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Recurrent Pneumococcal Community-Acquired Pneumonia in Hospitalised Patients

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RECURRENT PNEUMOCOCCAL COMMUNTY AQCUIRED PNEUMONIA IN HOSPITALISED PATIENTS

Matema Mangwadi Seabela

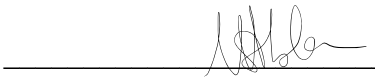
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A research report submitted to the Faculty of Health Sciences, University of Witwatersrand, in fulfilment of the requirements of the degree of Master of Science (Medicine) in Vaccinology.

Johannesburg, 2025

DECLARATION

I Matema Mangwadi Seabela declare that this Research Report is my own, unaided work. It is being submitted for the Degree of Master of Science (Medicine) in 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)

_____20th_____day of__February_____2025__in___Johannesburg____

DEDICATION

To my mother Prescilla Tshella Seabela, my late grandfather and grandmother; Benjamin Ngaka Seabela and Mapula Eversina "Betty" Seabela.

My daughter Thoriso.

And the rest of my family. The ones that came before me. "*Batlokwa ba Machaka Maimela. Dinkwe!" (The batlokwa of the Machaka Maimela, the leopards)*

ABSTRACT

Recurrent pneumococcal CAP refers to episodes of pneumococcal CAP that occur at least 30 days apart, constituting 60% of recurrent invasive pneumococcal diseases. HIV increases the risk of severe pneumococcal CAP. Given South Africa's high HIV prevalence, understanding the burden and vaccine preventability of recurrent pneumococcal CAP is crucial. The objective was to investigate the incidence of recurrent pneumococcal CAP, identify causal serotypes, and evaluate mortality among hospitalised patients with pneumococcal CAP. We retrospectively collected data from 1573 patients hospitalised with pneumococcal CAP at three hospitals between 2019 and 2021, including a 1-year follow-up period. Among 1573 patients, there were 63 episodes of recurrent pneumococcal CAP in 27 (1.72%) patients. The incidence was 18.53 per 1000 person-years with IRR=0.925 [95% CI: (0.608-1.352)]. Of the 27 patients with recurrent pneumococcal CAP, 19 (70%) were PLWH, OR=2.06 [p=0.0598; 95% CI (0.854-5.47)]. PCV13 and PPSV23 causal serotypes were 54% and 88% respectively. In conclusion, at least 19 of 1000 pneumococcal CAP hospitalised patients will have vaccine preventable recurrent pneumococcal CAP within 1-year.

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Thanks to my supervisor Dr. Kennedy Otwombe and co-supervisor Dr. Firdaus Nabeemeeah.

Thank you to the staff at the Centre for Respiratory Disease and Meningitis for assisting me with the databases.

Last but certainly not least, To my family. Thank you.

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LIST OF ABBREVIATIONS AND SYMBOLS

1. ACHU- age ≥ 45 years (A), confusion on admission (C), HIV-infection (H), and serum blood urea nitrogen >7 mmol/L (U)
2. AMR- Antimicrobial Resistance
3. ARDS- acute respiratory distress syndrome
4. ART- Antiretroviral Therapy
5. BPP-Bacteraemic Pneumococcal Pneumonia
6. CAP – Community Acquired Pneumonia
7. CD4+ - Cluster of differentiation 4
8. CDC- Centers for Disease Control and Prevention
9. Cells/mm^3- cells per cubic millimeter
10. CHBAH- Chris Hani Baragwanath Academic Hospital
11. COPD- Chronic Obstructive Pulmonary Disease
12. CRB-65- Confusion, Respiratory Rate ≥30, Systolic Blood Pressure <90 mmHg, Age ≥65
13. CRP-C-reactive Protein
14. CSF- Cerebrospinal Fluid
15. Ct-value- Cycle Threshold
16. CURB-65- Confusion, Urea, Respiratory Rate ≥30, Systolic Blood Pressure <90 mmHg, Age ≥ 65.
17. CXR- Chest-X-Ray
18. DNA- Deoxynucleotide acid
19. DRSP- Drug Resistant *Streptococcus Pneumoniae*
20. EPI- Extended Programme for Immunisation
21. FDA- Food and Drug Administration
22. HIC- High Income Countries
23. HIV-Human Immunodeficiency Virus
24. HREC-Heman Research Ethics Committee
25. ICU-Intensive Care Unit
26. IgG- Immunoglobulin G
27. IPD- Invasive Pneumococcal Disease
28. KTHC-Klerksdorp-Tshepong Hospital Complex
29. LMIC-Low- and Middle-Income Countries
30. LRTI- Lower Respiratory Tract Infection

31. LytA-Autolysin gene
32. MDR- Multi-Drug Resistant
33. NHLS- National Health Laboratory Service
34. Non-BPP- non-Bacteraemic Pneumococcal Pneumonia
35. NPS- Nasopharyngeal Swab
36. NVTs- Non-vaccine Serotypes
37. OR- Odds Ratio
38. pCAP- Pneumocococcal Community Acquired Pneumonia
39. PCR- Polymerase Chain Reaction
40. PCT-Procalcitonin
41. PCV- Pneumococcal Conjugate Vaccine
42. PHRU-Perinatal HIV Research Unit
43. PLWH- People Living With HIV
44. ply- Pneumolysin gene.
45. POTPREV- The potentially preventable burden of community acquired pneumonia in South African Adults in the era of widespread paediatric PCV13 immunisation and antiretroviral therapy rollout.
46. PPSV- Pneumococcal Polysaccharide Vaccine
47. psa- Pneumococcal surface Adhesin Antigen
48. RDW-Red Cell Distribution
49. rpCAP- Recurrent pneumococcal CAP
50. rt-PCR- Real-Time Polymerase Chain Reaction
51. SA- South Africa
52. SMART-COP- Systolic blood pressure, multilobar infiltrates, albumin, respiratory rate, tachycardia, confusion, oxygen, and pH
53. UAD- Urinary Antigen Detection
54. USA- United States of America
55. VE-Vaccine Efficacy
56. VTs- Vaccine Serotypes
57. WHO- World Health Organization

1. INTRODUCTION

Pneumococcal CAP (community acquired pneumonia) is the leading cause of CAP globally and a well-researched infectious disease in both adults and children [1–3]. However, there limited research on recurrent pneumococcal diseases globally, especially non-IPD (recurrent non-invasive pneumococcal) CAP and the *S.pneumoniae (Streptococcus pneumoniae)* serotypes causing recurrent pneumococcal CAP episodes[4–6]. Recurrent IPD (invasive pneumococcal disease) is diagnosed when S. *pneumoniae* is isolated from a normally sterile site >30 days from an index episode of recurrent IPD (King et al., 2003). Approximately 60% of all recurrent IPD are pneumococcal CAP episodes, although rarely diagnosed [1,4]. The lacking consensus on a standardised pneumococcal CAP case definition is a challenge, resulting in different case definitions sourced from literature being used and presenting a challenge in diagnosing non-IPD pneumococcal CAP because *S.pneumoniae* is not isolated from sterile sites in these episodes[7]. The most common definition of recurrent pneumococcal CAP is the occurrence of at least 2 subsequent episodes of pneumococcal CAP within a year or 3 episodes of pneumococcal CAP in a lifetime with an interval of at least 30 days between the first and subsequent pneumococcal CAP episode [1,5,6]. Recurrent pneumococcal CAP episodes are categorised as relapses or reinfections, described by the onset of disease and the strain of *S.pneumoniae* causing the episode of pneumococcal CAP; pre-existing strains cause relapses and new strains cause reinfections [8]. The independent predictors for recurrent pneumococcal CAP in adults are an index episode of pneumococcal CAP, no pneumococcal vaccination, advanced age of ≥65-years-old, immune-suppression and dysfunction including HIV (Human Immunodeficiency Virus) and hypo- and asplenia [1,5,9]. Other predictors associated with recurrent pneumococcal CAP are COPD (Chronic Obstructive Pulmonary Disease) ,diabetes mellitus and smoking [10,11]. Pneumococcal CAP is vaccine preventable and we have three pneumococcal vaccines available in South Africa; PPSV 23 (23-valent pneumococcal polysaccharide vaccine), PCV13 (13-valent pneumococcal conjugate vaccine) and PCV10 (10-valent pneumococcal conjugate vaccine [12–14]. Vaccine serotypes (VTs) are the most common disease-causing *S.pneumoniae* serotypes also associated with severe disease [3,15,16]. PCV 10 was introduced in 2024 in the South African EPI(expanded programme for immunisation) as a cost effective alternative formulation to PCV13 for the immunisation of children under the age 2 years [14]. Prior to that, PCV13 was used for immunising children under2 years old, scheduled at 6 and at 14 weeks and 9 months ; whilst PCV13 and PPSV23 are recommended for PLWH (People Living With HIV) and the elderly [12,17] . The PCV13

paediatric immunisation coverage in South Africa increased from >80% in 2018 to 89% in 2022, a figure much higher than the 60% global PCV13 coverage [18]. Pneumococcal vaccine coverage in adults is very low, suggesting that the protective effects of pneumococcal vaccines are entirely dependent on indirect protective effects gained from the EPI [15]. A South African study demonstrated the effects of vaccinationwere70% of serotypes causing IPD were PCV13 preventable [3]. In the pre-COVID-19 era, PCV7 and PCV13 serotype associated IPD, carriage and infections caused by DRSPs (Drug Resistant *S.pneumoniae*) in both children and adults declined.[14] Although there is limited research on recurrent pneumococcal CAP, is evident that HIV is the most common predictor for recurrent pneumococcal CAP; making the burden of recurrent pneumococcal CAP important in the South African context because of our high burden of HIV[3,12,19]. Pneumococcal CAP is still the leading cause of mortality and hospitalisations in PLWH even in the post-PCV era and with widespread ART (antiretroviral therapy) rollout [3].In this study, we aim to determine the incidence of recurrent pneumococcal CAP and mortality in patients hospitalised with recurrent pneumococcal CAP, we also aim to determine if the serotypes that cause recurrent pneumococcal CAP in hospitalised adult patients with CAP are vaccine serotypes so that we can determine if recurrent pneumococcal CAP is also vaccine preventable. If the cause of recurrent pneumococcal community acquired pneumonia of hospitalized patients is vaccine preventable, recommendations regarding adult vaccination schedules for individuals at risk of recurrent pneumococcal CAP may be implemented as an intervention to prevent recurrent episodes of pneumococcal CAP and the mortality thereof.

2. LITERATURE REVIEW

This literature review on recurrent pneumococcal CAP in hospitalised patients with CAP was conducted by searching for publications from the following databases: (PubMed, Cochrane Library and Embase, Google Scholar) using the following terms: “Pneumococcal Pneumonia”, “Invasive Pneumococcal Disease”, “Pneumonia”, “Recurrent Pneumococcal Pneumonia” and “Recurrent Invasive Pneumococcal Pneumonia” which was conducted from June 2022 to November 2023. In this literature review, we explored literature on recurrent pneumococcal community acquired pneumonia pertaining to the diagnosis, case definition, treatment, risk factors, serotypes of *S. pneumoniae* in recurrent and pneumococcal pneumonia and pneumococcal vaccination. The literature review of these topics were used to analyse the findings of our study investigating the incidence of recurrent pneumococcal CAP in hospitalised patients.

2.1. Diagnosis Of Pneumococcal CAP

Pneumococcal CAP is classified as non-BPP (non-bacteraemic pneumonia) or BPP (bacteraemic pneumococcal pneumonia) [20,21]. Bacteraemic pneumococcal CAP, a form of IPD; is serious and commonly associated with poor clinical outcomes, high mortality rates in PLWH who are predisposed to increased *S.pneumoniae* nasopharyngeal colonization also worsening their risk of IPD [22]. Bacteraemic pneumococcal CAP case definition is the isolation of *S.pneumoniae* from a normally sterile site in the presence of CAP associated clinical symptoms associated [21]. Normally sterile sites are sites and fluids in the body with no microorganisms including commensal flora under normal circumstances [23]. Normally sterile sites constitute of blood, cerebrospinal fluid, pleural fluid, peritoneal fluid, pericardial fluid, bone, synovial fluid and internal body sites such as the lymph nodes, brain, heart, liver [23–25].The absence of a gold standard diagnostic technique for diagnosing definitive non-bacteraemic pneumococcal CAP episodes is a challenge [26–28]. CAP specific clinical symptoms are important in diagnosis, the most common clinical presentation and symptoms of pneumococcal CAP are dyspnoea, cough, sputum production and pleuritic chest pain as well as fever and new infiltrates on chest X-ray [29].A combination of diagnostic techniques and assessing CAP specific clinical symptoms is important in diagnosing definitive episodes of CAP [12].There is a general consensus that a CAP diagnosis is determined in the presence of 2 or more CAP clinical symptoms with an abnormal CXR [12,26]. However, the clinical presentation and symptoms associated with CAP in the elderly and PLWH may be atypical, presenting yet an another diagnostic challenge especially in our limited resource setting [27,29]. CAP symptoms tend to be significantly lower in PLWH than in HIV-uninfected individuals, whilst the elderly normally present with non-specific symptoms such as headache and diarrhoea.[27,29]. Relying on clinical symptoms is problematic in patients who are asymptomatic because a normal CXR doesn't exclude pneumococcal CAP diagnosis[7,30]. Laboratory diagnosis of pneumococcal CAP is difficult in episodes where sputum production is absent, NPS (Nasopharyngeal Swab) specimens have proven to be more effective even though the isolation of *S .pneumoniae* from the nasopharynx alone is also not sufficient to confirm and distinguish definitive from presumed episodes of pneumococcal CAP[12,31].A high nasopharyngeal *S.pneumoniae* colonisation density is of >3.9 log10 copies/mL is considered high colonisation density and indicative of pneumococcal CAP diagnosis [32].Furthermore, patients with invasive pneumococcal CAP tend to have a significantly higher nasopharyngeal *S.pneumoniae* colonisation density than those with non-invasive/non-bacteraemic CAP [32]. In the absence of a gold standard diagnostic technique, clinical symptoms of a patient with

pneumococcal CAP are a major determinant for diagnosis with or without confirmation of a suspected episode using other diagnostic methods [12,26,29]. In hospitalised patients with pneumococcal CAP, prognostic and predictive tools are helpful in determining the clinical progression of disease, risk of mortality and severity of the infection to predict mortality [33]. Considering the challenges associated with the diagnosis of pneumococcal CAP, the multimodal use of various diagnostic tools is pivotal in correctly diagnosing patients with pneumococcal CAP to limit misdiagnosis, underestimation of the burden of disease and mortality of hospitalised patients with pneumococcal CAP.

2.1.1. Clinical and radiological diagnosis of CAP

The South African guidelines for the management of CAP recommends clinical diagnosis of CAP by examination of clinical symptoms associated with CAP such as a cough, pleuritic chest pain, shortness of breath, sputum production in the absence of rhinorrhoea or sore throat [12]. The clinical symptoms mostly associated with pneumococcal CAP are an acute onset of disease, a high fever depicted by a temperature ≥38°C and chest pain [34]. Globally, the guidelines for all-cause pneumonia recommend that a CXR must be performed on all hospitalised patients to confirm pneumonia diagnosis [26]. In South Africa, CXR is the most commonly used radiological diagnostic tool used for CAP and it is available in secondary healthcare facilities [12]. Furthermore, all hospitalized CAP patients must have a CXR performed and a repeated CXR 24-48 hours performed following a negative CXR for confirmation of CAP according to the SA CAP guidelines [12]. In one study, the 30-day mortality in hospitalised CAP patients without a confirmed CXR was 47.2% whilst over 50% of patients with CAP were misdiagnosed [12,13]. The interpretation of an abnormal CXR examination is subjective, often leading to the misdiagnosis of CAP patients because distinguishing new or previous chest infiltrates in patients with a confirmed abnormal CXR is difficult [26,35]. Chest infiltrates of an abnormal CXR result are observed as a white shadow over the lung, unfortunately; PLWH tend to have a normal CXR finding even in the presence of respiratory disease and more specifically in those who are ART naive [35,36]. Because a normal CXR doesn't exclude the diagnosis of CAP and an abnormal CXR may be misinterpreted, CT (computed tomography) is preferred to confirm pneumonia and improves diagnosis accuracy, although not widely available and used in low-income setting including South Africa [12,13,37–39].

