized patients, with resistance rates exceeding 50% in some reports (Tuohy & Washington, 1997).

Resistance has also been reported in viridans streptococci colonizing healthy individuals (Guiot, Corel & Vossen, 1994). Resistance to penicillin in these organisms is due to the development of altered forms of penicillin binding proteins which have reduced affinity for the antibiotic. These high-molecular weight proteins are encoded by 'mosaic' genes that are produced by genetic recombination events between different strains or species of oral streptococci (Chalkley *et al.*, 1991). Several streptococcal species, including *S. oralis*, *S. mitis*, *S. sanguis* and *S. pneumoniae* are naturally transformable, and can easily transfer antibiotic resistance markers into closely related species (Potgieter & Chalkley, 1995).

Resistance to penicillin in viridans streptococci is also associated with resistance or decreased susceptibility to other β-lactam antibiotics, including cephalosporins (McWhinney *et al.*, 1993; Alcaide *et al.*, 1995; Doern *et al.*, 1996; Pfaller & Jones, 1997; Pfaller, Marshall & Jones, 1997; Marron *et al.*, 2001; Kennedy *et al.*, 2001).

The prevalence of resistance of viridans streptococci to erythromycin and other macrolide antibiotics has also increased (Maskell *et al.*, 1990). Resistance to macrolides in viridans streptococci is associated with the presence of an rRNA methylase gene, *ermB,* which confers resistance to macrolides, lincosamides and streptogramin B antibiotics (Clermont & Horaud, 1990). A different mechanism, conferring resistance to macrolides but not to lincosamides and streptogramin B, has more recently been described (Luna *et al.*, 1999; Arpin *et al.*, 1999). The gene responsible, *mef,* codes for a membrane bound efflux protein. Resistance to tetracycline in viridans streptococci is encoded by the *tetM* gene which is often found linked with *ermB* (Poutanen *et al.*, 1999). Resistance to chloramphenicol and kanamycin is encoded by the *cat* and *aphA* genes respectively. Several studies have described resistance to two or more different classes of antibiotic amongst viridans streptococci (Pfaller *et al.*, 1997).

Susceptibility of viridans streptococci to the glycopeptide antibiotics, vancomycin and teicoplanin has remained high (Potgieter *et al.*, 1992; McWhinney *et al.*, 1993; Tuohy & Washington, 1997; Teng *et al.*, 1998; Marron *et al.*, 2001; Kennedy *et al.*, 2001). However, the potential for nephrotoxicity associated with the use of vancomycin, results in this agent commonly being reserved until microbial documentation of infection is available.

During the early years of the present study, there existed some concern that the observed increase in resistance of viridans streptococci to β-lactam antibiotics such as penicillin, cefaclor and ceftazidime might escalate and possibly spread to include the more modern β-lactam agents such as ceftriaxone and the carbapenems.

Around this time, two new anti-Gram-positive drugs, the streptogramin antibiotic, quinupristin/dalfopristin and the oxazolidinone, linezolid were being evaluated in clinical trials. The *in vitro* activity of these agents was also being determined - however predominantly against established Gram-positive pathogens (Pepper & Bouanchaud, 1996), rather than against opportunistic pathogens such as viridans streptococci.

Information was required on the susceptibility of viridans streptococci to the more modern β-lactam antibiotics, such as the carbapenems and the fourth generation cephalosporins and to the new agents, quinupristin/dalfopristin and linezolid.

CHAPTER 2

CHILDHOOD CANCER AND PREDISPOSITION TO INFECTION

CHILDHOOD CANCER AND PREDISPOSITION TO INFECTION

2.1 Introduction

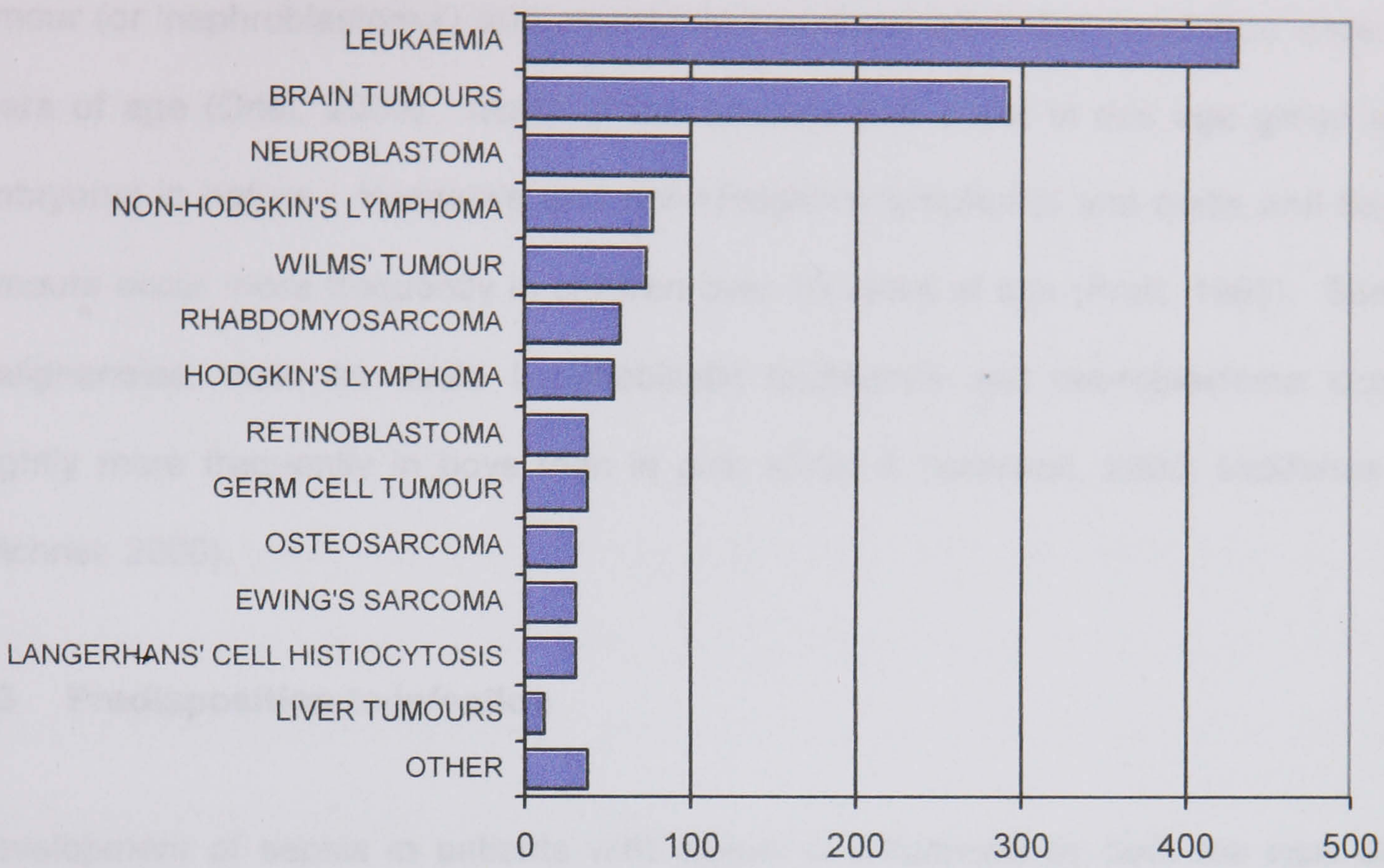
In the United Kingdom one in every 10,000 children under the age of 15 years develops cancer each year. Childhood malignancies differ markedly from those in adults, in type, site and incidence. Enormous progress has been made in the treatment of childhood cancer, with more than 60% of children now being cured – a much higher rate than that for adults. Because paediatric neoplasms are characterized by a high growth fraction with a propensity for frequent, early micrometastases, chemotherapy has become the cornerstone of treatment.

With the wider use of chemotherapy, more intensive treatment schedules have been developed, with or without bone marrow transplantation. Accordingly, infection has become an expected sequela of the therapy of paediatric malignancy. The patients described in the present study were receiving therapy for a wide range of malignancies. The following section provides a short description of the major types of childhood cancer.

2.2 Childhood cancer

Leukaemias comprise approximately one third of all paediatric malignancies with approximately 400 new cases occurring every year in the United Kingdom (Figure 2.1). Around 85% of these cases are acute lymphoblastic leukaemia (ALL). Acute myeloid leukaemia (AML) accounts for 10-15% of childhood leukaemia but is the commonest leukaemia of adulthood (Liesner & Goldstone, 1997). Chronic myeloid leukaemia (CML) occurs occasionally in young adults and is even less common in children.

Figure 2.1 Annual incidence of paediatric cancer in the U.K.



Source: Childhood Cancer Research Group, Oxford, 1996

Tumours of the central nervous system constitute the largest group of solid neoplasms in children (Figure 2.1). The overall distribution of childhood brain tumours according to their histologic appearance differs markedly from the pattern seen in adults as do the sites in which they arise. The predominant brain tumours in children are primitive neuroectodermal tumour (medulloblastoma) and astrocytoma.

Lymphomas are malignant tumours of the lymph nodes and are the third most common group of cancers in children and adolescents (Sandlund, Downing & Crist, 1996). There are two groups of lymphomas: Hodgkin's and non-Hodgkin's lymphomas (NHL). The presence of large binucleate cells (Reed-Sternberg cells) in the cancerous lymph nodes distinguishes the former from the latter.

Incidence and mortality rates of specific paediatric malignancies vary with age. Acute lymphoblastic leukaemia is commonest in the age range 2-10 years with a peak at 3-4

years (Liesner & Goldstone, 1997). The majority of cases of neuroblastoma, Wilms' tumour (or 'nephroblastoma') and retinoblastoma occur within the population under 5 years of age (Crist, 2000). Many of the tumours that occur in this age group are embryonal in nature. Hodgkin's and non-Hodgkin's lymphoma and testis and bone tumours occur more frequently in children over 10 years of age (Pratt, 1985). Some malignancies, such as acute lymphoblastic leukaemia and neuroblastoma occur slightly more frequently in boys than in girls (Crist & Smithson, 2000; McManus & Gilchrist, 2000).

2.3 Predisposition to infection

Development of sepsis in patients with cancer is influenced by both the type and extent of host predisposition to infection and by the individual's exposure to potentially pathogenic organisms. Predisposing factors include the malignancy itself, specific therapy directed against it, and supportive care.

2.3.1 Immunosuppression in malignancy and predisposition to infection

An intact and functioning immune system, along with physical barriers is necessary for protection against infecting micro-organisms. Immunosuppression in patients with cancer takes many forms and although one aspect may predominate, others may also be involved at more subtle levels. This section will describe the major causes of immune system dysfunction in patients with malignant disease.

2.3.1.1 Immunosuppression associated with malignancy

Granulocytopenia associated with tumour alone occurs most often with haematological malignancies intrinsic to marrow. However lymphomas, neuro-blastoma, malignant melanoma and certain carcinomas may also manifest

marrow involvement. In most situations, low cell counts appear due to marrow replacement with tumour. Defects in cell mediated immunity are recognized in patients with advanced Hodgkin's disease and to a lesser extent other lymphomas and acute and chronic leukaemias (Feld & Sutcliffe, 1987; Schimpff, 1995).

2.3.1.2 Immunosuppression associated with anti-cancer therapy

The tumoricidal effect of most anti-cancer drugs depends on their ability to kill dividing cells. This action inevitably damages normal cell populations in the skin, mucosal surfaces, gonads and bone marrow, resulting clinically in alopecia, mucositis, nausea, diarrhoea, infertility and myelosuppression.

Myelosuppression is the most serious complication of anti-cancer therapy. Anaemia and thrombocytopenia can usually be corrected with transfusion, but the problems associated with neutropenia can still be formidable. It is generally recognized that in cancer patients the risk of infection is highest during episodes of neutropenia following intensive chemotherapy for acute leukaemia or following allogeneic bone marrow transplantation. Other patients at high risk include those receiving intensive chemotherapy for relapsed lymphoma, because they frequently have severely compromised bone marrow reserve and other defects in immune function. Commonly used chemotherapeutic agents with dose limiting haematologic toxicity are listed in Table 2.1.

Table 2.1 Chemotherapeutic agents and associated haematologic toxicity/mucositis

	Neutropenia	Mucositis
Antimetabolites		
Methotrexate (high-dose)	+++*	+++*
Cytosine arabinoside (high-dose)	+++	+++
Fludarabine	+/++	+/-
6-mercaptopurine	+++	+/-
Alkylating agents		
Cyclophosphamide	+++	-
Melphalan (high-dose)	+++	+++
Anti tumour antibiotics		
Doxorubicin	+++	+++
Daunorubicin	+++	+++
Mitozantrone	+++	+++
Vinca alkaloids & Etoposide		
Vincristine	-	-
Vinblastine	++	+
Etoposide	++	++
Platinum analogues		
Carboplatin	++	-
Cisplatin	+	-

Sources: Perren, 1992; McGeer & Feld, 1994

(* +++, ++, +, -, *etc*: Interpretative severity guides)

After administration of chemotherapy, the neutrophil count nadir typically occurs 1-2 weeks later, although this can vary to some extent, depending on the particular agents used. Both the depth and duration of neutropenia are related to the development of infection (Bodey et al., 1966; Brown, 1984).

The frequency of infection begins to rise as the neutrophil count drops below 0.5 x 10^9/L. The absolute level of neutropenia is a useful index of infection risk, however

additional factors should be considered *e.g.* a rapidly decreasing neutrophil count is much more likely to be associated with an increased risk of infection than is the slow decline observed with syndromes such as cyclic neutropenia.

In addition to well-defined quantitative defects, qualitative abnormalities in neutrophil function can occur in leukaemia and lymphoma patients, including defects in chemotaxis, phagocytosis, and bactericidal activity (Curnette & Boxer, 1985). Corticosteroids, for example, can decrease phagocytosis and migration, and a number of antineoplastic agents, including vinca alkaloids, asparaginase, 6-mercaptopurine, methotrexate and anthracyclines can significantly decrease phagocytic and bactericidal activity (Pickering, Ericsson & Kohl, 1978). Corticosteroids can also alter macrophage function, diminishing host defence against fungi and other pathogens including mycobacteria, *Listeria* and *Brucella* as well as protozoans and viruses (Schaffner, Douglas & Braude, 1982).

The introduction of halogenated purine analogues, such as fludarabine, to certain drug regimens has added a new dimension to the infection risk associated with chemotherapy. These compounds do produce neutropenia, but they are more specifically lymphocytotoxic. There are reports of the use of these agents and the development of infections usually associated with defects in cell-mediated immunity *e.g. P. carinii* pneumonia and herpes virus infections (Schilling & Vadhan-Raj, 1990; Spielberger, 1993).

Radiation therapy, used alone, is generally associated with the least predisposition to infection. Radiation fields which include large volumes of bone marrow (for instance total craniospinal irradiation) are associated with some degree of reversible neutropenia; however the nadir neutrophil count is generally above 1.0 X 10^9/L, and defined infections and febrile neutropenia are rare. Extended field radiation therapy,

such as that employed in the treatment of Hodgkin's disease can produce long-lived lymphopenia and alterations in T lymphocyte subsets (Job *et al.*, 1984).

2.3.1.3 Immunosuppression associated with bone marrow transplantation

Risks of infection for bone marrow transplant (BMT) patients include the underlying disease and its remission status at the time of transplant, the ablative radiotherapy and/or chemotherapy used to prepare the recipient for transplantation, the prior infections of the donor, graft-versus-host disease (GVHD) and the immuno-suppressive therapy used for prevention or treatment of it. Patients who receive syngeneic or autologous BMT have fewer infectious complications than recipients of allogeneic transplants (Hunter, Haynes & Russell, 1995).

During the period before engraftment, patients undergoing any form of BMT are at risk of developing bacterial or fungal infections. This period usually lasts for the first 21 to 28 days after BMT, however the risk continues if complications such as acute GVHD occur. Acute GVHD may induce ulceration of the gastrointestinal tract and abnormalities of granulocyte function (Meyers, Flournoy & Thomas, 1986), however treatment of acute GVHD also enhances the risk of infection.

As the neutrophil count recovers, the pattern of infection changes, particularly for allogeneic transplant recipients, who remain profoundly immunosuppressed. Cellular immunity is particularly compromised and patients may develop life-threatening viral infections (Griffiths, 1995). Major pathogens after the initial period of engraftment include cytomegalovirus (CMV), adenovirus and fungi (including *P. carinii*).

The late phase of infection risk post BMT begins approximately three months after transplantation, around the time when chronic GVHD may develop. Sinopulmonary

and cutaneous infections, probably related to IgA deficiency may occur. Pneumonia caused by CMV and *P. carinii* may develop. Months, or even years after engraftment, encapsulated organisms such as *S. pneumoniae* may cause severe respiratory infections and bacteraemia attributable to the inability to make opsonizing antibody (Meyers, Flournoy & Thomas, 1986).

2.3.2 Splenectomy and predisposition to infection

Patients who have undergone splenectomy have a life-long increased risk of sepsis due to *S. pneumoniae*, *Haemophilus influenzae* and *Neisseria meningitidis*. This occurs because the spleen is of critical importance in clearing non-opsonized pathogens from the bloodstream early in infection (Bohnsack & Brown, 1986). Adults are at lower risk than children because they often have antibodies which can at least partially recognize pathogens.

2.3.3 Malnutrition and predisposition to infection

The effects of malnutrition on the immune system are complex and widespread; neutrophil migration, phagocytosis and bactericidal activity are all reduced, delayed type hypersensitivity is impaired, antibody responses to specific antigens are reduced, and cytokine secretion is abnormal (Chandra, 1983). Altered nutrition also contributes to the loss of integrity of the integument and mucosa. However it is difficult to estimate the size of these effects in predisposing to infection because malnutrition does not occur in isolation.

2.3.4 Disruption of physical defence barriers and predisposition to infection

Integumentary and mucosal physical defence barriers can be altered by tumour (*e.g.* obstruction of drainage). Chemotherapy may produce oral or gastrointestinal mucositis (Table 2.1), which provides a nidus for colonization and a portal for systemic

infection (Fayle & Curzon, 1991; Blijlevens, Donnelly & De Pauw, 2000). The use of implanted intravenous catheters such as Hickman lines offers sites for local as well as systemic infection (Elliot & Tebbs, 1998; Raad, 2000).

2.3.5 Disturbance of normal microbial flora and predisposition to infection

Administration of antimicrobial agents may alter natural colonization resistance which, may in turn, predispose to infection by exogenous pathogens. Repeated courses of empirical therapy or prophylaxis with antimicrobial agents may be associated with resistance amongst endogenous organisms (Carratala *et al.*, 1995, Spanik *et al.*, 1997, Kennedy *et al.*, 2001).

2.4 Infection in patients with malignancy

Infection is an expected sequela of the therapy of many forms of cancer. Although the fundamental principle of treatment of infection is to treat only sites that have been microbiologically defined, the standard practice for febrile neutropenic patients is to use broad spectrum antibiotics empirically (Kibbler, 1995). This approach originated in the late 1960s and early 1970s and was based on the observations of Schimpff, Bodey and others who demonstrated that empirical therapy could reduce the early morbidity and mortality associated with undiagnosed and untreated infections (Schimpff *et al.*, 1971; Bodey *et al.*, 1972).

Neutropenia not only increases the likelihood of infection developing - it also influences the severity of infection and the rapidity of its progression if left untreated (Schimpff *et al.*, 1971). In the late 1960s and early 1970s Gram-negative infections predominated and suitable antibiotics available for therapy were more limited than today. The literature of that period shows that infectious complications were an overwhelming problem for the haematologist/oncologist (Hersh *et al.*, 1965). The mortality from Gram-negative sepsis was reported to be 70-80% and reached 100% in

patients presenting with shock (Bryant *et al.*, 1971). This desperate situation has changed significantly with the availability of a wider range of potent antibiotics and the recognition that they should be started as soon as the neutropenic patient becomes febrile (Schimpff, 1986).

However, while the principles of empirical therapy have remained central to treatment of the febrile neutropenic patient, management of infection remains a dynamic field, as the challenges encountered continue to change as a function of the cancer treated, the antineoplastic regimens used, the hospital environment and the medical devices used. New and/or resistant pathogens have emerged. In particular, with the increasing use of bone marrow transplantation, many and varied factors interact to cause serious risk of infection, which may require several modifications in antimicrobial regimens, together with adjunctive cytokine therapy, to maximize patient outcome.

2.4.1 Micro-organisms associated with infection in patients with malignancy

Within the last 25 years there has been a major shift in the pattern of infection in febrile neutropenia. The studies of the International Antimicrobial Therapy Cooperative Group (IATCG) of the European Organization for Research and Treatment of Cancer (EORTC) demonstrated that in the mid-late 1970s, around 70% of cases of single-organism bacteraemia were caused by Gram-negative bacilli, predominantly *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* (EORTC Antimicrobial Therapy Project Group, 1978). Throughout the 1980s, the proportion of infections caused by Gram-negative bacteria declined while Gram-positive pathogens started to become more prominent. By the 1990s, a reversal of proportions relative to 1970s figures, had become established, with Gram-positive

bacteria representing around 70% of isolates responsible for bacteraemia (Table 2.2) (Cometta, *et al.*, 1995; Cometta *et al.*, 1996).

Table 2.2 The changing pattern of bacteraemia – EORTC trials 1973 - 94

Trial No.	Dates	Total No. of cases of single-organism bacteraemia	No. of cases (%) of Gram-positive bacteraemia	No. of cases(%) of Gram-negative bacteraemia
I	1973-6	145	42 (29)	103 (71)
II	1977-80	111	37 (33)	74 (67)
III	1980-3	141	58 (41)	83 (59)
IV	1986-7	219	90 (41)	129 (59)
VIII	1988-90	151	104 (69)	47 (31)
IX	1991-2	161	108 (67)	53 (33)
XI	1993-4	199	138 (69)	61 (31)

Sources: EORTC Antimicrobial Therapy Project Group, 1978; EORTC Antimicrobial Therapy Group, 1983; Klatersky *et al.*, 1986; EORTC International Antimicrobial Therapy Cooperative Group, 1987; EORTC International Antimicrobial Therapy Cooperative Group, 1993; Cometta *et al.*, 1995; Cometta *et al.*, 1996.

Bacteria have remained the most common pathogens in neutropenic patients, accounting for approximately 90% of the culture-documented infections (Pizzo, 1989). Although bacteria tend to be the initial cause of infection, fungi, such as *Candida* and *Aspergillus* spp. can emerge as important pathogens in patients who have protracted periods of neutropenia. In some cases, particularly post BMT, viruses such as herpes simplex virus (HSV), cytomegalovirus (CMV), adenovirus and varicella zoster virus (VZV) may cause serious infectious complications. Pathogens associated with infection in immunocompromised patients vary, to some extent, from centre to centre,

depending on differences in anti-tumour regimens, employment of prophylactic and therapeutic antibacterials, use of central lines, and the patient's environment.

Of the organisms responsible for bacteraemia, Gram-negative bacilli remain a major threat in view of their pathogenicity. *E. coli* is generally the most frequently isolated member of the Enterobacteriaceae, and although *Pseudomonas aeruginosa* is isolated less frequently than in the 1970s, it can still produce very rapidly progressing sepsis with significant morbidity and mortality. Other Gram-negatives associated with bacteraemia include *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Serratia* spp. Selective antibiotic pressure and the use of indwelling IV catheters may be contributory factors associated with the emergence of Gram-negative 'environmental' organisms, such as *Stenotrophomonas maltophilia*, as causes of bacteraemia (Spencer, 1995).

The frequency of anaerobic infections in neutropenic patients has remained relatively stable throughout the years, accounting for 3-5% of isolated organisms (Brown *et al.*, 1989). The most commonly isolated anaerobes are *Clostridium* and *Bacteroides* spp.

As mentioned in the preceding section, Gram-positive bacteria are now the commonest cause of bacteraemia in adult and paediatric neutropenic patients, and there is evidence that the shift to Gram-positives may have been more marked in the latter patient group (Langley & Gold, 1988; Viscoli, 1988). The reason for the prominence of Gram-positives is probably multifactorial, and includes the use of antibiotics targeted predominantly against Gram-negative bacteria, the use of antimicrobial prophylaxis, the use of indwelling venous access devices and therapy with more intensive chemotherapy causing severe mucositis (Schimpff, Scott & Wade, 1994; Rupp & Archer, 1994; Oppenheim, 1998).

The predominant Gram-positive pathogens are coagulase-negative staphylococci, mainly *Staphylococcus epidermidis*. It is generally accepted that the skin is an important source of these organisms and that the use of indwelling central venous catheters provides a surface to which bacteria can adhere, form a biofilm and persist (Pfaller & Herwaldt, 1988). However, certain centres noticed an increase in coagulase - negative staphylococcal bacteraemias a few years before the introduction of these catheters (Schimpff, Scott & Wade, 1994). Changing antibiotic prophylaxis and therapy may have been contributory, exerting selection pressure, resulting in colonization of the oral cavity and gastrointestinal tract with these organisms. In 1993, using molecular biological techniques, Wade demonstrated that strains of *S. epidermidis* found in blood cultures of a patient with leukaemia matched strains found in the alimentary tract.

The relative frequency of *Staphylococcus aureus* bacteraemia in neutropenic patients has decreased. However while coagulase-negative staphylococcal bacteraemia is usually a somewhat indolent infection, that associated with *S. aureus* can be life-threatening.

Over the last 2 decades, *Enterococcus* spp. (including glycopeptide-resistant isolates), *Corynebacterium* spp., *Bacillus* spp. and *Rhodococcus* spp. have been reported as causes of bacteraemia in neutropenic patients. However, of greatest clinical significance, throughout the 1980s and 1990s in some centres, and from the early 1990s in others, was the emergence of viridans streptococci as important pathogens (Cohen *et al.*, 1983; Henslee *et al.*, 1984; Groot-Loonen *et al.*, 1987; Menichetti *et al.*, 1987; Dybedal & Lamvik, 1989; Sotiropoulos *et al.*, 1989; Classen *et al.*, 1990; Villablanca *et al.*, 1990; Guiot *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; McWhinney *et al.*, 1991; Burden *et al.*, 1991; Elting, Bodey & Keefe, 1992; Awada *et al.*, 1992; Steiner *et al.*, 1993; Bochud *et al.*, 1994; Richard *et al.*, 1995). The course

of viridans streptococcal bacteraemia was reported to be variable, but could include a severe form with acute respiratory distress and septic shock (Cohen *et al.*, 1983; Groot-Loonen *et al.*, 1987; Menichetti *et al.*, 1987; Dybedahl & Lamvik, 1989; Guiot *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Steiner *et al.*, 1993; Bochud, Calandra & Francioli, 1994). Amongst paediatric patients, reported manifestations of the severe form included pulmonary or cardiac failure, shock, encephalopathy, pneumonia and renal involvement (Leblanc *et al.*, 1989; Sotiropoulos *et al.*, 1989). The median fatality rate was around 10% (Bochud, Calandra & Francioli, 1994). The epidemiology, aetiology, pathogenesis and management of viridans streptococcal bacteraemia will be discussed further in Chapter 3.

2.4.2 Management of infection

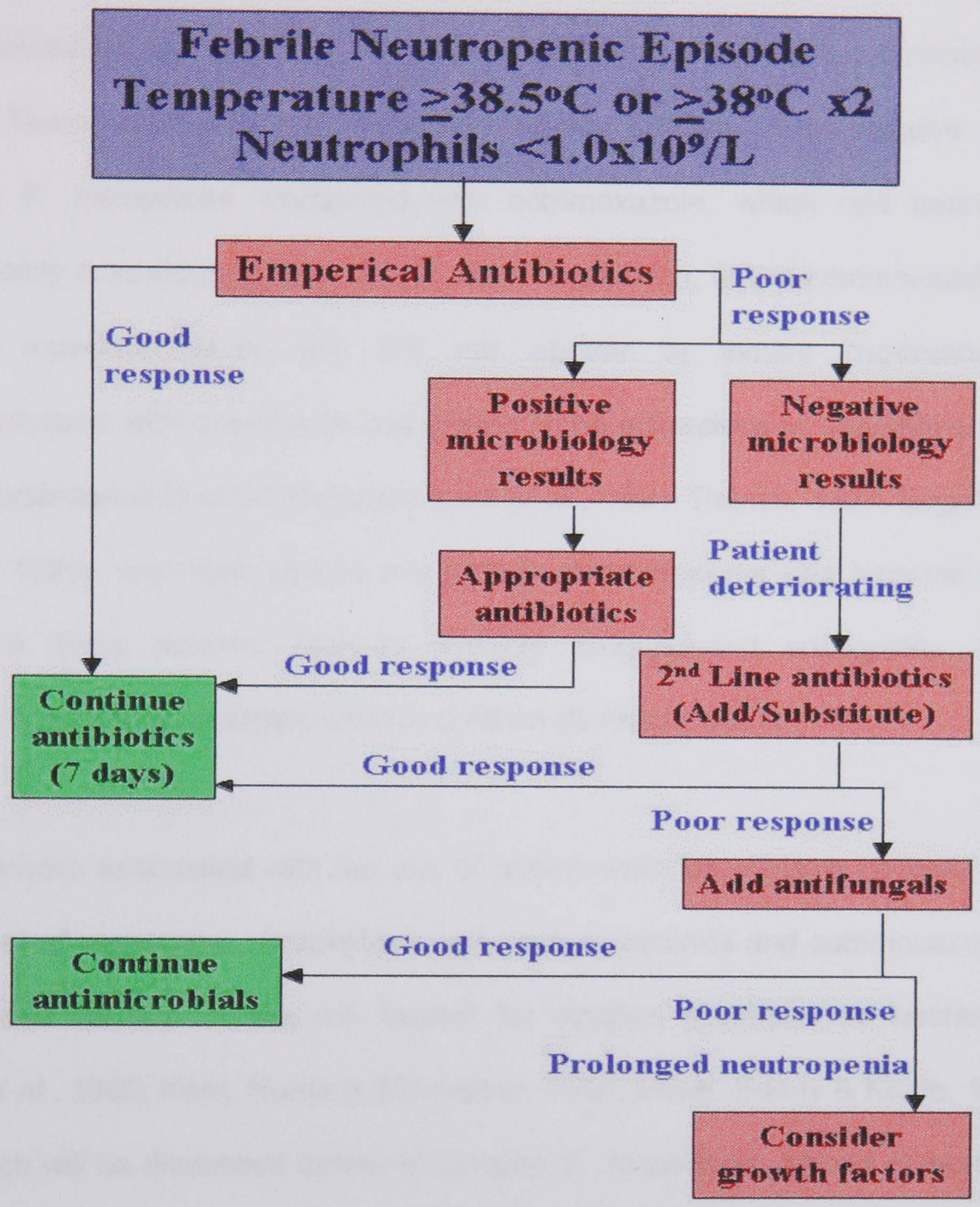
Although many febrile neutropenic patients do not initially have a proven site of infection, the prompt use of empirical antimicrobial therapy is crucial to a successful outcome. Selection of first line empirical therapy should be based on the local pattern of clinical isolates and their antimicrobial susceptibilities. The individual patient's infection history, results of surveillance cultures, and the presence of indwelling catheters, *etc.* should also be considered. First line empirical antibiotic therapy for episodes of febrile neutropenia usually comprises combination therapy of a β-lactam plus an aminoglycoside, or monotherapy with a very broad-spectrum β-lactam agent *e.g.* a carbapenem. A benefit of using synergistic combination therapy has been confirmed in the group of leukaemic patients with Gram-negative bacteraemia and severe protracted neutropenia (EORTC International Antimicrobial Therapy Cooperative Group, 1987). Other studies of empirical therapy, such as EORTC Trial XI, comparing meropenem monotherapy with combination therapy of amikacin plus

ceftazidime suggested that each approach was equivalent in efficacy (Cometta *et al.*, 1996).

The question of whether the predominant Gram-positive organisms should be fully covered by empirical therapy remains controversial. Most studies of the introduction of first-line Gram-positive cover used the glycopeptide antibiotics, vancomycin or teicoplanin, as these antibiotics historically have been the most effective of all against a wide range of Gram-positive pathogens (Karp *et al.*, 1986; Shenep *et al.*, 1988; EORTC International Antimicrobial Therapy Cooperative Group, 1991). However, some authors argue that infections caused by coagulase-negative staphylococci are quite indolent and carry a very low mortality rate, therefore it would be appropriate to wait for microbiological documentation before adding or substituting vancomycin (Rubin *et al.*, 1988). The potential for nephrotoxicity with vancomycin, particularly in combination with aminoglycosides (Kibbler *et al.*, 1989; Wood, 1996), and the development of glycopeptide resistance amongst *Enterococcus* spp. and reduced susceptibility amongst some strains of *S. aureus* (Witte, 1999), also lend support to the option of withholding glycopeptide therapy until the infection is microbiologically documented.

Algorithms are traditionally used to guide the antimicrobial therapy of febrile neutropenia (Figure 2.2). If the infecting organism can be cultured, therapy can be modified to suit, if necessary. However in cases of fever unresponsive to first-line empirical therapy, alternative or additional antibiotic cover is generally considered. If neutropenia is prolonged, the development of fungal infection becomes more likely.

Figure 2.2 Example of an algorithm for the treatment of febrile neutropenia



The recombinant haemopoietic growth factors, granulocyte colony-stimulating factor (G-CSF) and granulocyte macrophage colony-stimulating factor (GM-CSF) offer the potential of decreasing the duration of neutropenia after chemotherapy or bone marrow transplantation. In cases of neutropenic sepsis, as an adjunct to antibiotics, their use has been shown to augment the host response. However the majority of studies indicate that there is no significant reduction in mortality or decrease in duration of hospitalization (American Society of Clinical Oncology, 1994).

2.4.3 Preventative strategies

Shortly after their introduction in the early-mid 1980s, the fluoroquinolone antibiotics were considered useful agents for antibacterial prophylaxis of infection in neutropenic patients. These antibiotics had increased activity against Gram-negative bacilli, particularly *P. aeruginosa*, compared with cotrimoxazole, which had been used prophylactically in some units prior to this time. In addition, unlike cotrimoxazole they were not myelosuppresive and did not appear to induce hypersensitivity. Chemoprophylaxis with quinolones has proved to be efficacious in preventing Gram-negative bacteraemia in several studies (Lew *et al.*, 1991; Patrick, 1997; Engels, Lau & Baraza, 1998), and their use in the setting of neutropenia has become widely accepted in many centres. Due to possible drug-induced arthropathy, fluoroquinolones have not been widely used in children (Schluter, 1987).

A major concern associated with the use of antimicrobial prophylaxis however is the development of resistance. Prophylaxis with both quinolones and cotrimoxazole has been reported as one of the risk factors for viridans streptococcal bacteraemia (Classen *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992) - a topic which will be discussed further in Chapter 3. In addition, reports of increasing resistance of Gram-negative bacilli to these agents (Carratala *et al.*, 1995; Spanik *et al.*, 1996) may necessitate a general re-assessment of the prophylaxis of bacterial infections in neutropenic patients (Kerr, 1999).

Other preventative approaches include the use of high-efficiency particulate air (HEPA) filtration systems, good hospital hygiene, dietary advice, mouth care protocols and meticulous care of intravenous catheters.

CHAPTER 3

VIRIDANS STREPTOCOCCAL BACTERAEMIA IN IMMUNOCOMPROMISED PATIENTS WITH CANCER

VIRIDANS STREPTOCOCCAL BACTERAEMIA IN IMMUNOCOMPROMISED PATIENTS WITH CANCER

3.1 Historical perspective

In 1978, two groups in the United States produced the first reports of viridans streptococci as pathogens in immunocompromised patients with cancer. The publication of Hoecker and colleagues (1978) described 6 children with malignant disease in whom *S. salivarius* bacteraemia was diagnosed. Their ages ranged from 6-14 years. Information on their specific underlying neoplastic diseases was not provided. The portal of entry in all patients but one was thought to be the mouth, pharynx or respiratory tract. Three patients, who were profoundly neutropenic, died in spite of antibiotic treatment with intravenous penicillin. The authors stated that "penicillin G is the antibiotic of choice in treating infections due to *S. salivarius*" and added that "these organisms are sensitive to the majority of antibiotics except aminoglycosides and tetracycline occasionally".

The study of Pizzo and co-workers (Pizzo, Ladisch & Witebsky, 1978), of 29 episodes of viridans streptococcal bacteraemia in 27 patients, suggested that these organisms may cause clinically significant systemic infection in cancer patients, even in the absence of recent dental treatment. The median age of the patients was 16 years (range 2-57 years). Underlying diseases included leukaemia (18), lymphoma (7), and solid tumours (2). Twenty-six of the episodes (90%) occurred when the patient's malignancy was in relapse, and 75% occurred during episodes of neutropenia. Six of the patients had clinically apparent mucositis and two had a tooth extraction immediately prior to the onset of chills and fever.

Viridans streptococci were the only organisms isolated in 17 of the episodes (59%), whereas they were isolated as part of polymicrobial sepsis in twelve. The penicillin minimum inhibitory concentrations (MICs) were, < 0.8 mg/L for 27 isolates (93%), 1.6 mg/L for one and 3mg/L for the remaining strain. The authors proposed that it was probable that chemotherapy-induced oral mucositis provided a portal of entry into the bloodstream and that neutropenia allowed viridans streptococci to persist. They concluded that the isolation of viridans streptococci from the blood of patients with cancer should not automatically be dismissed as contamination.

In the 1980s, reports from other centres followed. In the U.K., the article by Cohen and colleagues, published in 1983, evoked considerable interest, and prompted groups elsewhere in Europe as well as in North America, to describe their recent clinical experience of viridans streptococcal sepsis in cancer patients (Henslee *et al.*, 1984; Ringden *et al.*, 1984; Bostrom & Weisdorf, 1984; Mascret *et al.*, 1984). Substantial morbidity and mortality, associated with a severe form of viridans streptococcal bacteraemia became documented, with complications such as septic shock and acute respiratory distress syndrome (Cohen *et al.*, 1983; Henslee *et al.*, 1984; Groot-Loonen *et al.*, 1987; Dybedal & Lamvik, 1989; Sotiropoulos *et al.*, 1989; Leblanc *et al.*, 1989). In certain cases, in spite of the use of appropriate antibiotics, clinical response required the recovery of neutrophil counts (Cohen *et al.*, 1983). High mortality rates were associated with the severe form of viridans streptococcal sepsis. During the 1980s, in addition to reports involving patients receiving conventional chemotherapy, several articles described viridans streptococcal bacteraemia in bone marrow transplant patients (Henslee *et al.*, 1984; Ringden *et al.*, 1984; Bostrom & Weisdorf, 1984). Around this time, as referred to in the previous chapter, the trials of the IATCG of the EORTC demonstrated a shift towards a predominance of Gram-positive organisms causing bacteraemia in febrile neutropenia, with the main organisms responsible, being coagulase-negative staphylococci, *Staphylococcus*

aureus and viridans streptococci (EORTC IATCG, 1990). Other groups documented a similar pattern (Pizzo *et al.*, 1986; Del Favero *et al.*, 1988; Shenep *et al.*, 1988; Viscoli *et al.*, 1988). Identification schemes used at that time, suggested that *S. mitis* and *S. sanguis* were the most common species of viridans streptococci causing bacteraemia (Cohen *et al.*, 1983; Henslee *et al.*, 1984; Leblanc *et al.*, 1989; Sotiropoulos *et al.*, 1989).

In the late 1980s, the association between chemotherapy with high-dose cytosine arabinoside and viridans streptococcal bacteraemia was proposed (Kern, Kurrle & Vanek, 1987; Weisman *et al.*, 1989; Dybedal & Lamvik, 1989; Sotiropoulos *et al.*, 1989). Several groups described viridans streptococcal infection in paediatric patients (Henslee *et al.*, 1984; Mascret *et al.*, 1984; Leblanc *et al.*, 1989; Sotiropoulos *et al.*, 1989) and one study suggested that this infection was more common in children than in adults (Mascret *et al.*, 1984).

During the following decade, further studies describing viridans streptococcal bacteraemia in paediatric immunocompromised patients were published. Weisman and colleagues (1990) described a predominance of Gram-positive bacteria (coagulase-negative staphylococci: 35.8% and viridans streptococci: 28.4%) from 109 consecutive episodes of microbiologically documented bloodstream infection in paediatric patients. Underlying diseases were predominantly leukaemias with a smaller proportion of solid tumours. Various chemotherapeutic regimens were used. A significantly higher incidence of viridans streptococcal bacteraemia was found among those patients who had received high-dose cytosine arabinoside.

The study of Villablanca and co-workers (1990), of a total of 832 paediatric and adult BMT (autologous: 214, allogeneic: 618) patients, demonstrated that age less than 18 years was a significant risk factor for viridans streptococcal infection. Valteau and

colleagues (1991) described 33 episodes of viridans streptococcal bacteraemia of a total of 91 cases of bloodstream infection in paediatric patients post autologous BMT. No prophylactic antibacterial treatment was given. The high rate of infection by viridans streptococci and the poor outcome for patients with prolonged and profound neutropenia led this group to modify their choice of first-line empirical antibiotic therapy from cefotaxime plus an aminoglycoside to ceftazidime plus teicoplanin. The antibiotic sensitivity pattern of the viridans streptococci isolated, showed that three of 34 (9%) were resistant to penicillin and 41% were intermediately susceptible. These investigators suggested that "the previous use of antibiotics, even though different from penicillin, might increase the rate of the resistant strains".

Similar clinical experiences resulted in other groups reviewing empirical therapy (Rossetti *et al.*, 1995), or employing or considering prophylaxis against viridans streptococcal infection (Guiot *et al.*, 1990; Broun *et al.*, 1994; Rolston *et al.*, 1995). Children were shown to be at higher risk of developing viridans streptococcal shock syndrome than adults (Steiner *et al.*, 1993; Martino *et al.*, 1995).

During the early 1990s, several papers discussed risk factors for viridans streptococcal bacteraemia (Villablanca *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992; Steiner *et al.*, 1993; Bochud *et al.*, 1994; Donnelly *et al.*, 1995; Engelhard *et al.*, 1995; Richard *et al.*, 1995) and some case control studies were published (Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994; Richard *et al.*, 1995). Several authors associated the prophylactic use of certain antimicrobial agents, to be discussed in Section 3.2, with the development of viridans streptococcal bacteraemia (Classen *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992).

The majority of papers published at the beginning of the 1990s, again suggested that *S. mitis* and *S. sanguis* were the predominant species causing viridans streptococcal bacteraemia (Guiot *et al.*, 1990; McWhinney *et al.*, 1991; Classen *et al.*, 1990; Burden *et al.*, 1991; Awada *et al.*, 1992; Elting, Bodey & Keefe, 1992; Steiner *et al.*, 1993; Bochud *et al.*, 1994). However, two groups of investigators found a different pattern. McWhinney and colleagues identified 47 sequential blood culture isolates of viridans streptococci from febrile neutropenic patients (McWhinney *et al.*, 1993) according to the scheme described by Beighton and co-workers (Beighton, Hardie & Whiley, 1991) and also using the commercial system, API 20 Strep (BioMerieux). The former system, which accommodated the recent taxonomic changes of that time, identified 39 isolates of *S. oralis*, 5 of *S. mitis* and 1 of *S. parasanguis*. Two isolates could not be identified to species level. In contrast, the results obtained using the older and more limited commercial system followed the pattern described earlier, with *S. mitis* and *S. sanguis* predominating. One year later, the report by Beighton and colleagues, utilizing the same two identification schemes, also demonstrated that the more modern and comprehensive system identified *S. oralis* as the predominant strain associated with bacteraemia in a group of neutropenic patients with cancer (Beighton, Carr & Oppenheim, 1994).

3.2 Established Risk Factors

As mentioned earlier, a number of case control studies have been performed (Villablanca *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994; Richard *et al.*, 1995). However, risk factors vary to some extent from study to study, because different control populations were used and, in some cases, different potential predisposing factors were considered.

Without doubt, profound neutropenia, produced by cytotoxic chemotherapy predisposes to viridans streptococcal infection (Elting, Bodey & Keefe, 1992). Many investigators proposed that chemotherapy-induced oral mucositis, was frequently involved (Cohen *et al.*, 1983; Sotiropoulos *et al.*, 1989; Classen *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Burden *et al.*, 1991) and this has been shown to be a significant predisposing factor in two case control studies (Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994). However at the commencement of the present study, there existed no definitive proof that the oral cavity could be the source of viridans streptococci causing bacteraemia. Ringden and colleagues suggested that oral ulceration caused by *Herpes simplex* virus could also provide a portal of entry (Ringden *et al.*, 1984). However other investigators found no association with oral herpes infection (Bostrom & Weisdorf, 1984).

The use of high-doses of cytosine arabinoside, as therapy for various forms of leukaemia has been shown to be a significant risk factor for the development of viridans streptococcal bacteraemia (Bochud *et al.*, 1994; Richard *et al.*, 1995). Used in high-doses, this agent produces profound neutropenia and severe mucositis. It has been hypothesized that oral and gastrointestinal mucositis produced by this agent provides a portal of entry for bacteria into the bloodstream and that neutropenia allows the persistence of the organisms.

Administration of high doses of this agent has also been associated with accompanying respiratory complications (Sotiropoulos *et al.*, 1989; Dybedal & Lamvik, 1989; Kern, Kurrle & Schmeiser, 1990; Guiot *et al.*, 1990; Bochud *et al.*, 1994). In addition to its toxic effects on the marrow and mucosa, high-dose cytosine arabinoside produces significant pulmonary toxicity. It has been proposed that immune-mediated effects, resulting from viridans streptococcal bacteraemia, have a role in the development of the acute respiratory distress syndrome (ARDS) in patients

whose pulmonary function has already been compromised by therapy with cytosine arabinoside (Guiot *et al.*, 1990; Bochud, Calandra & Francioli, 1994; Bochud, Cometta & Francioli, 1997). This topic will be discussed further in Section 3.6.

