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ESTIMATION OF SHEDDING TIME IN LABORATORY-CONFIRMED COVID-19 CASES IN SOUTH AFRICA: A POPULATION-BASED RECORD LINKAGE STUDY, MARCH-DECEMBER 2020

By

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A research report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the degree of Master of Science in Epidemiology (Field epidemiology)

This research work was supervised by:

Dr Lazarus Kuonza

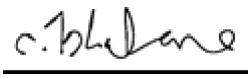
Dr Nkengafac Villyen Motaze

Mrs Hetani Mdose

SEPTEMBER 2022

DECLARATION

I, Carroll Tshabane, declare that this Research Report is my own unaided work. It is being submitted for the Degree of Master of Science in Epidemiology (Field Epidemiology) at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination to any other University.



(Signature of candidate)

2nd day of August 2022 in Johannesburg, South Africa

DEDICATION

In loving memory of my grandmother

Shalati N'wa Hlupheka Xikoxa

1921- 2021

CONFERENCE PRESENTATION

An abstract for this research was submitted to the 1st International Conference on Public Health in Africa (CPHIA 2021) and was accepted for an oral presentation (**Appendix 2)**. The conference was held virtually from 14-16 December 2021. The abstract was titled: Estimation of shedding time in laboratory-confirmed COVID-19 cases in South Africa, 2020.

ABSTRACT

Background

Since the announcement of the first confirmed COVID-19 case in March 2020, South Africa has seen an increase in cases. Determining shedding time is important to inform public health interventions. We aimed to estimate viral shedding time among laboratory-confirmed COVID-19 cases in South Africa.

Methods

A cross-sectional analytic study was conducted using COVID-19 data from 5 March-31 December 2020, obtained from the NMClist and the DATCOV system. These platforms report on laboratory-confirmed and hospitalized COVID-19 patients, respectively. The study consisted of COVID-19 patients with repeat positive PCR tests (at least two positive PCR test results) and who subsequently tested negative. To determine the association between shedding time and predictor variables, multiple linear regression analysis was conducted and a p-value <0.05 was considered statistically significant.

Results

We included 2752 cases that met the inclusion criteria. The 2752 included participants had a total of 8 642 PCR positive test results with a median of 3 positive tests per participant (range: 2 – 11). The majority (74.7%: 2,057/2752) of the participants had 3 consecutive positive tests. About 39.9% (1099/2752) of participants were inpatients and 60.1% (1653/2752) were outpatients. The median shedding time was 17 days(range: 1–128). No significant difference in shedding time was found between males (median:16 days, range:1-128) and females (median:17 days, range:1–94) and between inpatients (median: 16 days, range 1–108) and outpatients (median:17 days, range:1–128). Individuals aged 0-4 years hadthe lowest shedding time (median:14 days, range:1–72). The shortest shedding time was observed in Eastern Cape with a median of 15 days (range: 1-56) and the longest shedding time was observed in Limpopo province with a median of 21 days (range: 1-39). After adjusting for age, sex and province,shedding time was shorter for hospitalized patients compared to outpatients (coefficient: -0.14, CI:
-0.24 ─ -0.03, P-value: 0.014).

Conclusion

Shedding time varies with admission status (inpatients versus outpatients). We found a significant association between the hospital admission status of COVID-19 patients and shedding time. Further studies are required to explore the association between other comorbidities (such as heart disease, obesity and diabetes mellitus), socio-economic status, level of education, vaccination status and SARS-CoV-2 shedding. In addition, different approaches that can address transmission risk effectively in asymptomatic patients are required to control SARS-CoV-2 infection.

Keywords: COVID-19, South Africa, viral shedding time, laboratory-confirmed, population-based, SARS-COV-2

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LIST OF ABBREVIATIONS

CDW	Corporate Data Warehouse
CDC	Centers for Disease Control and Prevention
COVID-19	Coronavirus disease 2019
HIV/AIDS	Human immunodeficiency virus/acquired immune deficiency syndrome
NICD	National Institute for Communicable Diseases
NMC	Notifiable Medical Condition
PHEIC	Public Health Emergency of International Concern
POCT	point-of-care tests
RDT	Rapid Diagnostic Test
RNA	Ribonucleic acid
RT-PCR	Real-time reverse transcription-polymerase chain reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SAFETP	South African Field Epidemiology Training Programme
WHO	World Health Organization

CHAPTER 1: INTRODUCTION

This research report presents findings from a quantitative study that was conducted on coronavirus disease 2019 (COVID-19) patients in South Africa (SA). It provides an overview of COVID-19 viral shedding time and factors associated with shedding time in laboratory-confirmed COVID-19 cases. The introductory chapter will provide background information on COVID-19, describing the burden of disease, disease patterns and its epidemiology. We will further provide a literature review, providing context on shedding time on COVID-19 patients globally. The importance of estimating shedding time and gaps in knowledge will be discussed to justify the research study. To summarize the chapter, the research question, aim and objectives of the study will be listed.

1.1. Background

Wuhan, a city in Hubei province of China recorded its first COVID-19 cases in December 2019. COVID-19 is a respiratory disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus[1]. In early January 2020, COVID-19 cases started to show a gradual increase and by mid-January 2020, the disease had spread to other countries such as Japan, Thailand, and the United States of America. Following the spread of SARS-CoV-2 infections in many countries globally, the disease was announced as a public health emergency of international concern (PHEIC) in January 2020 and was later declared a pandemic in March 2020[2,3]. As of 5 January 2021, there were 84,233,579 reported COVID-19 cases and 1,843,293 reported deaths globally[4]. To date, there is no cure for COVID-19, however, it can be prevented and controlled through vaccinations, social distancing, wearing of masks and washing or sanitizing hands. As of 05 January 2021, the African region reported 2,852,491 confirmed COVID-19 cases and 67 943 deaths, with a case fatality ratio of 2.4%[3]. The highest number of SARS-CoV-2 infections in the African region were reported in South Africa.

1.1.1. Epidemiology of COVID-19

COVID-19 spreads through contact with a person infected with SARS-CoV-2, via larger respiratory droplets, through touching contaminated surfaces, and by inhaling small airborne droplets[5,6]. COVID-19 can stay in the air for some time and can spread through the airborne route however, the contribution of air transmission to the pandemic is unknown[7,8,9]. Studies have shown that poorly ventilated spaces can act as a potential for airborne transmission[10,11]. SARS-CoV-2

have also been discovered in specimens such as sperms, blood and stools. Although the virus has been identified in these non-respiratory sites, the role these sites play in COVID-19 transmission is unknown. Different transmission patterns have been reported across Africa, with the majority of the countries reporting community transmissions and some observing clusters and sporadic transmissions. Compared to other continents, Africa is one of the least affected, representing 3.4% of the total reported global cases[12].

COVID-19 affects people of all ages. However, individuals with comorbidities (such as heart disease, obesity and diabetes mellitus) and older people (above 60 years of age) are more vulnerable to infection and have a higher risk of developing severe illness[13]. Signs and symptoms of COVID-19 include body aches, coughing, sore throat, shortness of breath, chills, fever, headache, nausea, diarrhoea, and loss of smell or taste[14]. Some people remain asymptomatic after acquiring the infection. However, most people develop mild symptoms of the disease and recover from the illness without medical intervention[15]. One in six people have presented with more severe symptoms such as shortness of breath[13]. Nevertheless, the virus can effectively be prevented by wearing a face mask, hand sanitizing, vaccinating and social distancing.

1.1.2. COVID-19 in South Africa

South Africa ranks 15th globally with COVID-19 cases and is the most affected country in the African region even though it has relatively low numbers of deaths[12,16]. The first COVID-19 case in South Africa was confirmed on 5 March 2020, and on 17 March 2020, the president declared a national state of disaster[17]. Since then, lockdown control measures were put in place. These measures included compulsory wearing of face masks, closure of businesses except businesses providing essential services, travel ban, closure of schools and religious centers. Quarantine and isolation measures were also put in place. Asymptomatic people who may have been infected with COVID-19 are placed in quarantine. While isolation is for people who have been infected with SARS-CoV-2 but are not hospitalized[18]. Although these control measures were in place, the virus continued to spread throughout the country. By 05 January 2021, there were 1,127,759 cases of COVID-19 and 30 524 deaths in SA. Gauteng province had reported the highest number of cases, representing 27% of total cases, followed by Western Cape at 19.9% and KwaZulu-Natal at 19.8%[19].

COVID-19 continues to have severe economic impacts on the healthcare system[20]. Healthcare workers have a key role in fighting the COVID-19 pandemic however, they are at a very high risk of being infected by the virus. There is a gradual increase of infections amongst health workers throughout the African continent. Since the beginning of the outbreak, there had been 44 055 reported cases of healthcare workers by 20 October 2020. The most affected healthcare workers were from South Africa, with 27 360 infections[12].

