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SARS-CoV-2 Co-infections with Bacteria and Fungi in Symptomatic and Asymptomatic COVID-19 Patients in Rivers State, Nigeria

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Abstract

Background: Ever since the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-COV-2) which caused COVID-19 disease began to spread, the globe has been dealing with an unparalleled public health calamity. The entire health system is under strain as a result of the pandemic. Above all, microbiologists have experienced significant challenges in terms of diagnosis. The intriguing part that was long overlooked at this time was the contribution of bacterial and fungal infections to the severity of COVID-19 infection. Patients with SARS-CoV-2 infection are more predisposed to co-infections with bacteria and fungi as a result of immune system impairment, airway epithelium degradation, reduced mucociliary clearance, and virus-induced airway damage thereby increasing mortality and morbidity.

Methods: A total of 201 COVID-19-positive nasopharyngeal oropharyngeal samples were diagnosed using a rapid diagnostic test kit and confirmed with Reverse transcriptase real-time polymerase chain reaction (RtPCR) with cycle threshold of <=25 from three COVID-19 accredited laboratories in Rivers State was plated out on different culture media and isolates were identified using standard methods.

Results: Seventy-two (36.0%) of the SARS-CoV-2 patients overall exhibited pooled proportions of laboratory-confirmed bacterial and fungal infections. Among the three-study areas, Rivers State University Teaching Hospital (RSUTH) has the highest number of isolates (51.4%) followed by the University of Port Harcourt Teaching Hospital (UPTH) (37.5%) and then the University of Port Harcourt (UPH) (11.10%). Bacteria had a total number of 35.3% and fungi 0.5%. Bacterial isolates identified were *Staphylococcus aureus* (65.3%), *Klebsiella spp* (22.2%), *Pseudomonas aeruginosa* (11.1%) and fungi isolate *Candia albicans* (1.4%). *Staphylococcus aureus* was the most common bacteria co-infection pathogens isolated. Men and single individuals had the highest prevalence while those between the age group of 41-50 also showed the highest occurrence of bacterial and fungi isolates. This study has further confirmed the high coinfection rates of SARS-CoV-2, bacterial and fungi in Rivers State, Nigeria.

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1. Introduction

After the emergence of SARS-COV-2 in 2003, another beta-coronavirus was identified as (SARS-CoV-2) Severe Acute Respiratory Syndrome Coronavirus 2 which causes Coronavirus disease 2019 (COVID-19) emerged. it was initially found in a seafood wholesale market, In Wuhan, China in 2019. Various reports have proposed a number of creatures, including birds, bats, and snakes, as potential intermediate hosts and reservoirs (Rothan et al., 2020). Pangolins could be the intermediate hosts of SARS-CoV-2, but bats are thought to be the most likely original hosts (Shi et al., 2020). Following its global expansion, the outbreak was classified as a Public Health Emergency of International Concern on 30th of January, 2020, after which it was deemed a pandemic by the World Health Organization (WHO) on March 11, 2020 (Sohrabi et al., 2020; WHO, 2020). Across the globe, there have been 603,711,760 documented cases of COVID-19, resulting in 6,484,136 fatalities, leading to a case fatality rate of 1.1% (WHO, 2020).

Africa, being the most recent continent impacted by the Coronavirus, comprises only 3% of the total global infections. COVID-19 was initially confirmed in Africa with the first cases reported in Egypt on February 14, 2020. However, it did not become widespread across the continent until mid-March 2020. Since the confirmation of the first COVID-19 case in Nigeria, both cases and fatalities have been steadily increasing across all 36 states of the country. Between 2021 and 2022, Port Harcourt Rivers State, in Nigeria rose to rank third to fourth among the nation Nigeria impacted areas. Following the Federal Capital Territory (FCT) with 28,763 cases and Rivers State with 16,826 cases, Lagos State has registered 100,641 infections out of the 257,637 cases documented in Nigeria since the pandemic began in February 2020 (NCDC, 2022).

Human to human transmission appears to be the most important route of spread for this virus by direct, indirect, or close contact with infected people through saliva and respiratory secretions expelled by the mouth and the nose as they breathe, talk, cough, sneeze, or sing (Stadnytskyi et al., 2020; Luo et al., 2020: Ghinai et al.,2020; Huang et al., 2020). Indirect transmission occurs when secretions land on surfaces and are touched by a healthy individual who may then touch their nose, mouth, or eyes, allowing the virus entry into the body (Guo et al.,2020). Given that the SARS-CoV-2 virus has been detected in patient faces, stool-based transmission through the faecal-oral pathway may also be feasible (Xu et al., 2020). Some COVID-19 patients frequently experience diarrhoea, which if appropriate sanitation and personal hygiene requirements are not addressed, can develop into a significant mode of transmission (Yeo et al., 2020).

