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STREPTOCOCCUS PNEUMONIAE SEROTYPE DISTRIBUTION AMONG ADULTS ADMITTED WITH COMMUNITY ACQUIRED PNEUMONIA ACROSS SOUTH AFRICA IN THE COVID-19 ERA

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DECLARATION

I **Grant Munkwase** declare that this research report is my own, unaided work. It is being submitted for the Degree of Master of Science in Medicine in the field of Vaccinology at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

(Signature of candidate)

On __27____day of___June___2024_

DEDICATION

Dedicated to my lovely wife Betty Munkwase, my dear children Gabriel Jireh Muhumuza & Gianna Shalom Natukunda, my beloved mother Godliver Katushabe, and the entire family of the late Rwamunyonyore Vincent.

ABSTRACT

Introduction

Recommendations for pneumococcal vaccination require periodic updating considering the ever-changing pneumococcal epidemiology and licensure of new vaccines. Pneumococcal epidemiology especially oronasopharyngeal (ONP) carriage in the coronavirus disease 2019 (COVID-19) era remains unclear in South Africa (SA). This study investigated pneumococcal serotype distribution among adults hospitalized with Community Acquired Pneumonia (CAP) in the COVID-19 era in SA.

Methods

Secondary analysis of ONP and blood serotypes from autolysin gene polymerase chain reaction (*lytA* PCR) positive samples of 317 adults admitted between March 2020 and October 2021 and followed up for two years. Prevalence and incidence rates (IR) of pneumococcal serotypes were calculated, and incidence drivers were explored by Poisson regression. Serotype-associated mortality rates were calculated and mortality predictors were assessed using logistic regression, Kaplan Meier survival analysis, and Cox regression.

Results

Of the 317 participants enrolled in this study, 171 (54%) were males, 299 (94%) were blacks, 126 (40%) were aged 35-49 years, 143 (45%) had no source of income, 125 (39%) were alcohol users, 99 (31%) were current smokers and 203 (64%) were people living with HIV (PLWH) of which 122 (60%) were on antiretroviral therapy (ART). Pneumococcal CAP prevalence was 22,638 cases per 100,000 persons (95% CI: 20,098 -25,338). Serotypes 1, 3, 4, 5, 8, 9A/V, 12A/B/F/44/46, 19F, 22A/F, 33A/F/37 had leading prevalence and IR in the oronasopharynx while serotypes 1, 3, 4, 8, 6A/B, 11A/D, 19A had leading prevalence in blood. The oronasopharyngeal prevalence for all serotypes was 20,888 serotypes per 100,000 persons (95% CI: 18,919-22,964) and that of the 13-valent pneumococcal conjugate vaccine (PCV13) serotypes alone was 13,571 serotypes per 100,000 persons (95% CI: 11,929 - 15,348). The ONP carriage prevalence for all serotypes was significantly higher in males (70%), participants aged 35-49 years (75%), current smokers (73%) and alcohol users (72%) while prevalence for the 23-valent pneumococcal polysaccharide vaccine (PPSV23) serotypes was significantly higher in PLWH on ART (66%). IR varied with the period of testing with the lowest all-serotype IR in March 2020-October 2020 when compared to March 2021-June 2021 (IRR= 0.30, 95% CI: 0.07-

1.20). ONP serotypes 1, 3, 4, 5, 15A/F, 18A/B/C/F, 23A, 33A/F/37 were associated with leading mortality. Mortality was significantly higher in participants with CURB-65 score 1 (adjusted OR= 3.30, 95% CI:1.38-7.90, p=0.007) and in underweight participants (adjusted OR=4.82, 95% CI: 1.76-13.15, p=0.002). There was increased mortality risk among participants carrying ONP serotype 1 (adjusted HR=2.56, 95% CI=1.24- 5.30, p= 0.011) and those who required oxygen supplementation (adjusted HR= 1.98, 95% CI=1.11-3.54, p= 0.021).

Conclusion

S. pneumoniae remains important in CAP epidemiology in SA although there was a big decline in incidence in 2020 following the COVID-19 outbreak. Most of the serotypes circulating among adults in the COVID-19 era are included in PCV20, which is not used in SA.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
CAP	Community-Acquired Pneumonia
CHBAH	Chris Hani Baragwanath Academic Hospital
COVID-19	Coronavirus Disease 2019
CURB-65	Confusion, urea >7 mmol/l, respiratory rate ≥30 breaths/min, low blood pressure (systolic <90 mmHg and/or diastolic ≤60 mmHg) and age ≥ 65 years
CXR	Chest X-Ray
CXR+ CAP	Chest X-Ray Confirmed Community Acquired Pneumonia
EPI	Expanded Programme on Immunization
GERMS-SA	Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
IPD	Invasive Pneumococcal Disease
IR	Incident Rate
IRR	Incident Rate Ratio
KTHC	Klerksdorp Tshepong Hospital Complex
LRTIs	Lower Respiratory Tract Infections
lytA PCR	Autolysin gene Polymerase chain reaction
NICD	National Institute for Communicable Diseases
NVT	Non-Vaccine Type
ONP	Oronasopharyngeal
PCV	Pneumococcal Conjugate Vaccine
PHEIC	Public Health Emergency of International Concern
PHRU	Perinatal HIV Research Unit
PLWH	People Living with HIV
PMH	Polokwane/Mankweng Hospital
PPSV	Pneumococcal Polysaccharide Vaccine

VT	Vaccine Type

CHAPTER ONE: INTRODUCTION

1.1 General introduction

Community acquired pneumonia (CAP) continues to be one of the world's major cause of morbidity and mortality despite the recent advances in disease prevention through immunisation, new diagnostic tests, and antimicrobial therapy (1,2). The greater burden of CAP is borne by younger children (<5 years), the elderly, and persons with comorbidities including human immunodeficiency virus (HIV) (2–5).

Several microbes including bacteria, fungi, viruses and mycobacteria play a role in the etiology of CAP with *Streptococcus pneumoniae* (pneumococci) being the major bacterial cause (3,6–8). Over 100 serotypes of pneumococci are known and they contribute disproportionately to the burden of pneumococcal pneumonia in different ages and geographical regions (8).

Globally there were about 488 million incident cases of lower respiratory tract infections (LRTIs) with CAP inclusive among all age groups in 2019 (9). These cases were associated with over two million deaths among all age groups and pneumococci were associated with more deaths than all other CAP etiologies combined (9). Sub-Saharan Africa bears the highest burden of LRTIs across all age groups (6,10).

The high HIV/AIDS prevalence in South Africa (13.7 % in the general population and about 19.5% in individuals aged 15-49 years) contributes to the disproportionate burden of CAP in South Africa (11). It has been observed recently that 46.4% of all patients hospitalized with LRTI in KwaZulu-Natal in South Africa had CAP (12). The incidence of the invasive pneumococcal disease (IPD) in South Africa in 2020 was about two cases per 100,000 persons (13). According to the 2020 statistical release from the Department of Statistics of South Africa, respiratory diseases were responsible for 42,202 deaths in 2017, of which pneumonia accounted for 4.2 % (14).

Vaccination is arguably the most efficient approach for preventing morbidity and mortality associated with CAP. In South Africa, pneumococcal vaccination for children is part of the expanded programme on immunization (EPI). Pneumococcal vaccination was rolled out in South Africa in 2009 using the 7-valent pneumococcal conjugate vaccine (PCV7) (15) which was replaced two years later with PCV13 (16). Pneumococcal vaccination with PCV13 and/or PPSV23 has been recommended in South Africa since 2017 for all adults above 50 years of age and for younger adults (≥18 years) who have severe comorbidities or are immunocompromised

(17). However, it is currently not being implemented in South Africa's public health system. In addition to pneumococcal vaccination, vaccination against influenza has been reported to offer protection against pneumococcal disease (18,19). Although influenza vaccination is recommended for adults with comorbidities and for individuals above 65 years of age in SA (20), it is also not yet part of the public health system like adult pneumococcal vaccination.
Childhood pneumococcal vaccination with good coverage has been reported to offer indirect protection to the unvaccinated population including adults at high risk and the elderly (8,21,22). Herd immunity may however reach a ceiling just as it has been reported for PCV13 and this calls for vaccination of high-risk adult populations (8).
With increasing pneumococcal vaccination coverage, pneumococcal pneumonia epidemiology tends to change over time in both the vaccinated and the unvaccinated individuals. The non-vaccine type (NVT) serotypes and the non-encapsulated pneumococci may become major causes of CAP (2,8). Moreover, the outbreak of Coronavirus Disease 2019 (COVID-19) in 2019 and the public health restrictions that were subsequently implemented are believed to have affected pneumococcal vaccination coverage and altered CAP epidemiology during and post COVID-19 era in many parts of the world including South Africa.
The National Institute for Communicable Diseases (NICD) has been conducting laboratory-based IPD surveillance in South Africa (Group for Enteric, Respiratory and Meningeal Diseases Surveillance in South Africa - GERMS-SA) since 1999. GERMS-SA conducts serotyping of pneumococci from positive cultures of samples taken from the normally sterile sites (majorly blood) and hence does not provide information on oronasopharyngeal carriage in adults hospitalized with CAP (13). Though microbiological culturing is associated with higher specificity, it is associated with low yields, low sensitivity and may not be useful in individuals previously treated with antibacterial medicines (23,24). IPD surveillance does not provide information on non-bacteremic pneumococcal CAP which may be up to 10 times higher than bacteremic pneumococcal CAP (25).

1.2 Problem statement

Recommendations for pneumococcal vaccination require periodic updating considering the ever-changing pneumococci epidemiology and the global licensure of new pneumococcal vaccines. The recommendations for adult pneumococcal vaccination have not been updated since 2017 in South Africa despite the high burden of CAP among adults. The outbreak of COVID-19 in South

Africa in March 2020 and the subsequent implementation of various COVID-19 preventive and control measures are believed to have significantly impacted the pneumococci epidemiology in South Africa although this has not been adequately evaluated. The NICD laboratory based IPD surveillance in South Africa has limitations and only provides data on bacteremic pneumococcal CAP. Therefore, there remains a paucity of data on nasopharyngeal colonization among adults at the time of a CAP admission in South Africa particularly in the COVID-19 era.

1.3 Justification for the study

South Africa is yet to incorporate adult pneumococcal vaccination in its public health system although the burden of pneumococcal CAP and mortality among adults remains high. There is limited data on pneumococcal CAP and pneumococcal carriage epidemiology among adults hospitalized with CAP in the COVID-19 era in South Africa. This study will identify the most prevalent pneumococcal serotypes among adults following COVID-19 outbreak which will be useful to immunization policy makers and relevant stakeholders while deciding on the optimal pneumococcal vaccine for adult vaccination in SA. Establishment of factors associated with pneumococcal serotype distribution will also help in identifying priority groups for adult pneumococcal vaccination in SA.

1.4 Research questions

1. What is the pneumococcal serotype prevalence among adults hospitalized with CAP in the COVID-19 era in South Africa?
2. What are the pneumococcal serotype incidence rates among adults hospitalized with CAP in the COVID-19 era in South Africa?
3. What are the determinants of pneumococcal serotype distribution among adults hospitalized with CAP in the COVID-19 era in South Africa?
4. What is the pneumococcal serotype associated mortality rate among adults hospitalized with CAP in the COVID-19 era in South Africa?

1.5 Study aim

This study aimed at investigating *S. pneumoniae* serotype distribution in South Africa among adults hospitalized with CAP in the era of COVID-19. In addition, the association of different pneumococcal serotypes with mortality was explored.

It is hypothesized that different *S. pneumoniae* serotypes at initial hospital admission carry the same mortality risk and/or associated with the same survival time among *S. pneumoniae* Chest X-Ray Confirmed Community Acquired Pneumonia (CXR+ CAP)

1.6 Study objectives

1. To establish pneumococcal serotype prevalence across the provinces of Limpopo, Gauteng and North West in South Africa among adults hospitalized with CAP in the era of COVID-19.
2. To establish pneumococcal serotype incidence rates across the provinces of Limpopo, Gauteng and North West in South Africa among adults hospitalized with CAP in the era of COVID-19.
3. To investigate differences in pneumococcal serotype distribution across provinces of Limpopo, Gauteng and North West in South Africa by age, gender, and HIV serostatus among adults hospitalized with CAP in the era of COVID-19.
4. To examine the relationship between pneumococcal serotypes and mortality across provinces of Limpopo, Gauteng and North West in South Africa among adults hospitalized with CAP in the era of COVID-19.

CHAPTER TWO: LITERATURE REVIEW

2.1 Community acquired pneumonia (CAP) among adults

Community acquired pneumonia is an infection of the lung parenchyma that is acquired outside of a hospital or an extended-care facility (26). CAP is part of the pneumococcal disease and the clinical presentation of CAP varies from the non-invasive mild pneumonia characterized by fever and productive cough to the invasive severe pneumonia complicated by respiratory distress and sepsis (27). The risk of CAP may be influenced by age, comorbidities, immunosuppression, asplenia, smoking, alcohol intake, pregnancy and other life style factors such as overcrowding (20,27) There are multiple microbial etiologies for CAP which include viruses, fungi, mycobacteria, and bacteria though in some cases of CAP, no pathogens have been isolated (28,29).

Among the bacterial causes of CAP among adults, the gram-positive *S. pneumoniae* remains the most common cause (3,6–8,27). Pneumococci are estimated to be responsible for 10 to 40% of all cases of CAP among adults (30–34). Although there are over 100 known serotypes of *S. pneumoniae*, only a few of them account for most observed pneumococcal disease. Different serotypes are known to cause pneumococcal disease across different age groups, time periods and geographical regions (8,30). Different serotypes show variable virulence leading to variable severity of the pneumococcal disease. Similarly, different serotypes exhibit variable susceptibility to antimicrobial therapy making resistant serotypes of unique importance in the era of widespread antimicrobial resistance (35). ONP colonization (carriage) is a prerequisite to pneumococcal disease. In adults alone pneumococcal carriage may range from 5 to 10% which is lower than for school children where it ranges from 20-60% (30). *S. pneumoniae* is transmitted by direct contact with respiratory secretions from patients of pneumococcal disease and from healthy carriers (35).

As per the current guidelines of management of CAP in South Africa, it is required that the disease be appropriately diagnosed for effective management. The severity of the disease is taken into consideration while determining the site of care, the level of microbiological investigations and the choice of initial antibiotic therapy (20).

2.2 Vaccination against pneumococcal pneumonia in South Africa

In the era of widespread antibiotic resistance and the high mortality associated with community acquired pneumonia, pneumococcal vaccination is key in the prevention and control of the

spread of the pneumococcal disease. Though there is high vaccination coverage against pneumococcal disease in children (30), vaccination in other high-risk groups remains very low in many low- and middle-income countries (LMICs) including SA. In SA, vaccination coverage against vaccine preventable diseases including pneumococcal disease also varies across provinces (36).

Pneumococcal vaccines belong to two broad categories, that is the pneumococcal capsular polysaccharide vaccines and the conjugate pneumococcal vaccines. The currently approved pneumococcal capsular polysaccharide vaccine is 23-valent (PPSV23). Several pneumococcal conjugate vaccines have also been licensed which includes PCV7, PCV10, PCV13, 15-valent pneumococcal conjugate vaccine (PCV15) and the 20-valent pneumococcal conjugate vaccine (PCV20) (30).

Pneumococcal capsular polysaccharide vaccines were the first to be developed. The first pneumococcal capsular polysaccharide vaccine was authorized in the United States of America (USA) in 1977 and it contained 14 serotypes. This was later replaced in 1983 by the currently in use PPSV23 which contains 23 serotypes (30). Pneumococcal capsular polysaccharide vaccines elicit only a T-cell independent antibody immune response and as such they are ineffective in children less than two years but effective in other age groups. They reduce the burden of the invasive pneumococcal disease caused by the serotypes contained in the pneumococcal vaccines. There is still uncertainty on the role of pneumococcal capsular polysaccharide vaccine in reducing the non-invasive pneumococcal disease (37–39). These vaccines are not associated with immune memory, do not contribute to mucosal immunity, have no effect on pneumococcal carriage and do not offer herd protection to the unvaccinated individuals (40).

In a bid to overcome the limitations associated with the pneumococcal capsular polysaccharide vaccine, the pneumococcal conjugate vaccines were developed in which the capsular polysaccharide is conjugated to a carrier protein. The non-toxic mutant of diphtheria toxin CMR197 is one of the carrier proteins used almost all the time in the pneumococcal conjugate vaccines. Pneumococcal conjugate vaccines elicit a T-cell dependent antibody immune response and as such they are effective in all ages including children under two years of age. They reduce the burden of both the non-invasive and invasive pneumococcal disease caused by serotypes contained in the pneumococcal vaccines. These vaccines reduce pneumococcal carriage and offer herd protection to unvaccinated individuals (30,40,41).

