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Microbial Organisms in the Lower Respiratory Tract Associated with SARS-CoV-2 Infection: A Cross-Sectional Study in Northern Ghana

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Abstract: Colonization of SARS-CoV-2 with specific bacteria may either protect or increase the risk of disease severity. This study aimed to identify microbial organisms in the lower respiratory tract and their association with SARS-CoV-2 infection. This was a cross-sectional study conducted between May 2020 and August 2021 at the Tamale Public Health Laboratory in the Northern part of Ghana. RT-PCRs for SARS-CoV-2 and bacteriological cultures were performed on sputum samples collected from suspected COVID-19 patients. Biochemical identification and antimicrobial susceptibility tests were performed on the bacterial isolates. A total of 380 participants were recruited into the study. Most participants were within the 21–30 years age group (29.6%). RT-PCR testing detected SARS-CoV-2 in 118 (31.1%) patients. Headache was found to be associated with SARS-CoV-2 ($p = 0.033$). Sputum cultures yielded 187 (49.2%) positive bacteria growths. *Klebsiella* spp. (20.5%), *Moraxella catarrhalis* (7.9%), and *Pseudomonas* spp. (6.3%) were the most commonly isolated bacteria. *M. catarrhalis*, *Serratia* spp., and *Stenotrophomonas maltophilia* were significantly associated with SARS-CoV-2 infection. Most of the isolates were resistant to 3rd generation cephalosporins. This study has demonstrated the association between specific bacteria and SARS-CoV-2 infection. Clinicians should investigate possible bacterial co-infections in the management of COVID-19 cases.

Keywords: SARS-CoV-2; COVID-19; bacteria; lower respiratory tract; Northern Ghana

1. Introduction

Coronaviruses are a big family of zoonotic viruses that cause diseases ranging from the common cold to severe respiratory diseases [1]. Coronaviruses are diverse with the potential to infect a wide range of animals [2]. The novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most recently known coronavirus to cause human infections and can spread from person to person through droplets from infected hosts. The virus has an incubation period between 2 and 14 days, with an average of five days [3]. The

coronavirus disease 2019 (COVID-19) outbreak was first documented in Wuhan, Hubei Province, China in December 2019 and has now spread across the world [4].

Ghana recorded its first case of COVID-19 on the 12 March 2020 and has already experienced four waves of the pandemic with cumulated cases of 171,152 and deaths of 1462 as of 17 February 2023 [5]. The country is currently in the decline phase with 12 active cases and an average of three new infections daily as of 17 February 2023 [5]. Clinical presentation of patients with COVID-19 has varied from severe to critical illness with few unexplained conditions [6]. New variants of SARS-CoV-2 are causing life-threatening pneumonia and acute respiratory distress syndrome in light of COVID-19 vaccination [7]. The rapid spread of SARS-CoV-2 from one host to another forms part of the basis of acquiring mutations and the emergence of new variants. Identifying factors or determinants such as microbial co-infections in the respiratory tract associated with SARS-CoV-2 infections could be a key mechanism to reducing SARS-CoV-2 spread as well as the emergence of new variants.

Beyond the pathogenesis of SARS-CoV-2, bacterial co-infection may play an important role in the occurrence and development of COVID-19. SARS-CoV-2 infection can cause damage to ciliated cells resulting in deterioration of mucociliary clearance which may augment the colonization of bacteria in the airway [8]. Colonized bacteria could either protect the respiratory tract from subsequent infection or promote the pathogenesis of respiratory tract infection, resulting in severe pneumonia and death [9]. Although smoking can also cause damage to ciliated cells in the respiratory tract [10], we adopted the Ghana Health Service (GHS) national questionnaire for COVID-19 data collection which did not consider smokers. Additionally, the use of antibiotics for the management of COVID-19 patients may further affect the ongoing global fight against antimicrobial resistance. To date, few studies have investigated bacterial co-infections in COVID-19 patients and data on this is lacking in sub-Saharan Africa. This study, therefore, sought to investigate microbial organisms in the lower respiratory tract that may be associated with SARS-CoV-2 infection in the Ghanaian population.

