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Original Article

Bacteriological Profile of Clinical Isolates from COVID-19 Hospitalised and Non-Hospitalised Patients in Bloemfontein

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ABSTRACT

The COVID-19 literature is limited regarding bacterial infections. If bacterial infections drive mortality in respiratory viruses, this has clear implications for patient management. To address this limited literature problem, we review current knowledge on bacterial infections in COVID-19, assess information from past viral respiratory pandemics, and determine the most prevalent infections. We also study antibiotic misuse during the COVID-19 pandemic. The findings of this study conclude that there is a need to consider presence or absence of bacterial infection in COVID-19 management. Early determination and antibiotic treatment are of importance, however not all patients who are tested for COVID -19 are also tested for bacterial infections which may exacerbate the disease. Physicians utilised antibiotics as a treatment for COVID -19 for various reasons, this posed a risk of antibiotic overuse and antimicrobial resistance when administered in patients with no bacterial infection. While lack of access to antibiotics could be dangerous in the same vein as its misuse, it is of importance to ensure that these life-saving agents are preserved and used with utmost care.

Keywords: COVID-19, Antibiotics, Bacterial Infections, Patients.

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INTRODUCTION

Background

The study was performed during the COVID-19 period when it was considered a novel public health concern. The seventh human coronavirus, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARSCoV-2), was identified in January 2020 during the current pneumonia outbreak in Wuhan, Hubei province, China. Since then, the virus has spread over the whole planet, Globally, as of 11 November 2022, there have been 630,832,131 confirmed cases of COVID-19, including 6,584,104 deaths, reported to WHO.[1] Middle East Respiratory Syndrome Coronavirus (MERS-CoV), SARS-CoV, and SARS-CoV-2 all cause severe pneumonia with mortality rates of 2.9%, 9.6%, and 36%, respectively.[1]

Origin of SARS-CoV-2

Scientists have argued over the origin of the new coronavirus SARS-CoV-2 ever since it was discovered. It has been hypothesized that SARS-CoV-2 was created by manipulations in a laboratory. Genetic evidence, however, refutes this theory and demonstrates that SARS-CoV-2 did not originate from a previously identified viral backbone.[2]

Source of infection

The infectious sources of SARS-CoV-2 are infected animal hosts and other humans. Bats are considered to be the most likely initial hosts of SARSCoV-2, while pangolins may be the intermediate hosts ref. Likewise, both symptomatic and asymptomatic patients are known to be contagious. However, it is not clear how long virus shedding persists and how transmission might be altered during the natural history of the disease.[2]

Routes of transmission

Respiratory droplet and contact transmission are the main transmission routes for person-to-person spread of SARS-CoV-2. [2] Other potential routes include aerosol and faecal-oral transmissions, which have not yet been confirmed.[2]

Research questions.

1. Why is it important to study bacterial infections in COVID-19 patients?

It is documented that seasonal viral respiratory tract infections have long been linked to an increased risk of bacterial coinfection. Bacterial co-infections are common in viral respiratory infections, and they are a major cause of morbidity and mortality. The incidence of bacterial co-infection in individuals infected with the coronavirus that causes severe acute respiratory syndrome 2 (SARS-CoV-2) is not documented in Bloemfontein which was the epicentre of the Free State province during the pandemic.

2. What does this study add to existing literature?

This study will close the gap by documenting the organisms that were commonly co-infecting COVID-19 patients and their antibiotic profiling in coronavirus patients. There is a need to consider co-infection of SARS-CoV-2 with other pathogens to understand the type of treatment which was offered and optimized at that time.

The aim of the study was to determine the bacteriological profile so as to determine the most common bacterial infections in COVID- 19 patients. The objectiives of the study was to analyse bacterial profiling and identify the most common ones, secondly to assess antibiotic susceptibility profile associated with profiled organisms' isolates.

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LITERATURE REVIEW

Influenza and COVID-19

Bacterial co-infections are known to contribute negatively to the prognosis of patients with respiratory viral infections.[3] Their severity was well studied in pandemics caused by influenza virus but are not entirely comprehended in COVID-19. Influenza, studies have shown that bacterial infections contributed to longer mechanical ventilation, shock and even mortality. Establishing prevalence of bacterial infection in viral respiratory infections is paramount to aid empirical antibiotic therapy and to differentiate whether the pathogenesis is of a bacterial or viral nature.[3]

Annual influenza epidemics are reported to infect about 20% of the population, which leads to severe morbidity and mortality.[4] A well-studied case from the influenza pandemic of 1918.[4] proved that a greater percentage of deaths were due to bacterial coinfections rather than the virus itself. Cases of bacterial coinfection in influenza patients are difficult to deduce clinically due to the similar symptoms of both these diseases. Establishing presence of a bacterial infection is paramount to aid patient outcome and deduce necessity of correct empirical antibiotic therapy.[4]

A systematic review and meta-analysis evaluated bacterial co-infections in influenza pandemics. This review highlights that millions of deaths in influenza pandemics were a result of bacterial co-infection rather than the virus itself.[5] This proves the dire contribution of bacterial co-infections towards mortality and morbidity. This meta-analysis notes that the bacterial co-infections ranged from 2% to 65%, with the most common coinfecting pathogens being *Streptococcus pneumoniae* and *Staphylococcus aureus.*[5]

Dawood et al listed the investigated complications of bacterial coinfections in children as pneumonia, asthma exacerbation, dehydration, empyema, encelopathy, sepsis, acute renal failure, and myocarditis.[6] An alarming finding of this study was the reorted death toll of 40 patients which the cause was linked to bacterial infections. Dawood and colleagues also proved the substantial contribution of bacterial co-infections in influenza patients.[6] The limitations of this study, however, were that of representation by age. This study focused on hospitalized children only. Our study aims to showcase complications in a wide variety of age groups.