Pneumococcal vaccination in children had been implemented in 148 countries out of the 194 WHO member countries by the end of 2020 (30). South Africa rolled out pneumococcal vaccination for children in 2009 as part of the EPI. Vaccination for children started with PCV7 in 2009 but was later replaced with PCV13 in 2011 (15). The current PCV13 immunization coverage among children less than one year stands at 87% in South Africa (42). By 2016, pneumococcal vaccination alone was responsible for up to 33% reduction for all-cause pneumonia deaths in children aged one month to 18 years in South Africa (43).

On the other hand, vaccination against pneumococcal disease in other high-risk populations other than young children has remained low. The high-risk populations include persons older than 65 years, adults (18-64 years) with comorbidities or immunocompromising conditions and adults with functional or anatomical asplenia. In such high-risk groups, the risk of CAP can even be more than 20 times higher than in the general population (44,45).

Policies and programs for adult vaccination exist mainly in high-income countries and are nearly absent in all LMICs. Even where adult vaccination is part of the routine vaccination programs in high income countries such as USA, vaccination coverage remains low (46).

Different guidelines and recommendations exist for adult pneumococcal vaccination in different jurisdictions. These recommendations take into consideration the burden of CAP in the geographical area, pneumococcal serotype distribution and the cost-effectiveness of the vaccination program.

In South Africa, recommendations are made for pneumococcal vaccination of adults aged over 50 years with PCV13 and/or PPSV23 depending on their vaccination history and presence of comorbidities (17,20). It is recommended that individuals 50-64 years who are naïve to pneumococcal vaccines should receive one dose of PCV13. All individuals above 50 years with PPSV23 vaccination history should receive one dose of PCV13 at least one year later. Individuals who are 65 years and above that are naïve to pneumococcal vaccines should receive one dose of PCV13 followed by one dose of PPSV23 one year later. Similarly, all individuals who are 18 years and above with severe comorbid or immunocompromising conditions and are naïve to pneumococcal vaccines should receive one dose of PCV13 followed by one dose of PPSV23 at least two months later. If they have received PPSV23 in the past, they should receive one dose of PCV13 at least one year later (20). Despite these recommendations, adult pneumococcal vaccination is not yet incorporated in the public health sector of South Africa.

It remains unclear if PCV13 and/or PPSV23 are still optimal vaccines for adult pneumococcal vaccination in the context of South Africa six years later after the publication of the current guideline. The epidemiology of CAP changes with time and this change is believed to have been greatly impacted by the COVID-19 pandemic. Furthermore, new vaccines covering more serotypes such as PCV15 and PCV 20 have been licensed since the publication of the current guideline. PCV13 is no longer recommended by the Advisory Committee on Immunization Practices (ACIP) for adult vaccination in the USA (47) though it remains unknown if the epidemiology of pneumococcal CAP in South Africa significantly differs from that of USA. It is therefore necessary that the current epidemiology of CAP in South Africa be established, and the pneumococcal vaccination recommendations be updated appropriately for optimal health outcomes.

2.3 COVID-19 public health restrictions in South Africa and impact on pneumococcal pneumonia epidemiology

Following an outbreak of COVID-19 in Wuhan City, China in 2019, it quickly spread to all countries. WHO declared COVID-19 a public health emergency of international concern (PHEIC) on 30 January 2020 and South Africa registered its index case on 05 March 2020 (48,49). According to WHO, South Africa had registered 4,060,385 confirmed cases of COVID-19 with 102,595 deaths by 28 February 2023 (50).

In a bid to control the spread of COVID-19 in South Africa, the Department of Health (DoH) issued several public health measures and restrictions. These included night-time curfews, restrictions on public gatherings, restrictions on both international and local travels, use of face masks, vaccination, hand washing and physical distancing among others (51). These measures had far reaching effects to almost every sector of the society including the economy, social aspects, and health. These measures were either eased or tightened across the pandemic depending on COVID-19 status in South Africa leading to different alert levels (Figure 1) but the impact of this on CAP epidemiology across these different time periods remains unclear. The higher the alert level, the stricter the COVI9-19 restrictions that were implemented.

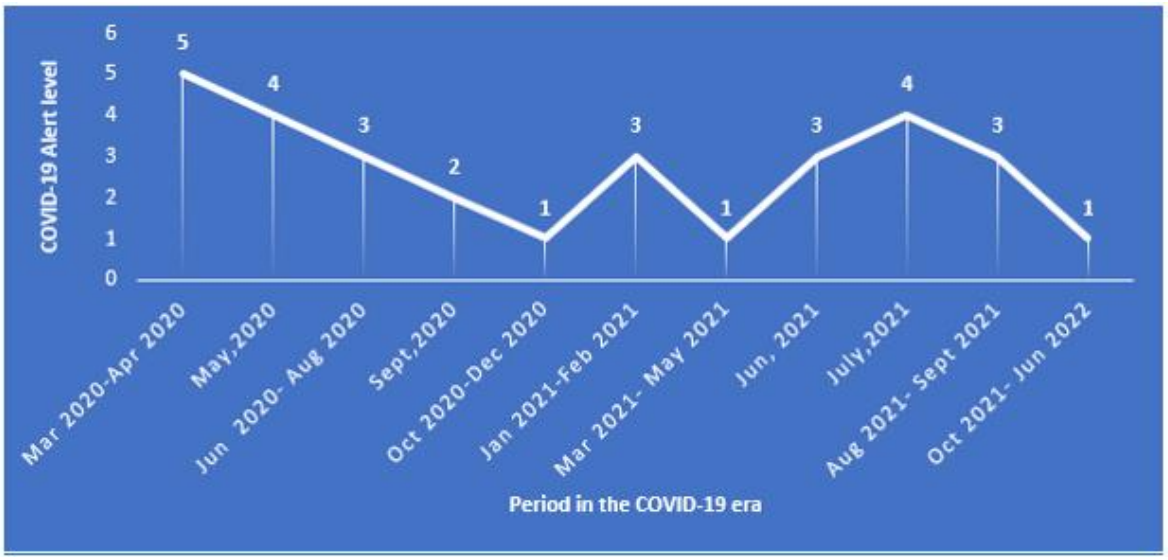

Figure 1: COVID-19 alert levels in South Africa in the COVID-19 era (52).

COVID-19 restrictions had a negative impact on access to health services including immunization services in various parts of the world. COVID-19 restrictions caused disruptions in access to immunization services and as such there was a decline in immunization coverage for a number of routine vaccines in several parts of the world (53–56). In South Africa, it is estimated that children who are fully immunized at one year dropped by about 4.3% between 2019 and 2020 (57). The immunization coverage for PCV13 alone among children aged one year in South Africa dropped by 3% in 2020 (42). It is postulated that changes in the vaccination coverage for routine vaccines including pneumococcal vaccines during the COVID-19 era had an impact on pneumococcal CAP epidemiology though it has not been evaluated in South Africa.

It is known that viral infections such as influenza predispose affected individuals to secondary bacterial infections such as IPD. It was therefore theoretically expected that severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections would increase the risk of IPD although the study in England found contrary results where the risk of IPD declined in England in the early months of the COVID-19 pandemic (58). Another study that analyzed surveillance data during the COVID-19 pandemic from 26 countries also reported a decline in IPD cases (59). A study in England found out that the *S. pneumoniae* serotypes associated with IPD remained unchanged in the pre-COVID-19 era and within the first six months of COVID-19 outbreak though it remains unknown if the serotypes changed as the pandemic progressed (58). The decline in the incidence rates of IPD could be attributed to COVID-19 public health restrictions

that reduced social mixing and other public health measures like hand washing, use of sanitizers and masks that are generally used for infection control and prevention.

Similarly, the NICD via GERM-SA reported a decline in IPD cases in 2020 and 2021 in South Africa. GERMS-SA involves serotyping of pneumococci isolated from positive cultures of samples from the normally sterile sites (13). Although blood culture is associated with higher specificity, it is limited by the low yields, low sensitivity and prior antibacterial treatment (23,24). IPD surveillance covers bacteremic pneumococcal CAP, meningitis, sepsis, osteomyelitis, and infections of other sterile body sites. It should be noted that bacteremic pneumococcal CAP represents just a small fraction of all pneumococcal CAP cases. Non-bacteremic pneumococcal CAP can be up to 10 times higher than bacteremic pneumococcal CAP (25). The current study addressed these limitations by serotyping pneumococci in the oronasopharyngeal swabs in addition to blood samples that tested positive for pneumococci by *lytA* PCR. *lytA* PCR used for identifying pneumococci in this study is highly sensitive and specific but it also remains useful even when individuals have been previously treated with antibiotics (60–64).

2.4 Pneumococcal serotype distribution (prevalence and incidence rates) among adults in the COVID-19 era in South Africa

Although there is vast information on the epidemiology of CAP without considering the etiology in all age groups from all over the word (2,5,6,27), there remains a paucity of information on the epidemiology of pneumococcal CAP among adults in many parts of the world including South Africa. Much of the available information is on the epidemiology of bacteremic pneumococcal CAP coming from IPD surveillance that is done in many parts of the world for monitoring the effectiveness of pneumococcal vaccination programs (65). On the other hand, the epidemiology of non-bacteremic pneumococcal pneumonia in adults remains poorly documented.

A study in South Korea reported an incidence rate of 44.3 cases per 100,000 persons for pneumococcal pneumonia compared to 4.8 cases per 100,000 persons for IPD. The incidence rates increased for both pneumococcal pneumonia and IPD with age with the highest rates observed in individuals over 80years (66). This further indicates that IPD surveillance underestimates the burden of pneumococcal disease.

The number of IPD cases in individuals older than five years has been on the decline in South Africa since the roll out of PCV13 in 2011 (67). The decline was even greater in 2020 and 2021

and this could be attributed to the COVID-19 public health restrictions that reduced social mixing, and other preventive and control measures for COVID-19 that reduce the risk of communicable diseases. It remains unknown if the decline in IPD cases in 2020 and 2021 was associated with a parallel decline in bacteremic pneumococcal CAP cases in the different age groups (especially for 18-49 years, 50-64 years and the elderly ≥65 years). Similarly, no information is available on the pattern of non-bacteremic pneumococcal CAP and pneumococcal carriage in the same period for the same age groups.

The number of IPD cases due to serotypes in PCV13 has declined significantly in South Africa with annual cumulative cases less than 150 by the end of 2021. On the other hand, the number of IPD cases due to NVT serotypes remained high by the end of 2021 (almost three times higher) (67). For example among the top five serotypes (8,3,19A,12F and 4) responsible for IPD in individuals older than 5 years in South Africa, serotypes 8 and 12F are not included in PCV13 (68). It was considered of great importance to characterize the burden of each of the pneumococcal serotype associated with CAP as this will help in deciding the optimal pneumococcal vaccine in the context of South Africa since there are newer licensed vaccines globally that may provide wider serotype coverage.

2.5 Determinants of *S. pneumoniae* serotype distribution and serotype associated mortality in the COVID-19 era in South Africa

Several factors have been associated with pneumococci distribution either in mere carriage or pneumococcal disease in adults but largely from studies conducted in the pre-COVID-19 era. In a systematic review by Neal *et al.*, factors associated with higher pneumococcal carriage included young age, poverty, ethnicity, lower respiratory tract infection symptoms, living with younger children, season and co-colonization with other respiratory pathogens while lower carriage was observed among breastfeeding mothers and users of antibiotics (69).

Gender has shown conflicting results on pneumococcal carriage with some studies reporting higher carriage in males than females (70–72), others reporting higher carriage in females than males (73) though other studies did not find any difference (74,75).

There seems to be consensus from previous studies that smoking or exposure to smoke increases pneumococcal carriage and pneumococcal disease (69,71,75–77).

Higher pneumococcal carriage was reported among PLWH on ART than those not on ART in a study in Malawi (78). Another study in the Netherlands demonstrated that CAP among adult PLWH increased with old age and low CD4 cell count (79).

Presence of comorbidities such as asthma, chronic obstructive pulmonary disease, HIV, heart disease and diabetes have been shown to increase the risk for pneumococcal carriage and more importantly for the invasive disease (68,80,81)

In a systematic review for lifestyle risk factors for IPD, smoking and alcohol-intake were largely associated with increased risk for IPD though one study showed that alcohol intake significantly reduced the risk of IPD (82). It remains unknown if alcohol consumption truly reduced the risk for IPD in this study or there were confounding factors in the study.

Few studies have evaluated factors associated with pneumococci distribution in the COVID-19 era. Though some factors have been reported in more than one study, other factors have only been reported from single studies and to the best of our knowledge no studies were available from South Africa.

In Thailand it was observed that pneumococcal carriage was associated with age with higher carriage in individuals above 65 years (75). In the same study it was shown that pneumococcal carriage did not vary by SARS-CoV-2 infection status (75) which was different in Turkey where carriage was higher among those with COVID-19 (83).

A study in the USA revealed that carriage in the COVID-19 era was higher among individuals that continued to interact with pre-school or school going children similar to what had been shown in the pre-COVID-19 era (84).

In Fiji, it was found out that carriage reduced with time elapsed post roll out of the PCV vaccine in the country while carriage increased with younger age, iTaukei ethnicity, urban residence and living with children below five years, low income, and upper respiratory tract infection symptoms (85).

The few available studies on pneumococcal epidemiology during the COVID-19 era have limited information on individual serotype distribution among adults. In this study we explored pneumococcal serotype distribution in South Africa in the COVID-19 era and investigated the factors associated with serotype distribution.

Similarly, there are a few studies where the association of pneumococci serotype with mortality have been investigated and the available data is limited to the pre-COVID-19 era.

Surveillance data from 2006 to 2013 in North-East England showed that serotypes associated with mortality varied with the period post the IPD episode. The study showed that mortality within 30 days was associated with serotypes 3, 6A, 9N and 19F while mortality at 36 months was associated with serotypes 1 and 9N (86).

Adults above 18 years in France who were hospitalized with IPD between 2013 and 2015 were likely to die within 30 days if they had any of the serotypes with known high mortality potential (1, 5, 12F, 24F, 38, 7F) (87).

From the review of IPD mortality data in South Africa from 2012 to 2018, it was observed that serotype 15B/C in addition to male gender, HIV infection and older age (≥45 years) were associated with higher IPD mortality in individuals above 15 years (88). However, analysis of mortality data in a period before introduction of PCV in South Africa (2003 to 2008) showed serotype 1 and 19F to be associated with higher mortality in addition to increasing age (89).

There is paucity of data on serotypes associated with pneumococcal pneumonia mortality in the COVID-19 era. This study explored pneumococci serotype associated mortality among individuals with presumed CAP and investigated factors associated with mortality as well.

CHAPTER THREE: METHODS AND MATERIALS

3.1 Study design

In this study, we retrospectively abstracted data from a dataset of a cohort study titled "The potentially preventable burden of community-acquired pneumonia in South African adults, in the era of widespread paediatric PCV13 immunisation and antiretroviral therapy rollout" (abbreviated as "Pot Prev Study"). The "Pot Prev Study" was conducted by the Perinatal HIV Research Unit (PHRU) of the University of the Witwatersrand between 2019 and 2022. The data extraction sheet used to obtain data for this study is provided in Appendix 1 while the brief description of the primary cohort study is provided in Appendix 2.

3.2 Study population

Our study-targeted adults who were hospitalized for presumed CAP, were enrolled into sub-study 2 of the "Pot Prev Study" and followed up prospectively for approximately two years.
A participant was considered to have presumed CAP if the physician clinically suspected pneumonia in addition to the presence of at least two of the following signs and symptoms: fever or hypothermia within 24 hours before enrolment, chest pain, coughing, expectoration, difficulty in breathing, rapid breathing, abnormal auscultatory findings, and a positive chest X-Ray.
The "Pot Prev Study" was conducted at the following study sites.

1. Polokwane/Mankweng Hospital (PMH) in Limpopo Province.
2. Chris Hani Baragwanath Academic Hospital (CHBAH) in Gauteng Province.
3. Klerksdorp Tshepong Hospital Complex (KTHC) in North West Province.

The "Pot Prev Study" enrolled individuals ≥18 years who were hospitalized for presumed CAP, provided informed consent, and were living in the relevant catchment area. A blood sample, urine sample, nasopharyngeal swab and oropharyngeal swab were taken from each participant at the time of enrolment. Participants were prospectively followed up for two years after enrolment for recurrent CAP and mortality. The follow up involved telephone calls and face to face visits. A urine sample, nasopharyngeal and oropharyngeal swabs were also collected from participants with presumed pneumonia at the clinic visit during the follow-up period. After collection, swabs and blood samples were transported to the NICD for processing for pneumococcus identification and serotyping. *S. pneumoniae* identification in blood and oronasopharyngeal (ONP) specimens was done at NICD by *lytA* PCR which is based on the method described by Carvalho *et al.* (63). Serotyping was also done for positive samples at NICD by triplex real time

PCR assay based on the methods described by Pimenta *et al.*, and Azzari *et al.* (90,91). Detailed description of these laboratory methods is provided in Appendix 2. Urine samples collected in the primary study were processed and shipped to USA for BinaxNOW® and Urinary Antigen Detection (UAD) analysis but their results were not included in this study.