The development of viridans streptococcal bacteraemia has also been associated with the use of antimicrobial prophylaxis with the quinolone antibiotics (Kern, Kurrle & Schmeiser, 1990; Classen *et al.*, 1990) and with cotrimoxazole (Elting, Bodey & Keefe, 1992). Both the earlier quinolone antibiotics and cotrimoxazole have poor activity against viridans streptococci and could thus select for resistant populations (Lew *et al.*, 1995). The study of Donnelly and co-workers (1995) added a further dimension to this topic, by demonstrating that two different cytostatic regimens - one containing idarubicin and the other high-dose cytosine arabinoside, had a much greater influence on the development of viridans streptococcal bacteraemia than did prophylactic antibiotics.

The use of antacids and H_2-antagonists in the treatment of gastritis, has also been associated with viridans streptococcal bacteraemia in immunocompromised patients (Elting, Bodey & Keefe, 1992). The resulting increase in gastric pH may favour the multiplication of these organisms.

3.3 Clinical features

Pyrexia is generally the first clinical feature of viridans streptococcal bacteraemia. Bloodstream infection occurs early in the course of neutropenia. The more common and less severe form of viridans streptococcal bacteraemia responds to appropriate antibiotic therapy, with resolution of symptoms. As discussed previously, a small sub-set of patients progresses to fulminant sepsis associated with prolonged fever and severe respiratory distress (Guiot *et al.*, 1990; McWhinney *et al.*, 1991; Bochud *et al.*,

1994). In some cases septic shock accompanies this form (Henslee *et al.*, 1984; Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994), with increased morbidity and mortality (Bochud, Calandra & Francioli, 1994). Endocarditis is not a common complication of viridans streptoccoccal bacteraemia in neutropenic patients, although some cases have been reported (Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994).

3.4 Management of viridans streptococcal bacteraemia

Empirical antibiotic therapy for episodes of febrile neutropenia should ideally have activity against Gram-negative pathogens and against potentially life-threatening Gram-positive organisms. Today, first-line empirical therapy usually consists of the combination of an aminoglycoside antibiotic plus either a ureidopenicillin (+/- a β-lactamase inhibitor) or a third generation cephalosporin, or monotherapy using a very broad-spectrum β-lactam agent, such as a carbapenem.

Historically, viridans streptococci were considered to be uniformly sensitive to penicillin, however, as discussed in chapter 1, this is no longer the case (Mogi *et al.*, 1997; Ghaffar *et al.*, 1999). One report comparing penicillin-resistant viridans streptococcal colonization in both healthy children who had not recently received antibiotics, those with malignant haematological disorders and adult leukaemia patients, found that both paediatric groups had a significantly higher prevalence than adults with leukaemia (Guiot, Corel & Vossen, 1994).

If viridans streptococci are regarded as organisms with potentially reduced susceptibility or resistance to penicillin (Carratala & Gudiol, 1995), the choice of ß-lactam agent for empirical therapy of febrile neutropenia must be made with care – particularly in settings where viridans streptococcal bacteraemia is likely. Some

centres have reported high resistance rates to ceftazidime, a third generation cephalosporin commonly used as empirical therapy, necessitating review of therapeutic options (Carratala & Gudiol, 1995). Carefully conducted studies are required to establish whether the use of certain ß-lactam agents is associated with selection of antibiotic-resistant strains of viridans streptococci.

Previously, at institutions where infection by ß-lactam-resistant viridans streptococci had become a clinical problem, vancomycin has been considered as an option as part of initial empirical therapy (McWhinney *et al.*, 1993; Carratala & Gudiol, 1995). However this practice may encourage development of glycopeptide resistance and vancomycin is potentially nephrotoxic – as discussed in Chapter 2 (Section 2.4.2). A more extensive range of antibiotics specifically directed against Gram-positive bacteria would be useful. The present study assessed two such novel agents (Chapter 6).

If viridans streptococci are isolated from blood culture, antimicrobial susceptibility testing should be performed to guide therapy. If the organism is resistant to first-line therapy and/or clinical response is sub-optimal, change of therapy or addition of a glycopeptide antibiotic is recommended. However, it should be noted that in the severe form of viridans streptococcal bacteraemia, even with appropriate antimicrobial therapy, total resolution of symptoms may not be achieved until neutrophil recovery (Cohen, 1983). If viridans streptococcal shock syndrome develops, ITU support may be required, with inotropic support for severe hypotension along with vasodilators to improve peripheral perfusion, and ventilation for respiratory complications.

3.5 Preventative strategies

Various antibiotics have been used as prophylaxis against viridans streptococcal bacteraemia in neutropenic patients. While the use of penicillin in this setting has been shown to reduce the incidence of viridans streptococcal bacteraemia, it also became associated with the emergence of some strains with reduced susceptibility or resistance to this antibiotic (Broun *et al.*, 1994; Bochud *et al.*, 1994; Krcmery & Trupl, 1995). The study of Bilgrami and colleagues (1998) demonstrated that the prophylactic use of ampicillin resulted in selection for organisms that were resistant to β-lactam antibiotics and failed to reduce the incidence of viridans streptococcal sepsis. Vancomycin has been used successfully as prophylaxis (Broun *et al.*, 1994; Rolston, Elting & Bodey, 1995; Arns da Cunha *et al.*, 1998), however again there is concern that such use of vancomycin may accelerate the emergence of glycopeptide-resistant bacteria.

As oral compromise may be associated with viridans streptococcal bacteraemia, the establishment and maintenance of good oral hygiene both before and during the period of neutropenia may be beneficial. The oral care protocol should ideally suit the needs of the individual patient, depending on the findings of pre-treatment oral assessment, and the type and duration of chemotherapy to be used (Gibson, Horsford & Nelson, 1997).

3.6 Theories on the pathogenesis of viridans streptococcal bacteraemia in immunocompromised patients

The pathogenesis of viridans streptococcal bacteraemia in immunocompromised patients has not, as yet, been fully elucidated. The outcome of any infection is the end-product of a complex set of interactions between the host and the pathogen. In the development of viridans streptococcal bacteraemia in cancer patients, medical-

and immunocompromise of the host play a major part. In contrast to infection in these patients, viridans streptococcal bacteraemia in the immunocompetent host is usually of little clinical significance.

Viridans streptococci reside in an ideal habitat for invasion with resultant bacteraemia in patients with mucosal damage. Factors such as adherence, their general stability within the oral ecosystem and their ability to degrade salivary glycoproteins for nutrition as discussed in Chapter 1, may result in their effective persistence on mucosal surfaces. Damage to such surfaces could potentially provide a portal of entry into the bloodstream.

Once in the bloodstream of a patient with cancer, neutropenia may allow these organisms to persist. However in the majority of cases, when appropriate antibiotic therapy is administered, viridans streptococci are eliminated readily, with resolution of symptoms.

By what mechanisms then, might the severe form of viridans streptococcal bacteraemia develop? Some investigators have proposed that a particularly high bacterial load may be cleared less readily from the bloodstream, allowing these organisms to elicit the production of cytokines and thus increase severity of clinical symptoms (Donnelly *et al.*, 1995; Bochud, Cometta & Francioli, 1997). An overgrowth of bacteria at source - in the oral cavity or gastrointestinal tract may conceivably develop if selection pressure is exerted by prior use of prophylactic or therapeutic antibiotics with poor activity against these organisms (Carratala *et al.*, 1995).

As mentioned earlier, viridans streptococci have generally been regarded as 'low-grade' pathogens, sometimes described in the same category as coagulase-negative staphylococci. However the latter organisms do not cause shock and ARDS – even in

immunocompromised patients. Nevertheless, the extent of oral and gastrointestinal colonization by viridans streptococci probably far exceeds that of coagulase-negative staphylococci, lending support to the theory that the magnitude of the bacterial load may be important.

Gram-negative septic shock and that produced by the more potentially pathogenic Gram-positive organisms, *Staphylococcus aureus* and *Streptococcus pyogenes*, involve the induction of pro-inflammatory cytokines. Several studies have been performed to investigate whether viridans streptococci might also have the ability to induce these agents. It has been demonstrated that cell wall components of viridans streptococci, such as lipoteichoic acid or peptidoglycan can stimulate synthesis of proinflammatory mediators, such as tumour necrosis factor or nitric oxide *ex vivo* (Bhakdi *et al.*, 1991; Huemann *et al.*, 1994; Standiford *et al.*, 1994; English *et al.*, 1996). However the extent of cytokine induction was generally found to be less than that associated with lipopolysaccharide from Gram-negative bacteria. Lipoteichoic acid from viridans streptococci has also been shown to activate complement *in vitro* (Monefeldt, Helgeland & Tollefsen, 1994).

Engel and colleagues (1996) measured cytokine levels in serum from neutropenic patients, and demonstrated that, in both viridans streptococcal shock syndrome and in Gram-negative bacteraemia, IL-6 levels increased to a similarly high level. In contrast, using serum from patients with uncomplicated Gram-positive bacteraemia, levels of IL-6 were much lower. In patients with viridans streptococcal shock, TNF-α levels were slightly elevated, whereas from those with uncomplicated Gram-positive bacteraemia, TNF-α was undetectable. This study involved a small number of patients, with only two per group, but it did demonstrate an association between viridans streptococci and induction of cytokines *in vivo*, a finding worth investigating further in a larger group.

Some investigators have compared viridans streptococcal shock syndrome with that of streptococcal toxic shock. Superantigenic bacterial toxins such as streptococcal pyrogenic exotoxin A can cause profound hypotension, inflammation and organ failure in animal models (deAzavedo, 1989) and it is believed that strains of *Streptococcus pyogenes* which produce this toxin cause toxic shock syndrome in humans. By circumventing the usual rules of antigen presentation, superantigens can elicit massive and often destructive immune responses, involving substantial release of pro-inflammatory cytokines (Kotb, 1992). To date however, although several investigators have shown that extracellular fractions of viridans streptococci can induce pro-inflammatory cytokines (Takada et al., 1993; Soto, Evans & Cohen, 1996; Soto *et al.*, 1998) there is no convincing evidence that the production of superantigenic toxin is involved in viridans streptococcal shock syndrome (Soto *et al.*, 1998).

As mentioned previously, ARDS is a predominant feature of the severe form of viridans streptococcal bacteraemia. This respiratory complication is defined as a form of non-cardiogenic pulmonary oedema that results from acute damage to the alveoli (Tobin, 2000). Clinical and experimental studies have provided circumstantial evidence of the occurrence of neutrophil-mediated injury in ARDS (Ware & Matthay, 2000), therefore its development in neutropenic patients (Braude *et al.*, 1985; Ognibene *et al.*, 1986; Sivan *et al.*, 1990) is somewhat anomalous. Interleukin-8 (IL-8) has an important association with neutrophil accumulation and lung damage in non-neutropenic patients with ARDS (Donnelly *et al.*, 1993), and isolates of viridans streptococci have been shown to induce this cytokine from human peripheral blood mononuclear cells *in vitro* (Soto *et al.*, 1998), however the clinical significance of this finding with regards to neutropenic patients is unclear.

The observation that ARDS may develop in the absence of neutrophil involvement emphasizes the heterogeneity of this condition. Several investigators have proposed that multiple factors may influence its development in this patient group. The administration of chemotherapeutic agents such as cytosine arabinoside has been shown to increase alveolar capillary permeability resulting in acute pulmonary oedema, manifested by the onset of respiratory failure (Haupt, Hutchins & Moore, 1981). The study of Guiot and co-workers (1990) suggested that streptococcal infection may also play a part. As prophylaxis against streptococcal bacteraemia, twenty patients about to receive intermediate high-dose cytosine arabinoside were given penicillin G. The incidence of streptococcal infection following chemotherapy decreased from 0.76 per episode for controls who did not receive penicillin G to 0.11 per episode in the prophylaxis group. Simultaneously, a decrease in the incidence of respiratory failure was observed. It was hypothesized that bacteraemia caused by streptococci may trigger the development of respiratory distress in patients with pre-existing damage to the lungs due to treatment with cytosine arabinoside.

It has also been shown that the incidence of respiratory complications associated with viridans streptococcal bacteraemia was higher than that associated with bacteraemia caused by other micro-organisms (Weisman *et al.*, 1990). Some investigators have suggested that *S. mitis* has a greater predilection to cause shock and ARDS than other species of viridans streptococci (Cohen *et al.*, 1983; McWhinney *et al.*, 1991; Steiner *et al.*, 1993; Bochud, Calandra & Francioli, 1994).

3.7 Viridans streptococcal bacteraemia in paediatric immunocompromised patients attending the Royal Hospital for Sick Children, Glasgow.

3.7.1 Background to the investigation

During 1994, the author noticed that the number of cases of viridans streptococcal bacteraemia in immunocompromised patients at RHSC appeared to be increasing. Anecdotally, some of these episodes were associated with greater morbidity than had been observed in the past. All episodes did resolve, but in many cases, only after the addition of vancomycin to the standard empirical antibiotic therapy at that time - ceftazidime plus amikacin. Towards the end of 1994, the annual prevalence of viridans streptococcal bacteraemia was compared with that of the previous year. In 1993, there had been 10 episodes of viridans streptococcal bacteraemia, of a total of 81 episodes of culture-proven bloodstream infection *(i.e.* 12%), compared with 18 episodes of a total of 83 (*i.e.* 22%) in 1994. These findings prompted a literature search on viridans streptococcal bacteraemia in immunocompromised patients with cancer, to obtain details of this infection in patients in other haematology/oncology units. It was decided that from December 1994 all isolates of viridans streptococci from blood culture should be collected and stored at –70°C and the present study commenced.

3.7.2 Aims of the study

1. To collect and identify to species level, all significant isolates of viridans streptococci isolated from blood culture throughout the study period (1st December 1994 – 31st December 2000), and to determine whether particular species were associated with this infection or severity of symptoms.

2. To collect epidemiological information regarding the frequency of, and range of clinical features associated with viridans streptococcal bacteraemia in paediatric immunocompromised patients with cancer attending The Royal Hospital for Sick Children, Glasgow and to make interim analyses of data obtained. From commencement, it was decided that the epidemiological study should take the form of a descriptive report using the inclusion criteria and definitions detailed in Section 4.1.3.

3. To record details of all co-infecting micro-organisms from blood culture and any concomitant viral infections, and to determine whether or not mixed infection was associated with greater morbidity.

4. To determine the susceptibility of all blood culture isolates to a wide range of antibiotics, including those commonly used in the empirical therapy of febrile neutropenia.

5. To investigate factors which might influence resistance patterns in viridans streptococci. Based on the early results of antibiotic susceptibility testing, first-line empirical therapy was changed from ceftazidime plus amikacin to piperacillin/ tazobactam plus amikacin in July 1996. This provided an opportunity to compare, in a retrospective sequential study, the antibiotic susceptibilities of viridans streptococci isolated from blood culture prior to this change with those following it.

6. To determine susceptibility of all blood culture isolates to novel antibiotics which were undergoing clinical trials at the commencement of the study. This information could potentially provide suitable alternative therapies for the future.

7. To investigate, using phenotypic and genotypic analyses, whether the mouth was a potential source of viridans streptococci causing bacteraemia.

8. To investigate whether tools used for mouth care could potentially become vectors of viridans streptococcal infection.

9. To improve management of the infection and, if possible, to reduce its incidence.

3.7.3 Plan of investigation

1. Throughout the period of the study, as cases of viridans streptococcal bacteraemia occurred, isolates from blood cultures were subcultured, identified and stored at -70°C, as were isolates from mouth swabs.

2. Clinical details of infection were recorded from case notes.

3. Extensive antibiotic susceptibility testing was performed on batches of stored organisms at approximately 6 monthly intervals.

4. Three cases were selected, and pulsed-field gel electrophoresis (PFGE) was performed using DNA extracted from viridans streptococci isolated from blood culture and from mouth swabs, to determine whether the oral cavity was a possible source of organisms causing bacteraemia.

5. Throughout the period of this study, at weekly ward round meetings with clinical staff of the department of haematology/oncology, relevant findings were discussed and management of viridans streptococcal bacteraemia was reassessed as appropriate.

From the outset it was hoped that the results of this study would enhance knowledge and understanding of the epidemiology, aetiology and clinical progression of viridans streptococcal bacteraemia, as well as provide measures by which this serious infection could be optimally treated or even prevented. The following chapters describe how these goals were achieved.

CHAPTER 4

METHODS

METHODS

4.1 Epidemiological analysis

4.1.1 Application for ethics committee approval

At the commencement of this study, an application was submitted to the Ethics Committee of Yorkhill NHS Trust. Full approval was granted.

4.1.2 Construction of case record forms

Case record forms were constructed to facilitate transcription of patient demographics, antimicrobial therapy, the clinical course of viridans streptococcal bacteraemia and any potential predisposing factors (Figure 4.1).

4.1.3 Definitions

A case was defined as any paediatric patient with malignant disease, admitted to the haematology/oncology unit of RHSC from 1st December 1994 to 31st December 2000, with two or more temporally spaced blood cultures positive for viridans streptococci or with one blood culture positive for viridans streptococci in conjunction with clinical illness compatible with viridans streptococcal bacteraemia. Viridans streptococci were considered to be probable contaminants and of no clinical significance if isolated from one blood culture from patients without compatible clinical findings, where recovery occurred without anti-streptococcal therapy and repeat blood cultures were negative. Polymicrobial blood stream infection was defined as the isolation of additional micro-organisms (bacteria or fungi) in addition to viridans streptococci from blood culture, or the isolation of more than one strain or species of viridans streptococcus from the same blood culture during a febrile

Figure 4.1 Case record form

VIRIDANS STREPTOCOCCAL INFECTION STUDY **CASE RECORD FORM**

PATIENT NAME: ..... HOSPITAL No: .....SEX: .....

DATE OF BIRTH: .....WEIGHT: .....Kg SURFACE AREA: .....m^2

DIAGNOSIS: .....

ANTI CANCER THERAPY (including BMT if appropriate) WITH DATES: .....

.....

.....

DATE OF EPISODE OF BACTERAEMIA: .....

	YES	NO	COMMENTS
HICKMAN LINE *IN SITU*:	☐	☐	.....
RECENT DENTAL MANIPULATION:	☐	☐	.....
ORAL HSV-1 INFECTION:	☐	☐	.....
OTHER RELEVANT INFECTION:	☐	☐	.....
ANTIMICROBIAL PROPHYLAXIS:	☐	☐	.....

	MILD	MOD	SEVERE	COMMENTS
SIGNS & SYMPTOMS OF INFECTION:	☐	☐	☐	.....
MUCOSITIS:	☐	☐	☐	.....
DIARRHOEA:	☐	☐	☐	.....
RESPIRATORY COMPLICATIONS:	☐	☐	☐	.....

No. OF PYREXIAL DAYS: .....

MOUTH CARE: .....

NEUTROPENIA: .....

ANTIMICROBIAL THERAPY & LEVELS: .....

.....

CRP LEVELS: .....

episode. Bacteraemia was defined as the presence of bacteria in the blood. Neutropenia was defined as a neutrophil count of < 1.0 x 10^9/L and fever as an oral temperature of ≥ 38.5°C once or ≥ 38°C on two or more occasions during a 12 hour period. Septic shock was defined as sepsis-induced hypotension (systolic blood pressure <90 mm Hg) or the requirement for vasopressors/inotropes to maintain blood pressure despite adequate fluid resuscitation along with the presence of perfusion abnormalities that included, but were not limited to, lactic acidosis, oliguria, or acute alteration in mental status (Bone *et al.*, 1992). Acute respiratory distress syndrome (ARDS) was defined as a condition involving impaired oxygenation with evidence of new, bilateral, diffuse, patchy or homogeneous pulmonary infiltrates on chest radiograph and a pulmonary artery occlusion pressure ≤ 18 mm Hg when measured or no clinical evidence of left atrial hypertension (Bernard *et al.*, 1994). Oral mucositis was defined as inflammation with or without ulceration of the oral mucosa (Lavelle, Jackin & Morry, 1984).

4.1.4 Compilation of data

To accommodate the total data collected in the case record forms, two spreadsheets were constructed using Excel. Spreadsheet 1 contained patients' names and underlying malignancies, most recent chemotherapy, dates of episodes of viridans streptococcal bacteraemia and episode reference numbers, the presence or absence of neutropenia, oral complications and/or diarrhoea and clinical features associated with infection – duration of pyrexia, development of septic shock (with blood pressure readings) and/or respiratory distress and clinical outcome.

Details of severity of oral mucositis were recorded directly from case notes. Oral examination was performed daily by various physicians, and mucositis recorded according to the ward scoring system, whereby '+', '++' and '+++', corresponded to

mild, moderate and severe symptoms respectively. The spectrum of symptoms ranged from erythema with few or no ulcers and some discomfort (mild), through to extensive erythema and severe ulceration with pain and dysphagia leading to the inability to consume food by the oral route (severe). This mucositis scoring system, although relatively simple, and influenced to some extent by subjective interpretation, provided useful indications of the severity of symptoms.

Degree of respiratory distress was also recorded. Mild symptoms were scored as '+' and corresponded to symptoms of respiratory compromise with chest X-ray changes, and a requirement of up to 3L of oxygen. Moderate symptoms were scored as '++' and corresponded to respiratory compromise with chest X-ray changes and a requirement of up to 10L of oxygen and severe symptoms were scored as '+++' corresponding to ARDS as defined in Section 4.1.3.

Spreadsheet 2 contained patients' names, dates of episodes of viridans streptococcal bacteraemia and episode reference numbers, as in spreadsheet 1, plus species of viridans streptococcus isolated from blood culture with Rapid ID 32 Strep (BioMerieux) profile. From this information, the total number of episodes of viridans streptococcal bacteraemia was calculated, as was the prevalence of bacteraemia by particular species. Relationships between recent chemotherapeutic regimens, potential predisposing factors and viridans streptococcal bacteraemia were investigated, as were the various associated clinical features.

For comparison of frequency of viridans streptococcal bacteraemia with that caused by other bacteria in immunocompromised patients, records routinely compiled by the author were consulted. These records are prepared on an annual basis, as part of an audit programme to monitor the pattern of infection within this patient population.

4.2 Isolation of viridans streptococci from clinical specimens

Throughout the study period, from 1st December 1994 until 31st December 2000, all significant isolates of viridans streptococci isolated from blood culture of paediatric haematology/oncology patients were subcultured and stored. The composition of media used for isolation and culture is presented in Table 4.1.

Table 4.1 Composition of culture media

Medium	**Composition**
BacTAlert aerobic culture bottles	Pancreatic digest of casein (1.95% w/v) Papaic digest of soybean meal (0.3% w/v) Sodium polyanetholesulfonate (0.035% w/v) Pyridoxine HCl (0.001% w/v) (Other complex amino acids and carbohydrates in purified water)
BacTAlert anaerobic culture bottles	Pancreatic digest of casein (1.95% w/v) Papaic digest of soybean meal (0.3% w/v) Sodium polyanetholesulfonate (0.035% w/v) Menadione (0.00005% w/v), Haemin (0.0005% w/v) Reducing agents 0.(Other complex amino acids and carbohydrates in purified water)
Columbia blood agar	Special peptone (23.0 g/L), Starch (1.0 g/L) Sodium chloride (5.0 g/L), Agar (10.0 g/L) Sterile defibrinated blood (5%) (pH 7.3 ± 0.2)
Columbia blood agar with Crystal Violet	As above except: Sterile defibrinated blood (7%) with Crystal Violet 0.003g/L (pH 7.3 ± 0.2)
Diagnostic Sensitivity Test Agar	Proteose peptone, Veal infusion solids Glucose (2.0 g/L), Sodium chloride (3.0 g/L) Disodium phosphate (2.0 g/L), Sodium acetate (1.0 g/L), Adenine sulphate (0.01 g/L) Guanine hydrochloride (0.01 g/L), Uracil (0.01 g/L), Xanthine (0.01 g/L), Aneurine (0.00002 g/L), Agar No. 1 (12.0 g/L) (pH 7.4 ± 0.2)
Brain heart infusion broth	Calf brain infusion solids (12.5 g/L) Beef heart infusion solids (5.0 g/L) Proteose peptone (10.0 g/L), Glucose (2.0 g/L) Sodium chloride (5.0 g/L), Disodium phosphate (2.5 g/L) (pH 7.4 ± 0.2)

4.2.1 Collection of blood specimens for culture

Blood was collected (generally via Hickman line), from febrile patients in the haematology/ oncology ward and transferred into BacTAlert culture bottles (5 to 10 ml of blood for each of the aerobic and anaerobic bottles) using aseptic technique. After receipt at the Diagnostic Microbiology Laboratory, inoculated bottles were incubated in the BacTAlert blood culture system (Organon Teknika Limited, Cambridge, U.K.).

4.2.2 Processing of positive blood cultures

Any blood culture bottles which signalled positive, were removed from the BacTAlert incubation cabinet, and an aliquot of fluid was removed and Gram stained (Table 4.2). If the bacteria were Gram-positive cocci resembling streptococci, an aliquot of blood culture fluid was tested using the Streptex latex agglutination test (Murex Biotech Limited, Dartford, U.K.). Although this test is designed for identification of streptococci possessing Lancefield group antigens using colonies from a culture plate (Table 4.2), useful results have also been obtained from liquid cultures ((Facklam, Cooksey & Wortham, 1979). A negative reaction with Streptex from blood culture fluid may suggest, by elimination, the possibility of the presence of viridans streptococci rather than that of *Strep pyogenes*, *Strep agalactiae* or *Enterococcus* spp. Columbia blood agar and chocolate blood agar plates (E & O Laboratories, Bonnybridge, U.K.) were inoculated with an aliquot of blood culture fluid, as were direct sensitivity plates (Diagnostic Sensitivity Test Agar with 5% lysed horse blood (E & O Laboratories, Bonnybridge, U.K.)) according to the Standard Operating Procedures of the Diagnostic Microbiology Laboratory. An optochin disc was placed on the inoculated blood agar plate. Cultures of all isolates of viridans streptococci from blood cultures were collected for the present study.

Table 4.2 Tests used routinely for identification of streptococci

Test	Method
Gram stain	The prepared slide was flooded with crystal violet and stained for 30 seconds. The slide was rinsed with iodine and then stained with iodine for 30 seconds, followed by rinsing with water. Acetone or alcohol was applied (for 4-5 seconds) to decolourise. The slide was flooded with dilute carbol fuchsin counterstain. After 30 seconds the slide was washed in water and then dried on a slide dryer. One drop of immersion oil was placed on the area of the slide and examined using the X 100 oil immersion lens of a light microscope. Bacteria stained a deep purple colour were termed 'Gram-positive'. (Bacteria stained pink were termed 'Gram-negative'.)
Catalase	A few drops of hydrogen peroxide were added to a test tube. Using a capillary tube, part of an isolated colony was added. The evolution of gas bubbles indicated catalase activity.
Streptex (Murex)	400 µl of Extraction Enzyme were added to a labelled test tube. A single sweep of growth from the test streptococcal culture was added to the tube to form a light suspension, which was then incubated at 37°C in a water bath for between 10 and 60 minutes. After 5 minutes incubation, the tube was shaken. One drop (20 µL) of each latex suspension was dropped on to a separate circle on a Reaction Card. Using a pipette, one drop (40 µL) of prepared extract was placed in each of the six circles on the card. The contents of each circle were mixed with a separate mixing stick. A positive result was indicated by the development of an agglutinated pattern showing clearly visible clumping of the latex particles.

Any organisms isolated concomitantly with viridans streptococci were identified using standard methods (Murray *et al.*, 1999). Three isolates of viridans streptococci were later excluded as probable contaminants after discussion of laboratory and clinical findings with a Consultant Haematologist and repeating blood culture.

4.2.3 Collection of oral specimens for culture

Viridans streptococci were also collected from mouth swabs. After discussion with clinical staff, it was decided that although oral rinses would probably provide a superior yield of bacteria (Spijkervet, 1991), swabs were practically more appropriate - particularly for very young patients and for some of the more seriously ill children. In order to cause minimal disruption to established practice in the busy haematology/ oncology ward, it was agreed that the nursing staff who routinely swabbed the patients' mouths as part of the general microbiology surveillance scheme should continue to do so. A single swab was used to sample buccal mucosa, gingivae, teeth, hard palate and tongue. Mouth swabs were dispatched to the Department of Microbiology for culture. Each mouth swab was plated out on to a Columbia blood agar plate, a Sabouraud dextrose agar plate (both incubated in 5% CO_2) and a Columbia blood agar plate with Crystal Violet (incubated in anaerobic conditions).

4.2.3.1 Surveillance cultures

Routine surveillance swabs were collected from neutropenic patients weekly. These specimens usually included a combined mouth/throat swab. Blood agar plates with evidence of viridans streptococci from these specimens were stored at 4°C for 2 weeks. The relevant patient's name was marked on the base of the plates. If the patient developed viridans streptococcal bacteraemia within the 2-week period, the stored culture plates from mouth/throat swabs were retrieved and subculture of isolates of viridans streptococci performed. On suspicion of viridans streptococcal bacteraemia from Gram-stain of blood culture fluid, a fresh mouth swab was also requested. For some patients, surveillance cultures were not available before viridans streptococcal bacteraemia was confirmed. In these cases, a mouth swab taken shortly after the blood culture signalled positive was the only oral specimen available.

4.3 Storage of isolates of viridans streptococci

All isolates of viridans streptococci were stored at –70°C in Microbank vials containing cryopreservative fluid and porous beads (Pro-Lab Diagnostics, Cheshire, U.K.). Vials were labelled with isolate reference number and date of specimen. Cryopreservative fluid was inoculated with young colonial growth (18-24 hours) picked from a pure culture to a density of a 4 McFarland standard (BioMerieux, Basingstoke, U.K.). After swirling the contents of the vial for thirty seconds, the cryopreservative fluid was removed using a syringe and needle to leave the inoculated beads as free of liquid as possible. The vials of inoculated beads were then placed in polystyrene containers with appropriately labelled inserts and stored at –70°C.

4.4 Recovery of isolates of viridans streptococci

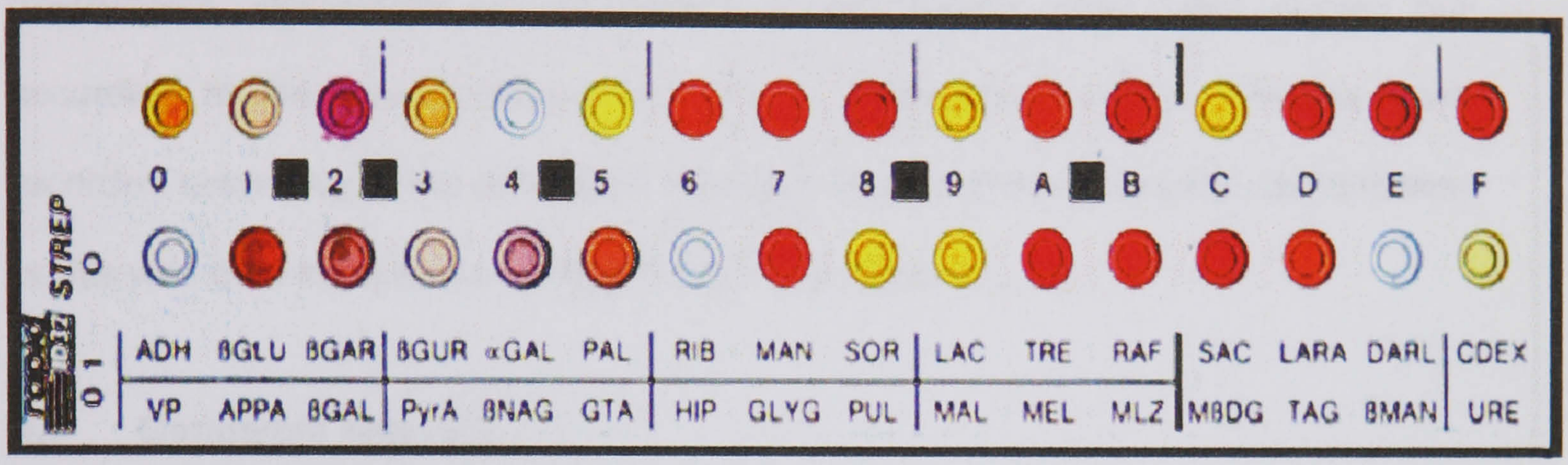
When required for identification tests, antibiotic susceptibility tests or pulsed- field gel electrophoretic analysis, the appropriate cryovials were removed from storage. One bead was removed from each vial using a sterile needle and directly streaked on to the surface of a Columbia blood agar plate, which was then incubated overnight at 37°C in 5% CO_2. From these cultures a further subculture (on Columbia blood agar) was performed before any tests were carried out.

4.5 Identification of viridans streptococci

Preliminary identification of viridans streptococci was performed by visual inspection of colonial morphology on Columbia blood agar. All organisms studied were catalase negative, Gram-positive cocci, resistant to optochin. The Rapid ID 32 Strep system (BioMerieux, Basingstoke, U.K) was then used according to the manufacturer's instructions (BioMerieux, 1994 &1998) to identify further each isolate. After inoculation of the Rapid ID 32 Strep strip with bacterial suspension, followed by

incubation for 4 hours, reactions were read, with positive tests indicated by colour change (Figure 4.2). A numerical identification profile was obtained which was then interpreted using API LAB Plus software.

Figure 4.2 Rapid ID 32 Strep strip following inoculation and incubation



Until March 1998, the API 1.1 database was used for the present study. When the updated database (Version 2.0) became available, it was used to identify all new strains and, for continuity, to re-identify all earlier isolates.

In instances when a low identification probability (< 90%) or an equivocal result was obtained or when no species result was acceptable, the organism was subcultured on to a fresh blood agar plate, incubated overnight and identification repeated. If definitive identification was still not achieved, a culture was dispatched to the Streptococcal Reference Laboratory, PHLS Central Public Health Laboratory, London, U.K. for further tests and identification. In total, seven isolates of viridans streptococci from blood cultures were sent.

4.6 Identification of coagulase-negative staphylococci

Staphylococci were initially identified visually by colonial morphology. Gram-positive cocci with a positive catalase reaction were tested using the Staphaurex slide

agglutination test (Murex Biotech Limited, Dartford, U.K.), which differentiates staphylococci which possess coagulase and/or protein A from those which produce neither of these factors. The staphylococcal isolates from both blood culture and mouth swab of the present study were Staphaurex negative. The organisms were then speciated using the API Staph system (BioMerieux, Basingstoke, U.K.). Preparation, inoculation and incubation of API Staph strips were carried out according to the manufacturer's instructions (BioMerieux, 2000). Results were recorded according to the API Staph Reading Table and the numerical identification profile was then interpreted using API LAB Plus software.

4.7 Genotypic analysis

The following procedures were used for all strains of viridans streptococci investigated in Chapter 7 and for the isolates of *Staphylococcus epidermidis* isolated concomitantly with one of these.

4.7.1 Preparation of cells and agarose blocks

Cultures were grown overnight in brain heart infusion broth, 0.5 ml was washed with NET buffer (10mM Tris, 1 mM EDTA, 10 mM NaCl) and resuspended in 0.25 ml of NET buffer. Lysozyme (1 mg), mutanolysin (100 units) and RNAase (25 µg) were added to the cell suspension and mixed with an equal volume of SeaPlaque GTG agarose (Flowgen) 2% at 50°C. The cell/agarose suspension was pipetted into a block mould and allowed to solidify at 4°C.

Cells were lysed by dispensing blocks into lysis buffer (lysozyme 1 mg/ml, RNAase 25 µg/ml, 6 mM Tris, 100 mM EDTA, 1 M NaCl, Brij 58 0.5%, sodium deoxycholate 0.2%, lauroyl sarcosine 0.5%) and incubating for 2-3 hours at 37°C. Lysis buffer was then removed, and 1 ml of proteolysis buffer (proteinase K 100 µg/ml, lauroyl

sarcosine 1% in 0.5 M EDTA) was added and incubation continued for 16-24 hours at 50°C. After incubation, the blocks were washed three times for 10 minutes each in TE buffer (10 mM Tris, 1 mM EDTA).

4.7.2 Digestion of DNA with restriction endonuclease

Gel plugs were cut from the blocks and digested with 30 units of *Sma* I at 30°C for 3 hours in a total volume of 100 µL of the appropriate restriction buffer (87 µL sterile distilled water + 10 µL 10 X restriction buffer).

4.7.3 Pulsed-field gel electrophoresis

Gels were prepared as 1% (w/v) PFGE grade agarose (BioRad Laboratories, Herts, U.K.) in 0.5 x TBE buffer (44.5 mM Tris, 44.5 mM boric acid, 1 mM EDTA). Gel plugs containing *Sma* I digested DNA were loaded and sealed with 1% SeaPlaque agarose (Flowgen).

Electrophoresis was carried out in 0.5 x TBE buffer using the contour-clamped homogeneous electric field (CHEF) method with a CHEF-Mapper drive module (BioRad Laboratories). The gel was run for 23 hours with a linear ramped pulse time of 6.75-63.8 seconds. A lambda ladder was used as a molecular size marker. After the electrophoresis run was complete the gel was removed, stained with ethidium bromide 1 µg/ml for 30 minutes and photographed under ultraviolet transillumination.

4.7.4 Analysis of PFGE profiles

Analysis of PFGE profiles was performed by visual inspection of band patterns. The total numbers of visible bands were counted for each isolate, and patterns were

compared. Isolates which differed by more than three bands were considered unrelated (Rudolf, Parkinson & Roberts, 1998).

4.8 Antibiotic studies

4.8.1 Determination of antibiotic MICs using the Etest method

MICs were determined on Diagnostic Sensitivity Test (DST) Agar with 5% lysed horse blood (E & O Laboratories, Bonnybridge, U.K.) using antibiotic Etests (AB Biodisk, Solna, Sweden). The Etest has been shown to be a reliable method for determination of antibiotic susceptibility of viridans streptococci (Hall, Heimdahl & Nord, 1998; Lewis *et al.*, 2000). Prior to this, its reliability versus those of reference methods for susceptibility testing of *S. pneumoniae* had been established (Macias *et al.*, 1994; Tenover, Baker & Swenson, 1996).

Antimicrobial agents tested in the present study were ceftazidime, piperacillin/ tazobactam, meropenem, penicillin G, cefaclor, cefpirome, vancomycin, quinupristin/dalfopristin and linezolid. Cefpirome and quinupristin/dalfopristin Etest strips were kindly supplied by Aventis Pharma (Kent, U.K.) and Linezolid Etest strips were supplied by Pharmacia Limited (Milton Keynes, U.K.).

From an overnight plate culture, viridans streptococci were suspended in 4 ml of sterile distilled water to achieve 0.5 McFarland turbidity. The suspension was used within 15 minutes of preparation. A sterile swab was dipped into the suspension and excess fluid was then removed by pressing the swab against the inside wall of the tube. The surface of a DST agar plate (90mm diameter) was swabbed three times, rotating the plate through approximately 90 degrees each time to ensure an even distribution of inoculum. Inoculated plates were left for 15 minutes to ensure that the surface of the agar was dry before applying Etest strips. Using a pair of forceps, the

appropriate Etest strip was applied to the inoculated agar surface. Care was taken to ensure that the whole length of the strip was in contact with the agar surface and any air pockets were removed by gently pressing on the strip with forceps, moving from the minimum concentration upwards. Plates were then incubated in aerobic conditions at 37°C for 24 hours.

After incubation, the MIC value was read at the point of intersection between the inhibition ellipse edge and the Etest strip. *Staphylococcus aureus* NCTC 6571 was used as control organism. MIC results were presented as number (and percentage) of strains inhibited at stated MICs (mg/L) and geometric mean MICs were calculated.

4.8.2 Determination of antibiotic susceptibility using the modified Stokes' disc diffusion method

At the commencement of this study, the modified Stokes' disc diffusion method (Stokes & Ridgway, 1980) was used routinely in the clinical microbiology laboratory at RHSC, as was the case in the majority of laboratories in the U.K. The use of this method allowed each isolate to be compared with a sensitive control organism which was subjected to the same technical conditions of medium, incubation time, atmosphere, temperature and disc content. As control organisms were adjacent on the same plate, the difference between respective zone sizes could be measured directly. Antibiotic susceptibilities of all isolates of viridans streptococci from blood culture were determined using the Stokes' method with *S. aureus* NCTC 6571 as control organism (as recommended in the BSAC Guide to Sensitivity Testing, 1991).

4.8.2.1 Preparation of inoculum

An inoculum which resulted in semi-confluent growth of colonies following overnight incubation, was used. For antibiotic disc susceptibility tests using viridans

streptococci, colonies were taken directly from a blood agar plate into sterile distilled water to produce a suspension with a density equivalent to a 0.5 McFarland standard. This suspension was then used directly to inoculate the sensitivity test plate. In a minority of cases, when the resulting inoculum was too heavy, the test was repeated using a 1:10 dilution of a freshly prepared bacterial suspension.

For antibiotic disc susceptibility tests using coagulase-negative staphylococci and for the control organism, *S. aureus* NCTC 6571 a suspension with a density equivalent to a 0.5 McFarland standard was diluted 1:10 to result in semi-confluent growth following overnight incubation.

4.8.2.2 Inoculation of agar plates

A sterile cotton-wool swab was dipped into the test bacterial suspension and excess liquid removed by turning the swab against the side of the tube. The swab was then placed on the surface of a DST agar plate on the platform of a rotary plater and was moved at an even pace from the periphery inwards to form a 1.5 cm inoculated band. The control organism was then applied to the centre of the plate. After the inoculum had dried, antibiotic discs were applied to the surface of the plate. Antibiotic discs tested against viridans streptococci were penicillin G (1 unit), cefaclor (30 µg), ceftazidime (30 µg), piperacillin (30 µg and 75 µg), piperacillin/tazobactam (75 + 10 µg), meropenem (10 µg), vancomycin (30 µg) and trimethoprim/ sulphamethoxazole (25 µg). Two inoculated plates for each isolate were used to accommodate the above discs. Antibiotic discs used for coagulase-negative staphylococci were vancomycin (30 µg), clindamycin (2 µg), rifampicin (2 µg), fusidic acid (10 µg), amikacin (30 µg), and ciprofloxacin (1 µg). Methicillin susceptibility was tested with 25 µg strips on inoculated Columbia blood agar plates, incubated

overnight at 30°C. All other inoculated sensitivity plates were incubated overnight at 37°C in aerobic conditions.

4.8.2.3 Measurement of zones and interpretation of results

Zone sizes were measured using dividers with a ruler and were interpreted according to the criteria of the Stokes' method as follows:

Sensitive: zone radius equal to, wider than, or not more than 3 mm smaller than the control.

Intermediate: zone radius greater than 2 mm but smaller than the control by more than 3 mm.

Resistant: zone radius 2 mm or less.

Percentage susceptibility figures were calculated and compared with those generated using MIC methods.

4.9 Statistical tests

The following statistical tests were employed in this thesis.

The Chi-squared (X^2) test

Differences between categorical variables were tested for significance using the X^2 test. P values of < 0.05 were considered statistically significant.

Exact probability test

When the sample size was too small for the X^2 test to be appropriate, Fisher's exact test was used.

Arithmetic and geometric means

The arithmetic mean was calculated when the distribution of a set of figures was symmetrical and unimodal. The geometric mean was calculated when the distribution was skewed, as may be observed for antibiotic MICs.

The *t* test

The *t* test was used to compare the means of two samples in order to determine whether the samples were from the same or different populations (Statworks – Cricket Software). *P* values of < 0.05 were considered statistically significant.

Correlation and linear regression

Statistical software StatWorks (Cricket Software) was used to analyse correlation (which measures the closeness of an association between different variables), and linear regression (which produces the equation of the straight line that best describes the association).

RESULTS

CHAPTER 5

EPIDEMIOLOGY OF VIRIDANS STREPTOCOCCAL BACTERAEMIA IN PAEDIATRIC IMMUNOCOMPROMISED PATIENTS WITH MALIGNANT DISEASE

EPIDEMIOLOGY OF VIRIDANS STREPTOCOCCAL BACTERAEMIA IN PAEDIATRIC IMMUNOCOMPROMISED PATIENTS WITH MALIGNANT DISEASE

5.1 Introduction

Throughout the period of this study, the number of episodes of viridans streptococcal bacteraemia was recorded and compared with that of total episodes of microbiologically documented bloodstream infection (bacteraemia plus fungaemia) in paediatric immunocompromised patients at RHSC. In addition, the frequency of isolation of viridans streptococci as a group was compared with that of other major groups of organisms responsible for infection in this patient population. Episodes of viridans streptococcal bacteraemia were also expressed as proportion of total febrile episodes.

The distribution of different species of viridans streptococci from blood culture was determined to investigate whether particular species were associated with this infection. Cases of polymicrobial bacteraemia were monitored to investigate the possible role of co-infecting bacteria or yeasts with viridans streptococci. Episodes of streptococcal bacteraemia with concomitant viral infection were also evaluated to determine whether this combination influenced clinical outcome.

This study included all paediatric patients with malignant disease who developed viridans streptococcal bacteraemia. It should be appreciated that this in itself is a fairly heterogeneous group. A diverse range of malignancies was involved and many different chemotherapeutic protocols and modalities of therapy were used. However the major advantage in studying this broad group was that the maximum number of organisms could be obtained for *in vitro* studies. It should be appreciated however, that the number of episodes involved in this study (69) still represents a fairly small sample size. For analysis of some of the findings from this study, a more

uniform sample population was required therefore specific patients or episodes were considered *e.g.* all episodes of viridans streptococcal bacteraemia following a particular course of chemotherapy containing high-dose cytosine arabinoside. While this approach provided a more narrowly defined population for analysis, the disadvantage was that it further reduced the sample size. However limited statistical analysis of this group could still be performed to provide useful information.

The epidemiological study presented in this thesis was descriptive rather than case-controlled. Earlier investigators had completed case control studies before the present work was started and several of their findings will be discussed (Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994). The main purpose of this part of the work was to determine the recent epidemiology of viridans streptococcal bacteraemia in a paediatric group and to provide a background to the practical aspects of the study.