SARS-CoV-2 has been changing over time through mutations causing new variants of the virus. Multiple SARS-CoV-2 variants have been discovered in different countries globally. The variants reported include the Alpha variant (B.1.1.7) which was identified in the United Kingdom (UK); Beta (B.1.351) which was discovered in SA, Delta (B.1.617.2) which was discovered in India and the Gamma variant (P.1) which emerged in Brazil[21,22]. The Delta variant has been reported to spread faster than other variants and may cause more severe cases compared to other variants[23]. The Beta variant was identified in October 2020 in the Eastern Cape, South Africa. Since then, cases caused by this variant has been reported in other countries. Due to significant genetic changes, which is associated with an increased transmission, the Beta variant is a variant of concern[24]. Variants could be the leading cause of an increase in COVID-19 cases in South Africa which led to a resurgence and affected younger populations during the second wave. The development of SAR-CoV-2 variants has caused an increased risk to public health globally.

COVID-19 vaccines have been developed and rolled out in response to the pandemic to offer protection against the virus to the masses. The Johnson and Johnson vaccine was first administered to healthcare professionals in South Africa in 2020[25]. A COVID-19 national vaccination program started in February 2021 and the rollout was done in phases covering people aged 12 years and older. Johnson and Johnson, Pfizer-BioNTech and Oxford-AstraZeneca are the only vaccines being administered because they have been reported to provide protection against the Beta variant and other variants[26]. As of 02 November 2021, 21.1% of the population was fully vaccinated. Studies show that vaccines may reduce the risk of infection and reduce the severity of COVID-19 illness among those that get infected[7,27].

1.1.3. COVID-19 Viral shedding time

Various molecular techniques including point-of-care tests (POCT) and central laboratory tests have been made available to diagnose and manage COVID-19 patients. This includes viral and

antibody tests. The World Health Organization (WHO) has recommended that COVID-19 be diagnosed using real-time reverse transcription-polymerase chain reaction (RT-PCR) test[28]. The PCR test is a molecular test used to detect viral Ribonucleic acid (RNA) which in people infected with SARS-CoV-2 can be detected before, during and after a person presents with symptoms. Studies have used the PCR test to study viral shedding in COVID-19 patients[29,30]. Viral shedding happens when a virus in an infected person's body is replicated and released into the environment[31]. An individual who is infected with a viral disease is infectious if they are shedding the virus. Therefore, the time and rate at which an infected person sheds the virus are important. For SARS-CoV-2, people shed the virus when they sneeze, talk, cough and through their stools.

During the period of infection, viral shedding time or its duration is used to determine the suitable period of isolation. Viral shedding time is a useful guide for infectivity since it estimates the period of infectiousness. Furthermore, it can be used to establish guidelines for the duration of the isolation period for COVID-19 positive patients, and quarantine for those exposed to COVID-19[32]. As at the time of reporting, COVID-19 guidelines require asymptomatic patients to isolate for 10 days after testing whereas people with mild disease are required to isolate for 10 days after symptoms onset. Also, people with severe COVID-19 should isolate for 10 days after showing clinical stability[33]. Research shows that SARS-CoV-2 transmission begins before symptom onset and peaks early as the diseasedevelops and the risk of transmission reduces thereafter[34,7]. During the early period of the pandemic, patients infected with COVID-19 were assumed to remain infectious no longer than 14 days after symptom onset. Research shows that the virus can begin shedding before a person starts to show symptoms and shedding increases during or after the onset of symptoms[34]. Understanding viral shedding time in detail can assist in decreasing the rate of SARS-COV-2 transmission. Therefore, there is a need to estimate shedding time in laboratory-confirmed COVID-19 cases in South Africa.

1.2. Literature Review

This literature review examines various studies that have assessed COVID-19 viral shedding time. It reviews studies that have examined the contributing factors that affect viral shedding time. It also evaluates studies on the importance of estimating COVID-19 shedding time and laboratory diagnosis.

1.2.1. COVID-19 viral shedding time

Viral shedding time is the period during which the virus is released from the body of a person with an infectious disease[34]. The shedding time of COVID-19 in the population of South Africa has not been studied[35]. In China, RT-PCR was used to diagnose COVID-19 among hospitalized patients to determine the duration of shedding time. They defined viral shedding time as "the number of days from the onset of the symptoms until the successive negative detection of SARS-CoV-2 RNA [36]." To determine shedding time among COVID-19 patients with mild disease in South Korea, viral shedding time was defined as "the duration from the day of diagnosis to the day when a patient displayed negative results for two series of RT-PCR tests, performed at least at a 24-hour interval apart[37]." With COVID-19 patients, viral shedding could start before the onset of symptoms[34]. The median shedding time amongst patients with COVID-19 in China was 11 days[38.] This was different from Japan where the median viral shedding time of COVID-19 patients was found to be 19 days, ranging between 6-37 days after the first viral detection[39]. The inconsistency in viral shedding time from different studies across the world may be due to differences in the definition of the RT-PCR test results (negative or positive)[40].

Studies show that both symptomatic and asymptomatic patients have the potential of transmitting COVID-19 since they can possess similar viral loads[41,42]. This is evident from a study conducted in Saudi Arabia which found that the mean shedding time of COVID-19 asymptomatic patients was 13.6 days and 16.9 days for symptomatic patients[43]. Age and comorbidities have been reported to have an impact on symptom severity and clinical outcome[44]. However, pre-symptomatic and asymptomatic cases have been reported to transmit the virus to close contacts and have led to secondary cases[45]. Asymptomatic cases make it difficult to control the transmission of the virus since the duration of their infectiousness is hard to measure.

1.2.2. Factors affecting viral shedding time

Different factors affect viral shedding time in COVID-19 patients. These factors could either slow or increase viral shedding in an individual. Prolonged viral shedding is associated with severe COVID-19 disease which may require ventilation. Studies have reported that shedding time among COVID-19 patients is affected by corticosteroid treatment, fever, long hospital stay and time from onset to hospitalization[36,46]. Evidence shows that early interventions such as early treatment of COVID-19 patients with symptoms can reduce viral shedding time[46]. Although COVID-19 can

infect anyone, younger age was associated with shorter shedding time whereas older age was associated with longer shedding time[30]. A study conducted in South Korea reported that comorbidities delay shedding time, also, females shed the virus more than males, although the difference was found to be marginal[37].

1.2.3. Importance of estimating COVID-19 shedding time

Several studies have shown that viral shedding time is significant in determining disease transmission[39,46]. Acquiring more knowledge on the importance of shedding time is important for public health planning, clinical purposes and could benefit society. Considering South Africa's unique epidemiological profile, the population has high HIV/AIDS prevalence, high Tuberculosis incidence and high death rates from non-communicable diseases such as diabetes, making it of study interest. As identified in this literature review, there are some noticeable gaps around shedding time in different contexts and in a large population[32]. Guidelines for isolation and the duration of quarantine in different countries are made to reflect viral shedding duration.

1.2.4. Laboratory diagnosis

It is fundamental to have rapid and accurate detection of SARS-CoV-2 for treatment and control of the virus. Different diagnostic tests are used to diagnose COVID-19, including molecular tests (such as RT-PCR tests), rapid diagnostic tests (RDT) and antigen tests. The signs and symptoms of the COVID-19 infection are similar to some other infectious respiratory diseases such as influenza, therefore performing laboratory tests on symptomatic people is crucial to identify SAR-CoV-2 infections. Moreover, it is estimated that up to 40% of infected individuals may have no symptoms (subclinical infection) or mild symptoms, yet are still capable of transmitting the virus to others[47]. This means that individuals without obvious signs or symptoms of SARS-CoV-2 infection also require testing. COVID-19 testing can be done in different ways, including detection of viral RNA, detection of viral antigen and detection of antibodies. The PCR test is the standard test for COVID-19 however, it is costly and may take time to receive results. The antigen test is cheaper compared to the PCR test, however, the test is not sensitive and may provide inaccurate results. Antibody tests can identify people who previously tested positive for COVID-19 but were asymptomatic, however, it can miss individuals whose IgM or IgG antibodies are not detectable. The WHO has recommended that laboratories use RT-PCR assays for COVID-19 diagnosis, as it is considered the best test at the moment[48].

1.3. Problem Statement

To inform infection control, prevention measures and public health policies, it is vital to understand the transmission dynamics of SARS-CoV-2. Population-level information on shedding time and factors affecting the duration of SARS-CoV-2 infection is lacking. This information could be used to understand the association between viral shedding and infectivity and the effectiveness of using a PCR test for infectiousness or re-infection. Estimating viral shedding time is vital since shedding can be utilized as a proxy for infectiousness[46]. Control measures such as quarantine and wearing of masks have been put in place in South Africa. However, the average shedding time in the South African population is unknown and there is a lack of thorough research on the duration of infectiousness for SARS-CoV-2[35]. Also, there is no routine test available to measure infectiousness. This means that determining viral shedding time and understanding the associated predictors will play a significant role in informing control and prevention measures in COVID-19 patients.

1.4. Justification

Numerous studies have been conducted globally to estimate the duration of SARS-CoV-2 viral shedding however, no study has estimated viral shedding time for laboratory-confirmed COVID-19 cases in the South African population. Since SA reported the highest numbers of COVID-19 infections on the African continent, it is important to better understand the transmission dynamics of the virus in the population. The results from this study will assist in refining infection control strategies such as contact tracing and isolation or quarantine, and assist in understanding the potential difference of positive PCR tests over time. The results may further be utilized in facilitating early treatment and intervention, leading to a decrease in the incidence and mortality due to coronavirus disease.