3.3 Eligibility criteria

3.3.1 Inclusion criteria

1) Adults (≥18 years) hospitalized with presumed CAP from the time South Africa reported its first case of COVID-19 (05 March 2020) to October 2021.
2) Having a positive *lytA* PCR on blood sample or oronasopharyngeal swab.

3.3.2 Exclusion criteria

1) Missing pneumococci serotype data.

3.4 Sample size

Data was collected from each participant in sub-study 2 of the "Pot Prev Study" who met our eligibility criteria. Sub-study 2 of the "Pot Prev Study" enrolled approximately 2000 patients of whom 1599 are included in this study between 05 March 2020 and October 2021.

The prevalence of S. *pneumoniae* CAP in adults varies from study to study and as such a prevalence of 50% was considered in sample size calculation since it gives the highest sample size. Considering a prevalence of 50%, margin of error of 5%, confidence interval of 95% and population of 1599 participants in sub-study 2, a minimum of 310 participants with positive *lytA* PCR blood sample or oronasopharyngeal swab were considered of sufficient statistical power for the analysis of our outcomes.

3.5 Variables

Variables collected in this study are provided in Table 1.

Table 1: Study variables

Predictor variables	**Outcome variables**
✓ Age ✓ Gender ✓ Province ✓ HIV serostatus ✓ PCV vaccine received ✓ Antiretroviral therapy (ART) status ✓ Other relevant variables [including race, alcohol in-take, smoking, BMI, employment status, income level, underlying comorbidities, pneumonia severity score based on "confusion, urea >7 mmol/l, respiratory rate ≥30 breaths/min, low blood pressure (systolic <90 mmHg and/or diastolic ≤60 mmHg) and age ≥65 years" (CURB-65 score)]	✓ Pneumococcal serotype at initial hospital admission and in the follow-up period. ✓ Death (mortality) ✓ Time to death since initial hospital admission (survival time)

3.6 Data management and analysis

Following extraction of data from the "Pot Prev Study" dataset, it was cleaned and analysed with StataSE 17.0., StataCorp LLC, Texas, USA.

The socio-demographic features were summarized using frequencies (percentages) for categorical variables and medians (interquartile range) for continuous measures.

The prevalence of CXR+ CAP associated with *S. pneumoniae* was calculated as follows.

$$\text{Prevalence of } S.\ pneumoniae \text{ CXR+ CAP} = \frac{\text{Counts of } S.\ pneumoniae \text{ CXR+ CAP}}{\text{Total number of CXR+ CAP participants}} \times 100{,}000$$

The analysis included all the 22 serotypes/serogroups in the study population. The analysis was stratified by serotypes/serogroups from blood or from oronasopharyngeal swabs.

In addition to individual serotype analysis, vaccine serotype analysis was conducted by grouping together all the serotypes included in a particular licensed pneumococcal vaccine. A serotype that

was detected in our study but is not included in any of the globally licensed pneumococcal vaccines was classified as “non-vaccine serotype”. The list of the current globally licensed pneumococcal vaccines is provided in Table 2.

Table 2: Globally licensed pneumococcal vaccines.

Vaccine type	**Trade name**	**Serotypes included.**
PCV7	Prevnar®	4, 6B, 9V, 14, 18C, 19F and 23F
PCV10	Synflorix®	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, and 23F
PCV13	Prevnar13®	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F
PCV15	Vaxneuvance®	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F
PCV20	Prevnar20®	1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F
PPSV23	Pneumovax 23®	1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F.

The statistical analyses conducted in this study are summarized in Table 3.

Table 3:Summary of statistical analyses done in this study.

Objective	**Outcome**	**Analysis**
Objective 1 To establish pneumococcal serotype prevalence across the provinces of Limpopo, Gauteng and North West in South Africa among adults hospitalized with CAP in the era of COVID-19.	Pneumococcal serotype prevalence	<u>Specific serotype prevalence</u> The counts of each serotype in the study population at initial hospital admission was divided by the number of participants enrolled in sub-study 2 between 05 March 2020 and October 2021. <u>Vaccine serotype prevalence</u> The counts of all serotypes (as included in a particular pneumococcal vaccine) in the study population at initial hospital admission was divided by the number of participants enrolled in sub-study 2 between 05 March 2020 and October 2021.

		Non-vaccine serotype prevalence The counts of all serotypes (not included in any pneumococcal vaccine) in the study population at initial hospital admission was divided by the number of participants enrolled in sub-study 2 between 05 March 2020 and October 2021. *Note: All prevalence values were expressed per 100,000 persons by multiplying each calculated prevalence value by 100,000 and all prevalence values were reported with 95% confidence intervals.* *Comparisons of serotype prevalence across different variables (such as province, age-group, gender, HIV status, ART status) were done using Pearson's chi-square test and p-values were reported.*
Objective 2 To establish pneumococcal serotype incidence rates across the provinces of Limpopo, Gauteng and North West in South Africa among adults hospitalized with CAP in the era of COVID-19.	Pneumococcal serotype incidence rates	Specific serotype incidence rate The counts of individual serotypes in the study population during follow up period was divided by the total person time of follow-up for eligible participants in sub-study 2 enrolled between 05 March 2020 and October 2021 and had a sample tested. Vaccine serotype incidence rate The counts of all serotypes (as included in a particular pneumococcal vaccine) in the study population during follow up period was divided by the total person time of follow-up for eligible participants in sub-study 2 enrolled between 05 March 2020 and October 2021 and had a sample tested.

		Non-vaccine serotype incidence rate The counts of all serotypes (not included in any pneumococcal vaccine) in the study population during follow up period was divided by the total person time of follow-up for eligible participants in sub-study 2 enrolled between 05 March 2020 and October 2021 and had a sample tested. Poisson regression modelling The serotype incidence rates were modelled for different predictor variables using Poisson regression modelling. The dependent (response) variable (Y) was the serotype counts with participant follow-up time as the offset variable. The goodness of fit of the Poisson model was assessed by the deviance goodness-of-fit and the Pearson goodness-of-fit. *Note: All incident rate values were expressed per 100,000 person years by multiplying each calculated incident rate value by 100,000 and all incident rate values were reported with the 95% confidence intervals.* *Participants were not censored when serotype(s) is identified at any point of follow-up. Only death was the basis to permanently censor participants. For participants lost to follow-up, their time was included up to when they were last contacted.*
Objective 3 To investigate differences in pneumococcal serotype distribution	Pneumococcal serotype distribution	Frequency counts and percentages The counts of each serotype and percentages were presented by province, age group, gender, HIV serostatus, ART status among other variables.

across the provinces of Limpopo, Gauteng and North West in South Africa by gender, age, and HIV serostatus among adults hospitalized with CAP in the era of COVID-19.		<u>Run-sequence plots (run charts)</u> The frequency counts of each individual serotype or grouped vaccine serotypes were plotted against different time points of follow-up to examine trends in serotype distribution.
Objective 4 To examine the relationship between pneumococcal serotypes and mortality across the provinces of Limpopo, Gauteng and North West in South Africa among adults hospitalized with CAP in the era of COVID-19.	Death	<u>Specific serotype mortality rate</u> The counts of deaths around initial hospital admission that are associated with each serotype in the study population were divided by person-years at risk post 05 March 2020 in sub-study 2. Mortality was assessed at 3 days, 7 days, and 14 days from the date of initial hospital admission. <u>Vaccine type mortality rate</u> The counts of deaths around initial hospital admission that are associated with serotypes included in a particular pneumococcal vaccine was divided by person-years at risk post 05 March 2020 in sub-study 2. Mortality was assessed at 3 days, 7 days, and 14 days from the date of initial hospital admission. <u>Non-vaccine type mortality rate</u> The counts of deaths around initial hospital admission that are associated with serotypes not included in any pneumococcal vaccine was divided by person-years at risk post 05 March 2020 in sub-study 2. Mortality was assessed at 3 days, 7 days,

		and 14 days from the date of initial hospital admission. *Note: All mortality rate values were expressed per 1000 person years by multiplying the calculated values by 1000 and all mortality rates were reported with 95% confidence intervals.*
	Death	**Hypothesis.** We hypothesized that different *S. pneumoniae* serotypes at initial hospital admission carry the same mortality risk among *S. pneumoniae* CXR+ CAP participants. <u>Binary logistic regression</u> The risk of *S. pneumoniae* serotype and other factors (including province, age, gender, HIV status, ART status) on death was analysed using binary logistic regression at 3 days, 7 days, and 14 days from the date of initial hospital admission. The multivariate logistic regression model was fitted using a stepwise approach with the probability of a predictor variable entering the model being less than 0.05. Only the predictor variables that were significant at bivariate analysis were considered for multivariate modelling. Odds ratios, 95% Confidence Intervals and p-values were reported for different risk factors. Goodness of fit of the models was assessed by the Pearson Chi2, Hosmer–Lemeshow Chi2 and the Area under ROC curve tests.
	Survival time (time to death since initial	**Hypothesis.** We hypothesized that different *S. pneumoniae* serotypes at initial hospital admission are associated

	hospital admission)	with the same survival time among *S. pneumoniae* CXR+ CAP participants. <u>Kaplan Meier analysis</u> The effect of individual *S. pneumoniae* serotype or pneumococcal vaccine serotypes on survival time was evaluated using Kaplan Meier estimates. Survival was evaluated at 3 days, 7 days, and 14 days from the date of initial hospital admission. Kaplan-Meier survival curves were generated for different variables, and the log-rank test was used for testing statistical significance. The p-values for the Log rank X^2 were reported. <u>Cox proportional hazards regression model.</u> The effect of specific *S. pneumoniae* serotype or pneumococcal vaccine serotypes and other relevant factors on survival time was evaluated using Cox proportional hazards regression model. Survival was evaluated at 3 days, 7 days, and 14 days from the date of initial hospital admission. The multivariate Cox regression model was fitted using a stepwise approach with the probability of a predictor variable entering the model being less than 0.05. The proportional hazard assumption was tested using the Schoenfeld residuals. Only the predictor variables that were significantly associated with survival under Kaplan Meier survival analysis were considered for multivariate Cox modelling. Hazard ratios with 95% confidence intervals and p-values were reported from the model at the corresponding time points.

3.7 Ethics

Ethics approval was obtained from the Witwatersrand University HREC for this study vide HREC (Medical) Clearance Certificate No. M221110 (Appendix 3).

CHAPTER FOUR: RESULTS

4.1 Demographic characteristics of study participants

Of the 1599 participants who were admitted with presumed CAP under sub-study 2 of the "Pot Prev Study" between 05 March 2020 and October 2021, only 317 participants were eligible for our study. Serotypes identified in the ONP swabs and blood samples of the 317 participants at baseline were used in calculation of pneumococcal serotype prevalence. Serotypes identified among participants who had a *lytA* PCR test done during the follow-up period (165 participants) were used in calculation of pneumococcal serotype incidence rates. Pneumococcal serotype associated mortality rates were calculated for the 126 participants who had a documented outcome over the follow-up period within the COVID-19 era (reason for termination from the "Pot Prev Study") (Figure 2).

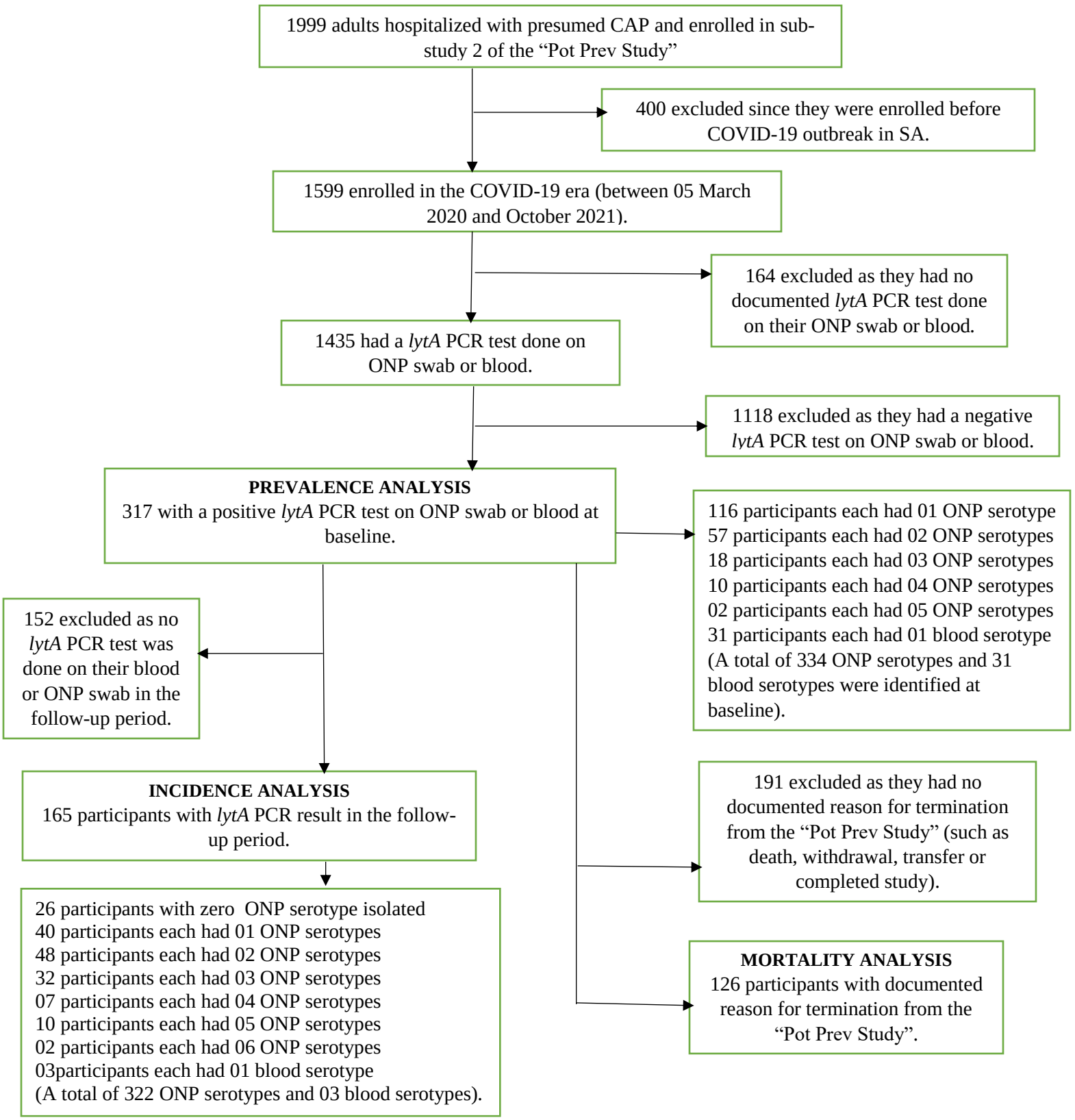

Figure 2: Flow chart showing enrolment of participants in this study.

Most participants were black people (94%), and almost half of the participants were from Gauteng Province. Only two participants (0.63%) reported having received a pneumococcal vaccine. Majority of the participants were aged between 35-49 years (40%) and were living with HIV (64%). Table 4 below gives a summary of demographic characteristics for our study.

Table 4:Demographic characteristics of study participants.

Sample characteristic	Frequency(N=317)	Percent	Median	IQR
Province				
Gauteng	156	49.2		
North West	114	36.0		
Limpopo	47	14.8		
Gender				
Female	146	46.1		
Male	171	53.9		
Race				
Black	299	94.3		
Caucasian	3	0.95		
Coloured	10	3.2		
Indian	4	1.3		
Other	1	0.32		
BMI (kg/m^2)			21.8	18.5 – 28.0
Underweight (<18.5)	77	24.3		
Normal weight (18.5 - <25)	117	36.9		
Overweight (25-<30)	50	15.8		
Obese (≥30)	58	18.3		
Unknown	15	4.7		
Age (years)			42	34 - 55
18-24	27	8.5		
25-34	56	17.7		
35-49	126	39.8		
50-64	75	23.7		
65-85	33	10.4		
HIV serostatus				
HIV Negative	114	36.0		
PLWH	203	64.0		
ART status				
PLWH not on ART	81	39.9		
PLWH on ART	122	60.1		
Presence of chronic comorbidities other than HIV				
No	248	78.2		
Yes	69	21.8		
Pneumococcal vaccination status				

No	315	99.4
Yes	2	0.63
Monthly income level		
No income (R0)	143	45.1
Low income (R1-R4999)	130	41.0
Moderate to high income(≥R5000)	44	13.9
Smoking		
Never smoked	173	54.6
Ex-smoker	45	14.2
Active smoker	99	31.2
Alcohol consumption		
Does not consume alcohol	192	60.6
Consumes alcohol	125	39.4

IQR: Interquartile range

4.2 Prevalence of CAP associated with *S. pneumoniae*

There were 1016 participants in sub-study 2 of the "Pot Prev Study" between 05 March 2020 and October 2021 who had CXR+ CAP as their final diagnosis and only 230 participants of these tested positive for pneumococci by *lytA* PCR on oronasopharyngeal or blood specimens. The calculated prevalence of CAP associated with *S. pneumoniae* (pneumococcal CAP) was about 22638 cases per 100,000 persons (given by (230/1016) X100,000) (95% CI: 20098 -25338).