5.2 The haematology/oncology unit

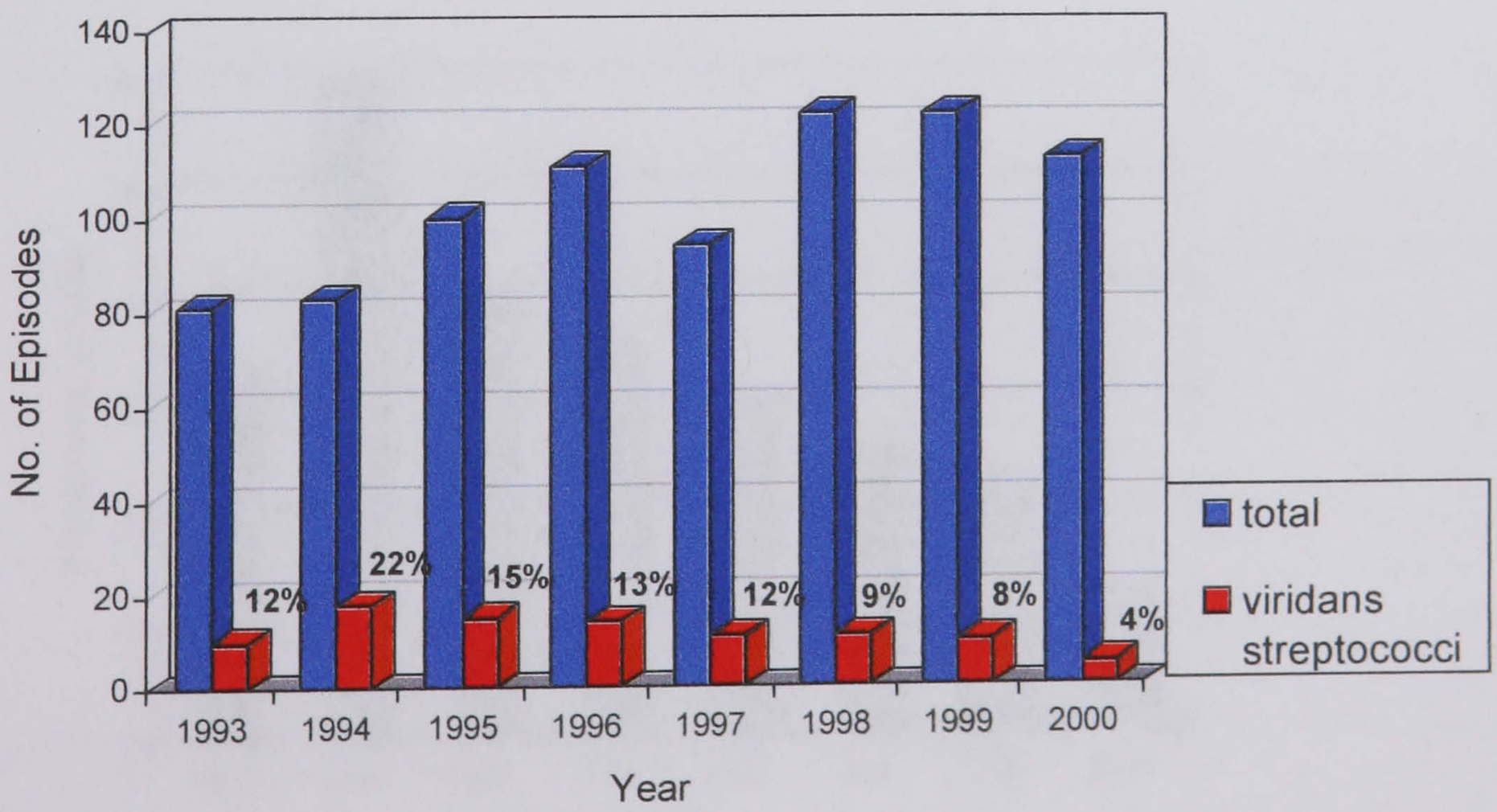
The Royal Hospital for Sick Children, Glasgow, is the largest paediatric hospital in Scotland (320 beds) with an in-patient population representing a variety of medical and surgical specialities, including haematology, oncology, renal transplantation and cardiac surgery.

The haematology/oncology clinical service is located on the first-floor of the hospital in a dedicated unit - 'Schiehallion'. The ward area has facilities for 21 inpatients and an adjacent day-care facility. The unit provides a comprehensive service for the diagnosis and management of children with leukaemia, solid tumours or benign haematological conditions. It houses the National Paediatric Bone Marrow Transplantation Unit and the Regional Haemophilia Centre. The department cares

for approximately two thirds of the children in Scotland with malignant disease or benign haematological conditions, with a catchment area covering the West of Scotland, with shared care arrangements with Dumfries and Inverness. Ward admissions average 1000 per year, representing around 350 patients, 35-40% of whom have leukaemia. At present, approximately 25 new cases of leukaemia and 50 new cases with solid tumours are referred annually. Around 750 children with various haematological/oncological disorders are on regular treatment or follow-up. The average annual increase in patient numbers is greater than 10%. Children with malignancy are treated on national Medical Research Council (MRC), United Kingdom Children Cancer Study Group (UKCCSG) or International Paediatric Oncology Society (SIOP) trials.

5.3 Episodes of viridans streptococcal bacteraemia.

Figure 5.1 Annual episodes of microbiologically documented bloodstream infection, with annual episodes of viridans streptococci bacteraemia, 1993-2000*

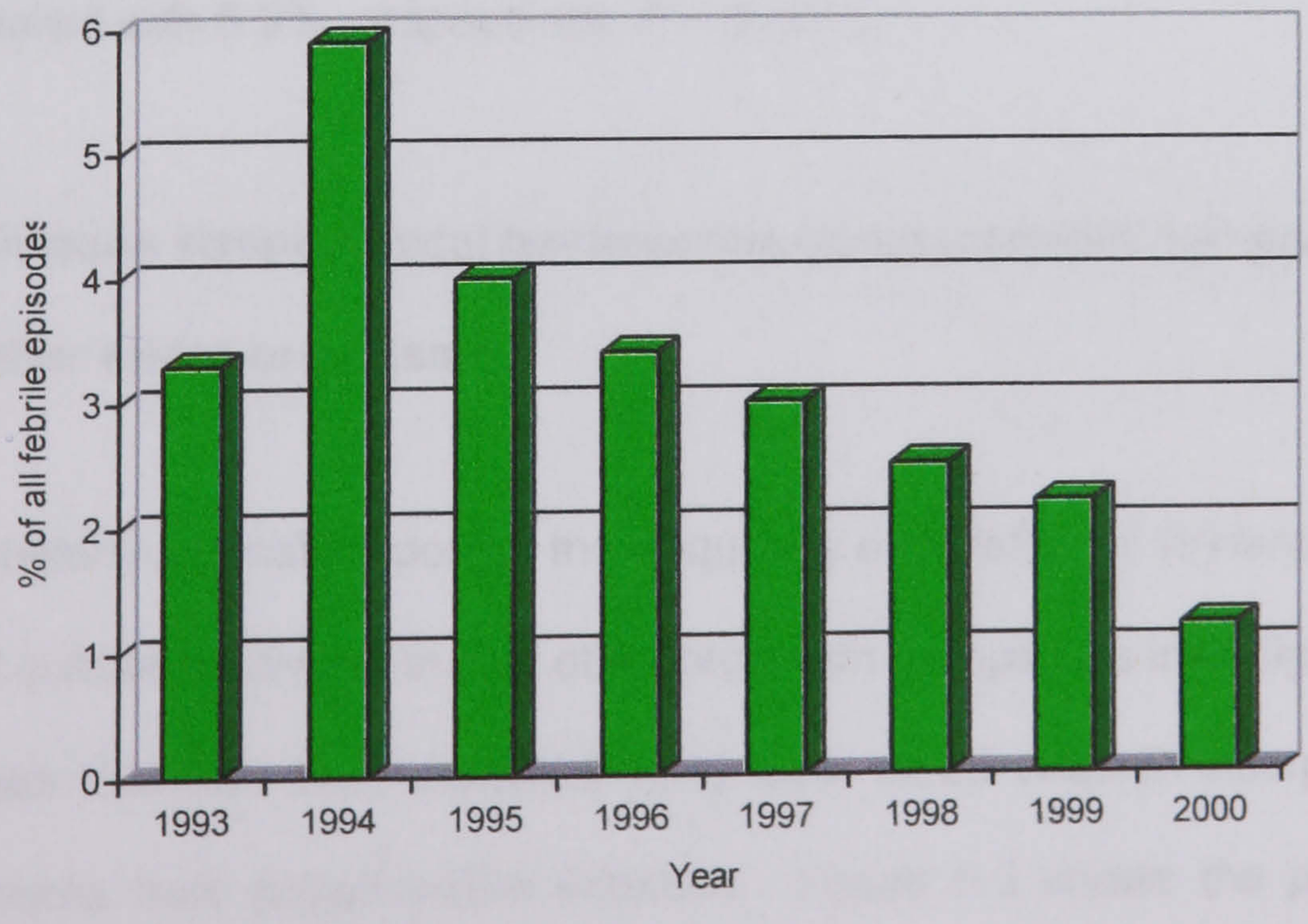


*: Figures for 1993 and '94 prior to the start of the study are included for reference.

Figure 5.1 shows the annual total of microbiologically documented bloodstream infections in paediatric patients with cancer at RHSC from 1993 to 2000 inclusive. Also presented are annual episodes of viridans streptococcal bacteraemia throughout the same period. In 1993 there were 10 episodes of viridans streptococcal bacteraemia accounting for 12% of all cases of microbiologically documented bloodstream infection. In 1994 there was an increase to 18 episodes of this infection (representing 22% of total). In 1995 there were 15 episodes (15% of total), followed by a continued decrease throughout the time interval of the study. By the end of the year 2000, annual episodes of viridans streptococcal bacteraemia, as proportion of microbiologically documented bloodstream infections, had decreased to around one-fifth of that for 1994 ($P < 0.001$).

Figure 5.2 shows episodes of viridans streptococcal bacteraemia with total febrile episodes as denominator.

Figure 5.2 Annual episodes of viridans streptococcal bacteraemia as percentage of annual total febrile episodes, 1993-2000 *



*: Estimated figures for total febrile episodes for 1993-96 (see over)

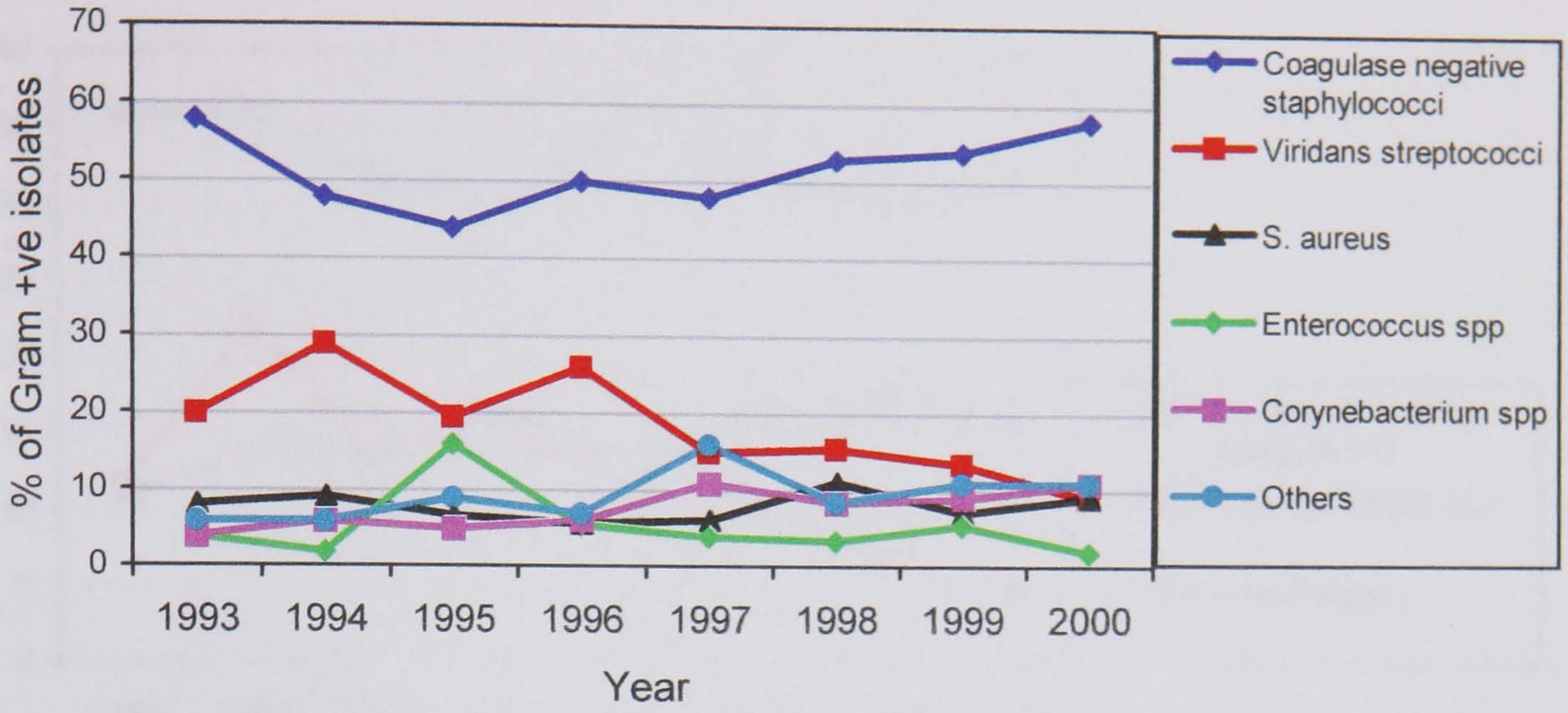
Unfortunately, exact figures for total febrile episodes for the early years of this project were not available. However, it was felt acceptable to obtain estimated figures for these years (1993-96) by calculation based on the consistency of rates of microbiologically documented bloodstream infection from febrile episodes for 1997-2000 (26% of all febrile episodes in 1997, 28% in 1998, 26% in 1999 and 27% in 2000). The majority of febrile episodes amongst the patients of the haematology/ oncology unit were associated with neutropenia.

Figure 5.2, in common with the previous figure, demonstrates a maximum frequency of viridans streptococcal bacteraemia in 1994, decreasing to a minimum in 2000. Although there were more episodes of infection in 1994 than in 1995 (5.9% versus 4.0%, of all febrile episodes, respectively), the most severe cases of the entire study occurred during 1995, and these included two fatalities. In 1996, there were 14 episodes of viridans streptococcal bacteraemia (3.4% of all febrile episodes), associated with less severe symptoms than during the previous year. By the end of the year 2000, episodes of viridans streptococcal bacteraemia as proportion of total febrile episodes had decreased to around one-fifth of those for 1994 (1.2% compared with 5.9%, respectively, $P < 0.001$).

5.4 Viridans streptococcal bacteraemia compared with bacteraemia caused by other micro-organisms

For epidemiological purposes, the frequency of isolation of viridans streptococci from blood culture relative to that of other organism groups was investigated. This form of analysis included total microbial yield from blood culture, incorporating individual organisms from polymicrobial infection. Figure 5.3 shows the position of viridans streptococci in the context of bacteraemia due to other Gram-positive organisms, from 1993 to 2000.

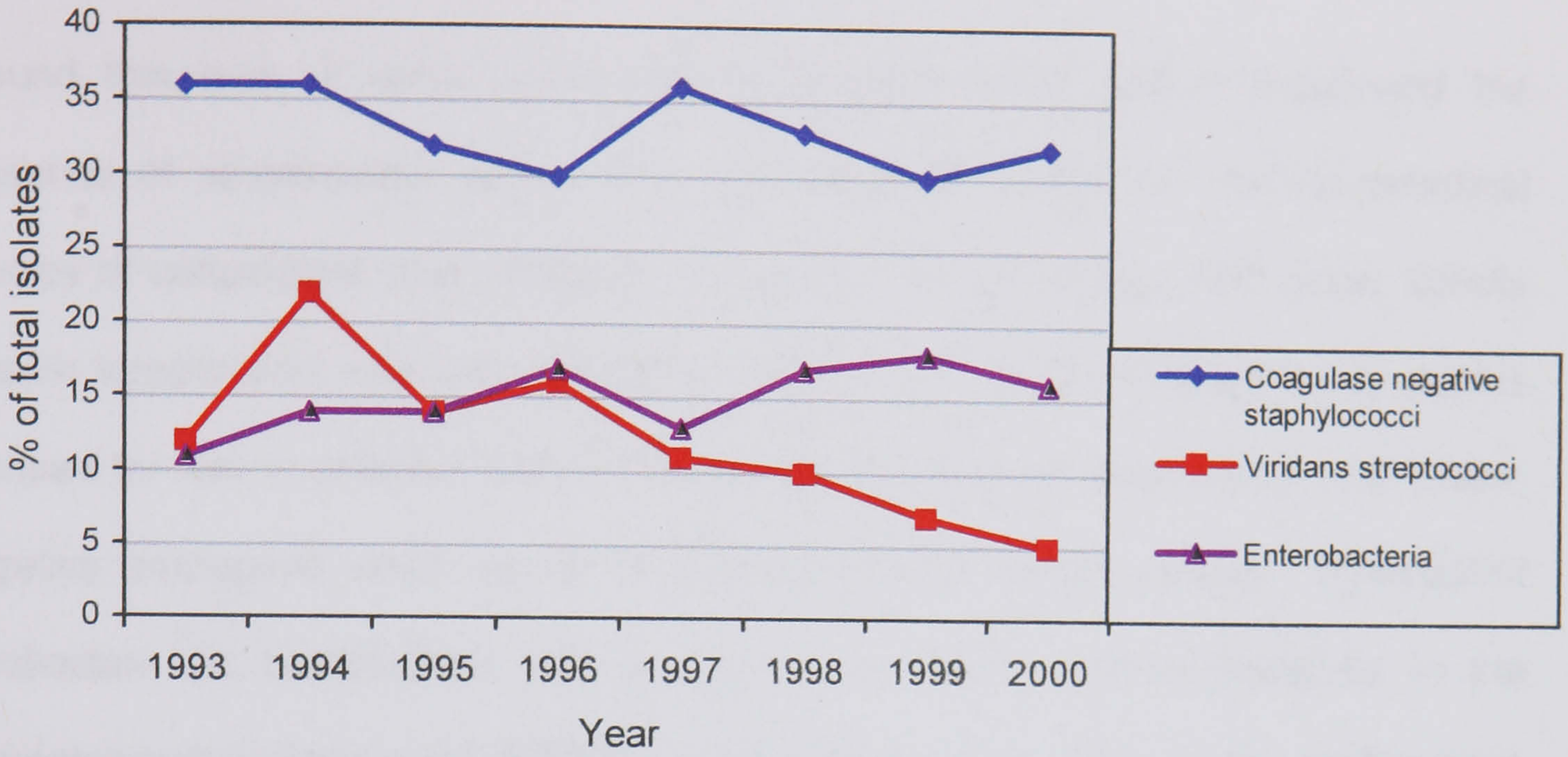
Figure 5.3 Gram-positive bacteria isolated from blood culture of paediatric patients with cancer, 1993-2000



Coagulase-negative staphylococci were consistently the most commonly isolated Gram-positive bacteria. Until the year 2000, viridans streptococci comprised the second most commonly isolated group. In 1994, these organisms represented 29% of total Gram-positives, decreasing to 13.5% in 1999. In the year 2000, viridans streptococci represented 9% of total isolates of Gram-positive bacteria. During this year, the proportion of isolates of viridans streptococci decreased to become identical to that of *Staphylococcus aureus,* and was exceeded by that of *Corynebacterium* spp. and related genera.

The position of viridans streptococcal isolates relative to other major groups of organisms causing bacteraemia in paediatric patients with cancer is presented in Figure 5.4. This demonstrates once more that the bacteria most commonly isolated from blood culture of patients with malignant disease were coagulase-negative staphylococci. Of the Gram-negative bacteria, those belonging to the tribe Enterobacteriaceae are generally encountered most frequently.

Figure 5.4 Major groups of bacteria isolated from blood culture of paediatric patients with cancer, 1993-2000



During 1994, total isolates of viridans streptococci exceeded those of Enterobacteriaceae (22% versus 14%), while the relative proportions of both groups were either identical or similar during 1993, '95, '96 and '97. Throughout the last three years of the study, the proportion of Enterobacteriaceae remained fairly constant at 17% of total organisms in 1998, 18% in 1999 and 16% in 2000, while viridans streptococcal isolates decreased in percentage (10%, 7% and 5% of total isolates respectively).

5.5 Viridans streptococcal bacteraemia and interventions associated with the present study

In late 1994, subsequent to the occurrence of several cases of viridans streptococcal bacteraemia, action was taken to improve the situation. The importance of mouth care procedures was emphasized, as the most likely source of viridans streptococci was the oral cavity. It was reasoned that good oral hygiene might reduce mucosal and dental complications associated with anti-cancer therapy and possibly prevent overgrowth of viridans streptococci in patients' mouths. However, in 1995, as

mentioned earlier, severity of symptoms associated with viridans streptococci was greatest and there were two fatalities.

Around this time, if initial Gram-stain of positive blood culture suggested the presence of streptococci, vancomycin was generally added to first-line empirical therapy of ceftazidime plus amikacin. However first-line therapy with better activity against streptococci was also desirable. In the mid -1990s there were few agents licensed for use in children, with good activity against both streptococci and Gram-negative pathogens which would be suitable in this clinical setting. Piperacillin/ tazobactam (in combination with amikacin) had been used successfully in the haematology/oncology ward of RHSC on a named-patient basis during participation in IATCG/EORTC Trial IX. Preliminary susceptibility test results of the present study indicated that its activity against viridans streptococci was superior to that of ceftazidime, and disc diffusion tests performed in the diagnostic laboratory indicated that it was active against piperacillin-resistant strains of *E. coli.* In July 1996, piperacillin/tazobactam was substituted for ceftazidime, as empirical β-lactam therapy.

5.6 Patient numbers and episodes of viridans streptococcal bacteraemia

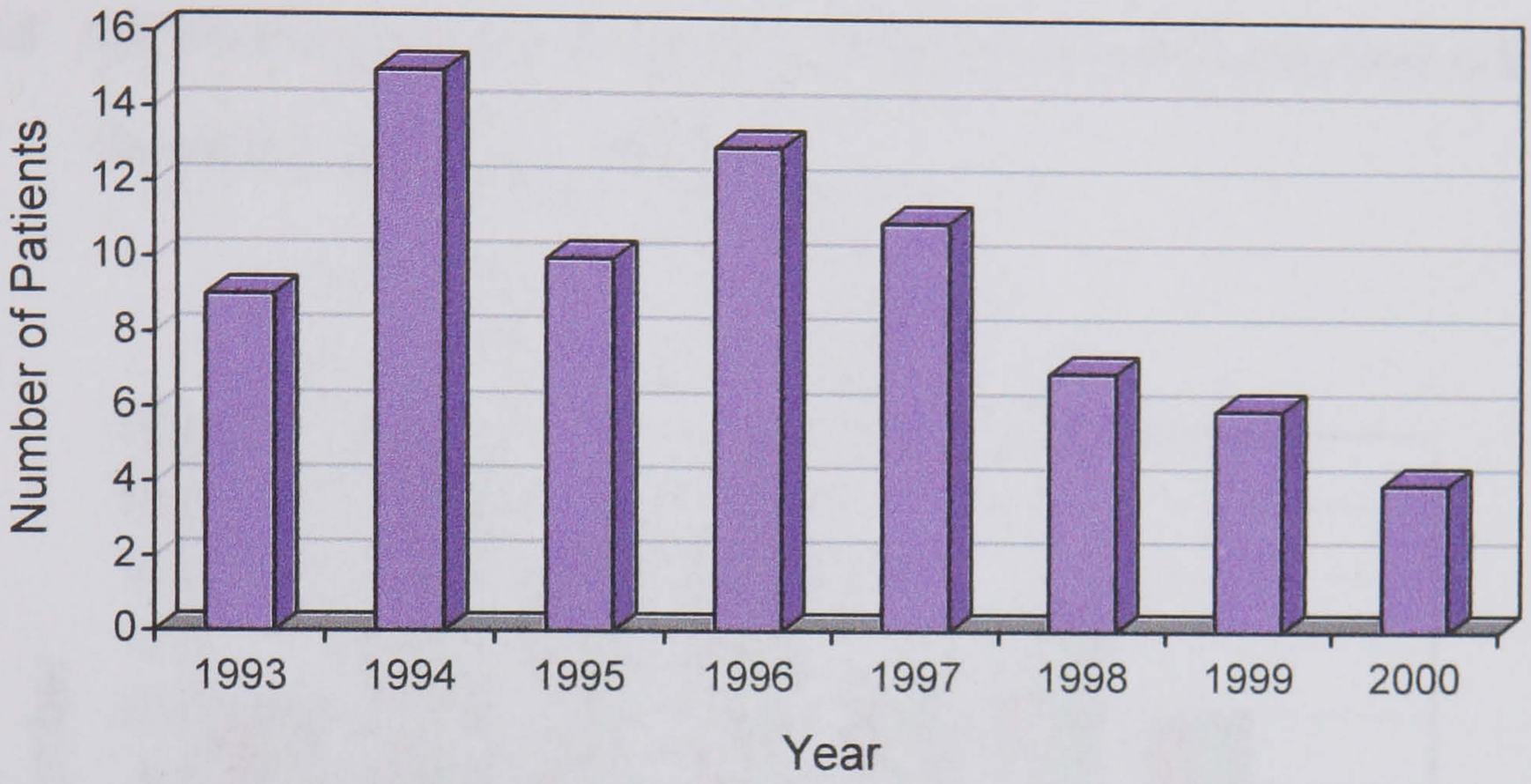
Fifty-four patients developed viridans streptococcal bacteraemia throughout the study period (*i.e.* between the beginning of December 1994 and the end of December 2000). Patient details are presented in Table 5.1.

Table 5.1 Patient demographics (and episodes of viridans streptococcal bacteraemia)

Patient Number *(No. of episodes if > 1)*	**Age**	**Gender**	**Underlying Malignancy**
1 *(4)*	3 months	Female	AML
2 *(2)*	11 years	Male	AML
3	1 year	Male	Neuroblastoma
4	4 years	Female	T-cell lymphoma
5	2 years	Male	AML
6	1 year	Male	ALL
7	14 years	Male	ALL
8 *(2)*	12 years	Female	AML
9	5 years	Female	ALL
10	6 years	Female	B-cell lymphoma
11	14 years	Female	AML
12	11 years	Female	AML
13 *(2)*	3 years	Male	ALL
14	2 years	Male	ALL
15	3 years	Male	B-cell lymphoma
16	14 years	Male	T-cell lymphoma
17	3 years	Male	ALL
18	3 years	Male	ALL
19	1 year	Male	ALL
20	1 year	Male	AML
21	10 years	Male	ALL
22	11years	Male	ALL
23	8 years	Female	ALL
24	4 years	Female	ALL
25	14 years	Male	CML
26	2 years	Female	Wilms' tumour
27	1 year	Female	ALL
28	3 years	Male	ALL
29	7 years	Male	ALL
30	2 years	Male	ALL
31	12 years	Female	ALL
32	12 years	Male	AML
33	6 years	Female	Rhabdomyosarcoma
34 *(3)*	11 years	Female	ALL
35	12 years	Female	ALL
36	12 years	Male	AML
37	7 years	Male	ALL
38	3 years	Female	ALL
39 *(3)*	14 years	Male	AML
40 *(2)*	12 years	Male	ALL
41	13 years	Female	ALL
42	2 years	Female	ALL
43	3 years	Female	ALL
44 *(4)*	2 years	Male	AML
45 *(2)*	7 years	Male	ALL
46	14 years	Male	AML
47	10 years	Male	AML
48	1 year	Female	AML
49	1 year	Male	AML
50	8 years	Female	ALL
51	14 years	Female	Rhabdomyosarcoma
52	10 years	Male	ALL
53	7 years	Male	Osteosarcoma
54	6 years	Male	L.C.A.L

Figure 5.5 shows the annual number of patients developing one or more episodes of viridans streptococcal bacteraemia.

Figure 5.5 Patients with viridans streptococcal bacteraemia, 1993 –2000*



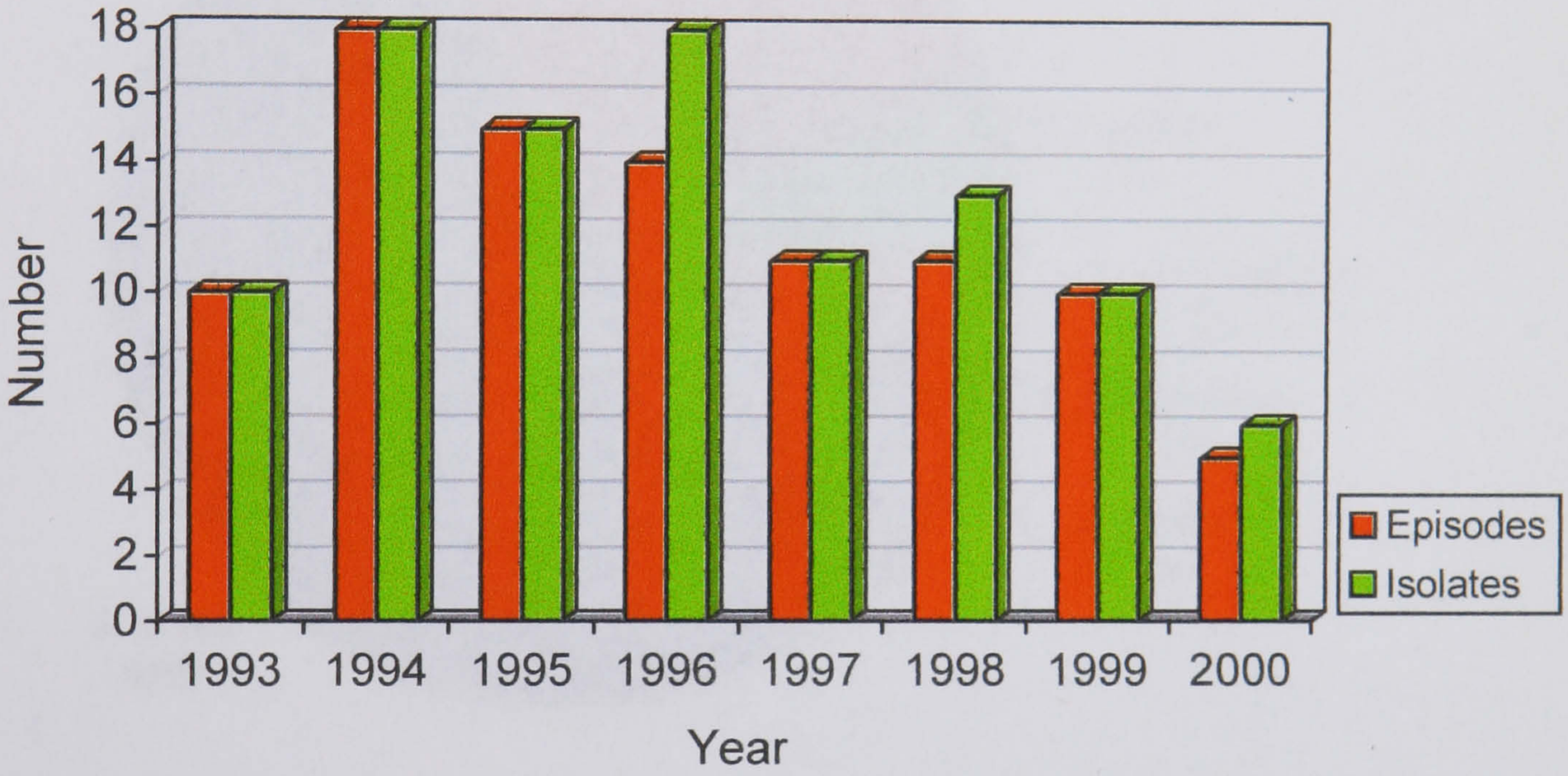
*: Figures for 1993 and '94 prior to the start of the study are included for reference.

Patients with multiple episodes of viridans streptococcal infection are represented only once in Figure 5.5 – by the first episode of infection. From the start of the study period (1st December 1994), fifty-four patients developed viridans streptococcal bacteraemia. Although there was a decrease in the number of individual patients who developed one or more episodes of viridans streptococcal bacteraemia from 1994 to 1995, an increase followed in 1996. From 1997 onwards, the number of patients with viridans streptococcal bacteraemia decreased steadily.

During the study period, forty-five patients experienced one episode of infection. Five patients experienced two separate episodes, two experienced three episodes and two experienced four. For the group of patients experiencing multiple episodes of viridans streptococcal bacteraemia, there elapsed a time interval of between 1 to 6 months between each episode, with negative blood cultures intervening.

Figure 5.6 demonstrates that in 1993, '94, '95, '97 and '99 the number of episodes of viridans streptococcal bacteraemia was identical to the number of strains of viridans streptococci cultured from blood. However during 1996,'98 and 2000, five episodes were associated with multiple strains of these organisms.

Figure 5.6 Episodes of viridans streptococcal bacteraemia and total isolates of the causative organisms, 1993-2000*



*: Figures for 1993 and '94 prior to the start of the study are included for reference.

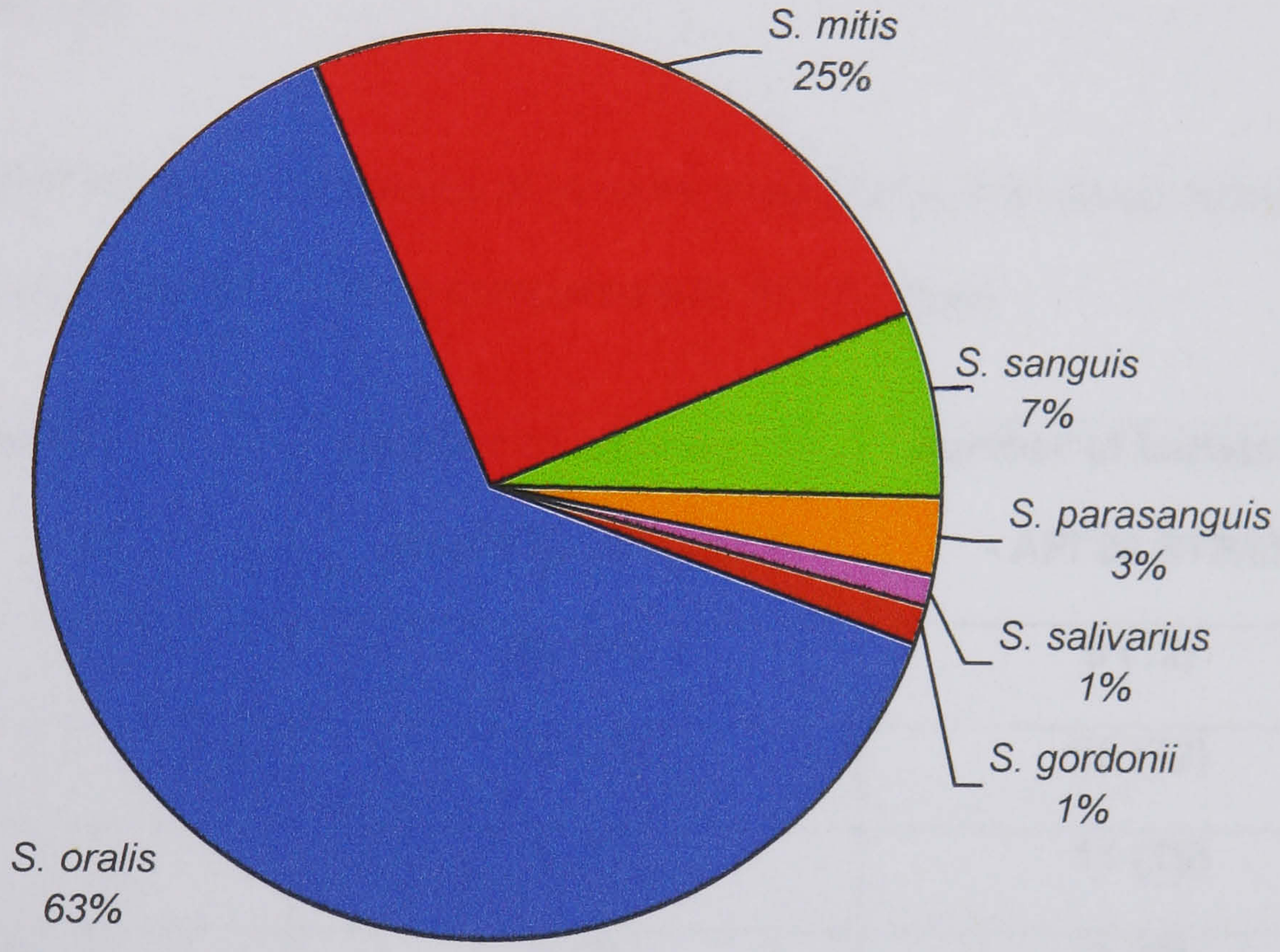
In total, 69 episodes of viridans streptococcal bacteraemia occurred during the period of this study, with a maximum of 15 in 1995 and a minimum of 5 in 2000. In three cases, 2 different species of viridans streptococci were isolated from a single blood culture and in two cases, 3 different species or strains were cultured, resulting in a final total of 76 organisms.

5.7 Species of viridans streptococci causing bacteraemia

The Rapid ID 32 Strep method (BioMerieux, Basingstoke, U.K.) was used to identify isolates of viridans streptococci to species level (Figure 5.7). On 7 occasions an

equivocal identification was obtained or no species result was acceptable, therefore a fresh culture was prepared and sent to the Streptococcal Reference Laboratory, PHLS Central Public Health Laboratory, London for further tests and identification.

Figure 5.7 Species of viridans streptococci from blood cultures



The results of the present study differed from those of several earlier publications which cited *S. mitis* and *S. sanguis* as the most commonly isolated species of viridans streptococcus from blood cultures of neutropenic patients (Cohen *et al.*, 1983; Henslee *et al.*, 1984; Leblanc *et al.*, 1989; Guiot *et al.*, 1990; Classen *et al.*, 1990; McWhinney *et al.*, 1991; Burden *et al.*, 1991; Awada *et al.*, 1992; Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994). Only two studies from the early 1990s, demonstrated a predominance of *S. oralis* from cases of viridans streptococcal bacteraemia (McWhinney *et al.*, 1993; Beighton, Carr & Oppenheim, 1994).

The earlier studies used a variety of streptococcal identification schemes, both conventional and commercial, however the most commonly used system was API 20 Strep (BioMerieux). Throughout the 1980s and '90s this commercial method was also used in the diagnostic microbiology laboratory of RHSC. The species identification results of the present study, could therefore be compared retrospectively with those obtained for the same isolates using the API 20 Strep method (Table 5.2).

Table 5.2 Identification of 76 isolates of viridans streptococci from blood culture using Rapid ID 32 Strep compared with API 20 Strep

Species	**Number of isolates (%) - Rapid ID 32 STREP**	**Number of isolates (%) - API 20 STREP**
S. oralis	48 (63)	9 (12)
S. mitis	19 (25)	55 (72)
S. sanguis	5 (7)	11 (15)
S. parasanguis	2 (3)	0 (0)
S. salivarius	1 (1)	1 (1)
S. gordonii	1 (1)	0 (0)
TOTAL	76 (100)	76 (100)

Table 5.2 demonstrates that the proportion of isolates identified as *S. oralis* and *S. mitis* by the two systems differed significantly, to the extent that the numbers of *S. oralis* identified by the more modern system approached the proportion of *S. mitis* identified by the older system. The Rapid ID 32 Strep system incorporates more tests and can identify a wider range of species than the API 20 Strep system and the

revised BioMerieux identification database, used in the present study, now incorporates some of the recent revisions in the taxonomy of viridans streptococci.

5.8 Polymicrobial bloodstream infection

In 16 of the 69 episodes of viridans streptococcal bacteraemia (*i.e.* 23%), co-infecting micro-organisms were present (sometimes more than one strain or species of viridans streptococcus). Yeasts were present in three of these episodes. Details of these organisms are presented in Table 5.3.

Table 5.3 Micro-organisms causing polymicrobial bloodstream infection

Patient No.	Organisms causing polymicrobial infection
10	*S. oralis* II, *Enterococcus faecalis*
13	*S. mitis* I, *S. mitis* II
17	*S. sanguis*, *Micrococcus* sp.
18	*S. oralis* II (2 strains), *S mitis* I, *Haemophilus parainfluenzae*, *Moraxella catarrhalis*
22	*S. oralis* II, *S. parasanguis*
24	*S. mitis* I, *Candida albicans*
25	*S. oralis* I, Coagulase-negative staphylococci
28	*S. oralis* II, *Rhodotorula rubra*
30	*S. mitis* I, *Candida lusitaniae*
34	*S. oralis* II, Microaerophilic streptococci
35	*S. oralis* II, *Enterobacter cloacae*
38	*S. sanguis* (2 strains), *S. salivarius*
40	*S. sanguis*, Coagulase-negative staphylococci
41	*S. oralis* II, *Bacillus* sp., Coagulase-negative staphylococci
49	*S. oralis* I, *Enterococcus faecium*
53	*S. oralis* I, *S. mitis* I

In each of four episodes, two species of viridans streptococci were isolated from blood culture (Table 5.3). If the mouth were the source of organisms causing bacteraemia through oral mucositis, it was possible that more than one species or strain of viridans streptococcus could have reached the bloodstream. A further episode involved *S. mitis*, 2 different strains of *S. oralis*, *Haemophilus parainfluenzae* and *Moraxella catarrhalis* - all organisms associated with the oropharynx. Three episodes involved viridans streptococci and coagulase-negative staphylococci, one of which will be investigated further in Chapter 7.

In two cases, the combination of *S. oralis* and *Enterococcus* spp. was isolated from blood cultures of patients with oral compromise and diarrhoea, suggesting a common source in the oropharynx or gastro-intestinal tract. A single episode involved the combination of viridans streptococci and *Enterobacter cloacae* – a coliform most commonly associated with the gastro-intestinal tract, but also isolated from the mouths of cancer patients. One episode involved the combination of *S. oralis*, coagulase-negative staphylococci and *Bacillus* sp. *Bacillus* spp. are most commonly found in the environment rather than colonizing humans. Repeat blood cultures from the patient in question, again yielded this organism, excluding the possibility of contamination. It is possible that the origin of infection was the patient's Hickman line.

Two episodes involved viridans streptococci and *Candida* spp., both of which can be found in the oral cavity and gastrointestinal tract of cancer patients. *Candida* spp. can also cause line-associated fungaemia in this patient group. Another episode featured the combination of *Rhodotorula rubra* and *S. oralis* from blood culture. The habitat of *R. rubra* is most often the environment.

5.9 Viridans streptococcal bacteraemia and concomitant viral infection

Concomitant infection with viruses occurred in 6 episodes (Table 5.4). The respiratory viruses, Parainfluenza virus and Influenza A virus were diagnosed from nasopharyngeal aspirates (NPA) by rapid immunofluorescence assay. Picorna virus infection was diagnosed from NPA by PCR. In one case, oral mucosal infection by *Herpes simplex* virus type1 (HSV-1) was diagnosed by shell vial culture, and in another, pulmonary cytomegalovirus (CMV) infection was diagnosed post-mortem.

Table 5.4 Cases of concomitant viral infection in patients with viridans streptococcal bacteraemia

Patient No.	Viral infection
1	Influenzae A
9	CMV
19	Parainfluenzae
41	Parainfluenzae
52	HSV1
54	Picorna

5.10 Viridans streptococcal bacteraemia and concomitant infection with *Pneumocystis carinii*

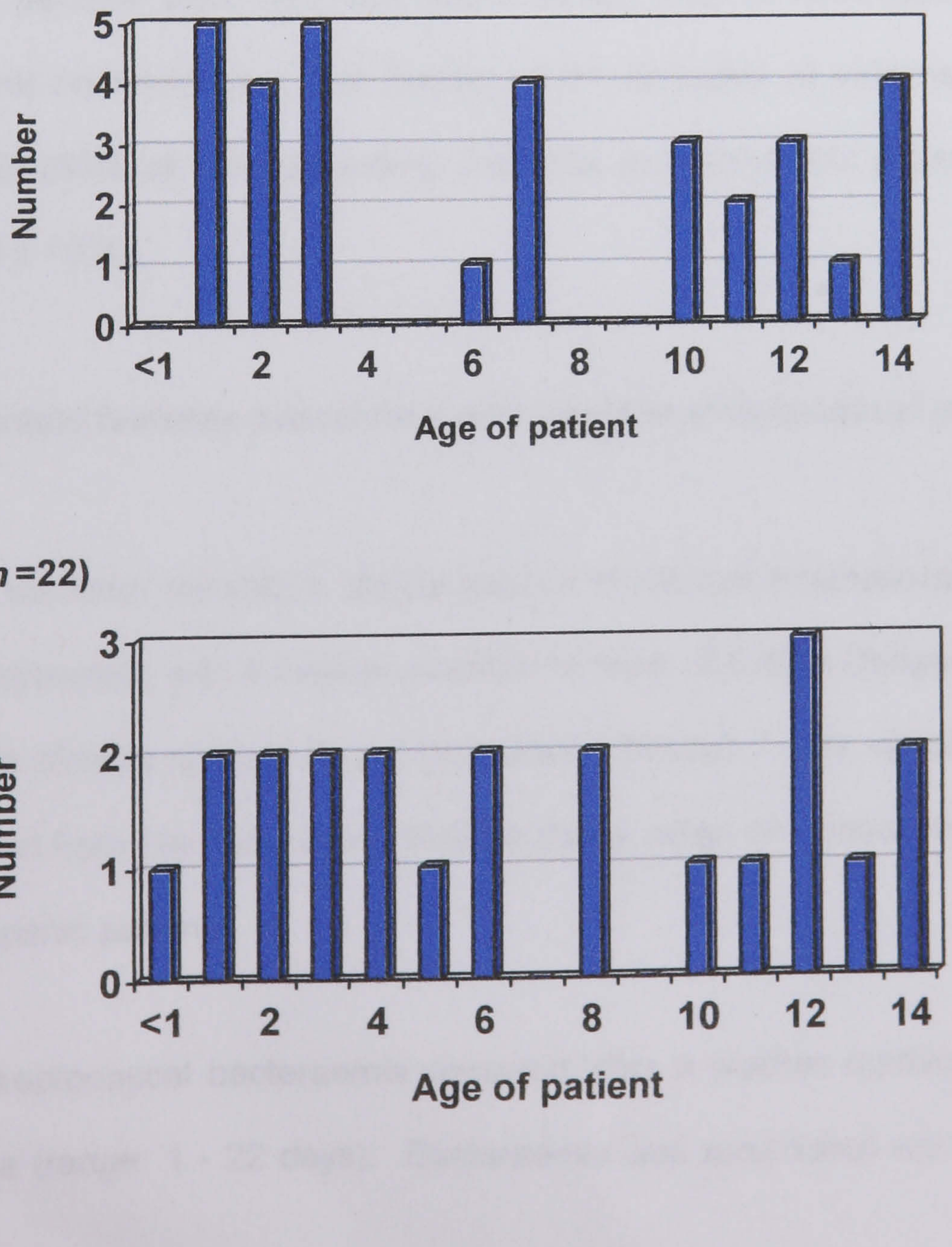
One episode of viridans streptococcal infection occurred in a patient from whom *P. carinii* was also detected (by immunofluorescence), from endo-tracheal secretions (patient No. 10).