1.5. Research Question

What is the viral shedding time of SARS-CoV-2 among laboratory-confirmed COVID-19 cases in South Africa?

1.6. Aim

To estimate the viral shedding time among laboratory-confirmed COVID-19 cases in South Africa.

1.7. Objectives

Objective 1: To describe the clinical and epidemiological characteristics of patients with COVID-19 in South Africa, 2020.

Objective 2: To determine the shedding time of laboratory-confirmed COVID-19 cases in South Africa, 2020.

Objective 3: To determine factors affecting viral shedding time in COVID-19 patients in South Africa.

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CHAPTER 2 – MANUSCRIPT

This chapter presents a manuscript written using the journal author guidelines for Clinical Infectious Diseases. The formatting of the journal article may differ from the rest of the research report to comply with the author guidelines of the journal to which we intend to submit the article. The journal guidelines are included in the appendices (**Appendix 6**).

Estimation of shedding time in laboratory-confirmed COVID-19 cases in South Africa: A population-based record linkage study, March-December 2020

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Summary: This cross-sectional analytic study reports shedding time among PCR-confirmed COVID-19 cases in South Africa from March to December 2020. The median shedding time was 17 days (range: 1–128). Disease severity (admission status) was associated with shedding time.

ABSTRACT

Background

In South Africa, COVID-19 cases are notified through the NMCList platform while hospitalized cases are reported on the DATCOV platform. It is crucial to estimate the duration of SARS-CoV-2 shedding to inform public health interventions. We aimed to estimate viral shedding time among laboratory-confirmed COVID-19 cases in South Africa.

Methods

We analyzed COVID-19 PCR results reported on the NMCList and DATCOV platforms from 5 March to 31 December 2020. We included cases with at least 2 consecutive positive PCR tests and a subsequent negative test. We performed multiple linear regression to determine the association between shedding time and predictor variables (age, sex, admission status and province). A p-value below 0.05 was considered to be statistically significant.

Results

We included 2752 cases that met the inclusion criteria. About 39.9% (1099/2752) of participants were inpatients and 60.1% (1653/2752) were outpatients. The median shedding time was 17 days (range: 1–128). There was no significant difference in shedding time between males (median:16 days, range:1-128) and females (median:17 days, range:1–94) and between hospitalized patients (median: 16 days, range 1–108) and outpatients (median:17 days, range:1–128). Individuals aged 0-4 years had the lowest shedding time (median:14 days, range:1–72). After adjusting for age, sex and province, shedding time was shorter for hospitalized patients compared to outpatients (coefficient: -0.14, CI: -0.24 ─ -0.03, P-value: 0.014).

Conclusion

Shedding time differs between hospitalized and outpatients. Further studies are required to explore the association between comorbidities and SARS-CoV-2 shedding time.

Keywords: COVID-19, SARS-CoV-2, viral shedding time, South Africa, population-based

INTRODUCTION

Coronavirus disease 2019 (COVID-19) is an illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1]. The virus was first identified in Wuhan, China in December 2019. COVID-19 has spread globally, causing a global pandemic [2]. On 5 March 2020, South Africa reported its first confirmed COVID-19 case [3]. Despite strict measures such as lockdown and compulsory wearing of masks put in place to control the epidemic, the virus has continued to spread. Viral shedding time is a useful guide for infectivity and can be used to establish the duration of isolation, quarantine and inform contact tracing guidelines [4]. With the polymerase chain reaction (PCR) test being the best test available for COVD-19 diagnosis, several studies have used it to estimate viral shedding time [5,6,7]. The Republic of Korea reported a median shedding time of 15 days among COVID-19 patients [8]. This was different from Japan where the median viral shedding time of COVID-19 patients was reported to be 19 days [9]. Studies show that both symptomatic and asymptomatic patients have the potential of transmitting the COVID-19 [8,10]. This is evident from a study conducted in Saudi Arabia which found that the mean shedding time of COVID-19 asymptomatic patients was 13.6 days and 16.9 days for symptomatic patients [11]. Factors affecting shedding time include the severity of disease, age, sex, comorbidity, timeliness of treatment and COVID-19 symptoms such as fever [6,7,12,13]. Age and comorbidities have been reported to have an impact on symptom severity and clinical outcome [14].

South Africa has reported the highest number of SAR-CoV-2 infections in Africa, therefore determining viral shedding time is important to understand the transmission dynamics of the disease within the population. This study aimed to estimate viral shedding time among laboratory-confirmed COVID-19 cases in South Africa.

METHODS AND MATERIALS

Study design and procedures

We performed a cross-sectional analytic study using COVID-19 data obtained from the COVID-19 Notifiable Condition List (NMCList). The study included laboratory results from public and private clinical laboratories in South Africa that reported COVID-19 test results to the NMCList database. The study population consisted of all laboratory-confirmed COVID-19 patients reported to the NMCList database from March to December 2020. We included laboratory-confirmed COVID-19 patients with repeat positive PCR tests (at least two positive PCR test results) and subsequently tested negative. A person should have done consecutive testing no longer than 14 days between the repeat positive PCR tests. We excluded positive cases that were tested using antigen tests and cases that took a repeat test after 14 days of continuing positivity.

Data sources

COVID-19 test results are reported to the NMCList database hosted by the National Institute for Communicable Diseases (NICD). COVID-19 is a category 1 Notifiable Medical Condition (NMC) with a requirement of immediate reporting upon clinical suspicion or laboratory diagnosis followed by a written notification within 24 hours. Clinical data of patients tested for COVID-19 are collected using the NMC reporting platform, which is a national reporting system in South Africa. Both RT-PCR and antigen COVID-19 test results are stored in the NMCList database and reported to the National Department of Health (NDoH). Individuals who have multiple test results are identified using a unique identifier (case_id). Other variables stored in the NMCList include personal identifiers (name, surname, date of birth, sex, national identity number), demographic information (physical address), and health facility information. Data from the NMCList is stored in the NICD surveillance data warehouse.

The DATCOV system is an active, prospective sentinel surveillance programme for COVID-19 in South Africa. This online platform enables private and public healthcare facilities to submit hospital admissions data for COVID-19 patients. COVID-19 admissions trends are monitored and the epidemiology of the disease in hospitalized patients within the country is described. Admission status (hospitalized or outpatient) was used as a proxy to determine disease severity. Hospitalized patients were defined as patients with severe disease and outpatients as patients with mild disease.

Data collection

We extracted data from the NMCList and DATCOV databases. It was assumed that COVID-19 cases that were not in the DATCOV database were not hospitalized. The patients' national identity number was used to link cases between the NMCList and DATCOV databases.

Case Definitions

COVID-19 case: "A confirmed case of COVID-19 is a person with laboratory confirmation of SARS-CoV-2 infection (using an RT-PCR assay), irrespective of clinical signs and symptoms between March and December 2020" [15].

Negative result: A person with a negative SARS-CoV-2 laboratory test using an RT-PCR assay.

Variables

Outcome Variable: The outcome variable is COVID-19 viral shedding time measured in days.

Viral shedding time (days) was defined as the period from the date of specimen collection for the first positive PCR test to the date of specimen collection for the last positive test prior to a first negative PCR test. The date of specimen collection for the first positive test was subtracted from

the date of specimen collection for the last positive test to obtain the shedding time (in days). The date of onset of viral shedding was defined as the day the specimen of a case was collected since laboratory data had no variable for the date of symptom onset. This approach catered for individuals who would have been symptomatic or asymptomatic.

Explanatory variables included sex, age, province, number of tests done and the severity of COVID-19 (hospitalized patients versus outpatient).

Statistical analysis

Data were analyzed using STATA 15 (Version 15, StataCorp, College Station, Texas). Characteristics of study participants were reported using descriptive statistics. Categorical variables (age groups, sex, admission status and province) were presented using absolute numbers and percentages. Normally distributed continuous variables (age) were reported using means and standard deviations while medians and ranges were used when continuous variables were not normally distributed (shedding time). Initially, simple linear regression was done to assess the association between the dependent variable (shedding time) and each predictor variable. Predictor variables with a p-value of <0.1 from the simple linear regression were added to the multiple linear regression model. We determined differences in shedding time by age, sex, hospital admission status and province. We reported effect estimates and 95% confidence intervals. We considered a p-value of less than 0.05 as statistically significant.

Ethical Considerations

We received ethics approval from the Human Research Ethics Committee at the University of the Witwatersrand (Clearance Certificate No. M210443).