4.3 Pneumococci serotype prevalence

A total of 334 individual serotypes were isolated in ONP swabs collected from 317 participants in our study. The counts of individual serotypes in oronasopharyngeal swabs were used to calculate prevalence and the corresponding 95% CI. The denominator used for the calculation of prevalence was 1599 (the number of participants admitted with presumed CAP under sub-study 2 of the "Pot Prev Study" at baseline between 05 March 2020 and October 2021).

Similarly, the counts of vaccine type or non-vaccine type serotypes were used in the calculation of the corresponding vaccine type or non-vaccine type serotype prevalence using the same denominator. Serotypes/serogroups 1, 3, 4, 5, 8, 9A/V, 12A/B/F/44/46, 19F and 33A/F/37 had leading individual pneumococci serotype prevalence in oronasopharyngeal swabs with serotype 4 having the highest prevalence. On the other hand, the seven serotypes with the leading prevalence in blood included 1, 3, 4, 6A/B, 8, 11A/D and 19A with serotype 3 having the highest prevalence.

The prevalence of pneumococcal serotypes expressed per 100,000 persons was obtained by dividing the counts of serotypes (numerator) by 1599 (denominator) and the calculated proportion was multiplied by 100,000.

The prevalence for oronasopharyngeal PCV13 serotypes, PPSV23 serotypes and non-vaccine serotypes were 13,571 serotypes per 100,000 persons (given by (217/1599) X100,000) (95% CI: 11,929 - 15,348), 18,889 serotypes per 100,000 persons (given by (302/1599) X100,000) (95% CI: 16,996 -20,893) and 2001 serotypes per 100,000 persons (given by (32/1599) X100,000) (95% CI: 1373 -2814) respectively. The prevalence for blood PCV13 serotypes, PPSV23 serotypes and non-vaccine serotypes were 1376 serotypes per 100,000 persons (given by (22/1599) X100,000) (95% CI: 864 - 2076), 1751 serotypes per 100,000 persons (given by (28/1599) X100,000) (95% CI: 1167-2521) and 188 serotypes per 100,000 persons (given by (3/1599) X100,000) (95% CI: 39 -547) respectively. The oronasopharyngeal and blood vaccine type and non-vaccine type pneumococci serotype prevalence results are summarised in Table 5, Figure 3, and Figure 4.

Table 5: Pneumococci serotype prevalence in oronasopharyngeal swabs or blood among adults admitted with presumed CAP in the COVID-19 era in South Africa.

Individual serotype	Oronasopharyngeal swabs		Blood	
	Serotype count	Prevalence per 100,000 persons (95% CI) [β]	Serotype count	Prevalence per 100,000 persons (95% CI) [β]
1	20	1251(766-1925)	2	125(15-451)
2	3	188(39-547)	0	0 (0-230) *
3	38	2376(1687-3247)	9	563(258-1066)
4	56	3502(2656-4524)	3	188(39-547)
5	23	1438(914-2151)	0	0 (0-230) *
6A/B	5	313(102-728)	3	188(39-547)
6C/D	12	751(388-1307)	2	125(15-451)
7A/F	5	313(102-728)	1	63(2-348)
8	18	1126(668-1773)	2	125(15-451)
9A/V	27	1689(1116-2447)	0	0 (0-230) *
11A/D	8	500(216-983)	3	188(39-547)
12A/B/F/44/46	19	1188(717-1849)	1	63(2-348)
14	1	63(2-348)	0	0 (0-230) *
15A/F	6	375(138-815)	0	0 (0-230) *
16F	7	438(176-900)	1	63(2-348)
18A/B/C/F	8	500(216-983)	0	0 (0-230) *
19A	11	688(344-1228)	4	250(68-639)
19F	21	1313(815-2001)	0	0 (0-230) *

22A/F	3	188(39-547)	0	0 (0-230) *
23A	7	438(176-900)	0	0 (0-230) *
23F	2	125(15-451)	0	0 (0-230) *
33A/F/37	34	2126(1477-2959)	0	0 (0-230) *
Vaccine type serotypes				
PCV7	120	7505(6261-8907)	6	375(138-815)
PCV10	168	10507(9046-12114)	9	563(258-1066)
PCV13	217	13571(11929-15348)	22	1376(864-2076)
PCV15	254	15885(14126-17770)	22	1376(864-2076)
PCV20	299	18699(16816-20698)	28	1751(1167-2521)
PPSV23[#]	302	18889(16996-20893)	28	1751(1167-2521)
Non-vaccine type serotypes[$]	32	2001(1373-2814)	3	188(39-547)
All serotypes	334	20888 (18919-22964)	31	1938(1321-2741)

** one-sided, 97.5% confidence interval*

[β] Prevalence per 100,000 persons = (serotype counts/1599) X 100,000.

[#] PPSV23 contains all the serotypes in all the licensed pneumococcal vaccines and hence in our study PPSV23 serotypes represent "all vaccine serotypes".

[$] refers to serotypes absent in all pneumococcal vaccines and included 6C/D, 15A/F, 16F, 23A.

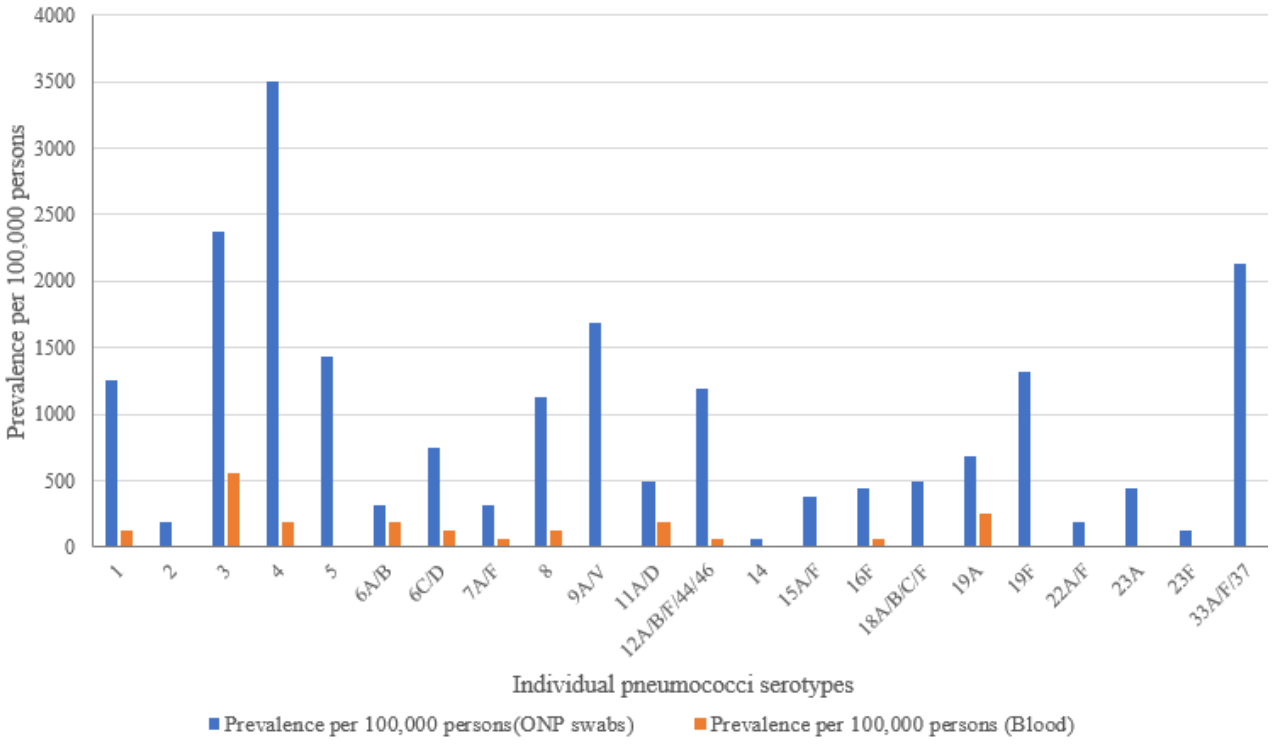

Figure 3: Prevalence of individual pneumococci serotypes in oronasopharyngeal swabs or blood among adults admitted with presumed CAP in the era of COVID-19 in South Africa.

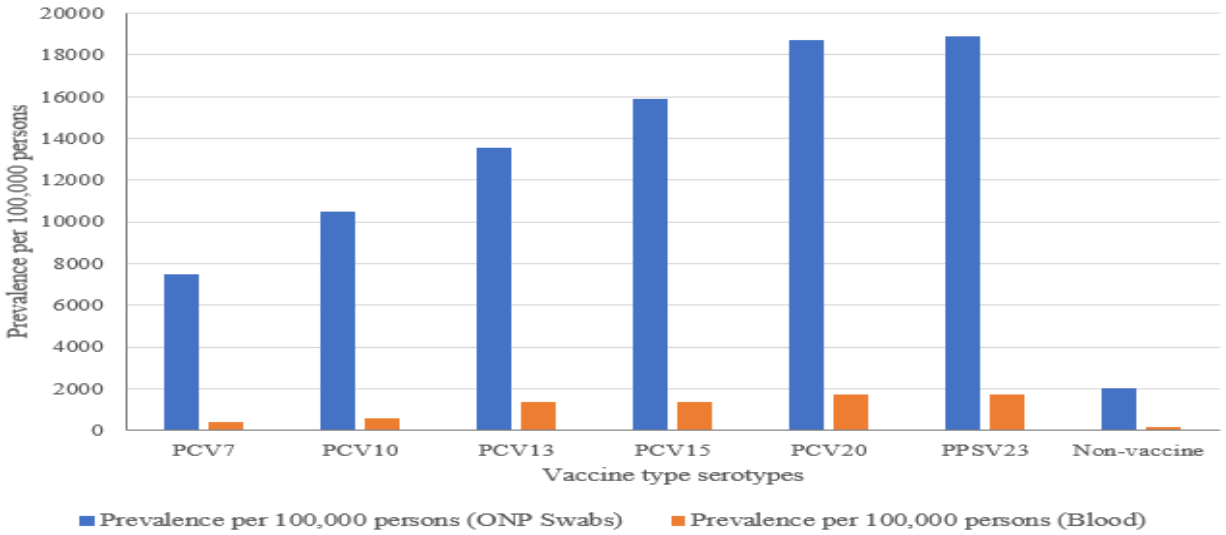

Figure 4: Prevalence of vaccine type or non-vaccine type serotypes in oronasopharynx or blood among adults admitted with presumed CAP in the era of COVID-19 in South Africa.

4.4 Serotype distribution by different variables at initial hospital admission

Due to very low frequency counts of pneumococci serotypes in blood, further analysis of the distribution of serotypes among participants for different variables was limited to serotypes in the oronasopharyngeal swabs grouped together by different pneumococcal vaccine types. The distribution for pneumococci serotypes (whether vaccine type or non-vaccine type) did not significantly differ by province, BMI, comorbidities (including HIV and COVID-19) and monthly income levels.

Oronasopharyngeal carriage prevalence for all serotypes was significantly higher in males (120/171 [70%]), participants aged 35-49 years (94/126 [75%]), active smokers(72/99 [73%]) and alcohol consumers (90/125 [72%]) while the prevalence for vaccine serotypes (represented by PPSV23 serotypes) was significantly higher in PLWH on ART (81/122 [66%]) and in participants not on chronic medication for comorbidities (12/15 [80%]) (Table 6).

Table 6: ONP serotype distribution by different variables at initial hospital admission.

Variable	All serotypes			Vaccine serotypes			Non-vaccine serotypes		
	No n (%)	Yes n (%)	p-value	No n (%)	Yes n (%)	p-value	No n (%)	Yes n (%)	p-value
Age (years)									
18-24	11(40.7)	16(59.3)	0.030	11(40.7)	16(59.3)	0.036	25(92.6)	2(7.4)	0.285
25-34	23(41.1)	33(58.9)		26(46.4)	30(53.6)		52(92.9)	4(7.1)	
35-49	32(25.4)	94(74.6)		39(30.9)	87(69.1)		108(85.7)	18(14.3)	
50-64	32(42.7)	43(57.3)		34(45.3)	41(54.7)		71(94.7)	4(5.3)	
≥ 65	16(48.5)	17(51.5)		19(57.6)	14(42.4)		29(87.9)	4(12.1)	
Gender									
Female	63(43.1)	83(56.9)	0.014	71(48.6)	75(51.4)	0.008	128(87.7)	18(12.3)	0.222
Male	51(29.8)	120(70.2)		58(33.9)	113(66.1)		157(91.8)	14(8.2)	
ART intake									
Yes	38(31.2)	84(68.8)	0.160	41(33.6)	81(66.4)	0.025	110(90.2)	12(9.8)	0.992
No	33(40.7)	48(59.3)		40(49.4)	41(50.6)		73(90.1)	8(9.9)	
Smoking									
Never smoked	65(37.6)	108(62.4)	0.035	72(41.6)	101(58.4)	0.003	158(91.3)	15(8.7)	0.601
Ex-smoker	22 (48.9)	23(51.1)		27(60.0)	18(40.0)		39(86.7)	6(13.3)	
Active smoker	27(27.3)	72(72.7)		30(30.3)	69(69.7)		88(88.9)	11(11.1)	
Alcohol intake									
Yes	35(28.0)	90(72.0)	0.017	43(34.4)	82(65.6)	0.066	111(88.8)	14(11.2)	0.598
No	79(41.1)	113(58.9)		86(44.8)	106(55.2)		174(90.6)	18(9.4)	
Chronic Medication									
Yes	24(44.4)	30(55.6)	0.086	27(50.0)	27(50.0)	0.045	47(87.0)	7(13.0)	0.333
No	3(20.0)	12(80.0)		3(20.0)	12(80.0)		15(100.0)	0(0.00)	

4.5 Pneumococci serotype incidence rates

A total of 322 individual serotypes were identified in ONP swabs that were tested. The swabs were collected in the follow-up period from 165 eligible participants who were enrolled in sub-study 2 of the "Pot Prev Study" between 05 March 2020 and October 2021. The total follow-up time for these participants was 232.45 person years. Incident rates were calculated by dividing serotype counts in oronasopharyngeal swabs (numerator) by 232.45 person years (denominator) and the calculated proportions were multiplied by 100,000 to express the incident rate values per 100,000 person years.

Serotypes/serogroups 1, 3, 4, 5, 9A/V, 12A/B/F/44/46, 19F, 22A/F and 33A/F/37 were among those with leading individual pneumococci serotype incidence rates in oronasopharyngeal swabs over the entire follow-up period. Serotype 4 was associated with the highest serotype incidence rate.

Similarly, the counts of vaccine type or non-vaccine type serotypes (numerator) were divided by the same denominator (232.45 person years) and then multiplied by 100,000 to obtain the corresponding vaccine type or non-vaccine type serotype incidence rates expressed per 100,000 person years. The incidence rates for oronasopharyngeal PCV13 serotypes, PPSV23 serotypes and non-vaccine serotypes were 90,772 serotypes per 100,000 person years (given by (211/232.45) X100,000) (95% CI: 78,937- 103,882), 130,781 serotypes per 100,000 person years (given by (304/232.45) X100,000) (95% CI: 116,491-146,340) and 8,604 serotypes per 100,000 person years (given by (20/232.45) X100,000) (95% CI: 5,256-13,288) respectively. The individual ONP pneumococcal serotype, vaccine type and non-vaccine type serotype incidence rates are summarised in Table 7.

Table 7: Pneumococci serotype incidence rates in ONP swabs among adults admitted with presumed CAP across the 2-year follow-up period in the era of COVID-19 in South Africa.

Serotype	Serotype count	Incidence rate per 100,000 person years (95% CI)[#]
1	19	8174(4921-12764)
2	1	430(11-2397)
3	19	8174(4921-12764)
4	56	24091(181981-31284)
5	35	15057(10488-20941)
6A/B	5	2151(698-5020)
6C/D	10	4302(2063-7912)
7A/F	5	2151(698-5020)

8	6	2581(947-5618)
9A/V	50	21510(15965-28358)
11A/D	10	4302(2063-7912)
12A/B/F/44/46	32	13766(9416-19434)
14	0	0(0-1587) *
15A/F	3	1291(266-3772)
16F	2	860(104-3108)
18A/B/C/F	13	5593(2978-9564)
19A	10	4302(2063-7912)
19F	17	7313(4260-11709)
22A/F	16	6883(3934-11178)
23A	4	1721(469-4406)
23F	4	1721(469-4406)
33A/F/37	36	15487(10847-21441)
Vaccine type serotypes		
PCV7	128	55066(45940-65473)
PCV10	185	79587(68531-91918)
PCV13	211	90772(78937-103882)
PCV15	258	110992(97860-125394)
PCV20	303	130351(116085-145885)
PPSV23	304	130781(116491-146340)
Non-vaccine serotypes	18	7744(4589-12238)
All serotypes	322	138524(123806-154512)

[#] *Incident rate per 100,000 person years= (serotype counts/232.45 person years) X 100,000.*

** one-sided, 97.5% confidence interval.*

The serotype incidence rates in blood were similarly calculated by diving blood serotype count (numerator) by the total follow-up period (232.45 person years) (denominator) and the obtained proportion was multiplied by 100,000 to express the incidence rate per 100,000 person years. Only serotype 3 and serogroup 6A/B were isolated in blood specimens during the follow-up period and these corresponded to the incidence rates of 860 serotypes per 100,000 person years (given by (2/232.45) X100,000) (95% CI:104-3108) and 430 serotypes per 100,000 person years (given by (1/232.45) X100,000) (95% CI:11-2397) respectively.