5.11 Patient characteristics and clinical features of infection

5.11.1 Gender and age

Thirty-two boys developed viridans streptococcal bacteraemia compared with 22 girls. The median age of the patients was 6.5 years (range 3 months to 14 years). Male and female age distribution graphs (Figure 5.8) indicate that, while the majority of cases of viridans streptococcal bacteraemia in boys occurred between the ages of 1 and 3 years (44%) or between the ages of 10 and 14 (41%), a more even distribution of cases with age was observed for girls.

Figure 5. 8 Age distribution of patients developing viridans streptococcal bacteraemia



5.11.2 General clinical characteristics

Only one patient (proceeding through ALL induction therapy) did not have a Hickman line. Patients were receiving or recovering from chemotherapy with the exception of two. In the first of these, viridans streptococcal bacteraemia with concomitant candidaemia occurred in a febrile, non-neutropenic patient several weeks after the completion of a block of chemotherapy. In the second (also non-neutropenic patient), an assessment pre-high dose chemotherapy and stem cell rescue revealed viridans streptococci from blood cultured from Hickman line on two consecutive days. This finding raised the possibility of line-associated colonization. Unfortunately peripheral blood specimens could not be obtained. (This particular case will be discussed further in Section 7.3.3.)

Only three patients were not neutropenic at the time of development of viridans streptococcal bacteraemia. The remaining 66 episodes of viridans streptococcal bacteraemia (96% of total episodes) occurred in neutropenic patients (neutrophil count < 1.0×10^9/L).

5.11.3 Clinical features associated with viridans streptococcal bacteraemia

Fever was the most consistent clinical feature of viridans streptococcal bacteraemia (98.6% of episodes) with a median duration of fever of 6 days (range: 1 to 27 days). In the single afebrile episode (to be discussed in Section 7.3.3), viridans streptococci were isolated from two sets of line blood cultures taken on consecutive days from a non-neutropenic patient.

Viridans streptococcal bacteraemia occurred after a median duration of 3 days of neutropenia (range: 1 - 22 days). Bacteraemia was associated with an increase in

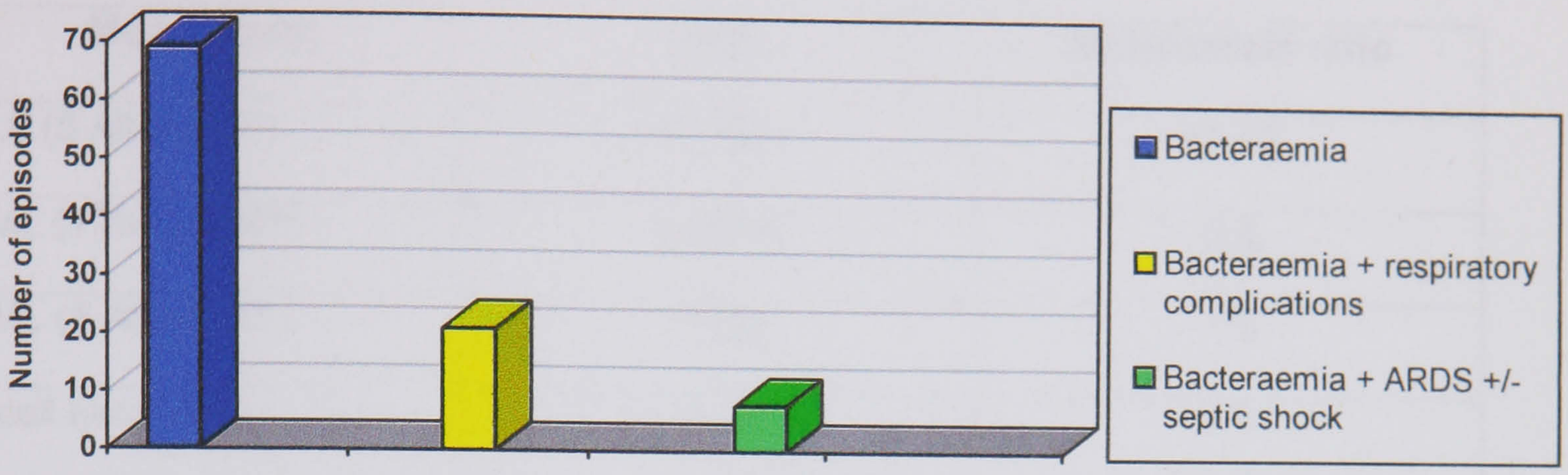
C-reactive protein, with a median level of 206 mg/L (range: 20-480mg/L). In the majority of cases (82%), maximum CRP level was reached within 3 days of positive blood culture.

In 22 cases (32%), a maculo-papular rash developed, however drug allergies could not be excluded as the cause. In 48 cases (70% of total), patients responded to antimicrobial therapy without developing further clinical complications. However, in 21 cases (30% of total), pulmonary complications developed, with 8 of these requiring mechanical ventilation and supplemental oxygen. Five of these 8 cases also developed septic shock, one of which was further complicated by meningitis. Clinical characteristics associated with cases with respiratory compromise and septic shock will be discussed further in Section 5.12. Two deaths were associated with viridans streptococcal bacteraemia. One girl died of the severe form of viridans streptococcal bacteraemia with meningitis. The other, who had a history of recurrent chest infections, and was being treated for a second malignancy, developed ARDS and septic shock and died a few hours later following cardiopulmonary arrest.

Viridans streptococcal endocarditis has been reported as a complication of bacteraemia in neutropenic patients by other investigators (Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994). None of the patients in the present study developed this condition. Clinical features were not helpful in distinguishing infections caused by one species of viridans streptococcus from those caused by another.

Figure 5.9 shows the spectrum of severity of symptoms associated with viridans streptococcal bacteraemia in the patients of this study.

Figure 5.9 Episodes of viridans streptococcal bacteraemia and spectrum of symptoms.



5.11.4 Underlying malignancies

Viridans streptococcal bacteraemia occurred more commonly in children suffering from acute leukaemia than in those with other malignancies (Table 5.5). The median age of patients with ALL (*n* = 29) was 6 years (range: 1 – 14 years) and for those with AML (*n* = 14) was 10 years (range: 3 months – 14 years). In the study population, ALL and AML were both more common in boys than in girls (17 versus 12 for the former and 9 versus 5 for the latter).

Although there were approximately twice as many ALL patients with viridans streptococcal bacteraemia as AML patients (29 versus 14), it is important to acknowledge that the latter form comprises only 15-20% of all childhood acute leukaemias. As a consequence, viridans streptococcal bacteraemia appears to be more strongly associated with AML than with ALL. However, the very intensive therapy for AML, more than the disease itself, may be the predisposing factor among this group of patients - as discussed in the following section.

Table 5.5 Underlying malignancies

Malignancy	*n* (%)	Male/female ratio
ALL (5 Allo. BMT)	29 (54)	17:12
AML (1 Auto. BMT)	14 (26)	9:5
CML (1 Allo. BMT)	1 (2)	1:0
T cell lymphoma	2 (4)	1:1
B cell lymphoma	2 (4)	1:1
Large cell anaplastic lymphoma	1 (2)	1:0
Rhabdomyosarcoma	2 (4)	0:2
Neuroblastoma	1 (2)	1:0
Osteosarcoma	1 (2)	1:0
Wilms' tumour	1 (2)	0:1

One teenage boy (14 years) developed viridans streptococcal bacteraemia post allogeneic bone marrow transplantation as therapy for CML. Five patients were being treated for lymphoma (3 boys and 2 girls). Two girls were undergoing therapy for Rhabdomyosarcoma and one for Wilms' tumour. There was one case of Osteosarcoma and one of Neuroblastoma (both boys).

5.11.5 Chemotherapeutic protocols and bone marrow transplantation

The cytotoxic chemotherapy regimens used to treat the above malignancies are listed in Table 5.6. Full details of the individual protocols are provided in Appendix I.

Table 5.6 Chemotherapeutic protocols and bone marrow transplantation prior to the development of viridans streptococcal bacteraemia

Chemotherapy Regimen	**Number of episodes of viridans streptococcal bacteraemia**
Induction (ALL)	1
DATES	11
3rd Intensification block (ALL)	3
Regimen B consolidation (ALL)	1
Relapse protocol (ALL)	4
ADE	2
MAE	4
MACE	2
MidAC	7
FLAG	7
CLASP	11
Allogeneic BMT	6
Autologous BMT	1
CYT/ETOP	1
COPADM2	1
CYM	1
OPEC	1
CDDP + DOX	1
H.D. Cyclophosphamide (MMT 98)	1
'mini' - BEAM	1

The DATES regimen comprised standard consolidation blocks 1 and 2 of the MRC protocols for treatment of childhood ALL until the end of 1999. As ALL is the most common childhood malignancy, DATES was the regimen of intensive chemotherapy used most often in the haematology/oncology unit during the course of this study. From the beginning of December 1994 to the end of December 1999, in excess of 200 courses of this form of chemotherapy were administered. Viridans streptococcal

bacteraemia developed following administration of 11 courses (Table 5.6). This represented < 5% of total courses of the DATES regimen.

Other components of standard therapy for ALL which featured in this study, were induction, the 3rd intensification block and the consolidation phase of one of the more recently introduced MRC ALL protocols - MRC ALL 97 (modified 1999) Regimen B (introduced at RHSC at the beginning of 2000). Induction and consolidation (B) preceded viridans streptococcal bacteraemia on one occasion only and 3rd block preceded this infection three times. Four episodes of viridans streptococcal bacteraemia were associated with the ALL relapse protocol.

Standard courses of induction therapy for childhood AML generally consist of ADE or MAE. Patients who have completed two courses of induction chemotherapy and are then in complete remission, generally receive one course of MACE consolidation chemotherapy. For patients who are considered good risk, a second course of consolidation chemotherapy, usually - MidAC is administered, to complete a total treatment course of 4 blocks. For AML patients in poorer prognosis categories, who require a total of 5 blocks of chemotherapy, the CLASP regimen may also be administered. MidAC and CLASP are also used to treat some cases of refractory ALL. The FLAG regimen may be used for therapy of acute leukaemia, when conventional therapy is not appropriate.

Episodes of viridans streptococcal bacteraemia occurred, following each one of the above courses of chemotherapy; 2 episodes following ADE, 4 following MAE, 2 following MACE, 7 following MidAC, 7 following FLAG and 11 following CLASP. However, to determine whether there is an association between viridans streptococcal bacteraemia and one of these chemotherapeutic regimens, the total number of courses of each chemotherapy schedule administered to all patients

receiving 'AML-type therapy' throughout the study period was required. The ratio of episodes of viridans streptococci to total courses of each chemotherapeutic regimen is expressed as a percentage in Figure 5.10. The number of courses of each regimen administered was within a fairly narrow range (22 – 34). Viridans streptococcal bacteraemia occurred after 9% of total courses of ADE, after 14% of total courses of MAE, and after 8%, 23%, 23% and 32% of total courses of MACE, MidAC, FLAG and CLASP respectively. The resulting trend (Figure 5.10) indicates that viridans streptococcal bacteraemia occurred more often after therapy with high doses of cytosine arabinoside than after the lower dose schedules (See Table 5.7 for cytosine arabinoside dose per regimen).

Figure 5.10 Viridans streptococcal bacteraemia following individual courses of chemotherapy for AML - as proportion (%) of total courses of each regimen administered

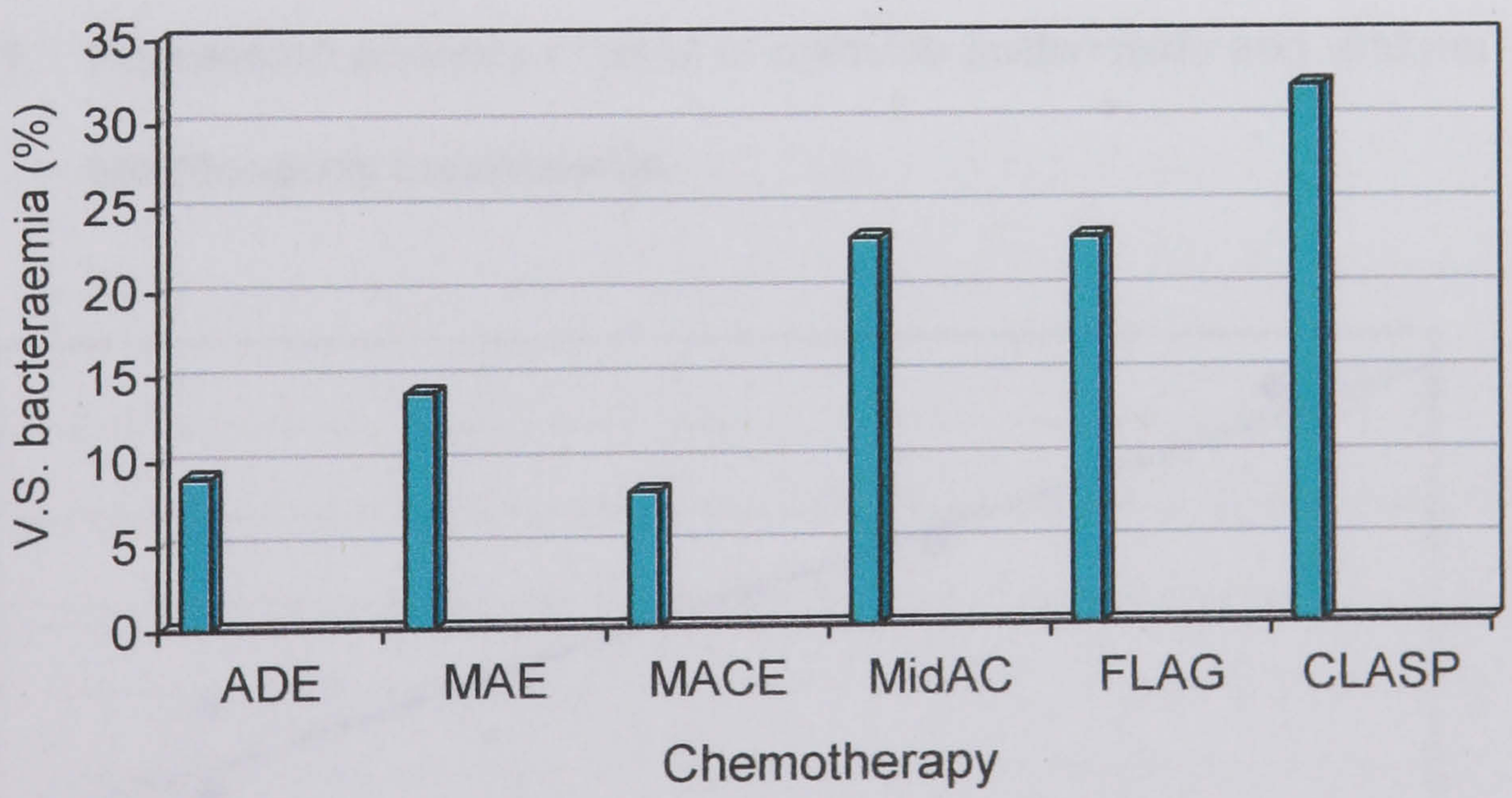
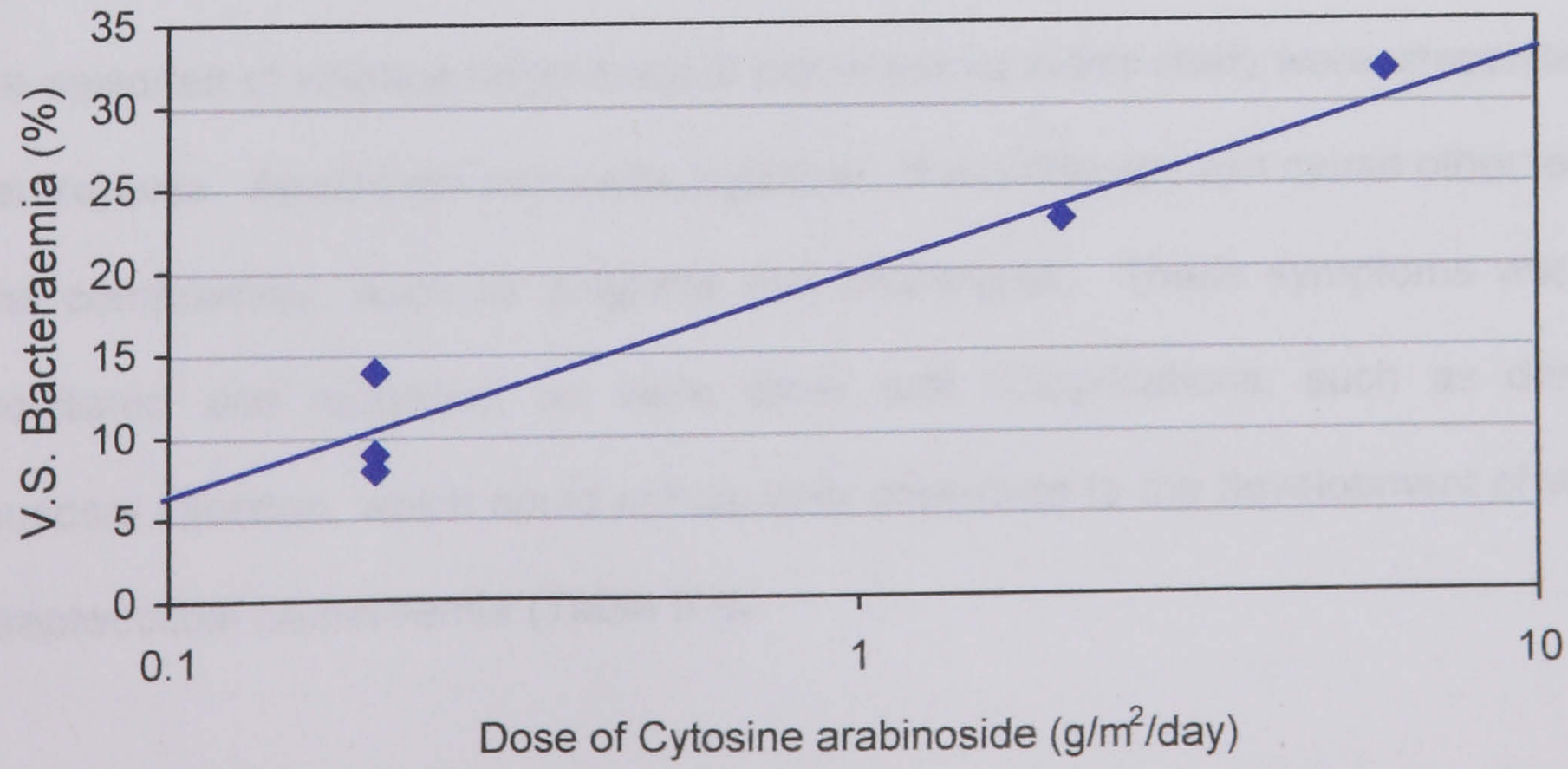


Table 5.7 Chemotherapeutic protocols with dose of cytosine arabinoside

Chemotherapy regimen	Dose of cytosine arabinoside
ADE	100 mg/m^2, 12-hourly
MAE	100 mg/m^2, 12-hourly
MACE	200 mg/m^2, daily
MidAC	1.0 g/m^2, 12-hourly ('intermediate-high dose')
FLAG	2.0 g/m^2, daily ('intermediate-high dose')
CLASP	3.0 g/m^2, 12-hourly ('high-dose')

It has been proposed previously that there is a relationship between the use of high-doses of this agent and the incidence of viridans streptococcal bacteraemia (Bochud *et al.*, 1994; Richard *et al.*, 1995). The regression analysis of Figure 5.11 demonstrates the influence of increasing doses of cytosine arabinoside on frequency of viridans streptococcal bacteraemia.

Figure 5.11 Regression analysis of dose of cytosine arabinoside and viridans streptococcal bacteraemia



Coefficient of correlation = 0.972, $P < 0.001$

The development of viridans streptococcal bacteraemia was less common following chemotherapy for solid tumours. One episode of infection occurred following each of CYT/ETOP, COPADM2, CYM, OPEC, CDDP + DOX, high-dose cyclophosphamide (MMT '98) and 'mini'- BEAM.

Seven episodes of viridans streptococcal bacteraemia followed bone marrow transplantation, predominantly in recipients of allogeneic rather than autologous transplants (6 versus 1). The ratio of episodes of viridans streptococcal bacteraemia to total transplants performed throughout the study period were 12.5% (6/48) following allogeneic transplantation and 9.1% (1/11) following autologous transplantation. Of the allogeneic transplants, viridans streptococcal bacteraemia was more common following transplantation using a matched unrelated donor (16% of a total of 25), than following transplantation using marrow from a sibling donor (8.7% of a total of 23).

5.11.6 Adverse effects of cytotoxic chemotherapy

Adverse effects of chemotherapeutic agents, such as neutropenia and mucositis have been described previously in Sections 2.3.1.2 and 2.3.4. Ninety-six percent of the episodes of viridans streptococcal bacteraemia in this study were associated with neutropenia. Apart from mucositis, cytotoxic chemotherapy can cause other forms of oral compromise, such as gingivitis and pharyngitis. These symptoms were also monitored and recorded, as were other oral complications, such as dental or mucosal infection, which could conceivably contribute to the development of viridans streptococcal bacteraemia (Table 5.8).

Classical chemotherapy-induced mucositis was associated with 33 episodes *i.e.* 48% of total episodes. (Severe mucositis was associated with 7 episodes, moderate

mucositis with 14 and mild mucositis with 12.) However if the other clinical features of Table 5.8 are included, oral complications were associated with 45 episodes (65% of total).

Table 5.8 Oral compromise and episodes of viridans streptococcal bacteraemia

Oral compromise	**Description**	**No. of episodes (% of total episodes)**
Mucositis	Inflammation +/- ulceration of oral mucosa	33 (48)
Gingivitis	Inflammation of the gingivae +/- spontaneous gingival bleeding	6 (9)
Bleeding Lips	-	4 (6)
Pharyngitis	-	2 (3)
Caries	-	2 (3)
Oozing sockets	- following tooth extraction	3 (4)
Loose teeth	-	1 (1)
'Teething'	-	1 (1)
Candidiasis	Clinical oral thrush with isolation of *Candida* spp from culture.	6 (9)
Herpes simplex Infection	Ulceration, with HSV isolated from viral culture	1 (1)

Chemotherapy-induced damage to gastro-intestinal mucosal barriers is more difficult to monitor. Symptoms include vomiting, abdominal pain and diarrhoea, sometimes accompanied by bleeding. Obviously, infection has to be excluded as a cause of diarrhoea. Symptoms, which may have been related to intestinal mucosal injury were associated with 32 episodes of viridans streptococcal bacteraemia (Table 5.9).

In total, 84% (58/69) of episodes were associated with one or more signs of oral compromise +/- gut mucosal damage.

Table 5.9 Gastro-intestinal symptoms of patients with episodes of viridans streptococcal bacteraemia

Symptom	**No. of episodes (% of total episodes)**
Diarrhoea	29 (42)
G.I. bleeding	2 (3)
Rectal mucositis	1 (1)

In addition to producing profound and protracted neutropenia with severe mucositis, high-dose cytosine arabinoside can also produce pulmonary toxic effects which have been associated with the development of ARDS in neutropenic patients with viridans streptococcal bacteraemia. The association of this agent with respiratory complications and the severe form of viridans streptococcal sepsis in the patients of the present study will be examined further in Sections 5.12.3 and 5.12.5.

It should also be acknowledged that the severity of adverse effects associated with chemotherapy, and consequently the contribution to infectious risk, may also depend on the combined effects of the different drugs employed in the same protocol. Daunorubicin, Mitozantrone and Amsacrine (Appendix I), which feature in some of the regimens of Table 5.7, also produce the adverse effects of neutropenia and mucositis.

5.11.7 Antibiotic prophylaxis and empirical therapy

Patients with viridans streptococcal bacteraemia who had received conventional cytotoxic chemotherapy for leukaemia and the two patients with T-cell lymphoma received cotrimoxazole prophylaxis as per protocol. Bone marrow transplantation patients also received cotrimoxazole prophylaxis - from Day –8 to Day –1, and post transplantation once neutrophil counts > 0.5 x 10^9/L. The remaining patients with viridans streptococcal bacteraemia did not receive antibiotic prophylaxis.

Empirical therapy for episodes of febrile neutropenia consisted of a β-lactam antibiotic plus an aminoglycoside throughout the period of this study. Until summer of 1996 this comprised ceftazidime plus amikacin. After this time the combination of piperacillin/tazobactam plus amikacin was used. Patient number 23 (Table 5.1) was the first to receive the new combination. Vancomycin was used as second-line empirical therapy throughout. This antibiotic was also added to first-line therapy for cases of viridans streptococcal bacteraemia if clinical response was sub-optimal and/or in cases of *in vitro* resistance to first-line antibiotics. Findings from this investigation in terms of the antimicrobial susceptibility of viridans streptococcal isolates will be presented in Chapter 6.

5.12 Investigation of episodes of viridans streptococcal bacteraemia with accompanying respiratory complications +/- septic shock

The uncommon, but severe form of viridans streptococcal sepsis has been termed 'viridans streptococcal shock syndrome' (Steiner *et al.*, 1993), and may result in severe hypotension requiring intravascular expansion and/or vasopressor support. Acute respiratory distress is usually an accompanying feature - described as

respiratory insufficiency with diffuse bilateral pulmonary infiltrates on chest radiography necessitating oxygen supplementation and ventilation.

However, there is a group of patients with viridans streptococcal bacteraemia who neither develop septic shock nor require mechanical ventilation, but do exhibit some degree of respiratory distress requiring supplemental oxygen (Figure 5.9). This particular group has been studied to a lesser extent in the past. The present study sought to investigate why there is such a spectrum of severity of clinical symptoms associated with viridans streptococcal bacteraemia and included patients with mild to severe pulmonary complications. Previously proposed risk factors will be considered, but the effect of other possible contributory factors will also be explored.

5.12.1 Respiratory complications associated with viridans streptococcal bacteraemia

Some form of respiratory distress was associated temporally with 21 episodes of viridans streptococcal bacteraemia, (21/69 =30% of total episodes). These symptoms varied from an increased respiratory rate with the requirement of 1litre of supplemental oxygen, to ARDS requiring mechanical ventilation. It is difficult to separate the range of symptoms into specific categories of severity, however cases were divided into three fairly broad groups according to the definitions of Sections 4.1.3 and 4.1.4. Mild respiratory symptoms were associated with 10 episodes, moderate with 3 and severe with 8. (One case with severe respiratory distress also featured cardiomyopathy and thus did not satisfy the definition of ARDS.) Patient demograhics and respiratory complications are presented in Table 5.10.

Table 5.10 Viridans streptococcal bacteraemia and respiratory complications following chemotherapy

Patient Number (Gender, Age)	Underlying malignancy	Chemotherapy regimen prior to episode of bacteraemia	Severity of respiratory complications	Additional factors
1 (F, 3 months)	AML	ADE	+++ (ARDS)	Septic shock. Immaturity of lungs
1 (F, 6 months)	"	CLASP	+++ (ARDS)	Septic shock
1 (F, 11months)	"	MACE	+	-
1 (F, 1 year)	"	Auto. BMT	+	Influenza A virus infection
5 (M, 2 years)	AML	CLASP	+++ (ARDS)	-
8 (F, 12 years)	AML	MidAC	+	-
9 (F, 5 years)	ALL	CLASP	+++ (ARDS)	Septic shock, meningitis, Pulmonary CMV infection
10 (F, 6 years)	B-cell Lymphoma	CYT/ETOP	+++ (ARDS)	Septic shock, *P. carinii* infection
11 (F, 14 years)	AML	CLASP	+	-
12 (F, 11 years)	AML	ADE	+++ (ARDS)	Septic shock. Recurrent chest infections. 2nd malignancy.
13 (M, 3 years)	ALL	Allo. (sibling) BMT	+	-
16 (M, 14 years)	T-cell lymphoma	CLASP	++	-
18 (M, 3 years)	ALL	3rd Block	++	-
19 (M, 1 year)	ALL	MidAC	+	Parainfluenza virus infection
29 (M, 1 year)	ALL	FLAG	+	-
32 (M, 12 years)	AML	MidAC	+	-
39 (M, 14 years)	AML	MidAC	+++	Cardiomyopathy
43 (F, 3 years)	ALL	DATES	+	-
44 (M, 2 years)	AML	CLASP	+	-
51 (F, 14 years)	Rhabdo-myosarcoma	High-dose cyclo-phosphamide	++	-
54 (M, 6 years)	LCAL	'Mini'-BEAM	+++ (ARDS)	Probable infiltration of lymphoma. Picorna virus infection

M: Male, F: Female

Eight girls and ten boys developed some degree of respiratory compromise shortly after the development of viridans streptococcal bacteraemia (Table 5.10). Their ages ranged from 3 months to 14 years (with a median age of 5 years). Malignancies comprised AML (11 episodes, 8 patients), ALL (6 episodes), B-cell lymphoma (1 episode), T-cell lymphoma (1 episode), large cell anaplastic lymphoma (1 episode) and rhabdomyosarcoma (1 episode). All patients were neutropenic. In 19 cases (90%) there was evidence of chemotherapy - induced oral compromise or possible gastro-intestinal toxicity. Of these, 4 episodes were associated with evidence of mild oral mucositis, 4 with moderate mucositis and 2 with severe mucositis. Five patients developed septic shock (Table 5.10).

Of the total episodes with respiratory complications (21), a single strain of *S. oralis* was cultured from blood in 14 (67%) and *S. mitis* from 5 (24%) (Table 5.11). In one case, 2 different strains of *S. mitis* were isolated and in one, 2 strains of *S. oralis* and 1 strain of *S. mitis* were involved. Overall, *S. oralis* represented 67% of total isolates of viridans streptococci associated with respiratory complications, while *S. mitis* represented 33%. In common with its predominance amongst total blood culture isolates (63%), *S. oralis* was the species most often associated with patients with respiratory compromise.

Table 5.11 Species of viridans streptococci from blood culture of patients with bacteraemia and respiratory complications

Species	**No. of episodes (% of total episodes with respiratory symptoms) *n* = 21**
S. oralis (single strain)	14 (67%)
S. mitis (single strain)	5 (24%)
Multiple strains or species ((a) *S. mitis* – 2 strains (b) *S. oralis* (2 strains) + *S.mitis*)	2 (9%)

On two occasions, blood cultures yielding organisms of different genera were associated with episodes of bacteraemia with respiratory complications. These represented 12.5% of all polymicrobial blood cultures. From one patient (No. 10), *S. oralis* and *Enterococcus faecalis* were isolated, and from the other (No. 18) *S.oralis* (2 strains), *S. mitis*, *Haemophilus parainfluenza* and *Moraxella catarrhalis* were isolated. Both patients suffered from oral mucositis and diarrhoea.

5.12.2 Viruses, *Pneumocystis carinii,* respiratory complications and viridans streptococcal bacteraemia

In four of the five cases of viridans streptococcal bacteraemia with concomitant respiratory viral infection some form of respiratory distress (2 ARDS, 1 moderate and 1 mild) developed - as presented in Table 5.10. Patient No. 10, with viridans streptococcal bacteraemia and *P. carinii* infection developed ARDS.

PCR-based detection of respiratory viruses was not available as a routine diagnostic service until late 2000, therefore prior to this, some patients may have been infected by respiratory viruses not detectable by immunofluorescence alone.

5.12.3 Chemotherapeutic regimens and respiratory symptoms

Respiratory complications were associated temporally with six of the eleven episodes of viridans streptococcal bacteraemia following the CLASP regimen (54%). In three of these episodes, patients' clinical symptoms and radiographic findings were consistent with ARDS, and mechanical ventilation and supplemental oxygen were required. CMV pulmonary infection also occurred in one of the episodes with ARDS. This was diagnosed post-mortem. The remaining three patients suffered from less severe respiratory complications, but required supplemental oxygen. (Table 5.12).

Table 5.12 Respiratory complications following chemotherapy

Chemotherapy regimen administered prior to episode of infection	No. of cases with respiratory complications	Severity of respiratory symptoms (No. of episodes)
CLASP	6	+++ (x 3) (ARDS) ++ (x 1) + (x 2)
MidAC	4	+++ (x 1) * + (x 3)
ADE	2	+++ (x 2) (ARDS)
FLAG	1	+
MACE	1	+
DATES	1	+
3rd Block	1	++
H.D. cyclophosphamide	1	++
Mini-BEAM	1	+++ (ARDS)
CYT/ETOP	1	+++ (ARDS)
Sibling BMT	1	+
Auto BMT	1	+

*: Associated with cardiomyopathy

Four of the seven episodes of viridans streptococcal bacteraemia following MidAC chemotherapy (57%) were associated with respiratory complications – 1 with severe symptoms (associated, in part, with cardiomyopathy) and 3 with milder symptoms (Table 5.12). One of the milder cases also featured infection by parainfluenza virus (Table 5.10). Two cases of ARDS occurred following ADE chemotherapy.

The remaining courses of chemotherapy preceding single episodes with respiratory complications were FLAG, MACE, DATES, 3rd Intensification block (for ALL), CYT/ETOP, high-dose cyclophosphamide and 'mini'-BEAM (Table 5.12). Two cases of mild respiratory symptoms occurred following bone marrow transplantation.

Several of the chemotherapy regimens of Table 5.12 contain agents with side effects which include pulmonary toxicity (*e.g.*high-dose cytosine arabinoside, high-dose cyclophosphamide and BCNU (in BEAM chemotherapy).

One child (patient No. 1) developed respiratory complications associated with four different episodes of viridans streptococcal bacteraemia. This patient was 3 months old when she received her first course of chemotherapy (ADE) for AML. Following this course, and following the subsequent one (CLASP – 3 months later), she developed ARDS and septic shock. After a further course (MACE) at 11 months of age and following autologous bone marrow transplantation at 14 months, she again developed respiratory complications, but of a milder nature on both of these occasions. The combination of lung immaturity in this infant, drug toxicity and severe sepsis may all have contributed to such marked respiratory problems. The final patient of this study, a five-year old boy receiving treatment for large cell anaplastic lymphoma developed severe respiratory failure with clinical features and chest X-ray findings consistent with ARDS. In addition to viridans streptococcal bacteraemia, this child had concomitant picorna virus infection, which although not usually associated with lower respiratory tract symptoms, may have played a minor part. A major complicating factor was probable pulmonary relapse of lymphoma, which may be indistinguishable from other causes of ARDS on radiography.

5.12.4 Viridans streptococcal bacteraemia and septic shock

Five episodes of viridans streptococcal bacteraemia (in 4 patients) were associated with the development of septic shock (Table 5.13). All four patients were female and their ages ranged from 3 months to 11years. One patient (No.1) developed two episodes of septic shock. Two were receiving therapy for AML and one for ALL (which was refractory to conventional chemotherapy). The fourth patient was being

treated for disseminated B-cell lymphoma with central nervous system involvement. All suffered from oral mucositis (mild symptoms associated with two episodes, moderate symptoms with two and severe symptoms with one), with probable gastrointestinal involvement and all were profoundly neutropenic when viridans streptococcal bacteraemia occurred. One child (patient No. 9) also developed viridans streptococcal meningitis. All received ITU support and monitoring, but in spite of this two died (patients 9 and 12).

Table 5.13 Episodes of viridans streptococcal bacteraemia with septic shock

Patient No. (Age)	**Malignancy**	**Chemotherapy**	**Mucosal damage**	**Additional symptoms**	**Additional factors**
1 1st episode (3 months)	AML	ADE	Mild	ARDS	Immaturity of lungs.
1 2nd episode (6 months)	AML	CLASP	Mild	ARDS	"
9 (5 years)	ALL	CLASP	Severe	ARDS Meningitis	CMV pulmonary infection
10 (6 years)	B-cell lymphoma	CYT/ETOP	Moderate	ARDS	*P. carinii* infection.
12 (11 years)	AML	ADE	Moderate	ARDS	Recurrent chest infections. 2nd malignancy.

The species isolated from blood culture in all five episodes of septic shock, was *S. oralis*. As discussed in Section 5.8, one of the cases of septic shock (patient No. 10) was associated with the isolation of *S. oralis* plus *E. faecalis* from blood culture. *E. faecalis* is rarely associated with the development of severe sepsis. Even in immunocompromised patients, *E. faecalis* bacteraemia generally runs a fairly benign course.

5.12.5 Chemotherapeutic regimens and viridans streptococcal septic shock

Following the CLASP regimen, two episodes of viridans streptococcal bacteraemia progressed to septic shock with ARDS. The other courses of chemotherapy associated with the combination of these symptoms were ADE in 2 cases, and CYT/ETOP in the other (Table 5.13). Pulmonary toxicity associated with the administration of cytosine arabinoside in these cases may have directly contributed to the development of ARDS, however cytokine mediated respiratory compromise may also have resulted as a consequence of septic shock. All of these patients suffered from oral mucositis and possible gastrointestinal mucositis (all suffered from diarrhoea) therefore if a particularly high bacterial load of viridans streptococci accessed the blood stream, this may have elicited the release of sufficient cytokines to cause septic shock and to trigger the development of ARDS.

5.13 Summary

Episodes of viridans streptococcal bacteraemia decreased in frequency over the period of this study, from 22% of all microbiologically documented episodes of bloodstream infection in 1994 to 4% in 2000 ($P < 0.001$). In 1994, viridans streptococcal bacteraemia was associated with 5.9% of all febrile episodes compared with 1.2% in 2000 ($P < 0.001$). *S. oralis* was the species of viridans streptococcus most commonly isolated from blood culture (63% of total isolates of viridans streptococci). *S. mitis* represented 25% of total isolates. Polymicrobial bloodstream infection occurred in 23% of episodes, however the combination of viridans streptococci plus other micro-organisms was not significantly associated with inferior outcome.

Patients with haematological malignancy (particularly AML) were more likely to develop viridans streptococcal bacteraemia, than those with solid tumours. Some form of oral compromise +/- possible gastrointestinal mucositis was associated with 58 episodes. In 48 cases, (70% of total), patients responded readily to antimicrobial therapy without developing further clinical complications. Mild to severe respiratory complications were associated with the remainder, with mechanical ventilation and supplemental oxygen required in eight episodes. Five of these also featured septic shock. *S. oralis* again predominated in cases of bacteraemia with respiratory complications (67%) and was the species isolated from blood culture of all 5 patients with septic shock.

Viridans streptococcal bacteraemia was associated with cytotoxic chemotherapy regimens containing high doses of cytosine arabinoside, particularly the CLASP regimen; 32% of all courses administered during the study period (11/34) preceded this infection. This pattern is consistent with the proposal by other investigators that intensive chemotherapy with high-dose cytosine arabinoside, used particularly in the therapy of AML, predisposes to viridans streptococcal bacteraemia. CLASP chemotherapy was also associated with the development of respiratory complications in 6 of the 11 cases mentioned above (54%), with 2 of these also developing septic shock.

Concomitant infection with respiratory viruses or *P. carinii,* occurred in 6 cases of viridans streptococcal bacteraemia (9% of total cases) and 5 of these exhibited respiratory complications, with 3 progressing to ARDS.

These findings indicate that the aetiology of complications associated with viridans streptococcal bacteraemia is complex and may be multifactorial - with sepsis, toxicity of chemotherapeutic agents, and concomitant viral infections playing a part.

CHAPTER 6

ANTIBIOTIC SUSCEPTIBILITIES OF VIRIDANS STREPTOCOCCI ISOLATED FROM BLOOD CULTURE OF PAEDIATRIC IMMUNOCOMPROMISED PATIENTS

ANTIBIOTIC SUSCEPTIBILITIES OF VIRIDANS STREPTOCOCCI ISOLATED FROM BLOOD CULTURE OF PAEDIATRIC IMMUNOCOMPROMISED PATIENTS

6.1 Introduction

Viridans streptococci were traditionally regarded as being almost uniformly susceptible to penicillin and a wide variety of other antibiotics. At the commencement of the present study (late 1994), a limited number of reports of reduced susceptibility or resistance amongst these organisms existed in the literature (Krumweide, 1949; Sprunt, Redman & Leidy, 1968; Phillips *et al.*, 1976; Southall *et al.*, 1983; Woodman *et al.*, 1985; Kern, Linzmeier & Kurle, 1989; Potgieter *et al.*, 1992; McWhinney *et al.*, 1993; Bochud *et al.*, 1994; Guiot, Corel & Vossen, 1994). Today, resistance rates to penicillins are generally higher, with some strains also resistant to cephalosporins, tetracyclines, macrolides, quinolones, chloramphenicol and cotrimoxazole. The emergence of resistance to multiple antibiotics has complicated the therapy of infection by viridans streptococci, particularly in the immunocompromised host.

This study examined the *in vitro* activity of several antibiotics, including those commonly used for empirical therapy of febrile neutropenia and newer agents which may be useful in the future. In the first category, the antibiotics chosen were the broad-spectrum, β-lactam agents, ceftazidime, piperacillin/tazobactam and meropenem. Susceptibility to vancomycin was also investigated, as this antibiotic is often used to treat infection by various Gram-positive bacteria causing bacteraemia in neutropenic patients. Two of the older β-lactam agents, penicillin, and the first generation cephalosporin, cefaclor were also included, for comparison with the newer, broader spectrum agents. More recently introduced antimicrobials which were tested, comprised the fourth generation cephalosporin, cefpirome and two agents reported to have particularly good activity against multiply-resistant Gram-

positive bacteria - quinupristin/dalfopristin and linezolid. Susceptibility of the isolates of viridans streptococci to cotrimoxazole was also determined as the majority of patients (85%) received this agent as prophylaxis against *P. carinii* pneumonia.

An investigation into species-specific susceptibility was performed, in an attempt to establish whether *S. oralis* and *S. mitis* differed significantly in antibiotic susceptibility patterns.

Results of both MIC methods and disc methods were compared for all isolates of viridans streptococci from blood cultures and interpretative aspects of these tests are discussed.

Antibiotic susceptibility of endogenous flora can be influenced by prior antimicrobial therapy. As many immunocompromised patients require several courses of antibiotics to treat different episodes of febrile neutropenia, the potential exists for selection of resistant strains. This study investigated the antimicrobial susceptibility of viridans streptococci isolated from blood culture while empirical therapy at RHSC was ceftazidime plus amikacin and compared this with the susceptibility of viridans streptococci isolated from blood culture after a change to piperacillin/tazobactam plus amikacin. The majority of patients from both time intervals had been exposed to prior empirical therapy.

Based on the observation that both the prevalence of and the severity of symptoms associated with viridans streptococcal bacteraemia decreased after the change in empirical therapy (Chapter 5), a further study attempted to determine whether resistance to antibiotics used as empirical therapy might influence clinical course of infection.

6.2 Susceptibility of 76 isolates of viridans streptococci to six antibiotics

Antibiotic susceptibilities of all isolates of viridans streptococci from blood cultures were determined as described in Chapter 4 and the results presented in Table 6.1.