The vertical transfer of the virus from the mother to the foetus or neonate is another contentious element related to the spread of

SARS-CoV-2. Through intrauterine vertical transmission, pregnant women who contracted SARS-CoV-2 in the late stages of their pregnancy, particularly in the last trimester, can pass the virus to their unborn child (Egloff et al., 2020; Hu et al., 2020; Mahyuddin et al., 2020; Vivanti et al., 2020). Human to human transmission has been found to occur not only from symptomatic patients but also from asymptomatic individuals who may not be even aware that they harbour the viral infection (Ahn et al., 2020). studies revealed that there is no difference in the viral load between asymptomatic and symptomatic COVID-19 patients, indicating a potential risk of transmission from asymptomatic individuals.

Patients may be more vulnerable to gastrointestinal and respiratory tract secondary pathogen infections if they have SARSCoV-2. They are sometimes responsible for the increasing COVID-19- morbidity and mortality rate (Cox et al., 2020). Dysbiosis of the gut microbiota has been linked to the intensity and course of this disease. The degree of COVID-19 infection has been positively connected with the reduction of commensals in the gut.

Less research has been done on the effect of concomitant bacterial and fungal infections on the severity of COVID-19 prognosis in the field of COVID-19 infection diagnosis. SARS-CoV-2 and other respiratory viral infections put patients at risk for co-infections, which worsen the condition and increase death. It's plausible that many victims of SARS-COV-2 die due to co-infection with bacteria and fungi instead of the virus itself. Among COVID-19 patients who did not survive, nearly half had co-infections with bacterial, fungal or both. We now know that SARS-COV-2 can compromise human mucosal immunity, which can lead to the emergence of viral-bacterial-fungal co-infection and an inability to control bacterial and fungal replication. Typically, viral infections have the potential to damage both the structure and function of the respiratory tract as they spread through the body.

Additionally, we should be aware of the common bacterial and fungal infections that could exacerbate COVID-19, as well as their expected antibiograms, and closely monitor the rate at which resistant bacterial strains are developing, given the long-term effects of the development of antimicrobial resistance brought on by the needless use of antimicrobial agents (Rawson et al., 2020). In order to treat the majority of COVID-19 patients, it is critical to quickly characterize co-infection. This will enhance antibiotic stewardship in the event of another outbreak and could potentially save lives. In light of recent research assessing co-infections in SARS-CoV-2 patients, we set out to re-assess the existence and incidence of bacterial and fungal co-infections in SARS-CoV-2 infection in Rivers State, Nigeria

2. Material and Methods

2.1. Study area

This analysis was conducted among symptomatic and asymptomatic confirmed COVID-19 patient that visited three major COVID-19 diagnostic centers accredited by Nigeria Centre for Disease Control and Prevention (NCDC) in Port Harcourt, Rivers State, Nigeria. Namely; The University of Port Harcourt Teaching Hospital (UPTH), University of Port Harcourt COVID-19 laboratory (UPH) and Rivers State University Teaching Hospital (RSUTH), all in Rivers State Nigeria. Socio-demographic information of the patients was also obtained using a well-structured questionnaire open data kit (ODK) approved by NCDC (NCDC, 2020).

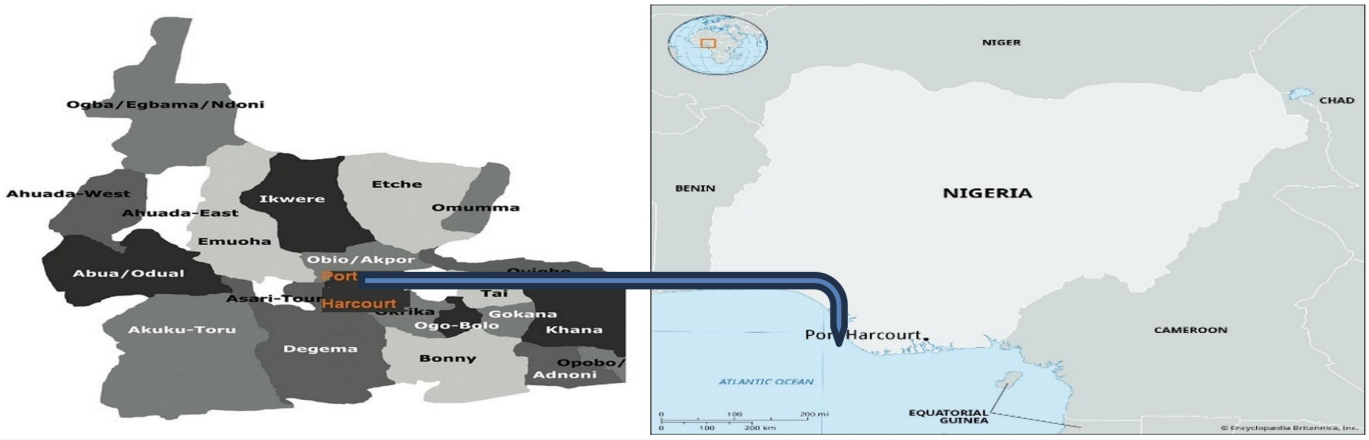

Figure 1. Map of Rivers State (Nigeria) showing study area Source:www.google.com/map of Rivers State (Retrieved on 01/01/2024)