A study by Chertow and Memoli demonstrated that bacterial coinfections and superinfections in influenza pandemics both had negative consequences for the patients.[7] Further noting that indication of empirical antibiotic therapy within four to eight hours of admission lead to reduced mortality. Empirical antibiotic therapy was much necessary in influenza patients, noting the high bacterial infections, this study will evaluate if this approach was indeed followed in COVID-19 patients or the pandemic dictated otherwise.[7]

Effects of bacterial infections are not well understood in patients with COVID-19. In this review we dissect with no opposing interest, studies with objectives to establish the prevalence of bacterial infections in COVID-19 patients to evaluate the burden and extent of this infections in relation to the patient's prognosis. Reviewed hereon are studies with objectives of showcasing a threat of antibiotic misuse and resistance in cases of COVID-19.

A systematic review and meta-analysis by Lansbury evaluated the risks factors, characteristics, prevalence, and etiologies linked to COVID-19 co-infections.[8] In this review, thirty cases from across the globe, spanning from China, Spain, Thailand, and Singapore were included in this review. Thirty-seven of these cases reported for hospitalized patients, two cases were reporting for deceased patients and one for a non-hospitalized patient.[8]

An important finding in this review and analysis, is that overall, 7% of the hospitalized patients had a bacterial infection upon admission.[8] The number increasing to 14% in cases of ICU-only studies. Lansbury compares findings to that of influenza pandemics, noting that bacterial co-infections were less prevalent in COVID-19 than in influenza, where 1 in 4 severe cases of influenza was associated with a bacterial infection, which lead to increased morbidity and mortality.[8]

Lansbury furthermore discerns the bacteria commonly associated with COVID 19 to those associated with influenza pandemics. Highlighting that in influenza the common bacteria included *Streptococcus pneumoniae, Staphylococcus aureus* and *Streptococcus pyogenes*.[8] These are bacteria well known to colonize the nasopharynx. This review had indicated that the common bacteria isolated in COVID-19 included *Mycoplasma pneumoniae, Pseudomonas aeruginosa, Haemophilus influenzae* and *Klebsiella pneumoniae*. These are bacteria well known to cause bacterial pneumonia.[8]

These findings were consistent with similar studies investigating bacterial coinfections in COVID-19 patients, with less cases of MRSA, *S. pneumoniae* and *S. pyogenes*.[9] This review by Lansbury however does not include any country from the continent of Africa. With our small study located in Southern Africa, accessing the poorest, difficulty in accessing health care and most hygiene deprived communities, our study is therefore representing for under-developed countries.[8]

Recently, several observational and cohort studies reported that pulmonary complications occurred in (51·2%) COVID-19 patients, of which 82.6% accounted for deaths, and independent risk factors for mortality were male sex, age 65years or older. However, the occurrence of co-infection in death across age and sex cohorts of COVID-19 patients has not been studied yet.[9] The basis of our study data analysis includes analysis by age and sex grouping, to bring about better understanding off the statistical standing of COVID-19. The analyses also include the outcomes of the patients as either discharged, longer hospitalization and death.[9]

SARS-CoV-2, SARS-CoV, and MERS-CoV

Two major coronavirus outbreaks have been witnessed in the past twenty years, the SARS-CoV of 2002 and MERS-CoV of 2012. The most recent outbreak being the 2019-nCoV pandemic renamed as SARS-CoV-2 or COVID-19.[10] According to Raban (2020) there are notable similarities between SARS-CoV and SARS-CoV-2, however the latter is proving to be rapidly spreading. Studies suggest that this is due to the structural differences among the coronaviruses.[10] Coronaviruses were believed to only cause mild and self-limiting respiratory infections in humans until the outbreak of SARS-CoV-2 or COVID-19.[10]

Raban reported that the Severe Acute Respiratory Syndrome (SARS) epidemic in the Guangdong Province of China in 2002–2003 was caused by the SARS-CoV virus. It is regarded as the most serious illness brought on by any coronavirus.[10] The fatality rate during the SARS-CoV epidemic was 9%. A total of 8098 SARS cases were documented during this epidemic, and 774 of these individuals succumbed to the virus. The death rate among the older population was higher (50%) (over 60 years). In addition to greater mortality, this outbreak had a shockingly large economic impact, costing over $40 billion globally, mostly in Southeast Asia and Toronto, Canada.[10]

The MERS-CoV outbreak followed the SARS-CoV outbreak as the subsequent coronavirus outbreak.[10] 2012 saw an outbreak of this disease in the Middle East (Saudi Arabia) as indicated in table 1. In Saudi Arabia and other Middle Eastern nations, MERS-CoV caused serious illnesses in the respiratory system of those who contracted it. MERS-CoV had an early fatality rate of roughly 50%.[10] However, by 2013, the outbreak had not worsened and just a few rare cases had appeared. Over 200 new cases were recorded in April 2014, and there

Table 1: The notable differences of three novel coronavirus outbreaks.

