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The clinical spectrum of viridans group streptococcus bacteraemia in paediatric patients at Chris Hani Baragwanath Academic Hospital.

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Declaration

I **Nkosinathi Sifiso Shongwe,** declare that this MMED research report is my own original research done under the supervision of Prof Karen Petersen and Dr Fikile Mabena, it is being submitted in partial fulfilment of Master of Medicine in paediatrics degree in the department of Paediatrics and Child Health University of the Witwatersrand, Johannesburg. This document has not been for any degree or examination at this or any other University.



20/11/2023

Dedication

I dedicate this work to my wife Nokuphila Shongwe who shows me love and patience everyday and has supported me throughout. My children Lunathi and Lwaluhle who have kept me sane and have shown me unconditional love. My mother for her prayers and emotional support. To all my siblings and in-laws who always have me in their prayers.

In memory of my dad who always believed in me and inspired me.

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The National Health Laboratory Services NHLS for providing the laboratory data which was required for the study.

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Abstract

Background: Viridans group streptococci (VGS) are often considered organisms of low virulence; however, infection can result in clinically significant sepsis and life-threatening complications in paediatric patients.

Objectives: To describe the spectrum of clinical presentation of VGS bacteraemia in paediatric patients, to analyse risk factors, and to describe the antibiotics resistance patterns of VGS.

Methods: Cultures of VGS in paediatric patients admitted to Chris Hani Baragwanath Academic Hospital in 2019 were retrieved from NHLS data base. Data was extracted from archived clinical records and analysed. Sepsis scores were calculated at the time of bacteraemia.

Results: 133 cultures were identified; 64 (48.1%) polymicrobial cultures (64) and no records 4 (0.03%) were excluded; 65 (48.9%) were analysed. The median age was 1.5 months (range 0.03 to 168, Interquartile range (IQR) 0.2 to 13.25), 27/65 (42%) were neonates. The median duration of hospitalisation was seven days (IQR 3 to 21). The commonest diagnoses were neonatal sepsis 30.8% (n=20) and pneumonia 28% (n=18). The systemic inflammatory response syndrome (SIRS) score was ≥2 in 57% (16/28) patients; paediatric sequential organ failure assessment (pSOFA) score was >2 in 10/24 (42%). Fifty-seven (88%) patients were discharged; three (5%) required ICU admission and 8/65 (12.3%) died. Malnutrition was present in 50% of patients who died. Cephalosporins and penicillin had susceptibility of 89% and 55% respectively.

Conclusion: VGS bacteraemia was common in neonates, and pneumonia was a common presentation in this cohort outside the neonatal period. VGS bacteraemia was associated with morbidity and deaths in this cohort.

Contribution: VGS should be considered a significant organism when cultured and routine antibiotic susceptibility testing should be performed. Prospective studies are recommended.

Introduction

The viridans group streptococci (VGS) are Gram-positive cocci in chains (1,2) with at least 30 recognised species (2). They are part of the normal flora of the oropharyngeal, urogenital, gastrointestinal tracts (2) and skin (3) . When cultured from sterile sites the question of their pathogenicity often occurs (4). Although still largely considered to be a contaminant, it can cause life-threatening conditions in neonates, children and even adults (1,5); as many as 32% isolates have been shown to be of clinical significance (5). It can cause invasive disease in immunocompromised paediatric patients (5), such as HIV infected patients, neonates and cancer patients undergoing chemotherapy or stem cell transplant (2,6,7). There is very limited literature on this organism other than in patients with malignancies undergoing chemotherapy or stem cell transplant.

Methods to determine if VGS cultured are true pathogens or contaminants include; i) the number of positive blood cultures in a set, (in true bacteraemia there is often multiple positive cultures of the same organism) (8), ii) the time to positivity of cultures (cultures which become positive after three to five days are more likely to be contaminants) (9), and iii) clinical features like fever, hypothermia, low or high white cell count and hypotension predict true infection as opposed to contaminant (10).

There is ongoing debate on the definition of sepsis. The third consensus for sepsis which was published in 2016 defines sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection (11). Clinical sepsis scores may be used as indicators of sepsis in patients. The systemic inflammatory response syndrome (SIRS) score requires two of four criteria and proven infection, the criteria being: i) fever or hypothermia, ii) tachycardia, iii) tachypnoea and iv) leucocytosis or leukopenia (11) . Infection is defined by laboratory documentation of a pathogen by positive culture, tissue stain or PCR test, or a clinical syndrome associated with a high probability of infection (12). The paediatric sequential organ failure assessment (pSOFA) is a better predictor for mortality and has a higher sensitivity for severe infection compared to SIRS (13). The pSOFA score has six organ specific criteria, with each criterion scored from 0 to 4 according to severity with a maximum score of 24. The scoring criteria are i) renal function

(creatinine), ii) cardiovascular (mean arterial pressure), iii) respiratory (PaO2:FiO2 or SpO2:FiO2), iv) haematological (platelets), v) hepatic (bilirubin), and vi) neurological (Glasgow coma scale). The pSOFA score showed excellent discrimination for in-hospital mortality in a general PICU (paediatric intensive care unit) population and in the subgroup of patients with suspected or confirmed infection (14). However, pSOFA is not well validated especially for patients outside the PICU setting (11).

Biomarkers are often used in the diagnosis of sepsis, but there are limitations. C-reactive protein (CRP) as a sepsis biomarker has poor specificity to differentiate between bacterial, viral, and non-infectious inflammatory conditions (12). It is useful when used in combination with other biomarkers and to assess response to therapy. White blood cell (WBC) count increase is indicative of inflammation and infection (15). However, both WBC count and CRP can be altered in several clinical conditions, such as any blood disorders and in inflammatory non-infective disorders. Additionally, the WBC count could be normal or even decreased in some cases of sepsis. Thus, total WBC has a poor specificity, which limits its usefulness as a biomarker of sepsis (15,16) . The test performance of total white blood cell count have been variable, with the reported sensitivities and specificities for the commonly used total white blood cell count threshold in paediatric patients of greater than 15×10^9 /L ranging from 38% to 86% and 53% to 85%, respectively (17). The normal range of WBC count in neonates is often higher at 9 to 30×10^9 (18).

The aim of this study was to determine the clinical spectrum of presentation and clinical significance of VGS bacteraemia in paediatric patients using sepsis scores and biomarkers, and to determine the resistance patterns of VGS in these patients.

Methods

This was a retrospective descriptive study for the period 01 January 2019 to 31 December 2019. All paediatric patients from birth to 14 years of age admitted to the Chris Hani Baragwanath Academic Hospital (CHBAH) during this period and who cultured VGS from bodily fluids such as cerebrospinal fluid (CSF), blood and abscess aspirate were considered eligible. Children with polymicrobial cultures on the same sample and those whose clinical records could not be traced were excluded from the study. Lists of positive cultures were obtained with permission from the National Health Laboratory Service (NHLS) microbiology department. Archived inpatient files

and electronic discharge summaries were retrieved with permission to obtain clinical data. Information on susceptibility of the organisms was obtained from NHLS LIS (laboratory information system). SIRS and pSOFA scores were calculated for each patient at the time of positive culture. A CRP value of 10mg/L or more was suggestive of infection. Data was analysed using Statistica.

Ethical considerations

The study was approved by the Human Research Ethics Committee (HREC) at the University of the Witwatersrand, approval number: M210538; approval was also obtained from the hospital.

Definitions

Underweight: classified according to world health organization (WHO) growth chart as weight for age less than -2 standard deviation (SD).

Stunted: classified according to WHO growth charts as height for age z score less than -2 SD.

Wasted: classified according to WHO growth charts, weight for height Z score less than -2SD.

Nosocomial sepsis: VGS isolated in patients with sepsis more than 48 hours post admission.

Probable meningitis: CSF with elevated cell count and/or protein and/or CSF glucose <60% blood glucose, with no organism identified on culture and/or PCR(19).

Confirmed meningitis: CSF with elevated cell count and/or protein and/or CSF glucose <60% blood glucose, and an organism identified on culture and/or PCR(19).

Results

VGS was cultured in 133 specimens from paediatric patients during the study period, 65/133 (48.9%) patients were included in the study after excluding polymicrobial cultures 64/133 (48.1%) and those with no clinical records 4/133(3%), figure 1.