2.1.2. Laboratory diagnostic testing for *S. pneumoniae*

Laboratory diagnosis of *S.pneumoniae* is conducted through conventional, molecular and antigen-based techniques [12,32,33]. Conventional laboratory and molecular techniques use sputum, blood, CSF (cerebrospinal fluid), NPS (nasopharyngeal swab) specimens and antigen-based techniques such as UAD (Urinary Antigen Detection) which detects *S.pneumoniae* in urine by identifying antigens specific to the bacteria [12,20,33]. Conventional laboratory techniques involve inoculation of a specimen on culture media to determine the presence of a particular microorganism in that specimen [40]. Molecular detection of *S.pneumoniae* is conducted primarily with PCR (polymerase chain reaction), this is used to identify unique gene sequences of bacteria [33].LytA-PCR, psa-PCR or ply-PCR are the common PCR techniques used in which specific *S. pneumoniae* genes are amplified; these are the LytA (autolysin) , PSA (pneumococcal surface adhesin) and the PLY (pneumolysin) genes respectively [33].The autolysin gene is the most specific to *S.pneumoniae*, increasing the proportion of episodes attributable to *S.pneumoniae* from 27.1% to 52.5% with a specificity and sensitivity of >80% even in afebrile and asymptomatic patients, this is however limited by the fact that *S.pneumoniae* is common colonizer of the nasopharynx [31]. Furthermore, LytA-rtPCR (autolysin gene real-time PCR) can identify 34.4% more cases of pneumococcal CAP compared to conventional diagnostic methods, reducing the underestimation of the incidence of pneumococcal CAP in adult patients [31]. UAD is a multiplex technique which uses 24 unique *S.pneumoniae* serotype-specific monoclonal and 24 different rabbit polyclonal antibodies as well purified polysaccharides to detect the 13 serotypes in PCV 13 and the 11 non-PCV 13 serotypes in PPSV 23 using UAD-1 and UAD-2 respectively [41]. UAD is a. highly sensitive technique making definitive pneumococcal CAP diagnosis possible in non-invasive and sputum-unproductive episodes [12,20,33]. The major limitations of molecular techniques in diagnosing pneumococcal CAP is the inability to distinguish between viable and non-viable bacteria [20]. Another limitation for both conventional and molecular techniques is that high quality blood and sputum specimens are difficult to obtain, this is challenging because invasive pneumococcal CAP is rare, only up to 25% of pneumococcal CAP cases in adults are invasive and an unproductive sputum is common[31,33]. Culture results depend on high quality specimens and inappropriately collected specimens lead to false negatives, this was as observed in a prospective study conducted in Spain, where 22% of the PLWH cohort who had died from CAP had not obtained positive culture results [42]. The absence of positive cultures presents a challenge for CAP diagnosis in our setting and is not recommended for use in isolation in diagnosing CAP but rather as a tool to direct antimicrobial therapy [12,33].

2.1.3. CAP predictive tools.

Predictive tools are used to determine disease severity and prognosis, establishing disease severity and site of care of patients for hospitalisation or out-patient management [12]. These tools are the CURB-65 (confusion , urea >7mmol/L, respiratory rate ≥30/min ,systolic blood pressure <90mmgHg and/or diastolic blood pressure ≤60mmHg and the age ≥65 years), SMART-COP (Systolic blood pressure, multi-lobar infiltrates, albumin, respiratory rate, tachycardia, confusion, oxygen and pH) , PSI (Pneumonia Severity Index) and ACHU (age ≥ 45 years , confusion on admission , HIV-infection , and serum blood urea nitrogen >7 mmol/L) scores [12,13,38,43]. CURB-65 is a useful 30-day mortality prediction tool and determines the prognosis of patients with CAP [38]. CURB-65 constitutes of 5 features in which a score of 1 is attributed to each feature, the features considered are new onset of confusion , urea >7mmol/L, respiratory rate ≥30/min ,systolic blood pressure <90mmgHg and/or diastolic blood pressure ≤60mmHg and the age ≥65 years [12]. Suspected CAP patients with a CURB-65 severity score of 2 or more require hospitalisation and patients with a CURB-65 score between 3 and 5 are at high risk for CAP requiring high care or ICU (Intensive Care Unit) admission [12,38]. Although CURB-65 is an effective tool; there is poor prediction in patients with radiographically diagnosed CAP and this requires that CURB-65 score be used in combination with the SMART-COP severity score (Systolic blood pressure, multi-lobar infiltrates, albumin, respiratory rate, tachycardia, confusion, oxygen and pH) to improve diagnosis of CAP in HIV patients [13]. The South African CAP guidelines also recommends the use of CURB-65 to determine the prognosis of a CAP patient and also emphasises the importance of using other scores as adjuncts to improve clinical examination of CAP [12]. In a resource limited setting with a high prevalence of HIV, the CURB-65 is not useful even when used in combination with the PSI (Pneumonia Severity Index), this is because both scores are not validated in PLWH [31]. PSI is used to identify patients that are low risk for pneumonia by calculating a score using 20 variables, this is conducted by using a point system to predict mortality [44]. The variables used in PSI consist of 5 categories which are sex, demographic factors, age, comorbidities, physical examination and laboratory/radiographic findings, the PSI score is categorised from Class I which determines a mortality prediction rate of 0.1% to Class V with a mortality prediction rate of 27% [45]. Locally, the ACHU score is highly recommended for use in PLWH as it constitutes major risk factors for LRTI (Low Respiratory Tract Infections) specific to our setting, these risk factors are age ≥ 45 years (A), confusion on admission (C), HIV-infection (H), and serum blood urea nitrogen >7 mmol/L

(U), these were used to create the seven-point ACHU score which is more suitable for use in pneumococcal CAP diagnosis when compared with CURB-65 for LRTI in PLWH [37].

2.2. Case Definition of Pneumococcal Cap in Hospitalised Patients

The case definition of BPP is homogenous as opposed to non-BPP which is still a challenge to diagnose and define [31,46]. The WHO (World Health Organization) defines severe pneumonia requiring hospitalization as the presence of cough or difficulty in breathing and tachypnoea, plus one or more of the general danger signs, which excludes lower chest indrawing [47]. The CDC (centres for disease control and prevention) only describes the case definition for IPD (invasive pneumococcal disease) and not for non-IPD [48]. According to the CDC; a probable case of IPD occurs when *S. pneumoniae* is identified from a normally sterile body site by CIDT (culture independent diagnostic tests) such as PCR (polymerase chain reaction) without isolation of the bacteria, whilst a definitive case of IPD is diagnosed when *S.pneumoniae* is isolated from a normally sterile site [48]. The confirmation of a case of pneumococcal CAP is dependent on positive *S. pneumoniae* respiratory, blood cultures and an abnormal chest X-ray and in the absence of these, a case of pneumococcal CAP is not considered a definitive case even though a normal CXR and negative sputum, nasopharyngeal swab and blood cultures do not exclude pneumococcal CAP diagnosis [12,27]. LytA-rt PCR has proven to be useful for non-BPP diagnosis because of the higher density of *S.pneumoniae* found in the nasopharynx of patients with non-BPP than in patients with BPP, this can be used in combination with clinical diagnosis and confirmed CXR to define an episode of non-BPP [31,46].The literature illustrates that none of the diagnostic tools recommended for diagnosis of CAP should be used in isolation to define a case of pneumococcal CAP and a combination of these tools as well as severity scores should be used for diagnosis of pneumococcal CAP in the absence of a homogenous pneumococcal CAP case definition. However, a positive UAD can be considered in the case definition of pneumococcal CAP as it detects 24 serotypes in the urine of both invasive and non-invasive pneumococcal CAP [12,20,33].

2.3. Treatment Of Pneumococcal CAP

The South African guidelines for the management of community acquired pneumonia in adults provides recommendations to clinicians treating and vaccinating adults was updated in 2022 [15,49].However, there is no local public health policy on adult pneumococcal vaccination compared to most countries in high income settings with established policies

[15,50]. The lack of an adult pneumococcal immunisation policy here may lead to lower vaccine uptake compared to high-income settings with established programmes[15]. The low vaccine uptake among adults leads to increased reliance on antibiotic treatment in managing pneumococcal CAP, which increases the burden of AMR (Antimicrobial Resistance) [12].

2.3.1. Antimicrobial resistance in pneumococcal CAP.

AMR is a major global public health burden reducing the effectiveness of antimicrobials in treating infectious diseases, this leads to worsened disease severity, morbidity and mortality [51]. In 2019, an estimated 5 million deaths were associated with AMR; with 1.3million of these attributed to antimicrobial resistant infections [52]. According to the CDC, *S.pneumoniae* is an important antimicrobial resistant bacteria contributing to high healthcare costs because of the emergence of DRSPs [53]. DRSPs are driven by the evolution of recombination hotspots related to genes encoding antimicrobial targets, capsular biosynthesis and adhesins of *S. pneumoniae* as a response to exertion of selective pressure by the immune system's response to vaccines and antimicrobials [54]. This selective pressure leads to serotype switching of *S. pneumoniae*, these factors may be the leading contributors to the incidence of recurrent pneumococcal CAP [54]. Serotype switching allows a specific serotype of *S.pneumoniae* to change to a different serotype or a serotype clone equipping the bacteria to evade vaccine-induced immunity[55]. DRSPs are also driven by the inappropriate use of antibiotics, the low vaccine coverage in adults who are above the age of 65 and other high-risk populations for pneumococcal diseases, the difficulty in the identification and definition of DRSPs and the lack of vaccines that are protective against all serotypes of *S. pneumoniae* [49,53]. Although there are recommended antibiotics for the treatment of pneumococcal CAP, AMR continues to present a public health threat especially in the absence of adult pneumococcal immunisation policy and the low adult pneumococcal vaccine coverage in South Africa [15]. The management of patients with DRSPs is economically burdensome, firstly because patients with DRSPs require new and costly antibiotics because of the limited effective antibiotics, secondly because of the increased need for investment in research and development of new antibiotics [53]. Furthermore, there are also increased costs associated with recurrent disease because of treatment failure, investment in surveillance of resistance patterns as well as the education of health care providers and patients [53].

2.3.2. Drug Resistant *S. Pneumoniae* Serotypes.

Globally prevalence of DRSP from blood and respiratory specimens in patients with CAP is around 1.3% with a much higher DRSP prevalence of approximately 7.0% in Africa [56]. The higher doses of antimicrobial usage common in African countries have led to the observed prevalence in DRSPs [57]. DRSPs in pneumococcal CAP in Africa are driven by the high burden of infectious diseases, acute respiratory illnesses, Tuberculosis, HIV and AIDS [52]. The highest burden of deaths attributable to AMR globally are in Western and Sub Saharan African countries, these also have the highest HIV burden globally [52]. MDR (Multi-Drug Resistance) of *S.pneumoniae* is common in NVTs (non-vaccine serotypes) than VTs because of pneumococcal vaccine pressure[53,58].In a retrospective pre-PCV era cohort study in Malawi, serotype 1 was the most common serotype with an MDR prevalence of 81.9% and serotype 12F, a non-vaccine serotype; was second with an MDR prevalence of 57.9% [58]. This demonstrates the potential natural capsule switching in the absence of selective vaccine pressure exerted and the serotype replacement because of the increased prevalence of serotype 12F and MDR [58]. DRSPs are commonly resistant to cotrimoxazole, tetracycline, chloramphenicol, penicillin and had a low resistance to erythromycin and ceftriaxone [58]. These antibiotics are associated with AMR prevalence and prescribed for prophylaxis and empiric antibiotic treatment of pneumococcal CAP in high risk patients[59]. Amoxicillin is widely used in primary health care facilities for several LRTI, this has increased amoxicillin resistance in non-BPP while penicillin and macrolides resistance is common in elderly patients where 11A is the most antimicrobial resistant serotype [12,60,61]. There is evidence of a high incidence of AMR in *S.pneumoniae* serotypes with high intra-serotype sequence diversity in the post-PCV13 era such as 6A, 19A and 18B/C [58]. The emergence of DRSPs is a major public health threat and the availability of higher valency pneumococcal vaccines covering more serotypes are important to limit the effects of AMR.

2.4. Predictors Of Recurrent Pneumococcal CAP

The most important risk factor for recurrent pneumococcal disease is an index episode which shows the inherent predisposition to disease, thus; the predictors of recurrent pneumococcal CAP will be the similar to those of pneumococcal CAP [5]. Recurrence of pneumococcal disease is not uncommon in adults especially in the elderly, PLWH , COPD , smokers, patients with immune and spleen dysfunction [1,5,62].

2.4.1. Chronic Obstructive Pulmonary Disease

The prevalence of COPD is increasing globally, this increase is attributed to the global increase in tobacco smoking; the leading cause of COPD and an independent risk factor of recurrent pneumococcal CAP [63,64]. COPD is characterised by lung inflammation due to the activation of inflammatory processes and the remodelling of the lower airways and lung parenchyma resulting in lung dysfunction, injury and susceptibility to infection because of previous TB infection, respiratory exposure to toxic particles and gases from occupational dust, indoor pollution and smoking [64,65]. The lung parenchyma is the section of the lung that is involved in gaseous exchange, this mainly constitutes of alveoli [66–68]. The deterioration of humoral immunity and inadequate functioning of the lung because of inflammation exacerbates the risk of recurrent pneumococcal pneumonia in COPD patients , hence these patients are up to 9 times at risk of recurrent pneumococcal pneumonia including CAP [62]. In patients with COPD, recurrent pneumococcal pneumonia occurs because of the loss of humoral immunity and it is specifically as a result of the deterioration of the nucleotide-binding domain-like receptor protein-3, associated with enhancing response in the clearance of the lungs as well as reducing lung injury caused by inflammation [54]. Globally, there are approximately 380 million cases and a COPD prevalence of 12% , 13.4% in Africa and 20% in South Africa [65,69]. Locally, the Western Cape Province has the highest prevalence of COPD at 19% for clinically significant or Stage ≥2 COPD [65]. The global prevalence of tobacco smoking was 22% in 2020 ,this is the equal to the South African smoking prevalence of 22% with the highest prevalence in the Western Cape at 39% [69,70]. During the COVID-19 pandemic a tobacco sales ban was introduced in South Africa to encourage smoking cessation, unfortunately only 2% of smokers stopped smoking following this intervention[71].

2.4.2. People Living With HIV

South Africa has the largest ART (Antiretroviral Therapy) programme in the world and approximately 5.5 million people >15 years on ART in 2021 [19]. In 2020, 7.8million adults and children were PLWH, this is an underestimated figure because there were 3.4million less tests performed in 2020 compared to 2019 [72]. The HIV prevalence in South Africa has remained relatively unchanged at 18% from 2016 to 2021 [3,19]. PLWH are at risk of opportunistic infections such as tuberculosis,(TB) and pneumocystis pneumonia (PCP)[73,74]. The immunosuppression caused by HIV infection predisposes PLWH to opportunistic infections more frequently and severely, thus contributing high morbidity and mortality in this cohort[73].