4.6 Pneumococcal serotype incidence rates in different periods in the COVID-19 era in SA

The incidence rates were lowest in the first eight months of the COVID-19 pandemic (about March 2020 to October 2020). After the first eight months post COVID-19 outbreak in South Africa, the incidence rates increased and generally peaked between 13 to 16 months (about March 2021 to June 2021) before they declined again (but to levels higher than those observed in the first eight months) (Figure 5).

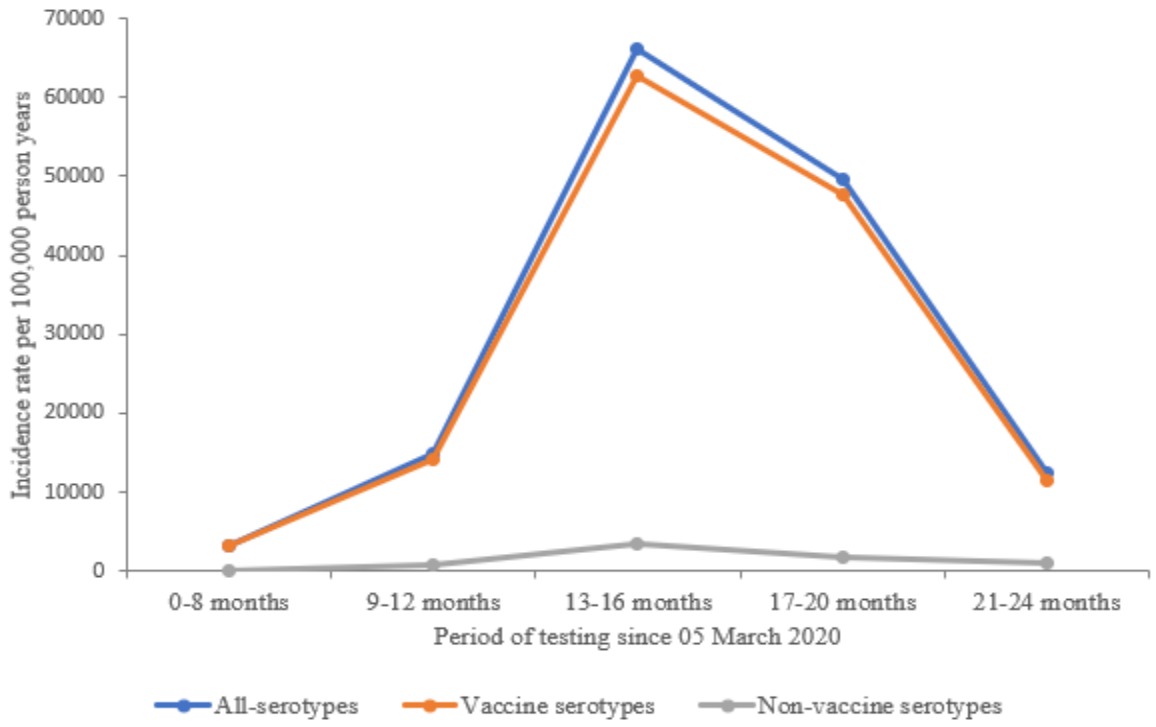

Figure 5: Incidence rates of all pneumococcal serotypes, vaccine type serotypes and non-vaccine serotypes by different time periods in the era of COVID-19 in South Africa.

4.7 Pneumococci serotype distribution by different variables during follow-up

Due to very low frequency counts of pneumococci serotypes in blood, further analysis of the distribution of serotypes among participants for different variables was limited to serotypes in the oronasopharyngeal swabs grouped together by different pneumococcal vaccine types. There were no significant differences in the incidence rates of vaccine serotypes and non-vaccine serotypes in the oronasopharyngeal swabs by province, gender, BMI, smoking, alcohol consumption, ART intake, comorbidities (including COVID-19) and monthly income at bivariate analysis. At bivariate analysis, the oronasopharyngeal incidence rates for "all serotypes" was significantly higher in HIV negative participants (97%) and in the period of 13-16 months post COVID-19 outbreak in South Africa (92%) (Table 8). In the Poisson regression model, only the period during which the participant samples were collected during participant follow-up was the only significant predictor of incidence rates for vaccine serotypes while participant age was significant predictor for non-vaccine serotypes.

Table 8: Serotype distribution by different variables during follow-up of adults initially admitted with presumed CAP in the era of COVID-19 in South Africa.

Variable	All serotypes			Vaccine serotypes[#]			Non-vaccine serotypes		
	No n (%)	Yes n (%)	p-value	No n (%)	Yes n (%)	p-value	No n (%)	Yes n (%)	p-value
Age (years)									
18-24	1(6.7)	14(93.3)	0.230	1(6.7)	14(93.3)	0.654	11(73.3)	4(26.7)	0.009
25-34	2(7.1)	26(92.9)		4(14.3)	24(85.7)		22(78.6)	6(21.4)	
35-49	10(17.9)	46(82.1)		10(17.9)	46(82.1)		53(94.6)	3(5.4)	
50-64	12(24.0)	38(76.0)		12(24.0)	38(76.0)		48(96.0)	2(4.0)	
≥ 65	1(6.2)	15(93.8)		3(18.8)	13(81.2)		13(81.3)	3(18.7)	
HIV Status									
Negative	2(3.2)	60(96.8)	0.000	4 (6.4)	58(93.6)	0.003	55(88.7)	7(11.3)	0.903
Positive	24(23.3)	79(76.7)		26(25.2)	77(74.8)		92(89.3)	11(10.7)	
Period of testing (months)*									
0-8	5(71.4)	2(28.6)	0.002	5(71.4)	2(28.6)	0.003	7(100.0)	0(0.00)	0.741
9-12	3(21.4)	11(78.6)		3(21.4)	11(78.6)		12(85.7)	2(14.3)	
13-16	5(8.1)	57(91.9)		6(9.7)	56(90.3)		54(87.1)	8(12.9)	
17-20	11(17.7)	51(82.3)		14(22.6)	48(77.4)		57(91.9)	5(8.1)	
21-24	2(10.0)	18(90.0)		2(10.0)	18(90.0)		17(85.0)	3(15.0)	

**Refers to the period in months since 05 March 2020.*
[#]refers to all serotypes in licensed pneumococcal vaccines (represented by PPSV23 serotypes).

The Poisson model that predicted the incidence rate for "all serotypes" in the oronasopharynx was represented by the equation; Log [ONP all- serotypes] =-1.902-1.214*Period (=0-8months) + 0.008*Period (=9-12months)-0.381*Period (=17-20months)-0.687* Period (=21-24months). In this model, the first eight months after 05 March 2020 was associated with the lowest incidence rate though it was not statistically significant. The incidence rate for "all serotypes" in the oronasopharynx in the period of 9-12 months was the same as that in the reference period of 13-16 months (IRR=1.00, 95% CI: 0.65-1.55, p=0.970). The incidence rate for "all serotypes" in the oronasopharynx in the first eight months was 70% lower than that for the reference period (IRR=0.30, 95% CI=0.07-1.20, p=0.088). The incidence rate for "all serotypes" in the oronasopharynx were significantly lower in the periods of 17-20 and 21-24 months than in the reference period (IRR=0.68, 95% CI=0.54-0.87, p=0.002 and IRR=0.50, 95% CI=0.35-0.73, p<0.0001 respectively).

The Poisson model that predicted the incidence rates for PPSV23 serotypes (all vaccine serotypes) in the oronasopharynx is represented by the equation; Log [ONP PPSV23 serotypes]

=-1.960-1.156*Period (=0-8months) -0.021*Period (=9-12months)-0.365*Period (=17-20months)-0.721* Period (=21-24months). In this model, the first eight months from 05 March 2020 was associated with the lowest incidence rate though it was not statistically significant. The incidence rate for PPSV23 serotypes in the oronasopharynx in the first eight months was 69% lower than that for the reference period of 13-16 months (IRR=0.31, 95% CI=0.08-1.27, p=0.105). The incidence rates for PPSV23 serotypes in the oronasopharynx were significantly lower in the periods of 17-20 and 21-24 months than in the reference period (IRR=0.69, 95% CI=0.54-0.89, p=0.004 and IRR=0.48, 95% CI=0.33-0.72, p<0.0001 respectively).

The Poisson model that predicted the incidence rates for the oronasopharyngeal non-vaccine serotypes is represented by the equation; Log [ONP non-vaccine serotypes] =5.730+1.557*Age (=18-24yrs) +1.364*Age (=25-34yrs)-0.311*Age (=50-64yrs) +1.160* Age (=65-85yrs). In this model, the age of the participant being 18-24 years was associated with a five times higher incidence rate for non-vaccine serotypes in ONP swabs than for the reference age of 35-49 years (IRR=4.75, 95% CI=1.06-21.20, p=0.041).

4.8 Mortality among adults admitted with presumed CAP that was associated with pneumococci serotypes in the era of COVID-19 in South Africa

For the 126 participants who had a documented outcome over the follow-up period within the COVID-19 era (reason for termination from the "Pot Prev Study"), no mortality was observed among individuals carrying pneumococci serotypes 2, 7A/F, 8, 14, 22A/F and 23F in their ONP swabs.

Mortality rates were calculated by dividing the counts of deaths associated with each serotype in the study population (numerator) by person-years at risk (denominator). The obtained proportions were multiplied by 1000 to express mortality rates per 1000 person years. For example, the mortality rate per 1000 person years associated with serotype 1 at 3 days from the date of initial hospital admission (8494 deaths per 1000 person years) was given by (1 / 0.1177) X 1000.

Mortality rates associated with serotypes/serogroups in the ONP swabs were observed to be higher on the 14th day of follow-up than on other periods of follow-up. The serotypes/serogroups associated with leading mortality rates at 14 days were 1, 3, 4, 5, 15A/F, 18A/B/C/F, 23A and 33A/F/37. Only three serotypes in the ONP swabs (1, 3 and 4) were associated with mortality within three days post admission.

No mortality was observed for six serotypes/serogroups (6A/B, 12A/B/F/44/46, 9A/V, 11A/D, 16F and 19F) before 14 days post admission. Mortality rates associated with serotypes in ONP swabs are presented in Table 9 and Figure 6.

Table 9: Mortality rates (MR) among adults admitted with presumed CAP associated with pneumococci serotypes in ONP swabs in the COVID-19 era in South Africa

	MR at 3 days			MR at 7 days			MR at 14 days			MR over entire follow-up period		
	Deaths (n)	PY	MR (95% CI) *	Deaths (n)	PY	MR (95% CI) *	Deaths (n)	PY	MR (95% CI) *	Deaths (n)	PY	MR (95% CI) *
Individual serotype												
1	1	0.1177	8494(1197-60300)	2	0.2245	8909(2228-35620)	3	0.0493	60875(19600-189000)	9	5.0595	1779(926-3419)
2	0	0	0	0	0	0	0	0	0	0	0	0
3	1	0.1013	9872(1391-70100)	3	0.1862	16114(5197-50000)	4	0.0548	73050(27400-195000)	6	7.2580	827(371-1840)
4	2	0.2136	9365(2342-37400)	4	0.4025	9939(3730-26500)	6	0.1177	50965(22900-113000)	14	16.2683	861(510-1453)
5	0	0.1205	0	1	0.2355	4247(598-30200)	1	0.0164	60875(8575-432000)	3	14.9678	200(65-621)
6A/B	0	0.0110	0	0	0.0219	0	0	0	0	1	0.0575	17393(2450-123000)
6C/D	0	0.0329	0	0	0.0657	0	1	0.0356	28096(3958-199000)	1	3.5428	282(40-2004)
7A/F	0	0.0219	0	0	0.0438	0	0	0	0	0	3.2416	0
8	0	0.0548	0	0	0.1095	0	0	0	0	0	8.6051	0
9A/V	0	0.0986	0	0	0.1971	0	0	0	0	1	14.2286	70(10-499)
11A/D	0	0.0329	0	0	0.0657	0	0	0	0	2	3.3785	592(148-2367)
12A/B/F/44/46	0	0.0767	0	0	0.1533	0	0	0	0	2	7.4031	270(68-1080)
14	0	0	0	0	0	0	0	0	0	0	0	0
15A/F	0	0.0548	0	1	0.1013	9872(1391-70100)	1	0.0137	73050(10300-519000)	2	4.5339	441(110-1764)
16F	0	0.0329	0	0	0.0657	0	0	0	0	1	4.0411	247(35 – 1757)
18A/B/C/F	0	0.0329	0	1	0.0602	16602(2339-118000)	1	0.0164	60875(8575-432000)	2	2.1246	941(235-3764).
19A	0	0.0548	0	1	0.1040	9612(1354-68200)	2	0.0520	38447(9616-154000)	3	4.2710	702(227- 2178)
19F	0	0.0767	0	0	0.1533	0	0	0	0	6	4.6023	1304(586-2902)
22A/F	0	0.0110	0	0	0.0219	0	0	0	0	0	0.8624	0
23A	0	0.0219	0	1	0.0411	24350(3430-173000)	1	0.0192	52179(7350-370000)	2	0.1971	10146(2537-40600)
23F	0	0	0	0	0	0	0	0	0	0	0	0
33A/F/37	0	0.1314	0	2	0.2491	8027(2008-32100)	2	0.0301	66409(16600-266000)	4	11.7125	342(128-910)

Vaccine type serotypes												
PCV7	2	0.3778	5293(1323-21200)	4	0.7310	5472(2054-14600)	6	0.1177	50965(22900-113000)	22	30.7789	715(471- 1086)
PCV10	2	0.4983	4014(1004-16000)	6	0.9637	6226(2797-13900)	9	0.1752	51363(26700-98700)	27	40.3723	669(459-975)
PCV13	3	0.6105	4914(1585-15200)	8	1.1773	6795(3398-13600)	12	0.2272	52807(30000-93000)	33	49.8453	662(471-931)
PCV15	3	0.6762	4436(1431-13800)	10	1.2950	7722(4155-14400)	14	0.2574	54399(32200-91900)	35	54.5489	642(461- 894)
PCV20	3	0.7858	3818(1231-11800)	10	1.5140	6605(3554-12300)	14	0.2574	54399(32200-91900)	38	68.5722	554(403-762)
PPSV23	3	0.7858	3818(1231-11800)	10	1.5140	6605(3554-12300)	14	0.2574	54399(32200-91900)	38	68.5722	554(403-762)
Non-vaccine serotypes	0	0.1424	0	2	0.2738	7305(1827-29200)	3	0.0684	43830(14100-136000)	6	12.3149	487(219-1084)
All serotypes	3	0.8624	3479(1122-10800)	10	1.6674	5998(3227-11100)	15	0.2930	51203(30900-84900)	41	74.7625	575(451-734)

**Mortality rates per 1000 person years =* $\frac{\text{Number of deaths (n) X1000}}{\text{Person years at risk (PY)}}$

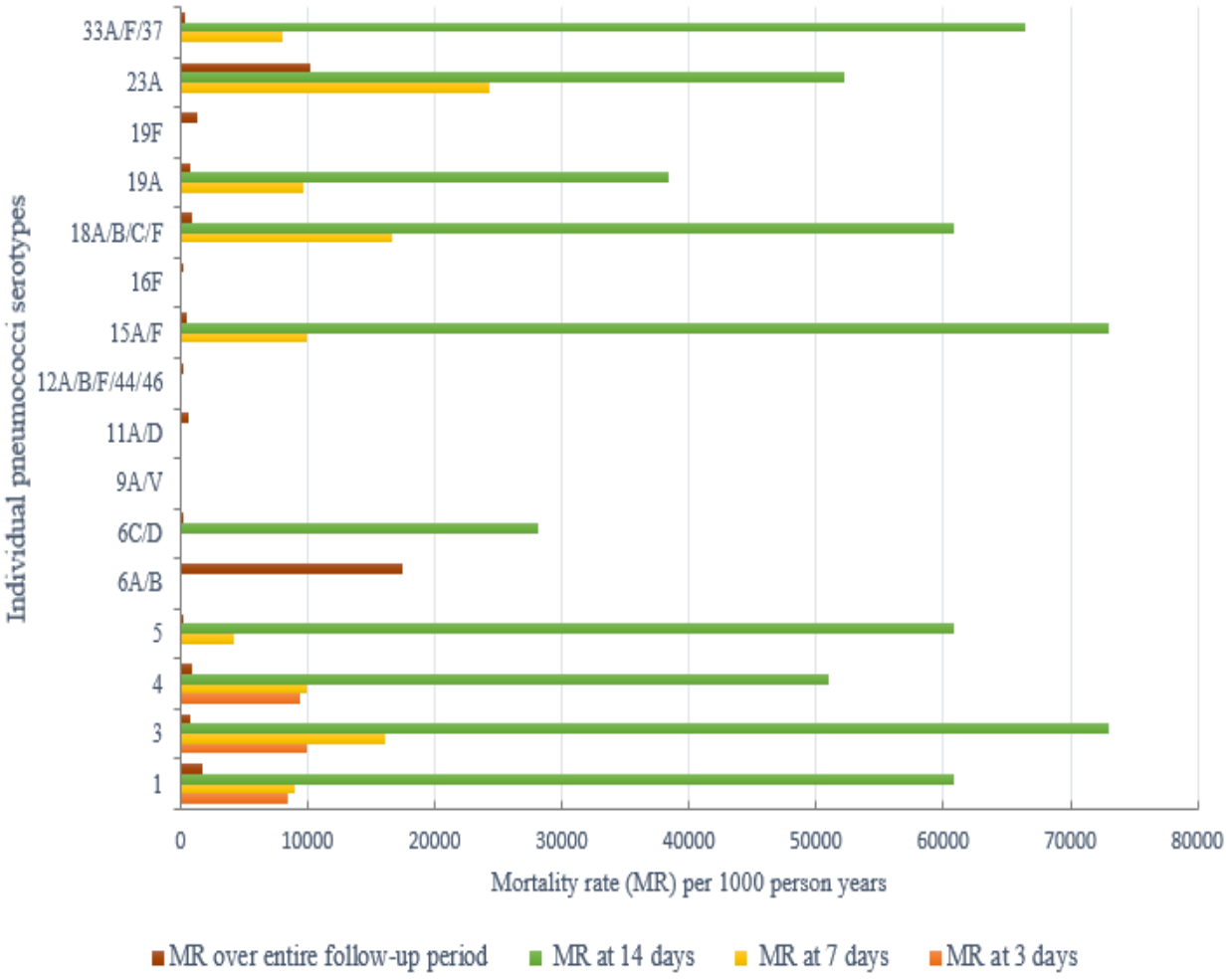

Figure 6: Mortality rates among adults admitted with presumed CAP associated individual pneumococci serotypes in ONP swabs in the era of COVID-19 in South Africa.