2.2. Study design

This study involves a prospective and a retrospective sample collection of nasopharyngeal and oropharyngeal Swab of symptomatic and asymptomatic confirmed COVID-19 from three major COVID-19 diagnostic center in Port Harcourt Rivers State, Nigeria; University of Port Harcourt, University of Port Harcourt Teaching Hospital and Rivers State University Teaching Hospital. The sample me*et al*-inclusive and exclusive criteria. Information of participants was collected in request forms and was pseudonymized. This cross-sectional study was carried out between the year 2021 to 2022.

2.3. Ethical consideration

Ethical committee of University of Port Harcourt referenced UPH/CEREMAD/REC/MM88/056 and Rivers State University Teaching Hospital referenced RUSTH/REC/2022190 gave approval for this study. Consents were also sought from each of the participants prior to tests and questionnaire administration, and only those individuals that have given full consent were used in the study.

2.4. Inclusion and Exclusion Criteria

Individuals within the ages of 20-70 years who present COVID-19 symptoms as well as those who do not was included in the study. Individuals within the ages of 0- 19 years were excluded from the study. Pregnant and Nursing mothers were also excluded.

2.5. Study Population

Study variables such as: Age, sex, marital status, with or with symptoms, educational background and existing medical condition were collected from patients' data log.

2.6. Data Analysis

All data were collated on excel sheet. Graphs were generated using Excel sheet.

2.7. Sample collection, handling and storage

Nasopharyngeal and Oropharyngeal swab was collected from the mouth and nostril of symptomatic and asymptomatic COVID-19 patient with the aid of flexible synthetic nasopharyngeal and oropharyngeal swab stick respectively. The Nasopharyngeal swab stick was inserted into the nostril and rotated 360^0 c while the oropharyngeal swab stick was inserted into the pharynx, the swab was used to wiped the bilateral pharynx tonsil and the posterior pharynx for 3 second and both placed in a 3 ml Viral Transport Medium (VTM). It was aliquoted in to 2ml Eppendorf Tube placed in a Ziploc Park (Enitan, 2020), transported to the laboratory in a cold chain (NCDC, 2020) and stored in 2-8^0C refrigerator (Zou et al., 2020). The refrigerated sample was allowed to thaw and was diagnosed for SARS-COV-2 using Rapid diagnostic test kits (RDT) and confirmed positive for SARS-COV-2 using Reverse transcriptase real time polymerase chain reaction (RtPCR) in a biosafety cabinet of class II.

2.8. Isolation and Identification of microorganism

Two hundred and one sample (201) confirmed positive for SARS-COV-2 were plated on culture media to identify bacterial and fungi isolates.

2.9. Isolation

Two loop full of 201 positive nasopharyngeal and oropharyngeal sample centrifuged at 1000g for 10 minutes with Cycle threshold (CT) value less than or equal to 25 (<=25) was plated out on the surface of the various prepared dried media plates. All the plates were incubated at 37^oC for 24 hours aerobically with the exception of chocolate agar plate that was incubated anaerobically in a candle jar. At the end of the incubation, the plates were examined for the presence of significant pathogens. Haemolysis was identified on chocolate agar and blood agar plates. Discrete and pure colonies of bacterial isolates were obtained and checked for macroscopic features, stored in Bijour bottles and was used for Biochemical test for proper identification tests.

2.10. Identification and characterization of isolates

The identification of the bacterial and fungi isolates was carried by standard bacteriological methods, which include, the Colony morphology, Gram staining reaction and Biochemical tests. The shape and arrangements of the cell were observed by the use of microscope. Colonial morphology include; the form(shape), the diameter, elevation, surface, Opacity, consistency, surface topography, texture, pigmentation on the reverse, haemolysis and colour (Luis et al., 2020). Bacterial Isolates were subjected to Gram staining, Spore staining, Motility test and biochemical test such while fungi isolates were identified microscopically using Lactophenol cotton blue and germ tube test.

3. Result

The data represented in Table 1 shows the percentage distribution of the isolates and the number of the isolates obtained in each study location. RSUTH has the highest percentage occurrence of isolate (51.4%) and then UPTH (37.5%). The least was seen in UPH (11.0%). *Staphylococcus aureus* has the highest occurrence among the micro-organisms isolated (65.3%). Followed by *Klebsiella* sp (22.2%), then *Pseudomonas aeruginosa* (11.1%). The least was *Candida albicans* (1.4%).