More than 50% PLWH are diagnosed with bacterial infections caused by *S. pneumoniae*, the most common being pneumonia[42]. There are many patients newly HIV diagnosed when presenting with pneumococcal CAP, approximately 35% of CAP patients are newly diagnosed PLWH while around 17% of CAP patients are known PLWH receiving ART, 75% of the newly diagnosed patients have advanced HIV (Aston et al., 2019). The South African population of PLWH is comprised mainly of young adults between the age of 15 and 49 years who are as high as 100 times more at risk of pneumococcal pneumonia than their HIV-uninfected counterparts, 25% these die from pneumococcal pneumonia [3,27,75]. PLWH are also at high risk of disease recurrence and approximately 1 in 4 of PLWH individuals are reported to have had a case of recurrent pneumococcal pneumonia within 12 months[3,4,15,49].PLWH in low-income settings are up to 8 times more at risk of pneumococcal CAP than PLWH in high-income settings when compared to their HIV-uninfected counterparts [76] . Pneumococcal CAP is a predictor for HIV diagnosis, and newly diagnosed PLWH normally present with a CD4+ count<500 cells/mm3 and more commonly a CD4+ count <200cells/mm^3 when diagnosed with pneumococcal CAP [76]. The incidence of BPP in South Africa has remained unchanged in the era of widespread ART, although PLWH with a CD4+ count of <100 cells/mm^{3}have a higher incidence of IPD [77]. PLWH with a normal CD4+ count of over 500 cells/mm3,on ART and have achieved viral suppression are also at high risk of pneumococcal CAP because there is no significant difference in the incidence of pneumococcal CAP than PLWH with a lower CD4+ count [76,78]. Although the pneumococcal CAP risk is in PLWH is similar irrespective CD4+ count, the incidence of IPD including that of BPP is much higher in PLWH with a CD4+ of <100 cells/mm^{3}or less [79]. The insignificant difference in the risk of pneumococcal CAP may be attributed to the B-cell defects in PLWH as well as the persistence of HIV infection in the spleen because of low ART exposure in the spleen [76].With an increased risk of PLWH to pneumococcal CAP in low-income settings than high income settings and the existing diagnostic challenges in PLWH, there is an increased risk in morbidity and mortality caused by recurrent pneumococcal CAP PLWH in South Africa.

2.4.3 Splenectomised patients

Individuals who have undergone a splenectomy, with congenital asplenia or hypo-splenia are susceptible to infections caused by encapsulated bacteria such as *S.pneumoniae* which are poorly opsonized [9]. *S.pneumoniae* is the most common cause of disease in this cohort and the absence or inadequate function of the spleen results in a reduced facilitation of opsonization

and phagocytosis as well as a reduced production of immunoglobulins and clearance of polysaccharide encapsulated bacteria mediated by antibodies and more specifically IgM (immunoglobulin M) [80]. Pneumococcal vaccination and antimicrobial prophylaxis are highly recommended in these individuals to prevent pneumococcal disease, although when vaccinated; this cohort tends to lose 30% of their immunity against *S.pneumoniae* within 2-years post-immunisation observed by inadequate protective anti-pneumococcal (IgG) immunoglobulin titres predisposing them to recurrent pneumococcal disease including CAP [62]. The spleen develops in the first 20 years of life and its function deteriorates in advanced age, this contributes to the high risk of pneumococcal pneumonia in elderly patients who may develop hypo-splenia [80].

2.4.4. Elderly patients

Globally, pneumococcal CAP is mostly attributed to the advancement in age where an increased risk exists in individuals who are above the age of 65 years in which pneumococcal pneumonia is the leading cause of death with a mortality rate that is 3 times more than the mortality rate attributed to other pathogens [81]. In the USA (United States of America), almost a million elderly patients were hospitalized because of CAP and approximately 33% of these fatalities occurred within a year following hospitalization in 2019 [82]. Furthermore, the annual rates of hospitalization in patients with CAP over the age of 65 years ranged from 847 -3500 per 100 000 persons compared to 89-1130 per 100 000 persons in patients below 65 years of age resulting in an incidence of CAP hospitalization up to 10 times higher in elderly patients. [83]. Although the prevalence of CAP in low income settings differs significantly from high income settings with regards to age, this could become more comparable in future [84]. In 2020, 9.1% of the South African population were persons over the age of 60 this increased from 7.2% in 2002 ; this population group remain vulnerable to the same age-related factors associated with CAP in the elderly in high income settings such as a high prevalence of comorbidities, poor nutrition, a weak immune system, immune dysregulation and dysphagia; these are further exacerbated by the social and healthcare dependencies of the elderly locally [82,85,86]. The population growth rate in the elderly increased from 1.1% in 2002 to a growth rate of 3.0% in 2020, this illustrates that the South African population is aging [86]. Countries in high income settings tend to have larger proportions of elderly persons than countries in middle to low income settings, 9% of the world's population were over 65 years in 2019 [84]. This is projected to increase to 16% by 2050 during which 25% of people living in Europe and North America will be over the age of 65 by 2050, contributing to an increase in age

associated mortality including pneumococcal CAP [84]. In South Africa, the only vaccine offered to the elderly in the public health sector is the influenza vaccine whilst the pneumococcal vaccines are not yet offered but only recommended [87]. Influenza vaccination is associated with reducing hospitalisation due to all-cause CAP including pneumococcal CAP by approximately 30% in elderly patients in which an annual influenza vaccine is recommended [88,89]. This also results to low mortality rates, decreased disease severity and treatment failures associated with resistance in patients with pneumococcal CAP, especially in high risk individuals [90]. The impact of influenza vaccination in reducing CAP during the influenza season in the elderly is dependent on the matching of vaccine and circulating strains in combination with non-pharmaceutical interventions such as social distancing and wearing a mask [89]. The projected growth in the elderly population and the high HIV prevalence may lead to an increased burden of pneumococcal CAP on the public health system in South Africa because of a combination of these independent risk factors.

2.4.5. Immune disorders

Immune disorders are a major risk factor recurrent pneumococcal disease and those of specific importance in recurrent pneumococcal pneumonia are complement dysfunction and anti-pneumococcal antibody deficiencies which may either be acquired or congenital [91–93]. The risk of recurrent pneumococcal disease is mainly researched in children where congenital immune disorders are the most common [91–93]. The complement system is important augmenting the functioning of phagocytes and antibodies in the innate and adaptive immune systems respectively, thus it is essential in the elimination of initial and recurrent pneumococcal CAP cases [94]. Complement dysfunction can either be congenital or acquired as a result of hepatic disease, this is because most of the complement system proteins are produced in the liver [95]. Acquired hepatic disease in adults is mostly attributed to excessive alcohol consumption, this leads to the development of alcohol associated hepatitis which results in the acquired dysfunction of the complement system [96]. Antibody deficiencies are also associated with recurrent pneumococcal pneumonia, this occurs because of the deficiency in anti-pneumococcal polysaccharide antibodies and more specifically IgG (Immunoglobulin G) which results in the lack of acquired immunity against subsequent cases of recurrent pneumococcal CAP [97].

2.4.4 Smoking

Tobacco smoking has an independent and dose-dependent association to all-cause severe CAP, it also leads to a high incidence of recurrent CAP and accounts for a 42% increased risk of recurrent pneumonia while a quarter of smokers with recurrent pneumonia die within 30 days increasing by 3-folds over a year compared to non-smokers [98]. Smokers and ex-smokers are at high risk of severe and recurrent pneumococcal CAP because tobacco smoking suppresses the inflammatory responses of the upper respiratory tract, this increases the density nasopharyngeal *S.pneumoniae* colonisation resulting from the inherent development of lung injury and ARDS (acute respiratory distress syndrome)from long-term smoking [99,100]. ARDS is severe lung injury which requires ventilation to limit the resulting low blood oxygen [101,102]. Although several studies have shown that smoking is a risk factor for invasive pneumococcal pneumonia, some studies in mice have shown that tobacco smoking may accelerate lung bacterial clearance and be protective for severe lung injury following lung infection [103].The risk of recurrent pneumococcal CAP in smokers may also be exacerbated by antibiotic treatment, this was observed in mice exposed to tobacco smoking which presented with more severe lung injury following antibiotic treatment for pneumococcal CAP [100].This phenomena may have occurred because of the elevated concentration of monocyte-derived chemokines and monocytes driven by the increase in bacterial cell-wall products over a short period following antibiotic treatment worsening lung inflammation [100] . The worsening of lung inflammation following antibiotic treatment has not been studied in humans, however; this study may suggest that this phenomenon may happen in humans which may make antibiotic treatment in smokers a predisposing factor for recurrent pneumococcal CAP. Lastly, studies have shown that patients tend to deny their smoking habits leading to low prevalence in smoking than when serum cotinine was used to validify smoking history, the prevalence of smoking as a risk factor may be underestimated in self-reported patients [104].

2.5 Factors Contributing to Recurrent Pneumococcal Pneumonia

There are factors that predispose high risk individuals to recurrent pneumococcal disease outside of the identified risk factors for pneumococcal disease and CAP. Other than antimicrobial resistance; bacterial-viral co-infection, *S.pneumoniae* serotype replacement and genetic variations that exist within the same serotype of *S.pneumoniae* contribute to recurrent pneumococcal disease in high risk populations because of the lack of adequate immune response to recurrent disease [10,11]

2.5.1. Bacterial-viral co-infection

Bacterial and viral lung co-infections are a common predisposing factor for pneumococcal CAP because multiple organisms may cause CAP [12]. Lower respiratory viral co-infection with the influenza virus, adenovirus and rhinovirus are associated with an increase in *S.pneumoniae* nasopharyngeal colonization [105]. Viral infections of the LRT (Lower Respiratory Tract) are a predisposing factor for secondary bacterial lung infections leading to worsened patient clinical outcomes [106]. Animal models have illustrated that pneumococcal pneumonia is enhanced by primary influenza viral infection; this may suggest that in high risk populations with an adequate immune system, a case of recurrent pneumococcal pneumonia and influenza may occur concurrently [106]. This is supported by a study in Malawi, where 20% of PLWH had *S.pneumoniae* co-infection with the influenza virus, while MTB (*Mycobacterium Tuberculosis*) was isolated in 9.6% of those with pneumococcal CAP where 23% of these patients had a previous case of pneumonia; highlighting the possibility of bacterial/viral/ MTB co-infection as a risk factor for recurrent pneumococcal CAP in PLWH [13]. The identification of all causal agents for CAP is costly in our resource limited setting, this increases the risk for underestimating pneumococcal CAP [75]. In a retrospective study of adults admitted with severe CAP over a 1-year period at Tygerberg hospital in Cape Town, South Africa; 43% of patients had no identified causal agent for CAP but in those who died, *S.pneumoniae* was attributed to 23% of the fatalities [75]. These data suggests that there is a high risk of missing recurrent pneumococcal CAP in patients presenting with other bacterial and viral respiratory infections, it should be noted that these patients have a high risk of co-infection with *S. pneumoniae* as a complication of the primary non-pneumococcal respiratory infection and that recurrent pneumococcal pneumonia may be more common.

2.5.2. Serotype replacement

Serotype replacement occurs when the prevalence of serotypes changes under vaccine pressure and VTs are replaced with commonly more virulent NVTs [2,107]. In the USA, the introduction of PPSV23 resulted in the reduction of serotype 1,the most prevalent serotype at the time and mostly associated with invasive disease and a high mortality rate [107]. In South Africa, according to national surveillance data collected from 2003 to 2008 during the pre-PCV era; in patients with pneumococcal pneumonia, serotype 19F and 14 were the most prevalent and associated with a higher in-hospital mortality[2]. During the pre-PCV period in South Africa, serotype 1 was the most prevalent vaccine-serotype amongst adults who were HIV-uninfected and 19F was the most common VT in PLWH associated with a higher

mortality, while serotype 1 was more associated with invasive disease [2]. Globally, serotype 14 and 19F are the most prevalent serotypes in IPD while serotypes 1 and 3 are the most isolated serotypes in pneumococcal pneumonia in the post-PCV era (Scelfo et al., 2021). Following the introduction of PCV7 in the USA, infections caused by serotype-19A increased, while the introduction of PCV 13 led to an increase in serotype-35A, both these serotypes were non-vaccine serotypes at the time of serotype replacement [107].In addition, the introduction of PCV13 in South Africa, led to the decline in the incidence of Serotype 1 pneumococcal disease while common colonisers; 23B and 35B were identified as frequent NVTs in healthy infants in at two clinics in Gauteng Province, South Africa [108,109]. In another local study conducted between 2012-2018 , serotypes 8 and 19A were the most common cause of IPD, although serotype 15B/C were associated with increased mortality in individuals over the age of 15 years which 40.7% of the IPD cases in 25–44-year-old adults with 67% PLWH [110]. Furthermore, a study conducted in the UK from 2006 to 2018 found that 19A, 19F and 3 were the most isolated vaccine-preventable serotypes in the period of 2017/18 and 15B/C was the most isolated non-VT [110,111].Overall, during the post-vaccine era; there was a decrease in the prevalence of PCV7 vaccine serotypes from >50% to 2% following the introduction of PCV7 and a decrease in the prevalence of PCV13 vaccine serotypes from 20% to approximately 5% in the post-PCV 13 era vaccine, however; because of serotype replacement, the prevalence of NVTs have increased to >50% [111].

2.6. Serotype Prevalence of *S. Pneumoniae*

There is a difference in the prevalence of *S.pneumoniae* serotypes based on age, HIV-status, IPD vs non-IPD and the site of infection[2,58]. There has also been a difference in Sero-prevalence prior and post-pneumococcal vaccine introduction in South Africa [2]. Serotypes causing recurrent pneumococcal CAP may also be different in demographic groups.

2.6.1. Serotype prevalence according to HIV status.

The distribution of pneumococcal serotypes amongst patients aged ≥15 years with IPD (invasive pneumococcal disease) in South Africa from 2003–2008 showed that VTs, 12F and 9V were significantly more common in PLWH while Serotype 1 was less common in this population group when compared to HIV negative individuals in a South African study conducted during the pre-pneumococcal vaccine era [2]. Serotypes that cause pneumococcal pneumonia in PLWH are often found in children and are penicillin- resistant [3] . Hibbs et.al reported that PLWH were more likely to be infected with DRSPs, although serotype

prevalence was not significantly different in PLWH and HIV uninfected adults [112]. These data is supported by a South African study in the post-vaccine era in which 71% of serotypes in PLWH and 73% of serotypes in HIV uninfected persons were PCV 13 serotypes with cotrimoxazole resistant serotypes more common in HIV positive individuals, cotrimoxazole is recommended for IPD prophylaxis in PLWH [3,112].

2.6.2. Serotype distribution according to age.

Several studies have reported a slight difference in serotype distribution in age groups in the post-PCV in children, adults who are18-64 years old and the elderly who are >65years [110,113–115]. Globally, the most prevalent serotypes in pneumococcal pneumonia in adults are 12F, 8 and 3 [115,116]. In a prospective study in Spain conducted between 2012 and 2016, PCV 13 serotypes were reported to be more common in children <17 years, these consisted mainly of serotypes 1,3, 19A, 14 and all the PCV13 serotypes [114]. In addition, this study found that PCV13 serotypes 1 and 3 were more common in pneumonia than other IPDs where overall pneumonia and complicated pneumonia were caused by 76.4% and 82% of PCV 13 serotypes respectively. In adults 55-65 years of age, PCV13 serotypes caused 21-61.4% and PPSV23 caused 72.4-97.9% cases of pneumonia between 2015-2019 in Brazil, England, Malawi and South Africa [117]. In South Africa, according to national surveillance data collected from 2012 to 2018; IPD cases of all ages were mostly caused by serotype 8, 19A, 12F and 3 at 11.9%, 8.9%, 8.8% and 6.8% respectively [110]. In addition, when comparing serotype prevalence according to age, serotype 8, 1, 19A and 19F were most common in individuals <15years with a decrease in 12F, 3, 1 and 6A within this age group in the same time period in comparison to those who are >15years, in which serotypes 8, 12F, 19A and 3 were the most common [110].

2.7. Recurrent Pneumococcal CAP Serotypes

DRSP serotypes are the leading cause of recurrent IPD but are subject to change because of serotype replacement , although the prevalence of certain VTs have not declined even after the introduction of both PCV13 and PPSV23 [62,118]. Domenech et.al observed that Serotypes 9V, 15A, 19F and 11A were associated with recurrent pneumococcal pneumonia in patients with COPD of which 60% of relapses were caused by 9V and 19F, these strains are more resistant to antimicrobials [8]. Serotypes that cause reinfection episodes of recurrent pneumonia are often treatable with antimicrobials as only 34% are DRSP whilst 74.3% of serotypes causing relapses are resistant serotypes likely because of increased antibiotic exposure [8] . Serotypes causing relapses and reinfections in COPD patients may also cause disease in other

individuals at high risk of pneumococcal CAP because of the similar lung dysbiosis influencing disease progression of lung diseases and infections [106,119]. On the contrary, in a case study of a splenectomised patient who had been vaccinated with PPSV23, serotype 12F was the reported cause of recurrent IPD despite adequate immunization [62].This specific case of a serotype 12F associated recurrent IPD in a PPSV23 vaccinated splenectomised patient occurred because adequate PPSV23 immunisation does not correlate with serotype 12F protection because the lack of anti-pneumococcal IgG titre cutoffs in 11 of the 23 vaccine serotypes in PPSV23 including serotype 12F [62]. PLWH with a dysfunctional spleen have reduced PPSV23 vaccine efficacy and are at risk of recurrent pneumococcal disease caused by the 11 identified PPSV23 serotypes with no correlates of protection even in the event of adequate immunisation because the only anti-pneumococcal immunoglobulin G included for IgG titre detection are for serotypes 1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F [62]. Another challenge in pneumococcal vaccination of PLWH is the inadequate correlation between total and specific immunoglobulin serum levels against *S.pneumoniae* as a consequence of polyclonal B-cell activation, thus making the determination of vaccine effectiveness challenging [120]. Pneumococcal serotypes causing adult CAP are also dependent on child contact because the most common serotypes are influenced by the vaccination status of children, thus adults who aren't in contact with children are likely to present with NVTs they are not vaccinated, this is a major contributing factor in the elderly who may not have contact with children especially those in old-age care facilities or homes [121].