2. Materials and Methods

2.1. Ethics Statement

This study was approved by the Ethics Review Committee of the Ghana Health Service (ERC-GHS) with approval number GHS-ERC 026/12/20. Written informed consent was sought from adult participants after explaining the purpose and relevance of the study to them. Children above 7 years, but younger than 18 years gave their assent together with their parental/guardian consent (written) before they were enrolled in the study. The findings of this research were anonymously presented and kept confidential.

2.2. Study Area

The study was conducted at the Zonal Tamale Public Health Laboratory (ZTPHL) situated in the Northern Region of Ghana. ZTPHL serves as the public health referral laboratory for the Northern, Savannah, Upper West, Upper East, North East, Bono East, Bono, and the Ahafo regions in Ghana. The laboratory has molecular testing capacity for meningitis diagnosis, HIV viral load/diagnosis, and COVID-19. ZTPHL is also well-equipped to perform bacteriological testing. Tamale is located at the center of the Northern region, which has a population of 2,310,939 inhabitants [11].

2.3. Study Design and Sample Collection

This was a cross-sectional study conducted between May 2020 and August 2021. Sputum samples were collected from 380 participants in the Northern part of Ghana who presented to healthcare facilities with signs and symptoms of COVID-19 as defined in the COVID-19 clinical guidelines for the management of patients [12]. Participants were instructed to rinse their mouth (with water) without using antiseptic mouthwash, take a deep breath, and cough in an open area to produce sputum into a sterile sputum container.

The samples were packaged using the principles of triple packaging techniques and transported immediately on ice to ZTPHL. All safety precautions were observed during sample collection, packaging, and transportation.

2.4. Laboratory Methods

Bacteria Culture

Sputum samples were cultured on blood agar, chocolate agar, and MacConkey agar plates in a level two bio-safety cabinet, incubated at 37 °C in a humidified 5% CO_2 incubator for 18–24 h, and macroscopically observed for bacterial growth. Agar plates with bacterial growth were considered positive cultures taking into consideration resident normal flora and those with no bacterial growth, considered negative cultures. Gram staining, biochemical testing, and analytical profile index (API) were used for bacteria identification. Antimicrobial susceptibility testing (AST) was performed using the disc diffusion method (Kirby–Bauer method) and following Clinical Laboratory Standards Institute guidelines [13] in the selection of appropriate antibiotics. Antibiotic discs were quality controlled using appropriate America Type Culture Collection (ATCC) strains (ATCC 25922—*Escherichia coli* and ATCC 25923—*Staphylococcus aureus*).

2.5. Detection of SARS-CoV-2

Viral RNA extraction was performed using the careGENETM nucleic acid extraction kit (WELLS BIO Inc., Seoul, Republic of Korea) following the manufacturer's protocol. Real-time polymerase chain reaction (RT-PCR) which targets the ORF1ab and nucleocapsid regions of SARS-CoV-2 was performed on the samples using a DaAnGene PCR Detection kit for 2019-nCoV (DaAnGene Co., Ltd., Guangzhou, China). The AriaMx-Real-time PCR System (Agilent Technologies, Santa Clara, CA, USA) was used to run all the samples. The PCR cycling conditions consisted of reverse transcription at 50 °C for 15 min, denaturation at 95 °C for 1 min, and 45 cycles of 94 °C for 15 s and 55 °C for 45 s. All cases with cycle threshold (Ct) values of 40 and below were interpreted as positive and greater than 40 or not detected were interpreted as negative as stated by the DaAnGene RT-PCR protocol. In all cases, positive, negative, and internal controls were included in the PCRs for validation.