	SARS-CoV-2	SARS-Cov	MERS-Cov
Outbreak beginning date	December 2019	November 2002	April 2012
Location of the first case	Wuhan, China	Guangdong, China	Saudi Arabia
Confirmed cases	595 800 (Mar 27,2020)	80%	2519 (from 2012 until January 31,2020)
Mortality	27.324%	744 (10%)	866 (34.4%)
Time to infect 1000 people (days)	48	130	903
Incubation period (Days)	7-14	2-7	5-6
Transmission	Touching, eating an infected, yet unidentified animal. Human-to-human transmission occurs through close contact	Believed to have spread from bats, which infected civets. Transmitted between humans through close contact	From touching infected camels or consuming their milk or meat, Limited transmission between humans through close contact

#Differences between SARS-CoV-2, SARS-CoV, and MERS-CoV [10]

were roughly 40 fatalities.[10] This was brought on by better case reporting and diagnosis, as well as a rise in camel births that year. According to estimates from the European Center for Disease Prevention and Control, there were 855 cases of MERS-CoV as of August 27, 2014, and 333 of those cases resulted in death, representing a mortality rate of roughly 40% (CDC,2020). According to the most recent information from the WHO, 866 MERS-CoV patients died out of the 2519 cases that were recorded globally, for a death rate of 34.4%.[10]

The world is facing the third coronavirus outbreak but literature regarding bacterial coinfections in SARS-CoV and MERS-CoV is lacking.[10] Potential literature which could have contributed to the in-depth understanding of the coronavirus family, aided the management of COVID-19, and reduce its rapid spreading.[10] This study therefore proves to be necessary to prevent misinformation and antimicrobial misuse in future.

Antibiotic misuse

Evidence of antibiotic misuse & overuse in the current COVID-19 pandemic

The capacity of bacteria to grow in the presence of drugs that are typically active against them is known as antibiotic resistance, which is a subset of the larger AMR.[1] The main cause of antibiotic resistance is antibiotic usage, and both community and hospital settings are important ecological niches for its formation in human health. In truth, the selection of resistant bacteria is influenced by the selective pressure applied anytime an antibiotic is taken, whether rationally or irrationally. [1] Resistant bacteria may persist in an organism for at least a year after being selected, and they can transmit from person to person directly or indirectly through the food chain and the environment. [1]Antimicrobial resistance has become a global calamity that has gone unaddressed. The ideas of antimicrobial stewardship activities are supported by proper prescription and effective use of antibiotics, together with superior diagnosis and treatment, infection reduction, and prevention.[3] During the ongoing coronavirus disease 2019 (COVID-19) pandemic, there are several worries that might affect antimicrobial stewardship efforts and encourage drug resistance.[3] For instance, a lot of patients who have a mild sickness without pneumonia or a moderate illness with pneumonia are given antibiotics.[3]

A review which aimed to identify the frequency and etiologies of bacterial infection presented upon admission (coinfection) and acquired during hospital stay for COVID-19 patients.[11] This review established that bacterial coinfections were present in less than 4% of the patients. In this review, ten studies with a minimum of 100 patients were evaluated, majority of these studies demonstrated that less than 4% of coinfections but 57% of patients in these studies received empirical antibiotic therapy targeting MRSA and a further 15% received therapy targeting *P. aeruginosa*. The gap between patients presenting with a bacterial infection and patient receiving bacterial therapy proves substantial unnecessary use of antibiotics.[11]

Another study of interest which reiterated on both bacterial infections and secondary infections, antibiotic misuse, and threat of antibiotic resistance .[12] It is deduced by Langford and colleagues those bacterial co-infections accounted for 3.5 % cases of COVID-19 patients.[12] Whereas infections acquired during hospitalization occurred in 15% of COVID-19 patients. This study is insightful and precise as the infections were discerned as to whether they were present on admission or acquired during hospital stay.[12] This allows for more accurate observation and analysis. An important finding in the Langford study, is that 71% of the patients were treated with antibiotics despite the rate of bacterial coinfection being so minute.[12]

Antimicrobial resistance has become a global calamity that has gone unaddressed .[13] A tiny number of published data, especially from Chinese institutes, that demonstrated that COVID-19 patients frequently get antibiotics have been used to support this argument.[13] According to a recent study, 93 and 100% of ICU and non-ICU patients, respectively, really used antibiotics and antivirals.[13] The results of the study indicate a 90% overuse rate for antibiotics, with just four (10%) of the 41 individuals in the study developing a secondary disease.[13]

In a manner like this, a retrospective cohort study found that antibiotics were given to 95 and 93% of all patients, respectively, non-survivors, and survivors (REF). Surprisingly, only 21% of patients received antiviral medicine, with survivors and non-survivors receiving 22 and 1%, respectively. In addition, the investigators noted that 50% of non-survivor patients experienced recurrent (bacterial) illnesses.[14]

Chen et al. provided evidence of similar findings, showing that 71% of patients got antibiotic treatment, with 45% of these patients receiving combination medication as a preventative measure against common infections.[14] Only 1% of patients had bacteria identified in the lab, with one patient having *Klebsiella pneumoniae, Acinetobacter baumannii,* and *Aspergillus flavus*.[14] The study also revealed that this A. baumannii had a high level of antibiotic resistance.