2.8. Pneumococcal Vaccination

PPSV (pneumococcal polysaccharide vaccine) and the PCVs (pneumococcal conjugate vaccines) are the two categories of pneumococcal vaccines available [49]. These pneumococcal vaccines are recommended for use in different age groups and differ in their efficacy and immunogenicity because differing vaccine properties and the vaccine serotype constituents [122].PPSV is a T-independent pneumococcal vaccine that constitutes of capsular polysaccharides of *S.pneumoniae* serotypes that are responsible for the highest proportion of pneumococcal disease [49].PCVs are a T-cell dependent pneumococcal vaccines which constitute of *S.pneumoniae* capsular polysaccharide covalently linked to a protein [49,122].Globally, the pneumococcal vaccines that are available are PPSV23 (23-valent pneumococcal polysaccharide vaccine), PCV10 (10-valent pneumococcal conjugate vaccine), PCV13 (13-valent pneumococcal conjugate vaccine), PCV15 (15-valent conjugate vaccine) and the PCV20 (20-valent pneumococcal conjugate vaccine) which was recently approved by

the FDA (Food and Drug Administration) and available in the USA [49,123,124]. In South Africa, pneumococcal vaccines were first introduced in 1992 with PPV23, in 2009; PCV7 (Pneumococcal Conjugate Vaccine 7-valent) was the first to be introduced then it was replaced in 2011 with the PCV13 (Pneumococcal Conjugate Vaccine 13-valent) in the national EPI for children. PPV23 (Pneumococcal Polysaccharide Vaccine 23 valent) is recommended for PLWH, elderly and adults with high risk conditions. [2,110]. The PCV20 is not yet available in South Africa and it is expected to be registered by 2025 [125]. Table 1. depicts a summary of the vaccine available globally.

Table 1. Summary of available pneumococcal vaccines

Pneumococcal vaccine	Serotypes	Age Group Indicated
PPSV23(Pneumovax®)	1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V,10A, 11A, 12F, 14, 15B, 7F, 18C, 19F, 19A, 20, 22F, 23F, 3F.	Children >2years old and adults.
PCV10(Synflorix®)	1, 4, 5A, 6B, 7F 9V, 14, 18C, 19F and 23F.	Children >5 years old.
PCV13(Prevnar13®)	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 19A, 19F, 18C and 23F.	Children <5years. Adults 65years or older and with risk factors.
PCV15(Vaxneuvance®)	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, 22F, 23F, and 33F.	Infants and children <5 years and children who are 5-18 years old with risk factors. Adults 65 years or older and 19-64 years old with risk factors.
PCV20(Prevnar20®)	1, 3, 4, 5, 6A, 6B, 7F, 8, 9V, 10A, 11A, 12F, 14, 15B, 18C, 19A, 19F, 22F, 23F, and 33F.	Adults 65 years or older and 19-64 years old with risk factors.

PPSV23 (23-valent pneumococcal polysaccharide vaccine), PCV10 (10-valent pneumococcal conjugate vaccine), PCV13 (13-valent pneumococcal conjugate vaccine), PCV15 (15-valent conjugate vaccine), PCV20 (20-valent pneumococcal conjugate vaccine). [12,49,107,122,124,126]

2.8.1. Pneumococcal vaccine cost effectiveness

Pneumococcal diseases exert a socio-economic burden in both children and adults globally, vaccines evidently improve clinical outcomes and reduce the economic burden on health systems than when populations are not vaccinated [127,128]. The impact of pneumococcal non-

vaccination has detrimental effects on the economy because of medical costs incurred directly or indirectly as a consequence of decreased or lost in economically active populations, incremental cost-effectiveness and cost-utility ratios [127,128]. Pneumococcal Conjugate Vaccines are more cost effective than PPSV23 because of the efficacy in children, the higher valency pneumococcal conjugate vaccines are associated with an improvement in cost-effectiveness and health-related QALY (quality-adjusted life-years) in adults because of the higher number of serotypes covered [125,127,128]. Furthermore, vaccines may be critical in the aversion of incremental costs arising from AMR-related costs because of the need for more expensive antibiotics, longer hospital length of stay because of severe and complicated illness caused by DRSPs in the context of pneumococcal diseases [129].

2.8.2. Pneumococcal vaccine coverage

The pneumococcal vaccine coverage in children increased pneumococcal disease herd immunity and reduced the burden of pneumococcal diseases in children resulting in the indirect vaccine protection effects in non-vaccinated adults in South Africa [2]. Pneumococcal vaccination in children has also proven to be cost effective, especially with the introduction of higher valency pneumococcal conjugate vaccines but these are not yet available locally [125].Unfortunately, further reduction in the burden of pneumococcal diseases in high risk adults is hindered by an inadequate pneumococcal vaccine coverage in adults, especially in PLWH and the elderly globally [130]. In a study conducted in the USA, the vaccine coverage in PLWH adults above 19 years was only 20-25%, while in a study in China; 94% of PLWH were not vaccinated which was attributed to the lack of information on the protective effects of PPSV23 and public education on the benefits of pneumococcal vaccination [130,131]. In a South African study, the estimated immunisation uptake was 0.18% in the public healthcare sector and 3.38% in the private healthcare sector while the PCV13 uptake in PLWH adults 0.64% in the public sector and 25.51% in the private sector [15]. As seen in this study, the pneumococcal vaccine uptake in PLWH in the private sector is comparable to the vaccine uptake in the study in the USA by Isturiz, et.al , possibly because of the similar access to improved healthcare resources [130].

A low pneumococcal vaccine coverage increases the burden of pneumococcal diseases on the healthcare system, driving the dependency on antimicrobial treatment to manage pneumococcal diseases. Unlike low-income settings, countries in high income settings in USA and Europe have established adult pneumococcal vaccination policies[130]. The influenza

vaccine is the only vaccine that is offered to the elderly in the public health sector in South Africa while the PCV13 is only included in the EPI for paediatric immunisation [50,87].The benefits of vaccination for adults have not been adequately explored and pneumococcal CAP remains high and mainly associated with HIV in South Africa [15].Pneumococcal vaccines have proven to reduce morbidity, mortality and healthcare costs associated with the management of IPD patients on antibiotics, hospital costs and human resources [15,131]. In an era where AMR limits the available antimicrobials used for pneumococcal CAP therapy and the high cost of newer antibiotics, vaccination is the most cost-effective solution.

2.8.3. Vaccine efficacy & effectiveness

Both PPSV23 and PCV13 have shown efficacy, effectiveness, safety, tolerability, and immunogenicity in HIV-infected and HIV-uninfected adults, though the vaccine uptake is low in both developing and developed countries, even in countries with mandatory pneumococcal vaccination policies [15,131]. VE (Vaccine efficacy) tends to be better in HIV-uninfected individuals than their HIV-infected counterparts, however; there are studies that also prove pneumococcal vaccines' VE in PLWH [76]. In a prospective observational study conducted in China, PPSV23 vaccine effectiveness was 81% in vaccine naïve PLWH on ART followed up over a 1 -year period (Zou et al., 2022). In the CAPITA study, which is the largest pneumococcal VE randomized-double blind study which evaluated PCV13 efficacy in adults above the 65 years, PCV13(13-valent pneumococcal conjugate vaccine) vaccine efficacy for pneumococcal pneumonia in this population was 45.6%, this VE was influenced by the high rates of influenza vaccination at the time of the study [132].

In another study evaluating the PPSV23 effectiveness in the elderly population, only 50% of the participants were diagnosed with pneumococcal CAP, proving that PPSV23 is effective in preventing pneumococcal CAP in settings with limited resources and where PCV13 may not be available in the public health sector [11]. However, Feldman et.al have suggested that PCV13 is not only more cost effective but is more effective in the reduction of mortality of IPD thus recommending the use of PCV13 over PPSV23 in adults including PLWH in the private and public sector [15]. There is a difference is the immunogenicity of PPSV23 and PCV13, the latter has potential prolonged immunity whilst immunity following PPSV23 immunisation may decline after 5-years in the elderly and adults with underlying conditions and children while over the age of 2 years have poor response [49]. Though both vaccines are effective against disease, PCVs reduce *S.pneumoniae* nasopharyngeal carriage whilst

PPSV23 has shown no indirect protective benefits towards nasopharyngeal carriage, a known pre-requisite for disease. [49,122]

2.8.4. Timing of pneumococcal vaccination in adults

Considering the impact of pneumococcal vaccines on the immune system, especially in PLWH and the elderly, vaccine effectiveness is dependent on the immunization schedule.

2.8.4.1.Pneumococcal vaccination timing in PLWH

Globally, the recommended immunization schedule for newly diagnosed PLWH, suggests that vaccination with PCV13 should be delayed until the CD4+ count is above 200 cells/mm^3 and followed by PPSV23 within 1-year followed by a booster every 5-years [76]. The delay of PCV13 vaccination in PLWH with a CD4+ count <200cells/mm3 is recommended because of the low vaccine immunogenicity, ART initiation is recommended until the CD4+ recovers to above 200cells/mm3[76]. According to the 2017 South African CAP guidelines, PLWH who have had PPV23 vaccination; PCV13 should also be received 1-year later and PCV naïve PLWH, a single dose of PCV13 followed by a dose of PPV23 at least 2 months later is recommended [12]. Additionally, in pregnant mothers LWH; PPSV23 reportedly has better immunogenicity than PCV10, this might also be the case withPCV13[133]. However, the updated South African CAP guidelines recommends pneumococcal vaccination in all PLWH regardless of their CD4+ count or ART treatment when vaccinated with PCV13, but recommends that vaccination is preferable when the HIV viral load is <1,000 copies/ml and PPSV23 vaccination is only recommended in PLWH on ART [49]. This recommendation is supported by the fact that PLWH remain at high risk of IPD even when on ART and having achieved virological suppression [49]. A high proportion of PLWH are diagnosed at an advanced stage of HIV infection when the CD4+count is <200cells/mm^3, this was worsened by the COVID-19 pandemic in 2020 leading to a drastic decline in HIV testing and an increase ART defaulting [134]. The initiation of ART is imperative in increasing virological suppression and restoring immune function which improves the immunogenicity of pneumococcal vaccines[135]. Pneumococcal vaccination and ART adherence are both important in preventing pneumococcal diseases in PLWH because the ART improves immune function which improves the immunogenicity of the pneumococcal vaccines thus pneumococcal vaccination is dependent on ART in PLWH.

2.8.4.2.Pneumococcal vaccination timing in the elderly population.

The risk of pneumococcal disease increases with age from 50 years with increased disease severity from the age of 65years where the most cost benefits of pneumococcal vaccination are observed [117,133]. In high resource settings and countries with established pneumococcal adult immunisation policies, PCV13, PCV15 or PCV20 with PPSV23 are recommended in adults >65years old as well as adults <64 years at high risk of pneumococcal disease infection [136]. In the USA where the PCV20 was registered in 2021, it is recommended that elderly persons >65years who had previously received the PCV13/PPSV23 combination should also receive PCV20 [136]. Further vaccination with PPSV23 is recommended over a period of 5-10years because waning immunity, PCVs have not shown any evidence of waning immunity over 4 years according to studies conducted [133].The South African CAP guidelines recommends the PCV13/PPSV23 combined vaccination for the elderly; a single dose of PCV13 is recommended for unvaccinated adults aged >50 years and if there is prior PPV23 vaccination, the single PCV13 dose may be given a year later [12]. In limited resource settings, the optimal age of pneumococcal vaccination is determined to be 55 years and 70 years in high resource settings; because most cases of hospitalisation and fatalities associated with pneumococcal diseases are prevented if vaccination is initiated at these ages [117].

3. STUDY AIM AND OBJECTIVES

The aim of this study was to determine the incidence of recurrent pneumococcal CAP in hospitalised patients with pneumococcal CAP.

This has been achieved through the following objectives:

I. To determine two or more consecutive cases of pneumococcal pneumonia ≥30 days from an initial case.
II. To determine the *S. pneumoniae* serotypes that caused initial cases of pneumococcal CAP and recurrent pneumococcal CAP.
III. To establish whether the serotypes that cause recurrent pneumococcal CAP are vaccine serotypes or non-vaccine serotypes.
IV. To determine the incidence of mortality of patients with recurrent pneumococcal CAP.
V. To determine the time to recurrent pneumococcal CAP cases.
VI. To determine the predictors for recurrent pneumococcal CAP.

4. METHODOLOGY

'Our study' is a sub-study that's nested in "The potentially preventable burden of community acquired pneumonia in South African Adults in the era of widespread paediatric PCV13 immunisation and antiretroviral therapy rollout" (POTPREV) study. The methodology of our study describes the methods and rationale used for the selection of the study design and approach, study site and population, sampling technique, the demographics of the study sample, the procedure of data collection, statistical analysis of the collected data, risk management and ethical considerations.

4.1. Study Design

Our research study design is a retrospective observational study design in which the quantitative study approach is adopted. The research study is observational as it is not experimental because there is no introduction of an intervention [137]. Furthermore, this study is retrospective as the data collected are existing data that were collected prior to the commencement of the study and takes into consideration the outcome of recurrent pneumococcal pneumonia of hospitalised patients with an index case of CAP [138]. The quantitative study approach was adopted for our study because we needed to meet our study objectives by presenting data that are measured and represented in numerical values, the data were explored for the purpose of achieving our objective to determine the incidence of recurrent pneumococcal pneumonia [139,140].

The quantitative data in our study are the incidence of pneumococcal CAP cases occurring >30days from an initial case of pneumococcal CAP, the incidence of mortality of pneumococcal recurrent CAP in patients and lastly, the proportion of the described *S. pneumoniae* serotypes that cause recurrent pneumoniae which were measured as a proportion of the total recurrent pneumococcal CAP cases per serotype. The incidence of recurrent pneumococcal CAP was described as the number of new recurrent pneumococcal CAP cases in our study sample over a follow-up period of 1-year, this made it measurable because the results generated a numerical value which substantiated the selection of the quantitative study approach [141]. The proportion of the total recurrent pneumococcal CAP cases per serotype was described as the quantity of the serotypes in relation to the cases of recurrent pneumococcal CAP and was represented as a percentage [142].

4.2. Study Population and Study Sites

The study population of this research study comprised of adult patients hospitalised from May 2019 to October 2021 between the ages of 18 and 92 years at 3 study sites in South Africa, these were CHBAH (Chris Hani Baragwanath Academic Hospital) in Gauteng Province, PMHC (Polokwane/Mankweng Hospital Complex) in Limpopo Province and KTHC (Klerksdorp Tshepong Hospital Complex) in North-West province.

4.2.1. Eligibility criteria

The study participants included in our study sample were:

- Adults ≥ 18 years of age enrolled in the POTPREV study.
- Adults hospitalised with a clinical diagnosis of CAP on admission.
- A positive NPS or blood LytA-rt PCR.
- Abnormal CXR.

The participants excluded in our study were:

- Participants who died < 30 days of the study.
- Participants who were lost to follow up, relocated, terminated following PI decision or withdrew informed consent.