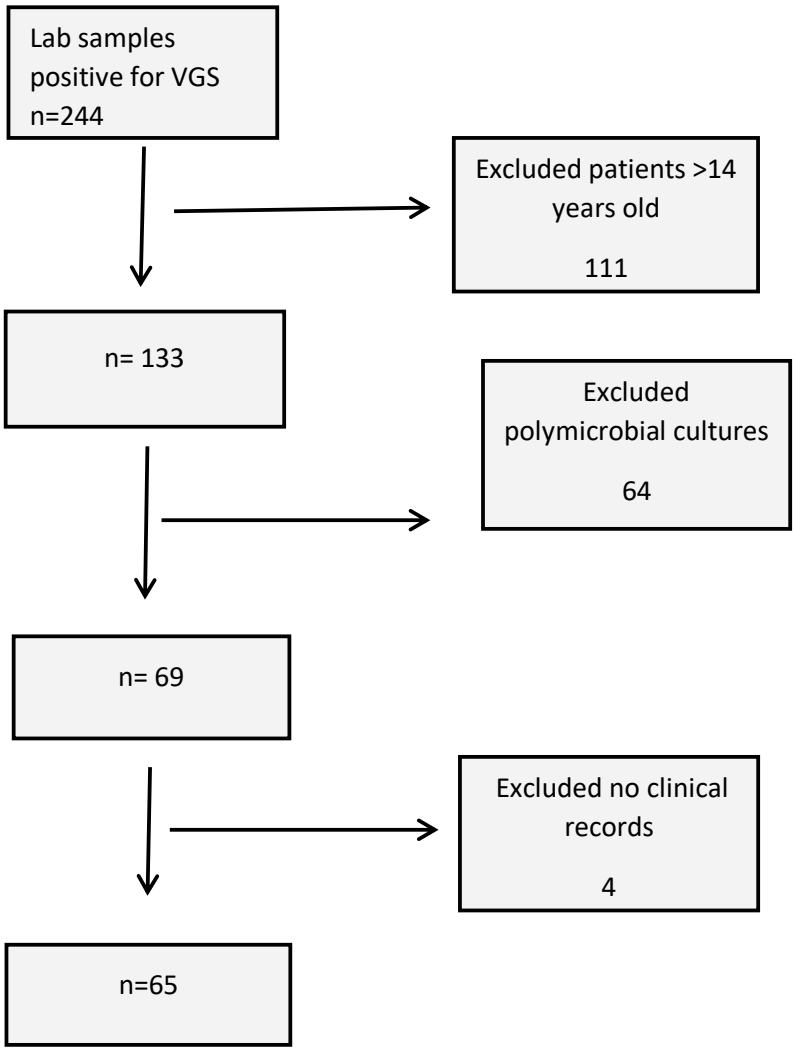


Figure 1. Flow diagram of case selection

There were 35 (53.8%) female patients. The median age of the patients was 1.5 months (IQR 0.2 to 13.3), and 27 (41.5%) patients were neonates. Ten (15%) patients were HIV exposed with one HIV infected. The HIV infected patient was newly diagnosed and antiretroviral treatment naive. Sixty-two (98.4%) patients were HIV uninfected. Four patients (6.2%) were oncology patients receiving chemotherapy. The median weight for age (WFA) Z score in 61/65 was -1.0 (IQR -2.0 to 0.0); 19 (29.2%) patients were stunted, and median weight for height (WFH) in 51/65 was 0.0 Z score (IQR -1.5 to 0.0) with 12 (18.5%) patients wasted, table 1.

Table 1: Patients' clinical demographics

Gender	
Male, n (%)	29 (44.6)
Female, n (%)	35 (53.8)
Unknown, n (%)	1 (1.5)
Age (months), Median (IQR)	1.5 (0.2-13.25)
Neonates (≤28 days), n (%)	27 (41.5)
Infants (>28 days to ≤1 year), n (%)	19 (29.2)
Children (>1 year to ≤14 years), n (%)	19 (29.2)
HIV status	
Uninfected, n (%)	62 (95.4)
Unknown, n (%)	2 (3.1)
Infected, n (%)	1 (1.53)
WFA z-score, Median (IQR)	-1.0 (-2.0; 0.0)
Normal, n (%)	42 (64.6)
Underweight, n (%)	19 (29.2)
Unknown, n (%)	4 (6.2)
HFA z- score, Median (IQR)	-1.0 (-2.0; 0.0)
Normal, n (%)	32 (49.2)
Stunted, n (%)	19 (29.2)
Unknown, n (%)	14 (21.5)
WFH z- score, Median (IQR)	0.0 (-1.5; 0.0)
Normal, n (%)	39 (60)
Unknown, n (%)	14 (21.5)
Wasted, n (%)	12 (18.5)
Clinical diagnosis	
Neonatal sepsis, n (%)	20 (30.8)
Pneumonia, n (%)	18 (27.7)
Nosocomial sepsis, n (%)	10 (15.4)
Acute gastroenteritis, n (%)	8 (12.3)

Malignancy, n (%)	4 (6.2)
Meningitis, n (%)	2 (3.1)
Submandibular abscess, n (%)	1 (1.5)
Tonsillitis, n (%)	1 (1.5)
Seizures, n (%)	1 (1.5)
Outcomes	
Discharged, n (%)	57 (87.7)
Demised, n (%)	8 (12.3)
Admitted ICU, n (%)	3 (4.6)

WFA= weight for age; HFA= height for age; WFH= weight for height

The most common clinical diagnoses were neonatal sepsis in 20 (30.8%); and pneumonia was documented in 18 (27.7%) patients outside the neonatal period. Nosocomial sepsis and acute gastroenteritis occurred in 10 (15.4%) and 8 (12.3%) patients respectively, table 1. The median duration of hospital stay was seven days (IQR 3 to 21). VGS were isolated from blood cultures in 62 (95.3%), 2 (3.1%) from cerebrospinal fluid (CSF) and 1 (1.5%) from abscess aspirate. The median time to positivity of the blood cultures was 12.5 hours (IQR 8 to 20). Fifteen (55.6%) of the neonatal cultures were performed in the first 72 hours of life. CRP was elevated within 48 hours of culture in 53.7% (22/41) with a median value of 10 (IQR 1 to 27; range 0 to 304). In neonates, the median white cell count was 12.08 (IQR 8.9 to 14.9) and for children post neonatal period 10.2 (IQR 7.58 to 15.4) (see table 2). Five (7.7%) of the patients in this study were neutropenic (neutrophil count $<1.5\times10^9$) of which 2/5 (40%) were oncology patients receiving chemotherapy with nosocomial neutropenic sepsis and one was a neonate with neonatal sepsis. All the neutropenic patients survived the sepsis episode. VGS meningitis occurred in two infants, both were hospital acquired, one died in ICU and the other survived to discharge. Three patients had presumed meningitis with VGS bacteraemia and CSF chemistry suggestive of meningitis, but the organism was not cultured in the CSF. Antibiotic susceptibility testing was performed in 57/65 (87.7%). Antibiotic susceptibility for third generation cephalosporins was 50/56 (89%) susceptible and five (8.9%) resistant and one intermediate susceptibility. Penicillin resistance was 45.8% (5/11). Ten of sixty-five (15.4%) of the specimens were tested for vancomycin susceptibility and were all sensitive.

Table 2: Laboratory investigations

Specimen type	
Blood culture n (%)	62 (95.3%)
CSF culture n (%)	2 (3.1%)
Abscess aspirate n (%)	1 (1.53%)
Time to positivity of cultures (hours), median (IQR)	12.5 (8 to 20)
Leucocytes ($x10^9$/L), median (IQR),	
Neonates, n=22	12.1 (8.9 to 14.9)
Children >28 days, n=32	10.2 (7.58 to 15.4)
Neutrophils ($\times10^9$/L), median (IQR), n=54	6.07 (2.45 to 7.56)
Lymphocytes($x10^9$/L), median (IQR), n=54	4.1 (2.67 to 5.68)
Platelets ($x10^9$/L), median (IQR), range n=54	279 (195 to 421)
CRP (mg/L), median (IQR), n=41	10 (1 to 27), range 0 to 304
CSF, n=26	
Normal	21
Probable meningitis	3
Confirmed meningitis	2

The SIRS score could be assessed for 28 (43%) patients; mean SIRS score was 1.7, range 0 to 4. Sixteen (57.1%) had a significant SIRS score of two or more. The pSOFA could be calculated in 28 (43%) patients, median score was 1 (IQR 0 to 4). Severe complications occurred in 10 (15.4%) of the patients, three required intensive care unit (ICU) admission and 8/65 (12.3%) patients died. Of the patients who died, 4/8 (50%) had pneumonia and 4/8 (50%) had nosocomial sepsis (table 3). Antibiotic use was documented in 32 (49.2%) patients. The median duration of antibiotics use was 5 days (IQR 5 to 7). Table 3 shows the characteristics of the patients who died.

Table 3. Clinical and laboratory characteristics of deaths associated with VGS bacteraemia.