Table 1. Occurrence of co-infected isolates among SARS-CoV-2 infected participants based on study area

ISOLATES	TOTAL	LOCATIONS (%)		
		UPH	RSUTH	UPTH
Staphylococcus aureus	47 (65.3)	6 (12.8)	24(521.1)	17(36.2)
Pseudomonas aeruginosa	8 (11.1)	2(25.0)	0(0.0)	6(75.0)
Klebsiella sp	16 (22.2)	0(0.0)	13(81.3)	3(18.8)
Candida albicans	1 (1.4)	0(0.0)	0(0.0)	1(100.0)
Total	**72 (100.0)**	**8 (11.1)**	**37 (51.4)**	**27 (37.5)**

The data represented in Table 2 demonstrates a notable distribution of the isolates based on the age brackets of the participants. Co-infection of SARS-CoV-2 and other microorganisms were isolated from all age groups. The highest occurrence was seen in age groups 41-50 (34.7%). This was followed by 31-40 (33.3%) and 51-60 (21.0%). The least prevalence occurred among ages 20-30 and 61-70 which had 6.0% rates, respectively.

Table 2 demonstrates occurrence of the isolate based on gender of the participants. The male counterpart (83.3%) has more occurrence of isolates compared to the women (16.7%).

The data represented in Table 2 shows the distribution of the isolate in terms of marital status. The singles have the highest occurrence (52.8%) followed by the married (34.7%) and the least was seen among the widow (12.5%).

Revealed in the Table 2 is the occurrence of isolates based on the level of education of the participants. Those with primary level of education had the highest occurrence (52.8%) followed by the those with secondary level of education (31.9%). The least occurrence was seen among those with tertiary level of education (15.3%).

Table 2. Distribution of occurrence of isolates by sociodemographic characteristics of SARS-CoV-2 infected participants

Variables	No. (%)	*Staphylococcus aureus* (%)	*Pseudomonas aeruginosa* (%)	*Klebsiella* sp (%)	*Candida albicans* (%)
Age groups (years)					
20-30	4 (6.0)	3(75.0)	1(25.0)	0(0.0)	0(0.0)
31-40	24(33.3)	16(66.7)	3(12.5)	5(20.8)	0(0.0)
41-50	25(34.7)	14(56.0)	2(8.0)	8(32.0)	1(4.0)
51-60	15(21.0)	11(73.3)	2(13.3)	2(13.3)	0(0.0)
61-70	4 (6.0)	3(75.0)	0(0.0)	1(25.0)	0(0.0)
Gender					
Males	60 (83.3)	41(68.3)	6(10.0)	13(21.7)	0(0.0)
Females	12 (16.7)	6(50.0)	2(16.7)	3(25.0)	1(8.3)
Marital Status					
Married	25(34.7)	14(56.0)	1(4.0)	9(36.0)	1(4.0)
Singles	38(52.8)	29(76.3)	6(15.8)	3(7.9)	0(0.0)
Widowed/Divorced	9(12.5)	4(44.4)	1(11.1)	4(44.4)	0(0.0)
Level of Education					
Primary	38(52.8)	33(86.8)	1(2.6)	4(10.5)	0(0.0)
Secondary	23(31.9)	10(43.5)	6(26.1)	6(26.1)	1(4.3)
Tertiary	11(15.3)	4(36.4)	1(9.1)	6(54.5)	0(0.0)
Total	**72(100.0)**	**47(65.3)**	**8(11.1)**	**16(22.2)**	**1(1.4)**

Table 3 indicated that those with existing medical condition has more confirmed isolate of bacteria and fungi co-infection (88.9%) than those without existing medical condition (11.1%). Illustration from the Table 3 shows distribution of the isolates based on those that had symptoms of SARS-COV-2 and those without symptoms. Those that develop symptoms had more isolates (83.3%) than those without symptoms (16.7%).

Table 3. Distribution of occurrence of co-infected isolates in SARS-CoV-2 infected participants by medical/clinical conditions

Variables	No. (%)	*Staphylococcus aureus* (%)	*Pseudomonas aeruginosa* (%)	*Klebsiella* sp (%)	*Candida albicans* (%)
Existing Medical Conditions					
Yes	64 (88.9)	43(67.2)	6(9.4)	14(21.9)	1(1.6)
No	8(11.1)	4(50.0)	2(25.0)	2(25.0)	0(0.0)
Clinical Conditions					
Symptomatic	60 (83.3)	43(71.7)	5(8.3)	11(18.3)	1(1.7)
Asymptomatic	12 (16.7)	4(33.3)	3(25.0)	5(41.7)	0(0.0)
Total	**72(100.0)**	**47(65.3)**	**8(11.1)**	**16(22.2)**	**1(1.4)**

4. Discussion

The diagnosis of bacterial and fungal co-infection in the nasopharyngeal and oropharyngeal swab of COVID-19 positive sample was proved through the isolation of bacteria and fungi in this study. Of the 201 positive SARS-COV-2 nasopharyngeal and oropharyngeal sample with CT value < 25 detected using Abbort and Standard Q Rapid Diagnostic Test kits and confirming using

Reverse transcriptase Polymerase Chain Reaction RT-PCR) plated out on the various prepared dried media plates. The overall pooled proportions of SARS-CoV-2 sample confirmed for bacterial and fungal growth were 72 (36.0%). The lesser the CT value the higher the viral load, the more susceptible to Co-infections.