4.2.2. Study sites

4.2.2.1.Chris Hani Baragwanath Academic Hospital

The CHBAH is a hospital in the Diepkloof section of the township of Soweto in Johannesburg, Gauteng Province, South Africa and it is an academic hospital for the University of Witwatersrand [143]. It is the biggest hospital in Africa and the 3rd biggest hospital in the world with an area of 0.70km^2 with approximately 3200 beds and an annual case registrations of approximately 150 000 outpatients and 500 000 inpatients annually [143].The hospital is financed by the South African Department of Health and mostly serves the residents of Johannesburg South, South African citizens, and citizens of neighbouring countries [143].

4.2.2.2.Soweto

Soweto is a township located in the southern part of Johannesburg, with a total population of 1,271,628 in which 24,7%, 71% and 4.2% of the population are 0-14, 15-64 and 65+ years respectively and 98.5% of these are black African with 1.0% coloured and <0.5% white and Indian [144]. According to a study based on data from NHLS (National Health Laboratory Service) at CHBAH of laboratory blood samples collected from inpatient, outpatient, and casualty facilities at CHBAH, as well as primary healthcare clinics in Soweto, 54.7% of these participants had their HIV status documented, 37.9% were PLWH inpatients with 71% of these on ART [145].

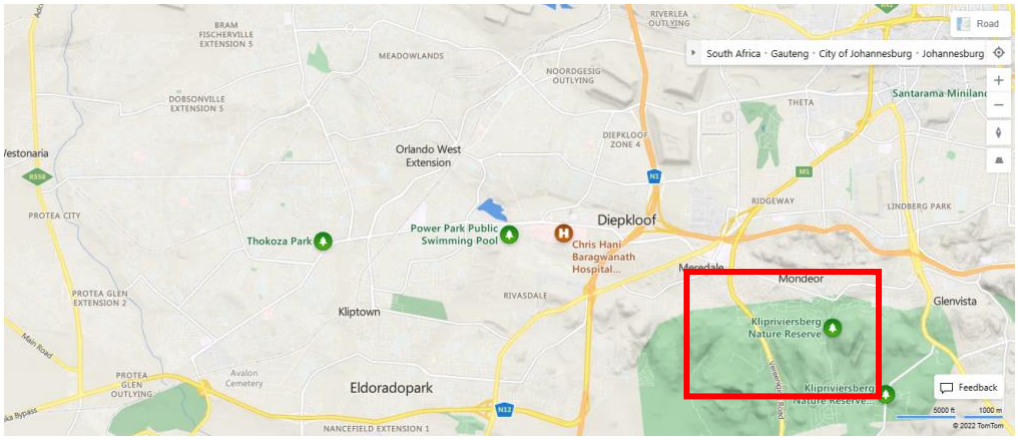


Figure 1: Map of CHBAH and Soweto [146]

4.2.2.3.Polokwane/Mankweng Hospital Complex (PMHC)

The PMHC (Polokwane/Mankweng Hospital Complex) consists of two hospitals situated in the Limpopo Province which are the Pietersburg Hospital and Mankweng Hospital [147]. The Limpopo province has a total population of 5.8million people and the Polokwane Municipality; in which the PMHC is located; has a total population of 628,999 which comprises of 30.1%, 64.8% and 5.1% in the ages of 0-14, 15-64 and 65+ respectively (Africa, 2023). Limpopo Province has 42 hospitals which includes 2 mental health institutions, 8 regional hospitals and 32 district hospitals which refer patients to PMHC [148]. The Pietersburg hospital is situated in the Polokwane city of the province and serves as a tertiary hospital in the province thus serving patients from district and regional hospitals in the province with a 450-bed capacity [147]. Mankweng hospital is in the Mankweng Township in Polokwane, 29km from Pietersburg Hospital [146,149]. The PMHC provides specialised services to patients in the province, however; there are services that are only provided by one hospital and not the other,

in these cases patients are referred between the two hospitals thus allowing the strategic configuration of services of a complex [147]. In instances where there is a need for super-specialist expertise; patients are referred to Steve Biko Academic Hospital or Dr. George Mukhari Hospital which are both in Pretoria, in the Gauteng Province [147].

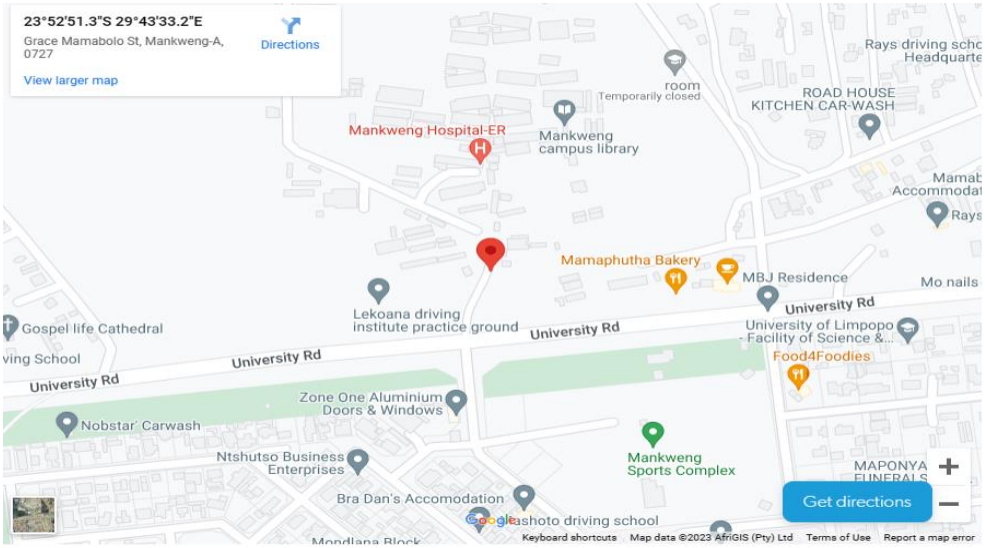


Figure 2: Map of Mankweng Hospital and surrounds[146]

4.2.2.4. Klerksdorp Tshepong Hospital Complex

KTHC is in the town of Klerksdorp in the Northwest province and provides tertiary health services in the two sites [150]. The North-West province is a popular mining area in South Africa with gold mines mostly in Klerksdorp [151]. Northwest had a population of 3.7 million in 2016 and an HIV prevalence of 13.59% [144,152]. Klerksdorp has a total population of 186,515 with 27.2%, 66.7% and 6.1% in the ages 0-14, 15-64 and 65+ respectively [144].



Figure 3: Map of KTHC and surrounds[146]

4.2.3. Sample size

The sample size of this study was determined using STATA/IC version 17.0 to generate a sample size that will be reflective of the study population with meaningful results [153] . We used an estimated the OR (odds ratio) for recurrent pneumococcal CAP which was estimated at 1.7 by other studies, this OR was used for our study to generate the sample size [5]. The OR of 1.7 identified the risk of recurrent CAP in high risk patients vs immune competent patients hospitalised with an initial case of CAP [5]. From the estimated OR of 1.7, a sample size of at least 1520 participants from the POTPREV study needed to be enrolled in our study to achieve sample size power of 90%. The sample size of at least 1520 will lead to the observation of recurrent pneumococcal CAP incidence rate of 3.0/100 person-years which is comparable to other studies.

4.2.4. Sampling strategy

The purposive sampling technique was used because POTPREV study enrolled 1999 participants who were hospitalised and a sample size of 1520 was required as an adequate sample size to observe an incidence rate of at least 3.0/100 person-years of recurrent pneumococcal CAP. Our sample of 1573 study participants were participants purposively sampled on condition of meeting the eligibility criteria. The sample was further stratified in

demographic strata to control for confounding factors. to an exposure [154]. The purposive stratified sampling was selected because our study sample consisted of hospitalised patients with pneumococcal CAP to meet the objectives of our study [155].The final study sample excluded participants from the POTPREV study who did not meet the eligibility criteria. The final study sample consisted of 1573/1999 of the study population from the POTPREV study.

4.2.5. Data collection and tools

Secondary data of the POTPREV were accessed from the PHRU (Perinatal HIV Research Unit). A data agreement was signed by the researchers before the access to data was granted by the principal investigator of the POTPREV study. The data agreement was issued to ensure that the data was secure and kept confidential. The patients included in the study had provided written consent and were selected according to the eligibility criteria. The cohort was made up of a sample size of 1573 adults who were admitted to hospital with LytA real-time PCR nasopharyngeal swab or blood confirmed pneumococcal CAP. Demographic data collected at the time of admission to hospital included age, sex, BMI (Body Mass Index), smoking history, alcohol intake, comorbidities (hypertension, diabetes, Asthma, COPD), HIV status, (including CD4+ count) and employment status Data on the incidence of recurrent cases of pneumonia amongst the participants, *S. pneumoniae* serotypes causing a case of recurrent pneumococcal pneumonia, mortality in the case that a participant demises before the end of the study were collected during the period when the patients were followed up. The incidence of positive nasopharyngeal or blood LytA rt-PCR with an abnormal CXR was observed over a 1- year follow up.

4.3. Determination Of Recurrent Pneumococcal CAP Episodes.

For the purposes of our study, hospitalised patients with 2 or more subsequent episodes of pneumococcal CAP as part of the admission diagnosis, a positive NPS or blood LytA-rtPCR >30 days from an initial episode with an abnormal CXR over a follow-up period of a year were defined as cases of recurrent pneumococcal CAP. Although a normal CXR does not exclude pneumococcal CAP, we included CXR for the purposes of estimating the incidence of confirmed episodes of recurrent pneumococcal CAP.

4.3.1. Serotyping of *S. pneumoniae*

Serotyping of *S. pneumoniae* from nasopharyngeal and blood specimens was conducted using triplex rt-PCR (real-time PCR) as per the NHLS SOP (National Health Laboratory Services Standard Operation Procedure) document number NIC1019, version 7, this is the SOP used by the NICD for serotyping of *S. pneumoniae*. The NICD is a specialised institution of the NHLS which is public health entity providing laboratory services in the South African national department of health [156].Real-time PCR is an enzymatic process used in-vitro for the amplification of targeted DNA sequences through numerous magnitude orders resulting in the generation of up to millions of copies of the targeted DNA sequence and fluorescence that occurs simultaneously to measure the magnitude of the amplified sequences which results in the identification of the DNA sequence coding for an interest gene or protein [28]. Triplex real-time PCR constitutes of three amplification reactions which need three primer pairs and three probes, the three probes anneal with the corresponding LytA-gene DNA sequence which results in a PCR reaction with the primers [157]. As per the NHLS SOP document number NIC1019, version 7; triplex rt-PCR is recommended because of the ability to detect 21 *S. pneumoniae* serotypes responsible for the highest burden of pneumococcal disease. The clinical specimens that were serotyped were those with a positive LytA rt-PCR, this was determined by a Ct-value (cycle threshold value) of <40; usually only clinical specimens with a LytA rt-PCR Ct-value of ≤35 should be serotyped using rt- PCR. LytA is the autolysin protein located intracellularly as well as extracellularly of the *S.pneumoniae* bacterium and serves as a virulence factor that causes autolysis, penicillin induced and fratricidal lysis [158]. The Ct-value is the number of PCR cycles in which the fluorescence level is higher than the minimum required fluorescence level for the detection of a targeted DNA sequence, a sample with an initially higher number of nucleic acid templates leads to a lower number of PCR cycles and a fluorescent level that's higher than the required minimum level for detection [158].The Lyta rt-PCR Ct-value of ≤35 is recommended for serotyping at the NICD because specimens with a higher Ct-value have a reduced likelihood of being accurately serotyped [159]. The triplex rt-PCR assay consists of eight reactions which detect 38 serotypes which are the most prevalent serotypes in South Africa are usually detected in the first 3 reactions. Each of the 8 reactions detects at least 2 serotypes of *S.pneumoniae*, the 38 serotypes that are detected are: 1,5,23F,4, 6A/6B/6C/6D,9V/9A, 14, 18C/18A/18B/18F, 19F, 3, 7F/7A, 12F/12A/12B/44/46, 22F/22A, 15A/15F, 23A, 33F/33A/37, 2,11A/11D,16F, 8 and 19A. Serotypes represented with "/" are serotypes that can't be distinguished because of the genetic similarity in this serotypes where only one base pair in the capsular sequence of these

serotypes is different [160] . Nasopharyngeal swab specimens were tested in all the 8 reactions because the nasopharynx is a non-sterile site and all serotypes that were detected in the NPS were reported. For blood specimens, the detection of a serotype with a Ct-value <35 resulted in the discontinuation of the remaining reactions. Blood specimens with two or more serotypes had the serotypes with the lowest Ct-value reported as the final serotype detected. Serotyping of blood specimens was repeated using a new DNA extract when the difference in Ct-values was ≥10. Specimens which tested negative in all 8 reactions of the LytA rt-PCR were recorded as Neg 38 as these serotypes were not part of the 38 serotypes that are detected by the triplex LytA rt-PCR assay.

4.4.Outcome Measures

We determined the incidence of recurrent pneumococcal CAP over a period of 1-year of follow-up, this was done by determining 2 or more positive nasopharyngeal LytA rt-PCR tests occurring >30 days or more from an initial positive LytA rt-PCR test and an abnormal CXR, these were identified as definitive cases of recurrent pneumococcal CAP. NPS LytA rt-PCR was used to determine the cases of recurrent pneumococcal CAP because only one possible case of recurrent CAP was detected from blood LytA rt-PCR. We also estimated the time it took for patients identified to have developed recurrent pneumococcal CAP within a year of follow-up and stratified this by study site. We further determined the predictors associated with recurrent pneumococcal CAP; this was done by determining the incident rate ratio of recurrent pneumococcal CAP for each of the socio-economic demographic factors. Lastly, we determined the serotypes that cause the recurrent pneumococcal CAP cases by collecting the serotype data of the identified recurrent pneumococcal CAP case, these serotypes were analysed to determine if they include vaccine preventable serotypes by conducting a comparison with the serotypes included in PCV13 and PPSV 23.

4.5.Statistical Analysis

All statistical analysis was performed using STATA/IC version 17.0 [161]. The statistical significance was set at p-value of < 0.05 and effect estimates at 95% CI (confidence interval). We used a non-parametric statistical inferential approach; Kruskal-Wallis test to compare the medians of the categorical variables demonstrated by the Socio-demographic factors [162]. For the Kruskal-Wallis test, our null hypothesis (H_0) was that the medians of a socio-demographic characteristic are equal whilst the alternative hypothesis (H_1) was at least one differs across the three study sites [162]. The incidence of recurrent pneumococcal CAP was determined by

dividing the cases of recurrent pneumococcal CAP by the total person-years over a 1-year study period of follow-up amongst the final study sample of 1573 hospitalised patients, which excluded those who died <30 days from hospital admission and those who were lost to follow-up during the study duration. The recurrent pneumococcal CAP predictors of interest were identified from other studies on pneumococcal CAP, recurrent and non-recurrent IPD, all-cause recurrent CAP [4,5,32,98]. Univariate and multivariate Poisson regression analysis were used to determine the risk factors associated with cases of recurrent pneumococcal CAP. We used Poisson regression to determine the relationship between one or more predictor variables and an outcome variable which consists of count data [163]. The predictor variables included for the Poisson regression were factors known to predispose patients to hospitalisation, infectious diseases and LRTI. In this study, we evaluated the suitability and fit of the Poisson regression model for our count data using the Deviance and Chi-Square-Goodness-of-Fit test. This assessment is essential to verify if the observed frequencies align well with the frequencies predicted by the model. The test produced a chi-squared statistic of 18.91 with a p-value of 0.002, indicating that the model provides an adequate fit to data. The time to recurrent pneumococcal CAP was determine by Kaplan-Meier survival analysis.

4.6.Ethical Considerations

Ethical clearance for this research study was obtained from the HREC (Human Research Ethics Committee) of the University of the Witwatersrand (REF:171009). The ethics certificate for this research study was issued on 8 August 2022 (clearance certificate no: M220318).