RESULTS

Characteristics of the study population

There were 1 057 161 COVID-19 cases reported from 5 March to 31 December 2020 in South Africa. Out of these cases, 2752 (0.26%) met the inclusion criteria. **Figure 1** shows details of the participant selection process. The 2752 included participants had a total of 8 642 PCR positive test results with a median of 3 positive tests per participant (range: 2 – 11). The majority (74.7%: 2,057/2752) of the participants had 3 consecutive positive tests. **Table 1** describes the characteristics of the study population. There were more males than females (53% versus 47%). Participants ages ranged from 0-99 years, with a mean of 46.4 years (SD: 17.4). Individuals aged 35-44 years were the most frequently represented age group (22.9%) while 0-14 year-olds were the least frequent age group (1.2%). Of all the participants, 39.9% were inpatients and 60.1% were outpatients. Gauteng province had the highest number of participants with 987 (35.9%) cases and Northern Cape had the lowest with 39 (1.4%) cases.

Shedding time in COVID-19 patients

Table 2 summarizes the distribution of shedding time by age, sex, province, and admission status. The median duration of viral shedding was 17 days, ranging from 1 to 128 days from the date of specimen collection or symptoms onset date. There was no significant difference in shedding time between males (median:16 days, range:1-128) and females (median:17 days, range:1-94) **(Figure 2)**. Viral shedding time between inpatients (median: 16 days, range 1-108) and outpatients (median:17 days, range:1-128) did not differ much either. Also, there was no significant difference in shedding time between age groups. Individuals aged 0-14 years had the lowest shedding time (median:14 days, range:1-72) while those aged 25-34 years had the highest shedding time (median:18 days, range:1-128) **(Figure 3)**. The shortest shedding time was

observed in Eastern Cape with a median of 15 days (range: 1-56) and the longest shedding time was observedin Limpopo with a median of 21 days (range: 1-39).

Factors associated with COVID-19 shedding time

In **Table 3,** we assessed factors associated with shedding time in COVID-19 patients in South Africa. We applied simple and multiple linear regression to compare the association between predictor variables (age, sex, province and hospital admission status) and SARS-CoV-2 shedding time. Following unadjusted analysis, shedding time did not vary with sex and age group. Regarding province of residence, Eastern Cape was considered the baseline for comparisons. Shedding time in Free State and Northern Cape did not differ from shedding time in Eastern Cape. However, shedding time was longer in KwaZulu-Natal (coefficient: 0.3, CI: 0.1 ─ 0.5, P-value: <0.001), Limpopo (coefficient: 0.6, CI: 0.3 ─ 0.9, P-value: <0.001), Mpumalanga (coefficient: 0.5, CI: 0.2 ─ 0.8, P-value: 0.002), North West (coefficient:0.4, CI: 0.1 ─ 0.7, P-value: 0.012) and Western Cape (coefficient: 0.3, CI: 0.1 ─ 0.5, P-value: 0.001). Shedding time was shorter among hospitalized patients compared to outpatients (coefficient: -0.1: -0.2 ─ -0.04, P-value: 0.007).

After adjusting for age, sex and province, shedding time remained shorter for hospitalized patients compared to outpatients (coefficient: -0.14, CI: -0.24 ─ -0.03, P-value: 0.014). Province, sex and age, were not associated with shedding time.

DISCUSSION

This study aimed to estimate the viral shedding time among laboratory-confirmed COVID-19 cases in South Africa. We found that the median shedding time our sample was 17 days from symptoms onset date or date of specimen collection. A study conducted on the Canadian population [16] found that the median shedding time was 19 days which is 2 days more than

our findings among the South African population. Another study conducted in the United States of America reported a median shedding time of 21 days within their population, which is higher than the shedding time we found within our population [17]. The difference in shedding time between South Africa, Canada and the United States could be due to the difference in population characteristics in these geographic locations. Our findings are similar to those reported in a study conducted in China which found that the median shedding time among adults was 17 days [6]. Although our study shows a similar median shedding time to that of China, our study included both children and adults and covered a wider population whereas the study conducted in China only included adults.

Shedding time ranged from 1 to 128 days from the date of specimen collection or symptoms onset date. Our study did not show a significant difference in the median shedding time between males and females and between hospitalized and outpatients. Our study is supported by another study that found no significant difference in the median shedding time between males and females and further reported that prolonged shedding was not affected by signs and symptoms of COVID-19 [18]. Another study conducted on outpatients and inpatients in the United States of America found that the viral shedding time of inpatients was similar to that of outpatients [17].

Our study also shows no significant difference in shedding time between age groups. Age group 0-14 years had a median shedding time of 14 days which was the lowest among other age groups. Age group 25-34 reported the highest shedding time with a median shedding time of 18 days. The slight difference in shedding time between age groups might be due to the fact that people live in families and communities where people of different age groups live together, allowing for transmission to happen within different age groups. Our study found that South

African children had a lower shedding time compared to children in South Korea who were reported to have an average shedding time of 17.6 days [19]. Another study found that children less than 18 years had a shorter shedding time compared to other age groups [17]. The study further highlights that viral shedding can happen in children presenting with symptoms and those without symptoms, with a shedding time of up to 3 weeks (21 days). Children and young adults are more likely to have mild disease and less likely to have severe or fatal COVID-19 compared to adults [18]. The age-related difference in severity of COVID-19 in children and adults could be due to the fact that children have lower intensity of exposure to SARS-CoV-2. Furthermore, it is thought that children have a stronger innate immune system, which acts as the first line of defense against SARS-CoV-2 [19]. Older adults usually have a higher prevalence of comorbidities, such as obesity, hypertension and heart disease, which are associated with severe COVID-19 compared to children [20]. Although children can also experience severe clinical disease, they are less affected by COVID-19 than adults.

In general, testing of is patterns in children are different from those in adults and children are most often tested when they are symptomatic or a contact of a case. SARS-CoV-2 is shed when talking, coughing, sneezing, or even exhaling. Studies also show that the virus can be shed via feces and urine and can continue for several weeks [23,24]. This makes it possible for the virus to spread in schools where they share classrooms and objects and in pre-schools where children are taken care of by the same teacher.

Viral shedding can continue for longer periods in some patients. Two cases had a shedding time greater than 100 days. The cases were a 77-years-old inpatient male who shed the virus for 108 days and a 34-year-old outpatient male who shed the virus for up to 128 days. A study of 38 COVID-19 patients with prolonged shedding reported that the longest shedding time they observed was 118 days, and was a 77-year-old male [25]. It is difficult to determine why

certain individuals have such prolonged shedding and further research is needed to better understand this.

A study conducted on six COVID-19 patients shows that prolonged viral shedding can be observed in both asymptomatic and severe patients [25]. Severe disease, comorbidities, socioeconomic status, old age and male sex have been reported as factors associated with prolonged SARS-CoV-2 viral shedding [13,16,21,26]. Other studies have reported prolonged shedding, however, prolonged shedding was mainly reported in patients with severe disease [18]. The Centers for Disease Control and Prevention (CDC) recommends that people with possible COVID-19 exposure should quarantine for 14 days and people who test positive should isolate for 10 days from the date of symptoms onset [27]. South Africa however recommends 10 days of isolation or quarantine for asymptomatic patients, and patients with mild, moderate or severe disease [28]. Of people infected with COVID-19, only 20% experience moderate or severe disease and require hospitalization. Eighty per cent experience mild COVID- 19 and isolate from home. Also, it has been reported that asymptomatic or pre-symptomatic cases are the main drivers of COVID-19, accounting for about 50% of new COVID-19 infections[26].

Therefore, it is important to monitor viral shedding in patients with mild disease because they can shed the virus longer and could potentially be a source of future COVID-19 outbreaks. Our findings indicate that infection control strategies provided by CDC and the NDoH should take into account prolonged shedding and its impact on disease transmission.

Our study found a significant association between hospitalized patients and shedding time. Inpatients had 1 day less shedding time compared to hospitalized patients. Studies show that COVID-19 patients who are hospitalized usually have COVID-19 symptoms, comorbidities and

are more likely to be older [30,31,32,34]. In line with another study, our study found no association between age and shedding time [35]. However, some studies have reported that older age is associated with prolonged shedding [36,37]. Furthermore, male sex has been reported to have an association with prolonged shedding however, our study found no association between sex and viral shedding time [38]. Several studies have reported no association between viral shedding time and sex [37, 39,40]. Although the variable province was not statistically significant, the association between shedding time and province varied. KwaZulu-Natal, Gauteng, Limpopo, North West, Mpumalanga, and Western Cape had a significant association with shedding time. The differences in shedding time between different regions may be due to differences in comorbidities, socioeconomic status, type of test used, sample size and disease severity.

Our study had some limitations. Firstly, data received from the NMCList did not include clinical information about the cases. Secondly, due to the secondary nature of the study, we could not get certain variables that could have assisted in determining other factors affecting shedding time within the population. Lastly, viral shedding before symptom onset and before testing was not measurable using the data we obtained. It is likely that viral shedding occurs beyond the time points we have described. It is also possible that the shedding time presented in our study is likely to be an underestimate of the true shedding time in the population.

CONCLUSION

The duration of viral shedding within the population of South Africa varies from 1 to 128 days. Hospitalized patients are associated with SARS-CoV-2 shedding time. Our findings indicate that infection control strategies should take into account factors affecting shedding time such as disease severity.