Table 6.1 MICs of six antibiotics against 76 isolates of viridans streptococci

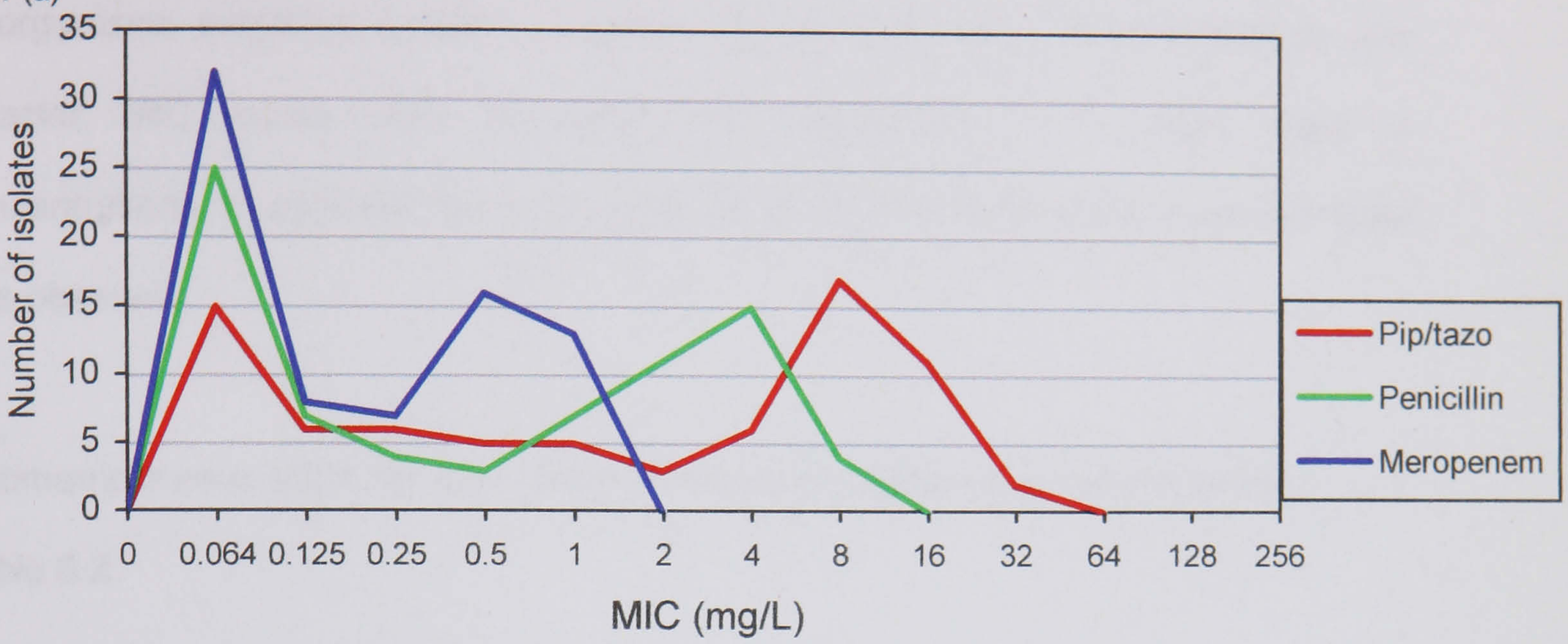
Antibiotic	Cumulative % of strains with stated MIC (mg/L)												
	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256
Ceftazidime	0	9	18	20	40	46	51	57	63	78	87	96	100
Piperacillin/ tazobactam	20	28	36	42	49	53	60	83	97	100	-	-	-
Meropenem	42	53	62	83	100	-	-	-	-	-	-	-	-
Penicillin	33	42	47	51	60	75	95	100	-	-	-	-	-
Cefaclor	0	0	3	8	25	37	42	46	50	50	53	55	100
Vancomycin	0	0	0	17	97	100	-	-	-	-	-	-	-

The results indicated that of the β-lactam antibiotics, meropenem had the greatest *in-vitro* activity, with an MIC_{50} of 0.125 mg/L and MIC_{90} of 1.0 mg/L. Vancomycin also showed considerable activity, over a narrow range, with both MIC_{50} and MIC_{90} of 1.0 mg/L. MICs of penicillin and piperacillin/ tazobactam covered a wider range - from 0.064 – 8.0 mg/L and 0.064-32 mg/L respectively, with MIC_{50} of 0.5 mg/L and MIC_{90} of 4 mg/L for the former and MIC_{50} of 2.0 mg/L and MIC_{90} of 16 mg/L for the latter. Of all antibiotics tested, the two cephalosporins demonstrated the poorest activity against viridans streptococci. MIC_{50} and MIC_{90} results for ceftazidime were 4 mg/L and 128 mg/L respectively, while those for cefaclor were 16 mg/L and ≥ 256 mg/L.

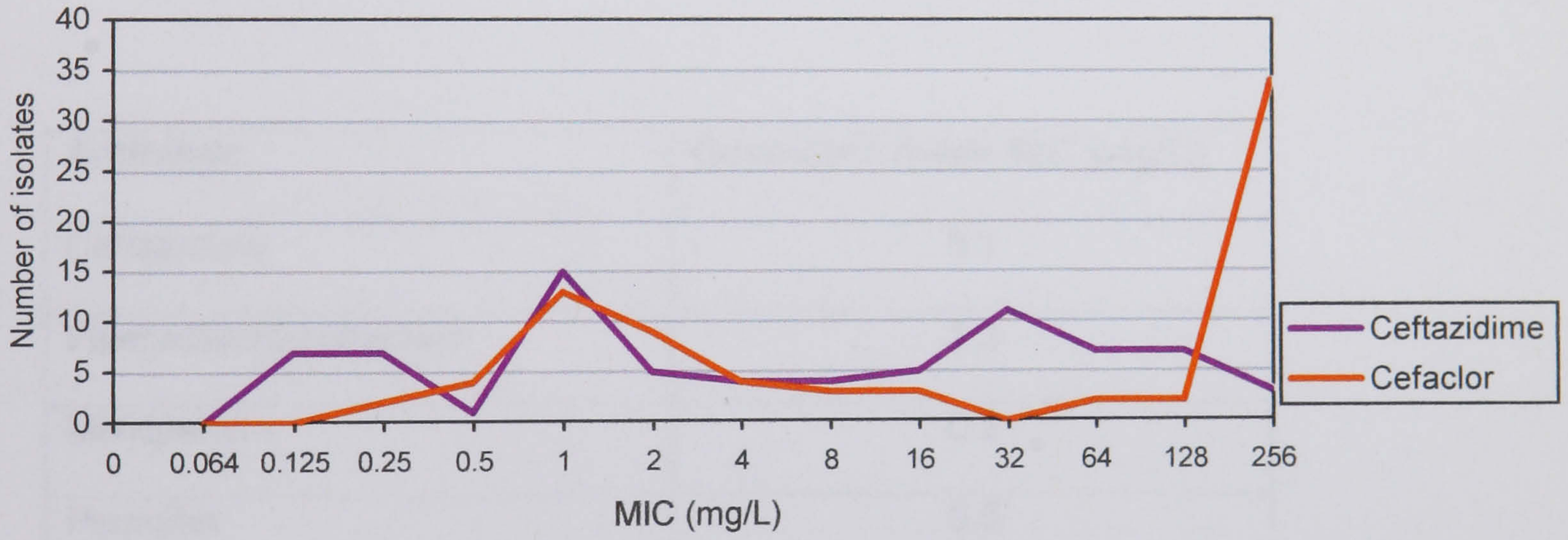
MIC distributions for the above antibiotics against viridans streptococci are presented graphically in Figure 6.1.

Figure 6.1 Distribution of MIC values for six antibiotics against 76 isolates of viridans streptococci

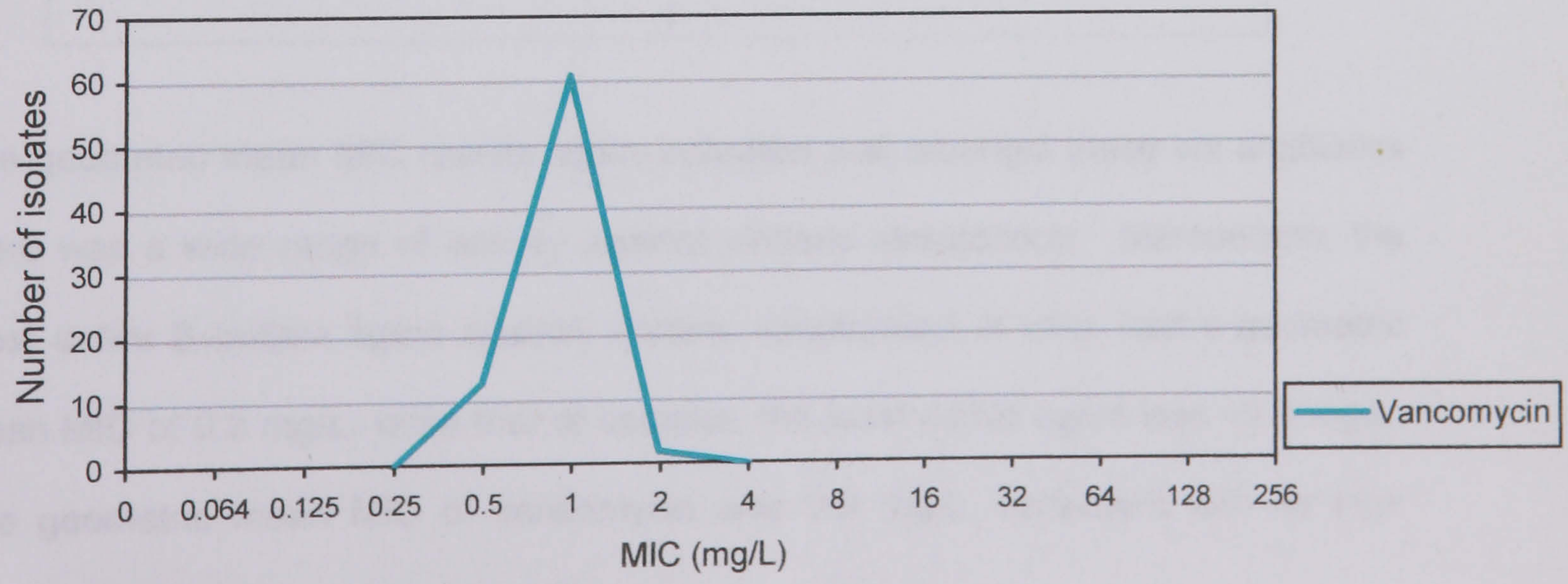
6.1 (a)



6.1 (b)



6.1 (c)



The MIC results for penicillin, piperacillin/tazobactam and meropenem against viridans streptococci followed a bimodal distribution – with one of the two populations of organisms exhibiting greater susceptibility than the other. For ceftazidime and cefaclor, MIC values were generally more dispersed over a wider range of concentrations. In contrast, for vancomycin a normal distribution over a narrow range was observed.

Geometric means MICs for each antibiotic were calculated and are presented in Table 6.2.

Table 6.2 Geometric mean MICs of six antibiotics against 76 isolates of viridans streptococci

Antibiotic	**Geometric mean MIC (mg/L)**
Ceftazidime	5.1
Piperacillin/tazobactam	1.3
Meropenem	0.2
Penicillin	0.5
Cefaclor	19.9
Vancomycin	0.9

The geometric mean MIC results again indicated that amongst these six antibiotics there was a wide range of activity against viridans streptococci. Meropenem, the most active β-lactam agent against viridans streptococci *in vitro*, had a geometric mean MIC of 0.2 mg/L, while that of cefaclor, the least active agent was 19.9 mg/L. The geometric mean MIC of vancomycin was 0.9 mg/L, consistent with its high activity against viridans streptococci.

6.3 Comparative susceptibilities of *S. oralis* and *S. mitis* to six antibiotics

It was important to determine whether certain species of viridans streptococci are generally more resistant to antibiotics than others. If this were the case, rapid identification could guide antimicrobial therapy – even before *in vitro* susceptibility results were available. MIC distributions were analysed for all isolates of *S. oralis* (*n*=48) from the present study and were compared with those for all isolates of *S. mitis* (*n*=19). There were too few isolates of *S. sanguis*, *S. parasanguis*, *S. salivarius* and *S. gordonii* for inclusion in this analysis. The results are presented in Table 6.3.

Table 6.3(a) MICs of six antibiotics against 48 isolates of *S.oralis*

Antibiotic	**Cumulative % of strains with stated MIC (mg/L)**												
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**
Ceftazidime	0	4	12	15	35	40	48	56	65	85	98	100	-
Piperacillin/ tazobactam	21	27	33	38	46	48	58	88	100	-	-	-	-
Meropenem	40	50	62	90	100	-	-	-	-	-	-	-	-
Penicillin	29	38	44	48	56	77	100	-	-	-	-	-	-
Cefaclor	0	0	4	8	23	33	40	44	48	48	50	50	100
Vancomycin	0	0	0	21	96	100	-	-	-	-	-	-	-

Table 6.3(b) MICs of six antibiotics against 19 isolates of *S. mitis*

Antibiotic	**Cumulative % of strains with stated MIC (mg/L)**												
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**
Ceftazidime	0	16	26	26	42	53	53	53	53	53	58	84	100
Piperacillin/ tazobactam	26	32	42	53	53	53	53	68	90	100	-	-	-
Meropenem	53	53	53	63	100	-	-	-	-	-	-	-	-
Penicillin	42	53	53	53	58	58	79	100	-	-	-	-	-
Cefaclor	0	0	0	10	32	47	53	53	53	53	53	53	100
Vancomycin	0	0	0	10	100	-	-	-	-	-	-	-	-

The isolates of *S. oralis* and *S. mitis* had similar ranges of MICs for all antibiotics tested with no more than one doubling dilution's difference between them.

The MIC ranges for piperacillin/tazobactam against *S. oralis* were 0.064 –16 mg/L versus 0.064 –32 mg/L for *S. mitis*, while those for penicillin were 0.064 – 4 mg/L versus 0.064 – 8 mg/L. Geometric mean MICs for piperacillin/ tazobactam were 1.3 mg/L for *S. oralis* versus 1.2 mg/L for *S. mitis,* while those for penicillin for both species were identical (0.5 mg/L) (Table 6.4).

The meropenem geometric mean MICs for both species were also identical (0.2 mg/L), as were the MIC ranges (0.064 – 1.0 mg/L). For ceftazidime, the MIC range for *S. oralis* was 0.25 – 128 mg/L compared with the range for *S. mitis* of 0.125 – ≥ 256 mg/L with geometric mean MICs of 5.3 and 7.2 mg/L respectively. For cefaclor the respective ranges were 0.25 - ≥ 256 mg/L versus 0.5 - ≥ 256 mg/L with geometric mean MICs of 23 and 15.4 mg/L. The vancomycin geometric mean MICs were identical (0.9 mg/L) with a range of 0.5 – 2.0 mg/L for *S. oralis* and a range of 0.5 – 1.0 mg/L for *S. mitis*.

Table 6.4 Geometric mean MICs of six antibiotics against 48 isolates of *S. oralis* and 19 isolates of *S. mitis*.

Antibiotic	**Geometric mean MIC (mg/L) against *S. oralis***	**Geometric mean MIC (mg/L) against *S. mitis***
Ceftazidime	5.3	7.2
Piperacillin/tazobactam	1.3	1.2
Meropenem	0.2	0.2
Penicillin	0.5	0.5
Cefaclor	23.0	15.4
Vancomycin	0.9	0.9

Larger sample sizes, particularly of *S. mitis* would have yielded more information, however these results suggest that identification of an isolate of viridans streptococcus as either *S. oralis* or *S. mitis* does not predict antibiotic susceptibility, and although *S. oralis* may appear less susceptible to cefaclor than *S. mitis* the difference in geometric mean MICs at these elevated levels is probably not clinically relevant, as the results suggest that this antibiotic would be ineffective against the majority of isolates of viridans streptococci tested.

6.4 Susceptibility of 76 isolates of viridans streptococci to cotrimoxazole

As detailed in Section 5.11.7, cotrimoxazole prophylaxis was administered to the majority of patients (85%) in this study. The exceptions were patients number: 3,10, 15, 26, 33, 51, 53 and 54. Susceptibility to this agent, of all isolates of viridans streptococci isolated from blood culture was determined using the Stokes' disc diffusion method. In total, 74% (56/76) of isolates were resistant to cotrimoxazole. Other investigators have also reported high rates of resistance to this antibiotic amongst viridans streptococci (Cohen *et al.*, 1983; Wisplinghoff *et al.*, 1999).

6. 5 Interpretation of *in vitro* sensitivity test results

The purpose of *in-vitro* sensitivity testing is to provide an indication of an organism's susceptibility to a particular antibiotic (*i.e.* whether it is sensitive, intermediately susceptible or resistant) to guide antimicrobial therapy. However, *in vivo*, there are considerable individual variations in antibiotic pharmacokinetics in health and disease. Immunocompetence of the patient may influence response to infection, such that an organism deemed sensitive to an antibiotic may be more easily eradicated in a healthy individual than in one who is profoundly neutropenic. The use of combination antibiotic therapy may further influence clinical response. The rationale for using a β-lactam antibiotic plus an aminoglycoside is based on the

argument that synergy will result in enhanced bacterial killing (Eliopoulos & Eliopoulos, 1988).

Antibiotic susceptibility reference points, such as MIC breakpoints provide guidance on the suitability of an antibiotic against a particular organism. However it must be appreciated that the MIC itself has the disadvantage of being a static end-point response to a defined concentration of antibiotic, dissimilar to conditions found *in vivo*. Differing techniques are used to determine antimicrobial susceptibility in different parts of the world and differing breakpoints are used (British Society for Antimicrobial Therapy, 1991 and 1996; National Committee for Clinical and Laboratory Standards, 1998). The following section will discuss the antibiotic MIC results against viridans streptococci relative to the breakpoint recommendations of the British Society for Antimicrobial Chemotherapy (BSAC).

The BSAC does not provide breakpoint references specifically for viridans streptococci (except for penicillin – in endocarditis (see below)), therefore those for *Streptococcus* spp. in general have been utilized (British Society for Antimicrobial Therapy, 1991 and 1996). The BSAC MIC breakpoint for both ceftazidime and piperacillin/tazobactam against *Streptococcus* spp. is 2 mg/L, while that for cefaclor is 1 mg/L and that for meropenem is 4 mg/L.

For penicillin against *Streptococcus* spp. the recommended breakpoint is 0.12 mg/L, and for cases of viridans streptococcal endocarditis, the Endocarditis Working Party of the BSAC (1998) recommends the penicillin MIC breakpoint of 0.1 mg/L. The MIC breakpoint for vancomycin against *Streptococcus* spp. is 4 mg/L.

Applying these interpretive criteria to the MIC results of this study provided the information presented in Table 6.5.

Table 6.5 Susceptibility of 76 isolates of viridans streptococci to 6 antibiotics according to MIC breakpoints recommended by the BSAC.

Antibiotic	**Number (%) of susceptible isolates**
Ceftazidime	35 (46)
Piperacillin/tazobactam	40 (53)
Meropenem	76 (100)
Penicillin	32 (42)
Cefaclor	19 (25)
Vancomycin	76 (100)

Using BSAC MIC breakpoint guidelines, all isolates of viridans streptococci were susceptible to meropenem and vancomycin (Tables 6.5). As expected from the MIC distribution results (Table 6.1) and geometric mean MIC results (Table 6.2), susceptibility rates to cefaclor were very low at only 25% (Table 6.5).

6.6 Interpretation of susceptibility of viridans streptococci to antibiotics using the Stokes' disc diffusion method

The relatively simple technique used in disc diffusion methods involves placing antimicrobial impregnated discs onto the surface of agar after inoculation of an organism. Antimicrobial activity is indicated by inhibition of bacterial growth around the disc. Although this may be a straightforward test, it is important that it is performed accurately with the correct inoculum and incubation conditions and interpreted with care, because these results often dictate choice of antimicrobial agent.

At the commencement of the present study, the disc diffusion method used in the clinical microbiology laboratory at RHSC, as in the majority of laboratories in the U.K. was that of Stokes' (Stokes & Ridgway, 1980). This method allowed each individual isolate to be compared with a sensitive control organism which was subjected to the same technical conditions of medium, incubation time, atmosphere, temperature and disc content. As control organisms were adjacent on the same plate the difference between respective zone sizes could be measured directly.

Antibiotic susceptibilities of all isolates of viridans streptococci from blood cultures were determined using the Stokes' method with *S. aureus* NCTC 6571 as control organism (Chapter 4). The proportions of total isolates determined as susceptible to each antibiotic using this technique are presented in Table 6.6.

Table 6.6 Susceptibility of 76 isolates of viridans streptococci to six antibiotics using the Stokes' disc diffusion method.

Antibiotic	**Code & concentration**	**Number (%) of susceptible isolates**
Ceftazidime	CAZ (30 µg)	45 (59)
Piperacillin/tazobactam	TZP (75 + 10 µg)	43 (57)
Meropenem	MEM (10 µg)	76 (100)
Penicillin	P (1 unit)	32 (42)
Cefaclor	CEC (30 µg)	31 (41)
Vancomycin	VA (30 µg)	76 (100)

Comparison of these results with the MIC results of Table 6.5 indicated that the Stokes' disc diffusion method corresponded exactly with results using BSAC MIC breakpoints for meropenem, penicillin and vancomycin. High levels of agreement were obtained for piperacillin/tazobactam.

For both ceftazidime and cefaclor, the proportion of susceptible isolates determined by disc diffusion exceeded those determined by the MIC method (59% versus 46% and 41% versus 25% respectively). For ceftazidime this difference represented 10 isolates of viridans streptococci and for cefaclor 12 isolates. The disagreements between MICs and disc tests for these two antibiotics and for piperacillin/tazobactam are detailed in Table 6.7.

Table 6.7 MIC values at which discordance between the MIC method and Stokes' disc diffusion method occurred, with frequency

Antibiotic	**MIC values (frequency of discordant results)**
Ceftazidime	4 mg/L (x 4) 8 mg/L (x 4) 16 mg/L (x 2)
Cefaclor	2 mg/L (x 9) 4 mg/L (x 3)
Piperacillin/tazobactam	4mg/L (x 3)

An unacceptably high proportion of isolates of viridans streptococci, determined as sensitive to cefaclor by disc diffusion tests were interpreted as resistant by MIC (*n* = 12 (16%)). The majority of these isolates (Table 6.7) possessed cefaclor MICs of 2 mg/L - one doubling dilution above the MIC breakpoint of 1 mg/L and the remainder had cefaclor MICs of 4mg/L.

However, the situation with ceftazidime is of greater concern. Four isolates termed sensitive by disc diffusion possessed ceftazidime MICs one doubling dilution above the breakpoint, 4 had MICs two doubling dilutions above and 2 had MICs three doubling dilutions above. At MICs of 16 mg/L the activity of ceftazidime would

certainly be sub-optimal, and reporting an organism with such MICs as sensitive by disc testing may adversely affect patient outcome.

Correlation between MIC and disc test results for piperacillin/tazobactam was much better. There were only 3 discordant results, all occurring at the MIC value of 4 mg/L. This level of discordance is less significant clinically than that described for ceftazidime, as considerable activity would still be expected against organisms with this MIC.

The generally good correlation between the MIC determinations and disc tests for piperacillin/tazobactam were somewhat unexpected in view of the high concentration of the TZP 75 + 10 µg disc. Disc tests were also performed using piperacillin discs at two different strengths: PRL 75 µg and PRL 30µg. As expected, the results using PRL 75 µg discs were superimposable on those using TZP 75 + 10 µg, because the addition of tazobactam as a β-lactamase inhibitor does not increase the activity of piperacillin against viridans streptococci. The use of PRL 30 µg discs made no difference to the interpreted results, however reduced susceptibility of viridans streptococci could be more readily detected by eye using this disc than using the PRL 75 µg or TZP 75 + 10 µg discs.

6.7 Antimicrobial susceptibility of blood culture isolates of viridans streptococci before and after a change in empirical antibiotic therapy for episodes of febrile neutropenia

As the study progressed into 1997, it appeared that the β-lactam MICs of isolates of viridans streptococci from blood cultures from around that time, tended to be lower than those of viridans streptococci from blood cultures from the beginning of the study. A possible temporal association with this trend, was the change of empirical

antibiotic therapy (in Summer 1996), from ceftazidime plus amikacin to piperacillin/tazobactam plus amikacin. An investigation was then conducted to determine whether or not there existed a statistically significant difference in antibiotic MICs for organisms isolated before and after this change. First, the *in vitro* antimicrobial susceptibilities of viridans streptococci isolated from blood cultures from the beginning of December 1994 to the end of June 1996 (period 1), were compared with those of strains isolated after the change, until the end of December 2000 (period 2). During period 1, a time interval of 19 months, 31 isolates of viridans streptococci were collected from blood culture from 28 episodes of bacteraemia. The MIC distributions for these organisms are shown in Table 6.8

Table 6.8 MIC distributions for viridans streptococci isolated during period 1 (ceftazidime + amikacin as empirical therapy) *n* = 31

Antibiotic	**Cumulative % of strains with stated MIC (mg/L)**												
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	**32**	**64**	**128**	**≥256**
Ceftazidime	0	3	10	10	29	39	42	42	52	71	84	94	100
Piperacillin/ tazobactam	13	19	23	32	39	45	45	71	97	100	-	-	-
Meropenem	29	42	52	90	100	-	-	-	-	-	-	-	-
Penicillin	23	32	42	42	55	81	90	100	-	-	-	-	-
Cefaclor	0	0	0	6	13	29	29	36	42	42	42	45	100
Vancomycin	0	0	0	23	100	-	-	-	-	-	-	-	-

During period 2, a time interval of 54 months, 45 isolates of viridans streptococci were collected from 41 episodes of bacteraemia, with MIC distributions shown in Table 6.9.

Table 6.9 MIC distributions for viridans streptococci isolated during period 2 (piperacillin/tazobactam + amikacin as empirical therapy) *n* = 45

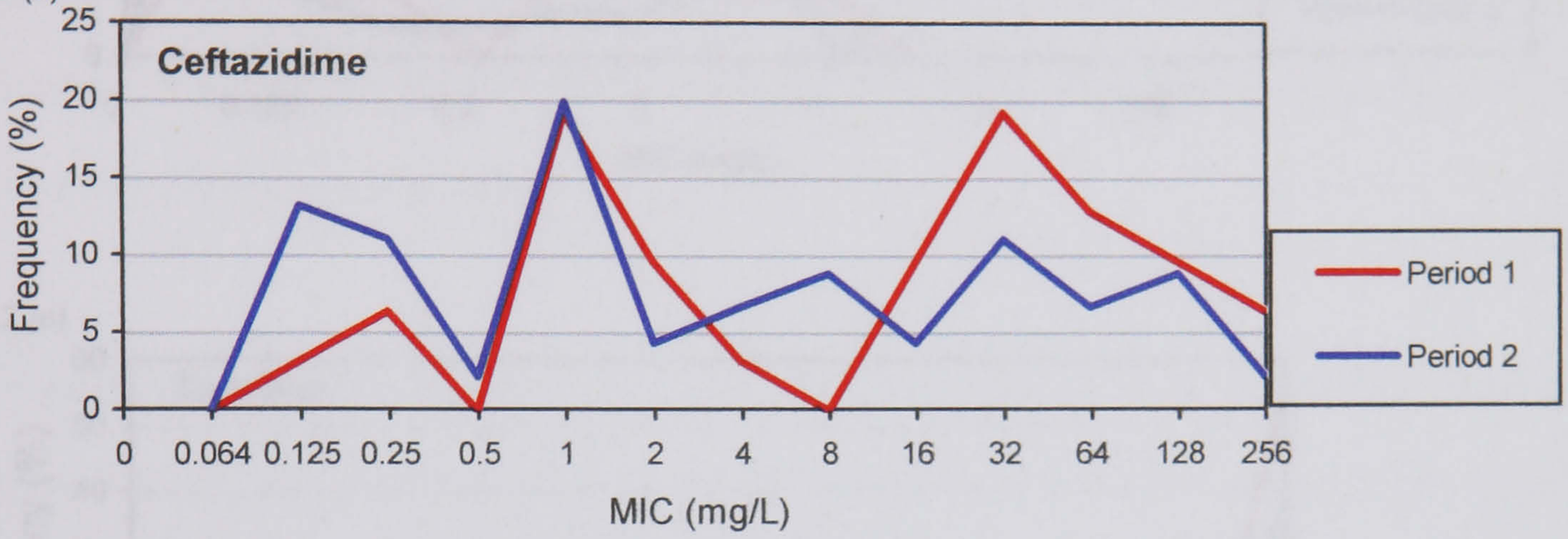
Antibiotic	Cumulative % of strains with stated MIC (mg/L)												
	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	≥256
Ceftazidime	0	13	24	27	47	51	58	67	71	82	89	98	100
Piperacillin/ tazobactam	24	33	44	49	56	58	71	91	98	100	-	-	-
Meropenem	51	60	69	78	100	-	-	-	-	-	-	-	-
Penicillin	40	49	52	58	64	71	98	100	-	-	-	-	-
Cefaclor	0	0	4	9	33	42	51	53	56	56	60	62	100
Vancomycin	0	0	0	13	96	100	-	-	-	-	-	-	-

Comparison of the results from these tables demonstrated that between periods 1 and 2, the MIC_{50} values for ceftazidime and piperacillin/tazobactam decreased eight-fold, from 16 mg/L to 2 mg/L and 8 mg/L to 1 mg/L respectively. The MIC_{50} values for penicillin and meropenem decreased four-fold, from 1 mg/L to 0.25 mg/L and 0.25 mg/L to 0.064 mg/L. The MIC_{50} for cefaclor decreased from ≥ 256 mg/L to 4 mg/L.

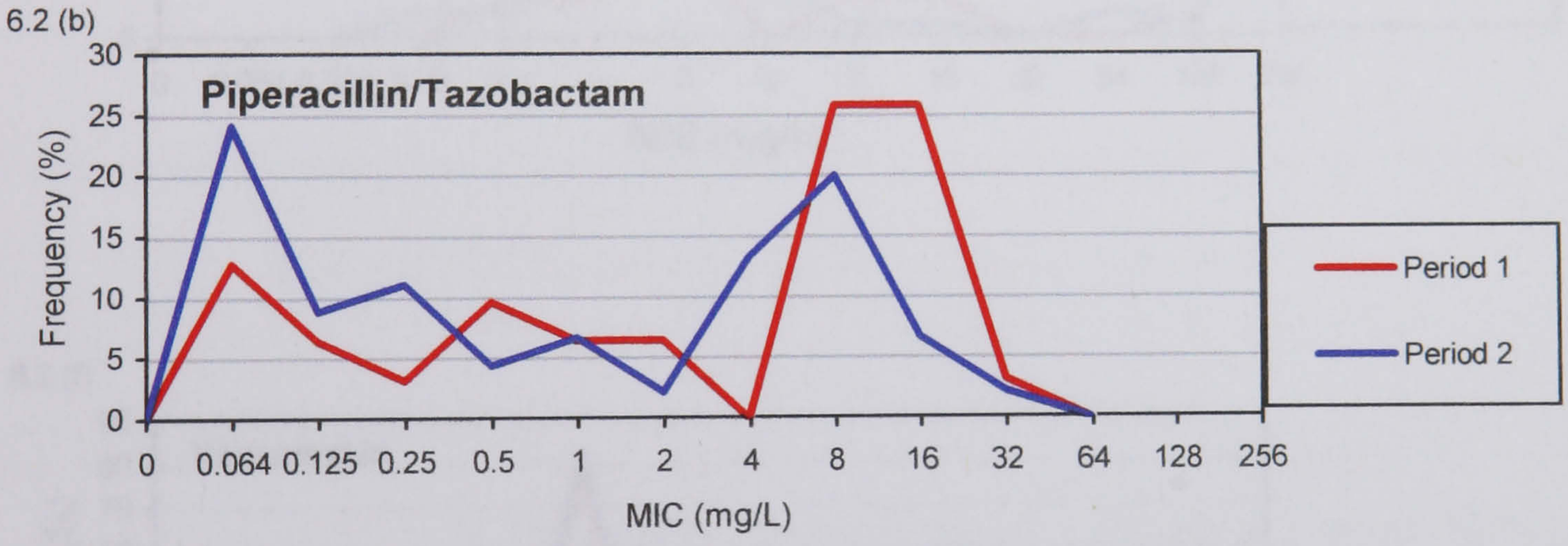
The shifts in MIC distributions for these antibiotics are presented graphically as frequency (%) of isolates with stated MICs (Figure 6.2 (a)–(e)), illustrating the increased frequency of isolates with lower β-lactam MICs when empirical therapy was changed to piperacillin/tazobactam plus amikacin.

Figure 6.2 MIC distributions of 31 isolates of viridans streptococci from blood culture prior to a change in empirical antibiotic therapy, compared with 45 isolates following this change

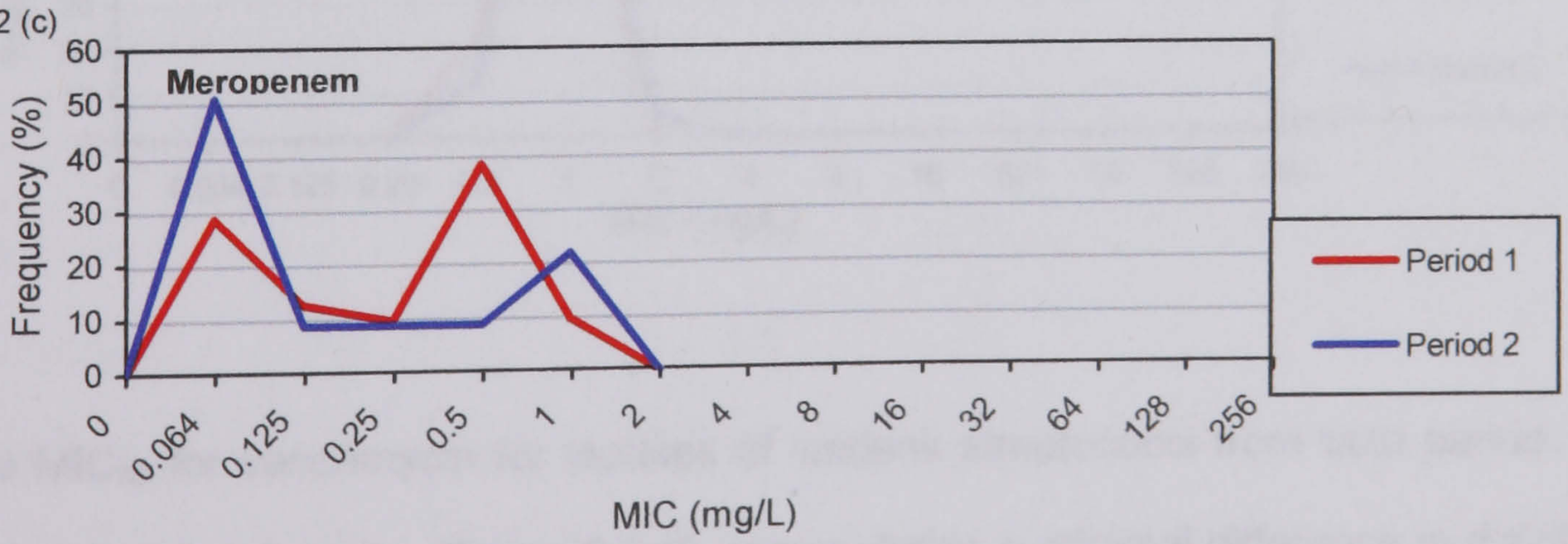
6.2 (a)



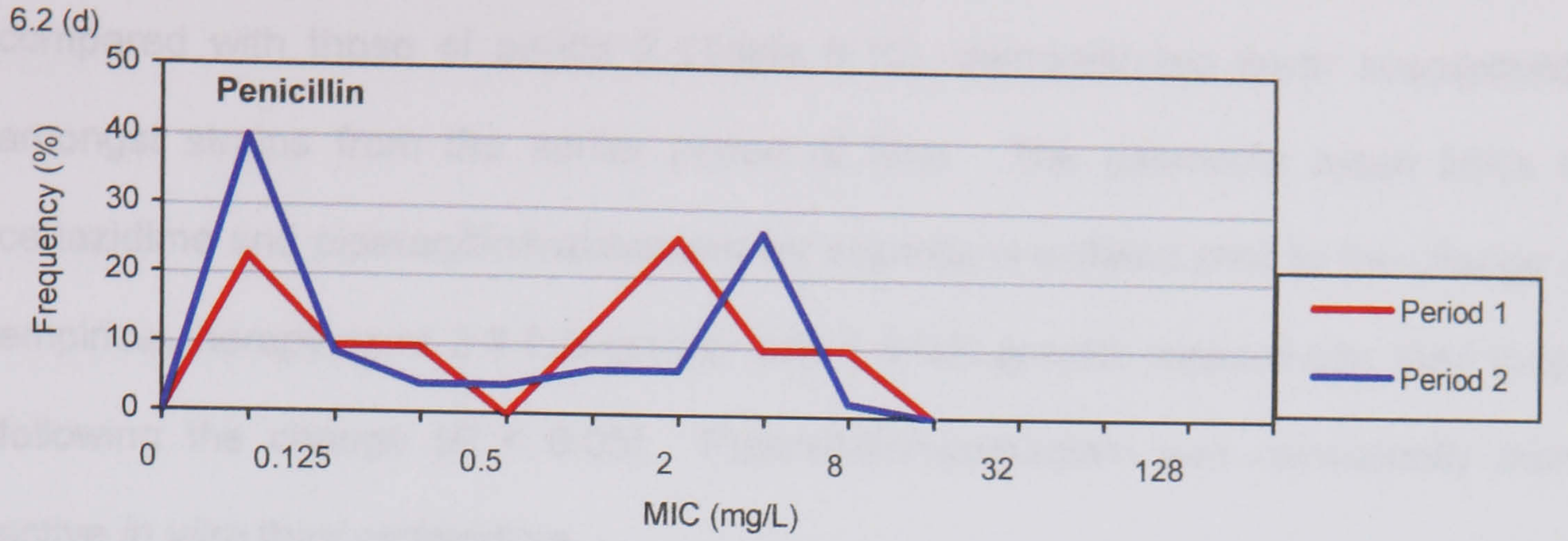
6.2 (b)



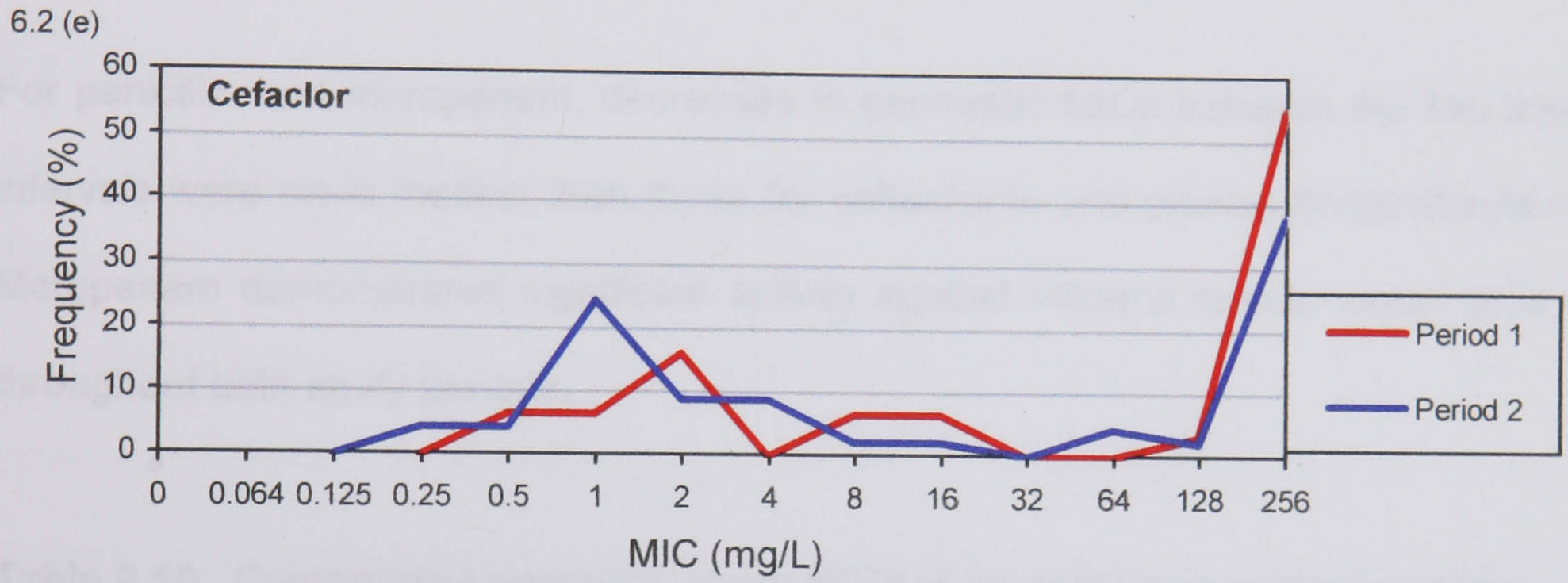
6.2 (c)



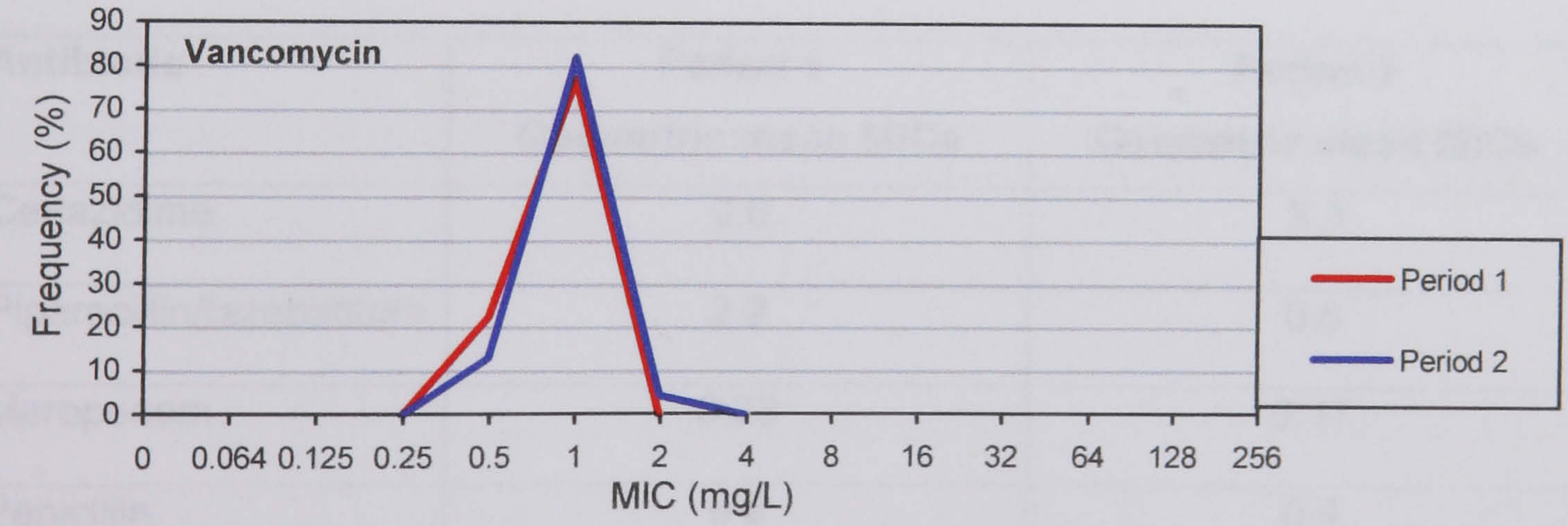
6.2 (d)



6.2 (e)



6.2 (f)



The MIC_{50} for vancomycin for isolates of viridans streptococci from both period 1 and period 2 was 1.0 mg/L. Figure 6.2 (f) demonstrates a minimal difference in distribution of vancomycin MICs between the two time intervals, indicating that change in empirical therapy had no influence on the susceptibility of viridans streptococci to this antibiotic.

For β-lactam agents, the geometric mean MICs for isolates collected during period 1 compared with those of period 2 (Table 6.10), demonstrated lower susceptibility amongst strains from the earlier period of time. The geometric mean MICs of ceftazidime and piperacillin/tazobactam for organisms isolated prior to the change in empirical therapy were 2.9-fold greater and 2.8-fold greater respectively, than those following the change ($P < 0.05$). Piperacillin/tazobactam was consistently more active *in vitro* than ceftazidime.

For penicillin and meropenem, decreases in geometric MICs between the two time intervals were more modest then those for ceftazidime and piperacillin/tazobactam. Meropenem demonstrated significant activity against viridans streptococcal strains throughout both study periods.

Table 6.10 Comparative geometric mean MICs of six antibiotics against viridans streptococci from blood cultures – periods 1 and 2

Antibiotic	**Period 1** **Geometric mean MICs**	**Period 2** **Geometric mean MICs**
Ceftazidime	9.6	3.3
Piperacillin/tazobactam	2.2	0.8
Meropenem	0.23	0.17
Penicillin	0.6	0.4
Cefaclor	35.8	13.3
Vancomycin	0.9	0.9

Period 1: ceftazidime + amikacin as empirical therapy
Period 2: piperacillin/tazobactam + amikacin as empirical therapy

With the demonstration that geometric mean MICs of all β-lactam agents tested against viridans streptococci isolated from blood culture during period 1 were higher than for those for the isolates of period 2, with MICs for ceftazidime and piperacillin/tazobactam significantly so, an investigation into possible contributory factors such as antimicrobial prophylaxis and prior antibiotic therapy, was conducted.

6.7.1 Comparison of study groups – period 1 versus period 2

The following data were collected for each patient with viridans streptococcal bacteraemia during periods 1 or 2: age, gender, underlying disease (as presented in Table 5.1), the presence or absence of a central venous catheter, neutropenia, mucocutaneous lesions, chemotherapy regimen administered, the use of antibacterial prophylaxis, and previous courses of empirical antibiotic therapy with agents used. During period 1, empirical therapy consisted of ceftazidime (50 mg/kg every 8 hours) plus amikacin (7.5 mg/kg every 12 hours). During period 2, empirical antibiotic therapy consisted of piperacillin/ tazobactam (90 mg/kg every 8 hours) plus amikacin (7.5 mg/kg every 12 hours). All antibiotics were administered by intravenous injection. The first patient to receive piperacillin/tazobactam plus amikacin was patient number 23 of Table 5.1.

The mean age of the 22 patients who developed viridans streptococcal bacteraemia during period 1 was 6.0 years (range 3 months - 14 years), and of the 32 patients of period 2 was 7.7 years (range 1 year – 14 years). The male to female ratio for period 1 was 2.1:1 and for period 2 was 1.1:1. All patients from period 1 had central venous catheters and only one child from Period 2 did not. Management of central venous lines remained essentially unchanged throughout the entire study period, as did the mouth care protocol (Appendix II). During period 1, episodes of viridans streptococcal bacteraemia occurred in patients with leukaemia on 23 occasions (=

82% of total episodes during period 1) compared with 36 occasions (= 88% of episodes) during period 2. During period 1, viridans streptococcal bacteraemia was associated with the presence of mucosal damage in 25 episodes (= 89% of total for period 1) and during period 2, with 33 episodes (= 80.5% of total episodes for period 2) ($P > 0.25$). Although a wide variety of chemotherapeutic regimens were employed during periods 1 and 2, there was no statistically significant difference between the proportion of episodes of viridans streptococcal bacteraemia associated with high-dose or intermediate-high-dose cytosine arabinoside or allogeneic BMT *i.e.* the therapies most associated with mucositis (54% of episodes during period 1, versus 42% during period 2, $P > 0.25$). During period 1, neutropenia was associated with 96.4% of total episodes and during period 2, with 95.1%. Throughout both time intervals, cotrimoxazole was the sole agent of antibiotic prophylaxis, associated with 93% of episodes in period 1 and 88% in period 2. Resistance rates to this agent, during the two time intervals, were 74% and 73% respectively. Prior empirical antibiotic therapy of ceftazidime + amikacin had been administered in 26 of the cases of period 1 (= 93% of total in period 1), and prior empirical antibiotic therapy of piperacillin/ tazobactam + amikacin had been administered in 35 of the cases of period 2 (85% of total in period 2). The first two patients receiving empirical therapy during period 2 had received recent prior empirical therapy with ceftazidime, which may have influenced susceptibility patterns of viridans streptococci isolated after the change. However the date of change was considered the most appropriate point of comparison of the two populations and any effects of prior therapy with ceftazidime on the second group would cease to exist with time.