Among the three-study areas, Rivers State University Teaching Hospital (RSUTH) has the highest number of isolates (51.4%), followed by the University of Port Harcourt Teaching Hospital (UPTH) which had 37.5%, then the University of Port Harcourt (UPH) having 11.1%. RSUTH is the reference hospital for almost all the health centres and General Hospitals in the 23 local Government area of Rivers State. It was one of the first Covid-19 diagnostic center in Rivers State accredited by National Centre for Disease Control (NCDC) where all the COVID samples in the state was run. Therefore, RSUTH had more isolates than other COVID-19 testing centres.

UPTH is also a major COVID -19 center and the UPH community COVID-19 lab which had the least acknowledging that it attends mostly to the University community and some oil rig works that has less crowd and low occurrence of SARS-COV-2 infection. Most people that come for Covid- 19 test in UPTH and RSUTH are sick while those that come to UPH are to confirm their status for travel purpose and to go back to rig work.

In this study, bacterial isolate identified were *Staphylococcus aureus* (65.3%), *Klebsiella spp* (22.2%), *Pseudomonas aeruginosa* (11.1%), and *Candia albicans* (1.4%). This is in line with the findings of Fan*et al.* (2023) who discovered that *Pseudomonas aeruginosa*, *Klebsiella spp* and *Staphylococcus aureus* were the most common co-infection pathogens that is relatively in high proportion of patients with COVID-19 (Fan et al., 2023).

Similarly, Ramadan et al. (2020) reported that *Candida albicans* and *Candida glabrata* were the most frequently isolated fungi in Egypt, whereas the most frequently isolated bacteria were *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Staphylococcus aureus*. This study reported Gram-negative bacteria in the majority of the bacteria isolated than Gram-positive. Fontana et al. (2021) found that *Staphylococcus aureus* was the predominant gram-positive species, representing 62.0% of the isolates, followed by gram-negative pathogens like *P. aeruginosa* and *Klebsiella* sp. Bacterial isolate was more prevalent than fungal. Bacteria gave prevalence rate of 98.6% and fungi was 1.4%. According to research by Saeed et al. (2021), co-infection rates with bacteria were significantly higher in critically ill COVID-19 patients (25.5%) than with fungi (10.9%). Comparably, Yang et al. (2021) identified bacteria and fungi from COVID-19 patients in the intensive care unit. Following Chen et al. (2020), bacterial co-infections were present in 50% of COVID-19-related deaths among people during the current pandemic.

Fungi and Bacteria Co-infection may arise from the viruses' ability to aid in the adhesion and colonization of bacteria in the respiratory tract, with SARS-CoV-2 being no exception (Sharifipour et al., 2020). Patients with SARS-CoV-2 infection are more vulnerable to microbial co-infections because of immune system impairment, airway epithelial degradation, decreased mucociliary clearance, and virus-induced airway damage (Mirzaei et al., 2020).

However, in this study, *Staphylococcus aureus* was the most common bacterial pathogens co-infecting SARS-CoV-2 patients. This discovery aligns with the research conducted by Massey et al. (2020), where they observed that 85.6% of all *Staphylococcus aureus* co-pathogens were found in COVID-19 patients. Staphylococcus aureus is recognized for its synergistic role in SARS-CoV-2 patients, contributing to pneumonia and exacerbating both the mortality rate and the severity of the disease (Cusumano et al., 2020). The suggested mechanisms underlying viral-induced Staphylococcus aureus co-infections involve viral alterations to airway structures, enhanced adherence of the organism to respiratory mucosa, and the initiation of immune-suppressive responses. A

poorer prognosis is associated with Staphylococcus aureus infection in COVID-19 patients (Hughes et al., 2020)

Klebsiella sp. was the second isolated bacterial specie in this study. In contrast to patients in critical care units, Montrucchio et al. (2020) found that patients in non-intensive care units had a greater prevalence of Klebsiella sp. Klebsiella pneumoniae was the second most frequent respiratory pathogen found in COVID-19 patients, according to a retrospective analysis *(*Zhu et al., 2020). This bacterium produces a variety of virulence factors and have high levels of antibiotic resistance to antibiotics that contribute to its high mortality rate. A decline in general health in COVID-19 patients was associated with co-infection with this bacterium.