NOTES

Conflict of interest

No conflict of interest declared

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Author contributions

Carroll Tshabane and NV Motaze conceived the study question. All authors reviewed and approved the study protocol.

Carroll Tshabane conducted the study, analyzed the data and drafted the manuscript. Co-Authors were responsible for study supervision and reviewing the manuscript.

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TABLES

Table 1: Baseline characteristics of COVID-19 patients in South Africa, 2020

	Number of cases (n)	Percentage (%)
Age Group (years)		
0-14	46	1.7
15-24	172	6.3
25-34	516	18.8
35-44	630	22.9
45-54	547	19.9
55-64	418	15.2
65+	419	15.3
Sex		
Male	1,456	52.9
Female	1,291	46.9
Unknown	5	0.2
Admission status		
Inpatient	1,099	39.9
Outpatient	1,653	60.1
Province		
Eastern Cape	283	10.3
Free State	70	2.5
Gauteng	987	35.9
KwaZulu-Natal	603	21.9
Limpopo	94	3.4
Mpumalanga	95	3.5
North West	94	3.4
Northern Cape	39	1.4

Western Cape	487	17.7
Number of positive tests		
2	255	9.3
3	2,057	74.7
4	314	11.4
5	86	3.1
6	20	0.7
7	12	0.4
8	3	0.1
9	4	0.2
11	1	0.04

Table 2: Shedding time of laboratory-confirmed COVID-19 cases in South Africa, 2020

	Number of cases n(%)	Median (Range)
Overall shedding time	2 752 (100)	17 (1-128)
Age Group (years)		
0-14	46 (1.7)	14 (1-72)
15-24	172 (6.3)	16 (1-56)
25-34	516 (18.8)	18 (1-128)
35-44	630 (22.9)	17 (1-78)
45-54	547 (19.9)	17 (1-84)
55-64	418(15.2)	15 (1-94)
65+	419 (15.3)	16 (1-108)
Sex		
Male	1,456 (53)	16 (1-128)
Female	1,291 (47)	17 (1-94)
Admission status		
Inpatient	1,099 (39.9)	16 (1-108)
Outpatient	1,653 (60.1)	17 (1-128)
Province		
Eastern Cape	283 (10.3)	15 (1-56)
Free State	70 (2.5)	16 (1-67)
Gauteng	987 (35.9)	17 (1-128)
KwaZulu-Natal	603 (21.9)	17 (1-84)
Limpopo	94 (3.4)	21 (1-39)
Mpumalanga	95 (3.5)	20 (1-78)
North West	94 (3.4)	16 (1-54)
Northern Cape	39 (1.4)	16 (1-51)
Western Cape	487 (17.7)	17 (1-84)

Table 3: Univariate and multivariate analysis of factors affecting viral shedding time in COVID-19 patients in South Africa, 2020

	Bivariate			Multivariate		
	Coefficient	**95%CI**	**P-value**	**Coefficient**	**95%CI**	**P-value**
Age Groups (years)						-
0-14		Ref*				
15-24	-0.2	-0.6 - 0.2	0.376			
25-34	-0.01	-0.4 - 0.4	0.952			
35-44	0.1	-0.3- 0.5	0.696			
45-54	0.03	-0.4 - 0.4	0.870			
55-64	-0.1	-0.5- 0.3	0.488			
65+	-0.1	- 0.5 – 0.3	0.506			
Sex						-
Male	-0.3	-0.1 - 0.07	0.576			
Female		Ref*				
Admission status				-0.1	-0.2- -0.03	**0.014**
Inpatient	-0.1	-0.2 - -0.04	**0.007**			
Outpatient		Ref*				
Province						-
Eastern Cape		Ref*				
Free State	0.2	-0.2 - 0.5	0.339			
Gauteng	0.4	0.2 - 0.6	**<0.001**			
KwaZulu-Natal	0.3	0.1 - 0.5	**<0.001**			
Limpopo	0.6	0.3 - 0.9	**<0.001**			
Mpumalanga	0.5	0.2 - 0.8	**0.002**			
North West	0.4	0.1 - 0.7	**0.012**			
Northern Cape	0.04	-0.4 - 0.5	0.857			

Western Cape	0.3	0.1 - 0.5	**0.001**			

*p-values – overall p-values for each exposure variable in the model, CI – Confidence Intervals

Bivariate analysis, Adjusted - Multivariable analysis, Ref* - Reference category

- Not included in the final model because it is not significant (p>0.005)

FIGURE LEGENDS

Figure 1: Flow chart showing the participant selection process.

Figure 2: Distribution of shedding time of COVID-19 cases in South Africa by sex, 2020

Figure 3: Distribution of shedding time by age group (years) in South Africa by sex, 2020

Figure 1

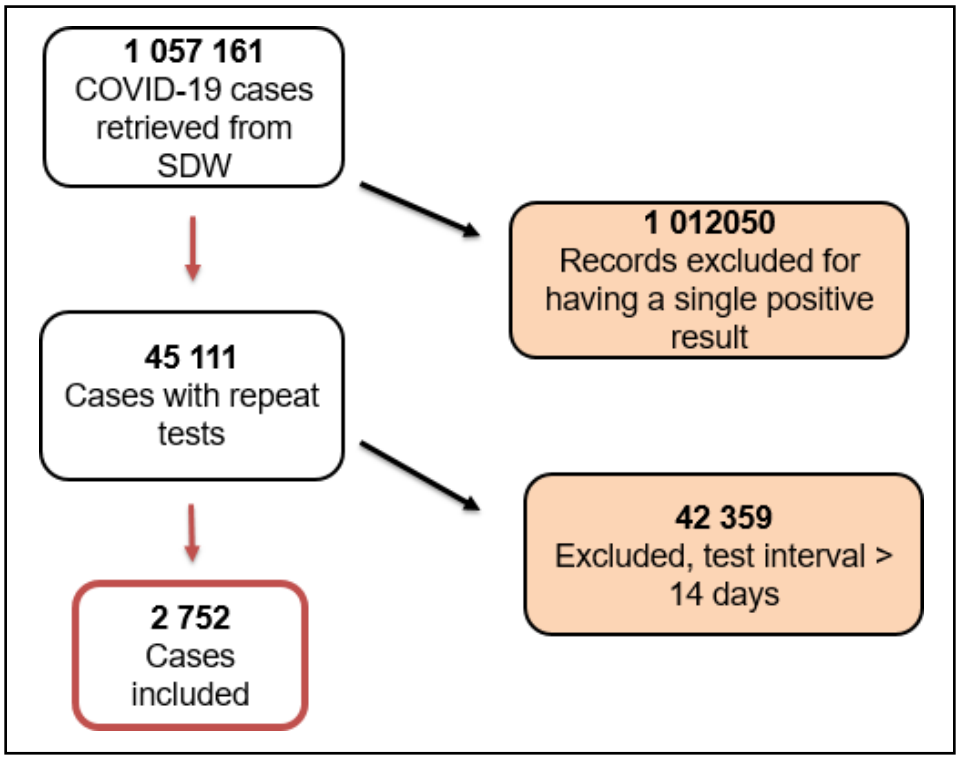


Figure 2

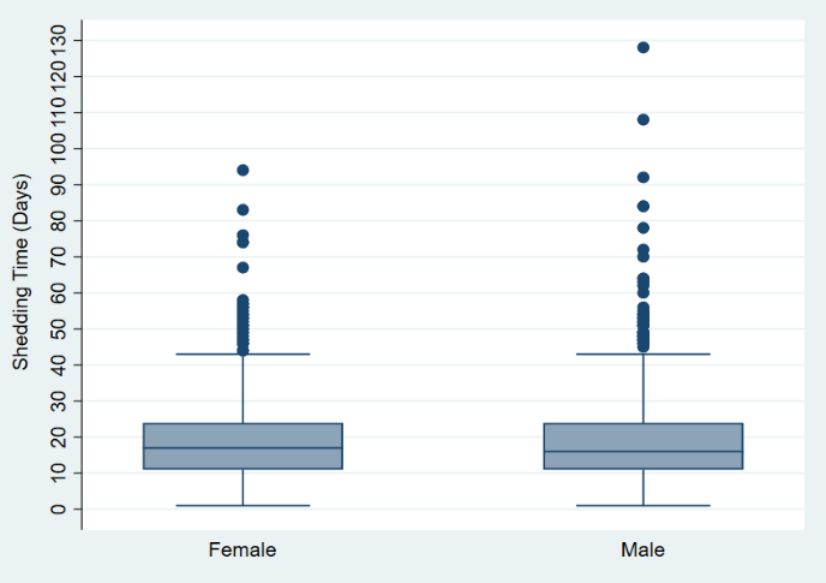
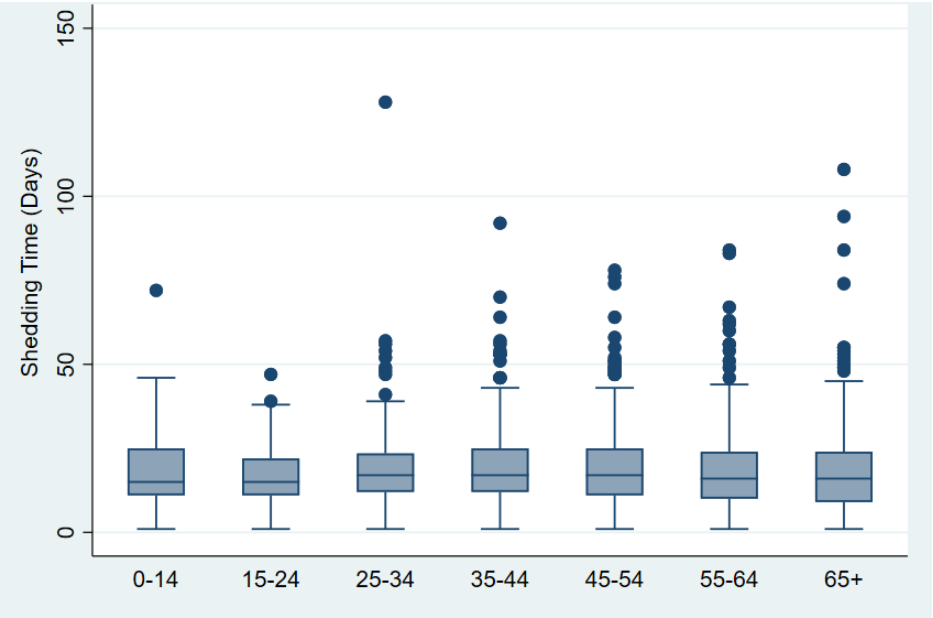