2.6. Data Collection

All sputum samples were collected from participants along with case-based forms. Demographic and clinical data including age, gender, and symptoms were extracted from the case-based forms and entered into a spreadsheet for further analysis.

2.7. Statistical Analysis

Data were entered and cleaned in Microsoft Excel 2016 (Microsoft Corp., Redmond, WA, USA). In computing descriptive statistics, categorical variables were summarized as frequencies and percentages, whilst continuous variables were summarized as means and standard deviations for parametric data and medians with interquartile ranges for non-parametric data. The Chi-square test was used to establish demographic and clinical characteristics and microbial isolates associated with SARS-CoV-2 infection. Univariate logistic regression was used to assess possible factors associated with SARS-CoV-2 infection. Multivariate logistic regression was used to compute independent factors associated with SARS-CoV-2 infection. For analysis purposes, cases were categorized as mild/moderate or severe as previously described [14] based on the extent of clinical symptoms. Mild or moderate cases were persons presenting with symptoms such as fatigue, fever, cough, anorexia, malaise, muscle pain, sore throat, or headache. Severe cases were persons presenting with mild or moderate symptoms plus shortness of breath. Antimicrobial resistance (R) was presented as frequencies and percentages for the commonly isolated bacteria in COVID-19. All statistical analyses were performed using GraphPad Prism, version 8.0 (GraphPad Software Inc., San Diego, CA, USA) and IBM SPSS Statistics for Windows, version 26.0(IBM Corp., Armonk, NY, USA). p-values less than 0.05 were considered statistically significant.

3. Results

3.1. Socio-Demographic and Clinical Characteristics of Study Participants

A total of 380 participants were tested for SARS-CoV-2 infection and bacterial culture and were included in the analysis. The study registered more males (n = 231, 60.8%) than females (n = 149, 39.2%). Most of the study participants fell within the age groups of 21–30 years (n = 112, 29.6%) and 31–40 years (n = 102, 27.0%), with children and teenagers (<20 years) being the least represented group (n = 26, 6.9%). Gender and age were not associated with SARS-CoV-2 infection ($p > 0.05$) [Table 1]. The majority of the study participants were symptomatic (n = 260, 68.4%). However, a significantly higher proportion of participants who tested negative for SARS-CoV-2 were symptomatic compared to those who tested positive for SARS-CoV-2 (77.1% vs. 49.2%, $p < 0.001$). Clinical symptoms such as cough ($p < 0.001$), headache ($p = 0.001$), and general weakness ($p = 0.03$) were significantly associated with SARS-CoV-2 infection. On the other hand, symptoms such as sore throat, runny nose, shortness of breath, fever, diarrhea, irritability, and body pain were not significantly associated with SARS-CoV-2 infection ($p > 0.05$). Table 1 displays the socio-demographic and clinical characteristics of study participants.

Table 1. Socio-demographic and clinical characteristics of study participants.