5. RESULTS

5.1.Socio-Demographic Characteristics of Study Participants

Table 2: Comparison of socio-demographic characteristics of study participants in three study sites

	CHBAH	KTHC	PMHC	Total	
	(N=707)	**(N=701)**	**(N=165)**	**(N=1573)**	**p-value**
Age of participant in years:					0.143
Median (IQR)	44.0 (36.0, 57.0)	46.0 (35.0, 58.0)	44.0 (33.0, 55.0)	45.0 (35.0, 57.0)	
Age group of participants					0.018
18-25	37 (5.2%)	48 (6.8%)	14 (8.5%)	99 (6.3%)	
26-35	136 (19.2%)	137 (19.5%)	37 (22.4%)	310 (19.7%)	
36-45	203 (28.7%)	155 (22.1%)	35 (21.2%)	393 (25.0%)	
46-60	192 (27.2%)	231 (33.0%)	57 (34.5%)	480 (30.5%)	
>60	139 (19.7%)	130 (18.5%)	22 (13.3%)	291 (18.5%)	
Sex of participant					0.521
Female	371 (52.5%)	349 (49.8%)	88 (53.3%)	808 (51.4%)	
Male	336 (47.5%)	352 (50.2%)	77 (46.7%)	765 (48.6%)	
HIV status of participant?					0.007
Negative	311 (44.0%)	320 (45.6%)	95 (57.6%)	726 (46.2%)	
Positive	396 (56.0%)	381 (54.4%)	70 (42.4%)	847 (53.8%)	
CD4 count (cells/mm^3)					<0.001
Median (IQR)	100.5 (27.5, 243.5)	163.5 (61.0, 338.0)	82.0 (27.0, 224.0)	123.0 (40.0, 292.0)	
CD4 count category					0.005
<100	194 (50.0%)	119 (34.8%)	38 (55.1%)	351 (43.9%)	
100-200	78 (20.1%)	72 (21.1%)	11 (15.9%)	161 (20.2%)	
>200-300	37 (9.5%)	52 (15.2%)	8 (11.6%)	97 (12.1%)	
>300-400	30 (7.7%)	32 (9.4%)	4 (5.8%)	66 (8.3%)	
>400-500	18 (4.6%)	21 (6.1%)	3 (4.3%)	42 (5.3%)	
>500	31 (8.0%)	46 (13.5%)	5 (7.2%)	82 (10.3%)	

Race as reported by patient					<0.001
Black	644 (91.1%)	665 (94.9%)	163 (98.8%)	1472 (93.6%)	
Caucasian	3 (0.4%)	9 (1.3%)	0 (0.0%)	12 (0.8%)	
Coloured	50 (7.1%)	25 (3.6%)	0 (0.0%)	75 (4.8%)	
Indian	9 (1.3%)	0 (0.0%)	0 (0.0%)	9 (0.6%)	
Other	1 (0.1%)	2 (0.3%)	2 (1.2%)	5 (0.3%)	
BMI					0.039
Median (IQR)	23.7 (19.2, 29.5)	23.9 (19.2, 31.9)	26.3 (21.1, 29.9)	24.0 (19.4, 30.4)	
BMI category					0.001
<18.5	145 (21.0%)	131 (19.8%)	24 (14.6%)	300 (19.8%)	
18.5-24.9	245 (35.6%)	221 (33.3%)	51 (31.1%)	517 (34.1%)	
25-29.9	137 (19.9%)	111 (16.7%)	48 (29.3%)	296 (19.5%)	
30-30.9	123 (17.9%)	136 (20.5%)	34 (20.7%)	293 (19.3%)	
>=40	39 (5.7%)	64 (9.7%)	7 (4.3%)	110 (7.3%)	
Smoking					<0.001
Never smoked	439 (62.1%)	375 (53.5%)	135 (81.8%)	949 (60.3%)	
No -Participant is an ex-smoker	136 (19.2%)	146 (20.8%)	10 (6.1%)	292 (18.6%)	
Yes	132 (18.7%)	180 (25.7%)	20 (12.1%)	332 (21.1%)	
Hypertension					<0.001
Yes	146 (20.7%)	206 (29.4%)	36 (21.8%)	388 (24.7%)	
No	561 (79.3%)	495 (70.6%)	129 (78.2%)	1185 (75.3%)	
Diabetes					<0.001
Yes	66 (9.3%)	64 (9.1%)	33 (20.0%)	163 (10.4%)	
No	641 (90.7%)	637 (90.9%)	132 (80.0%)	1410 (89.6%)	
Asthma					0.042
Yes	25 (3.5%)	44 (6.3%)	6 (3.6%)	75 (4.8%)	
No	682 (96.5%)	657 (93.7%)	159 (96.4%)	1498 (95.2%)	
Employment status					0.139
Currently employed	207 (29.3%)	181 (25.8%)	56 (33.9%)	444 (28.2%)	
Not employed	454 (64.2%)	473 (67.5%)	96 (58.2%)	1023 (65.0%)	
Self employed	39 (5.5%)	32 (4.6%)	9 (5.5%)	80 (5.1%)	
Student	7 (1.0%)	15 (2.1%)	4 (2.4%)	26 (1.7%)	

5.2.Incidence Of Recurrent Pneumococcal CAP

Our study consisted of 1573 eligible patients, a total of 426 patients from the POTPREV study were excluded from our study. There were 27/1573 patients with recurrent pneumococcal CAP over the study period with a total follow-up of 1457, 56 person years. The incidence rate ratio for recurrent pneumococcal CAP was 0.929 (95% CI: 0.929 [0.635-1.359]). The incidence of recurrent pneumococcal CAP was 18.5 per 1000 person-years. A total of 27/1573 (1.72%) patients in our study sample developed at least one episode of pneumococcal CAP 1-year follow up as determined from May 2019 to October 2021. Only 1/27 episode of pneumococcal CAP had a positive LytA rt-PCR blood sample and 27/27(100%) study participants with recurrent pneumococcal CAP had an abnormal Chest-X-Ray. Amongst the 27 participants with recurrent pneumococcal CAP, 26/27 were from KTHC and 1/27 from CHBAH and there were no episodes of recurrent pneumococcal CAP reported for participants at the PMHC site.

Table 3: Baseline characteristics of participants with recurrent pneumococcal CAP vs those without.

-	**No Recurrent pneumococcal CAP (N=1546)**	**Recurrent pneumococcal CAP (N=27)**	**Total (n=1573)**	**p-value**
Age of participant in years:				0.188
Median (IQR)	45.000 (35.000, 57.000)	41.000 (34.000, 54.000)	45.000 (35.000, 57.000)	
Age group of participants				0.440
18-25	97 (6.3%)	2 (7.4%)	99 (6.3%)	
26-35	304 (19.7%)	6 (22.2%)	310 (19.7%)	
36-45	383 (24.8%)	10 (37.0%)	393 (25.0%)	
46-60	473 (30.6%)	7 (25.9%)	480 (30.5%)	
>60	289 (18.7%)	2 (7.4%)	291 (18.5%)	
Sex of participant:				0.133
Female	798 (51.6%)	10 (37.0%)	808 (51.4%)	

Male	748 (48.4%)	17 (63.0%)	765 (48.6%)	
HIV status of participant?				0.082
Negative	718 (46.4%)	8 (29.6%)	726 (46.2%)	
Positive	828 (53.6%)	19 (70.4%)	847 (53.8%)	
CD4 count (cells/mm^3)				0.773
Median (IQR)	123.000 (40.000, 291.000)	119.500 (44.000, 338.000)	123.000 (40.000, 292.000)	
CD4 category (cells/mm^3)				0.847
<100	343 (43.9%)	8 (44.4%)	351 (43.9%)	
100-200	158 (20.2%)	3 (16.7%)	161 (20.2%)	
>200-300	95 (12.2%)	2 (11.1%)	97 (12.1%)	
>300-400	64 (8.2%)	2 (11.1%)	66 (8.3%)	
>400-500	42 (5.4%)	0 (0.0%)	42 (5.3%)	
>500	79 (10.1%)	3 (16.7%)	82 (10.3%)	
Race as reported by patient				0.757
Black	1445 (93.5%)	27 (100.0%)	1472 (93.6%)	
Caucasian	12 (0.8%)	0 (0.0%)	12 (0.8%)	
Coloured	75 (4.9%)	0 (0.0%)	75 (4.8%)	
Indian	9 (0.6%)	0 (0.0%)	9 (0.6%)	
Other	5 (0.3%)	0 (0.0%)	5 (0.3%)	
BMI				0.371
Median (Q1, Q3)	24.000 (19.400, 30.400)	21.100 (19.700, 28.200)	24.000 (19.400, 30.400)	
BMI				0.317
<18.5	295 (19.8%)	5 (18.5%)	300 (19.8%)	
18.5-24.9	504 (33.8%)	13 (48.1%)	517 (34.1%)	
25-29.9	293 (19.7%)	3 (11.1%)	296 (19.5%)	
30-30.9	287 (19.3%)	6 (22.2%)	293 (19.3%)	
>=40	110 (7.4%)	0 (0.0%)	110 (7.3%)	
Smoking				0.238
Never smoked	934 (60.4%)	15 (55.6%)	949 (60.3%)	

No -Participant is an ex-smoker	289 (18.7%)	3 (11.1%)	292 (18.6%)	
Yes	323 (20.9%)	9 (33.3%)	332 (21.1%)	
Hypertension				0.036
Yes	386 (25.0%)	2 (7.4%)	388 (24.7%)	
No	1160 (75.0%)	25 (92.6%)	1185 (75.3%)	
Diabetes				0.444
Yes	159 (10.3%)	4 (14.8%)	163 (10.4%)	
No	1387 (89.7%)	23 (85.2%)	1410 (89.6%)	
Asthma				0.241
Yes	75 (4.9%)	0 (0.0%)	75 (4.8%)	
No	1471 (95.1%)	27 (100.0%)	1498 (95.2%)	
Employment status				0.384
Currently employed	435 (28.1%)	9 (33.3%)	444 (28.2%)	
Not employed	1008 (65.2%)	15 (55.6%)	1023 (65.0%)	
Self employed	77 (5.0%)	3 (11.1%)	80 (5.1%)	
Student	26 (1.7%)	0 (0.0%)	26 (1.7%)	

5.3.S. Pneumoniae Serotypes in 1st And 2nd Episodes of Recurrent Pneumococcal CAP

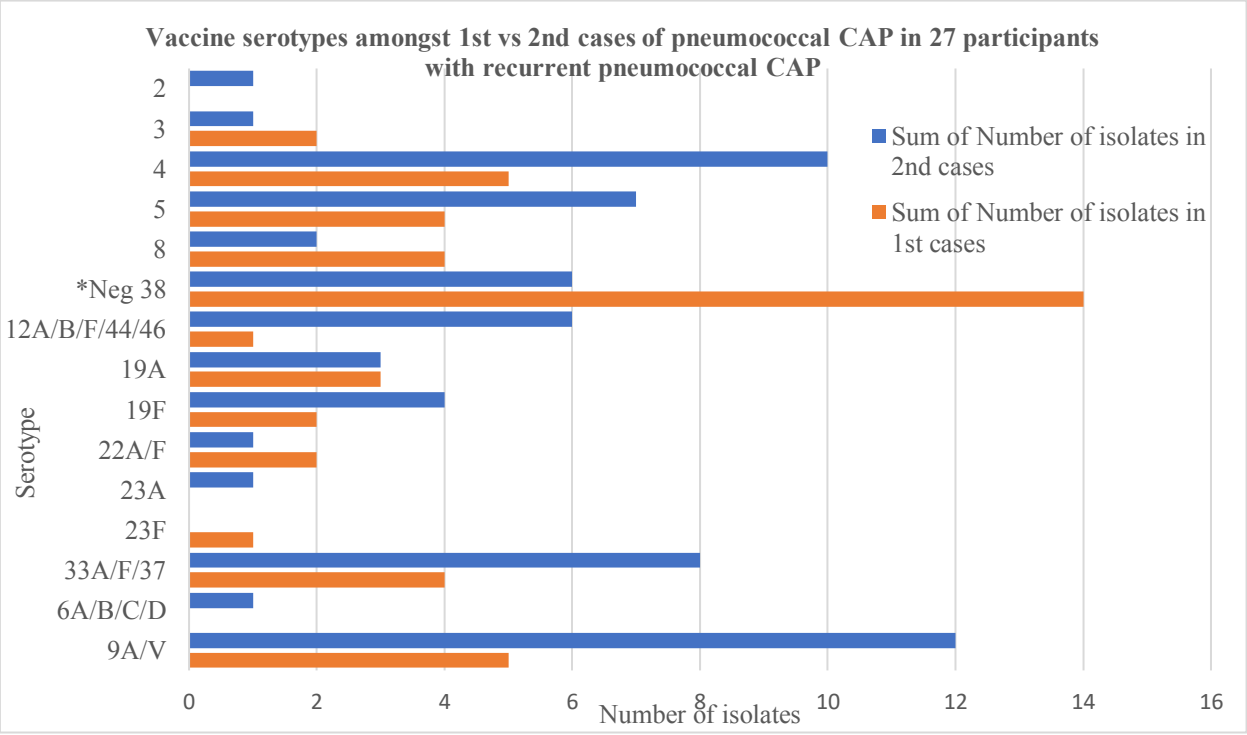


Figure 4: Serotypes in the 1st and 2nd episode of pneumococcal CAP amongst patients with recurrent pneumococcal CAP

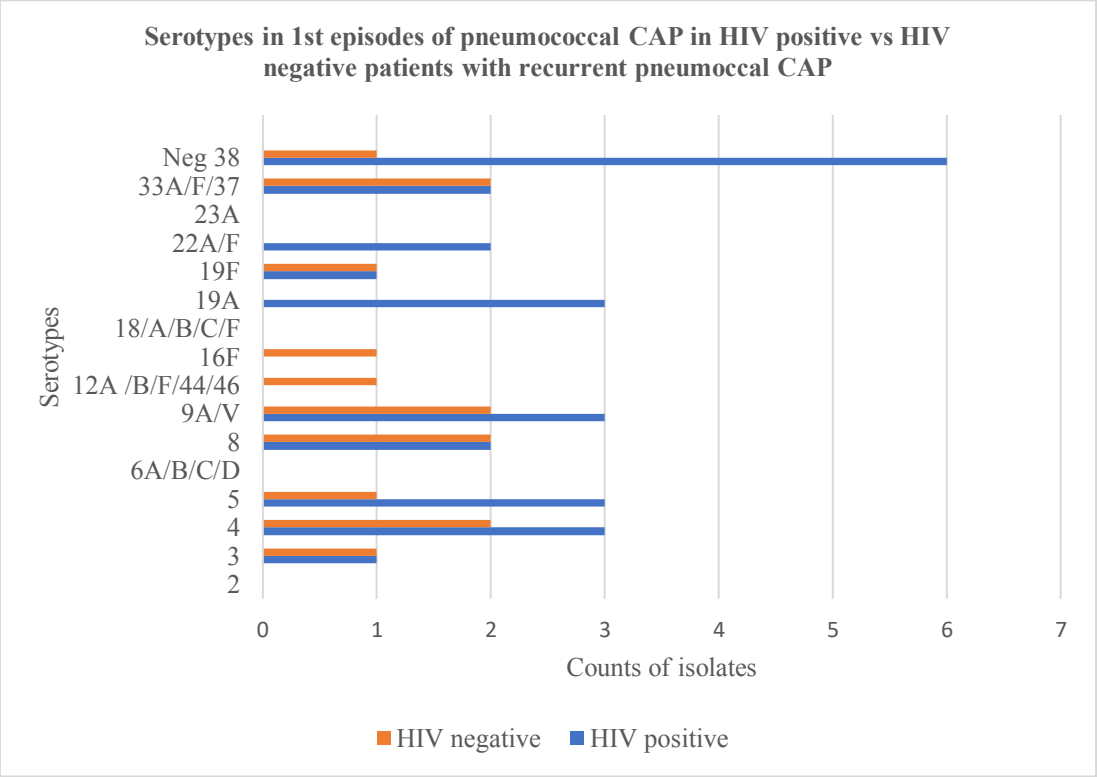


Figure 5: Serotypes in 1st episodes of pneumococcal CAP in HIV positive vs HIV negative patients with recurrent pneumococcal CAP

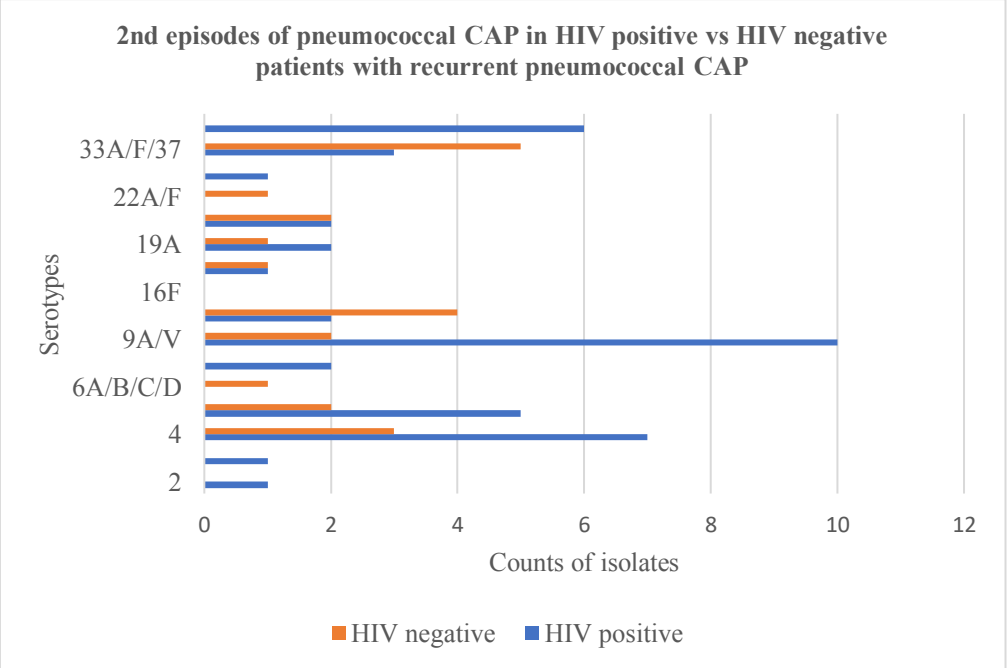


Figure 6: 2nd episodes of pneumococcal CAP in HIV positive vs HIV negative patients with recurrent pneumococcal CAP.