Figure 3


Shedding Time (Days)
0
50
100
150
0-14
15-24
25-34
35-44
45-54
55-64
65+

CHAPTER 3: EXTENDED METHODS

This chapter presents additional research materials and methods that were not included in the manuscript due to the manuscript word count. These include record linkage and ethical considerations.

3.1 Record linkage

We applied a deterministic method to identify repeat test results from the same individuals and to link patients between the NMCList and DATCOV databases. The national identity or passport number was used as the linking variable. The linkage helped us determine patients who were admitted and who were not.

3.2 Ethical Considerations

For further permission to use COVID-19 surveillance data, we obtained ethical approval for the NMC surveillance system from the Human Research Ethics Committee at the University of the Witwatersrand (Approval number: M160667). Privacy of participants was ensured by removing personal identifiers (name, surname, ID number, telephone number, address) and using the unique identifier in the NMCSS line list database. The study data was stored on a password-protected computer and data was only accessible to the study team.

CHAPTER 4: EXTENDED RESULTS

This chapter presents additional findings of the study. The results are presented in percentages and a chart. We provide analysis and interpretation of findings based on the results of the statistical analysis performed.

In the manuscript, we reported that the median number of tests done by COVID-19 patients in our study was 3. Patients who had tested 3 times accounted for 75% (2057/ 2752) of the study population. The highest number of tests done was 11 and were done by 1 patient who was an inpatient. Inpatients had the highest numbers of tests conducted compared to outpatients, with 6 tests done by 65%, 7 tests (91.7%), 8 tests (66.6%), 9 and 11 tests done by inpatients only (**Figure 4.1**).

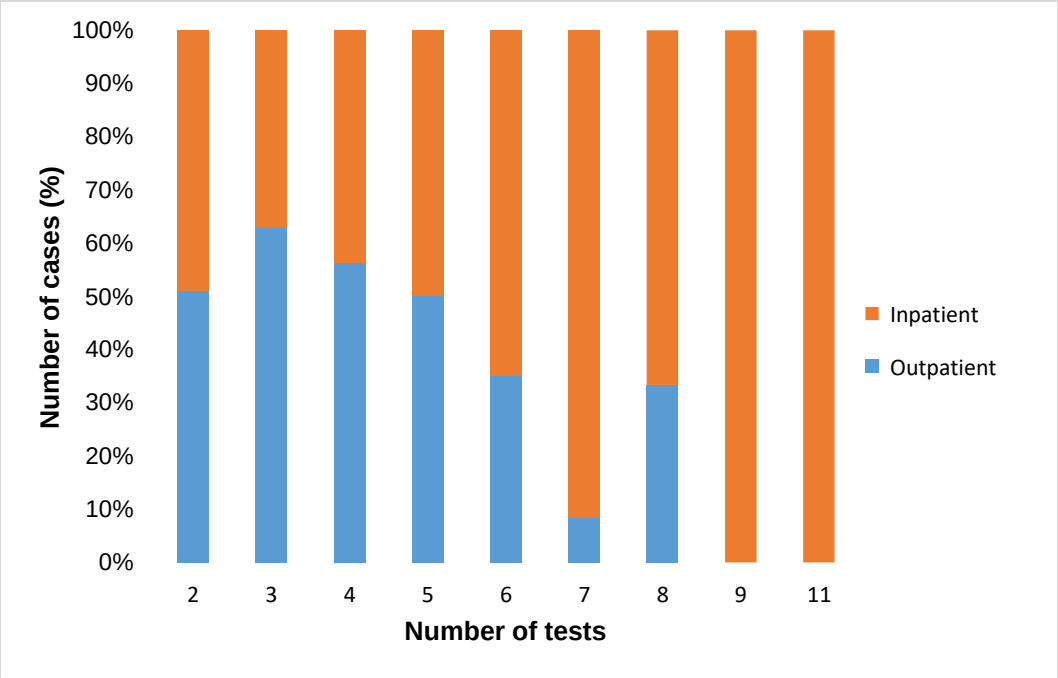


Figure 4.1: Number of positive test results by COVID-19 admission status (inpatients patients and Outpatients) in South Africa, 2020

CHAPTER 5: OVERALL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

This chapter presents the main findings of the study. The results are presented in percentages, charts and tables. We provide analysis and interpretation of findings based on the results of the statistical analysis performed.

5.1 DISCUSSION

Recent guidelines in South Africa states that COVID-19 retesting after the isolation period is not needed[1]. These guidelines were set on the basis that the presence of a detectable virus on a positive case does not necessarily indicate infectiousness. Even though continuous positivity from a PCR-test is not used to indicate continuous infectivity as assumed at the beginning of the pandemic, an RT-PCR test is still the most reliable for SARS-CoV-2 detection and diagnosis[2]. For infection control, symptom and test-based strategies are used in different settings. Symptoms based strategy is done by calculating time passed from symptom onset and test-based strategy makes the use of a negative SARS-CoV-2 RT-PCR result. Furthermore, other factors are used to determine virus transmissibility positivity besides the use of viral load.

Viral shedding time varied within provinces across the country. Eastern Cape observed the shortest shedding time with a median of 15 days. Free State, North West and Northern Cape had a shedding time with a median of 16 days. They were followed by Gauteng, KwaZulu-Natal and Western Cape with a median of 17 days. Mpumalanga and Limpopo observed the highest shedding times with a median of 20 and 21 days, respectively. From the literature explored, factors associated with shedding time may differ according to the setting. This is evident in studies that were done in other countries and regions where we observed varying shedding durations. For example, the median shedding time in Changsha was (17 days)[3], Singapore (12 days)[4], Ethiopia (19 days)[5], Tianjin (17 days)[6] and Netherlands (8 days)[7]. This finding is similar to a study conducted in Germany that reported an association of viral shedding with disease severity which was defined by whether a patient was on mechanical ventilation or not[8]. Whereas Free State and Northern Capewere not statistically significant. Eastern Cape was used as a reference group. The difference in shedding time among provinces could be due to varying socioeconomic status and age distribution.

5.2 LIMITATIONS

In the manuscript, we mentioned the lack of clinical information from the data obtained from the NMCList and the DATCOV system. Although our data contained inpatients and outpatients, we could not report disease severity due to the lack of supporting information such as treatment given to patients. Our study contained a small sample of children, making it hard to generalize the findings of shedding time among children. Since we could not measure viral shedding before testing or before symptoms onset, we assumed that the viral shedding began on the date of symptom onset or date of specimen collection. This could have led to an underestimation of viral shedding time. This study only included cases that tested using an RT-PCR test and excluded cases that were tested using antigen tests. As a result, it is possible that our study might have underestimated shedding time within the population. Furthermore, we only addressed viral shedding time from the upper respiratory specimen sampled from the oropharyngeal and nasopharyngeal. With studies showing that cases also shed the virus using other channels such as feces and urine, we may need to compare shedding time from our study with shedding time from other studies that estimated shedding time using other routes. Since our study used the RT-PCR test, we need to consider the fact that there is a possibility that the positive SAR-CoV-2 results detected on the test may not have a correlation with infectivity. Although our study had limitations, the study design used enabled us to obtain data across all provinces within the country and enabled us to obtain a bigger sample size that is representative of the population. Our study enabled us to explore multiple settings which reduces selection bias.