The above investigations demonstrated that there was no statistical difference between the patient groups with viridans streptococcal bacteraemia during period 1 compared with period 2 with regard to haematological versus solid malignancy, the presence of central lines, mucosal lesions or neutropenia, therapy with high- or

intermediate-high-dose cytosine arabinoside or allogeneic BMT, frequency of use of antimicrobial prophylaxis or resistance rates to cotrimoxazole, previous receipt of empirical antibiotic therapy or time intervals between administration of antibiotics. A major difference between the two patient groups was the change of β-lactam agent for empirical antibiotic therapy.

A second observation - that fewer cases of viridans streptococcal bacteraemia occurred during period 2 than during period 1 had also been made. The prevalence of viridans streptococcal bacteraemia as proportion of total microbiologically-documented episodes for period 1 was 17% versus 8% for period 2 ($P < 0.001$), and as proportion of all febrile episodes was 4.6% for period 1 versus 2.2% for period 2 ($P < 0.005$). Could there be any link between *in vitro* susceptibility to empirical antibiotics and development of infection?

To explore this possibility, parameters for <u>all</u> febrile patients in the haematology/ oncology unit during periods 1 and 2 should be compared in a retrospective sequential analysis. However, due, in part, to the very nature of such analyses, it is sometimes difficult or impossible to retrieve all the relevant information. Another possible complicating factor, in this instance, is the markedly different time span of periods 1 and 2. This, in turn, was due to the natural progression of the study itself; at the outset, one could not predict that such a comparison would be relevant. It is generally preferable to conduct this type of study with both time intervals of similar or identical duration. However, could such an analysis still be performed? Episodes of viridans streptococcal bacteraemia during 1993 and January–November 1994, prior to the commencement of this study were presented in Figure 5.1 for reference. Inclusion of specific details of all of these patients and episodes of infection would certainly produce a time interval ('extended period 1') with a duration closer to that of period 2. However, several complicating variables would also be introduced, due to

the multiple different empirical antibiotic regimens which were administered throughout 1993; ceftazidime + amikacin or meropenem for patients on IATCG/EORTC Trial IX and piperacillin + amikacin for non-trial patients. Consequently, this approach was not pursued.

In early 1994, by which time participation in IATCG/EORTC Trial IX was complete, RHSC standard empirical therapy was changed to ceftazidime plus amikacin. To compare prevalence of viridans streptococcal bacteraemia during two time intervals of acceptable and equivalent lengths, before and after change of empirical antibiotic therapy, figures for January 1994 - June 1996 inclusive, versus those for July 1996 – December 1998 inclusive were used. Between these two time intervals, episodes of viridans streptococcal bacteraemia as proportion of total febrile episodes decreased significantly from 4.4% to 2.2% ($P < 0.05$). This finding concurred with the decrease in prevalence between periods 1 and 2, of unequal duration, described previously.

Factors that may have influenced the development of bacteraemia, such as mouth care protocols and patients' line management, as discussed earlier, remained essentially unchanged throughout the entire period of this study. Although figures for total patients receiving antibiotic prophylaxis were not available, guidelines described previously would have determined which patients groups received such treatment, *i.e.* cotrimoxazole prophylaxis for high-risk groups only. No other form of antibiotic prophylaxis was generally used in the total patient population.

During period 1 the total number of blood cultures collected from febrile haematology/oncology patients in the unit was 1169 compared with 4539 during period 2. Although different numbers of blood cultures were collected each month, the average monthly figure for period 1 would be 62 and for period 2 would be 84.

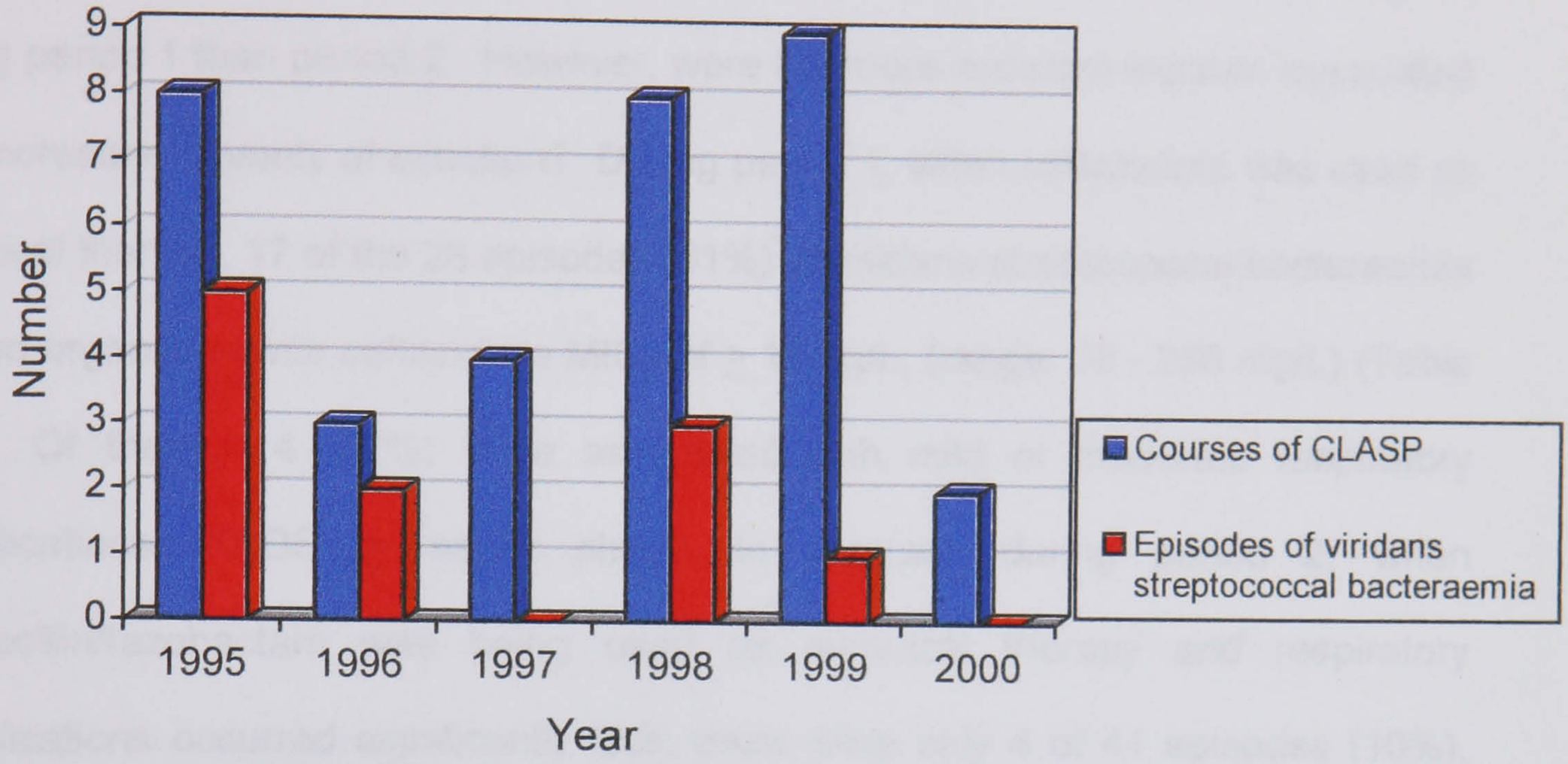
Figures for total admissions, or those for admissions of patients with specific malignancies during periods 1 and 2 were not available, however the general annual distribution of malignancies was not markedly different from one year of the study to another.

The same chemotherapy regimens were used in the haematology/oncology unit during periods 1 and 2, with the exception of the new MRC ALL treatment protocol, introduced at the beginning of 2000. Chemotherapy regimens commonly associated with mild to severe mucositis were used throughout both periods. However, the frequency of administration of courses of the various chemotherapeutic regimens, varied, to some extent, as one would expect. For this reason, to investigate whether the number of courses of intensive chemotherapy per time interval was related to the prevalence of viridans streptococcal bacteraemia, and to compare like with like, analysis of a distinct group - all patients in the haematology/ oncology unit who received the CLASP regimen was performed – as described in the following section.

6.7.2 The CLASP regimen and viridans streptococcal bacteraemia - period 1 versus period 2

All patients who received CLASP chemotherapy between the start of December 1994 and the end of December 2000 were selected because of all regimens administered, CLASP was associated with the greatest number of cases of viridans streptococcal bacteraemia and would therefore yield the largest sample sizes for statistical analysis. A total of 34 courses of CLASP were administered over the six years of this study. Viridans streptococcal bacteraemia developed following 11 of these. Comparison of cases throughout this time (Figure 6.3), revealed that there were more cases of viridans streptococcal bacteraemia following CLASP chemotherapy during the early years of the study than during the later years.

Figure 6.3 Courses of CLASP chemotherapy administered throughout the study period and episodes of viridans streptococcal bacteraemia



In the context of periods 1 and 2 of the earlier analyses, there occurred 7 cases of viridans streptococcal bacteraemia following CLASP chemotherapy during the former period, compared with 4 cases during the latter. The 7 episodes of viridans streptococcal bacteraemia of period 1 represented 64% of the 11 courses of CLASP chemotherapy administered during that time, compared with the 4 episodes of period 2 which represented 17% of 23 courses of CLASP. This difference is statistically significant ($P < 0.01$ Fisher's exact test).

6.7.3 Antibiotic susceptibility and severity of infection

During period 1, complications associated with viridans streptococcal bacteraemia occurred more frequently than during period 2, with half of all cases (14/28) during period 1 developing respiratory complications compared to 17% (7/41) of cases during period 2 (difference statistically significant, ($P< 0.005$). During period 1, 21% of total episodes (6/28) were temporally associated with ARDS +/- septic shock.

septic shock and only one of a total of 41 episodes of viridans streptococcal infection was temporally associated with ARDS ($P< 0.025$).

From previous results (Section 6.7), it was obvious that MICs generally, were higher during period 1 than period 2. However, were the more resistant isolates associated with increased severity of infection? During period 1, when ceftazidime was used as empirical therapy, 17 of the 28 episodes (61%) of viridans streptococcal bacteraemia yielded organisms with ceftazidime MICs of $\geq$ 16mg/L, (range: 16 - 256 mg/L) (Table 6.11). Of these, 14 (82%) were associated with mild or moderate respiratory complications, ARDS or septic shock. In contrast, during period 2, when piperacillin/tazobactam was being used as empirical therapy and respiratory complications occurred significantly less, there were only 4 of 41 episodes (10%), which featured isolates with piperacillin/tazobactam MICs of $\geq$ 16 mg/L (range: 16 – 32 mg/L), of which one was associated with mild respiratory complications (Table 6.11). The significance of these findings in relation to those preceding, will be discussed in Chapter 8.

Table 6.11 Episodes associated with isolates of viridans streptococci from blood culture with MICs of ≥ 16 mg/L for β -lactam agent being used as empirical therapy and severity of symptoms - periods 1 and 2

	Period 1 **Ceftazidime + amikacin as empirical therapy**	**Period 2** **Piperacillin/tazobactam + amikacin as empirical therapy**
Episodes of bacteraemia caused by viridans streptococci with MICs ≥ 16 mg/L for β -lactam agent	17 (61% of total episodes)	4 (10% of total episodes) ($P < 0.001$, X^2 test)
Proportion of isolates with MICs ≥ 16 mg/L associated with cases with some degree of respiratory compromise	14/17 (82%)	1/4 (25%) ($P = 0.05$, Fisher's Exact test)
Proportion of isolates with MICs ≥ 16 mg/L associated with cases with ARDS +/- septic shock	5/17 (29%) (ARDS + septic shock:4/17 ARDS alone: 1/17)	0

6.8 Susceptibility of viridans streptococci to more recently introduced antibiotics

Newer agents tested against viridans streptococci as part of the present study comprised cefpirome, quinupristin/dalfopristin and linezolid. Cefpirome is a fourth generation cephalosporin reported to possess significant activity against Gram-positive, as well as against Gram-negative bacteria (Spencer, 1993; Wiseman & Lamb, 1997; Fernandes *et al.*, 1998; Glauser, 1998). Quinupristin/dalfopristin is a streptogramin antibiotic complex of two synergistic components, and linezolid belongs to a new class of antimicrobial agents, the oxazolidinones.

Quinupristin/dalfopristin and linezolid were investigational antibiotics at the time of commencement of the present study. Limited reports available at that time, described the promising *in vitro* activity of quinupristin/dalfopristin against Gram-positive bacteria (Brumfitt, Hamilton-Hiller & Shah, 1992; Appelbaum, Spangler & Jacobs, 1993). Early reports of linezolid's *in vitro* activity were published during the second half of the 1990s (Zurenko *et al.*, 1996; Jones, Johnson & Erwin, 1996; Shinabarger *et al.*, 1997). Of particular note was the activity of both quinupristin/dalfopristin and linezolid against multiply-resistant Gram-positive bacteria. The majority of reports at this time concentrated on antimicrobial activity against *Staphylococcus* spp., *Enterococcus* spp. and *S. pneumoniae* (Johnson *et al.*, 1995; Mulazimoglu, Drenning & Yu, 1996; Pepper & Bouanchaud, 1996). Information on *in vitro* activity against viridans streptococci was lacking.

The MIC distributions and geometric mean MICs for these agents against the 76 isolates of viridans streptococci from the present study are presented in Table 6.12.

Table 6.12 MIC distributions for cefpirome, quinupristin/dalfopristin and linezolid against 76 isolates of viridans streptococci - with geometric mean MICs

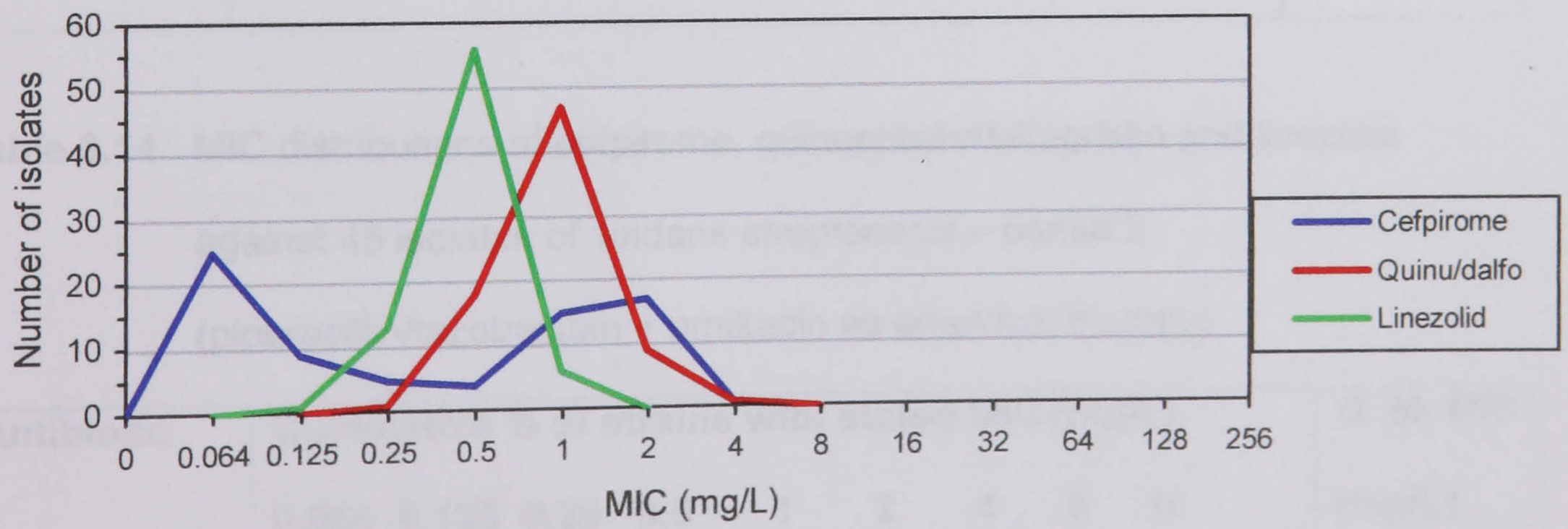
Antibiotic	**Cumulative % of strains with stated MIC (mg/L)**									**G. M. MIC (mg/L)**
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	
Cefpirome	33	45	51	57	76	99	100	-	-	0.33
Quinupristin/ dalfopristin	0	0	1	25	87	99	100	-	-	0.92
Linezolid	0	1	18	92	100	-	-	-	-	0.46

Cefpirome exhibited considerable *in-vitro* activity against these organisms with an MIC range of 0.064 mg/L to 4 mg/L, with MIC_{50} of 0.25 mg/L and MIC_{90} of 2 mg/L. The MIC breakpoint of the BSAC for cefpirome against *Streptococcus* spp. is 2 mg/L.

Only one isolate exceeded this MIC. The distribution of cefpirome MICs was bimodal (Figure 6.4).

The results demonstrated that quinupristin/dalfopristin was also highly active against the isolates of viridans streptococci, with an MIC_{50} of 1 mg/L and MIC_{90} of 2 mg/L. The BSAC MIC breakpoint for quinupristin/dalfopristin against *S. pneumoniae* is 2 mg/L. Only one isolate of viridans streptococcus exceeded this value. (A BSAC MIC breakpoint for *Streptococcus* spp is not available at present.) Figure 6.4 Indicates that the MIC distribution for quinupristin/dalfopristin against viridans streptococci was normal. Linezolid exhibited excellent *in vitro* activity against these organisms (with an MIC_{50} and MIC_{90} of 0.5 mg/L. The BSAC breakpoint for linezolid against *Streptococcus* spp is 2 mg/l, therefore all isolates would be termed sensitive. Geometric mean MICs for these three antibiotics are presented in Table 6.12. The low geometric mean MIC values for all three agents are indicative of their impressive *in vitro* activity.

Figure 6.4 Distribution of MICs for cefpirome, quinupristin/dalfopristin and linezolid against 76 isolates of viridans streptococci



6.8.1 Investigation to determine whether the *in vitro* susceptibility of viridans streptococci to newer antibiotics may be influenced by prior empirical antibiotic therapy

As cefpirome is a β-lactam antibiotic, susceptibility of endogenous organisms, such as viridans streptococci, may be affected by prior therapy with other, less active agents such as ceftazidime. To investigate whether this were the case, sensitivities of blood culture isolates to cefpirome during period 1 of this study were compared with blood culture isolates from period 2. Similar analyses were performed for quinupristin/dalfopristin and linezolid (Tables 6.13 and 6.14).

Table 6.13 MIC distributions of cefpirome, quinupristin/dalfopristin and linezolid against 31 isolates of viridans streptococci – period 1 (ceftazidime + amikacin as empirical therapy)

Antibiotic	**Cumulative % of strains with stated MIC (mg/L)**									**G. M. MIC (mg/L)**
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	
Cefpirome	26	32	42	45	77	97	100	-	-	0.4 (0.44)
Quinupristin/ dalfopristin	0	0	3	23	90	100	-	-	-	0.9 (0.89)
Linezolid	0	3	23	94	100	-	-	-	-	0.4 (0.44)

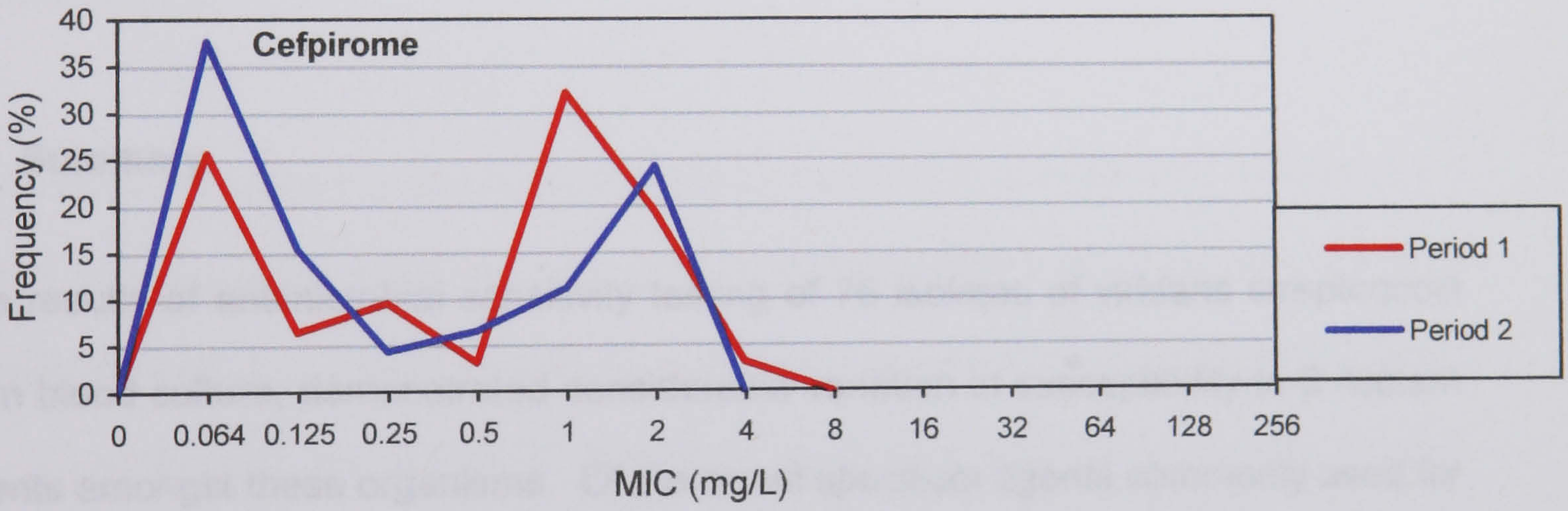
Table 6.14 MIC distributions of cefpirome, quinupristin/dalfopristin and linezolid against 45 isolates of viridans streptococci – period 2 (piperacillin/tazobactam + amikacin as empirical therapy)

Antibiotic	**Cumulative % of strains with stated MIC (mg/L)**									**G. M. MIC (mg/L)**
	0.064	**0.125**	**0.25**	**0.5**	**1**	**2**	**4**	**8**	**16**	
Cefpirome	38	53	58	64	76	100	-	-	-	0.3 (0.27)
Quinupristin/ dalfopristin	0	0	0	27	84	98	100	-	-	0.9 (0.94)
Linezolid	0	0	16	91	100	-	-	-	-	0.5 (0.48)

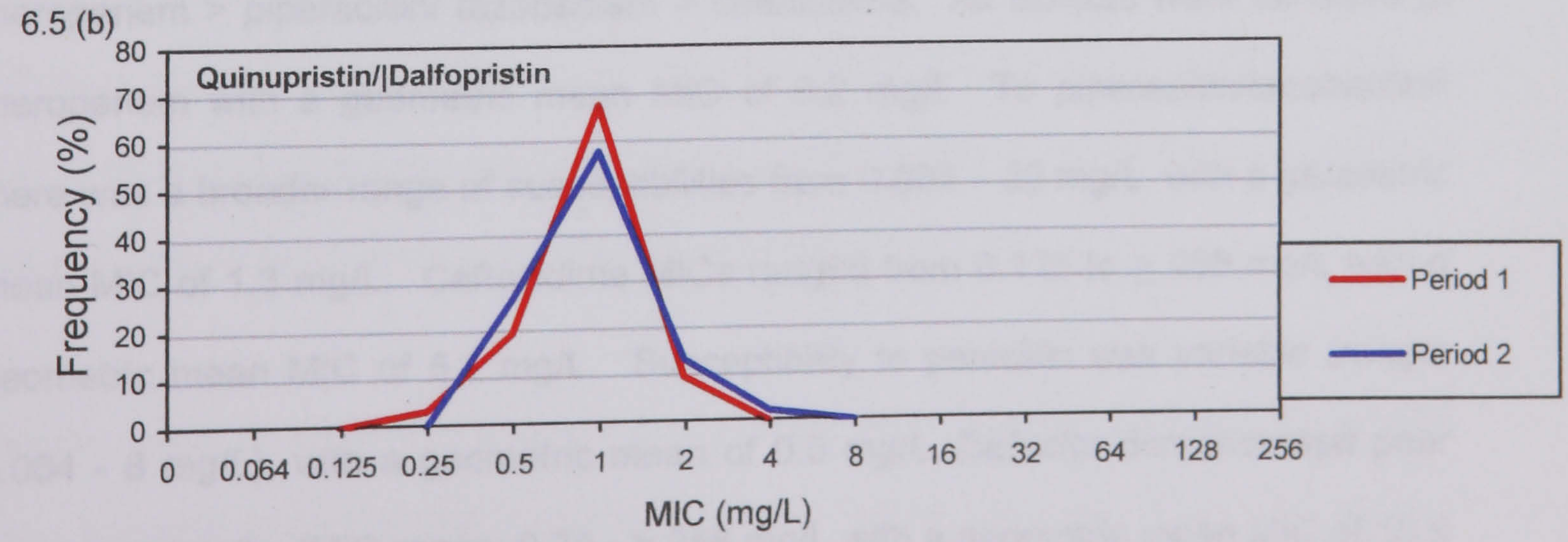
For cefpirome, the MIC_{50} during period 2 was 3 doubling-dilutions less than that for period 1, with the geometric mean MIC for the later period minimally less than that for the earlier period. The shift in MIC distribution is presented in Figure 6.5 (a). For quinupristin/dalfopristin and linezolid, MIC_{50} results were identical for isolates of viridans streptococci isolated during both periods of the study (1 mg/L and 0.5 mg/L respectively) and geometric mean MIC values were almost identical (0.89 mg/L versus 0.94 mg/L and 0.44 mg/L versus 0.48 respectively) (Tables 6.13 and 6.14).

Figure 6.5 Distributions of MICs for cefpirome, quinupristin/dalfopristin and linezolid against 31 isolates of viridans streptococci from blood culture prior to a change in empirical antibiotic therapy, compared with 45 isolates following this change

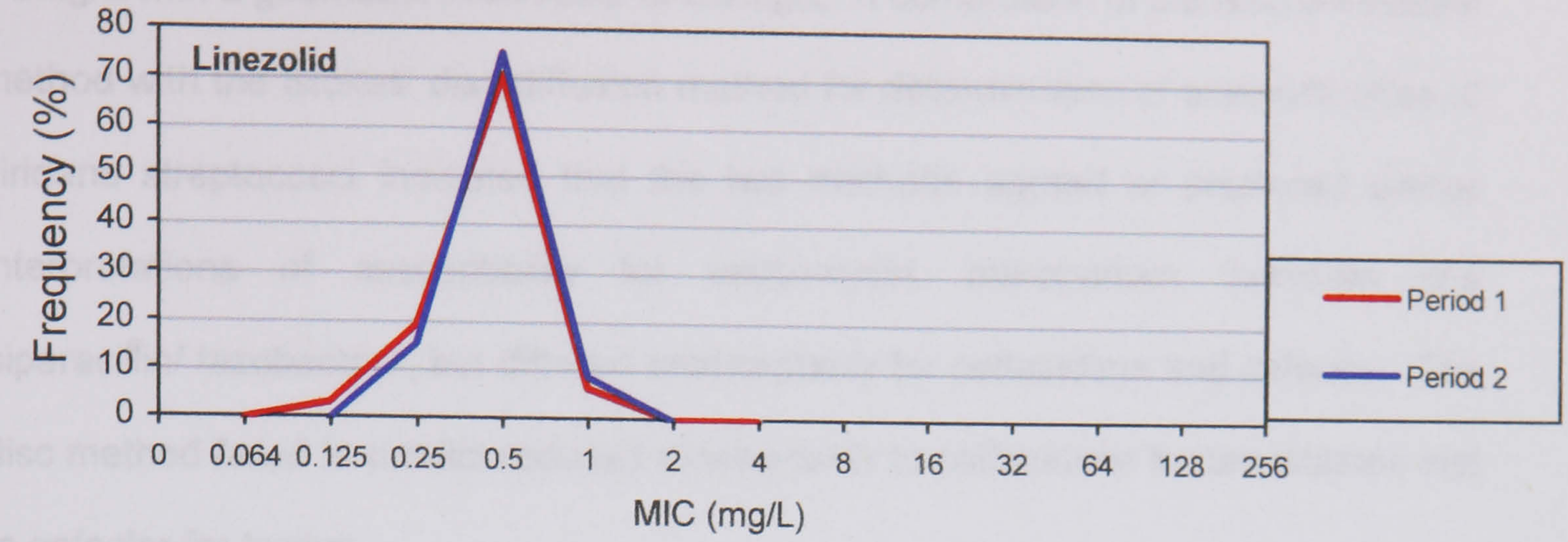
6.5 (a)



6.5 (b)



6.5 (c)



Graphical representation of MIC results for quinupristin/dalfopristin for periods 1 and 2 demonstrate distributions which are almost superimposable - as is also the case for the linezolid. (Figure 6.5 (b) and (c)). Resistance of viridans streptococci to the cell-wall active (β-lactam) agents tested in this study, does not appear to be associated with reduced susceptibility to these two new antibiotics.

6.9 Summary

The results of antimicrobial sensitivity testing of 76 isolates of viridans streptococci from blood culture, demonstrated considerable variation in susceptibility to β-lactam agents amongst these organisms. Of the broad spectrum agents commonly used for first-line empirical therapy of febrile neutropenia, the rank order of activity was meropenem > piperacillin/ tazobactam > ceftazidime. All isolates were sensitive to meropenem with a geometric mean MIC of 0.2 mg/l. To piperacillin/tazobactam there was a broader range of susceptibilities from 0.064 – 32 mg/L, with a geometric mean MIC of 1.3 mg/L. Ceftazidime MICs ranged from 0.125 to ≥ 256 mg/L with a geometric mean MIC of 5.1 mg/L. Susceptibility to penicillin was variable (range: 0.064 - 8 mg/L), with a geometric mean of 0.5 mg/L. Cefaclor demonstrated poor activity generally, (MIC range: 0.25 - ≥ 256 mg/L with a geometric mean MIC of 19.9 mg/L). All isolates of viridans streptococci were sensitive to vancomycin (range: 0.5

– 2mg/L with a geometric mean MIC of 0.9mg/L. A comparison of the MIC breakpoint method with the Stokes' disc diffusion method for determination of susceptibilities of viridans streptococci indicated that the two methods agreed or produced similar interpretations of susceptibility for vancomycin, meropenem, penicillin and piperacillin/ tazobactam, but differed unacceptably for ceftazidime and cefaclor. The disc method failed to predict reduced susceptibility to ceftazidime for ten isolates and to cefaclor for twelve.

The two predominant species represented in this study – *S. oralis* and *S. mitis* displayed an almost identical range of susceptibilities to piperacillin/ tazobactam, penicillin, ceftazidime, cefaclor, meropenem and vancomycin, indicating that identification of either to species level was not useful in predicting antibiotic sensitivity patterns.

This study monitored antibiotic susceptibility of viridans streptococci isolated from blood cultures while empirical antibiotic therapy consisted of (i) ceftazidime plus amikacin (period 1) compared with (ii) piperacillin/tazobactam plus amikacin (period 2). A significantly higher rate of resistance to chosen empirical β-lactam therapy occurred during period 1 than during period 2 ($P < 0.05$). Of note, resistance to all β-lactam antibiotics was higher during period 1 than period 2. The prevalence of viridans streptococcal bacteraemia was also higher during period 1 than period 2 (17% of all culture-proven episodes versus 8% respectively, $P < 0.001$, and 4.6% of all episodes of pyrexia versus 2.2% respectively, $P < 0.005$).

Finally, tests of the newer antimicrobial agents, cefpirome, quinupristin/ dalfopristin and linezolid indicated that these antibiotics possess considerable *in vitro* activity against viridans streptococci.

CHAPTER 7

INVESTIGATION TO DETERMINE THE ORIGIN OF VIRIDANS STREPTOCOCCI CAUSING BACTERAEMIA

INVESTIGATION TO DETERMINE THE ORIGIN OF VIRIDANS STREPTOCOCCI CAUSING BACTERAEMIA

7.1 Introduction

Historically it has been generally accepted that the oral cavity is the most likely source of viridans streptococci causing bacteraemia - for several reasons. Colonization by viridans streptococci is most dense at this site and it is well recognized that viridans streptococcal bacteraemia may develop following dental and oral procedures (Heimdahl *et al.*, 1990; Roberts *et al.*, 1997, Daly *et al.*, 1997). In the context of this study, it would also seem reasonable that in patients with cancer, chemotherapy-induced oral mucositis or other forms of oral compromise, could potentially provide a portal of entry for these organisms into the bloodstream.

An investigation was conducted to determine the distribution of different species of viridans streptococci in the mouths of paediatric patients with cancer. The patients studied comprised those who developed viridans streptococcal bacteraemia. Three cases were selected, and viridans streptococci isolated from both the mouth and blood of these patients were compared by phenotypic and genotypic methods. Finally, the ability of viridans streptococci to colonize tools used for mouth care was investigated. Viridans streptococci from a patient's toothbrush, teeth, oral mucosae and blood culture were compared phenotypically and genotypically to determine whether the toothbrush could potentially act as a vector of infection.

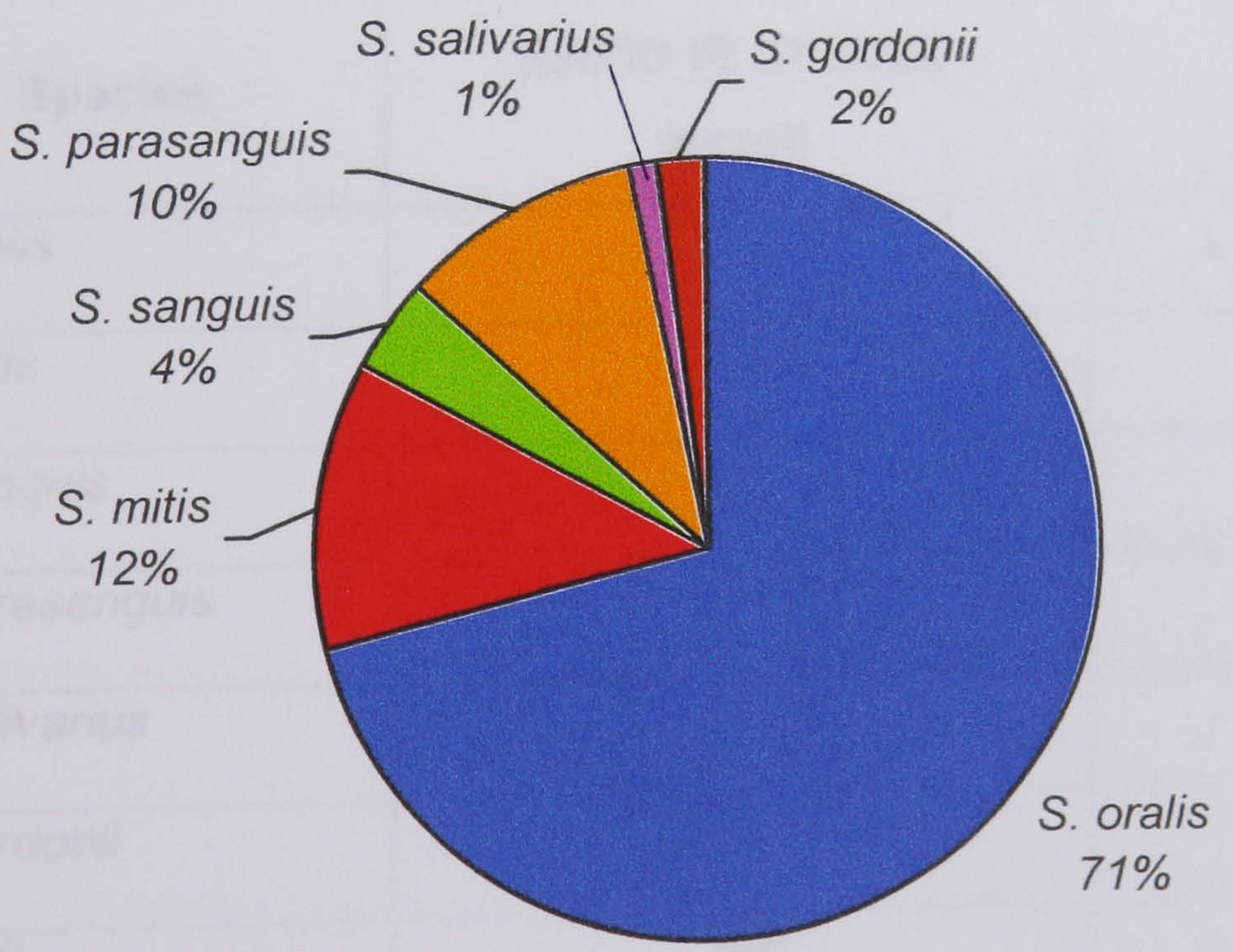
7.2 Distribution of species of viridans streptococci from oral swabs

To obtain information on the distribution of different species of viridans streptococci in the mouths of paediatric patients with cancer, mouth swabs were taken from

cases in this study for the first 4 and a half years – to obtain a sample size of 100 organisms (from 44 patients). For patients with multiple episodes of viridans streptococcal bacteraemia, isolates of viridans streptococci were cultured from one mouth swab only – taken around the time of the first episode.

The range of species of viridans streptococci isolated, is presented in Figure 7.1. *S. oralis* was the predominant species, representing 71% of total isolates. The proportion of *S. mitis*, the next most commonly isolated species was 12% - only slightly greater than that of *S. parasanguis* (10%). The remaining species, *S. sanguis, S. gordonii* and *S. salivarius*, each represented < 5% of total isolates.

Figure 7.1 Viridans streptococcal species isolated from the mouths of paediatric haematology/oncology patients



Viridans streptococci, $n = 100$. Patients, $n = 44$.

7.2.1 Comparison of species from oral swabs with species from blood cultures

The relative proportions of different species of viridans streptococci isolated from the oral cavity with those from blood cultures are presented in Table 7.1. Blood culture isolates were collected over a longer time interval than those from mouth swabs to obtain the largest possible collection. As mentioned earlier, the total number of blood culture isolates was 76 (Section 5.6). Although lesser in magnitude, a comparison with the species results for 100 isolates from mouth swabs provided some useful information.

Table 7.1 Comparison of species of viridans streptococci isolated from the mouth and blood of paediatric haematology/oncology patients.

Species	Number of isolates (=%) RAPID ID 32 Strep Mouth	Number of isolates (%) RAPID ID 32 Strep Blood
S. oralis	71	48 (63)
S. mitis	12	19 (25)
S. sanguis	4	5 (7)
S. parasanguis	10	2 (3)
S. salivarius	1	1 (1)
S. gordonii	2	1 (1)
TOTAL	**100**	**76(100%)**

From both sites, *S. oralis* was the predominant species, comprising 71% of total isolates from mouth swabs and 63% from blood. *S. mitis* was the second most commonly isolated species representing 12% from the oral cavity and 25% from

blood. Other species of viridans streptococci present in lower numbers at both sites, comprised *S. parasanguis* (10% in the mouth and 3% in the blood), *S. sanguis* (4% versus 7%), *S. gordonii* (2% versus 1%) and *S. salivarius* (1% at both sites).

If the mouth were the source of viridans streptococci causing bacteraemia, the predominance of *S. oralis* in the oral cavity of these patients corresponds well to its predominance amongst blood culture isolates (71% and 63% of total isolates respectively). *S. mitis*, while the second most common species from both sites was associated, to a greater extent with blood cultures than the mouth (25% versus 12%, $P < 0.05$). This may reflect some enhanced ability of *S. mitis* to access the blood stream, or may be related to the location of this species in the oral cavity of these patients. The difference may alternatively be related to sampling method. As mentioned previously, swabbing the mouth is not the most efficient method of obtaining total oral microbial yield. Oral rinse techniques are superior (Spijkervet, 1991), but would not have been suitable for some of the very young or very sick patients in this study. As a consequence, there is a possibility that some isolates of viridans streptococci may not have been detected, leading to an underestimation of their contribution to the overall composition of viridans streptococcal flora in the oral cavity. A further complicating factor may have been the inevitable variation in swabbing technique used by many different nurses over the period of this study. Finally, patient compliance, in a minority of cases, was not optimal.

7.3 Studies to determine the origin of viridans streptococci causing bacteraemia

This investigation involved phenotypic and genotypic analyses of isolates of viridans streptococci cultured from blood and oral specimens from several patients. Case 1 was selected because this represented an example of polymicrobial bacteraemia

caused by bacteria of two different genera, which also colonize the mouth. Case 2 was chosen to investigate whether oral compromise other than mucositis could potentially predispose to viridans streptococcal bacteraemia. Case 3 was selected because this patient differed from the majority of others; she was not neutropenic and was apyrexial, with no evidence of mucositis, but viridans streptococci were isolated from multiple blood cultures. The results are preceded by a short description of each case.

7.3.1 Case 1

A 15-year-old boy with chronic myeloid leukaemia was treated with hydroxyurea from diagnosis, followed by bone marrow transplantation from a matched unrelated donor at 9 months from presentation. On day 7 post-transplant, while neutropenic and suffering from severe oral mucositis, the patient became febrile. Viridans streptococci and coagulase-negative staphylococci were isolated from blood culture.

A mouth swab, taken 4 days post transplant yielded normal buccal flora (including viridans streptococci) plus coagulase-negative staphylococci. Surveillance cultures of the Hickman line exit site, faeces and urine yielded no evidence of viridans streptococci or coagulase-negative staphylococci. All distinct colonial types of viridans streptococci from the mouth swab were subcultured and identified to species level, as was the isolate from blood culture. The isolates of coagulase-negative staphylococci from both sites were also identified to species level using the API Staph method (BioMerieux). Antibiograms for all isolates were obtained.

The viridans streptococcal isolate from blood culture and one isolate from the mouth were identified as *S. oralis* I. A further isolate from the mouth was identified as *S. oralis* II. The biochemical identification profile of the *S. oralis* I from blood was identical to that of the oral isolate, while that of the isolate of *S. oralis* II was distinct

(Table 7.2). All isolates of viridans streptococci possessed the same antibiogram (Table 7.2).

Table 7.2 Case 1 - biotypes and antibiograms of viridans streptococci from blood culture and mouth

Site	Species	Biochemical ID Profile	Antibiogram						
			PEN	CEFT	PIP/T	MER	CEFA	AMI	VA
Blood	*Strep. oralis* I	46052643120	S	S	S	S	S	R	S
Mouth	*Strep. oralis* I	46052643120	S	S	S	S	S	R	S
Mouth	*Strep. oralis* II	44032441120	S	S	S	S	S	R	S

PEN: Penicillin, CEFT: Ceftazidime, PIP/T: Piperacillin/tazobactam,
MER: Meropenem, CEFA: Cefaclor, AMI: Amikacin, VA: Vancomycin
S: Sensitive, R: Resistant

Both isolates of coagulase-negative staphylococci were identified as *Staph epidermidis* with identical biochemical identification profiles and antibiograms (Table 7.3).

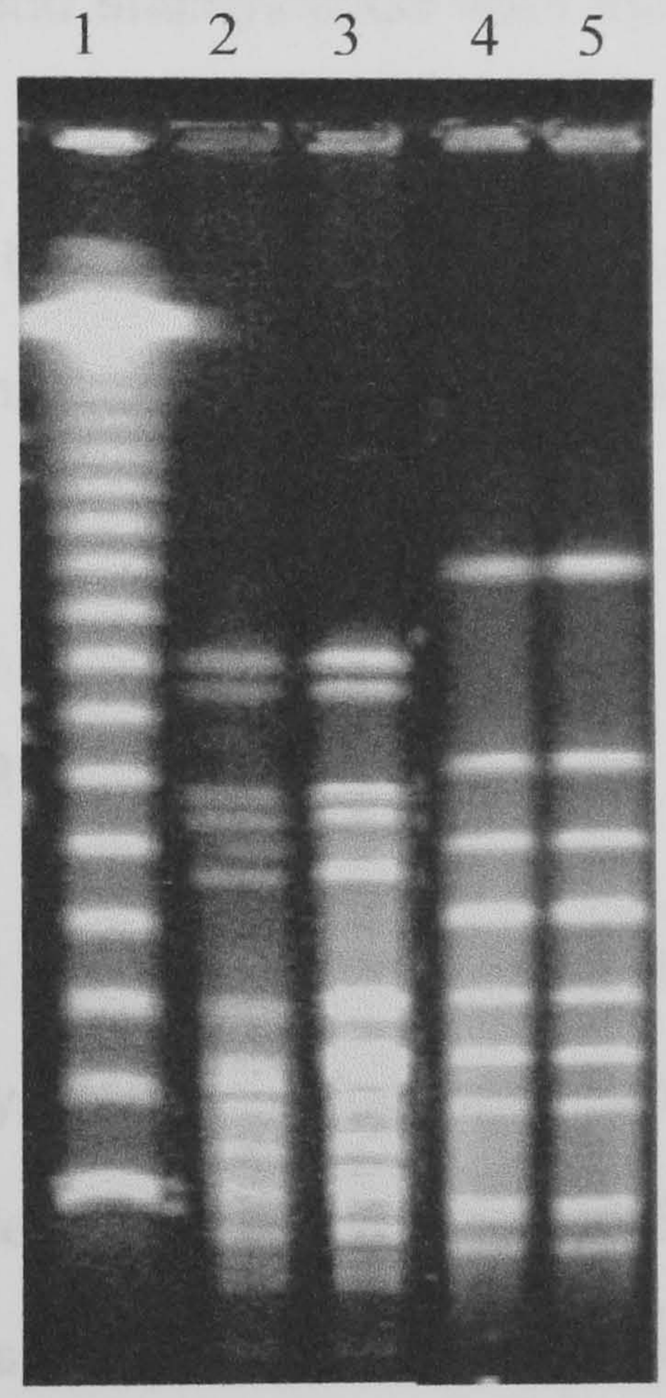
Table 7.3 Case 1 - biotypes and antibiograms of *Staph epidermidis* from blood culture and mouth

Site	Species	Biochemical ID profile	Antibiogram						
			VA	MET	CLI	RIF	FUS	AMI	CIP
Blood	*Staph epidermidis*	6706112	S	R	S	S	R	R	R
Mouth	*Staph epidermidis*	6706112	S	R	S	S	R	R	R

VA: Vancomycin, MET: Methicillin, CLI: Clindamycin, RIF: Rifampicin,
FUS: Fusidic acid, AMI: Amikacin, CIP: Ciprofloxacin
S: Sensitive, R: Resistant

Pulsed-field gel electrophoresis (PFGE) of chromosomal DNA digested with *Sma* I restriction endonuclease was used to compare the isolates of *Strep. oralis* I and *Staph. epidermidis* from blood culture with those from the mouth.