For mortality rates associated with vaccine type and non-vaccine type pneumococci serotypes, it followed the pattern; mortality rate at 14 days > mortality rate at 7 days > mortality rate at 3 days > mortality rate at 2 years (Figure 7).

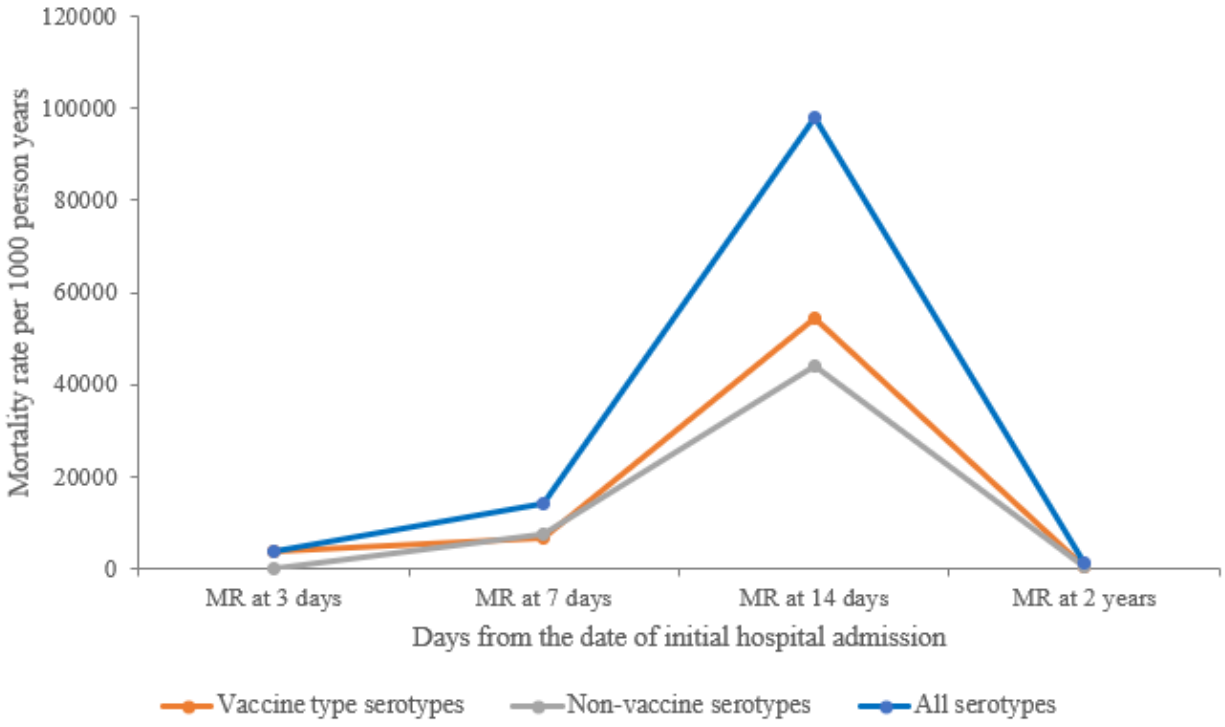

Figure 7: Mortality rates (MR) among adults admitted with presumed CAP associated with vaccine type pneumococci serotypes in ONP swabs in the era of COVID-19 in South Africa.

On the other hand, only five serotypes/serogroups (1, 3, 8, 11A/D and 19A) were identified in blood samples from seven participants within our study over the entire follow-up period. The associated mortality rates for individual blood serotypes, vaccine type and non-vaccine type serotypes at different time points are presented in Table 10. No mortality was associated with serotype 8, PCV7 serotypes and non-vaccine serotypes that were identified in blood in our study.

Table 10: Mortality rates (MR) among adults admitted with presumed CAP associated with pneumococci serotypes in blood in the COVID-19 era in South Africa.

	MR at 3 days			MR at 7 days			MR at 14 days			MR over entire follow-up period		
	Deaths (n)	PY	MR (95%CI) *	Deaths (n)	PY	MR (95%CI) *	Deaths (n)	PY	MR (95%CI) *	Deaths (n)	PY	MR (95%C) *
Individual serotype												
1	0	0.0110	0	1	0.0137	73050(10300-519000)	1	0.0137	73050(10300-519000)	1	0.0137	73050(10300-519000)
3	1	0.0137	73050(10300-519000)	2	0.0164	121800(30400-487000)	2	0.0164	121800(30400-487000)	2	0.0164	121800(30400-487000)
8	0	0.0110	0	0	0.0219	0	0	1.9713	0	0	1.9713	0
11A/D	1	0.0055	182600(25700-1300000)	1	0.0055	182600(25700-1300000)	1	0.0055	182600(25700-1300000)	1	0.0055	182600(25700-1300000)
19A	0	0.0219	0	0	0.0438	0	1	0.0246	40583(5716-288000)	1	2.0260	493(70-3504
Vaccine serotypes												
PCV7	0	0	0	0	0	0	0	0	0	0	0	0
PCV10	0	0.0110	0	1	0.0137	73050(10300-519000)	1	0.0137	73050(10300-519000)	1	0.0137	73050(10300-519000)
PCV13	1	0.0465	21485(3026-153000)	3	0.0739	40583(13100-126000)	4	0.0548	73050(27400-195000)	4	2.0561	1945(730-5183)
PCV15	1	0.0465	21485(3026-153000)	3	0.0739	40583(13100-126000)	4	0.0548	73050(27400-195000)	4	2.0561	1945(730-5183)
PCV20	1	0.0630	31761(7943-127000)	4	0.1013	39486(14800-105000)	5	0.0602	83011(34600-199000)	5	4.0329	1240(516-2979)
PPSV23	2	0.0630	31761(7943-127000)	4	0.1013	39486(14800-105000)	5	0.0602	83011(34600-199000)	5	4.0329	1240(516-2979)
NVT serotypes	0	0	0	0	0	0	0	0	0	0	0	0
All serotypes	2	0.0630	31760(7943-127000)	4	0.1013	39486(14800-105000)	5	0.0602	83011(34600-199000)	5	4.0329	1240(516-2979)

**Mortality rates (per 1000 person years) =* $\frac{\text{Number of deaths (n)} \times 1000}{\text{Person years at risk (PY)}}$

The mortality rates associated with vaccine type serotypes in blood was highest at 14 days and lowest at two years since initial participant hospital admission (Figure 8).

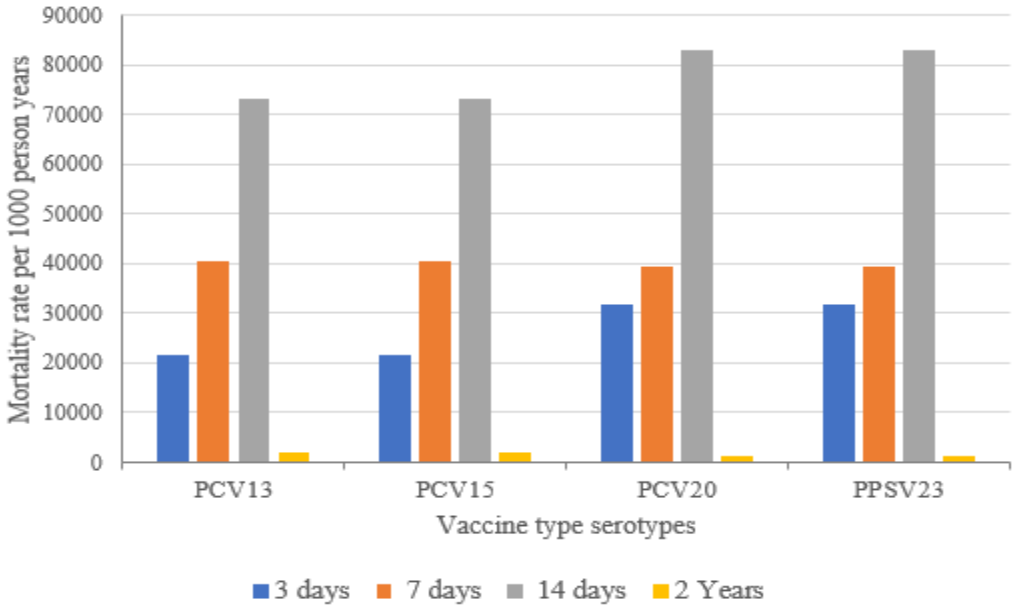

Figure 8: Mortality rates among adults admitted with presumed CAP associated with vaccine type pneumococci serotypes in blood in the era of COVID-19 in South Africa.

4.9 Predictors of pneumococci serotype associated mortality in logistic regression.

It was hypothesized that mortality risk among adults admitted with presumed CAP in the era of COVID-19 in South Africa was the same regardless of the pneumococci serotypes isolated in their oronasopharynx or blood. We limited our analysis to vaccine type or non-vaccine type serotypes in ONP swabs due to the very low serotype frequency counts in blood. The goodness of fit of our model was assessed by the Pearson Chi2 (p=0.332), Hosmer–Lemeshow Chi2 (p=0.357) and the Area under ROC curve (0.762). In our logistic model, the odds of death among our participants who had a CURB-score of 1 was 3.3 times that of CURB-score of 0 (Adjusted OR=3.298, 95% CI= 1.377-7.898, p=0.007). Similarly, the odds of death among our participants who were underweight was 4.9 times that of normal weight (Adjusted OR=4.816, 95% CI= 1.764- 13.149, p=0.002) (Table 11).

Table 11: Logistic regression results

Predictor variable	Unadjusted OR	95% CI	p-value	Adjusted OR	95% CI	p-value
Participant age	1.015	0.991- 1.040	0.211	1.015	0.985- 1.045	0.332
CURB-65 score						
0	Reference	Reference	Reference	Reference	Reference	Reference
1	3.285	1.486- 7.264	**0.003**	3.298	1.377-7.898	**0.007**
≥2	3.109	1.014- 9.530	**0.047**	2.304	0.637- 8.330	0.203
BMI (kg/m^2)						
Underweight (<18.5)	4.073	1.575-10.531	**0.004**	4.816	1.764- 13.149	**0.002**
Normal weight (18.5 - <25)	Reference	Reference	Reference	Reference	Reference	Reference
Overweight (25-<30)	1.600	0.495- 5.172	0.432	1.653	0.482-5.664	0.424
Obese (≥30)	1.173	0.427- 3.224	0.757	1.237	0.404- 3.788	0.710

4.10 Kaplan Meier survival analysis among adults admitted with presumed CAP in the era of COVID-19 in South Africa

From Kaplan Meier survival analysis, it was observed that mortality was significantly higher in adults carrying serotype 1 in the oronasopharynx (p= 0.011) but significantly lower in those carrying serotype 8 and 9A/V (p=0.049 and 0.030 respectively). Survival analysis was not done for pneumococci serotypes in blood because of the very low serotype frequency counts in blood (n=7). Mortality was significantly higher in underweight participants (p= 0.025), participants who required oxygen supplementation (p=0.024), length of time from initial hospital admission to death ≤14 days and CURB-65 score of ≥1 (Table 12).

Table 12: Participant survival by different factors in the era of COVID-19 in South Africa.

Variable	Level	Number by level	Number died	PY	Mortality Rate (95%CI) [#]	p-value for Log rank X2
Overall		126	65	112.93	576(451- 734)	
Serotype 1	No	115	56	107.87	519(400- 675)	0.011
	Yes	11	9	5.06	1779(926- 3419) *	
Serotype 8	No	121	65	104.33	623(489- 794) *	0.049
	Yes	5	0	8.61	0	
Serogroup 9A/V	No	117	64	98.71	648(508-828) *	0.030
	Yes	9	1	14.23	70(10- 499)	
BMI	Underweight	39	28	25.66	1091(753 -1580) *	0.025
	Normal weight	39	15	43.21	347(209-576)	
	Overweight	16	8	15.74	508(254-1016)	
	Obese	26	11	23.32	472(261-852)	
Duration post admission	0-3 days	4	4	0.03	160000(60927-430000) *	<0.0001
	4-7 days	13	13	0.20	64166(37258-110000) *	
	8-14 days	10	10	0.31	32039(17239-59547) *	
	Above 14 days	99	38	112.39	338(246-465)	
CURB-65 score	0	62	23	68.52	336(223- 505)	<0.0001
	1	47	31	32.03	968(681-1376) *	
	2	14	8	12.31	650(325-1300) *	
	3	3	3	0.079	37784(12186-120000) *	
Oxygen therapy	No	44	18	50.71	355(224- 563)	0.024
	Yes	82	47	62.23	755(568- 1005) *	

PY=person years at risk

[#] *Mortality rates were calculated by dividing the counts of deaths (numerator) by person-years at risk (denominator) and the obtained proportions were multiplied by 1000 to express mortality rates per 1000 person years. For example, the mortality rate per 1000 person years associated with all serotypes (576 deaths per 1000 person years) was given by (65/ 112.93) X 1000.*

**Group associated with higher observed mortality than expected.*

4.11 Cox proportional hazard regression among adults admitted with presumed CAP in the era of COVID-19 in South Africa

Variables that were associated with significant differences in survival under Kaplan Meier analysis (Table 12) and participant age were considered in the multivariate Cox proportional hazards modelling done at 3 days, 7 days, and 14 from the date of initial CAP hospital admission.

The Cox model at 3 days from the date of initial hospital admission is represented by the equation; log[h(ti)/h0(ti)] =0.845*CURB-65 score (=1) + 0.549*CURB-65 score (≥2)+ 1.025*BMI (=underweight)+0.421*BMI (=overweight)+0.286*BMI (=obese) + 0.683*Oxygen supplementation (=Yes), where h(ti) is the hazard function and h0(ti) is the baseline hazard. Based on the Schoenfeld residuals, the individual predictor variables in the model were not significant (p>0.05) and the global test was also not significant (p=0.332) indicating that the proportional hazards assumption was not violated. Having a CURB-65 score of 1 was significantly associated with higher mortality risk (adjusted HR= 2.327, 95% CI=1.322- 4.098, p= 0.003). Participants who required oxygen supplementation were at higher risk of mortality (adjusted HR= 1.980, 95% CI=1.109-3.535, p= 0.021) as well as underweight participants (adjusted HR= 2.788, 95% CI=1.441- 5.396, p= 0.002).

There was no predictor variable significantly associated with mortality at 7 days from the date of initial CAP hospital admission.

The Cox model at 14 days from the date of initial hospital admission is represented by the equation; log[h(ti)/h0(ti)] =-0.941*ONP serotype 1 (=Yes), where h(ti) is the hazard function and h0(ti) is the baseline hazard. Based on the Schoenfeld residuals, the global test was not significant (p=0.905) indicating that the proportional hazards assumption was not violated. Oronasopharyngeal carriage of serotype 1 was significantly associated with higher mortality risk when adjusted for participant age) at 14 days post initial CAP hospital admission (adjusted HR=2.562, 95% CI=1.239- 5.296, p= 0.011) (Table 13).