5.3 CONCLUSION

This study provided details on viral shedding time within the population of South Africa. Our study found a significant association between the hospital admission status of COVID-19 patients and shedding time. There was a 1-day difference in shedding time between hospitalized and outpatients. Therefore, there is a need to strengthen prevention and control strategies such as contact tracing as both hospitalized and outpatients can shed the virus for a similar duration. We discovered that factors such as province, age and sex were not associated with shedding time. The study showed that some people can shed the virus longer than others. Although prolonged shedding might not necessarily indicate infectiousness, individual patient monitoring and management is needed for patients with prolonged shedding. Furthermore, to control SARS-CoV-2 infection,

different approaches that can address transmission risk effectively in asymptomatic patients are required.

5.4 RECOMMENDATIONS

Our study only looked at four predictor variables (sex, admission status, age and province). We recommend that further studies be conducted, including more variables such as comorbidities, socio-economic status, level of education and vaccination status. Since continuous positivity might not necessarily mean infectivity, we recommend that future studies consider evaluating live viruses within the population and also do a following up on cases with prolonged shedding to assess their contacts. This can assist in figuring out infectivity and can be used to inform isolation and quarantine requirements. We also recommend that studies evaluate the impacts of possible symptom recrudescence on shedding time and its impacts on SARS-COV-2 transmission.

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APPENDICES

Appendix 1: Plagiarism declaration

SCHOOL OF PUBLIC HEALTH

STUDENT DECLARATION

PLAGIARISM

I Carroll Tshabane (student number: 2374447) am a post-graduate student registered for the degree / programme Master of Science in Field Epidemiology in the Wits School of Public Health.

I am submitting written work for assessment for the module COHM 7065A Field Based Research Project

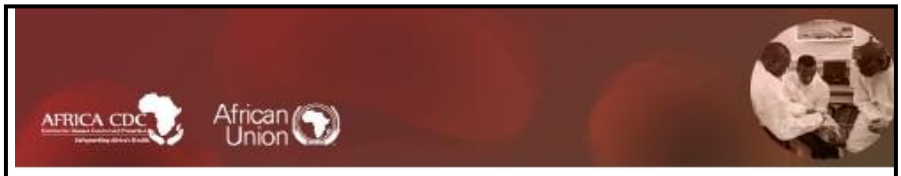
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- I am aware that plagiarism is the use of someone else's work without their permission and/or without acknowledging the original source.
- I am aware that plagiarism is wrong
- I confirm that the work submitted for the above course and module, is my own work, except where I have stated 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 if I have failed to acknowledge the ideas or writing.

Signature: C. Tshabane Date: 25/01/2022

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Appendix 2: Conference Abstract



CPHIA 2021 (2021)

193 Track 1- Epidemiology of SARS-COV-2, virology, prevention and clinical management

Estimation of Shedding Time in Laboratory-confirmed COVID-19 Cases in South Africa, 2020

Applicant details

Institutional Affiliation	National Institute for Communicable Diseases

Abstract details

I confirm that I have read the abstract submission instructions and agree to the terms and conditions for CPHIA 2021 abstract applicants.	✓
First Name	Carroll
Last Name	Tshabane
Title	Ms.
Gender	✓ Female
Age	✓ 25-35
Do you have a PhD?	✓ No
Highest Educational Qualification	Masters
Country	South Africa
Email	ctshabane@gmail.com
Phone Number	+27612588731

Abstract Title

Estimation of Shedding Time in Laboratory-confirmed COVID-19 Cases in South Africa, 2020

Has this abstract been presented or published before? ✓ No

Keywords

COVID-19, SARS-COV-2, laboratory-confirmed, shedding time, PCR

Lead Author: Carroll Tshabane

Co-author(s)

Nkengafac Villyen Motaze, Hetani Mdose, Lazarus Kuonza

Research Year ✓ 2021

Introduction/Background

South Africa recorded its first COVID-19 case on 5 March 2020 and continues to report the largest number of cases in Africa. Determining viral shedding time is crucial in understanding COVID-19 transmission dynamics in South Africa. We aimed to estimate viral shedding time among laboratory-confirmed COVID-19 cases in South Africa.

Methods

We performed a cross-sectional analytic study using COVID-19 data collected in 2020, obtained from the NMCList and DATCOV systems. These platforms report laboratory-confirmed and hospitalized COVID-19 patients, respectively. The study consisted of laboratory-confirmed COVID-19 patients with repeat positive PCR tests (at least two positive PCR test results) and who subsequently tested negative. We defined shedding time as the period from the first positive PCR test to the last positive test prior to a first negative PCR test result. To determine the association between shedding time and predictor variables, multivariate analysis was conducted and a p-value <0.05 was considered statistically significant.

Results

We included 2752 cases. About 39.9% (1099/2752) of participants were inpatients and 60.1% (1653/2752) outpatients. The median shedding time was 17 days (range: 1–128). There was no significant difference in shedding time between males (median: 16 days, range: 1-128) and females (median: 17 days, range: 1–94) and between inpatients (median: 16 days, range 1–108) and outpatients (median: 17 days, range: 1–128). Individuals aged 0-4 years had the lowest shedding time (median: 14 days, range: 1–72). After adjusting for age, sex and province, shedding time was shorter for admitted cases compared to outpatients (coefficient: -0.14, CI: -0.24 — -0.03, P-value: 0.014).

Impact

Our results will assist in refining COVID-19 infection control strategies and assist in the interpretation of repeat positive COVID-19 PCR tests over time. These results may also be used in facilitating early treatment and intervention, leading to a decrease in the incidence and mortality due to coronavirus disease.

Conclusion

The duration of viral shedding within the population of South Africa varies from 1 to 128 days. Admission status is associated with SARS-CoV-2 shedding time. Our findings indicate that infection control strategies should take into account factors affecting shedding time such as disease severity.

Appendix 3: Conference Attendance certificate



International CPHIA 2021 Conference on Public Health in Africa

African Union

AFRICA CDC Centres for Disease Control and Prevention Safeguarding Africa's Health

CERTIFICATE OF ATTENDANCE

This certificate is presented to:

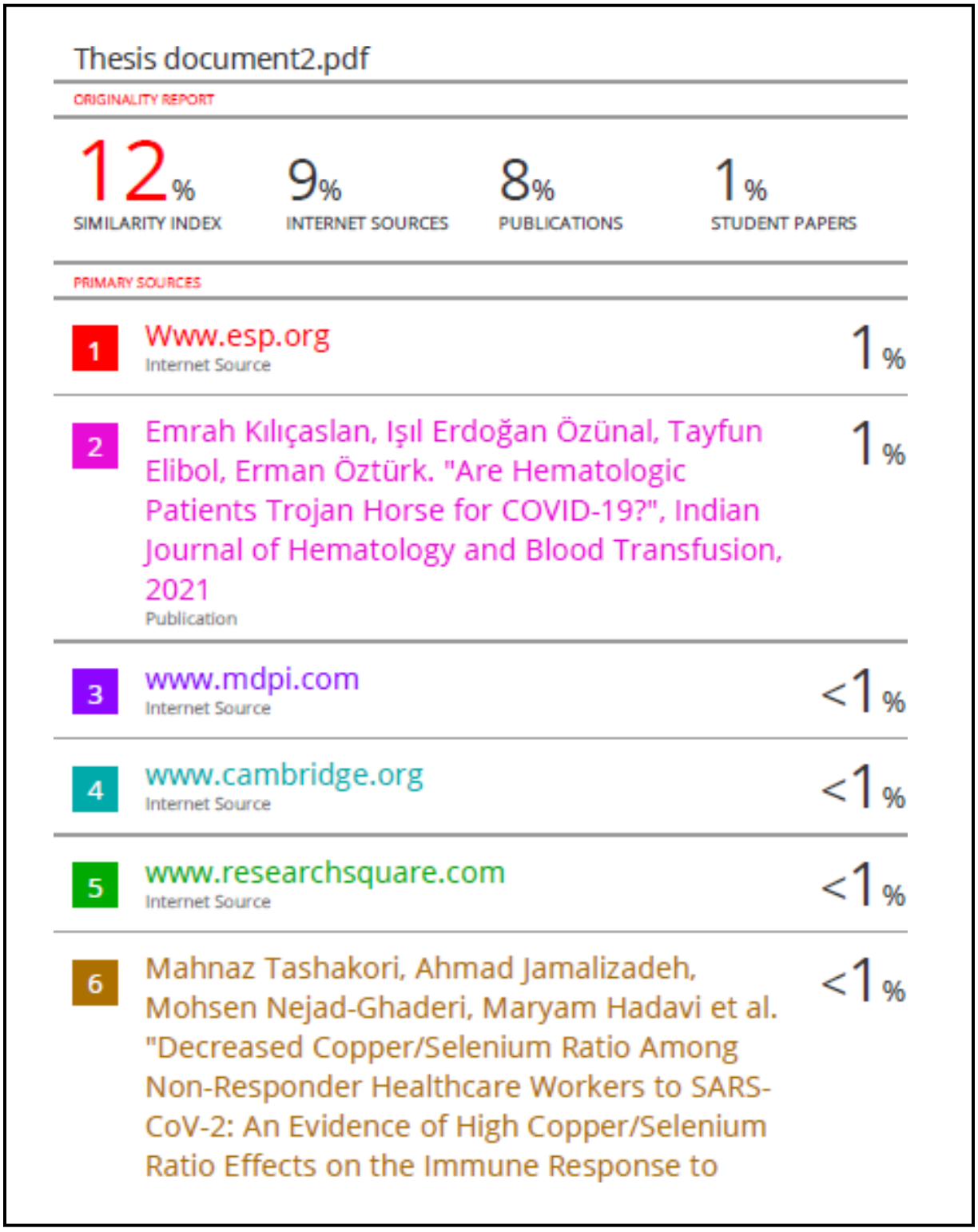
CARROLL TSHABANE

as a participant in the first international Conference on Public Health in Africa (CPHIA 2021) from 14 – 16 December 2021.