The ineffectiveness of the antibiotics may thus be attributed to resistant bacteria, even if it is conceivable that the timing and length of each patient's antibiotic therapy, as well as their co-morbidities, significantly influenced the results.[13] Similar to this, a meta-analysis by Clancy et al. found that 32% of COVID-19-positive individuals worldwide died as a result of bacterial lung superinfections.[13] The authors also demonstrated that 79% of patients received antibiotic treatment, with A. baumannii, P. aeruginosa, *K. pneumoniae, E. coli,* and *S. aureus* being the most common causative agents. These results sufficiently demonstrate the extremely undesired and seemingly ineffective antibiotic therapy in COVID-19 patients.[13]

Given that COVID-19 is typically brought on by a virus that results in a self-limiting infection, antibiotic use in COVID-19 patients,

particularly when bacterial infections are not laboratory confirmed, is a sign of a widely acknowledged global pattern of antibiotic misuse that has aided in the emergence and spread of AMR.[14] Even though it's unclear now whether COVID-19 or the ICU therapies it necessitates cause secondary bacterial infections, it's critical that these infections—in particular, bacterial pneumoniae—be thoroughly identified before antibiotics are given.[14] This is crucial to prevent the abuse and overuse of antibiotics, which leads to the development and spread of AMR.[14]

METHODOLOGY

Study location

The study was executed in one of the private laboratories in Bloemfontein, Mangaung. Within South Africa's central interior, in the province of Free State, sits the Mangaung Metropolitan Municipality. To its north, Mangaung has borders with the Districts of Lejweleputswa. To its north-east is Thabo Mofutsanyane, while to its south is Xhariep. Mangaung and Lesotho share a boundary to the south-east. Because the laboratory performed COVD-19 testing for the whole Mangaung region and not only Bloemfontein, but we also profiled Mangaung to cater for all patients.[15]

One of South Africa's eight metro areas, Mangaung is easily reachable thanks to the N1, N6, and N8 as well as Bram Fischer International Airport's national infrastructure. At this time, Mangaung is home to 276 905 households and 861 651 total residents. 36.6% of people live below the poverty line, according to statistics. This municipality is the most unequal, with a Gini Coefficient of 0.62 for the Metro. Due to its huge rural area, this is reflected. Lower than both the provincial and national averages, Mangaung's unemployment rate was 25.3%.[15]

Population

The population of Mangaung Metropolitan Municipality increased from 853 141 in 2018 to 861 651 in 2019. As of 2019, the growth rate has decreased from 1.6% in 2011 to 1.0%. Bloemfontein (63%), Botshabelo (24%), Thaba Nchu (9%), Dewetsdorp and Wepener (1.5%), Soutpan (0.8%), and Van Stadensrus (0.2%) are the areas with the highest concentrations of people.[15]

Gender, Age and Race

The median age of Mangaung is 25, which is comparable to the median age in South Africa. With a total of 274 400 people (31.8%), the young working age (25–44) age group makes up the greatest portion of the population. Young children are the age group with the second-highest population (0-14 years) the elder working age (45–64 years) age category came in second with 156 038 (18.1%), accounting for a total share of 25.6%. With only 64 378 (7.4%) individuals, the retired/old age group (65 years and older) has the lowest population. Mangaung's population consisted of 86% african, 11% white and 4% coloured .[15] Males make up 421 591 (48.9%) of the population, while females make up 440 060 (51.07%). Similar male proportions (48.9%) between the Mangaung metropolitan municipality and South Africa (48.96%) are normal in a stable population.[15]

Health Profile

In the metro, 129 198 persons are HIV positive. For those aged 5 to 14 (10.8%), 15 to 24, and 25 to 64 (18.1%), HIV/AIDS is the main cause of mortality. The top causes of death for people over 65 are cerebrovascular illness, such as strokes (15.4%), ischemic heart disease (10.1%), and lower respiratory infections (8.4%). In Mangaung, lower respiratory infections rank among the top 10 causes of death across all age groups. Preterm birth complications (13.8%), lower respiratory infections (13.3%), and diarrheal diseases (11.7%) are the three main causes of death in children under the age of five.[15]

Study population

The desired study population was patients diagnosed with COVID-19 by qRT-PCR, specifically at the laboratory where the study was executed, which is in Bloemfontein.

Study design

This quantitative study made use of the retrospective design as it involved analysis of Pathcare archived data that was originally collected for reasons other than research, this archived data included laboratory and diagnostic testing reports, and other clinical or administrative data.

This study followed a cross-sectional study design. The cross-sectional study design was best suited because the data was collected from a single point in time, with that point defined by the period the patient tested positive for COVID-19, the variables were identified as COVID-19 and bacterial infections, which were only be observed without influence, the design enabled identification of correlation between COVID-19 and bacterial infections and lastly in this study the frequency of bacterial infections in COVID-19 patients was investigated, qualifying this study as a prevalence study.This cross-sectional study design used of descriptive methods to analyze the data collected, which are measures of frequency, central tendency, dispersion or variation, and position to draw insight from the past data to make it more meaningful.