Table 13: Results from the Cox Proportional Hazards Regression

Predictor variable	Unadjusted HR	95% CI	p-value	Adjusted HR	95% CI	p-value
At 3 days from the date of initial CAP hospital admission						
Age (years)	1.013	0.997-1.030	0.122	1.010	0.991-1.028	0.300
CURB-65 score						
0	Reference	Reference	Reference	Reference	Reference	Reference
1	2.273	1.322-3.910	**0.003**	2.327	1.322-4.098	**0.003**
≥2	2.593	1.255-5.354	**0.010**	1.731	0.772-3.881	0.183
BMI (kg/m^2)						
Underweight (<18.5)	2.166	1.142-4.107	**0.018**	2.788	1.441-5.396	**0.002**
Normal weight (18.5 - <25)	Reference	Reference	Reference	Reference	Reference	Reference
Overweight (25-<30)	1.442	0.611-3.404	0.403	1.524	0.640-3.628	0.341
Obese (≥30)	1.248	0.573-2.719	0.578	1.331	0.597-2.968	0.485
Need for oxygen therapy						
Yes	1.869	1.084-3.222	**0.024**	1.980	1.109-3.535	**0.021**
At 14 days from the date of initial CAP hospital admission						
Age (years)	0.998	0.982-1.014	0.795	0.999	0.982-1.016	0.882
ONP serotype 1						
Yes	2.570	1.245-5.304	**0.011**	2.562	1.239-5.296	**0.011**

CHAPTER FIVE: DISCUSSION

The epidemiology of pneumococcal CAP is ever-changing. This study investigated the prevalence and incidence rates of *S. pneumoniae* serotypes in South Africa among adults hospitalized with CAP in the era of COVID-19. Furthermore, mortality associated with different serotypes was explored.

5.1 Pneumococci serotype prevalence and incidence rates in the COVID-19 era in South Africa

Our findings indicate that nearly a quarter of all pneumonia cases (about 23%) were associated with pneumococci in South Africa in the COVID-19 era. The prevalence of pneumococcal CAP is known to vary with the study method, season, age and region with recent studies showing that it ranges between 10% and 40% for adults (30–34). The prevalence of pneumococcal CAP in our study was within this range reported in literature. The fact that pneumococci are associated with about one case for every four cases of CAP, it is an indicator that pneumococci remained of significant importance in the etiology of CAP in South Africa even in the time of COVID-19. Vaccination of adults in South Africa is therefore likely to have a significant benefit in the control of pneumococcal disease including pneumococcal CAP in this population.

Nine serotypes/serogroups were associated with the leading prevalence in ONP swabs in our study (1, 3, 4, 5, 8, 9A/V, 12A/B/F/44/46, 19F and 33A/F/37). The same serotypes/serogroups were associated with leading oronasopharyngeal incidence rates except serotype 8. Serotype 4 was associated with the highest oronasopharyngeal prevalence and incidence rate.

Serotypes isolated in the ONP swabs could simply be associated with mere carriage or they could be associated with non-invasive/non-bacteremic pneumococcal CAP. There is limited information on serotypes associated with pneumococcal carriage and non-invasive pneumococcal CAP among adults before and after COVID-19 outbreak in South Africa since most of the focus has been on IPD surveillance in children. From our study, the leading oronasopharyngeal pneumococcal serotypes in South Africa in the COVID-19 era were different from those reported in other countries in the same period. The leading oronasopharyngeal pneumococcal serotypes in South Africa were different from those observed in Serbia in 2021 (6A,11A, 15B, 18C, 6C and 35F) (92). The disparity could be attributed to differences in age groups involved (≥18 years for our study vs ≥ 50 years for the Serbian study) and the small number of participants in the Serbian study (only 16). Similarly, the five leading serotypes/serogroups (19B/C, 6A/B/C/D, 24, 15A/F and 20) among adults above 60 years in

Thailand in the COVID-19 era were different from the leading serotypes/serogroups in our study(75). The variance could be attributed to the differences in the serotyping techniques used and participant ages.

Our data on oronasopharyngeal serotype prevalence and incidence rates suggest that PCV13 vaccine that is currently recommended for adult pneumococcal vaccination in South Africa does not cover four of the serotypes/serogroups associated with leading oronasopharyngeal prevalence/incidence rates in adults with CAP in our study (8, 12A/B/F/44/46, 22A/F, and 33A/F/37). On the other hand, the newly licensed PCV 15 does not cover two of the serotypes/serogroups (8 and 12A/B/F/44/46) while PCV20 and PPSV23 cover all the serotypes/serogroups with the leading oronasopharyngeal prevalence/incidence rates in our study. Since polysaccharide vaccines have been shown to have limited efficacy against the non-invasive pneumococcal disease and oronasopharyngeal carriage, our data suggests that PCV20 would be a preferred vaccine for adult pneumococcal vaccination in South Africa.

Among the seven serotypes/serogroups with leading prevalence in blood (1, 3, 4, 6A/B, 8, 11A/D and 19A), four of them (1, 3, 4, and 8) were also associated with leading prevalence/incidence rates in ONP swabs. Serotypes 1, 3, 4, 8,6A/B and 19A were reported among the leading serotypes associated with IPD in the pre-COVID period from 2012 to 2018 in individuals older than 15 years in South Africa (88). This finding supports the observation that major serotypes associated with IPD in the COVID-19 era have remained nearly the same as for the pre-COVID-19 era. The differences in serotype/serogroup prevalence in ONP swabs and blood among participants in our study may be attributed to variability in the invasiveness of different serotypes since carriage precedes the invasive disease (93,94).

Our blood serotype prevalence data is consistent with the NICD report of March 2023 that reported serotypes 3, 4 and 19A as the leading causes of IPD in South Africa (67). A previous report from NICD in early 2020 also showed serotype 8 as one of the leading causes of IPD for all age groups in South Africa (95). These data reflect the serotype coverage limitation of PCV13 and PCV15 vaccines against IPD (do not cover serotype 8 and serogroup 11A/D that are among the leading causes of IPD) in the context of South Africa. PCV20 and PPSV23 on the other hand cover all the seven serotypes/serogroups with leading prevalence in blood in our study.

The oronasopharyngeal prevalence for “all serotypes” among hospitalized adults with CAP was 20.9% while the prevalence for PCV13 serotypes alone was 13.6%. This implies that serotypes

detected in our study but absent in PCV13 (2, 8,11A,12F, 22F, 33F, 6C/D, 15A/F, 16F, 23A) contributed to a prevalence of about 7.3%. This finding indicates that PCV13 if used alone among adults in South Africa, it would leave vaccinated individuals at a considerably high risk to pneumococcal CAP since protection is serotype specific. The prevalence for PCV20 and PPSV23 vaccine serotypes were almost the same in ONP swabs (18.7% vs 18.9%) and identical in blood (1.8%) in our study. This implies that use of PPSV23 may only provide additional benefit if used together with lower valency PCV vaccines like PCV13 but not PCV20. This finding corroborates the current guidelines in South Africa that recommends the use of PCV13 with PPSV23 and the ACIP guidelines which recommends use of PCV20 alone without PPSV23 (47,68).

Non-vaccine serotypes were associated with the lowest prevalence (2% in ONP swabs and 0.2% in blood) in our study. Our findings contradict previous studies that showed increase in non-vaccine serotype prevalence in children and adults following the roll out of pneumococcal conjugate vaccines in children (2,8,96,97). The low prevalence of non-vaccine serotypes in our participants was due to the fewer serotypes that were considered non-vaccine in our study (6C/D, 15A/F, 16F, 23A). Our study considered a serotype as non-vaccine if it was detected but is not included in any of the commercial vaccines unlike other studies which considered a serotype as non-vaccine if it was not included in the pneumococcal vaccine that is in routine use in that country. Furthermore, the low pneumococcal vaccination coverage in South Africa may explain the continued high circulation of vaccine type pneumococcal serotypes and low circulation of non-vaccine serotypes.

5.2 Determinants of serotype distribution among adults admitted with presumed CAP in the era of COVID-19 in South Africa

Our study found out that oronasopharyngeal carriage prevalence for all serotypes was significantly higher in males (70%), 35-49 years old participants (75%), active smokers (73%) and alcohol consumers (72%) while the prevalence for vaccine serotypes only was significantly higher in PLWH on ART (66%) and in participants not on chronic medication for comorbidities (80%).

Previous studies have reported conflicting results on the role of gender on oronasopharyngeal pneumococcal carriage with some studies reporting higher prevalence in males than females (70–72), others reporting higher prevalence in females than males (73) while others have not reported

any difference (74,75). The higher pneumococcal carriage for “all serotypes” in males in our study may be attributed to a higher likelihood of socialization and smoking among males than females in South Africa.

Pneumococcal carriage for “all serotypes” increased with increasing age in our study among participants below 50 years but decreased with increasing age among participants above 50 years. Some studies have reported decreasing carriage with increasing age (similar to our study for participants above 50 years) (98,99). Increasing carriage with increasing age was also observed in individuals aged at least 20 years in a cross-sectional survey in the Gambia (70). Individuals older than 40 years were also associated with increasing carriage for increasing age in USA after 12 years of PCV7 rollout (100). It remains unclear as to why oronasopharyngeal pneumococcal carriage was significantly higher in participants aged 35-49 years in our study. This may however be explained by the fact that participants in this age stratum comprise most of the working class in South Africa who are likely to have continued working in the COVID-19 era and hence had higher chances of movement and interaction leading to higher pathogen transmission.

Similar to our study, the significant association of active smoking and alcohol consumption with increased risk for pneumococcal carriage and pneumococcal disease has been reported in several other studies (69,71,75–77,82,101–104). A study in mouse demonstrated that smoking attenuates nasal host immune responses to pneumococci leading to increased colonization and infection (105).

Our study showed that oronasopharyngeal pneumococcal carriage prevalence for PPSV23 serotypes was significantly higher among PLWH on ART (66%). This observation is supported by findings from a study in Malawi where higher pneumococcal carriage for serotypes not included in PCV13 was observed among PLWH on ART than those not on ART (78). It remains unclear if ART truly increases pneumococcal carriage or there are other underlying factors in PLWH on ART that are responsible for the higher carriage.

Participants who were not on chronic medication for comorbidities other than HIV were significantly associated with higher oronasopharyngeal carriage for PPSV23 serotypes (80%). It is not known which chronic medication our participants were taking during the study period, but it is possible that some of the medication taken had antibacterial activity leading to lower carriage in this group (69).

The period in which samples were collected during participant follow-up was the only significant predictor of the incidence rates for all serotypes in the oronasopharynx in the Poisson regression model. The highest serotype incidence rates were observed between March 2021 and June 2021 (13-16 months post COVID-19 outbreak in SA) and this was considered the reference period in our study. Except the period of November 2020 to February 2021 (9-12 months post COVID-19 outbreak in SA) which had the same ONP incidence rate for "all serotypes" as the reference period (IRR=1.00), all other periods of follow-up were associated with lower incidence rates than the reference period (IRR<1.0). However, only the period of July 2021-October 2021 (17-20 months post COVID-19 outbreak in SA) and the period of November 2021-February 2022 (21-24 months post COVID-19 outbreak in SA) were statistically significant.

The highest serotype incidence rates observed between March 2021 and June 2021 may be explained by the fact that this period coincided with the autumn/winter season in SA which is normally a peak season for pneumonia (95). Furthermore, this period was associated with lower COVID-19 alert levels (1 to 2) which may have facilitated higher pathogen transmission and colonization (52). On the other hand, a comparable period in 2020 was associated with the lowest pneumococcal serotype incidence rates (though it was statistically non-significant) because it coincided with higher COVID-19 alert levels (3 to 5). The stricter COVID-19 prevention and control measures disrupted sample collection in our study but are also believed to have reduced social mixing leading to a decline in pathogen transmission and colonization. The observation of low pneumococcal serotype incidence rates in the oronasopharynx in our study is consistent with the reduction in IPD incidence reported in South Africa during the COVID-19 pandemic especially in the early months of the pandemic 2020 (67).

The period of July 2021-October 2021 coincided with COVID-19 alert level 3 or level 4 which may explain the reduced incidence rates in this period compared to the reference period. The COVID-19 alert level 1 was implemented in November 2021-February 2022 even though it was significantly associated with lower incidence rates for "all vaccine" serotypes. The low serotype incidence rates in this period may be explained by the fact it coincided with the summer season in South Africa during which the incidence of pneumonia is usually at its lowest (67,106).

The general decline in pneumococcal serotype incidence rates in our study is consistent with the reported decline in incidence of respiratory infections such as those caused by influenza virus and respiratory syncytial virus (RSV) during COVID-19 pandemic especially in periods of strict

COVID-19 prevention and control measures (59,107–111). In fact South Africa had no defined influenza and RSV seasons in the COVID-19 era (112).

Unlike for vaccine serotypes, being 18-24 years old was significantly associated with a five times higher risk for oronasopharyngeal incidence rate for the non-vaccine serotypes than individuals aged 35-49yrs in the Poisson Regression model. This observation had not been observed in related studies to the best of our knowledge and it remains unclear why the incidence rates for non-vaccine serotypes was significantly higher in this age group. It could however be explained by the fact that this age group comprised mainly of students who had greater interaction with children at home in the COVID-19 era as schools had been closed and yet children are likely to have a higher carriage prevalence for pneumococci non-vaccine serotypes. Since young children in South Africa are vaccinated against pneumococcal disease using PCV13, this reduces carriage of vaccine serotypes in children but at the same time increases their risk for carriage of non-vaccine serotypes (96,113).

5.3 Pneumococci serotype associated mortality and determinants of mortality among adults admitted with presumed CAP in the era of COVID-19 in South Africa

In this study we explored the occurrence of mortality in participants carrying pneumococci serotypes in their blood or oronasopharynx but the serotype isolated was not necessarily the cause of death. Across the two-year follow-up period for 126 eligible participants in our study, leading mortality was observed in participants who carried serotypes 1, 3, 4, 5, 15A/F, 18A/B/C/F, 23A and 33A/F/37 in their oronasopharynx. The few serotypes isolated in blood (1, 3, 8, 11A/D and 19A) were all associated with mortality in our study except serotype 8.

On exploring the predictors of mortality in the logistic regression model, being underweight and having a CURB-65 score of 1 were the only significant predictors of increased mortality.

In the multivariate Cox model at 3 days from the date of initial hospital admission, CURB-65 score 1 (reference=CURB-65 score 0), being underweight (reference=normal weight), and need for oxygen supplementation (reference=no need for oxygen supplementation) were significantly associated with higher mortality risk. The CURB-65 score is a well-validated tool for assessing disease severity and mortality among CAP patients, with lower mortality risk at lower CURB-65 scores and higher mortality risk at higher CURB-65 scores (17,114–116). Therefore, the significant higher mortality for participants with CURB- 65 score 1 than those with CURB- 65 score 0 in our study is therefore consistent with this tool.

The need for oxygen supplementation reflects hypoxemia in pneumonia patients and this puts hypoxemic patients at increased mortality risk than non-hypoxemic patients (117). The higher CURB-65 score, and the requirement of oxygen therapy directly reflect a more severe disease and hence a higher mortality risk.

There have been conflicting results from previous studies on the relationship between BMI and mortality among adult CAP patients. In comparison to normal weight individuals, some studies have reported reduced mortality at high BMI values ($\geq 25 kg/m^2$) (118–122) while others found increased mortality at low BMI values ($\leq 18\ kg/m^2$) (123–125). The higher mortality among underweight individuals than normal weight individuals in our study suggests a need for interventions to restore normal weight for CAP patients for better clinical outcomes.

At 14 days from the date of initial hospital admission, only ONP carriage of serotype 1 was significantly associated with higher mortality risk in the Cox model when adjusted for participant age. We postulated that serotype 1 isolated in the oronasopharynx could also have been significantly associated with non-bacteremic pneumococcal CAP among our participants that contributed to increased mortality risk. Serotype 1 was demonstrated to be significantly associated with IPD mortality in South Africa in individuals older than 15 years and our study showed similar result (89).

5.4 Strengths of our study

Our study used both blood samples and oronasopharyngeal swabs to investigate pneumococci serotype distribution among adults hospitalized with CAP in South Africa in the COVID-19 era. This enabled us to explore serotypes that were associated with oronasopharyngeal pneumococcal carriage and those that may be associated with pneumococcal CAP.

Our study was conducted in a heterogenous cohort of adults with presumed CAP enrolled throughout all seasons and from three geographically and socio-economically distinct provinces in South Africa. This enabled us to evaluate multiple factors that were associated with pneumococci serotype prevalence, incidence rates and mortality.

The two-year follow-up period in our study was associated with different levels of stringency of implementation of COVID-19 public health restrictions which enabled us to explore the effect of COVID-19 restrictions on the incidence rates of *S. pneumoniae* serotypes.