Variable	All Participants n = 380 (%)	SARS-CoV-2 Negative n = 262 (%)	SARS-CoV-2 Positive n = 118 (%)	p-Value
Age group (years)				0.699
<20	26 (6.9)	18 (6.9)	8 (6.8)	
21–30	112 (29.6)	81 (31.2)	31 (26.3)	
31–40	102 (27.0)	65 (25.0)	37 (31.4)	
41–50	48 (12.7)	32 (12.3)	16 (13.6)	
51–94	90 (23.8)	64 (24.6)	26 (22.0)	
Gender				0.821
Female	149 (39.2)	104 (39.7)	45 (38.1)	
Male	231 (60.8)	158 (60.3)	73 (61.9)	
Clinical Symptoms				
Asymptomatic	120 (31.6)	60 (22.9)	60 (50.8)	**<0.001**
Symptomatic	260 (68.4)	202 (77.1)	58 (49.2)	
Cough				**<0.001**
No	146 (38.6)	69 (26.5)	77 (65.3)	
Yes	232 (61.4)	191 (73.5)	41 (34.7)	
Sore throat				0.354
No	343 (90.3)	239 (91.2)	104 (88.1)	
Yes	37 (9.7)	23 (8.8)	14 (11.9)	
Runny nose				0.104
No	364 (95.8)	254 (96.9)	100 (93.2)	
Yes	16 (4.2)	8 (3.1)	8 (6.8)	
Fever				0.181
No	346 (91.1)	242 (92.4)	104 (88.1)	
Yes	34 (8.9)	20 (7.6)	14 (11.9)	
Shortness of breath				0.650
No	357 (93.9)	247 (94.3)	110 (93.2)	
Yes	23 (6.1)	15 (5.7)	8 (6.8)	
Diarrhoea				0.648
No	375 (98.7)	259 (98.9)	116 (98.3)	
Yes	5 (1.3)	3 (1.1)	2 (1.7)	
Nausea/Vomiting				0.886
No	373 (98.2)	257 (98.1)	116 (98.3)	
Yes	7 (1.8)	5 (1.9)	2 (1.7)	
Headache				**<0.001**
No	365 (96.1)	258 (98.5)	107 (90.7)	
Yes	15 (3.9)	4 (1.5)	11 (9.30)	

Table 1. *Cont.*

Variable	All Participants n = 380 (%)	SARS-CoV-2 Negative n = 262 (%)	SARS-CoV-2 Positive n = 118 (%)	p-Value
Irritability				
No	380 (100.0)	262 (100.0)	118 (100.0)	0.999
Yes	0 (0.0)	0 (0.0)	0 (0.0)	
Pain				0.127
No	345 (90.8)	242 (92.4)	103 (87.3)	
Yes	35 (9.2)	20 (7.6)	15 (12.7)	
General weakness				**0.030**
No	347 (91.3)	245 (93.5)	102 (86.4)	
Yes	33 (8.7)	17 (6.5)	16 (13.6)	

p-Values were computed using the Chi-square/Fischer's exact test.

3.2. Clinical and Socio-Demographic Risk Factors Associated with SARS-CoV-2 Infection

Presenting with headache [OR = 6.63 (2.07–21.29), p = 0.001] and general weakness [OR = 2.26, 95% CI (1.10–4.65), p = 0.027] were significantly associated with increased odds of SARS-CoV-2 infection. After adjusting for possible confounders in multivariate logistic regression, headache [aOR = 4.41, 95% CI (1.13–17.19), p = 0.033] was independently associated with increased odds of testing positive to SARS-CoV-2 (Table 2).

Table 2. Clinical and socio-demographic risks factors associated with SARS-CoV-2 infection.

Variable	cOR (95% CI)	p-Value
Categories of age (years)		
<20	1 (Ref)	
21–30	0.86 (0.34–2.18)	0.753
31–40	1.28 (0.51–3.23)	0.6
41–50	1.13 (0.40–3.14)	0.822
51–94	0.91 (0.35–2.36)	0.853
Gender		
Female	1 (Ref)	
Male	1.07 (0.68–1.67)	0.773
Clinical Symptoms		
Asymptomatic	1 (Ref)	
Symptomatic	0.29 (0.18–0.46)	**<0.001**
Cough		
No	1 (Ref)	
Yes	0.19 (0.12–0.31)	**<0.001**
Sore throat		
No	1 (Ref)	
Yes	1.40 (0.69–2.83)	0.35
Runny nose		
No	1 (Ref)	
Yes	2.31 (0.85–6.31)	0.103
Fever		
No	1 (Ref)	
Yes	1.63 (0.79–3.45)	0.185
Shortness of breath		
No	1 (Ref)	
Yes	1.20 (0.49–2.91)	0.69
Diarrhea		
No	1 (Ref)	
Yes	1.49 (0.25–9.03)	0.665
Nausea/Vomiting		
No	1 (Ref)	
Yes	0.89 (0.17–4.64)	0.886