Stratified sampling was the preferred sampling method in which 217 elements (sample) was randomly selected from a list of all hospitalized COVID -19 patients (population). Stratified sampling was best suited as specific characteristics can be represented by means of dividing data into strata

Sample

From the population of patients diagnosed with COVID-19, the actual data collected was selected based on whether the patient tested for positive for COVID-19 and had also had an MC&S test at the time they tested for COVID-19, with the positive MC&S defined by bacterial growth negative MC&S defined by no bacterial growth.

Sample size

The sample size was calculated with aid of a sample size calculator. With confidence level of 95%, confidence interval of 5 and population of 500, the sample size was calculated to be 217.[16]

Ethical considerations

Ethical approval

This study was conducted with permission from the Faculty of Health and Environmental Sciences and Head of Department with approval by the Pathcare Research Committee.

Sample selection.

Sampling strategy

The sample was selected randomly from the population pool, ensuring all elements have an equal chance to be selected. The elements were chosen randomly then the data was stratified. Stratified sampling was the preferred sampling method in which 217 elements (sample) was randomly selected from a list of all COVID -19 positive patients (population).

PARTICIPATION CRITERIA

Inclusion criteria

The inclusion criteria were based firstly that all the patients must have been diagnosed with COVID-19 by RT-PCR methods and had MC&S results, both and women men between the ages of 18 and 90 years

were included. Another factor was that the patients' medical history is available including any medication administered.

Exclusion criteria

COVID-19 positive results from antigen or antibody serology tests which had no RT-PCR method confirmation was excluded, as well as patients with no captured MC&S results and medical history, and patients younger than 18 years and older than 90 years.

METHODS

Data collection

The Pathcare Laboratory Information Systems (LIS) was used for the data extraction. The system was protected by means of a password-only access to authorized users throughout the study. The Pathcare Information Technology (IT) department was responsible for performing regular maintenance and integrity checks to ensuring that patient data was protected at all times. Upon extraction and anonymisation, the data was recorded on Microsoft Excel on the data sheet that was password protected and did not include patient personal information.

Validity and Reliability

The validity and reliability of the data presented for this study was ensured by the selection of appropriate methods of measurement, sampling consistently and standardizing the conditions of the research. A test-rE-test method was used to ensure the results are the same when repeated. The data was generated from instruments which were regularly quality controlled, and the tests performed according to Pathcare's Standard Operating Procedures.

Statistical Data Analysis

For categorical data, absolute and relative frequencies were determined, and for continuous variables, means and standard deviations (SDs) were calculated. Using X^2 tests or independent t-tests, associations between participant variables (such age and sex) of the two groups and results were looked at. The presence of bacterial infection (yes = 1, no = 2), age (in years), sex (female = 1, male = 2), whether the patient has received empirical antibacterial therapy (yes = 1, no = 2), whether the patient is hospitalized (no = 1, yes = 2), the patient's prognosis in terms of whether the patient recovered (yes = 1, no = 2), and other variables were examined using linear regression.

Binary logistic regression was used to examine patient infections (Is patient infected? yes/no). The patient's bacterial infections type, susceptibility results, and age and sex were all considered. In each instance, odds ratios (ORs), 95% confidence intervals (CIs), and model fits were assessed using pseudo-R^2 (Nagelkerke's R^2) values. A p-value of 0.05 or less was regarded as significant. Data analytics software Microsoft Excel was used for the analyses.

Table 2: Summary of microscopy, culture and culture results among COVID-19 patients.

MC&S Results	Number of patients
Bacterial growth	90 (16 hospitalised; 74 non-hospitalised)
No bacterial growth	127
Total COVID-19 positive patients	217

Table 3: Bacterial infections identified in hospitalised and non-hospitalised patients.

Organism	Hospitalised	Non-hospitalised	Total
Acinetobacter baumanii	3	6	9
Citrobacter koseri	4	6	10
Enterobacter cloacae	2	6	8
Escherichia coli	0	6	6
Haemophilus parainfluenzae	2	8	10
Klebsiella oxytoca	0	8	8
Klebsiella pneumoniae (CPE)	0	6	6
Methicillin R S.aureus	0	3	3
Methicillin Sensitive S.aureus	0	6	6
Proteus mirabilis	0	10	10
Pseudomonas aeruginosa	5	7	12
Stenotrophomonas maltophilia	0	2	2
Sum	**16**	**74**	**90**

Table 3: Bacterial infections identified in hospitalised and non-hospitalised patients.

Organism	Percentage of non-hospitalised patients
Acinetobacter baumanii	8.11%
Citrobacter koseri	8.11%
Enterobacter cloacae	8.11%
Escherichia coli	8.11%
Haemophilus parainfluenzae	10.81%
Klebsiella oxytoca	10.81%
Klebsiella pneumoniae (CPE)	8.11%
Methicillin R S.aureus	4.05%
Methicillin Sensitive S.aureus	8.11%
Proteus mirabilis	13.51%
Pseudomonas aeruginosa	9.46%
Stenotrophomonas maltophilia	2,70%

RESULTS

Prevalence of bacterial infection in COVID-19 patients

In total 217 patients with COVID-19 were profiled, bacterial infections were identified in 41% (n=90) of which 17,78% (n=16) consisted of hospitalised patients and the remaining 82.2 % (n=74) consisted of non-hospitalised patients as indicated on table 2. The value of patients with bacterial infections were significantly lower than that of patients with no identified bacterial infection (both $p < 0.001$).