The *lytA* PCR used in our study to identify *S. pneumoniae* is highly sensitive and specific and unlike culture-based methods, it remains useful even when participants have received prior

antibiotic therapy. This minimized the risk of falsely excluding samples carrying pneumococci in our study.

5.5 Limitations of our study

The triplex real time PCR assay used for serotyping in our study could not detect some of the serotypes in the commercially available vaccines like PCV20 and PPV23 (such as 9N, 10A, 15B, 17F and 20). These serotypes were excluded in the analysis of pneumococci serotype epidemiology in South Africa, yet they could be in circulation. Furthermore, the triplex real time PCR assay could not distinguish between individual serotypes in every serogroup. We considered an individual serotype to be present in our study if its corresponding serogroup was isolated. This could have led to overestimation of the prevalence/incidence rates of some individual serotypes in our study.

For serotypes isolated in the ONP swabs in our study, we could not distinguish whether they were simply associated with mere oronasopharyngeal carriage, or they were associated with non-bacteremic/non-invasive pneumococcal CAP. The burden of non-bacteremic pneumococcal CAP has been reported to be up to 10 times higher than that of bacteremic pneumococcal CAP though only the prevalence of bacteremic pneumococcal CAP could be established with certainty using serotypes identified in blood. In our study, we largely considered serotypes isolated in the ONP swabs to mean oronasopharyngeal carriage and assumed positive blood samples were evidence of IPD.

Our study established incidence rates for pneumococcal serotypes in the COVID-19 era using serotypes identified in samples collected at different time points across the two-year follow-up period. We minimized this limitation in our study by grouping together different periods of follow-up in the analysis of incidence trends and we used person-years of follow-up for calculation of incidence rates.

Our study excluded pregnant women who are known to be at high risk for viral pneumonia especially influenza that in turn predisposes them to bacterial pneumonia.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

In this study we establish ten serotypes/serogroups with leading prevalence and/or incidence rates in the oronasopharynx among adults with presumed CAP in the COVID-19 era in South Africa (1, 3, 4, 5, 8, 9A/V, 12A/B/F/44/46, 19F, 22A/F and 33A/F/37). Four of these serotypes (1, 3, 4 and 8) in addition to serotypes/serogroup 6A/B, 11A/D and 19A had leading prevalence in blood. The difference in serotype prevalence and incidence rates in the oronasopharynx and blood suggests serotype variability in invasiveness and hence differences in serotypes associated with mere carriage, non-bacteremic and bacteremic pneumococcal CAP.

The big prevalence of serotypes not included in PCV13 (7.3%) suggests that PCV13 which is the currently recommended pneumococcal vaccine for adult vaccination has limited pneumococcal serotype coverage in the South African context. To maximize benefits from pneumococcal vaccination, PPSV23 should be used sequentially in all adults vaccinated with PCV13 or a higher valency pneumococcal vaccine like PCV20 should be considered.

This study establishes higher pneumococcal carriage in males, individuals aged 35-49 years, active smokers, alcohol consumers, ART and individuals on ART. The differences in pneumococcal carriage provide insights to policy makers in South Africa on who should be prioritized for adult pneumococcal vaccination.

Pneumococcal serotype incidence rates in the COVID-19 era were dependent on the stringency of COVID-19 public health restrictions implemented. Serotype incidence rates were lowest in periods of more stringent COVID-19 public health restrictions. Though public health restrictions may reduce pneumococci incidence rates, they are not sustainable and can't substitute pneumococcal vaccination.

Our study establishes that oronasopharyngeal carriage of serotypes/serogroups 1, 3, 4, 5, 15A/F, 18A/B/C/F, 23A and 33A/F/37 is associated with higher mortality. Mortality risk was highest within the first two weeks post hospital admission but the overall risk of death across the two-year follow-up period reduced with increasing time from initial hospital admission.

This study shows that mortality among adults with presumed CAP is predicted by serotype 1 (higher in ONP carriers), BMI (higher in underweight participants), CURB-65 score (higher for score 1) and need for oxygen supplementation (higher in oxygen supplementation recipients). These data strengthen the need for assessment of mortality risk of all CAP patients at hospital

admission and providing appropriate management for the modifiable risk factors for improved patient survival.

6.2 Recommendations

The higher prevalence of PCV20 serotypes in South Africa seems to suggest that PCV20 may be a more cost-effective vaccine than PCV13 in the context of South Africa. However, policy makers in immunization should evaluate the cost-effectiveness of PCV20 before introduction in South Africa for adult pneumococcal vaccination.

The high pneumococcal carriage prevalence in adults younger than 50 years (especially 35-49 years) for vaccine serotypes in South Africa suggests the need for extension of the target adult population for pneumococcal vaccination. Since pneumococcal carriage precedes pneumococcal disease, it will be beneficial to lower the priority age group for adult pneumococcal vaccination to at least 35 years of age. This being restricted by the cost of the vaccine; adult pneumococcal vaccination should be prioritized for the high-risk individuals such as pregnant women and those who are immunocompromised.

We recommend further studies in South Africa using serotyping techniques that can identify all the individual serotypes included in the globally licensed vaccines. This will help to establish with higher precision the prevalence/incidence rates of vaccine serotypes and non-vaccine serotypes in South Africa.

Since non-bacteremic pneumococcal CAP is responsible for the biggest burden of CAP, we suggest further studies in South Africa using novel approaches/techniques that can distinguish mere carriage (colonization) from non-bacteremic pneumococcal CAP to establish the true burden of non-bacteremic pneumococcal CAP.

Literature on the epidemiology of pneumococcal pneumonia remains sparse in high risk pregnant women especially in developing countries. We recommend further studies to evaluate the epidemiology of pneumococcal pneumonia in pregnant women in South Africa.

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8. APPENDICES

Appendix 1: Data extraction sheet

Variable	**Findings/Results**
Province	
Gender	
Age at enrolment	
HIV serostatus	
ART status	
lytA PCR results	
Triplex real time PCR assay results (serotype(s) identified on initial admission or follow-up)	
PCV vaccine received	
Death/Mortality	
Duration from enrolment to death	
Other relevant variables (including race, alcohol in-take, smoking, BMI, employment status, income level, underlying comorbidities, CURB-65 score)	

Appendix 2: Brief description of the primary study (HREC Reference: 171009)

The primary study was a 3-year, prospective, epidemiological, multicentre surveillance and cohort study involving four sub-studies. The current study used data from sub-study 2 of the primary study and hence only the information relevant to sub-study 2 is provided. The aim of sub-study 2 was to determine the incidence of CXR confirmed, hospitalized CAP and recurrent CAP among a prospectively followed cohort of adults.

Primary objectives of sub-study 2

- To estimate the incidence of CXR confirmed, hospitalized CAP.
- To estimate the recurrent CAP among a prospectively followed cohort of adults hospitalized at baseline for CXR confirmed CAP.

Secondary objectives of sub-study 2

- To estimate the population incidence (cases/100,000 adults) of CXR confirmed, hospitalized CAP with PCV13 *S. pneumoniae* detected by UAD assay and/or culture at baseline.
- To determine the incidence (cases/100,000 adults) of *S. pneumoniae* positive CAP patients with *S. pneumoniae* identified by culture, BinaxNOW®, and/or UAD assay(s) at baseline.
- To estimate the incidence (recurrent cases/100 patient years of follow up) of recurrent cases of CXR confirmed CAP.
- To estimate the proportion of patients with PCV13 *S. pneumoniae* detected by UAD assay and/or culture, among those recurrent CXR confirmed CAP.
- To determine the overall proportion of SP+ CAP patients with *S. pneumoniae* identified by culture, BinaxNOW®, and/or UAD assay(s), among those with recurrent CXR confirmed CAP.
- To determine the *S. pneumoniae* serotype distribution from UAD and culture isolates, among initial and recurrent CXR confirmed CAP cases.
- To determine the proportion of hospitalised patients with *S. pneumoniae* positive CAP who are identified by *lytA* PCR and determine its diagnostic value compared to blood culture, Urine for UAD or BinaxNOW®

Sub-study 2 enrolled adult patients (≥18 years) of both gender who were hospitalized for presumed CAP and had a positive chest X-Ray. Participants were enrolled at the following sites.

1. Chris Hani Baragwanath Academic Hospital (CHBAH) in Gauteng Province.

2. Klerksdorp Tshepong Hospital Complex (KTHC) in North West Province.
3. Polokwane/Mankweng Hospital (PMH) in Limpopo Province.

At enrolment, a blood sample, urine sample, oropharyngeal swab and nasopharyngeal swab were collected from each participant. The oropharyngeal swab and nasopharyngeal swab from each participant were combined to form the oronasopharyngeal swab. The blood samples and the oronasopharyngeal swabs were shipped to NICD where they were processed for pneumococcus identification and serotyping according. Enrolled participants were followed up at multiple regular time points over a period of two years. During the follow up period, it was planned to contact participants telephonically weekly in the first six months and/or in the influenza season and biweekly during other time points. Participants who reported respiratory illnesses during telephone calls were asked to visit the study clinics for further assessment. A urine sample, oropharyngeal swab and nasopharyngeal swab were taken from participants with presumed pneumonia during the clinic visit. If after the call, it was reported that the participant was hospitalized outside the study hospital, the participant was visited at his/her hospital of admission to gather the relevant study data. Furthermore, face–to-face follow-up research visits at the study clinics were scheduled every four months for all participants including the final 24-month visit. Advantage was taken during the telephone calls or during face-to-face visits to gather relevant study data including mortality data. However, not all planned telephone contacts were achieved, and some face-to-face visits did not take place as planned due to COVID-19 public health restrictions.

Data from participants in sub-study 2 was collected and entered in real time using eCRFs on Research Electronic Data Capture (REDCap) database management system. Data was collected on the following variables.

- Participant demographics.
- Participant detailed medical history including underlying medical conditions.
- Vaccination history (PPV23, PCV and influenza).
- Risk factors for both pneumococcal disease and influenza.
- Anthropometric measurements and vital signs.
- General habits including smoking and alcohol in-take.
- HIV-serostatus, ART status, and cotrimoxazole chemoprophylaxis status.

Laboratory methods

The oronasopharyngeal swabs and blood samples collected from participants were transported to NICD and tested by *lytA* PCR for *S. pneumoniae* identification. Serotyping was done on *lytA* PCR positive samples at NICD by triplex real time PCR assay.

***lytA* PCR**

The *lytA* PCR was done according to the NICD Standard Operating Procedure (SOP) for detection of *S. pneumoniae* (*lytA*) using real time PCR, Document number: NIC0605 Version number:6 (126). *lytA* PCR is a molecular method used to detect *S. pneumoniae* in different specimens including blood and oronasopharyngeal swabs. This molecular assay is based on the method described by Carvalho *et al*. (63). The method involves amplification of *lytA* gene target, using primers specific for *lytA* in *S. pneumoniae.* Details on the procedure for *lytA* PCR and how the results are interpreted are provided below.

Procedure of the real time PCR

1. Place the 96-well plate in real time PCR instrument.
2. Open the real time PCR software on the computer.
3. Select "new experiment" and name the plate. Select "7500 FAST (96 -wells)", "Quantification-Standard curve", "TaqMan reagents", and "standard (approximately 2 hours to complete run)" as the experiment properties.
4. Select the "plate setup" and define the targets and samples.
5. Select the "assign samples and targets" tab to assign samples to each well followed by the target.
6. Select "Run Method" and enter a reaction volume per well of 25 µl.
7. Save the run.
8. Press "start", the run will take approximately 1.5 hours.
9. Once the run is complete, select "Analysis" to view the amplification plot.
10. Print results and store in the file.

The universal cycling conditions during PCR involve stage 1 (50 °C for 2 min), stage 2 (95°C for 10 min and stage 3 (40 cycles involving 95 °C for 15sec and 60 °C for 1min).

Interpretation of results

A Ct value is generated for each positive well. A Ct value is the amplification cycle at which the fluorescence of the sample exceeded the threshold value. A sample is considered positive if the Ct value is <40. Negative samples are defined as having a Ct value ≥40.

Triplex Real time PCR assay

The triplex real time PCR was done according to the NICD Standard Operating Procedure (SOP) for serotyping of *S. pneumoniae* using triplex real time PCR assay, Document number: NIC1019 Version number:7 (127). Triplex real time PCR is a molecular method used for serotyping *S. pneumoniae*. The assay is based on the method described by Primenta *et al*.(91). This method was designed to detect 21 pneumococcal capsular serotypes that account for high burden of pneumonia globally. The method was modified to include a reaction for serotype 8 since it causes significant disease in South Africa. Due to challenges of detection of serotype 19A in triplex reaction 4, serotype 19A was removed from reaction 4 and is now part of reaction 8 (duplex reaction) that is validated as described by Azzari *et al.* (90).

The method consists of eight reactions detecting 22 serotypes (serogroups) as shown below.

Reaction	Serotype/ Serogroup detected
1	1, 5, 23F
2	4, 6A/6B/6C/6D, 9V/9A
3	14, 18C/18A/18B/18F, 19F
4	3, 7F/7A
5	6C/6D, 12F/12A/12B/44/46, 22F/22A
6	15A/15F, 23A, 33F/33A/37
7	2, 11A/11D, 16F
8	8, 19A

"/" implies that the method is not able to distinguish between serotypes.

This method is used for serotyping of clinical specimens and non-viable transport medium samples that test positive by *lytA* PCR. Only specimens with a Ct value of ≤35 are serotyped using triplex real time PCR assay. For samples testing positive with Ct value of ≤20, they are first diluted to higher Ct values to avoid possible inhibition by the high DNA concentration during serotyping.

Details on the procedure for triplex real time PCR assay and how the results are interpreted are provided below.

Procedure of the real time PCR

1. Place 96-well plate or strip in the Applied Biosystems real time PCR instrument.
2. Open the real-time PCR instrument icon on the computer.
3. Under "new experiment" Select absolute quantification (Standard curve) and name the plate with initials, test, and date.
4. Select "Absolute Quantification (Standard curve)" and "TaqMan reagents", and "standard (approximately 2 hours to complete run)" as the experiment properties.
5. Select the "plate setup" and define the targets and samples.
6. Select the "assign samples and targets" tab to assign samples to each well followed by the target.
7. Select the "instrument" tab and change final reaction volume to 25 µl.
8. Save the data in the appropriate folder.
9. Press "start", the run will take approximately 1h40min.
10. Following completion of the run, select the "Amplification plot" tab. Enter the appropriate threshold and press "analyse". A report will be generated.
11. Print and store in the pneumococcal serotyping file.

The universal cycling conditions during PCR involve stage 1 (50 °C for 2 min), stage 2 (95°C for 10 min and stage 3(40 cycles of 95°C for 15sec and 60°C for 1min).

Interpretation of results

If the reaction is positive for the target, a Ct value will be generated. A sample is considered positive if the Ct value is <40. Negative samples are defined as having a Ct value ≥40 in any of the reactions and if negative in all the eight reactions, it is labelled "neg38". Samples positive for serogroup 6 in reaction 2 must be tested under reaction 5. If the sample is positive under reaction 5, then the sample is considered to carry serotypes 6C/D and if negative under reaction 5, then it carries serotypes 6A/B.

For blood samples, If the serotype is detected with Ct value <35, then the remaining reactions may be discontinued for that sample. However, if the Ct value is ≥35, then the sample should proceed with testing in the subsequent reactions. If wo serotypes are identified in blood and the difference between their Ct values is ≥10, the serotype with the lowest Ct value is reported as

final. If the difference is <10, serotyping should be repeated from a new DNA extract. If similar results are obtained on repeat testing, all the identified serotypes are reported as final.
For non-sterile site samples including the oronasopharyngeal swabs, all eight reactions must be performed, and all serotypes detected are reported in the order of their increasing Ct values.

Appendix 3: Wits HREC (Medical) clearance certificate.

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R14/49 Dr Grant Munkwase

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M221110

NAME: (Principal Investigator)	Dr Grant Munkwase
DEPARTMENT:	School of Pathology Perinatal HIV Research Unit (PHRU)
PROJECT TITLE:	Streptococcus pneumoniae serotype distribution among adults admitted with Community Acquired Pneumonia across South Africa in the COVID-19 era
DATE CONSIDERED:	25/11/2022
DECISION:	Approved unconditionally
CONDITIONS:	N/A
NOTE:	N/A
SUPERVISOR:	Dr F. Nabeemeeah and Prof K. Otwombe
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/03/2023

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. 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 resubmit the application to the Committee. **I agree to submit a yearly progress report.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **November** and will therefore be due in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

munkwase
Principal Investigator Signature

06 March 2023
Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix 4: Plagiarism Declaration Form

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I ____**Grant Munkwase**____(Student number:__**2601111**_) am a student registered for the

degree of__**Master of Science in the field of Vaccinology**__in the academic year: **2024**

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/orwithout acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided workexcept where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:_ *munkwase* ___Date:_27 June 2024

Appendix 5: Turnitin Report (Excerpt)

Research_Report_09-Oct-2023-Body.docx

by Grant Munkwase

Submission date: 09-Oct-2023 01:02PM (UTC+0200)
Submission ID: 2190230026
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