Patient number	Age category: Neonate Infant Child	Gender	Nutrition Normal Underweight Stunted Wasted	HIV status	Diagnosis	Underlying condition	CRP	WBC	SIRS score	pSOFA score	Antibiotic susceptibility	Empiric Antibiotic used	Time interval in days (culture collection to outcome)	Time to positivity of culture(hours)
1	Neonate	F	Underweight	Neg	Pneumonia	Premature	1	8.73	2	8	Susceptible	Ampicillin+gentamycin	7	10
2	Neonate	M	Underweight	Neg	NOS	Premature	19	3.13	NC	NC	Resistant Ampicillin	Unknown	16	12
3	Neonate	F	Normal	Neg	NOS	DiGeorge/CHD	23	33.8	NC	NC	Not done	Unknown	19	10
4	Neonate	F	Normal	Neg	NOS	Spina bifida	8	11.8	NC	NC	Not done	Unknown	10	6
5	Infant	M	Underweight	Neg	Pneumonia	Trisomy21/CHD	1	9.95	2	5	Resistant cefotaxime	Meropenem	1	8
6	Infant	F	Underweight	Pos	NOS	BPD	102	8.96	0	5	Susceptible	Meropenem +vancomycin	44	CSF (no time stated)
7	Infant	F	Underweight	Neg	Pneumonia	Premature	18	7.32	NC	NC	Susceptible	Unknown	1	12
8	Child	F	Normal	Neg	Pneumonia	Cerebral palsy	300	15.4	NC	NC	Susceptible	Unknown	1	19

Neg= Negative; Pos= Positive; NOS= nosocomial sepsis; CHD= congenital heart disease; BPD= bronchopulmonary dysplasia; NC= Not calculable (missing data)

Discussion

The clinical significance of VGS bacteraemia in immunocompetent paediatric patients has not been documented before at this site. This study describes the clinical spectrum and outcomes in paediatric patients with VGS bacteraemia and the antibiotics resistance patterns of the organism over a one-year period. Except for the neonatal group, this study included immunocompetent children; HIV infection and malnutrition were not the predominant patient profile.

In this cohort, neonatal sepsis was the most common clinical presentation; this is similar to other studies (7), including two previous studies from the same site (20,21). Mangeni et al reported VGS was the most common isolated bacteria in neonates presenting from home with neonatal sepsis (21), while Velaphi et al reported VGS to be the most common bacterial cause of early onset neonatal sepsis after Group B streptococcus (20). VGS sepsis in neonates is most likely because of their immunocompromised state (22). Since VGS is not normal flora of the newborns skin (7), it is likely that the VGS cultured from the neonatal group in this cohort represent true infection. In our cohort, pneumonia was the second most frequent clinical manifestation; this clinical manifestation has been documented in other studies (7,23). Few cases of viral acute gastroenteritis (AGE) resulting in secondary VGS bacteraemia have been reported (24,25). We could not find any literature reports of VGS as primary cause of AGE. Also, we could not determine in our study whether the AGE was primary or secondary. In a large multicentre study of adult and paediatric patients in America, streptococcus species accounted for 5.9% of nosocomial infections with the most common being VGS.

, streptococcus species accounted for 5.9% of nosocomial infections with the most common being VGS (26). Contrary to other reports (6,7), the patients with oncological conditions recovered from VGS bacteraemia. However, this should be interpreted with caution because of the small sample size and the exclusion of polymicrobial cultures from this study. Similarly, the absence of cases with infective endocarditis may be because of the small sample size, decreased prevalence rates of the disease, improvements in oral hygiene and the use of prophylactic antibiotics for dental procedures (27). Risk factors for VGS bacteraemia have been described in neutropenic patients including mucositis, use of proton pump inhibitors and previous use of cotrimoxazole (2); this data was not collected in this study.

It is unlikely that this cohort of VGS bacteraemia were contaminants based on the selection criteria of single organism culture. In addition, the clinical findings and time to positivity of less than 24 hours support true infection (9). However, the CRP and WBC as biomarkers of infection were not routinely elevated in this cohort. The limitations of these tests have been discussed before (15,17). The sepsis scores could not be calculated for some patients because it requires tests which were not performed or documented; this is a limitation of its use in clinical practice especially in developing countries (28). The same study found pSOFA to be a better predictor of death than SIRS score (28). In our cohort the pSOFA and SIRS score yielded different results in patients in which it could be calculated, the SIRS score suggested sepsis in some but the pSOFA score was not suggestive of sepsis. The invasiveness of the organism as shown by the CSF results also supports true infection.

Some studies have reported a median case fatality rate of 10% (range 0 to 50%) (7). In this study, 12% of the patients died. As some of the deaths recorded in our study occurred a considerable time after the organism was cultured, not all of them could be definitively attributed to VGS bacteraemia. Prematurity, malnutrition and underlying chronic illnesses could be contributory factors in the causation of death.

Of concern is the high rate of penicillin resistance compared to previous reports from South Africa (29), particularly since this is Ampicillin is often used as the first line antibiotic for community-acquired infections. We recommend routine antibiotic susceptibility testing to inform antibiotic choice. The antibiotics susceptibility testing was inconsistent as it was performed at the discretion of the microbiologist and at times in consultation with the treating clinician.

Larger, prospective studies are recommended to analyse sepsis biomarkers and determine risk factors for death in paediatric patients with VGS bacteraemia.

Study limitations and strengths

This study is limited by its retrospective nature and sample size. It was not designed to show causation of disease or powered to analyse risk factors for mortality. In addition, not all specimens had susceptibility testing done, this limits recommendations. The absence of clinical information for some cases is a further notable factor that limits the interpretation of the data and of sepsis scores. Despite these limitations this study has some strengths. It is unlikely that some of the VGS

was missed since subspecies were included in the data provided by NHLS. Single pure cultures were analysed and polymicrobial cultures excluded. This study represents a diverse paediatric patient population with different age groups and includes oncology and non-oncology conditions.

Conclusion

VGS is often viewed as a contaminant when cultured. However, VGS bacteraemia is associated with serious complications including death. Further prospective studies with larger patient samples are required to be able to determine the significance of VGS.

Declaration

The research for this study was done in partial fulfilment of the requirements for NSS's MMed (Paed) degree from the University of the Witwatersrand.

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Wits VIDA for providing electronic discharge summaries for review. NHLS for the laboratory data used. CHBAH clerks for assisting with retrieving patient records. Wits University Biostatistics department for assisting with data analysis.

Author contributions

KLP conceptualized the research. NSS collection of data, analysis of results and writing of the article. NSS, KLP and FM design, analysis and interpretation of the data and editing of the article.

Funding

None.

Conflict of interest

None.

Data availability

The data that support the findings of this study are available from the corresponding author, NSS, upon reasonable request.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors, and the publisher.

Prior presentation

Preliminary results of this study were presented at the University of Witwatersrand Department of Paediatrics Research Day, December 2022.

References

1. Danilatou V, Mantadakis E, Galanakis E, Christidou A, Stiakaki E, Kalmanti M. Three Cases of Viridans Group Streptococcal Bacteremia in Children with Febrile Neutropenia and Literature Review. Scand J Infect Dis. 2003 Dec 1;35(11–12):873–6.

2. Dulanto Chiang A, Sinaii N, Palmore TN. Risk Factors for Viridans Group Streptococcal Bacteremia in Neutropenic and Non-neutropenic Patients: A Single Center Case-Case-Control Study. Open Forum Infect Dis. 2017 Dec 2;5(1):ofx260.

3. Gaudreau C, Delage G, Rousseau D, Cantor ED. Bacteremia caused by viridans streptococci in 71 children. Can Med Assoc J. 1981 Dec 1;125(11):1246–9.

4. Dwyer R, Ringertz S. Viridans streptococci in blood cultures. Can we see any patterns of species related to patient category? APMIS. 1997;105(7–12):972–4.

5. Molinaro J, Cohen G, Saudek K. Streptococcus Infection in a Newborn. :3.

6. Nielsen MJ, Claxton S, Pizer B, Lane S, Cooke RPD, Paulus S, et al. Viridans Group Streptococcal Infections in Children After Chemotherapy or Stem Cell Transplantation. Medicine (Baltimore). 2016 Mar 7;95(9):e2952.

7. Shenep JL. Viridans-group streptococcal infections in immunocompromised hosts. Int J Antimicrob Agents. 2000 Mar 1;14(2):129–35.

8. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and Outcome of Nosocomial and Community-Onset Bloodstream Infection. J Clin Microbiol. 2003 Aug;41(8):3655–60.

9. Hall KK, Lyman JA. Updated Review of Blood Culture Contamination. Clin Microbiol Rev. 2006 Oct;19(4):788–802.

10. Weinstein MP. Blood Culture Contamination: Persisting Problems and Partial Progress. J Clin Microbiol. 2003 Jun;41(6):2275–8.

11. Morin L, Hall M, de Souza D, Guoping L, Jabornisky R, Shime N, et al. The Current and Future State of Pediatric Sepsis Definitions: An International Survey. Pediatrics. 2022 May 25;149(6):e2021052565.

12. Standage SW, Wong HR. Biomarkers for pediatric sepsis and septic shock. Expert Rev Anti Infect Ther. 2011 Jan 1;9(1):71–9.

13. Wu Z, Liang Y, Li Z, Liu G, Zheng J, Zuo Y, et al. Accuracy Comparison Between Age-Adapted SOFA and SIRS in Predicting in-Hospital Mortality of Infected Children at China's PICU. Shock. 2019 Sep;52(3):347–52.

14. Matics TJ, Sanchez-Pinto LN. Adaptation and Validation of a Pediatric Sequential Organ Failure Assessment Score and Evaluation of the Sepsis-3 Definitions in Critically Ill Children. JAMA Pediatr. 2017 Oct 2;171(10):e172352.