Prevalence of bacterial infections in hospitalised and non-hospitalised patients

The group of patients with bacterial infections (n=90), were further analysed and all bacterial infections listed, and patients identified as either hospitalized or non-hospitalised. Hospitalised patients accounted for 17,78% (n=16) and non-hospitalised patients accounted for 82,22% (n=74).

Further analysing the hospitalised patient's group as shown on table 3, it is evident the majority of the infections were due to *Pseudomonas aeruginosa*, which was present in 31.25% (n=5) of the hospitalised patients. *Citrobacter koseri* was the second most prevalent at 25% (n=4) followed by *Acinetobacter baumanii* at 18,75% (n=3), both *Enterobacter cloacae* and *Haemophilus parainfluenzae* at 12.5% each (n=2).

The most prevalent bacterial infection in the category of non-hospitalized patients proved to be *Proteus mirabilis* 13.51% (n=10), followed by *Haemophilus parainfluenzae* and *Klebsiella oxytoca* at 10.81% (n=7) each as indicated in figure 1.

The distribution of bacterial infections in both the male and female gender was recorded in table 3. With males accounting for 52.22% (n=47) and females accounting for 47.78% (n=43). The same distribution pattern was noted for the non-hospitalised group as well. We furthermore went into detailed by analysing the prevalence of the

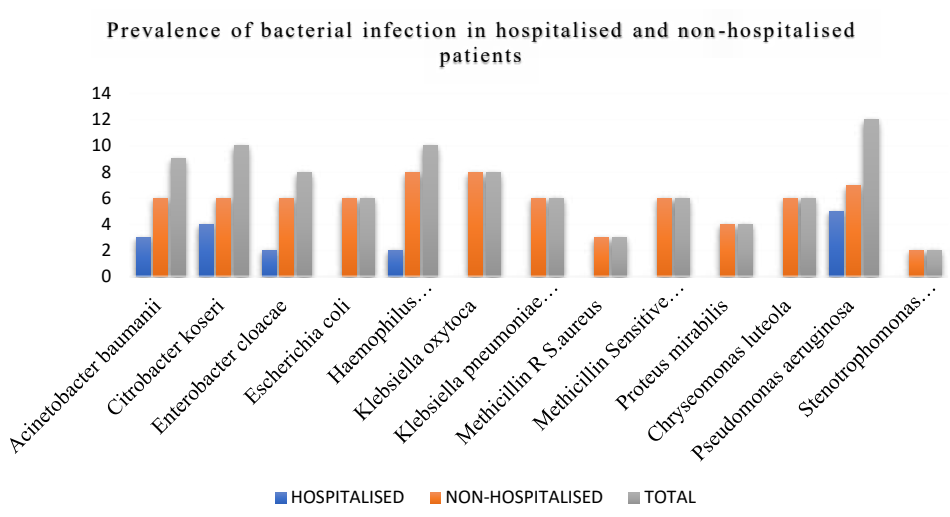

Figure 1: Prevalence of bacterial infections in hospitalised and non-hospitalised patients.

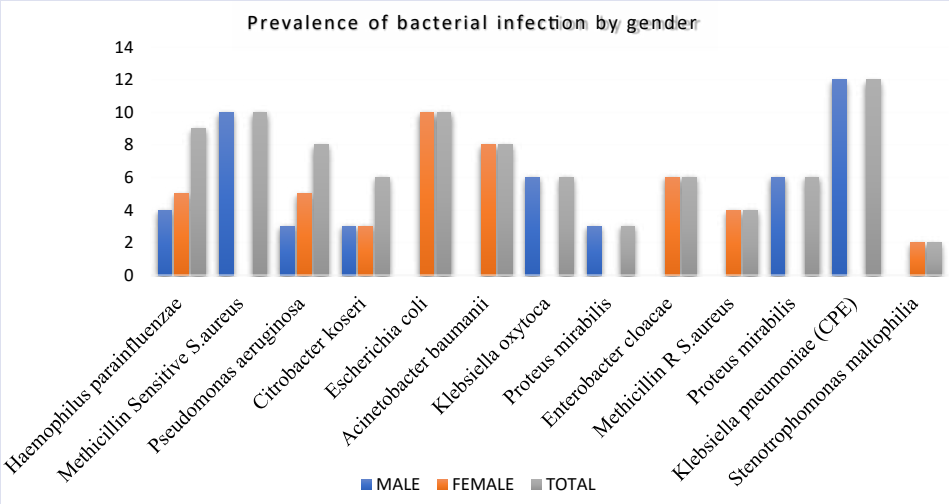

Figure 2: Prevalence of bacterial infection by gender in hospitalised patients.