5.4.Vaccine Serotypes in Recurrent Pneumococcal CAP Cases

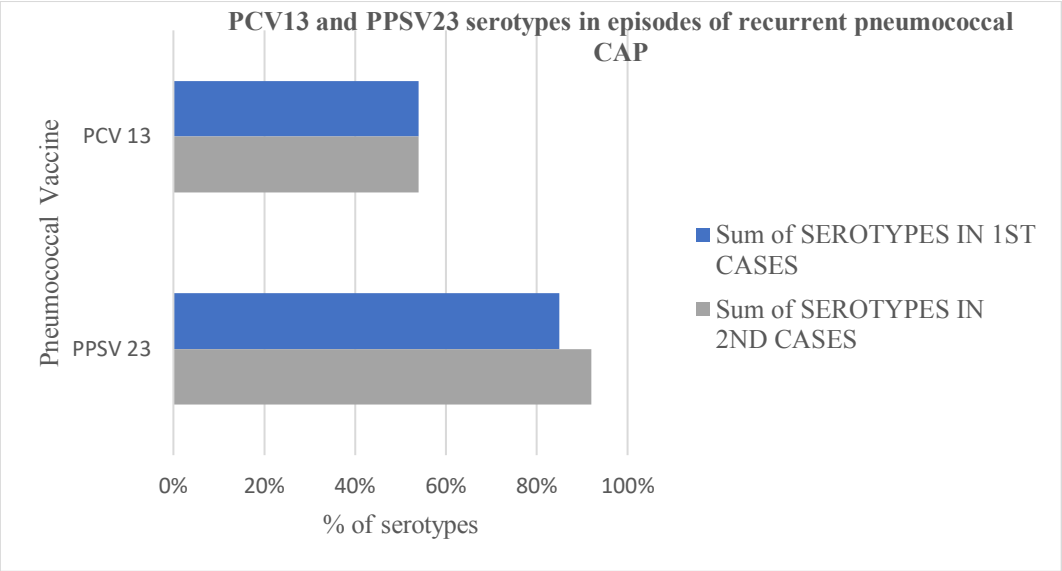


Figure 7: Vaccine serotypes in 1st vs 2nd case of pneumococcal CAP in 27 participants with recurrent pneumococcal CAP

Table 4: Vaccine categorisation of serotypes amongst the 1st cases of pneumococcal CAP in patients with recurrent pneumococcal CAP

Serotype	Number of isolates	PCV 13	PPSV 23	NVT
3	2	X	X	
4	5	X	X	
5	4	X	X	
8	4		X	
9A/V	5	X	X	
12A/B/F/44/46	1		X	
19A	3	X	X	
19F	2	X	X	
22A/F	2		X	
33A/F/37	4		X	
23F	1	X	X	
***Neg 38**	14			X

*Neg 38 represents serotypes which are not detected by the LytA rt-PCR assay.

Table 5: Vaccine categorisation of serotypes amongst the 2nd cases of pneumococcal CAP in patients with recurrent pneumococcal CAP

Serotype	Number of isolates	PCV 13	PPSV 23	NVT
2	1		X	
3	1	X	X	
4	10	X	X	
5	7	X	X	
6A/B/C/D	1	X	X	
8	2		X	
9A/V	12	X	X	
12A/B/F/44/46	6		X	
19A	3	X	X	
19F	4	X	X	
22A/F	1		X	
33A/F/37	8		X	
23A	1			X
***Neg 38**	6			X

Neg 38 represents serotypes which are not detected by the LytA rt-PCR assay.

Among 27 participants who had recurrent pneumococcal CAP within one year, patients were admitted to the hospital with pneumonia at least twice, 30 days apart, and had a positive NP LytA-rtPCR. The total number of *Streptococcus pneumoniae* serotype isolates identified for the first and second cases of recurrent pneumococcal community-acquired pneumonia (CAP) were 41 and 75, respectively. The "neg 38" isolates comprised 7 and 6 isolates for the first and second cases, respectively, with over 90% detected in people living with HIV (PLWH) and only one isolate detected in an HIV-negative participant. Serotypes 4 and 9A/V were common in first episodes of recurrent pneumococcal CAP, making up about a quarter of these cases. Serotype 9A/V was found in 12 patients during their second episode of recurrent pneumococcal CAP. Among PLWH, serotype 9A/V exhibited the highest occurrence, followed by serotype 5, with distributions of 23,3% and 16.3%, respectively. In the second cases of recurrent pneumococcal CAP, the most common serotype among HIV-negative participants was 33A/F/37, while 9V/A was common in PLWH. Unidentifiable *S. pneumoniae* serotypes were most common in PLWH. Among HIV negative participants, serotypes 33A/F/33 had the highest distribution at 22.7% in those with recurrent pneumococcal CAP. Overall, 64% (7/11) and 100% (11/11) of the serotypes that caused the initial episode in recurrent pneumococcal CAP cases were vaccine serotypes included in

PCV13 and PPSV23 vaccines respectively. The only NVT detected in one case was serotype 23A. In the second episodes of recurrent pneumococcal CAP, 54% (7/13) and 92% (12/13) of the serotypes were PCV13 and PPSV23 serotypes respectively.

5.5. Incidence Of Recurrent Pneumococcal CAP

Among the 1573 participants followed-up over a 1-year period, 27 patients experienced recurrent pneumococcal community-acquired pneumonia (CAP). The incidence of recurrent pneumococcal CAP between 2019 to 2021 in South Africa at CHBAH and KTHC was 18.53 per 1000 person years.

5.6. Incidence Of Mortality of Patients with Recurrent Pneumococcal CAP

0/27 participants with recurrent pneumococcal CAP died from the CAP episode during the study period of 1-year of follow up.

5.7. Time To Recurrent Pneumococcal CAP

Table 6: Statistical summary of time (days) to recurrent pneumococcal CAP

	CHBAH (N=1)	**KTHC (N=26)**	**Total (N=27)**	**p-value**
Days to recurrent pneumococcal CAP				0.404
Median (Q1, Q3)	134.0 (134.0, 134.0)	205. 5(134.0, 275.0)	205.0 (275.0, 682.0)	

Our analysis revealed no significant difference in the duration it took for participants to develop recurrent pneumococcal CAP between the two sites (p=0.404). The median time for participants to develop recurrent pneumococcal CAP within a year was 205 days, or approximately 6.8 months.

5.8.Predictors for recurrent pneumococcal CAP

Table 7: Univariate and multivariate analysis of risk factors of recurrent pneumococcal CAP

	Unadjusted		**Adjusted**		
	IRR (95% CI)	**p-value**	**IRR (95% CI)**	**p-value**	
Age group of participants					
18-25	1		1		
26-35	0.3 (0.1-1.0)	0.50			
36-45	0.5 (0.2-1.4)	0.60			
46-60	0.4 (0.2-1.1)	0.08			
>60					
Sex of participants					
Female Male	1 1.3 (0.4-1.4)	 0.41			
BMI category					
<18.5	1				
18.5-24.9	1.1 (0.1-1.0)	0.74			
25-29.9	0.4 (0.1-1.4)	0.09			
30-30.9	0.6 (0.5-3.2)	0.33			
>=40	0.2 (0.2-11.0)	0.18			
Smoking					
Never smoked	1			1	
No -Participant is an ex-smoker	1.1 (0.5-2.6)	0.76		1.0 (0.5-2.4)	0.9
Yes	2.61 (1.10-3.90)	0.032		1.90 (1.01-3.72)	0.051
Alcohol intake					
No	1				
Yes	1.6 (0.9-3.0)	0.09			
Hypertension					
Yes	1				
No	2.1 (0.9-4.9)	0.09			
Diabetes					

Yes	1				
No	0.9 (0.4-2.4)	0.81			
Asthma					
Yes	1				
No	0.7 (0.2-2.3)	0.56			
COPD					
Yes	1				
No					
HIV status of participant?					
Negative	1			1	
Positive	2.1 (1.1-4.1)	0.02		2.1 (1.1-3.9)	0.03 *
CD4 counts (cells/mm^3)					
<200	1				
>=200	1.4 (0.4-2.0)	0.36			

In the multivariate Poisson regression, participants who were smokers had a 1.9 increased risk of developing presumed recurrent pneumococcal CAP [adjusted IRR = 1.9; *P*=0.05; 95% CI (1.01–3.72)] compared to those who did not smoke. Participants who were HIV positive had 2.1 increased risk of developing presumed recurrent pneumococcal pneumonia [adjusted IRR = 2.1; *P*=0.03; 95% CI (1.1–3.9)] in an adjusted model than those who were HIV negative. The chi-square goodness of fit test showed that the data aligned with Poisson regression distribution assumptions.

Table 8: HIV prevalence in recurrent pneumococcal CAP

HIV status	**Frequency of recurrent episodes**	**%**
Negative	8	29.6
Positive	19	70.4
Total	**27**	**100**

Table 9: ART in PLWH with recurrent pneumococcal CAP

ART status	Number of patients	%
On ART	10	52.7
Not on ART	9	47.3
Total	**19**	**100**

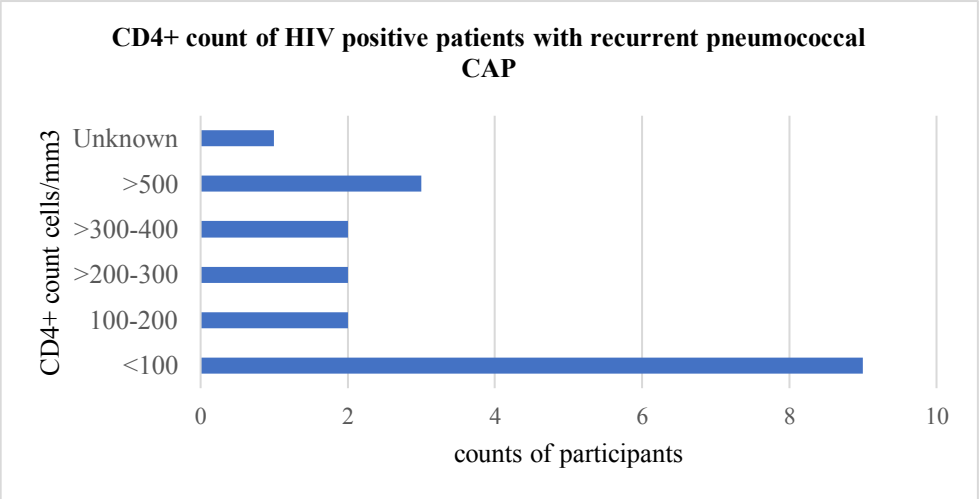


Figure 8: CD4+ count of PLWH with recurrent pneumococcal CAP

Table 10: Smoking in patients with recurrent pneumococcal CAP

Smoking	Frequency	%
Never smoked	15	55.00
Participant is an ex-smoker	3	11.11
Yes	9	33.33
Total	**27**	**100.00**

6. DISCUSSION

In this study, we aimed to determine incidence of recurrent pneumococcal CAP in hospitalised patients from 3 study sites in South Africa, to determine if the serotypes that cause recurrent pneumococcal CAP are VTs and to determine the incidence of mortality attributed to VTs. We included chest-X-ray to ascertain the 1st and 2nd cases of recurrent

pneumococcal CAP and considered 2 or more cases as confirmed cases of recurrent pneumococcal CAP.

6.1. Incidence Of Recurrent Pneumococcal CAP

A total of 27/1573 (1.72%) recurrent pneumococcal CAP cases were observed over a study period of 1 year with a total follow-up period of 1457,56 person years of follow-up from May 2019 to October 2021, the incidence of recurrent pneumococcal CAP was 18.5 per 1000 person-years. The findings of our study are comparable other studies in which a rate of approximately 25% of recurrent pneumococcal CAP was determined in a population based prospective cohort study with a follow-up of 5 years by Dang et.al in which the rate of recurrent pneumococcal CAP was 2% within 90 days and between 9-12% at 3-5 years of follow-up [5]. The majority of recurrent pneumococcal CAP at KTHC may indicate an association of recurrent pneumococcal CAP to mining activities. The incident rate of recurrent pneumococcal CAP seems to increases over a period, this is supported by a study that demonstrated that the mortality rate in survivors of pneumococcal pneumonia can increase to up to 35% over a 10 year period [164]. The increase of incidence in recurrent pneumococcal CAP suggests the need for longer follow-up periods to observe cases of recurrent pneumococcal CAP. Since our study excluded patients who died with 30 days of hospitalisation, it is not known if these patients had a case pneumococcal pneumonia prior to the commencement of the study. Early recurrent CAP which occurs >30 days of antimicrobial discontinuation has a recurrence rate of 5.4% in >75 years hospitalised patients and approximately 25% of these patients have early recurrent CAP had recurrent pneumococcal CAP [165]. Most studies on recurrent pneumococcal CAP exclude early recurrent pneumococcal CAP in the recurrent case definition and thus do not take into consideration the history of patients who die within 30 days of a CAP admission [5,166]. Inappropriate and incomplete antibiotic treatment are common risk factors for early recurrent and persistent pneumonia and the history of primary healthcare antibiotic treatment for respiratory tract infection symptoms prior to the commencement of our study is not known [60,167,168]. Long-term antibiotic use is the most common risk factor for early recurrent pneumonia, this is likely the treatment regimen in our primary healthcare sector where treatment guidelines are often not adhered to [168]. In South Africa, respiratory tract infections are the most common reason for antibiotic prescription in the primary healthcare setting in both the private and public sector which account for approximately 80% of antibiotic usage [60,168]. Considering the inappropriate usage of antibiotics in the primary healthcare setting in South Africa for respiratory tract infections;

this should be regarded as a risk factor for early recurrent pneumococcal CAP and highlights the importance of including participants who died <30 days on an initial case of pneumococcal CAP and thorough history of prior pneumococcal CAP cases as well antibiotic treatment prior to hospitalisation. Studies have shown that 60.4% of all-cause recurrent CAP episodes occur within 1-year from an initial episode in elderly patients, it is not known if this is the case in our study which mostly included adults with a median age of 45years.