15. Farkas JD. The complete blood count to diagnose septic shock. J Thorac Dis. 2020 Feb;12(Suppl 1):S16–21.

16. Agnello L, Giglio RV, Bivona G, Scazzone C, Gambino CM, Iacona A, et al. The Value of a Complete Blood Count (CBC) for Sepsis Diagnosis and Prognosis. Diagnostics. 2021 Oct;11(10):1881.

17. De S, Williams GJ, Hayen A, Macaskill P, McCaskill M, Isaacs D, et al. Republished: Value of white cell count in predicting serious bacterial infection in febrile children under 5 years of age. Postgrad Med J. 2015 Mar 1;91(1073):493–9.

18. Sushanth, Avabratha KS, Tauro KJ, Shwethadri GK. Hyperleukocytosis in a neonate: A diagnostic dilemma. Indian J Med Paediatr Oncol Off J Indian Soc Med Paediatr Oncol. 2010;31(3):86–8.

19. Shahan B, Choi EY, Nieves G. Cerebrospinal Fluid Analysis. Am Fam Physician. 2021 Apr 1;103(7):422–8.

20. Velaphi SC, Westercamp M, Moleleki M, Pondo T, Dangor Z, Wolter N, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. PLOS ONE. 2019 Apr 10;14(4):e0214077.

21. Mangeni NS, Solomon F, Velaphi S, Izu A, Madhi SA, Dangor Z, et al. Sepsis in previously healthy neonates discharged home after delivery in Soweto, South Africa. S Afr Med J. 2021 Apr 30;111(5):432–6.

22. Gervassi AL, Horton H. Is Infant Immunity Actively Suppressed or Immature? Virol Res Treat. 2014 Feb 24;5:1–9.

23. Freitas M, Castelo A, Petty G, Gomes CE, Carvalho E. Viridans streptococci causing community acquired pneumonia. Arch Dis Child. 2006 Sep 1;91(9):779–80.

24. Yao TC, Chiu CY, Tsai YC, Huang JL. Viridans streptococcal bacteremia secondary to viral gastroenteritis in a healthy infant. Pediatr Int. 2010 Apr;52(2):e108–10.

25. González-Carretero P, Noguera A, Fortuny C. Rotavirus Gastroenteritis Leading to Secondary Bacteremia in Previously Healthy Infants. Pediatrics. 2006 Nov 1;118(5):2255a–256.

26. Nosocomial streptococcal blood stream infections in the SCOPE program: Species occurrence and antimicrobial resistance - ScienceDirect [Internet]. [cited 2023 Nov 21]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0732889397001594

27. Lockhart PB, Brennan MT, Thornhill M, Michalowicz BS, Noll J, Bahrani-Mougeot FK, et al. Poor oral hygiene as a risk factor for infective endocarditis–related bacteremia. J Am Dent Assoc 1939. 2009 Oct;140(10):1238–44.

28. Comparing the precision of the pSOFA and SIRS scores in predicting sepsis-related deaths among hospitalized children: a multi-center retrospective cohort study.

29. Potgieter E, Carmichael M, Koornhof HJ, Chalkley LJ. In vitro antimicrobial susceptibility of viridans streptococci isolated from blood cultures. Eur J Clin Microbiol Infect Dis. 1992 Jun 1;11(6):543–6.

30. Eichberger J, Resch E, Resch B. Diagnosis of Neonatal Sepsis: The Role of Inflammatory Markers. Front Pediatr. 2022 Mar 8;10:840288.

31. Afran L, Garcia Knight M, Nduati E, Urban BC, Heyderman RS, Rowland-Jones SL. HIV-exposed uninfected children: a growing population with a vulnerable immune system? Clin Exp Immunol. 2014 Apr;176(1):11–22.

32. Wedderburn CJ, Evans C, Yeung S, Gibb DM, Donald KA, Prendergast AJ. Growth and Neurodevelopment of HIV-Exposed Uninfected Children: a Conceptual Framework. Curr HIV/AIDS Rep. 2019 Dec 1;16(6):501–13.

Appendix A: Research protocol

TITLE: The Clinical Spectrum of Viridans Group Streptococcus Bacteraemia in Paediatrics Patients at Chris Hani Baragwanath Academic Hospital.

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Protocol for MMed by submissible format

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1. Introduction

The viridans group streptococci (VGS) are gram positive cocci in chains (1,2) with at least 30 recognised species (2). They are considered to be normal flora in oropharyngeal, urogenital and gastrointestinal microbiota (2) and sometimes on skin, especially in children (3). They are often considered to be of low virulence because they do not produce endo and exotoxins, and in healthy individuals considered to be protective against colonization by pathogenic organisms (1,4).

However, they can cause life threatening conditions in neonates, children and even adults (1, 4), as many as 32% isolates have been shown to be of clinical significance in one study (4). They have been shown to cause clinically significant bacteraemia and other significant invasive disease in immunocompromised paediatric patients (4), these include HIV positive patients, neonates and cancer patients undergoing chemotherapy or stem cell transplant (2,5,6). JL Shapen suggests that viridans streptococci are a leading cause of neonatal sepsis and meningitis. The normal flora of the female genital tract contains *Streptococcus viridans*, which are a common cause of chorioamnionitis and septic abortion. In some institutions, the incidence of VGS bacteraemia and meningitis exceeds that seen from group B streptococci, although VGS infections are usually less severe (7).

A ten-year review done at a paediatric hospital in the UK showed that VGS bacteraemia can result in VGS septic shock syndrome which carries an ICU admission rate of 64% and up to 23% mortality rate (6). It is the third most common cause of bacteraemia in HIV patients (8) and the third most common cause of bacteraemia globally in patients undergoing chemotherapy and those with haematopoietic stem cell transplant (6). A study done in a children's hospital Edmonton Alberta over 19 years found *S viridans* to be the second most common cause of infective endocarditis in children (9). *S viridans* is responsible for 0.3%–3% of the cases of adult bacterial meningitis, and 1% of those occurring in children. *S viridans* is also responsible for: pulmonary infections (especially in patients with cystic fibrosis), abdominal abscesses, sepsis in immunocompromised patients, neonatal sepsis, and osteomyelitis (10).

There were previously 18 recognised species of VGS (11) the number has risen to at least 30 species (2). A molecular method is used to define taxonomy of VGS based on genetic relatedness (12). *S. mitis* is the most commonly isolated species (41%) in neutropenic patients followed by *S.*

sanguis (22%) (11). *S. mitis* is also the leading cause of viridans streptococci shock syndrome (9). *S viridans* endocarditis can be accounted for by four species *S. oralis, S. sanguis, S. gordonii* and *S. bovis* (12). *S viridans* on its own has rarely been described as a cause of pneumonia but has been isolated from empyema and lung abscesses. The *S. milleri* group is commonly described as a cause of suppurative infection (13). Viridans streptococci typically cause non-pyogenic infections such as bacteraemia. Streptococci intermedius group organisms cause invasive pyogenic infections including bone infections, brain abscesses, appendicitis, and pulmonary and abdominal abscesses (7).

VGS are thought to get into the blood stream when mucosal barriers are compromised which could be due to neutropenia, chemotherapy, mucositis, and alteration in stomach pH. Thus, the use of proton pump inhibitors, mucositis and some chemotherapy drugs were found to be risk factors in neutropenic patients. Nielsen et al found that in children with malignancy, relapsed acute lymphoblastic leukaemia has the highest risk for VGS bacteraemia (6). No risk factors were found in healthy patients (5). There is also increased risk of HIV patients getting VGS sepsis, with a study done in an academic hospital in Ethiopia showing 6% of patients younger than 18 years had VGS as the third most common infection after *Staphylococcus aureus* and coagulase negative staphylococcus (8). The use of quinolones increased the risk of VGS bacteraemia in children with acute myeloid leukaemia (14), especially in neutropenic patients (5). The prior use of vancomycin was found to be protective against *S Viridans* septicaemia (5).

VGS were previously considered to be susceptible to penicillin but recent studies have shown an increase in resistance globally. Some studies have found 20% resistance to penicillin (15). In a South African study, they found that there was up to 38% resistance of VGS to penicillin (16). There has also been an increase in resistance to erythromycin which is used as an alternative in patients who are allergic to penicillin (12). Tetracycline resistance has been reported as 41% in South Africa (16) and as high as 70% in Taiwan (15). Resistance to 4th generation cephalosporins ranged from 14% to 34% (16). Prior use of beta lactam antibiotics was the only factor significantly associated with cephalosporins resistance. Vancomycin, imipenem, ceftriaxone and cefotaxime had the best susceptibility in a South African study of 211 VGS isolates from blood cultures collected from three Johannesburg hospital laboratories (16). Vancomycin had 97% susceptibility in a study in USA (18). Cancer patients were also found to be more likely to culture resistant VGS

species, with trimethoprim-sulphamethaxazole resistance at 36% in this group of patients compared to 17% in the rest of the population (18).