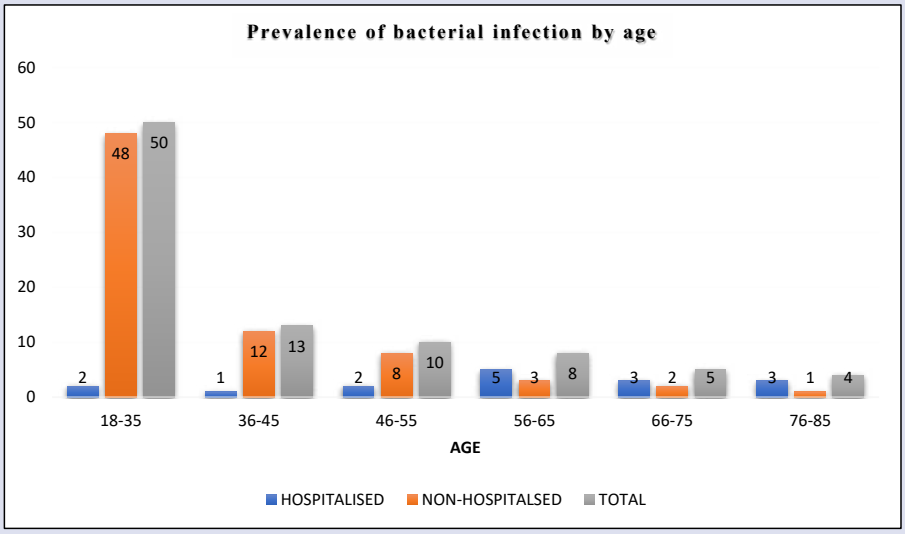

Figure 3: Prevalence of bacterial infection in different age groups.

Table 4: Prevalence of bacterial infection by gender in hospitalised patients.

Organism	Male	Female	Total
Haemophilus parainfluenzae	4	5	9
Methicillin Sensitive S.aureus	10	0	10
Pseudomonas aeruginosa	3	5	8
Citrobacter koseri	3	3	6
Escherichia coli	0	10	10
Acinetobacter baumanii	0	8	8
Klebsiella oxytoca	6	0	6
Proteus mirabilis	3	0	3
Enterobacter cloacae	0	6	6
Methicillin R S.aureus	0	4	4
Proteus mirabilis	6	0	6
Klebsiella pneumoniae (CPE)	12	0	12
Stenotrophomonas maltophilia	0	2	2
Sum	**47**	**43**	**90**

Table 5: Prevalence of bacterial infection in different age groups.

Age	Hospitalised	Non-hospitalised	Total
18-35	2	48	50
36-45	1	12	13
46-55	2	8	10
56-65	5	3	8
66-75	3	2	5
76-85	3	1	4
Sum	16	74	90

Table 6: Antibiotic susceptibility.

Antibiotic	%Sensitive	%Resistant	Antibiotic	%Sensitive	%Resistant
Amoxycillin-clavulanate	61.52	38.48	Erythromycin	46.15	53.85
Ampicillin/amoxycillin	45.15	53.85	Fucidin	15.38	84.62
Cefepime	30.77	69.23	Gentamicin	30.77	69.23
Cefotaxime/ceftriaxone	61.52	38.48	Imipenem	23.08	76.92
Cefoxitin	23.08	76.92	Imipenem E-test	15.38	84.62
Ceftazidime	53.85	46.15	Keftazidiem E-test	93.31	6.69
Ceftazidime-avibactam E-test	53.85	46.15	Levofloxacin E-test	7.69	92.31
Cefuroxime/cefprozil	23.08	76.92	Levofloxasien E-tes	7.69	92.31
Ciprofloxacin	30.77	69.23	Linezolid	15.38	84.62
Clindamycin	61.52	38.48	Meropenem	69.23	30.77
Cloxacillin/cefazolin	46.15	53.85	Meropenem E-test	76.92	23.08
Colistin/polymyxin B mic	46.15	53.85	Minocycline E-test	93.1	6.9
Daptomycin	23.08	76.92	Moxifloxacin	46.15	53.85
Doripenem E-test	7.69	92.31	Piperacillin-tazobactam	46.15	53.85
Ertapenem	23.08	76.08	Rifampicin	15.38	84.62
Ertapenem E-test	38.48	61.52	Sulfa/trimethoprim	69.23	30.77
Tigecycline	69.23	30.77	Teicoplanin	15.38	84.62
Tobramycin	84.62	15. 38	Tetracycline	15.38	84.62
Vancomycin	15.38	84.62			

infections by gender. It was noted that *Klebsiella pneumoniae* was the most prevalent infection in hospitalised males at a shocking 25.53% (n=12). Females were noted to have *Escherichia coli* infection as the most prevalent at 23.26% (n=23.26) as indicated in table 4 and on figure 2.

Another category studied was that of age for both hospitalised and non-hospitalised patients. With the most of infections found in the age group 56-65 years for the hospitalised group, accounting for 31.25% (n=5). It is evident in table 5 that this is the opposite for the non-hospitalised patients, with more infections in 18-35 years old patients, at 64.86% (n=48) as indicated.

Antibiotic susceptibility

Part of the MC&S results included antibiotic susceptibility tests for the identified bacteria. The results are listed in table 6 below. Indicating the percentage of bacteria resistant to these antibiotics is overwhelming.