Prof. Agnes Binagwaho
CPHIA 2021 Co-Chair

Dr. Senait Fisseha
CPHIA 2021 Co-Chair

Appendix 4: Turnitin Report



Thesis document2.pdf

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Appendix 5: Ethics clearance certificates

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Ms C Tshabane

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M210443

NAME: (Principal Investigator)	Ms C Tshabane
DEPARTMENT:	School of Public Health Division of Epidemiology and Biostatistics Medical School University
PROJECT TITLE:	*Estimation of shedding time in laboratory-confirmed COVID-19 cases in South Africa: A population-based record linkage study, March-December 2020*
DATE CONSIDERED:	2021/04/30
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Messrs V Motaze and L Kuonza
APPROVED BY:	Dr CB Penry, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2021/06/09

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

DECLARATION OF INVESTIGATORS

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

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

Signature of Principal Investigator

09 June 2021
Date

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG	HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research and Innovation)

TO: The Executive Director
National Institute for Communicable Diseases
Sandringham

E-mail: AdrianP@nicd.ac.za

CC: Supervisor: Not applicable
<>
and <HREC-Medical Research Office@wits.ac.za>

FROM: Mr Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 2021/08/05

REF: R14/49

PROTOCOL NO: **M210752** (This is your ethics application reference number. Please quote it in all enquiries, oral or written, relating to this study.)

PROJECT TITLE: *Essential communicable disease surveillance and outbreak investigation activities of the National Institute for Communicable Diseases (NICD)*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to Government funding of the University.

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Appendix 6: Clinical Infectious Diseases Guidelines

Available from:

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- Tables/Figures: Data in the text should not be repeated extensively in tables or figures.

Please refer to a recent issue of Clinical Infectious Diseases for guidance on style and layout of articles. Also refer to the Article type section for guidance on relevant information for each article type.

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Clinical Infectious Diseases: Open Access follows a prescribed set of article types with a specific format for manuscript. Type the entire manuscript, including figure legends, tables, and references, in font 12, in Times New Roman, double-spaced using Microsoft Word. Leave 1-inch margins on all sides. Manuscripts should be written succinctly and should cite select references that are directly relevant. For guidance on length of each article, see section on article types below.

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Manuscripts should be double-spaced throughout, including the references and the table and figure legends, with 1-inch margins on each side. All pages, except for the figures, should be numbered in the lower right-hand corner of the page, with the title page as page 1. The recommended layout is as follows: title page, abstract, text, acknowledgments, references, tables, figure legends, and figures.

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All manuscripts, including Correspondence, should have a title page that includes the following information:

1. A concise, informative title
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3. Up to 5 keywords
4. A running title of no more than 40 characters and spaces
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It is editorial policy to list only one author for correspondence. We do not accept co-first authors, nor co-corresponding authors. However, it is acceptable to state that "author X, author Y, etc. contributed equally to this manuscript."

Any changes of address may be given next to the Affiliations or in the Acknowledgments. Any deletions or additions to the author list after submission of the paper must be submitted in writing, and signed by all authors.

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The second page of the manuscript should contain the Abstract. Please refer to the Article Type for Abstract formats. The Abstract should be comprehensible to readers before they have read the paper and should not contain reference citations.

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Authors of the following article types are allowed to consider including a graphical abstract or video abstract as part of the article, in addition to the text abstract.

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Multimedia abstracts are a visual summary of the main article findings and often take the form of graphical images or videos. They display in addition to the text abstract and offer readers a quick browsing method, in addition to providing you with an asset to share and discuss your findings. The abstract is considered a part of the article and should be submitted with the full article; they are peer reviewed and published online and in PDF.

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Non-standard abbreviations should be kept to a minimum. They should be defined at the first occurrence and introduced only where multiple use is made.

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Authors are encouraged to follow the Uniform Requirements for Manuscripts Submitted to Biomedical Journals. They should strive for a concise article without excessive detail (word limits are specified under Categories of Articles). All but the shortest articles should have subheadings.

Funding

Details of all funding sources for the work in question should be given in a separate section entitled "Funding." This should appear before the "Acknowledgment" section.

The following rules should be followed:

- The sentence should begin: "This work was supported by …"
- The full official funding agency name should be given, ie "the National Cancer Institute at the National Institutes of Health" or simply "National Institutes of Health" not "NCI" (one of the 27 subinstitutions) or "NCI at NIH." Please see here for a full RIN-approved list of UK funding agencies.
- Grant numbers should be complete and accurate and provided in brackets as follows: "[grant number ABX CDXXXXXX]"
- Multiple grant numbers should be separated by a comma as follows: "[grant numbers ABX CDXXXXXX, EFX GHXXXXXX]"
- Agencies should be separated by a semi-colon (plus "and" before the last funding agency)
- Where individuals need to be specified for certain sources of funding the following text should be added after the relevant agency or grant number "to [author initials]."

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In order to meet your funding requirements authors are required to name their funding sources, or state if there are none, during the submission process. For further information on this process or to find out more about CHORUS, visit the CHORUS initiative.

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Personal acknowledgment should precede those of institutions of agencies. Any substantial assistance in preparing the manuscript—for example, in data retrieval or statistical analysis—other than by an author should be stated.

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- Gorecki DC, Monaco AP, Derry JMJ, Walker AP, Barnard, EA, Barnard, PJ. Expression of four alternative dystrophin transcripts in brain regions regulated by different promoters. Hum Mol Genet, 1995 ; 155: 505-511.
- Francis V, Bastin M. (2000) Gene targeting in rat embryo fibroblasts promoted by the polyomavirus large T antigen. Nucleic Acids Res [in press].
- Maniatis T, Fritsch EF, Sambrook J. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, 1982 .
- Huynh TV, Young RA, Davis RW. DNA Cloning. In: Glover DM. DNA Cloning - A Practical Approach. Vol 1. Oxford, UK: IRL Press; 1988 :49-78.
- Public Health Service Task Force. Recommendations for the use of antiretroviral drugs in pregnant HIV-1 infected women for maternal health and interventions to reduce perinatal HIV-1 transmission in the United States. Available at: http://www.aidsinfo.nih.org. Accessed 24 April 2002.
- Lyon DJ, Cheng AFB, Norrby SR. Mechanisms of cefotaxime resistance in blood culture isolates of Enterobacter high prevalence of extended-spectrum β-lactamases [abstract C43]. In: Program and abstracts of the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy (San Francisco). Washington, DC: American Society for Microbiology, 1995:47.
- Pre-print example: Bar DZ, Atkatsh K, Tavarez U, Erdos MR, Gruenbaum Y, Collins FS. Biotinylation by antibody recognition- A novel method for proximity labeling. BioRxiv 069187 [Preprint]. August 11, 2016 [cited 2017 Jan 12]. Available from: https://doi.org/10.1101/069187.

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Nomenclature

CID attempts to use the latest widely accepted nomenclature. See Bergey's Manual of Determinative Bacteriology (9th ed., revised, Williams & Wilkins, 1994) and Enzyme Nomenclature: Recommendations of the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology on the Nomenclature and Classification of Enzymes (Academic Press, 1992). Formal terms for virus families, genera, and species should be those approved by the International Committee on Taxonomy of Viruses; see Virus Taxonomy—The Classification and Nomenclature of Viruses: Seventh Report of the International Committee on Taxonomy of Viruses (Academic Press, 2000). This volume also includes standard abbreviations

for virus species. For names and abbreviations of chemical compounds, refer to the Merck Index (13th ed., Merck, 2001). The Editors appreciate the assistance of authors and readers who inform them of changes in nomenclature.

Human Genetic Nomenclature and Notation

For human genes, use genetic notation and symbols approved by the Human Gene Mapping Workshop (see Wain HM, Bruford EA, Lovering RC, Lush MJ, Wright MW, Povey S. Guidelines for human gene nomenclature. Genomics 2002; 79:464–70). Human gene names and loci should be written in italicized capital letters and Arabic numerals. Human protein product names are not italicized. For human mutation nomenclature, see Antonarakis et al. (Recommendations for a nomenclature system for human gene mutations. Hum Mutat 1998; 11:1–3).

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I, Carroll Tshabane, student number [2374447], declare that this Research Report is my own work and that I contributed adequately towards research findings published in the article which is included in my Research Report.

Signature of Student: C. Tshabane **Date:** 25 January 2022

Name of Primary Supervisor: Dr Lazarus Kuonza

Signature of Primary Supervisor: ……Date…28 March 2022……