Pseudomonas aeruginosa is the third most identified co-infecting bacterium among the positive COVID-19 sample that was diagnosed in this study (11.1%). This result is in conformity with the findings of Qu et al. (2021) who diagnosed *P aeruginosa* as the third most identified co-infecting bacterium among COVID-19 patients. About 5.1% of all the COVID-19 patients diagnosed with secondary infections, among which 23.8% (5/21, 4 critically illed and 1 severely illed patients) was infected with *P. aeruginosa.* Tissue damage caused by the SARS-CoV-2 virus includes broad alveolar destruction and alveolar epithelial cell shedding (Martines et al., 2020). The tissue impairments and diminished host immunity subsequent to viral infection may potentially provide an opportunity for *P. aeruginosa* to enhance its virulence.

P. aeruginosa, a Gram-negative bacillus frequently detected in lung infections, contributes significantly to morbidity and mortality, particularly in severe cases of chronic lung conditions such as COVID-19. Comparing this bacterium to others present in different medical materials, it is far more virulent. *P. aeruginosa* prefers a moist environment, which is why it is frequently discovered in the respiratory tract. (Eklöf et al.,2020). One of the main causes of the disease's severity is infections by potentially harmful bacteria *P. aeruginosa*, which mimic or block particular receptors and cause host-pathogen interactions as well as damage to the alveolar tree (Eklöf et al., 2019). In COVID-19 patients, *P. aeruginosa* can cause serious chronic infections including pneumonia. *P. aeruginosa* produces unusual but deformed mucoid colonies in SARS-CoV-2 infected patients because of the high amount of elastase generated, which damages host tissue and compromises normal lung function (Cigana et al., 2020).

The only fungal organism isolated in this study was *Candida albicans*. This finding agreed with the study done in Upper Egypt by Ramadan et al. (2020) who found that *Candida albicans* and *Candida glabrata* were the most common fungi isolated in COVID-19 patients in Bahrain. This observation also agrees with that of (Celaya et al., 2023) who also discovered that *Candida albicans* was the most prevalent fungus in his research which were present in 6.3% of the patients. According to other research, patients with COVID-19 had a two-to ten-fold higher prevalence of candidemia than individuals without the virus (Kayaaslan et al., 2021). Additionally, 83.3% of patients with SARS-CoV-2 candidemia had a high mortality rate, despite receiving the recommended antifungal treatment, according to a study (Bhatt et al., 2021). Based on these findings, it is fair to hypothesize that intestinal inflammation may be linked to the prevalence of *Candida albicans* in COVID-19 patients. *Candida albicans* primarily targets endothelium and epithelial cells. To determine how such fungi contribute to the worsening of COVID-19 patients' health conditions, it would be useful to understand their structure, pathogenicity, clinical signs and symptoms, and laboratory diagnosis.

The study showed bacteria and fungi isolate co-infection in SARS-CoV-2 positive samples in terms of age, gender, marital status, educational background, those with or without medical conditions and symptomatic and asymptomatic individuals. *Staphylococcus aureus* showed higher occurrence among age group 41-50 years and least in the age groups 61-70 and 20-30 years. *Pseudomonas aeroginosa* was seen to occur more in the age group 31-40 and least in the age group 20-30 years. None was isolated in the age group 61-70 years. *Klebsiella sp* was predominant in age group 41-50, least in age group 61-70 and none was seen in age group 20-30 years. *Candida albicans* was only isolated from age group 41-50.

In all, isolates were seen among middle age (31-61 years) and decreases in the older age groups (61-70 years). According to Zimmermann and Curtis (2021), younger individuals may have Angiotensin-converting enzyme 2 (ACE2) receptors with a reduced affinity for SARS-CoV-2 and a different distribution throughout body sites, which may make it more difficult for the virus to enter cells and decrease the invasion of co-infection. This incidence is not overlooked, though, even if the quantity and affinity of ACE2 receptors on epithelial cells rise with age and are impacted by a variety of other factors, such as nutrition, underlying medical conditions, smoking, gender, and heredity. It increases the potential for fungal and bacterial infections (Bunyavanich et al., 2020). Moreover, the decreased abundance of ACE2 receptors in the elderly explains the lower number of isolates observed in the older group.

Conditions that can lower immunity in adults, such as obesity, diabetes, hypertension, and chronic kidney, lung, and heart disease, increase the chance of inversion of co-infections (Zhou et al., 2020). People of younger age (20-30 years) have a lower prevalence of co-infections that have been associated with severe COVID-19. Also, younger people have greater proportions of lymphocytes and absolute quantities of T and B cells, whereas ageing is linked to decreased thymic activity and naïve T cells. Adults infected with SARS-CoV-2 usually have lower lymphocyte counts; however, it's possible that higher lymphocyte counts—particularly the large repertoire of naïve T cells that support a robust T cell-mediated immune response—protect the young against SARS-CoV-2, thereby lowering co-infection by bacteria and fungi.

The variations in their oropharyngeal, nasopharyngeal, lung, and/or gastrointestinal microbiota could also account for the less severe co-infection symptoms in younger age groups. In younger individuals, the microbiota is crucial for immune control, inflammatory management, mucosal homeostasis maintenance, and defence against bacterial and fungal infections (Xiao et al., 2020). Advanced age has been identified as an independent risk factor for mortality in patients with COVID-19-associated candidiasis (Kayaaslan et al., 2021).