6.2. Incidence Of Mortality in Recurrent Pneumococcal CAP

The incidence of mortality from recurrent pneumococcal CAP was 0/27 because none of the participants with recurrent pneumococcal CAP died during the 1-year study period. Although we excluded participants who died within 30 days, it is worth noting that is important for the patient's history to be considered to determine the risk of recurrent pneumococcal CAP because the most important risk factor is a previous episode of pneumococcal CAP. This particularly in PLWH with advanced immunosuppression. Other studies have shown that there is a high incidence of 30-day mortality in the elderly and those with comorbidities [165,169]. Studies suggest that mortality rate in patients with invasive pneumococcal pneumonia within 5 days of hospitalisation is 50%, this illustrates that most patients with recurrent pneumococcal CAP may have died within 30 days [170]. Data on the mortality of recurrent pneumococcal CAP in children is limited, with studies focusing more on recurrent IPD mortality which could not supplement our analysis in adult patients. In-hospital mortality of pneumococcal CAP can reach up to 32% within 1-year of an initial case of pneumococcal CAP with the highest burden of mortality risk in those admitted to ICU, likely to have severe pneumococcal CAP [171]. Due to the potential of underestimating the incidence of recurrent pneumococcal CAP, there is a possibility that fatalities from episodes of recurrent pneumococcal CAP not confirmed by CXR were missed, particularly since most recurrences occur within one year.

6.3. Serotype Distribution in Episodes of Recurrent Pneumococcal CAP

Over 70% of recurrent pneumococcal CAP episodes were caused by VTs in both the first and second episodes. Our findings indicate that the serotypes causing recurrent pneumococcal CAP are those included in PCV13 and PPSV23. Despite widespread pneumococcal vaccination, the prevalence of these serotypes remains significant with an average of 70% in both HIV-negative and HIV-positive participants which is comparable to other studies conducted post-PCV13 era. According to a previous study, serotypes 9V, 15A, 19F, 11A and 12 F were

associated with recurrent pneumococcal CAP; in our study we observed that these serotypes accounted for 30% of the 75 isolates detected in the 2nd cases of recurrent pneumococcal cases with 9A/V as the most prevalent serotype in recurrent pneumococcal pneumonia [8]. According to Domenech et.al, serotypes 9V and 19F are DRSPs and are VTs included in PCV13, PCV 10 PPSV 23 and the new PCV20. The prevalence in recurrent pneumococcal pneumonia caused by these serotypes is concerning because these serotypes are also resistant to antibiotics. Serotype 9V is a serotype of interest in as it is associated with invasive pneumococcal disease. Approximately 50% of pneumococcal pneumonia in adults is caused by multiple serotypes, serotypes 5, 9N/9L, 10A, 12/22/46, 17F and 35F have been associated with multiple-serotype pneumonia while serotypes 6A/6B, 23F, 11 and 6C/6D were associated with single-serotype pneumonia and there is a higher proportion of more invasive serotypes (serotypes 1, 5, 7F, 8) in multiple-serotype pneumonia [172].

6.3.1. Serotype 9A/V

Isolates of serotype 9A are independently derived from Serotype 9V resulting from the inexpression of the wcjE gene thus have genetic diversity which is a virulence factor commonly diverse amongst 9A/V isolates [173]. This serotype is known to cause invasive pneumococcal disease in children and is included in the PCV13 [174]. It is not surprising that serotype 9A/V was the most common serotype in recurrent pneumococcal CAP episodes in our study because of the high HIV prevalence amongst participants with recurrent pneumococcal CAP and the fact that serotypes in PLWH are those usually prevalent in children [175]. Serotype 9V has been associated with relapse recurrent pneumococcal pneumonia in COPD patients and identified as a DRSP. Serotype 9V was a common serotype in the pre-PCV era in South Africa and may have been more common in recurrent pneumococcal CAP in our study because of the antimicrobial resistance, the genetic diversity, and the prevalence of HIV in our cohort of recurrent pneumococcal CAP episodes.

6.3.2. Serotype 4

Globally, serotype 4 was detected in 10% IPD outbreaks between 2000-2017 in adults <65 years in the post-PCV-era and it is rarely carried by children [176–178]. Serotype 4 is an invasive *S.pneumoniae* serotype with strains that are genetically diverse, however; it has low antimicrobial resistance and is associated with smoking and homelessness [177]. We have not found data on the prevalence of serotype 4 in the pre- and post-PCV era. This serotype may be an emerging serotype in South Africa causing outbreaks as seen globally. The elderly in

old aged homes are at a high risk of pneumococcal disease outbreaks and Serotype 4 was identified to have caused an outbreak in unvaccinated elderly persons in old aged homes[179]. Of note, in this study; institutionalised people were excluded hence there is no data on the elderly from old age homes or prisoners.

6.3.3. Serotype 5

Globally, serotype 5 is associated with large outbreaks in all age groups in the post-PCV era especially in low resource settings [180,181]. This serotype is the 5th most common serotype in children and inclusion of it in the PCV 13 may not be effective as a result of degradation during isolation and conjugation which affects its stability and immunogenicity [182]. Serotype 5 is not commonly isolated from carriers, not considered a DRSP and not associated with high mortality and ICU admission [183,184].

6.3.4. Serotype 33A/F/37

Serotype 33F is a common global replacement serotype causing invasive pneumococcal disease following the introduction of the PCVs however it has been associated with lower mortality especially in pneumonia [185,186]**.** PCR doesn't discriminate between serotype 33A, 33F and 37, serotype 33F is an emerging serotype globally and it is included in the PCV-15 which is not available in South Africa **(**Gillis *et al.*, 2018**).** Serotype 33F has also been described as an emerging MDR serotype with an MDR rate of >5% in a study conducted in Canada where PCV-15 was available at the time of the study [188]**.** We have not found data on serotype 33A/F/37 in South Africa, however; it is not surprising that a non-vaccine serotype not included in the vaccines available in our setting was identified in recurrent pneumococcal CAP cases because of serotype prevalence. The possible emergence of this serotype in locally is in concordance with global data on the emergence of serotypes 33A/F/37 post-PCV introduction.

6.4. Time To Recurrent Pneumococcal CAP

The median time to recurrence of all cause CAP was 10,5 months in a different study, this is longer than the median of 6.8 months in our study which comprised of a sample with a high HIV prevalence of >70% [5]. In the aforementioned study, 43% of the patients were >75 years old, the study also only considered clinical symptoms and there these were no laboratory tests conducted. In addition, the time to recurrence may have been longer than

our study because patients included in the study were those who were free of pneumonia 90 days after discharge from hospital. The study participants in our study were enrolled at different times of the year, the seasonality of recurrent pneumococcal CAP should be interrogated.

6.5. Predictors Of Recurrent Pneumococcal Cap

We found HIV to be a risk factor for presumed recurrent pneumococcal cases, consistent with other studies identifying HIV as an independent risk factor for pneumococcal pneumonia and its recurrence.

6.5.1. Recurrent pneumococcal CAP in PLWH

Our study found that 53.8% of participants had HIV, with about 75% showing advanced immunosuppression (CD4+ count ≤ 200 cells/mm³). In recurrent pneumococcal pneumonia cases, 70.4% of patients were PLWH, with 47.3% either newly diagnosed or non-adherent to ARV treatment. Studies suggest that pneumococcal CAP occurs more frequently in PLWH with a CD4+ count of <500 cells/mm^3 [21]. According to a study conducted in Ethiopia, approximately 60% of patients are likely to have an increase in CD4+ cell count (>100 cells/mm^3) from baseline and about half of patients may achieve a CD4+ cell count (>500 cells/mm^3)1-year after ART initiation[189]. These data suggest that PLWH with advanced immunosuppression starting ART may not recover their CD4+ count enough to reduce the risk of pneumococcal CAP or recurrent episodes within 6 months. The HIV prevalence of 70.5% in our study is comparable with other studies in South Africa in with HIV prevalence of 89% and 74% in IPD and bacteraemia respectively during the PCV-era [3]. Our findings indicate that 47.3% of hospitalized patients with CAP were either newly diagnosed as PLWH or were not adherent to ARV treatment. This is comparable to the results of a study conducted by Aston et al., which found that 35% of patients with CAP were newly diagnosed with HIV.

6.5.2. Smoking in recurrent pneumococcal pneumonia

We expected smoking to be identified as a predictor for recurrent pneumococcal CAP in accordance with other studies, however this was not evident in our study. A total of 55% of self-reported non-smokers were diagnosed with recurrent pneumococcal CAP. Considering that smoking was found to be a predictor for IDP in approximately 60% of patients in which *S.pneumoniae* was the commonly identified bacteria, this included pneumococcal pneumonia in which patients were younger, immunocompetent and often required hospitalization as a result of the development of severe sepsis[190,191].The proportion of self-reported current or ex-

smokers is similar to that of non-smokers; however; self-reported smokers may not disclose their true smoking history and approximately 17% of self-reported non-smokers are current smokers [192]. The prevalence of smoking in our study which was based on self-reporting is likely not a true reflection of the smoking prevalence in the study and the prevalence may have been different if serum cotinine tests were considered in combination of self-reporting [104]. Furthermore, Baskaran et.al found that smokers were 1.42 times more to be hospitalised with recurrent pneumonia within a year, while this risk decreases in ex-smokers; smoking is an independent risk factor for recurrent pneumonia [98].

7. STUDY LIMITATIONS

The limitations of our study included a small sample size, with only three sites in three provinces, each consisting of one hospital. Consequently, the sample was not representative of South Africa as six other provinces were not included in the study. The follow-up period of one year also posed a limitation, potentially underreporting episodes of recurrent pneumococcal CAP. Additionally, the follow-up occurred during the COVID-19 pandemic, which disrupted health systems and led to missing information. During this period, participants had limited access to healthcare, affecting data collection. We could not perform a survival analysis to determine mortality incidence because of the small sample size of presumed pneumococcal cases over a year, and none of the followed-up participants died during the study period. The incidence of recurrent pneumococcal CAP was likely underestimated due to high participant mortality within 30 days, the low incidence of recurrent cases, and the small sample size. Lastly, self-reported smoking status was unreliable, potentially leading to an underestimation of smoking prevalence.

8. CONCLUSION

Our findings indicate that approximately 19 out of 1000 patients hospitalized with pneumococcal CAP will experience a recurrent episode within one year. HIV is identified as a significant risk factor for recurrent pneumococcal CAP, particularly in individuals with advanced immune suppression not on ART. A high prevalence of advanced immunosuppression was noted in HIV patients with recurrent pneumococcal CAP, with 70% exhibiting advanced immunosuppression. This suggests that newly diagnosed PLWH or those non-adherent to ART are at increased risk due to susceptibility to opportunistic infections at low CD4+ counts. Notably, recurrent pneumococcal CAP is primarily caused by *Streptococcus pneumoniae* serotypes covered by both PCV13 and PPSV23 vaccines, indicating it is vaccine preventable. It is recommended that PLWH hospitalized with

pneumococcal CAP should be initiated on ART as soon as possible if they are not already receiving it until their CD4+ count exceeds 200 cells/mm3 and their viral load is below 1000 copies/ml. These patients should also be prioritized for immunization with PPSV23 followed by PCV13 within two months, given the high prevalence of PPSV23 serotypes in recurrent cases. For PLWH with advanced immunosuppression, PPSV23 immunization should be administered after the CD4+ count recovers above 200 cells/mm3, followed by PCV13, contrary to other recommendations. Although South African CAP guidelines recommend administering PPSV23 within two months of a PCV13 dose in PLWH, our findings suggest that this regimen may predispose them to recurrent pneumococcal CAP caused by PPSV23 serotypes. Thus, the importance of both ART and pneumococcal vaccination in PLWH cannot be overstated. The observation that 96.2% of recurrent pneumococcal CAP cases occurred at the KTHC site suggests potential associations with adverse environmental effects from mining activities, as all patients with recurrent episodes had abnormal CXR with evidence of chronic lung disease. This observation might also result from regular medical screening of mine workers and higher HIV transmission rates in mining communities. The low or no incidence of recurrent pneumococcal CAP at CHBAH and PMHC points indicates possible missed episodes, especially since we focused on confirmed cases with positive blood samples and abnormal CXR. Literature indicates that bacteraemic pneumonia is uncommon, suggesting an underestimation of recurrent pneumococcal CAP in our study. To address this, we recommend using the ACHU score as a predictive tool for pneumococcal CAP in the absence of a positive blood test or abnormal CXR. Our findings imply that recurrent pneumococcal pneumonia in hospitalized patients is associated with underlying lung abnormalities, damage, or disease, and HIV infection. We advocate for larger studies to determine if recurrent pneumococcal CAP is more prevalent among PLWH across various provinces. Given the high smoking and COPD prevalence in the Western Cape (not included in our study), we also recommend investigating the incidence of recurrent pneumococcal pneumonia in PLWH with COPD to assess if this cohort has an increased risk. Finally, we advise conducting broader studies in South Africa, considering the high HIV prevalence, encompassing multiple sites nationwide to accurately determine the incidence of recurrent pneumococcal CAP using blood LytA PCR and UAD in hospitalized patients.

9. APPENDICES

9.1.APPENDIX A: Ethics Clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Matema Seabela

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M220318

NAME: (Principal Investigator)	Miss Matema Seabela
DEPARTMENT:	School of Public Health Perinatal HIV Research Unit
PROJECT TITLE:	*Recurrent Pneumococcal Community Acquired Pneumonia in hospitalized patients*
DATE CONSIDERED:	25/03/2022
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof K Otwombe
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	02/08/2022

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 **March** and will therefore be due in the month of **March** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature — Date: 30/08/2022

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

9.2.APPENDIX B: Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

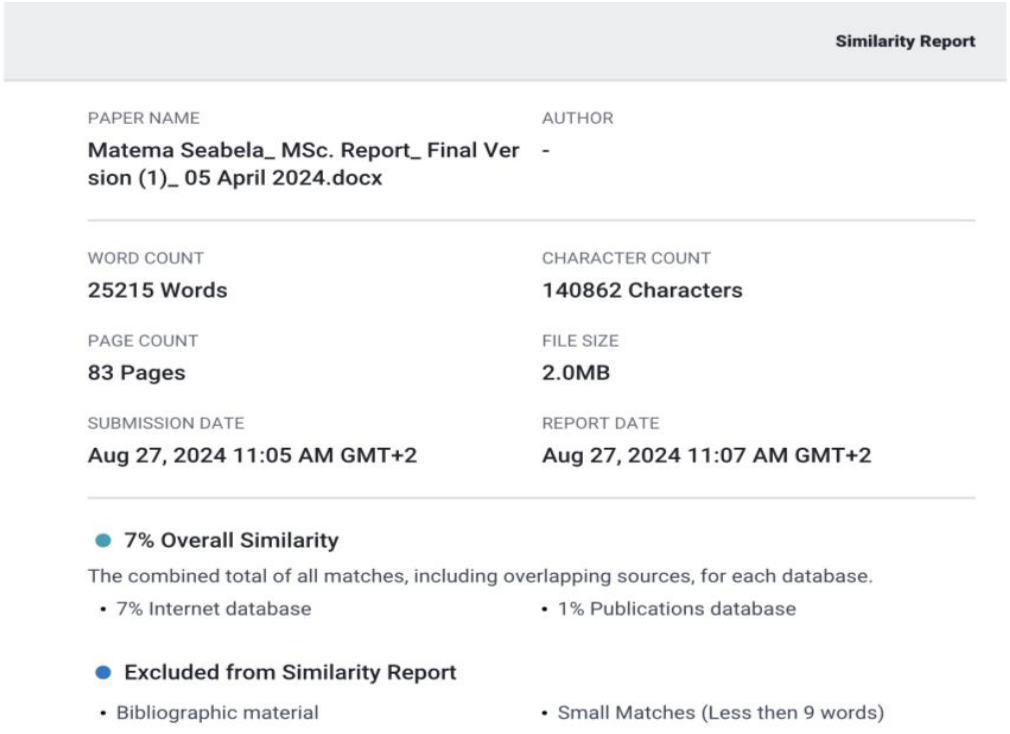
I MATEMA MANGWADI SEABELA (Student number: 1333276) am a student registered for the degree of MSc. Med. Vaccinology in the academic year 2025.

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except 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.

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9.3.APPENDIX C: Turnitin Plagiarism Report



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