Antibiotic Misuse

A total of 127 patients out of the 217 profiled patients presented with no bacterial growth, however, 41.73% patients (n=53) as indicted in table 7 were administered empirical antibiotic therapy. With the antibiotics being noted as broad spectrum antibiotics.

DISCUSSION

From analysis of the results obtained in this study, it can be deduced that 41.47% (n=90) of patients with COVID-19 also had a bacterial infection at the time they tested positive. A total of 16 patients of the 90 (17,78%) were hospitalised patients who depended on respiratory support machines. This alarming numbers are significant because bacterial co-infections are known to contribute negatively to the prognosis of patients with the respiratory viral infections. Establishing prevalence of bacterial infection in viral respiratory infections is paramount to aid empirical antibiotic therapy and to differentiate whether the pathogenesis is of a bacterial or viral nature.

Further analysing the hospitalised patient's group, it is evident the majority of the infections were due to *Pseudomonas aeruginosa,* which was present in 31.25% (n=5) of the hospitalised patients. The most prevalent bacterial infection in the category of non-hospitalized patients proved to be *Proteus mirabilis* 13.51% (n=10). *Pseudomonas aeruginosa* is a gram-negative, aerobic, non-spore producing rod that can infect both immunocompetent and immunocompromised hosts with a range of diseases. It is a very difficult organism to treat in modern medicine due to its propensity to infect immunocompromised hosts, high flexibility, antibiotic resistance, and a wide spectrum of dynamic defences.[17] The nature of this organism, an opportunistic organism, explains why it is the most prevalent in an elderly and hospitalised group which qualifies to be deemed as immunocompromised.

Proteus mirabilis is a Gram-negative bacterium which is well-known for its ability to robustly swarm across surfaces in a striking bulls'-eye pattern. Although it is debatable whether P. mirabilis is a commensal, a pathogen, or a transient organism, it is frequently isolated from the gastrointestinal system. While some *P. mirabilis* urinary tract infections (UTI) are known to be transmitted from person to person, the majority are assumed to be caused by germs rising from the gastrointestinal tract. This species can infect the respiratory system, eye, ear, and nose in addition to the urinary tract. According to Groff, 1 in 5 individuals who tested positive for COVID-19 experienced at least one gastrointestinal ailment, such as nausea, vomiting, or abdominal pain. 25.9% of patients who were hospitalized experienced digestive problems.[18]

Males accounted for the most bacterial infection as compared to females, it was noted that *Klebsiella pneumoniae* was the most prevalent infection in hospitalised males at a shocking 25.53% (n=12). Females were noted to have *Escherichia coli* infection as the most prevalent at 23.26% (n=23.26). Another category studied was that of age for both hospitalised and non-hospitalised patients. With the most of infections found in the age group 56-65 years for the hospitalised group, accounting for 31.25% (n=5). It is evident that this is the opposite for the non-hospitalised patients, with more infections in 18-35 years old patients, at 64.86% (n=48).

It is evident that the elderly was more affected and needed hospitalisation as compared to the younger population, this can be attributed to by immunocompromises and comorbidities, the young are strong enough to fight off these bacterial infections without the need for hospitalisation. Similar studies found that, perceived COVID-19 vulnerability seems to decline with age.[19] However, older persons believed that COVID-19 increased their risk of dying.[20] These significant findings prompted the inquiry as to what variables might account for older persons' perceptions of risk.[21] The research has shown that a variety of moderating factors, such as sociodemographic and sociopsychological characteristics, knowledge/experience, and others, can affect an individual's attitudes about hazards.[22]

An important finding from this study was that an alarming 41,73% patients were prescribed and administered antibiotics despite having had no bacterial infection identified by laboratory analysis. Given that COVID-19 is typically brought on by a virus that results in a self-limiting infection, antibiotic use in COVID-19 patients, particularly when bacterial infections are not laboratory confirmed, is a sign of a widely acknowledged global pattern of antibiotic misuse that has aided in the emergence and spread of AMR. The risk of spread of AMR can also be seen in the results of the antibiotic susceptibility testing, majority of organisms were resistant to antibiotics, and this can be due to misuse of antibiotics, incorrect use and self-medicating.

This study has achieved all its objectives, successfully profiling bacterial infections of the population at question, therefore determining prevalent bacterial infections in all categories. However, limitations to this study was that the infection cannot be classified as either co-infection or secondary infection, and we could not analyse the prognosis of this patients as such data was limited to the treating hospital only.

CONCLUSIONS

Bacterial infections are a significant cause of morbidity, mortality, and poor prognosis in patients with viral respiratory tract infections. Early determination and antibiotic treatment are of importance in this case, however not all patients who are tested for COVID -19 are also tested for bacterial infections which may exacerbate the disease. Physicians utilised antibiotics as a treatment for COVID -19 for various reasons, this posed a risk of antibiotic overuse and antimicrobial resistance when administered in patients with no bacterial infection. While lack of access to antibiotics could be dangerous in the same vein as its misuse, it is of importance to ensure that these life-saving agents are preserved and used with utmost care.

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CONFLICTS OF INTEREST

Author declares no conflict of interest. All authors contributed equally to the development of the manuscript.

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