In the overall, the results of this study showed that COVID-19 was most common in people aged 20 to 61 years, especially in those who had moderate symptoms when they first appeared. This is not surprising as the working-class group falls within this age category and they could have been predisposed to the virus through commuting to work in comparison with the younger age group as well as the elderly who were mostly at home during the partial lockdown period at the time.

Report from this study revealed that males had more occurrence of co-infection than females. This is in line with the report of Anton-Vazquez et al. (2021) who observed that compared to female individuals with SARS-CoV-2, males were more likely to have co-infections. Again, this also agrees with the findings of Sharifpour et al. (2020) where 58.0% of the COVID-19-positive patients with co-infection were male and 42.0% were female. This can be explained by the fact that women are more conscientious about following the guidelines for managing illnesses, attend healthcare facilities more frequently than men do, and have higher standards of sanitation. Another explanation for this might be that women have stronger immunological responses than men do (Takahashi et al., 2020).

Gender disparities also manifest in social behaviour, with gender-based lifestyles such as higher rates of smoking, excessive alcohol consumption, and poor dietary habits observed more frequently among men than women. Smoking affects the level of pre-existing diseases such as heart disease, chronic lung disease and cancer and has a huge impact on the outcome of Coronavirus infection. There may be contributing variables for women's more responsible approach to the COVID-19 pandemic than for men's, including their adherence to stay-at-home instructions and their frequent hand washing and face mask wearing (Bwire 2020). Alsan et al. (2020) revealed that in a nationwide US poll, males washed their hands 3.8 times fewer frequently than women. During

lockdown, men demonstrated a greater inclination to venture outside compared to women, potentially increasing their exposure to SARS-CoV-2 and heightening the risk of co-infection (Alsan et al., 2020).

Comorbidities such as obesity, diabetes and hypertension seen more in men may also affect the course of disease (Di Giosia et al., 2018). Also, in Lakbar et al. (2020) study, men were more likely than women to contract SARS-CoV, and when they do, they also appear to have worse sequelae. These clinical observations align with mouse models indicating that estrogen decreases susceptibility to and severity of SARS-CoV infection; After having their ovaries removed, female mice developed a more severe and contagious illness (Lakbar *et al.*, 2020). Women who contract SARS-CoV often have stronger immunity, which shields them against more severe strains of the virus and prevents co-infections. This is probably because activation of the X regulatory genes results in lower viral loads and greater CD4 T-cell numbers. Compared to men, women have extra immunological traits that provide them an advantage when exposed to viral infections (Conti & Younes, 2020). Toll-like receptor 7 (TLR7), which is known to identify viral RNA, is expressed more frequently in women.

Additionally, in animal models, they also create higher interferon-α, which is linked to lung tissue protection. Differences in the production of IL-6 have also been observed between men and women (Scully et al., 2020). Women's Estrogens are thought to boost the synthesis of antibodies and activate humoral and cell-mediated immunological responses. Angiotensin-converting enzyme 2 (ACE2), which is well-known for its protective effect against acute respiratory distress syndrome, is likewise expressed in response to estrogens. Estrogens might enhance the immune functions of vitamin D, thereby improving infection outcomes. Therefore, it is possible to use Estrogen to lessen the severity of COVID-19 condition (Pagano et al., 2020). Conversely, testosterone is recognized to have immunosuppressive properties and a decrease in testosterone synthesis is linked to an increase in pro-inflammatory cytokines (Bianchi, 2019). Therefore, whereas estrogen increases the rise in antibody and CD8 titers and is likely to boost the antiviral immune response, testosterone-related suppression of the inflammatory response may attenuate the antiviral response. On the other hand, men are more susceptible to COVID-19 and have a worse prognosis due to male sex hormones. First, they may encourage viral entrance by boosting the function of the SARS-CoV-2 Coronavirus's entry site, the ACE2 receptor thereby encouraging co-infection. Secondly, testosterone exerts immunosuppressive effects and also reduces the antibody response.

In terms of marital status, singles showed high level of Co-infection (52.8%) followed by the married (34.7%) and the least was seen among the widowed (12.5%). The married takes good care of themselves and most of them are in the market industry where health is a priority for efficient and effective working conditions. The widowed mostly are seen within age group 51-70 with experience of life tend to be careful and regularly visit the health facilities for check-ups. Most of the widowed have existing medical condition therefore are on medications which is liable to supress pathogens that can cause co-infections. The singles seen among age group 20-40 years are less concerned about their health and do not regularly visit the health facility. Also, their high social life accompanied by smoking and drinking increases the chance of COVID-19 infection and Co-infection.