Several studies have suggested that up to about 40% of positive blood cultures may represent contaminants (19), the numbers can be higher in infants and children (20). A study done by Weistein found that VGS was a pathogen in only 38% of the positive cultures (21). Several methods have been used to differentiate positive blood cultures due to pathogens and those due to contaminants. These include number of positive blood cultures in a set, in true bacteraemia there is often multiple positive cultures, this is not useful when a single culture was done (22). Time to growth is another important factor with several studies showing that cultures which become positive after 3 to 5 days are more likely to be contaminants (20). Weistein also found that clinical features like fever, hypothermia, low or high white cell count and hypotension predicted true infection as opposed to contaminant (21). VGS are not usually part of the flora of the newborns' skin and should therefore be considered as potential pathogens when isolated from normally sterile sites in newborns (7).

There is no literature in South Africa on the clinical presentation of VGS bacteraemia in children, and most international data is on neonates and oncology patients. Currently at Chris Hani Baragwanath Academic Hospital (CHBAH) it is often considered to be a contaminant than true indication of bacteraemia in most patients. This is a topic which needs exploration to see the clinical significance and spectrum of presentation of paediatric patients with blood cultures and other sterile fluids positive for VGS.

2. Aims and objectives

2.1 Research question

How common is VGS bacteraemia and what is the pattern of clinical presentation of children with VGS bacteraemia at CHBAH?

2.2 Aim

The aim of this study is to describe the spectrum of clinical presentation of VGS bacteraemia in paediatric patients and to analyse risk factors associated with morbidity and mortality from VGS.

2.3 Objectives

a) To define the spectrum of clinical presentation of VGS in paediatric patients (0 to 14 years) at CHBAH.
b) To describe the risk factors for VGS bacteraemia.
c) To describe the antibiotics resistance patterns of VGS.

2.4 Secondary objective

To compare white cell count (WCC) and C reactive protein (CRP) in immunocompromised compared to immune competent patients with VGS bacteraemia.

3. Methods

3.1 Study design

It will be a retrospective descriptive study over a one-year period from 01 January 2019 to 31 December 2019. The clinical records and National Health Laboratory Service (NHLS) data base will be reviewed to establish the clinical presentation and outcomes in children, and to describe the resistance patterns of Streptococci Viridans bacteraemia.

3.2 Testing and classification of VGS

Testing and speciating of VGS at NHLS is done using the Clinical & Laboratory Standards Institute (CLSI) guidelines. When gram-positive cocci in chains/pairs on gram stain its plated out onto 5% blood agar Incubate for 18-24 hours. Plate is observed for the type of haemolysis, VGS are generally alpha-haemolytic. Its then put on optochin disk using the Kirby-Bauer/disk diffusion method (*S.pneumoniae* is susceptible where VGS are resistant). On the same day if it is a sterile site specimen the organism is then put on an identification system, either Vitek-2 (biomerieux) or analytical profile system (API) (both are biochemical identification methods). Sensitivity testing is also done using CLSI guidelines (attached in appendix B). Identification of subspecies is only done for a small group of patients, like the haematology/oncology patients.

3.3 Sample size

Based on available data from NHLS there were about 200 positive cultures for VGS in children from 1 January to 31 December 2019.The clinical records of patients with positive cultures will be reviewed and that will determine the sample size.

3.4 Study population

Inclusion criteria

All inpatients from birth to 14 years with positive cultures for VGS from sterile bodily fluids (blood, CSF, urine, ascites, pleural and pericardial effusion) at CHBAH from 01 January 2019 to 31 December 2019.

Exclusion criteria

Children who have cultured more than one organism on the same culture specimen.

3.5 Variables

Variables which will be used in the study include age, gender, nutritional status, HIV status, admission diagnosis, fever, hypothermia, white cell count, differential count, C-reactive protein (CRP), oncology vs non-oncology, other co-morbidities, antibiotics susceptibility, antibiotics used (duration, intravenous or oral), duration of hospital stay and outcomes (death or discharge).

3.6 Procedures

Data will be collected with permission from NHLS microbiology for all sterile body fluids cultures positive for Streptococci Viridans for children from 0 to 14 years, over the period of one year. Files will be retrieved from records for all the patients identified to obtain the clinical data. Discharge summaries will be obtained from the electronic records from clinical units and the Vaccines and Infectious Diseases Analytical Unit (VIDA) formerly Respiratory and Meningeal Pathogens Research Unit (RMPRU). Information on susceptibility of the organisms will be obtained from NHLS.

4. Definitions

Bacteraemia: Presence of bacteria in the blood stream

Fever: temperature more than 38°C or history of feeling hot.

Gastro-enteritis: acute infectious syndrome of the stomach lining and the intestine. It is characterized by diarrhoea, vomiting, and abdominal cramps.

Immunocompromised: is a broad term which means that the immune system is weaker than expected and not functioning properly.

Infective endocarditis: infection of the endocardial surfaces of the heart primarily of one or more heart valves, the mural endocardium, or a septal defect.

Meningitis: inflammation of meninges secondary to an infection, will be defined by elevated cell counts, any polymorphonuclear cells or lymphocytes or bacterial identification on gram stain or positive culture.

Moderate acute malnutrition: weight for height between -2 and -3 Z score and/or mid upper arm circumference between 11.5cm and 12.5cm.

Pneumonia: a form of acute respiratory infection that affects the lungs. the alveoli are filled with pus and fluid, resulting in increased work of breathing and limits oxygen intake.

Sepsis: Infection in presence of systemic inflammatory response syndrome (SIRS).

Septic shock: Sepsis in the presence of cardiovascular dysfunction.

Severe acute malnutrition: weight for height less than -3 Z score and/or Mid upper arm circumference less than 11.5cm in children between 6 months and 6 years.

SIRS: Meets two or more of the following criteria, one of which must be temperature or WBC Pyrexia (>38°C) or hypothermia (<36°C).

- Age-dependent tachycardia or bradycardia
- Tachypnoea or need for mechanical ventilation
- Abnormal WBC count or >10% bands

Urinary tract infection (UTI): infection of any part of urinary system as evidenced by positive urine culture from sterile urine sample, or identification of organism on gram stain. Presence of leucocytes and nitrites on urine will also be considered as UTI.

5. Data analysis

The statistics will be descriptive and analytical. Data will be entered into an Excel spreadsheet. The data will then be analysed using Statistica (Statsoft USA, version 12). Categorical variables will be reported as numbers and percentages. Continuous variables will be reported as means and standard deviations if normally distributed, if not normally distributed the data will be represented as medians and interquartile ranges. The p-value will be used to determine statistical significance with p-value of <0.05 being statistically significant. The students T test and Mann-Whitney U test will be used.

6. Ethics

The protocol will be submitted with an application to the University of Witwatersrand Human Research Ethics Committee (HREC). Permission will be obtained from the hospital CEO and NHLS to do the study. No names will be used during the study. Consent will not be required since it is a retrospective study.

7. Timing

Year	2020	2021										
Activity	Jan to Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	NOV
Literature Review and Preparation of protocol												
Submission to Ethics												

Submission of protocol												
Data collection												
Data analysis												
Write up of research report												

8. Funding

Costs which would be incurred will be for printing data collection sheets and I will personally fund. No other costs are anticipated.

9. Strengths

The research will bring together laboratory and clinical data, it does not have a lot of costs and data retrieved can be generalized to the South African paediatrics population.

10. Problems and limitations

There may be difficulty retrieving all files after obtaining data from NHLS. It will be a retrospective study so information obtained will be dependent on clinical notes done by clinical practitioner and may be incomplete. Some files may not be found.

11. References

1. Danilatou V, Mantadakis E, Galanakis E, Christidou A, Stiakaki E, Kalmanti M. Three cases of viridans group streptococcal bacteremia in children with febrile neutropenia and literature review. Scand J Infect Dis. 2003;35(11–12):873–6.

2. Dulanto Chiang A, Sinaii N, Palmore TN. Risk factors for viridans group streptococcal bacteremia in neutropenic and non-neutropenic patients: A single center case-case-control study. Open Forum Infect Dis. 2018;5(1):ofx260.

3. Gaudreau C, Delage G, Rousseau D, Cantor ED. Bacteremia caused by viridans streptococci in 71 children. Can Med Assoc J. 1981;125(11):1246–9.

4. Molinaro J, Cohen G, Saudek K. Streptococcus infection in a newborn. WMJ. 2014;113(5):202–3.

5. Nielsen MJ, Claxton S, Pizer B, Lane S, Cooke RPD, Paulus S, et al. Viridans group streptococcal infections in children after chemotherapy or stem cell transplantation: A 10-year review from a tertiary pediatric hospital. Medicine (Baltimore). 2016;95(9):e2952.

6. Shenep JL. Viridans-group streptococcal infections in immunocompromised hosts. Int J Antimicrob Agents. 2000;14(2):129–35.

7. Alebachew G, Teka B, Endris M, Shiferaw Y, Tessema B. Etiologic agents of bacterial sepsis and their antibiotic susceptibility patterns among patients living with human immunodeficiency virus at Gondar university teaching hospital, northwest Ethiopia. Biomed Res Int. 2016;2016:5371875.