Figure 7.2 Case 1 - PFGE analysis of *Staph. epidermidis* and *Strep. oralis* I from blood culture and mouth swab



Lane 1 DNA molecular weight marker (48.5 kb Lambda ladder)

Lane 2 *Staph. epidermidis* from blood

Lane 3 *Staph. epidermidis* from mouth

Lane 4 *Strep. oralis* I from blood

Lane 5 *Strep. oralis* I from mouth

The results (Figure 7.2) indicated that both isolates of *Strep.oralis* I were indistinguishable and that both isolates of *Staph. epidermidis* were indistinguishable. These findings demonstrated that the patient's mouth was the most likely source of both *Strep. oralis* I and *Staph. epidermidis* responsible for polymicrobial bacteraemia and provided proof that classical chemotherapy-induced oral mucositis could provide a portal of entry. When the patient's Hickman line was removed, culture yielded no evidence of either of these strains (Kennedy *et al.*, 2000). The finding that both streptococci and staphylococci from the oral cavity could access the blood stream suggests that other combinations of micro-organisms from this source could cause polymicrobial bacteraemia. In this particular case, biochemical identification profiles were useful in distinguishing between different strains of *S. oralis*, but antibiograms were not.

7.3.2 Case 2

A 12-year-old boy with high-risk AML, had completed his second course of induction chemotherapy (MAE). Fourteen days later, while profoundly neutropenic, he became febrile. Blood cultures yielded *S. oralis* I. The patient was not suffering from oral mucositis, but did have severe haemorrhagic gingivitis and an oozing tooth socket. *S. oralis* I had been the only isolate of viridans streptococcus cultured from a surveillance mouth swab taken 2 days after the date of the positive blood culture. The isolate from the mouth and that from blood culture had identical biochemical identification profiles and antibiograms (Table 7.4). Surveillance cultures of the patient's Hickman line exit site, faeces and urine yielded no evidence of viridans streptococci.

Table 7.4 Case 2 - biotypes and antibiograms of viridans streptococci from blood culture and mouth

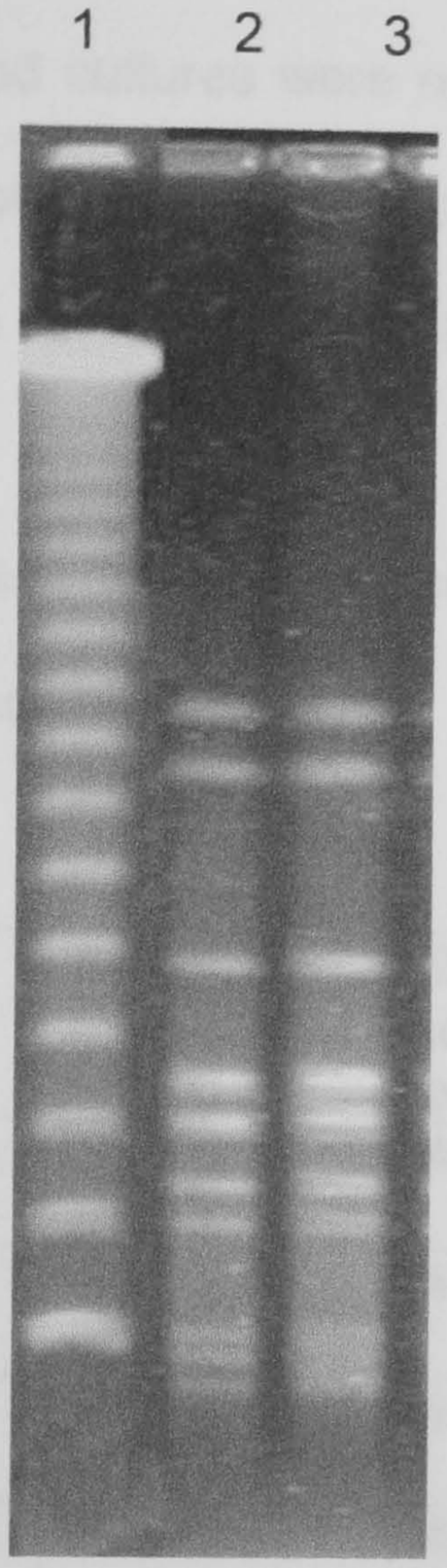
Site	Species	Biochemical ID Profile	Antibiogram PEN CEFT PIP/T MER CEFA AMI VA
Blood	*S. oralis* I	46052443120	R R I S R R S
Mouth	*S. oralis* I	46052443120	R R I S R R S

PEN: Penicillin, CEFT: Ceftazidime, PIP/T: Piperacillin/tazobactam,
MER: Meropenem, CEFA: Cefaclor, AMI: Amikacin, VA: Vancomycin

S: Sensitive, R: Resistant, I: Intermediately susceptible

PFGE analysis of chromosomal DNA digested with *Sma* I restriction endonuclease from both isolates of *S. oralis* I demonstrated that these organisms shared the same PFGE type, *i.e.* were genotypically indistinguishable (Figure 7.3), indicating that the mouth was the source of *S. oralis* I responsible for the episode of bacteraemia and that gingivitis had provided a portal of entry into the blood stream. This case thus provided evidence that oral compromise other than mucositis may be associated with the development of viridans streptococcal bacteraemia.

Figure 7.3 Case 2 - PFGE analysis of isolates of *S. oralis* I from blood culture and mouth swab



Lane 1 DNA molecular weight marker (48.5 kb Lambda ladder)

Lane 2 *S. oralis* I from blood

Lane 3 *S. oralis* I from mouth

7.3.3 Case 3

A 2-year-old girl with relapsed Wilms' tumour had received several courses of chemotherapy and had recently achieved remission. To complete her therapy she was due to receive high-dose Melphalan and peripheral blood stem cell rescue. Three days after stem cell harvest, blood cultures were performed as part of an assessment prior to high-dose chemotherapy. The patient was well, afebrile, was

not neutropenic, and had no symptoms suggestive of oral or gastrointestinal mucosal damage. *S. oralis* I was cultured from blood from both lumens of her Hickman line. When the initial microscopy results for these specimens were telephoned to the ward, line blood cultures were repeated to exclude the possibility of contamination. From repeat blood cultures *S. oralis* I was isolated once more. All three isolates possessed the same biochemical identification profile and antibiogram (Table 7.5)

Table 7.5 Case 3 - biotypes and antibiograms of viridans streptococci from blood culture and mouth

Site	Species	Biochemical identification profile	Antibiogram						
			PEN	CEFT	PIP/T	MER	CEFA	AMI	VA
Blood (day 1) red lumen	*S. oralis* I	46056643120	S	S	S	S	S	R	S
Blood (day 1) white lumen	*S. oralis* I	46056643120	S	S	S	S	S	R	S
Blood (day 2)	*S. oralis* I	46056643120	S	S	S	S	S	R	S
Mouth	*S. oralis* I	46056643120	R	R	S	S	R	S	R
	S. oralis II	44016601120	R	R	I	S	R	S	R
	S. oralis II	40016641120	R	R	I	S	R	S	R

PEN: penicillin, CEFT: ceftazidime, PIP/T: piperacillin/tazobactam,
MER: meropenem, CEFA: Cefaclor, AMI: amikacin, VA: vancomycin

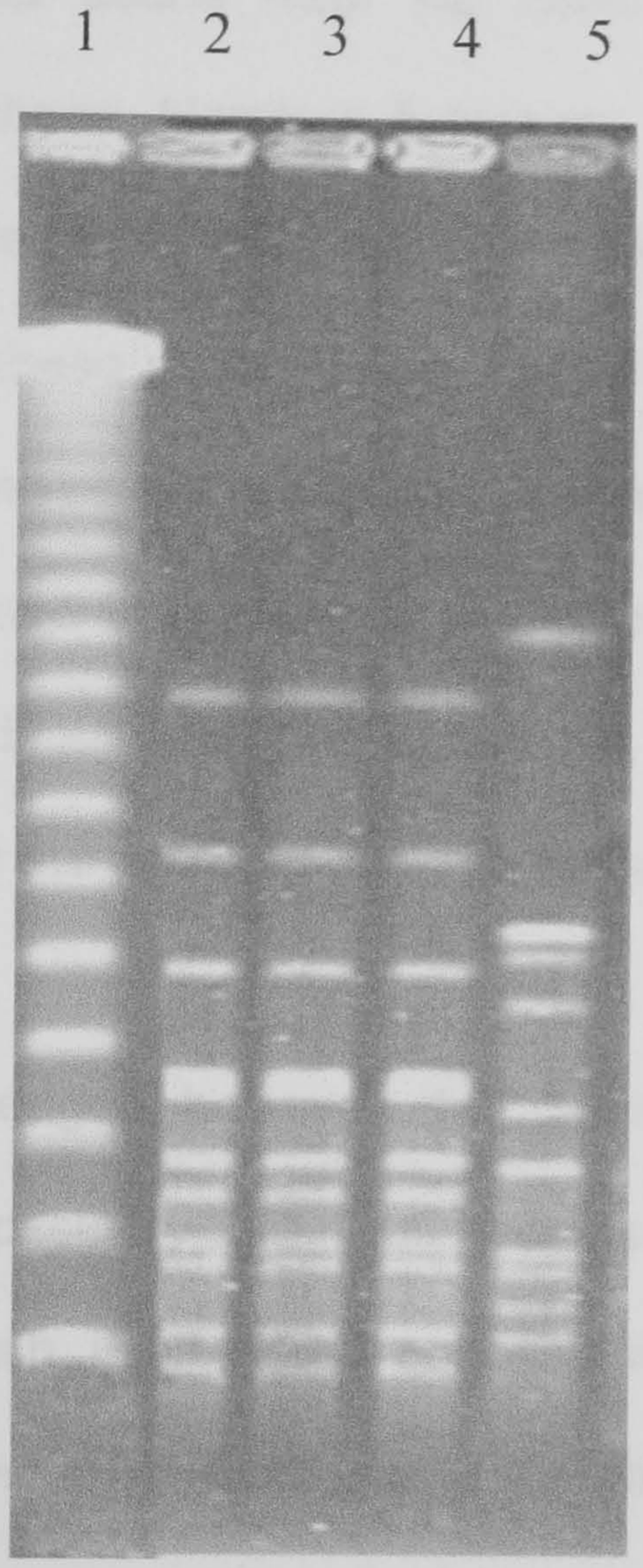
S: Sensitive, R: Resistant, I: Intermediately susceptible

From a mouth swab taken within the next 72 hours, one strain of *S. oralis* I and two of *S. oralis* II were cultured. The oral strain of *S. oralis* I had an identical biochemical identification profile to that of the *S. oralis* I cultured from blood and this was quite distinct from those of both isolates of *S. oralis* II from the mouth (Table 7.5). The antibiogram of the *S. oralis* I from blood culture differed from all isolates of viridans streptococci from the oral cavity (Table 7.5).

All isolates of *S. oralis* I (with identical biochemical identification profiles) from both sites were compared by PFGE. The results revealed that all three isolates of *S. oralis* I from blood were genotypically indistinguishable, while the isolate from the oral cavity was distinct (Figure 7.4). In this case, there were no clinical signs of infection. Culture of viridans streptococci from line blood may have represented catheter colonization +/- transient bacteraemia. Unfortunately, blood could not be obtained from a peripheral site to aid diagnosis. Confirmation that the strain of *S. oralis* I from the oral cavity was different from that in blood was in accord with the finding that the patient had no signs of oral or gastrointestinal tract mucositis and no other forms of oral compromise which might provide a portal of entry into the bloodstream.

Surveillance cultures of the patient's Hickman line site, faeces and urine yielded no evidence of viridans streptococci. Although line site culture was negative, it is likely, that in this particular case, the line itself was the source of viridans streptococci.

Figure 7.4 Case 3 - PFGE analysis of isolates of *S. oralis* I from blood culture and mouth swab



Lane 1 DNA molecular weight marker (48.5 kb Lambda ladder)

Lane 2 *S. oralis* I from blood day 1 (red lumen)

Lane 3 *S. oralis* I from blood day 1 (white lumen)

Lane 4 *S. oralis* I from blood day 2

Lane 5 *S. oralis* I from mouth

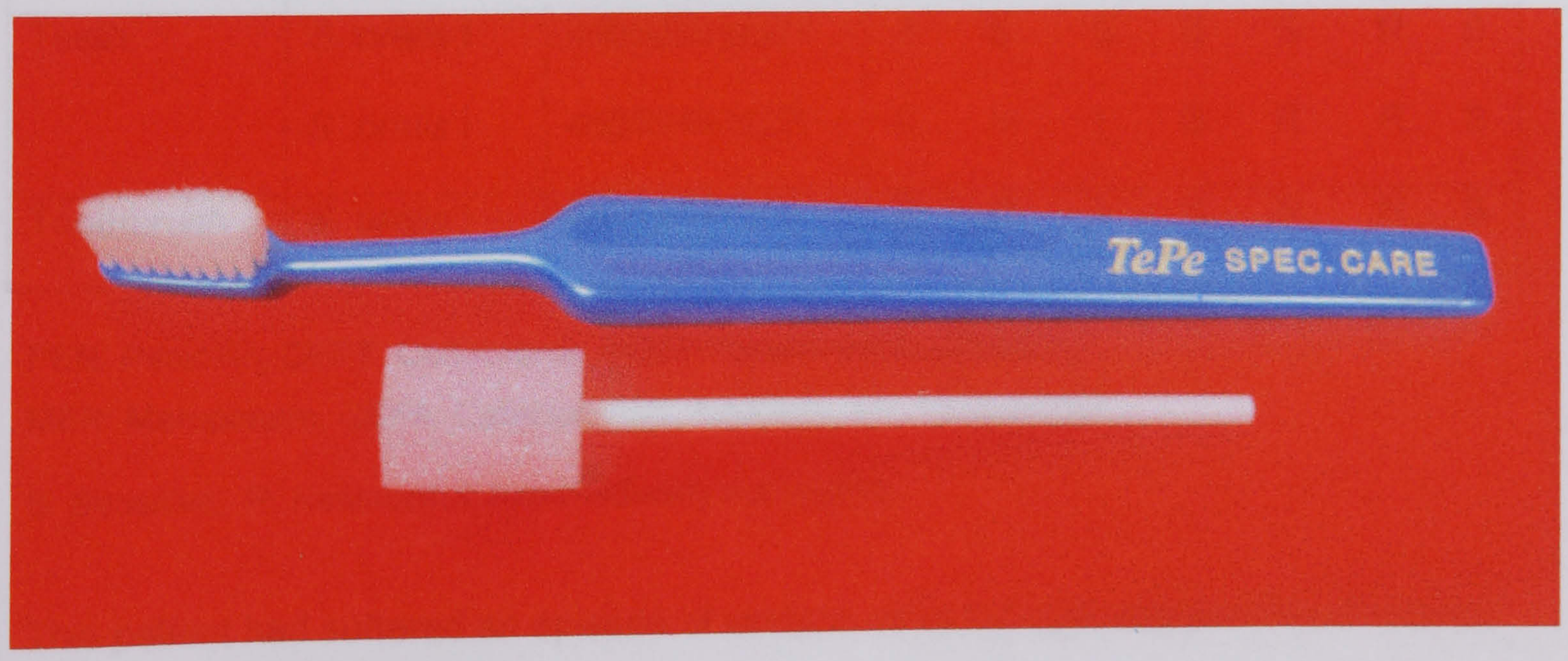
The results of this part of the study also demonstrated that isolates with the same biochemical identification profile may not necessarily be genotypically identical.

7.4 Tools for mouth care as potential vectors of infection.

It is generally agreed that a toothbrush is the most efficient (non-specialist) tool for removing plaque and debris from the teeth and mouth (Campbell, Evans & MacTavish, 1995; Gibson, Horsford & Nelson, 1997). However, because the oral tissues of cancer patients are particularly sensitive, foam toothettes have traditionally been used to clean these sites (Figure 7.5). Foam toothettes are used once and then discarded. However, today the staff of some paediatric haematology/ oncology units actively encourage their patients to use special soft toothbrushes (*e.g. TePe* Select Special Care, Molar Ltd., Mayfield, U.K.), with the view that superior cleaning will be achieved while still avoiding damage to fragile oral tissues (Figure 7.5).

It should be acknowledged, however, that unless the toothbrush is discarded after one use, there is a possibility that it will become colonized with oral bacteria. If this occurs, the toothbrush itself could conceivably become a vector of organisms capable of causing systemic infection in vulnerable patients.

Figure 7.5 Foam toothette and *TePe* Select Special Care toothbrush



The 12-year-old boy with AML (case 2) described earlier (Section 7.3.2), agreed to use a soft toothbrush for a short time to provide information to determine how quickly the toothbrush became colonized with oral bacteria. Regular swabbing of the brush, performed prior to mouth care, revealed that after 2 weeks, in spite of thorough rinsing after use, oral flora similar in general appearance to that isolated from his mouth could be cultured from the toothbrush. All distinct colony types of viridans streptococci from teeth swabs, general mouth swabs, and toothbrush swabs were cultured and identified. Biochemical identification indicated that two different strains of *S. oralis* I were present on the teeth and that a strain identical phenotypically to one of these was also detected from the mouth swab and from the toothbrush (Table 7.6).

Table 7.6 Biotypes and antibiograms of viridans streptococci from mouth, teeth and toothbrush swabs.

Site	Species	Biochemical identification profile	Antibiogram						
			PEN	CEFT	PIP/T	MER	CEFA	AMI	VA
Mouth	*S. oralis* I	46052443120	R	R	I	S	R	R	S
Teeth	*S. oralis* I	42052643120	S	S	S	S	S	R	S
	S. oralis I	46052443120	R	R	I	S	R	R	S
Toothbrush	*S. oralis* I	46052443120	R	R	I	S	R	R	S

PEN: Penicillin, CEFT: Ceftazidime, PIP/T: Piperacillin/tazobactam,
MER: Meropenem, CEFA: Cefaclor, AMI: Amikacin, VA: Vancomycin

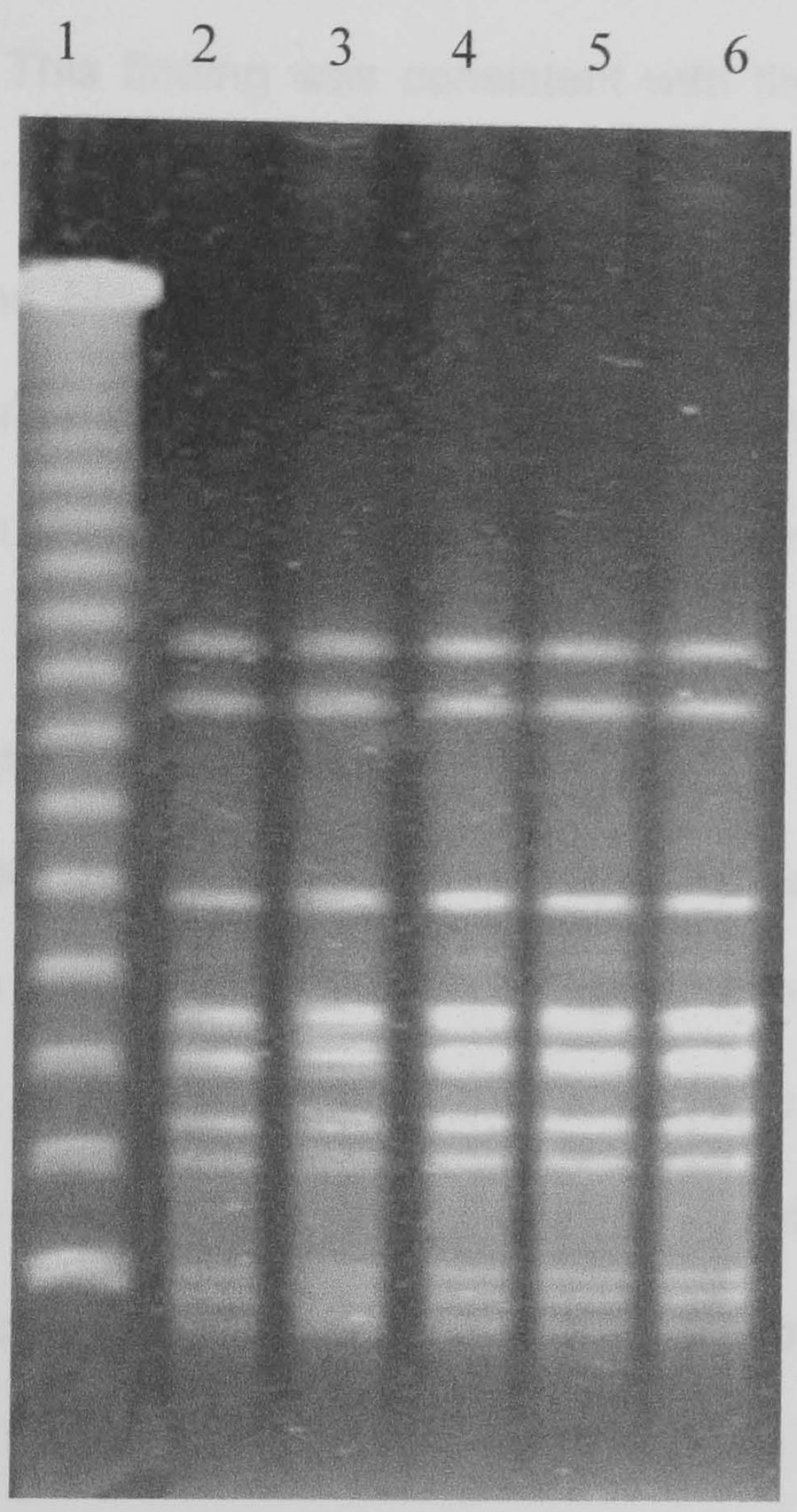
S: Sensitive, R: Resistant, I: Intermediately susceptible

This patient had suffered an episode of viridans streptococcal bacteraemia 2 months prior to starting to use the special soft toothbrush. The biochemical identification

profiles and antibiograms for viridans streptococci isolated from blood and mouth at that time were compared with those of viridans streptococci more recently isolated from mouth, teeth and toothbrush swabs. All isolates from both periods of time had been identified as *S. oralis* I and, with the exception of one of the two strains from teeth swabs, all organisms had the same biochemical identification profile and antibiotic susceptibility pattern (Tables 7.4 and 7.6).

PFGE of *Sma* I chromosomal DNA digests of the phenotypically identical isolates revealed that they were also indistinguishable genotypically (Figure 7.6). This suggested that certain strains may persist in the oral cavity, in spite of antimicrobial therapy and that the toothbrush may indeed harbour organisms with the potential to cause bacteraemia.

Figure 7.6 PFGE analysis of *S. oralis* I from blood culture, mouth, teeth and toothbrush swabs*



Lane 1 DNA molecular weight marker (48.5 kb Lambda ladder)

Lane 2 *S. oralis* I from blood 20.12.97

Lane 3 *S. oralis* I from mouth 22.12.97

Lane 4 *S. oralis* I from toothbrush 9.03.98

Lane 5 *S. oralis* I from teeth 9.03.98

Lane 6 *S. oralis* I from mouth 9.03.98

*: All isolates obtained from the patient described in Section 7.3.2 (Case 2)

7.5 Summary

The results of this part of the study indicated that *S. oralis* was the predominant species of viridans streptococcus in the oral cavity of this group of paediatric patients with cancer. This finding was consistent with the predominance of this species in blood culture. Phenotypic and genotypic analyses of viridans streptococci from oral specimens and blood cultures from two selected patients, indicated that viridans streptococci originating in the mouth may cause bloodstream infection in neutropenic patients. Both mucositis and gingivitis were shown to be likely portals of entry.

From analyses of the organisms from Case1, it was possible to demonstrate that isolates of both *Strep. oralis* I and *Staph. epidermidis* from the oral cavity were phenotypically and genotypically indistinguishable from isolates from blood culture, indicating that organisms responsible for polymicrobial bacteraemia can originate in the mouth. In contrast, analyses of viridans streptococci from Case 3 demonstrated that in this non-neutropenic patient, with no symptoms of mucositis, the mouth was unlikely to be the source of viridans streptococci which were isolated from multiple blood cultures.

All isolates of viridans streptococci from the present study with identical PFGE-types also possessed identical biochemical identification profiles and antibiograms. However the reverse association does not necessarily follow. Case 3 demonstrated that viridans streptococci with identical antibiograms possessed different biochemical identification profiles and that isolates with identical biochemical identification profiles had different PFGE-types. It can therefore be concluded that only genotypic methods such as PFGE can provide definitive proof of the relatedness of isolates.

Finally, phenotypic followed by genotypic analyses demonstrated that viridans streptococci colonizing the teeth and oral mucosae may also colonize toothbrushes, which may in turn become potential vectors of infection.

DISCUSSION

CHAPTER 8

AETIOLOGY OF VIRIDANS STREPTOCOCCAL BACTERAEMIA

AETIOLOGY OF VIRIDANS STREPTOCOCCAL BACTERAEMIA

8.1 Species of viridans streptococci causing bacteraemia

Viridans streptococci belonging to the mitis group were responsible for the majority of episodes of bacteraemia in this study – a finding which is in agreement with earlier investigations (Cohen *et* al., 1983; Henslee *et al.*, 1984; Sotiropoulos *et al.*, 1989; Classen *et al.*, 1990; Villablanca *et al.*, 1990; Guiot *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; McWhinney *et al.*, 1991; Burden *et al.*, 1991; Elting, Bodey & Keefe, 1992; Awada *et al.*, 1992; McWhinney *et al.*, 1993; Steiner *et al.*, 1993; Donnelly *et al.*, 1993; Broun *et al.*, 1994; Bochud *et al.*, 1994). From the present study, only one isolate from outwith this group was cultured from blood – one strain of *S. salivarius.*

The predominant species from the mitis group, identified using the Rapid ID 32 Strep system, was *S. oralis* (63%), while *S. mitis* represented 25% of total isolates. In contrast, studies carried out during the 1980s and early '90s described *S. mitis* or *S. sanguis* as the predominant organism in this clinical setting (Cohen *et* al., 1983; Henslee *et al.*, 1984; Sotiropoulos *et al.*, 1989; Classen *et al.*, 1990; Villablanca *et al.*, 1990; Guiot *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; McWhinney *et al.*, 1991; Burden *et al.*, 1991; Elting, Bodey & Keefe, 1992; Awada *et al.*, 1992; Steiner *et al.*, 1993; Donnelly *et al.*, 1993; Broun *et al.*, 1994; Bochud *et al.*, 1994). There were only 5 isolates of *S. sanguis* (7% of total isolates of viridans streptococci) from the present study.

Earlier investigations used a variety of methods for identification of viridans streptococci, but, by far, that most commonly employed was API 20 Strep (BioMerieux). The taxonomy of the viridans streptococci was less well defined at

that time, especially before the development of genotypic methods. API 20 Strep databases prior to version 5.1 (1995) did not even include *S. oralis*. This species was first proposed by Bridge and Sneath in 1982 and an amended description was proposed by Kilian, Mikkelsen & Henrichsen in 1989. However, it was rarely reported from clinical specimens before publication of the identification scheme of Beighton, Hardie & Whiley in 1991 and its introduction into the API profile registers some years later. As a consequence, some isolates identified by earlier commercial systems as *S. mitis* or *S. sanguis* would probably be more accurately identified today as *S. oralis*.

Even now, the current API 20 Strep system does not include certain critical tests which have been incorporated into the more recently introduced Rapid ID 32 Strep system which also possesses a more extensive identification database. As a result of this, *S. gordonii*, *S. parasanguis*, *S. vestibularis* and *S. downei/sobrinus* can be identified using the latter system but not the former.

Comparison of the identification results of the present study, using the Rapid ID 32 Strep system with those for the same organisms identified routinely in the diagnostic microbiology laboratory using the API 20 Strep system from 1994 to 2000, revealed a significant difference in proportions of individual species. Identification results using the older system, demonstrated a predominance of strains of *S. mitis* (72%) – in accord with several earlier studies.

As mentioned previously, in 1991 Beighton and co-workers published an identification scheme for viridans streptococci. They used a collection of strains representative of a wide range of viridans streptococcal species whose taxonomic position had been established by DNA-DNA hybridization or by analysis of SDS-PAGE cell-protein profiles (Whiley, 1987).

This identification scheme determined 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 (Beighton, Hardie & Whiley, 1991). Two years later, the publication of McWhinney and co-workers included the identification of 47 isolates of viridans streptococci from blood cultures from neutropenic patients, using this method (McWhinney *et al.*, 1993). *S. oralis* was the predominant species (*n*=39 (83% of total isolates)), followed by *S. mitis* (*n*=5 (11%)) and *S. parasanguis* (*n*=1 (2%)). Two isolates could not be identified further than '*Streptococcus* spp'. The following year, a further report using the identification method of Beighton *et al.*, described a predominance of *S. oralis* (Beighton, Carr & Oppenheim, 1994). This study identified 23 isolates of viridans streptococci from blood cultures of neutropenic patients with cancer. These streptococci consisted of 14 isolates of *S. oralis* (61% of total isolates), 5 of *S. mitis* (22%) and 2 of *S. salivarius*. Two isolates could not be identified definitively.

The predominance of *S. oralis* in the present study is in accord with both of the above studies. In particular, the proportions of isolates of *S. oralis* and *S. mitis* from the latter study are very similar to those of the present work, in which *S. oralis* represented 63% of total isolates and *S mitis* represented 25%.

There are few studies, published after the commencement of the present work, which describe the species of viridans streptococci associated with bacteraemia in neutropenic patients, and of those which exist, some do not state the precise method of identification. One study which utilized the Rapid ID 32 Strep system, that of Reinert and colleagues (2001), investigated both α-haemolytic and β-haemolytic streptococci isolated from blood culture, and demonstrated that *S. oralis* strains represented 26.3% of total isolates of streptococci, while *S. mitis* represented 9%. (Other predominant species, identified by other methods, were *S. pneumoniae*

(26.3%), *S. agalactiae* (11.5%), and *S. pyogenes* (5.8%)). Using Rapid ID 32 Strep, Wisplinghoff and co-workers (1999) identified 37 isolates of *S. mitis,* 19 of *S. oralis* and 2 of *S. salivarius* from blood culture of neutropenic patients. The study of Richard and colleagues of 1995 identified 15 strains of *S. mitis*, 8 of *S. oralis* and 2 of *S. sanguis*. However the particular method of identification used was not mentioned. In 1995, Donnelly and co-workers described bacteraemia caused by *S. oralis* in BMT patients and by *S. mitis* in non-BMT patients with malignancy using 'API Strep' for identification. The trend from the results of these studies using modern identification techniques and classification schemes, and those of the present work suggest that the species most commonly associated with sepsis in the immunocompromised population, are *S. oralis* and *S. mitis*.

Since the commencement of this study, the Rapid ID 32 Strep system has been adopted more widely and experience of its usefulness is accumulating. Nevertheless, this system should not be considered to be the definitive answer to identification of viridans streptococci. It cannot identify the more recently described species of viridans streptococci, such as *S. crista* (Handley *et al.*, 1991), *S.peroris* and *S. infantis* (Kawamura *et al.*, 1998) and some authors have reported problems in differentiating between certain strains of *S. mitis* and *S. oralis* (Kikuchi *et al*, 1995; Jensen, Konradsen & Bruun, 1999). These problems were encountered in the present study, and as described earlier, 7 of the isolates of viridans streptococci from blood cultures (9% of total) were sent to the Streptococcal Reference Laboratory, PHLS Central Public Health Laboratory, London, for further tests and identification.

8.2 Polymicrobial bacteraemia

During six episodes of bacteraemia, multiple strains or species of streptococci associated with the oral cavity were concomitantly isolated from blood culture. Few

other workers have reported similar experience (Groot-Loonen *et al.*, 1987; Burden *et al.*, 1991; Valteau *et al.*, 1991; Awada *et al.*, 1992; Arns da Cunha *et al.*, 1998; Graber *et al.*, 2001). However it seems reasonable that if chemotherapy-induced mucositis provides a portal of entry, more than one species of viridans streptococcus from the mouth or gastrointestinal tract could concomitantly access the blood stream. A further episode of polymicrobial bacteraemia involved *S. mitis*, 2 different strains of *S. oralis*, *Haemophilus parainfluenzae* and *Moraxella catarrhalis* – all organisms associated with the oropharynx.

Several other investigators have reported polymicrobial bacteraemia caused by viridans streptococci plus organisms of other genera (Pizzo, Ladisch & Witebsky, 1978; Groot-Loonen *et al.*, 1987; Kern, Kurrle & Schmeiser, 1990; Burden *et al.*, 1991; Awada *et al.*, 1992; Donnelly *et al.*, 1993; Bochud *et al.*, 1994; Engelhard *et al.*, 1995; Spanik *et al.*, 1997; Arns da Cunha *et al.*, 1998). Coagulase-negative staphylococci were the most common co-infecting bacteria (Groot-Loonen *et al.*, 1987; Burden *et al.*, 1991; Donnelly *et al.*, 1993; Bochud *et al.*, 1994; Spanik *et al.*, 1997), in accord with the findings of the present study, which featured three episodes of such polymicrobial bacteraemia. Historically it has become accepted that coagulase-negative staphylococcal bacteraemia is most commonly associated with line-related colonization in immunocompromised patients. However, the oral cavity of children with cancer may also be colonized by these organisms (Jackson *et al.*, 2000) and, as part of the present study, it has been shown that viridans streptococci with coagulase-negative staphylococci originating in the mouth may cause polymicrobial bacteraemia (Kennedy *et al.*, 2000).

There were two episodes of bacteraemia caused by viridans streptococci with *Enterococcus* spp, a finding rarely reported in earlier papers (Kern, Kurrle & Schmeiser, 1990). In both cases, the patients suffered from oral compromise and

diarrhoea, suggesting a source in the oropharynx or gastrointestinal tract. Several authors have described concomitant infection by viridans streptococci with Enterobacteria (Groot-Loonen *et al.*, 1987; Kern, Kurrle & Schmeiser, 1990; Burden *et al*, 1991; Awada *et al.*, 1992; Engelhard *et al.*, 1995; Spanik *et al.*, 1997), as was also observed in the present study (1 episode: *S. oralis* and *Enterobacter cloacae*). Coliforms are most commonly associated with the gastrointestinal tract, but may also be isolated from the mouths of cancer patients (Sixou, de Medeiros-Batista & Bonnaure-Mallet, 1996; Meurman *et al.*, 1997).

Concomitant isolation of viridans streptococci with *Candida* spp. from blood culture has rarely been described (Kern, Kurrle & Schmeiser, 1990; Awada *et al.*, 1992). In the present study *C. albicans* plus *S. mitis* caused one episode of bloodstream infection and *C. lusitaniae* plus *S.mitis*, another. *Candida* spp. commonly colonize the oral cavity of immunocompromised patients, causing opportunistic infection (Wray & Bagg, 1997). It is quite possible that damaged oral mucosal membranes may provide a portal of entry for such organisms into the bloodstream. In addition, candidaemia and infection by yeasts of other genera may also be associated with the use of central venous catheters (Stratov *et al.*, 1998; Krcmery, Kunova & Trupl, 1998). The bloodstream infection, described in the present study, involving the environmental yeast, *Rhodotorula rubra* with *S. oralis*, in a patient with no evidence of mucosal damage to the oral cavity or gastrointestinal tract was more likely to have been associated with such a device.

The present study found no relationship between polymicrobial bacteraemia and severity of infection in immunocompromised patients with cancer, in common with the findings of Burden and co-workers (1991) who reported that 55% of episodes of viridans streptococcal bacteraemia involved additional organisms. The study of Kern and colleagues (1990) revealed that the death rate was slightly higher in patients

with streptococci plus other organisms from blood culture than in patients with streptococci alone (5/15 versus 7/40), however the difference was not statistically significant. The report of Pizzo and co-workers, published considerably earlier (1978), revealed that, of a total of twelve patients with polymicrobial bacteraemia, all eight with Gram-negative organisms, isolated concomitantly with viridans streptococci, died. All eight showed evidence of Gram-negative sepsis at post mortem. Of the fifteen patients from whom viridans streptococcus was the sole isolate from blood culture, three died, and in none of these could death be directly related to viridans streptococcal sepsis.

8.3 Origins of viridans streptococci and portals of entry

As referred to previously, the presence of oropharyngeal mucositis, was observed in the majority of earlier studies of viridans streptococcal bacteraemia in neutropenic patients (Pizzo, Ladisch & Witebsky, 1978; Hoecker *et al.*, 1978; Cohen *et al.*, 1983; Groot-Loonen *et al.*, 1987; Sotiropoulos *et al.*, 1989; Classen *et al.*, 1990; Kern, Kurrle & Schmeiser, 1990; Burden *et al.*, 1991; Elting, Bodey & Keefe, 1992; Donnelly *et al.*, 1993; Bochud *et al.*, 1994; Donnelly *et al.*, 1995; Engelhard *et al.*, 1995; Gonzalez-Barca *et al.*, 1996), and was demonstrated to be a statistically significant risk factor in three case control studies (Kern, Kurrle & Schmeiser, 1990; Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994). More recently, a case control study has identified severe oral mucositis as a factor significantly associated with the development of complications related to viridans streptococcal bacteraemia (Marron *et al.*, 2000).

From the earliest reports of viridans streptococcal bacteraemia, it was accepted by many investigators, that the mouth was a likely source of these bacteria. However, in 1994, when the present study was commenced, there was still no definitive proof

that isolates of viridans streptococci from blood culture of neutropenic patients with bacteraemia were actually identical to those isolated from the oral cavity.

As discussed earlier, one of the aims of the present study was to address this issue by performing phenotypic followed by genotypic analyses of viridans streptococci from both sites. In preparation for this part of the present study, the range of species of viridans streptococci resident in the oral cavities of paediatric immuno-compromised patients was determined. As detailed in Chapter 7, *S. oralis* was the predominant species (71%) in the mouth, followed by *S. mitis* (12%). *S. parasanguis* represented 10% of total isolates, with the remaining species, *S. sanguis*, *S. gordonii* and *S. salivarius* each representing < 5%.

The work of Lucas and colleagues (1997), which utilized an oral rinse technique to investigate viridans streptococci from the mouths of 20 paediatric BMT patients demonstrated that 1 week post transplantation, *S. oralis* was the predominant species of viridans streptococcus. As part of their analysis, these investigators grouped *S. oralis* and *S. mitis* together, and termed them the '*S. oralis* group'. They reported that 13 of the 20 children were colonized with species belonging to this group alone. From the mouths of two children, S. *salivarius* was isolated in addition to species of the 'S. oralis group'. From two, *S. gordonii* was the additional species and from one, *S. gordonii* plus *S. parasanguis*. From two children no oral viridans streptococci were detected. Although the present study used a different method of assessing oral colonization by swabbing the mouth, the predominance of viridans streptococcal species of the '*S. oralis*' group is in agreement with the work of Lucas and colleagues. It has been shown by others that the oral flora of paediatric patients with cancer becomes less complex and that dominant organisms arise (Sixou *et al.*, 1998).

If the mouth were the source of viridans streptococci causing bacteraemia, the predominance of *S. oralis* in the oral cavity of the patients of the present study also corresponded to its predominance in blood cultures (71% of total isolates versus 63% respectively).

As described in Chapter 7, three patients were selected, and phenotypic, followed by genotypic investigations were performed to determine whether the oral cavity was a potential source of viridans streptococci causing bacteraemia in these individuals. The demonstration that from two patients (cases 1 & 2), isolates from the respective mouth swabs and blood cultures were indistinguishable by biochemical identification profile, antibiogram and also by PFGE-type fulfilled the aim of this part of the study. Of interest, shortly after the commencement of this work, another group published an article, using a different molecular typing technique which provided the first definitive evidence that the mouth was a potential source (Richard *et al.*, 1995). Ribotyping demonstrated that in all seven described cases of viridans streptococcal bacteraemia, the strain from blood culture had an identical ribotype to that recovered from the mouth of the respective patient and that similar ribotypes were never shared by strains from different patients. All seven patients in the study of Richard and co-workers suffered from oral mucositis.

As discussed in Section 8.2, viridans streptococci plus bacteria of different genera may cause polymicrobial bacteraemia in neutropenic patients. The present study demonstrated that *Staph. epidermidis* and *Strep. oralis* from mouth swab and blood culture of a BMT patient (case 1) with severe mucositis were identical by biochemical identification profile, antibiogram and PFGE-type. Previously, it had been demonstrated by others that *Staph. epidermidis* isolated from the throat of a neutropenic, bacteraemic patient had an identical PFGE profile to that isolated from blood culture (Lina *et al.*, 1994). However the work reported in this thesis is the first

to demonstrate that bacteria of different genera, originating in the oral cavity, may gain access to the blood stream concomitantly (Kennedy *et al.*, 2000).

Classical chemotherapy-induced oral mucositis was associated with just less than half (48%) of the total episodes of viridans streptococcal bacteraemia described in the present study. The simple scoring system used in the ward indicated that the majority of patients who experienced the more severe symptoms associated with viridans streptococcal bacteraemia did have some degree of mucositis. However, for the group of 4 patients (5 episodes) with both septic shock and ARDS, severity of oral mucositis varied. In 2 of these episodes, mucositis was described as mild, in one, as moderate and in 2 as severe. Moreover, four other patients who developed viridans streptococcal bacteraemia and who suffered from severe mucositis did not develop septic shock or any degree of respiratory distress. Therefore, severity of mucositis may not necessarily correlate with severity of clinical symptoms associated with viridans streptococcal bacteraemia. Nevertheless, it must be appreciated that the scoring system used was not particularly sophisticated and that interpretation was probably subject to some degree of inter-observer variation.

Historically, most investigators have focused on mucositis as providing a portal of entry for viridans streptococci into the bloodstream. Accordingly, various scoring systems have been devised to monitor this feature (World Health Organization, 1979; Weisdorf *et al.*, 1989; Donnelly *et al.*, 1992, Sonis *et al.*, 1999). However, other forms of oral compromise should also be considered in this context. As part of the present study, all oral complications were recorded, including mucositis, gingivitis, pharyngitis, caries, loose teeth, bleeding lips, oral candidiasis and herpetic lesions, increasing the total number of cases with oral symptoms to 65%.

It has been demonstrated that the frequency of mouth problems in children with cancer is high (Fayle & Curzon, 1991) and far exceeds that of adults (Sonis & Sonis, 1979). The higher mitotic index of mucosal cells of children renders these cells particularly sensitive to the effects of chemotherapy. As a consequence, they are lost faster and are replaced more slowly than those of the adult oral cavity (Campbell, Evans & MacTavish, 1995). The child's mouth is a particularly dynamic environment with loss of deciduous teeth and eruption of permanent teeth with associated gingival trauma.

In 1998, Lucas and colleagues demonstrated that plaque and gingival inflammation scores increased significantly in the mouths of paediatric patients during the intense period of immunosuppression following BMT. They suggested that inflamed gingival tissues may conceivably provide a portal of entry for oral organisms into the bloodstream. The findings of the present work suggested that gingivitis, in the absence of mucositis, could provide such a portal. Phenotypic followed by genotypic analyses of viridans streptococci isolated from the oral cavity and blood culture of a child with AML (Case 2) with gingivitis but no evidence of mucositis showed indistinguishable typing patterns.

Extension of this part of the study revealed another potential source of infection by viridans streptococci. Two months later, when not neutropenic, the same child was issued with a *TePE* Special Select soft toothbrush (Molar Ltd., Mayfield, U.K.) to control plaque levels without damaging delicate oral structures. After two weeks of use, the toothbrush was colonized with oral bacteria and an isolate of *S. oralis* from the brush was shown to be identical by biochemical identification profile, antibiogram and PFGE-type to one cultured from swabs of the buccal mucosa and teeth. As described in the Section 7.4, the strain of *S. oralis* isolated from this patient's blood

and mouth, two months earlier during a period of febrile neutropenia was also identical.

Earlier investigators had demonstrated that toothbrushes may become colonized readily by oral bacteria (Svanberg, 1978; Glass & Lare, 1986; Kozai, Iwai & Miura, 1989), and it has been shown that even simple toothbrushing may produce viridans streptococcal bacteraemia (Donley & Donley, 1988, Roberts *et al.*, 1997). However until now, genotypic analyses to compare toothbrush with blood culture isolates have not been performed.

These novel results demonstrated that strains of viridans streptococci can persist in the oral cavity and suggest that a heavily colonized toothbrush may be a potential vector of infection in neutropenic patients with oral compromise. Special soft toothbrushes are now recommended as more effective tools for mouth care for paediatric cancer patients (Gibson, Horsford & Nelson, 1997; Corbett, 1997), than the disposable foam toothettes, which were commonly used in the past. Children with painful mouths are perhaps unlikely to use aggressive techniques of toothbrushing. However, the results of this study demonstrate the importance of replacing such brushes regularly to prevent excessive accumulation of oral bacteria.