Again, those with Existing medical condition that is an underlying health condition in this study showed a high risk of bacteria and fungi co-infections (11.1%) compared to those without (88.9%). This is in line with Ssentongo et al. (2020) According to his research, individuals with comorbidities that is, pre-existing medical illnesses like diabetes, cardiovascular disease, hypertension, cancer and obesity have a greater mortality rate from COVID-19 infections and more venerable to co-infection. This medical disease that already exists in viral infections like SARS-COV-2 weakens the immune system and exposes it to other pathogens (Richardson et al., 2020). The COVID-19 patient's comorbidities contribute significantly to their considerable morbidity and death and put them in a vicious cycle of infection.

For instance, individuals with diabetes may have weakened immune systems, which makes it more difficult for the body to combat the coronavirus. This can result in chronic inflammation, impaired pancreatic function, and blood coagulability, all of which increase the risk of co-infection complications (Maddaloni & Buzzetti, 2020). Patient with cardiovascular disease infected with SARS-COV-2 are more likely to experience severe symptoms and have a worse result with co-infection (Onder et al., 2020). Reduced pro-inflammatory cytokines due to cardiovascular disease may lead to a weakened immune system (Zheng et al., 2020).

Among the participants with co-infection, 16.7% has Symptomatic and 83.3% are asymptomatic. When co-infection occurs with SARS-COV-2 infection, symptoms are exacerbated. The increased rate of virus shedding from infected host cells and compromised alveolar macrophage activity have been proposed as the reasons for the rise in viral load of SARS-COV-2 after co-infection with bacteria and fungi. However, co-infection with fungi and bacteria can change some aspects of the host's mucosal defence, making it more difficult to stop bacterial replication and enhance symptoms (Ramadan et al., 2020). Symptoms in SARS-CoV-2 individuals with bacterial and fungal co-infection were increased compared to SARS-CoV-2 patients with no co-infections or other respiratory viral coinfections (May et al., 2021). Several metabolic and infectious diseases especially preexisting medical conditions impact the severity of COVID-19 and play a pivotal role in establishing complex symptoms.

High educational attainment and low educational attainment groups influence the outcome of SARS-CoV-2 and co-infection. In this study, more isolates are seen among those with a primary level of education (52.8%) followed by a secondary level of education (31.9%) and then a tertiary level of education (15.3%). The least was seen with those with a tertiary level of education. To a degree, the educational difference diminished but high mortality in people with low educational attainment in all age groups. Pre-existing medical issues, poverty, and limited access to healthcare services could be the cause of this. We hypothesise that because they faced more financial hardships, those with less education were less able to remain in quarantine and continue working. Those with tertiary level of education are more aware of pathogens, have a high level of hygiene and visit the health facility early when they see signs of unwell compared to those with primary and secondary levels of education. Those with a primary level of education most times do not believe the issue of SARS-CoV-2, call it a scam and do not declare themselves to observe social distancing and other preventive measures thereby exposing them to other pathogens, especially in severe cases (Haftom et al., 2020; Bernard & Keiichi 2022).

Health status is greatly influenced by education in both wealthy and impoverished nations. Researchers have stated that education is strongly linked to health and helps sustain and promote healthy lifestyles. It also increases knowledge and creates a positive attitude regarding the causes, prevention, and management of diseases. The results indicate that education can have both negative and positive influences in alleviating the burden of COVID-19 infection and co-infection.

5. Conclusion

In this study, 72 (35.8 %) of bacterial and fungi co-infection was observed out of 201 SARS-COV-2 positive sample plated out. Bacteria and fungi isolated include *Staphylococcus aureus*, *Klebsiella spp, Pseudomonas aeruginosa and Candida albicans.* Among the study area, more pathogen was observed in Rivers State University Teaching Hospital. In all the most common pathogen isolated was *Staphylococcus aureus.* The only fungi isolated was *Candida albicans.* The Men, the single, age brackets 31-40, those with existing medical conditions, those with symptoms and those with a primary level of education showed the highest incidence of bacterial and fungi isolate. That is such co-infections are more reported in older people and those who have

underlying diseases. The results found that the level of “Education” is a strong positive predictor of health knowledge outcome; the higher the level of “Education,” the higher the health knowledge regarding COVID-19 and co-infections. Bacterial and fungal co-infections are common and pose a significant threat to the patient with COVID-19 disease. At the same time, COVID-19 disease increases the risk of bacterial and fungal co-infections. These entities' SARS-CoV-2 co-infections with bacterial and fungal pathogens have become another serious threat that should not be neglected.

Further research is required to better describe the biological, epidemiological, genetic and clinical characteristics of bacteria and fungi co-infections. In the current situation, appropriate and systematic analysis of COVID-19 patients diagnosed with bacterial co-infection should be implemented to choose proper antibiotics to increase the survival of patients and limit the spread of drug-resistant bacteria. For the precise diagnosis and evaluation of co-infections during the COVID-19 pandemic, samples for culture must be taken longitudinally throughout the disease course for culture to identify co-infections.

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