8. Alshammary A, Hervas-Malo M, Robinson JL. Pediatric infective endocarditis: Has Staphylococcus aureus overtaken viridans group streptococci as the predominant etiological agent? Can J Infect Dis Med Microbiol. 2008;19(1):63–8.

9. Birlutiu V, Birlutiu RM, Costache VS. Viridans streptococcal infective endocarditis associated with fixed orthodontic appliance managed surgically by mitral valve plasty: A case report. Medicine (Baltimore). 2018;97(27):e11260.

10. Bryskier A. Viridans group streptococci: a reservoir of resistant bacteria in oral cavities. Clin Microbiol Infect. 2002;8(2):65–9.

11. Tunkel AR, Sepkowitz KA. Infections caused by viridans streptococci in patients with neutropenia. Clin Infect Dis. 2002;34(11):1524–9.

12. Jerng JS, Hsueh PR, Teng LJ, Lee LN, Yang PC, Luh KT. Empyema thoracis and lung abscess caused by viridans streptococci. Am J Respir Crit Care Med. 1997;156(5):1508–14.

13. Doern CD, Burnham C-AD. It's not easy being green: the viridans group streptococci, with a focus on pediatric clinical manifestations. J Clin Microbiol. 2010;48(11):3829–35.

14. Teng LJ, Hsueh PR, Chen YC, Ho SW, Luh KT. Antimicrobial susceptibility of viridans group streptococci in Taiwan with an emphasis on the high rates of resistance to penicillin and macrolides in Streptococcus oralis. J Antimicrob Chemother. 1998;41(6):621–7.

15. Potgieter E, Carmichael M, Koornhof HJ, Chalkley LJ. In vitro antimicrobial susceptibility of viridans streptococci isolated from blood cultures. Eur J Clin Microbiol Infect Dis. 1992;11(6):543–6.

16. Lehrnbecher T, Varwig D, Kaiser J, Reinhardt D, Klingebiel T, Creutzig U. Infectious complications in pediatric acute myeloid leukemia: analysis of the prospective multi-institutional clinical trial AML-BFM 93. Leukemia. 2004;18(1):72–7.

17. Marron A, Carratalà J, Alcaide F, Fernández-Sevilla A, Gudiol F. High rates of resistance to cephalosporins among viridans-group streptococci causing bacteraemia in neutropenic cancer patients. J Antimicrob Chemother. 2001;47(1):87–91.

18. Diekema DJ, Beach ML, Pfaller MA, Jones RN, SENTRY Participants Group. Antimicrobial resistance in viridans group streptococci among patients with and without the diagnosis of cancer in the USA, Canada and Latin America. Clin Microbiol Infect. 2001;7(3):152–7.

19. Richter SS, Beekmann SE, Croco JL, Diekema DJ, Koontz FP, Pfaller MA, et al. Minimizing the workup of blood culture contaminants: implementation and evaluation of a laboratory-based algorithm. J Clin Microbiol. 2002;40(7):2437–44.

20. Hall KK, Lyman JA. Updated review of blood culture contamination. Clin Microbiol Rev. 2006;19(4):788–802.

21. Weinstein MP. Blood culture contamination: persisting problems and partial progress. J Clin Microbiol. 2003;41(6):2275–8.

22. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. J Clin Microbiol. 2003;41(8):3655–60.

Data collection sheet

File number

Gender

Age

Time period in hospital

Ward

HIV exposure (if less than 18 months)

HIV status negative or positive

If positive:

- HIV Viral load
- CD4 count
- HIV positive on antiretrovirals >6months
- HIV positive on antiretrovirals <6 months

Nutritional status

Co-morbid disease

Cancer

- On chemotherapy
- Not on chemotherapy

Previous hospital admission

Central line present or not

- If yes duration

Culture specimen

Blood

- Cerebrospinal fluid (CSF)
- Urine
- Pus
- Pleural fluid
- Other

Time to positivity of culture in hours:

Cultured from multiple sites: yes or no

Antibiotic susceptibility of culture:

Antibiotics used:

Duration of antibiotics:

Clinical presentation

- Fever
- Respiratory rate
- Heart rate
- Mean arterial pressure (MAP)
- Oxygen saturation
- Oxygen requirements (Nasal prongs, facemask oxygen, High flow oxygen intubated or other)
- Need for ionotropes (which ionotropes and dose if used)
- Glasgow coma scale
- Pneumonia
- Infective endocarditis
- Meningitis
- Neonatal sepsis
- Septic shock
- Gastro-enteritis
- Urinary tract infection
- Other

Blood results

- White cell count
- Differential count
- Platelet count
- CRP
- Total bilirubin
- Creatinine

Urine dipstix:

Urine results:

- White cell count
- Microscopy

CSF results

- Proteins
- Cell count
- Glucose
- Serum glucose
- Microscopy

Outcome

- Discharged home.
- Admitted to intensive care unit (ICU)
    - Duration of ICU stay
- Transferred to other hospital.
- Demised

CLSI guideline for antimicrobial susceptibility testing for VGS at NHLS

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Table 2H-2. Zone Diameter and MIC Breakpoints for *Streptococcus* spp. Viridans Group

Testing Conditions	
Medium:	Disk diffusion: MHA with 5% sheep blood Broth dilution: CAMHB with LHB (2.5% to 5% v/v); the CAMHB should be supplemented to 50 µg/mL calcium for daptomycin (see M07[1] for instructions for preparation of LHB) Agar dilution: MHA with sheep blood (5% v/v); recent studies using the agar dilution method have not been performed and reviewed by the subcommittee.
Inoculum:	Colony suspension, equivalent to a 0.5 McFarland standard using colonies from an overnight (18- to 20-hour) sheep blood agar plate
Incubation:	35°C ± 2°C Disk diffusion: 5% CO_2; 20–24 hours Dilution methods: ambient air; 20–24 hours (CO_2 if necessary for growth with agar dilution)

Routine QC Recommendations (see Tables 4B and 5B for acceptable QC ranges)

S. pneumoniae ATCC® 49619

When a commercial test system is used for susceptibility testing, refer to the manufacturer's instructions for QC test recommendations and QC ranges.

General Comments

(1) For disk diffusion, measure the diameter of the zones of complete inhibition (as judged by the unaided eye), including the diameter of the disk. The zone margin should be considered the area showing no obvious, visible growth that can be detected with the unaided eye. Do not measure the zone of inhibition of hemolysis. Measure the zones from the upper surface of the agar illuminated with reflected light, with the cover removed. Ignore faint growth of tiny colonies that can be detected only with a magnifying lens at the edge of the zone of inhibited growth.

(2) For viridans streptococci when testing chloramphenicol, clindamycin, erythromycin, linezolid, tedizolid, and tetracycline by broth microdilution MIC, trailing growth can make end-point determination difficult. In such cases, read the MIC at the lowest concentration where the trailing begins. Tiny buttons of growth should be ignored (see M07,[1] Figures 3 and 4).

(3) The viridans group of streptococci includes the following five groups, with several species within each group: *mutans* group, *salivarius* group, *bovis* group, *anginosus* group (previously "*S. milleri*" group), and *mitis* group. The *anginosus* group includes small colony–forming β-hemolytic strains with groups A, C, F, and G antigens. For detailed information on the species within the groups, please refer to recent literature.

(4) Breakpoints for *Streptococcus* spp. viridans group are proposed based on population distributions of various species, pharmacokinetics of the antimicrobial agents, previously published literature, and the clinical experience of subcommittee members. Systematically collected clinical data were not available for review with many of the antimicrobial agents in this table.

NOTE: Information in boldface type is new or modified since the previous edition.

Table 1. Pediatric Sequential Organ Failure Assessment Score

Variables	Score[a] 0	1	2	3	4
Respiratory					
Pao_2:Fio_2[b] or Spo_2:Fio_2[c]	≥400	300-399	200-299	100-199 With respiratory support	<100 With respiratory support
	≥292	264-291	221-264	148-220 With respiratory support	<148 With respiratory support
Coagulation					
Platelet count, $\times 10^3/\mu L$	≥150	100-149	50-99	20-49	<20
Hepatic					
Bilirubin, mg/dL	<1.2	1.2-1.9	2.0-5.9	6.0-11.9	>12.0
Cardiovascular					
MAP by age group or vasoactive infusion, mm Hg or μg/kg/min[d]					
<1 mo	≥46	<46	Dopamine hydrochloride ≤5 or dobutamine hydrochloride (any)	Dopamine hydrochloride >5 or epinephrine ≤0.1 or norepinephrine bitartrate ≤0.1	Dopamine hydrochloride >15 or epinephrine >0.1 or norepinephrine bitartrate >0.1
1-11 mo	≥55	<55			
12-23 mo	≥60	<60			
24-59 mo	≥62	<62			
60-143 mo	≥65	<65			
144-216 mo	≥67	<67			
>216 mo[e]	≥70	<70			
Neurologic					
Glasgow Coma Score[f]	15	13-14	10-12	6-9	<6
Renal					
Creatinine by age group, mg/dL					
<1 mo	<0.8	0.8-0.9	1.0-1.1	1.2-1.5	≥1.6
1-11 mo	<0.3	0.3-0.4	0.5-0.7	0.8-1.1	≥1.2
12-23 mo	<0.4	0.4-0.5	0.6-1.0	1.1-1.4	≥1.5
24-59 mo	<0.6	0.6-0.8	0.9-1.5	1.6-2.2	≥2.3
60-143 mo	<0.7	0.7-1.0	1.1-1.7	1.8-2.5	≥2.6
144-216 mo	<1.0	1.0-1.6	1.7-2.8	2.9-4.1	≥4.2
>216 mo[e]	<1.2	1.2-1.9	2.0-3.4	3.5-4.9	≥5

Abbreviations: Fio_2, fraction of inspired oxygen; MAP, mean arterial pressure; pSOFA, pediatric Sequential Organ Failure Assessment; Spo_2, peripheral oxygen saturation.