Damaged gastrointestinal mucosa should also be considered as a portal of entry for viridans streptococci. In the present study, when the total number of cases with some form of oral complication plus those with chemotherapy-related diarrhoea and/or rectal mucositis were considered together, a total of 58 cases (84%), could be included as a group with possible bacterial translocation from the oral cavity or gastrointestinal tract into the bloodstream.

In their case control study, Elting and colleagues (1992) described the various manifestations of gastrointestinal toxicity in adults treated for cancer. They found that treatment of chemotherapy-induced gastritis with antacids or with H_2 antagonists was associated with a sevenfold increase in risk of viridans streptococcal bacteraemia. They proposed that overgrowth of viridans streptococci was facilitated by the use of antacids and that gastrointestinal tract ulceration would allow access to the bloodstream.

It should still be appreciated that, in some instances, the mouth or gastrointestinal tract may not be the source of viridans streptococci. As demonstrated by case (3) of the present study, viridans streptococci cultured from line blood on two occasions, were not genotypically identical to any strain of viridans streptococcus isolated from the patient's oral cavity. In this particular case, the patient was not neutropenic and was afebrile. The source of viridans streptococci may have been the Hickman line.

8.4 The changing pattern of viridans streptococcal bacteraemia

8.4.1 Introduction

It was outwith the remit of this study to determine the reason for the increase in cases of viridans streptococcal bacteraemia at RHSC during the early 1990s - reaching a maximum of 18 (representing 22% of total episodes of culture-proven bloodstream infection and 5.9% of all febrile episodes) in 1994. Intensification of chemotherapy protocols, as reported by others (Sotiropoulos *et al.*, 1989; Kern, Kurrle & Schmeiser, 1990) may have been a contributory factor. With the success of such chemotherapeutic approaches in the treatment of certain malignancies, oral and gastro-intestinal mucositis became viewed as undesirable but inevitable side effects.

In 1995, while there were fewer episodes of viridans streptococcal bacteraemia, than in the previous year at RHSC (15 versus 18), the associated morbidity and mortality were unacceptable (Sections 5.12 and 5.13). Mouth care protocols were being followed satisfactorily, and a preliminary diagnosis of streptococcal bacteraemia from Gram staining of any positive blood cultures prompted the rapid addition of vancomycin to first line empirical therapy. However, at this time, in spite of such efforts, viridans streptococcal bacteraemia had become a potentially life-threatening infection.

Then, from late summer of 1996, in conjunction with a further decrease in the number of cases of viridans streptococcal bacteraemia, serious complications associated with this infection also declined. By the end of this study, in the year 2000, there occurred only 5 episodes of viridans streptococcal bacteraemia. However, with the continued use of intensive chemotherapeutic protocols, potentially providing a mucosal portal of entry for viridans streptococci into the bloodstream of neutropenic patients, how could the statistically significant decrease in cases from 1994 to 2000 be explained?

A decrease in incidence of viridans streptococcal bacteraemia was observed in EORTC trial IX (Cometta *et al.*, 1995) compared to trial VIII (EORTC International Antimicrobial Therapy Cooperative Group, 1993), however the precise reasons for this decrease were not proposed. During trial VIII the combination of ceftriaxone plus amikacin was compared with standard ceftazidime plus amikacin as empirical therapy and during trial IX the comparator regimen was piperacillin/tazobactam plus amikacin.

It is difficult to make firm conclusions regarding changing trends in incidence of microbial infection and influential factors, when relatively small sample sizes are

involved, and if a major intervention is involved at some point during a study, there is obviously no way of assessing definitively what the outcome would have been otherwise. However, in the following sections, the findings of this study will be discussed and evaluated, and possible reasons for and contributory factors to the decrease in viridans streptococcal bacteraemia will be proposed.

8.4.2 The influence of underlying malignancy, cytotoxic chemotherapy and empirical antibiotics

Cases of viridans streptococcal bacteraemia were more likely to be associated with a primary diagnosis of acute leukaemia than that of other malignancies (80% versus 20% in the present study), as described previously by others (Cohen *et al.*, 1983; Groot-Loonen *et al.*, 1987; Weisman *et al.*, 1990; Burden *et al.*, 1991; Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994; Richard *et al.*, 1995; Wisplinghoff *et al.*, 1999). Patients with acute leukaemia generally receive more intensive chemotherapy than those with solid tumours, therefore the former group would be expected to experience, to a greater extent, toxic side effects such as oral and/or gastrointestinal mucositis and profound neutropenia. This in turn, may more readily predispose them to viridans streptococcal bacteraemia. A logical conclusion therefore is that the therapy for the malignancy, more than the malignancy itself may be a predisposing factor.

More boys than girls (32 versus 22) developed viridans streptococcal bacteraemia. This finding may be related to the fact that males are affected more often by cancer than are females. Twenty-nine paediatric patients with ALL developed viridans streptococcal bacteraemia compared to 14 with AML. However, as mentioned earlier, AML comprises around 15-20% of all cases of childhood acute leukaemia, therefore children with this disease are at greater risk than those with ALL of

developing viridans streptococcal bacteraemia. Therapy for AML is more intensive than that for ALL, producing profound neutropenia with more severe mucosal toxicity, thus potentially increasing the predisposition to infection by oral organisms such as viridans streptococci. In particular, the frequency of viridans streptococcal bacteraemia was found to be associated with increasing dose of cytosine arabinoside (Section 5.11). Chemotherapy regimens containing high doses of this agent, such as CLASP, or 'intermediate-high' doses, such as MidAC and FLAG are used in the treatment of AML. As detailed in the results section, 32% of total courses of CLASP administered in the unit were associated with the development of viridans streptococcal bacteraemia. Twenty-three percent of courses of MidAC and similarly, 23% of courses of FLAG preceded viridans streptococcal bacteraemia, compared with an association with less than 5% of all courses of DATES (which contains a lower dose of cytosine arabinoside).

Earlier studies demonstrated the relationship between high or intermediate high-dose cytosine arabinoside and the incidence of viridans streptococcal bacteraemia (Sotiropoulos *et al.*, 1989; Kern, Kurrle & Schmeiser, 1990; Weisman *et al.*, 1990; Bochud *et al.*, 1994; Richard *et al.*, 1995). In 1989, Sotiropoulos and co-workers described 14 episodes of viridans streptococcal bacteraemia which occurred after therapy with either continuous or high-dose cytosine arabinoside. Prior to the introduction of these intensive chemotherapeutic protocols, only one case of viridans streptococcal bacteraemia had occurred in the previous 8 years. Several case control studies confirmed a statistically significant association between therapy with high doses of cytosine arabinoside and the incidence of viridans streptococcal bacteraemia (Kern, Kurrle & Schmeiser; Weisman *et al.*, 1990, Bochud *et al.*, 1994; Richard *et al.*, 1995). In a 5-year prospective study of the incidence of viridans streptococcal bacteraemia in bone marrow transplantation recipients (Engelhard *et*

al., 1995), the major risk factor identified was cytosine arabinoside administration in the conditioning regimen ($p \leq 0.01$).

However, an important new aspect of this association emerged as a result of the present work. Detailed analyses of the relationship between administration of the CLASP regimen and incidence of viridans streptococcal bacteraemia, described in Section 6.7.2, revealed an asymmetrical distribution of cases (Figure 6.3), with the association between these two factors much stronger during the first 2 years than during the last four. As mentioned previously, the only significant change in management of haematology/ oncology patients receiving the CLASP regimen (or any other form of chemotherapy), between these two time intervals was the change of empirical antibiotic therapy for episodes of febrile neutropenia, from the combination of ceftazidime plus amikacin to piperacillin/tazobactam plus amikacin. Cotrimoxazole prophylaxis was a constant factor for the majority of cases throughout the period of this study, with rates of resistance to this agent virtually identical prior to the change and following it (73% versus 74%).

All patients who had been treated with the CLASP regimen had received empirical antibiotics for previous episodes of febrile neutropenia. It is possible that the use of an antibiotic with poor activity against viridans streptococci, such as ceftazidime, may have selected for resistant strains which could readily colonize their oral cavities and gastrointestinal tracts. It has previously been discussed that in certain circumstances bacterial loading can occur, and that dominant organisms may become established in the mouths of patients with cancer (Sixou *et al.*, 1998). This phenomenon may have occurred in the patients of the present study, with resistant strains of *S. oralis* predominating.

In contrast, in the mouths and gastrointestinal tracts of patients receiving piperacillin/ tazobactam for empirical therapy, bacterial loading effects may be less likely to occur. Piperacillin/tazobactam is more active against viridans streptococci and would therefore not tend to select for overgrowth of these organisms. In the study of Bradley and colleagues (1999), which also compared ceftazidime with piperacillin/tazobactam as empirical therapy in febrile neutropenia, clinical cases of infection with glycopeptide-resistant enterococci were observed only when carriage rates were high and only during the ceftazidime phase of the study.

A further factor which may have influenced anti-bacterial activity by piperacillin/ tazobactam or ceftazidime is synergy with amikacin. The combination of piperacillin/ tazobactam plus the aminoglycoside antibiotic, gentamicin, has been shown to be synergistic *in vitro* against streptococci (Gould & Milne, 1997). Synergy has also been reported between the extended-spectrum cephalosporin, cefriaxone and the aminoglycoside, netilmicin in a rat model of streptococcal endocarditis (Francioli & Glauser, 1993). Although piperacillin/tazobactam itself demonstrated far superior *in vitro* activity against viridans streptococci to that of ceftazidime, it would have been interesting to determine whether differential synergy existed between the aminoglycoside/β-lactam combinations of the present study.

The recent study of Marron and colleagues (2001) described the prevalence of resistance to cephalosporins among viridans streptococci causing bacteraemia in neutropenic patients with cancer. They found that previous administration of a β-lactam antibiotic (predominantly ceftazidime) was the only factor significantly associated with bacteraemia due to cephalosporin-resistant strains. The present study revealed that higher geometric mean MICs of cephalosporins were associated with isolates of viridans streptococci from blood cultures while empirical therapy was ceftazidime plus amikacin rather than piperacillin/tazobactam plus amikacin. As the

majority of patients in whom viridans streptococcal bacteraemia occurred had received prior courses of ceftazidime during phase 1, this finding concurs with that of Marron and co-workers. The present study also demonstrated that susceptibility to other β-lactam antibiotics may be affected by prior empirical therapy with ceftazidime and also revealed that viridans streptococci isolated from blood cultures of patients after the change in empirical therapy had lower geometric mean MICs for all β-lactam antibiotics tested. Smith (1999) also described a reduction in resistance to antibiotics, associated with a change in prescription from third-generation cephalosporins to piperacillin/tazobactam. Over a four-year period, the overall bacterial resistance pattern in a U.S. hospital changed significantly, with reduction in antimicrobial resistance in Enterobacteriaceae, *Enterococcus* spp. and methicillin-resistant *Staphylococcus aureus.*

In summary, the findings of the present study indicated that viridans streptococcal bacteraemia occurred more often following chemotherapy with regimens containing high-dose cytosine arabinoside, such as CLASP. However the frequency of viridans streptococcal bacteraemia following this form of chemotherapy was significantly reduced when empirical therapy for episodes of febrile neutropenia was changed from ceftazidime plus amikacin to piperacillin/tazobactam plus amikacin, suggesting that multiple inter-related factors may contribute to the development of this infection. Chemotherapy-induced mucositis produces a portal of entry, but bacteraemia may be more likely to occur in the presence of high oral bacterial loads of resistant organisms. Although there were fewer episodes of viridans streptococcal bacteraemia in 1995 than in 1994, this decrease was not statistically significant, and while it is obviously impossible to determine the pattern of infection which would have followed in the absence of the change in empirical antibiotic therapy, the findings of this thesis suggest that the substitution of ceftazidime with piperacillin/tazobactam was influential in the consistent decline in viridans

streptococcal bacteraemia. Almost 15 years ago, Viscoli and colleagues, when discussing the increase in Gram-positive bacteraemia amongst immuno-compromised patients, commented that "Cyclical variation over several decades has been recognized in the type of offending pathogens and may be related to the selective pressure of antibiotics with a modified spectrum of action, such as the cephalosporins" (Viscoli, Van der Auwera & Meunier, 1988).

8.5 Spectrum of symptoms associated with viridans streptococcal bacteraemia

8.5.1 Introduction

The results of this study demonstrated that a variety of symptoms may accompany viridans streptococcal bacteraemia in immunocompromised paediatric patients. In the majority of episodes (70% of total), patients responded readily to antimicrobial therapy with no further complications. Varying degrees of respiratory compromise were associated with the remaining 21 episodes. In eight (12% of total), this feature became so severe that ITU support with mechanical ventilation was required. Septic shock accompanied ARDS in 5 of these severe cases.

As mentioned earlier, one aim of the present work was to identify why there existed such a spectrum of symptoms in patients suffering from viridans streptococcal bacteraemia. Previous authors had described a severe form of viridans streptococcal bacteraemia sometimes referred to as "viridans streptococcal shock syndrome" (Cohen *et al.*, 1983; Henslee *et al.*, 1984; Sotiropoulos *et al.*, 1989; Weisman *et al.*, 1990; Classen *et al.*, 1990; Villablanca *et al.*, 1990; Elting, Bodey & Keefe, 1992; Martino *et al.*, 1995). However, in contrast to the present study, most had identified *S. mitis* as the causative organism in the severe form of sepsis (Elting, Bodey & Keefe, 1992; Bochud *et al.*, 1994; Carratala *et al.*, 1995; Engel, Kern &

Kern, 1996; Kern, Kurrle & Schmeiser, 1990). This more recent work demonstrated a predominance of *S. oralis*, which may be related to the advances in identification and taxonomy of viridans streptococci. However the distribution of species of viridans streptococci in the oral cavity varies with age (as discussed in Section 1.3.1) and therefore species from blood culture may also vary from one study group to another. The present study demonstrated that the identification of *S. oralis per se* did not necessarily predict severity of infection.

8.5.2 Complications associated with viridans streptococcal bacteraemia: the influence of chemotherapeutic agents, empirical antibiotics and concomitant infections

In addition to its association with the actual incidence of viridans streptococcal bacteraemia (Sotiropoulos *et al.*, 1989; Kern, Kurrle & Schmeiser, 1990; Weisman *et al.*, 1990; Bochud *et al.*, 1994; Richard *et al.*, 1995), the use of chemotherapy regimens containing high-doses of cytosine arabinoside is also the most commonly quoted risk factor for development of the severe form of this infection (Sotiropoulos *et al.*, 1989; Kern, Kurrle & Schmeiser, 1990; Bochud *et al.*, 1994). Twelve of the 21 episodes (57%) of the present study with respiratory complications occurred following therapy with either high- or 'intermediate-high' dose cytosine arabinoside, with 3 of these progressing to ARDS and septic shock and two to ARDS alone. However, it must be acknowledged that not all patients treated with high or intermediate-high dose cytosine arabinoside developed viridans streptococcal bacteraemia and not all who did, developed respiratory complications or shock.

During the first two years of the study, both a higher frequency of viridans streptococcal bacteraemia and increased severity of associated symptoms were found amongst patients who received the CLASP regimen when compared with those receiving this regimen during the later 4 years. Five of these cases, from 1995

- 1996 developed moderate to severe respiratory complications, (with two also developing septic shock), compared with one case from 1997 - 2000 with mild respiratory symptoms only (patient No. 44).

It has been shown that even organisms with low pathogenic potential, such as coagulase-negative staphylococci can induce the release of cytokines and produce shock if inoculated experimentally in large doses (Wakabayashi *et al.*, 1991). The combination of large numbers of viridans streptococci and their persistence in the bloodstream due, in part, to resistance to first line empirical therapy in the early years of the present work, may have provided these organisms with a distinct pathogenic advantage.

Preparations of cell walls from viridans streptococci have been shown to induce the production of TNF-α and IL-6 from human monocytes (Heumann *et al.*, 1994). Lipoteichoic acids derived from viridans streptococci may also induce the production of proinflammatory cytokines (Bhakdi *et al.*, 1991). The study of Soto and co-workers (1998) demonstrated that cell free extracts of viridans streptococci could induce TNF-α, TNF-β and IL-8 from human peripheral blood mononuclear cells *in vitro*. In certain clinical settings, infecting viridans streptococci may also be able to elicit the release of proinflammatory cytokines *in vivo*, as suggested by the work of Engel and colleagues (1996), which demonstrated that TNF-α and IL-6 could be detected from serum of patients with "lethal viridans streptococcal sepsis". If the bacterial load is high, sufficient levels of cytokines may be produced to result in the development of septic shock. Septic shock did not accompany any episodes of viridans streptococcal bacteraemia in the present study, after empirical therapy was changed to piperacillin/tazobactam.

Additional factors however, must be considered when investigating the aetiology of ARDS in the patients of this study. The definition of the American-European Concensus Conference on ARDS requires two positive criteria – bilateral infiltrates on chest radiograph and arterial hypoxaemia, and one negative criterion – absence of clinical evidence of cardiogenic pulmonary oedema (Bernard *et al.*, 1994). Historically ARDS was considered to be a neutrophil mediated disease. When associated with septic shock, it was postulated that within the lung, cytokine induced changes led to profound hypoxaemia and respiratory failure. TNF-α, IL-8 and C5a all contributed to neutrophil chemotaxis, whilst the upregulation of endothelial cell adhesion molecules and neutrophil integrins facilitated the passage of fluid and leucocytes from the circulation into the lung interstitium and alveolar spaces (Sriskandan & Cohen, 1995).

However, ARDS also occurs in neutropenic patients (Braude *et al.*, 1985; Ognibene *et al.*, 1986; Sivan *et al.*, 1990) as in the present study, therefore there must also exist neutrophil independent mechanisms capable of producing this condition. A further complicating factor is that although sepsis is a major factor associated with the development of ARDS, other events such as aspiration, severe trauma and pneumonia can also predispose to this condition (Bagshaw & Gajraj, 1999; Ware & Matthay, 2000). It has also been reported that the risk of developing ARDS increases when more than one factor is present (Kollef & Schuster, 1995).

A variety of chemotherapeutic agents such as bleomycin, busulphan, carmustine, cyclophosphamide and cytosine arabinoside are known to induce pulmonary dysfunction (Haupt, Hutchins & Moore, 1981; Andersson *et al.*, 1985; Tjon A Tham *et al.*, 1987; Seibert & Lewis, 1992). Therefore, any one of these agents could potentially contribute to the development of ARDS in cancer patients. However,

sensitivity to these drugs may vary from patient to patient and what, if any is the role of viridans streptococcal bacteraemia?

The work of Guiot and colleagues (1990), demonstrated that prophylaxis with penicillin reduced not only the incidence of viridans streptococcal bacteraemia in patients treated with this agent, but also that of respiratory complications. This observation may, in part, relate to that of the present study, where a change to empirical therapy with superior activity against viridans streptococci was temporally associated with a decrease in incidence of viridans streptococcal bacteraemia and pulmonary complications following cytotoxic therapy with high-dose cytosine arabinoside. One explanation for these findings is that additional stress, such as viridans streptococcal bacteraemia is more likely to contribute to the development of respiratory distress in patients treated with agents with pulmonary toxicity. An extension of this hypothesis is that, the higher the bacterial load and the longer these organisms are allowed to persist in the bloodstream, the greater the additional stress and the more severe the clinical symptoms.

Five of the seven cases with ARDS in the present study also featured septic shock, therefore sepsis-driven induction of cytokines may have influenced the development of pulmonary complications. However, in all of these cases, multiple predisposing factors for respiratory compromise were present. Three had recently received therapy with high-doses of cytosine arabinoside. In one of these three, pulmonary CMV infection was diagnosed and in another, *P. carinii* infection. Another patient with ARDS and septic shock had a history of recurrent chest infections and had received chemotherapy some time earlier for a different malignancy. Finally, the development of ARDS in the baby with viridans streptococcal bacteraemia and septic shock on two occasions may also have been associated with pulmonary

immaturity, with increased sensitivity to the toxic effects of intensive chemotherapy for AML.

A variety of viruses may contribute to the development of severe respiratory complications in immunocompromised patients. On chest X-ray, viral pneumonitis or that produced by *P. carinii* may resemble the early ARDS-type pattern (Sivan *et al.*, 1990). Very few earlier reports of viridans streptococcal bacteraemia, included details of concomitant infection by these agents (Groot-Loonen *et al.*, 1987) or mentioned their exclusion as contributory to respiratory complications (Tjon A Tham, 1987; Dybedal & Lamvik, 1989). Today, immunofluorescence and molecular techniques, such as PCR have lead to improved diagnosis of respiratory pathogens. The findings of this study in which 5 of the 21 episodes (*i.e.* 24%) with respiratory complications featured concomitant infection with viruses or *P. carinii* demonstrate that these organisms may play a significant role.

One of the two patients with viridans streptococcal bacteraemia who developed ARDS in the absence of septic shock had received therapy with high-dose cytosine arabinoside and had no other predisposing factors. In the other, pulmonary infiltration by lymphoma was strongly suspected. Diffuse bilateral infiltrates on radiography may also result from lung infiltration by certain malignancies such as lymphoma (Seibert & Lewis, 1992). Histological confirmation could not be obtained because of the very poor clinical condition of the child. Infection by Picorna virus (diagnosed by viral PCR) in this compromised patient may also have contributed to respiratory complications.

The development of and range of severity of features accompanying viridans streptococcal bacteraemia, appear to depend on several highly interrelated elements which may co-exist in the immunocompromised host. An awareness of the complex

factors involved and the ability to identify them is crucial to the optimal management of these patients.

CHAPTER 9

MANAGEMENT AND PREVENTION OF VIRIDANS STREPTOCOCCAL BACTERAEMIA – TODAY AND TOMORROW

MANAGEMENT AND PREVENTION OF VIRIDANS STREPTOCOCCAL BACTERAEMIA – TODAY AND TOMORROW

9.1 Antibiotic therapy

Viridans streptococci have now become a rare cause of bacteraemia in immunocompromised patients at RHSC. Empirical antibiotic therapy for episodes of febrile neutropenia is still amikacin plus piperacillin/tazobactam, a combination which remains active against a wide variety of bacteria responsible for infection in this patient group.

As detailed in Chapter 6, since the change in empirical therapy for episodes of febrile neutropenia, MICs of all β-lactam antibiotics against isolates of viridans streptococci are generally lower. However if resistance to piperacillin/tazobactam were to develop, which new antimicrobial treatment options would be considered?

From the *in vitro* results of this study, one would expect that meropenem would be a useful option, because of its extremely low MIC values against all isolates of viridans streptococci. Other authors have also reported the excellent *in vitro* activity of carbapenem antibiotics against these organisms (Potgieter *et al.*, 1992; McWhinney *et al.*, 1993; Alcaide *et al.*, 1995; Teng *et al.*, 1998; Marron *et al.*, 2001). However it is unlikely that meropenem would become part of first line empirical therapy at RHSC. Its role at present is that of a reserve agent – for treatment of infection by coliforms which produce extended spectrum β-lactamases.

Cefpirome may be another option. This fourth generation cephalosporin antibiotic demonstrated considerable *in vitro* activity against viridans streptococci and has been used by others in the setting of febrile neutropenia (Paredes & South, 1997).

An evaluation of *in vitro* susceptibility of Gram-negative bacilli to cefpirome is currently being undertaken by the author for future reference.

The present study demonstrated the effective *in vitro* activity of vancomycin against viridans streptococci. However, because of its nephrotoxic potential (Kibbler *et al.*, 1989; EORTC, 1991) and the possibility that its widespread use may lead to the selection of glycopeptide-resistant *Enterococcus* spp. or *S. aureus* it is unlikely that this antibiotic would be considered as part of first line empirical therapy at RHSC. Rather, it will remain a reserve agent for treatment of microbiologically documented infection by certain Gram-positive bacteria.

The newer antibiotics tested in this study, quinupristin/dalfopristin and linezolid both displayed excellent *in vitro* activity against viridans streptococci with no cross resistance with the other antimicrobial agents tested. Several recent reports describe the considerable *in vitro* activity of these new agents against Gram-positive bacteria (Schouton & Hoogkamp-Korstanje, 1997; Barry, Fuchs & Brown, 1998; Wise *et al.*, 1998; Johnson, Warner & Livermore, 2000; Fines & Leclerq, 2000; Henwood *et al.*, 2000; Cercenado, Garcia-Garrote & Bouza, 2001; Gemmell *et al.*, 2001; Kennedy *et al.*, 2001; Tubau *et al.*, 2001). The few publications which include susceptibility testing against viridans streptococci confirm the findings of the work described in this thesis (Schouton & Hoogkamp-Korstanje, 1997; Barry, Fuchs & Brown, 1998; Johnson, Warner & Livermore, 2000; Cercenado, Garcia-Garrote & Bouza, 2001). At present only quinupristin/dalfopristin is licensed for treatment of infection in paediatrics.

It is impossible to predict whether these agents will ultimately be required for treatment of infection caused by Gram-positive bacteria other than strains of *Enterococcus* spp. or *S. aureus* which are resistant to multiple antibiotics. However it

is reassuring to know that over the time interval of this project two new antibiotics with excellent activity against Gram-positive bacteria have become available.

9.2 Antibiotic prophylaxis

Antibacterial prophylaxis is used less commonly in paediatric patients with malignant disease than in their adult counterparts. As mentioned earlier, the only form of antibiotic prophylaxis used routinely at RHSC, for high-risk groups, is cotrimoxazole, as prophylaxis against *P. carinii* pneumonia. As discussed previously, its use has been reported by others as a risk factor for the development of viridans streptococcal bacteraemia (Elting, Bodey & Keefe, 1992). Although the results of the present study suggest that the prophylactic administration of cotrimoxazole may be associated with high rates of resistance to this agent amongst viridans streptococci isolated from blood culture, its use will continue, as the benefits significantly outweigh the disadvantages. As this study demonstrated, both the incidence and severity of symptoms associated with viridans streptococcal bacteraemia decreased throughout the period of this study, in spite of the continuing use of cotrimoxazole.

Quinolones are not generally used for prophylaxis or treatment of infection in paediatric patients because of the arthropathogenic potential of these agents in juvenile animals (Schluter, 1987). Prophylaxis using these agents has been reported as a risk factor for viridans streptococcal bacteraemia in adult patients (Kern, Kurrle & Schmeiser, 1990; Classen *et al.*, 1990).

The results of this study demonstrated considerable rates of high-level resistance to penicillin in spite of the fact that penicillin is neither used for therapy or prophylaxis of infection in this patient population. Such resistance rates may be related, in part, to the use of other β-lactam agents as empirical therapy. In addition, it has been shown that even healthy children harbour more penicillin resistant-viridans

streptococci than adults with cancer (Guiot, Corel & Vosen, 1994). Again, in contrast to the situation with adult cancer patients (Spanik *et al.*, 1997), penicillin is unlikely to be used as antibacterial prophylaxis for paediatric patients with cancer at RHSC.

9.3 Pre-emptive antibiotic therapy

Future research in the field of viridans streptococcal infection in immunocompromised patients should attempt to determine why some patients develop several episodes of viridans streptococcal infection. The present study demonstrated that five patients developed 2 episodes of viridans streptococcal bacteraemia, two patients developed 3 and two developed four. As early as 1978, Pizzo and colleagues reported three discrete episodes of viridans streptococcal bacteraemia in one patient with cancer. Similar findings have been described by others (Sotiropolous *et al.*, 1989; Weisman *et al.*, 1990; Gamis *et al.*, 2000). Recent investigation has demonstrated that multiple episodes in the one patient may be due to either the same isolate or different isolates (Wisplinghoff *et al.*, 1999). Pre-emptive antibiotic therapy for such patients may be appropriate.

9.4 Dental and oral assessment

Several studies have focused on the oral health of children with malignancy, and have demonstrated that considerable dental and mucosal disease often exists at diagnosis, as well as during anti-cancer therapy (Berkowitz et al., 1987; Fayle & Curzon, 1991; Clarkson & Eden, 1998; Lucas, Roberts & Beighton, 1998). In view of the subsequent very high incidence of complications as a result of oral compromise in such patients, it is clear that preventative approaches are important. However, these should ideally be tailored to the individual patient and his/her treatment schedules, as elective dental procedures and manipulation of the oral tissues should not be undertaken when the patient is receiving chemotherapy or is

myelosuppressed. A thorough oral examination is important in the overall pre-chemotherapy evaluation. Existing and potential dental and oral disease should be identified and, if possible, eradicated and a plan devised for continuing management during, and following therapy.

9.5 Mouth care protocols

In the past, mouth care protocols for patients receiving cytotoxic chemotherapy tended to be ritualistic with the same regimen being used for patients with different levels of oral compromise or risks of infection. However, today, in many centres, procedures are being reviewed to suit individual patients or patient groups (Gibson, Horsford & Nelson, 1997). The mouth care protocol (Appendix II) used throughout the period of this study remained essentially unchanged, therefore were unlikely to have significantly influenced the decrease in incidence of viridans streptococcal bacteraemia. However, the demonstration, by PFGE, that the predominant strain of *S. oralis* which colonized the oral mucosa, gingivae and teeth of one patient, and which had caused one episode of bacteraemia, could also readily colonize his toothbrush, indicated that tools for mouth care could potentially become vectors of infection. Therefore, although soft toothbrushes may now be regarded as the most efficient (non-specialist) tool for removing plaque and debris from the teeth and mouth (Campbell, Evans & MacTavish, 1995; Gibson, Horsford & Nelson, 1997), they should be replaced regularly to prevent excessive accumulation of oral bacteria on their bristles.

9.6 Recent developments

The prevention and management of oral mucositis remains unsatisfactory. However, some promising results have been produced using sucralfate, a complex of sucrosulphate and aluminium hydroxide. In a recent double-blind randomized trial in

patients undergoing autologous or allogeneic bone marrow transplantation, the prophylactic administration of this agent was found to be associated with a lower frequency of severe mucositis and also of diarrhoea (Castagna *et al.*, 2001). In contrast, in earlier studies, sucralfate did not ameliorate radiation-induced mucositis (Makkonen *et al.*, 1994; Franzen *et al.*, 1995). Other recent studies have attempted to evaluate the efficacy of growth factors (G-CSF and GM-CSF), used locally as a mouth wash (Sprinzl *et al.*, 2001; Hejna *et al.*, 2001). However these studies were small, with conflicting results. Methods of detecting and monitoring mucosal barrier injury are now being developed and these will be useful in assessing new therapeutic agents (Blijlevens, Donnelly & De Pauw, 2000).

9.7 Concluding comments and a 'wider perspective'

During the time interval of this study, the prevalence of viridans streptococcal bacteraemia in paediatric cancer patients attending RHSC has decreased, as has the severity of symptoms associated with this infection. The intervention of a change in empirical antibiotic therapy in the present study may have played a part. Of interest, similar decreases in viridans streptococcal bacteraemia have occurred in other haematology/oncology centres in the U.K. and abroad. Perhaps the growing trend towards the use of carbapenems or piperacillin/tazobactam rather than ceftazidime for empirical therapy of febrile neutropenia, may be a factor in the general decline in cases of this infection. While the role of antibiotic prophylaxis in selection of resistant strains has been studied extensively, more investigations into the part played by previous courses of empirical antibiotics are required.

Studies to investigate possible virulence factors of viridans streptococci continue (Tarelli *et al.*, 1998; Byers *et al.*, 1999; Whatmore *et al.*, 2000). While subtle mechanisms, such as mannosidae and sialidase activities may contribute to

proliferation amongst members of the mitis group, there is no evidence of production of conventional bacterial exotoxins by these organisms.

The pathogenesis of viridans streptococcal bacteraemia in paediatric immuno-compromised patients is an excellent example of the interplay of the effects of cytotoxic chemotherapy, the prior use of antibacterial agents and a disturbance in microbial homeostasis. Thankfully, management of this particular infection is less of a challenge today. However as further advances in the management of malignant disease result in an ever-increasing number of immunocompromised patients, new challenges will arise.

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APPENDICES

APPENDIX I

CHEMOTHERAPY REGIMENS

The following is not a comprehensive list of the many chemotherapy protocols used at RHSC. Rather, it provides information on the treatment regimens relevant to the present study.

Treatment of AML (from MRC protocol - AML 12)

Induction Chemotherapy

Each induction schedule comprises two courses of chemotherapy – either ADE or MAE. Remission status is determined after each course. If remission is not confirmed after the second course, the patient is off protocol for standard MRC AML therapy and may be entered into the current MRC refractory/relapse trial or can receive alternative therapy.

<u>ADE schedule</u>

Course 1 ADE 10 + 3 + 5

Cytarabine 100 mg/m^2 12-hourly by i.v. push on days 1-10 inclusive.

Daunorubicin 50 mg/m^2 daily by 6 hour i.v. infusion on days 1, 3 and 5.

Etoposide 100 mg/m^2 daily by 4 hour i.v. infusion on days 1-5 inclusive.

Course 2 ADE 8 + 3 + 5

Cytarabine 100 mg/m^2 12-hourly by i.v. push on days 1-8 inclusive.

Daunorubicin 50 mg/m^2 daily by 6 hour i.v. infusion on days 1, 3 and 5.

Etoposide 100 mg/m^2 daily by 4 hour i.v. infusion on days 1-5 inclusive.

<u>MAE schedule</u>

Course 1 MAE 3 + 10 + 5

Mitozantrone 12 mg/m^2 daily by 6 hour i.v. infusion on days 1, 3 and 5.

Cytarabine 100 mg/m^2 12-hourly by i.v. push on days 1-10 inclusive.

Etoposide 100 mg/m^2 daily by 4 hour i.v. infusion on days 1-5 inclusive.

Course 2 MAE 3 + 8 + 5

Mitozantrone 12 mg/m^2 daily by 6 hour i.v. infusion on days 1, 3 and 5.

Cytarabine 100 mg/m^2 12-hourly by i.v. push on days 1-8 inclusive.

Etoposide 100 mg/m^2 daily by 4 hour i.v. infusion on days 1-5 inclusive.

In children less than 1 year old, the above doses are reduced by 25%.

Consolidation chemotherapy

Course 3 MACE

Amsacrine 100 mg/m^2 daily by 1 hour i.v. infusion (in 5% dextrose) on days 1-5 inclusive.

Cytarabine 200 mg/m^2 daily by continuous i.v. infusion on days 1-5 inclusive

Etoposide 100 mg/m^2 daily by 4 hour i.v. infusion on days 1-5 inclusive.

In children less than 1 year old, the above doses are reduced by 25%.

Additional consolidation chemotherapy is dependent on randomisation and the availability of a matched sibling donor. Courses may include the following:

MidAC

Mitozantrone 10 mg/m^2 by 6 hour i.v. infusion on days 1-5 inclusive.

Cytarabine 1.0 g/m^2 12-hourly by 2 hour i.v. infusion on days 1-3 inclusive.

CLASP

Cytarabine 3 g/m^2 12-hourly by 3 hour i.v. infusion on days 1, 2, 8 and 9.

Asparaginase 6000 units/m^2 s.c. on days 2 and 9, three hours after Completion of 4th and 8th doses of cytarabine.

MRC Chemotherapy Protocols for the Treatment of ALL
(UKALL XI, UKALL 97)

Standard Remission Induction Chemotherapy
(Days 1- 28)

(a) Allopurinol	100 mg/m^2 orally thrice daily should commence 24 hours before cytotoxic therapy is started and continue for 14 days.
(b) Prednisolone	40 mg/m^2 oral daily in three divided doses from the beginning of Week 1 to the end of week 4.
(c) Vincristine	1.5 mg/m^2 (maximum single dose 2 mg) i.v. weekly for five weeks, starting on day 1 of the first week.
(d) L-asparaginase	6000 units/m^2 s.c. three times a week, for 9 doses. (First dose on Day 4 of Week 1)
(e) I.T. Methotrexate	On days 1 and 8 (Dose related to CSF volume)

Consolidation Therapy

The 'DATES' regimen

'DATES' chemotherapy (two courses per patient) was used as intensification therapy for ALL patients at RHSC until the end of 1999.

Prednisolone	40 mg/m^2 oral daily for one week
Vincristine	1.5 mg/m^2 i.v. (maximum 2 mg) as a single dose on day 1
Daunorubicin	45 mg/m^2 i.v. as an infusion over 6 hours on both days 1 and 2.
Etoposide	100 mg/m^2 i.v. by 4 hour infusion on days 1-5 inclusive.
Cytarabine	100 mg/m^2 i.v. ,12 hourly, by bolus injection on days 1-5 inclusive.
Thioguanine	80 mg/m^2 orally, daily on days 1-5 inclusive.
i.t. Methotrexate	On day 1 (dose by age)

Third intensification block

(a) Dexamethasone	10 mg/m^2/day oral for 10 days and then tailed over 4 days.		
(b) Vincristine	1.5 mg/m^2 i.v. (maximum of 2 mg) on day 1 of weeks 35, 36, 37 and 38.		
(c) L-Asparaginase	6,000 u/m^2 s.c.	days 4, 6	(week 35)
		days 1, 3, 5	(week 36)
		days 1, 3, 5	(week 37)
		day 1	(week 38)
(d) I.T. Methotrexate	On day 1 of week 35 and 39.		
(e) Cyclophosphamide	600 mg/m^2 i.v. day 1 week 39 and 41.		
(f) Cytarabine	75 mg/m^2 i.v. or s.c. daily on days 1 – 4 of weeks 39, 40, 41, 42.		
(g) Thioguanine	60 mg/m^2 daily weeks 39-42 inclusive		

Extract from MRC ALL Regimen B (ALL 97 – modified 1999)

-Used at RHSC from the beginning of 2000.

Consolidation

Cyclophosphamide	1,000 mg/m^2 i.v. over 20-30 minutes on days 1 & 15.
Cytarabine	75 mg/m^2/day by i.v. push or subcutaneously. Sixteen doses in four pulses of 4 days each. Days 2-5, 9-12, 16-19 and 23-26.
Mercaptopurine	60 mg/m^2/day p.o. at least one hour after evening meals on days 1-28.
I.T. Methotrexate	once a week for 4 doses on days 1, 8, 15, and 22. (Dose by age.)

Extract from MRC ALL Relapse Protocol

Consolidation Phase

Days 1-15	
Vincristine	1.5 mg/m^2 i.v. (maximum 2 mg) on Day1.
Etoposide	150 mg/m^2 by 4 hour i.v. infusion. Days 1, 4, 8,11.
Cytarabine	300 mg/m^2 by 1 hour i.v. infusion. Days 1, 4, 8, 11.
I.T. Methotrexate	Day1 (Dose: age dependent)
Days 15-28	
Dexamethasone	10 mg/m^2 Days 15-28 orally.
	Tail over 48 hours.
Asparaginase	10,000 i.u./m^2 i.m./s.c. Days 15, 18, 22, 25.
Epirubicin	50 mg/m^2 i.v. Days 15 and 22.
Vincristine	1.5 mg/m^2 i.v. Days 15 and 22.
Days 29-42	
I.T. Methotrexate	Days 29 and 36 (dose : age dependent).
Thioguanine	60 mg/m^2 orally daily. Days 29-42 inclusive.
Cytarabine	75 mg/m^2 s.c./i.m./i.v. Days 29, 30, 31, 32.
	Days 36, 37, 38, 39.
Cyclophosphamide	1 g/m^2 i.v. given at the start of week 5, *i.e.* day 29.
	- infused over 1 hour.

Extract from the FLAG Regimen

Consolidation regimen

Fludarabine	25 mg/m^2 daily by 4 hour i.v. infusion for 4 days, (4 hours prior to each daily infusion of cytarabine).
Cytarabine	2 g/m^2 daily by 4 hour i.v. infusion for 4 days, (beginning 4 hours after the start of the infusion of fludarabine phosphate).
G-CSF	0.5 miu/kg daily by i.v./s.c. injection beginning 24 hours prior to the first administration of fludarabine until count recovery.

Extracts from UKCSSG NHL Protocols

COPADM 2 (Protocol 903)

Vincristine	2 mg/m^2 i.v. (maximum 2 mg) Days1and 6.
Methotrexate (H.D)	8 g/m^2 i.v. Day 1.
Folinic acid	Days 2-4.
I.T. Methotrexate	8-15 mg (by age). Days 1, 3, 5.
I.T. Hydrocortisone	8-15 mg (by age). Days 1, 3, 5.
I.T. Cytarabine	16-30 mg (by age). Days 1, 3, 5.
Cyclophosphamide	1 g/m^2/day i.v. (in 2 injections) Days 2-4.
Mesna infusion	500 mg/m^2/day i.v. Days 2-4.
	+ 100 mg/m^2 bolus i.v. Day 2.
Doxorubicin	60 mg/m^2 i.v. Day 2.
Prednisolone	60 mg/m^2/day p.o. Days 1-5.

CYT/ETOP (Protocol 903)

Cytarabine	(over 12 hours)	50 mg/m^2 i.v. Days 1-5 (from 20.00 – 08.00 hours).
Cytarabine	(over 3 hours)	3 g/m^2 i.v. Days 1-4 (from 08.00 – 11.00 hours).
Etoposide	(over 2 hours)	200 mg/m^2 i.v. Days 1-4 (from 14.00 – 16.00 hours).

CYM

Methotrexate	3000 mg/m^2 in 500mls/m^2 dextrose as i.v infusion over 3 hours on day 1.
Folinic acid	15 mg/m^2 orally every 6 hours for a total of 12 doses – starting at 24 hours from the start of the methotrexate infusion
Cytarabine	100mg/m^2 in 1000 mls/m^2 dextrose saline as infusion over 24 hours – days 2-6.
i.t. Methotrexate	8 – 15 mg by i.t. injection day 2 (dose varies with age)
i.t. Cytarabine	15 – 30 mg by i.t. injection on day 7 (dose varies with age)
i.t. Hydocortisone	8 – 15 mg by i.t. injection on day 2 and 7 (dose varies with age)

Extract from MRC Trial for Therapy of Osteosarcoma (MRC BO 06)

Conventional therapy arm

Regimen 1 – cisplatin (CDDP) + doxorubicin (DOX)

This therapy comprises 2 pre-operative cycles of CDDP + DOX at 3-weekly intervals followed by surgery scheduled for week 6. Two weeks after surgery a further 4 cycles of CDDP + DOX are given at 3-weekly intervals.

The following comprises one treatment cycle:

Doxorubicin 25 mg/m^2/day	Days 1-3 - i.v. 4 hour infusion.
Cisplatin 100 mg/m^2	Day 1. 24 hour infusion.

Extract from OPEC/OJEC protocol for therapy of neuroblastoma

OJEC

0 hours	Vincristine 1.5 mg/m^2 (maximum dose 2 mg) i.v. bolus
	Cyclophosphamide 600 mg/m^2 i.v. bolus
	Etoposide 200 mg/m^2 in 500 ml/m^2 0.9% sodium chloride infused i.v. over 4 hours
4 hours	Carboplatin 500 mg/m^2 in total volume of 100 ml/m^2 of 5% glucose infused i.v. over 1 hour.

Extract from UKCCSG Malignant Mesenchymal Tumour Study for metastatic disease (1998)

Week 6 - High dose cyclophosphamide (course 1 (Day 0))

Pre-hydration	-according to protocol
Cyclophosphamide	2 g/m^2 on days 1,2 and 3 of the monotherapy sequence as a 1 hour infusion with Mesna uroprotection

Mini-BEAM Salvage Chemotherapy for Relapsed Large Cell Anaplastic Lymphoma

BCNU 60 mg /m^2	Day 1.
Etoposide 75 mg/m^2	Days 2-5.
Cytarabine 100mg /m^2 q12 h	Days 2-5.
Melphalan 30 mg/m^2	Day 6.

APPENDIX II

MOUTH CARE PROTOCOL

The following is a copy of the protocol issued to patients/parents during the study period.

MOUTH CARE FOR YOUR CHILD

Following chemotherapy or radiotherapy your child may be at risk of developing a sore mouth, as the fast growing cells in his/her mouth are broken down.
The following **may** develop - Thrush (a fungal infection)
- Ulcers
- Cold sores (a viral infection called Herpes)
- Bleeding gums

It is important that good dental hygiene continues throughout your child's treatment. A dental hygienist will usually visit the ward once a week to discuss mouth care with you. Normal teeth brushing and mouth care should be continued unless you are instructed to do otherwise by a doctor or nurse.

When your child has a low blood count he/she will be started on a special mouth care regimen (see below). A low white cell count will mean that your child may be more likely to develop mouth infections and a low platelet count **may** cause the gums to bleed.

The following mouth care should be carried out by your child 4 times a day, that is, after meals and before going to bed at night.

1. Brush teeth with a pink sponge and a pea-sized amount of Corsodyl dental gel. The sponges will be supplied and they must be discarded after use.
2. Rinse the mouth with Corsodyl mouth wash. Swirl the mouth wash around the mouth. There are two flavours of mouth wash:
   - Original flavour (pink)
   - Mint flavour (clear)

   Corsodyl helps prevent bacterial infections.
3. **WAIT 20 MINUTES** before using Nystatin if this has been prescribed. This yellow medicine is swirled around the mouth and swallowed. Nystatin helps prevent fungal infections. This is also available in pastille form. Your child may prefer an orange flavoured gel called Daktarin which also helps prevent fungal infections. Ask a nurse if you think your child would prefer this.
4. If possible wait a further 20-30 minutes before giving your child anything to eat or drink.

Sometimes your child may be prescribed medications called fluconazole or itraconazole. These also help prevent fungal infections and Nystatin or Daktarin may not be required.

Watch for sore areas or white patches in your child's mouth and inform a doctor or nurse if any should develop.

If your child does get a sore mouth, Difflam mouth wash may be useful. This numbs the mouth to help relieve pain and discomfort. It may be useful to use it 15-20 minutes before meals. Pain killers will also be given if your child does develop a sore mouth.

Occasionally chemotherapy can distort the taste. This can mean a reduced sensitivity to taste, an unusual or unpleasant taste or absence of taste. These problems should resolve once the treatment has stopped.

Produced by Senior Staff Nurse Debbie McClure, Yorkhill NHS Trust.