SI conversion factors: To convert bilirubin to micromoles per liter, multiply by 17.104; creatinine to micromoles per liter, multiply by 88.4; and platelet count to $\times 10^9$/L, multiply by 1.

[a] The pSOFA score was calculated for every 24-hour period. The worst value for every variable in each 24-hour period was used to calculate the subscore for each of the 6 organ systems. If a variable was not recorded in a given 24-hour period, it was assumed to be normal and a score of 0 was used. Daily pSOFA score was the sum of the 6 subscores (range, 0-24 points; higher scores indicate a worse outcome).

[b] Pao_2 was measured in millimeters of mercury.

[c] Only Spo_2 measurements of 97% or lower were used in the calculation.

[d] MAP (measured in millimeters of mercury) was used for scores 0 and 1; vasoactive infusion (measured in micrograms per kiligram per minute), for scores 2 to 4. Maximum continuous vasoactive infusion was administered for at least 1 hour.

[e] Cutoffs for patients older than 18 years (216 months) were identical to the original SOFA score.

[f] Glasgow Coma Scale was calculated using the pediatric scale.

Appendix B: Supplementary

Not included in submission to journal due to word count and tables limitation.

Secondary objective

To compare white cell count (WCC) and C reactive protein (CRP) in immunocompromised compared to immune competent patients with VGS bacteraemia.

Table 4: Immunocompromised vs immunocompetent inflammatory markers

Condition	**WCC Median (IQR)**	**CRP median (IQR)**
HIV positive (n=1)	8.96 (absolute)	102 (absolute)
Nephrotic syndrome (n=1)	12.79 (absolute)	10 (absolute
Malignancy (n=4)	5.51 (2.09-8.96)	8.0 (4.0-12.0)
HIV exposed (n=10)	11.81 (8.97-13.98)	26.0 (8.5-66.5)
SAM (n=15)	11.8 (8.97-13.98)	26 (8.5-66.5)
Neonates (n=27)	12.09 (8.97-14.97)	5.0 (1.00-18.5)
Immunocompetent (n=27)	11.6 (7.58-18.1)	11.5 (1.0-51.0)

HIV=Human immunodeficiency virus; SAM =Severe acute malnutrition; WCC =White cell count

Neonates are often immunodeficient as their immune system is not fully developed with delayed maturation of specific humoral and cellular immune response B and T cells (30). Children with nephrotic syndrome are often immunodeficient as a result of immunoglobulins loss and due to steroids use, there was one patient in our study with nephrotic syndrome. We also had four children with malignancy undergoing chemotherapy and these patients had a lower white cell count compared to the other groups of patients. Children who are HIV exposed uninfected (HEU) also have lower immunity compared to those who are HIV unexposed uninfected (HUU), several factors have been proposed for these (31). HEU children have been found to have a higher CRP compared to HIV unexposed children as proinflammatory cytokines are upregulated in these children (32) and this correlates with the results in our study. Neonates had a lower CRP compared

to that of children with SAM or those HEU. CRP was also relatively lower in patient who were immunocompetent compared to those HEU and those with SAM. There was no difference in the white cell counts in patients who are immunocompetent or those immunodeficient. Further analysis could not be done to determine clinical significance of the differences in WCC and CRP values due to small patient numbers.

References

1. Danilatou V, Mantadakis E, Galanakis E, Christidou A, Stiakaki E, Kalmanti M. Three Cases of Viridans Group Streptococcal Bacteremia in Children with Febrile Neutropenia and Literature Review. Scand J Infect Dis. 2003 Dec 1;35(11–12):873–6.

2. Dulanto Chiang A, Sinaii N, Palmore TN. Risk Factors for Viridans Group Streptococcal Bacteremia in Neutropenic and Non-neutropenic Patients: A Single Center Case-Case-Control Study. Open Forum Infect Dis. 2017 Dec 2;5(1):ofx260.

3. Gaudreau C, Delage G, Rousseau D, Cantor ED. Bacteremia caused by viridans streptococci in 71 children. Can Med Assoc J. 1981 Dec 1;125(11):1246–9.

4. Dwyer R, Ringertz S. Viridans streptococci in blood cultures. Can we see any patterns of species related to patient category? APMIS. 1997;105(7–12):972–4.

5. Molinaro J, Cohen G, Saudek K. Streptococcus Infection in a Newborn. :3.

6. Nielsen MJ, Claxton S, Pizer B, Lane S, Cooke RPD, Paulus S, et al. Viridans Group Streptococcal Infections in Children After Chemotherapy or Stem Cell Transplantation. Medicine (Baltimore). 2016 Mar 7;95(9):e2952.

7. Shenep JL. Viridans-group streptococcal infections in immunocompromised hosts. Int J Antimicrob Agents. 2000 Mar 1;14(2):129–35.

8. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and Outcome of Nosocomial and Community-Onset Bloodstream Infection. J Clin Microbiol. 2003 Aug;41(8):3655–60.

9. Hall KK, Lyman JA. Updated Review of Blood Culture Contamination. Clin Microbiol Rev. 2006 Oct;19(4):788–802.

10. Weinstein MP. Blood Culture Contamination: Persisting Problems and Partial Progress. J Clin Microbiol. 2003 Jun;41(6):2275–8.

11. Morin L, Hall M, de Souza D, Guoping L, Jabornisky R, Shime N, et al. The Current and Future State of Pediatric Sepsis Definitions: An International Survey. Pediatrics. 2022 May 25;149(6):e2021052565.

12. Standage SW, Wong HR. Biomarkers for pediatric sepsis and septic shock. Expert Rev Anti Infect Ther. 2011 Jan 1;9(1):71–9.

13. Wu Z, Liang Y, Li Z, Liu G, Zheng J, Zuo Y, et al. Accuracy Comparison Between Age-Adapted SOFA and SIRS in Predicting in-Hospital Mortality of Infected Children at China's PICU. Shock. 2019 Sep;52(3):347–52.

14. Matics TJ, Sanchez-Pinto LN. Adaptation and Validation of a Pediatric Sequential Organ Failure Assessment Score and Evaluation of the Sepsis-3 Definitions in Critically Ill Children. JAMA Pediatr. 2017 Oct 2;171(10):e172352.

15. Farkas JD. The complete blood count to diagnose septic shock. J Thorac Dis. 2020 Feb;12(Suppl 1):S16–21.

16. Agnello L, Giglio RV, Bivona G, Scazzone C, Gambino CM, Iacona A, et al. The Value of a Complete Blood Count (CBC) for Sepsis Diagnosis and Prognosis. Diagnostics. 2021 Oct;11(10):1881.

17. De S, Williams GJ, Hayen A, Macaskill P, McCaskill M, Isaacs D, et al. Republished: Value of white cell count in predicting serious bacterial infection in febrile children under 5 years of age. Postgrad Med J. 2015 Mar 1;91(1073):493–9.

18. Sushanth, Avabratha KS, Tauro KJ, Shwethadri GK. Hyperleukocytosis in a neonate: A diagnostic dilemma. Indian J Med Paediatr Oncol Off J Indian Soc Med Paediatr Oncol. 2010;31(3):86–8.

19. Shahan B, Choi EY, Nieves G. Cerebrospinal Fluid Analysis. Am Fam Physician. 2021 Apr 1;103(7):422–8.

20. Velaphi SC, Westercamp M, Moleleki M, Pondo T, Dangor Z, Wolter N, et al. Surveillance for incidence and etiology of early-onset neonatal sepsis in Soweto, South Africa. PLOS ONE. 2019 Apr 10;14(4):e0214077.

21. Mangeni NS, Solomon F, Velaphi S, Izu A, Madhi SA, Dangor Z, et al. Sepsis in previously healthy neonates discharged home after delivery in Soweto, South Africa. S Afr Med J. 2021 Apr 30;111(5):432–6.

22. Gervassi AL, Horton H. Is Infant Immunity Actively Suppressed or Immature? Virol Res Treat. 2014 Feb 24;5:1–9.

23. Freitas M, Castelo A, Petty G, Gomes CE, Carvalho E. Viridans streptococci causing community acquired pneumonia. Arch Dis Child. 2006 Sep 1;91(9):779–80.

24. Yao TC, Chiu CY, Tsai YC, Huang JL. Viridans streptococcal bacteremia secondary to viral gastroenteritis in a healthy infant. Pediatr Int. 2010 Apr;52(2):e108–10.

25. González-Carretero P, Noguera A, Fortuny C. Rotavirus Gastroenteritis Leading to Secondary Bacteremia in Previously Healthy Infants. Pediatrics. 2006 Nov 1;118(5):2255a–256.

26. Nosocomial streptococcal blood stream infections in the SCOPE program: Species occurrence and antimicrobial resistance - ScienceDirect [Internet]. [cited 2023 Nov 21]. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0732889397001594

27. Lockhart PB, Brennan MT, Thornhill M, Michalowicz BS, Noll J, Bahrani-Mougeot FK, et al. Poor oral hygiene as a risk factor for infective endocarditis–related bacteremia. J Am Dent Assoc 1939. 2009 Oct;140(10):1238–44.

28. Comparing the precision of the pSOFA and SIRS scores in predicting sepsis-related deaths among hospitalized children: a multi-center retrospective cohort study.

29. Potgieter E, Carmichael M, Koornhof HJ, Chalkley LJ. In vitro antimicrobial susceptibility of viridans streptococci isolated from blood cultures. Eur J Clin Microbiol Infect Dis. 1992 Jun 1;11(6):543–6.

30. Eichberger J, Resch E, Resch B. Diagnosis of Neonatal Sepsis: The Role of Inflammatory Markers. Front Pediatr. 2022 Mar 8;10:840288.

31. Afran L, Garcia Knight M, Nduati E, Urban BC, Heyderman RS, Rowland-Jones SL. HIV-exposed uninfected children: a growing population with a vulnerable immune system? Clin Exp Immunol. 2014 Apr;176(1):11–22.

32. Wedderburn CJ, Evans C, Yeung S, Gibb DM, Donald KA, Prendergast AJ. Growth and Neurodevelopment of HIV-Exposed Uninfected Children: a Conceptual Framework. Curr HIV/AIDS Rep. 2019 Dec 1;16(6):501–13.

Appendix C: South African Journal of infectious diseases guidelines

South African Journal of Infectious Diseases Author Guidelines

Original Research Article

An original article provides an overview of innovative research in a particular field within or related to the focus and scope of the journal, presented according to a clear and well-structured format.

Submission status	open
Word limit	3500 words (excluding the abstract, tables, figures, graphs, and references)
Abstract	maximum: 250 words requires structural headings: Background, Objectives, Method, Results, Conclusion and Contribution
Main text	requires structural headings, refer to the full structure 'Ethical considerations' is a sub-section in the manuscript and must include: • Name of the ethical review committee • Study approval number • Manner of consent (written, oral) for human participants • Description of measures taken to maintain the confidentiality of data • If the study was not human or animal research or the study was determined to be non-human subjects research or exempt, the authors must provide a statement with those details in this section.
References	60 or less, adhere to the **Vancouver referencing style**
Tables, figures and graphs	7 or less, adhere to the Illustrations requirements found in the AOSIS House style guide
Formatting requirements	apply the guidelines located on the **Formatting requirements page** and the **AOSIS house style guide**
Compulsory supplementary file(s)	the **Authorship, disclosure statements, copyright, and license agreement form**, **Ethical Clearance/Waiver Documentation** and any other relevant form applicable to your submission
Ethical clearance/waiver documentation	evidence of ethical clearance for the study, such as the study approval letter or certificate from the Institutional Review Board (IRB), a waiver from the IRB et cetera

Original Research Article full structure

Title: The article's full title should contain a maximum of 95 characters (including spaces).

Abstract: The abstract, written in English, should be no longer than 250 words and must be written in the past tense. The abstract should give a succinct account of the objectives, methods, results and significance of the matter. The structured abstract for an Original Research article should consist of five paragraphs labelled Background, Methods, Results, Conclusion and Contribution.

- Background: Summarise the social value (importance, relevance) and scientific value (knowledge gap) that your study addresses.
- Methods: Clearly express the basic design of the study, and name or briefly describe the methods used without going into excessive detail.
- Results: State the main findings.
- Conclusion: State your conclusion and any key implications or recommendations.
- Contribution: What practical, scientific or theoretical gap did your research filled? How do these insights link to the focus and scope of the journal? It should be a concise statement of the primary contribution of the manuscript; and how it fits within the scope of the journal.

Do not cite references and do not use abbreviations excessively in the abstract.

Introduction: The introduction must contain your argument for the social and scientific value of the study, as well as the aim and objectives:

- Social value: The first part of the introduction should make a clear and logical argument for the importance or relevance of the study. Your argument should be supported by the use of evidence from the literature.
- Scientific value: The second part of the introduction should make a clear and logical argument for the originality of the study. This should include a summary of what is already known about the research question or specific topic and should clarify the knowledge gap that this study will address. Your argument should be supported by the use of evidence from the literature.
- Conceptual framework: In some research articles it will also be important to describe the underlying theoretical basis for the research and how these theories are linked together in a conceptual framework. The theoretical evidence used to construct the conceptual framework should be referenced from the literature.
- Aim and objectives: The introduction should conclude with a clear summary of the aim and objectives of this study.

Research methods and design: This must address the following:

- Study design: An outline of the type of study design.
- Setting: A description of the setting for the study; for example, the type of community from which the participants came or the nature of the health system and services in which the study is conducted.

- Study population and sampling strategy: Describe the study population and any inclusion or exclusion criteria. Describe the intended sample size and your sample size calculation or justification. Describe the sampling strategy used. Describe in practical terms how this was implemented.
- Intervention (if appropriate): If there were intervention and comparison groups, describe the intervention in detail and what happened to the comparison groups.
- Data collection: Define the data collection tools that were used and their validity. Describe in practical terms how data were collected and any key issues involved, e.g. language barriers.
- Data analysis: Describe how data were captured, checked and cleaned. Describe the analysis process, for example, the statistical tests used or steps followed in qualitative data analysis.
- Ethical considerations: Approval must have been obtained for all studies from the author's institution or other relevant ethics committee and the institution's name and permit numbers should be stated here.

Results: Present the results of your study in a logical sequence that addresses the aim and objectives of your study. Use tables and figures as required to present your findings. Use quotations as required to establish your interpretation of qualitative data. All units should conform to the **SI convention** and be abbreviated accordingly. Metric units and their international symbols are used throughout, as is the decimal point (not the decimal comma).

Discussion: The discussion section should address the following four elements:

- Key findings: Summarise the key findings without reiterating details of the results.
- Discussion of key findings: Explain how the key findings relate to previous research or to existing knowledge, practice or policy.
- Strengths and limitations: Describe the strengths and limitations of your methods and what the reader should take into account when interpreting your results.
- Implications or recommendations: State the implications of your study or recommendations for future research (questions that remain unanswered), policy or practice. Make sure that the recommendations flow directly from your findings.

Conclusion: Provide a brief conclusion that summarises the results and their meaning or significance in relation to each objective of the study.

Acknowledgements: Those who contributed to the work but do not meet our authorship criteria should be listed in the Acknowledgments with a description of the contribution. Authors are responsible for ensuring that anyone named in the Acknowledgments agrees to be named. Refer to the acknowledgement structure guide on our *Formatting Requirements* page.

Also provide the following, each under their own heading:

- Competing interests: This section should list specific competing interests associated with any of the authors. If authors declare that no competing interests exist, the article will include a statement to this effect: *The authors declare that they have no financial or*

personal relationship(s) that may have inappropriately influenced them in writing this article. Read our **policy on competing interests**.

- Author contributions: All authors must meet the criteria for authorship as outlined in the **authorship** policy and **author contribution** statement policies.
- Funding: Provide information on funding if relevant
- Data availability: All research articles are encouraged to have a data availability statement.
- Disclaimer: A statement that the views expressed in the submitted article are his or her own and not an official position of the institution or funder.

References: Authors should provide direct references to original research sources whenever possible. References should not be used by authors, editors, or peer reviewers to promote self-interests. Refer to the journal referencing style downloadable on our *Formatting Requirements* page.

Appendix D: Ethics clearance

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R49 Dr N Shongwe

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210538

NAME: **(Principal Investigator)**	Dr N Shongwe
DEPARTMENT:	School of Clinical Medicine Department of Paediatrics and Child Health Medical School University
PROJECT TITLE:	*The clinical spectrum of Viridans Group Streptococcus bactereamia in paediatrics patients at Chris Hani Baragwanath Academic Hospital* *Change of study title noted on 2021/09/20*
DATE CONSIDERED:	2021/05/28
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Professor K Petersen and Dr F Mabena
APPROVED BY:	CB Penny Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/08/24

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. **I agree to submit a yearly progress report**. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in May and therefore reports and re-certification will be due in the month of **May** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

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