Table 2. *Cont.*

Variable	cOR (95% CI)	*p*-Value
Headache		
No	1 (Ref)	
Yes	6.63 (2.07–21.29)	**0.001**
Pain		
No	1 (Ref)	
Yes	1.76 (0.87–3.58)	0.117
General weakness		
No	1 (Ref)	
Yes	2.26 (1.10–4.65)	**0.027**

3.3. Co-Infection/Microbial Isolates among Study Participants

Among the 380 participants, 215 microbes were isolated from the sputum of 187 (49.2%) participants. Some participants recorded more than one isolate. *Klebsiella* spp. (n = 78, 20.5%) was the most commonly isolated bacteria, followed by *Moraxella catarrhalis* (n = 30, 7.9%), and then *Pseudomonas* spp. (n = 24, 6.3%). *Citrobacter* spp. (n = 3, 0.8%) and *Streptococcus pneumoniae* (n = 1, 0.3%) were the least isolated bacteria (Table 3). *Moraxella catarrhalis* isolates were more prevalent among SARS-CoV-2-negative individuals compared to SARS-CoV-2-positive individuals [28 (10.7%) vs. 2 (1.7%), p = 0.002]. Conversely, *Serratia* spp. [8 (6.8%) vs. 16 (6.1%), $p < 0.001$] and *Stenotrophomonas maltophilia* [4 (3.4%) vs. 1 (0.4%), p = 0.017] were more prevalent among SARS-CoV-2-positive individuals compared to SARS-CoV-2-negative individuals. *Serratia* spp. and *Stenotrophomonas maltophilia* were significantly associated with SARS-CoV-2 infection ($p < 0.05$). Conversely, *Acinetobacter baumannii*, *Candida* spp., *Citrobacter* spp., *Enterobacter* spp., *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., and *Streptococcus pneumonia* were not associated with SARS-CoV-2 infection.

Table 3. Microbial isolates among study participants.

Microbial Isolate	All Participants n = 380 (%)	SARS-CoV-2 Negative n = 262 (%)	SARS-CoV-2 Positive n = 118 (%)	*p*-Value
Acinetobacter baumannii				0.862
No	368 (96.8)	254 (96.9)	114 (96.6)	
Yes	12 (3.2)	8 (3.1)	4 (3.4)	
***Candida* spp.**				0.733
No	355 (93.4)	244 (93.1)	111 (94.1)	
Yes	25 (6.6)	18 (6.9)	7 (5.9)	
***Citrobacter* spp.**				0.932
No	377 (99.2)	260 (99.2)	117 (99.2)	
Yes	3 (0.8)	2 (0.8)	1 (0.8)	
***Enterobacter* spp.**				0.496
No	373 (98.2)	258 (98.5)	115 (97.5)	
Yes	7 (1.8)	4 (1.5)	3 (2.5)	
Escherichia coli				0.084
No	368 (96.8)	251 (95.8)	117 (99.2)	
Yes	12 (3.2)	11 (4.2)	1 (0.8)	
***Klebsiella* spp.**				0.446
No	302 (79.5)	227 (86.6)	95 (80.5)	
Yes	78 (20.5)	35 (13.4)	23 (19.5)	
Moraxella catarrhalis				**0.002**
No	350 (92.1)	234 (89.3)	116 (98.3)	
Yes	30 (7.9)	28 (10.7)	2 (1.7)	
***Proteus* spp.**				0.312
No	374 (98.4)	259 (98.9)	115 (97.5)	
Yes	6 (1.6)	3 (1.1)	3 (2.5)	

Table 3. *Cont.*

Microbial Isolate	All Participants n = 380 (%)	SARS-CoV-2 Negative n = 262 (%)	SARS-CoV-2 Positive n = 118 (%)	p-Value
***Pseudomonas* spp.**				0.803
No	356 (93.7)	246 (93.9)	110 (93.2)	
Yes	24 (6.3)	16 (6.1)	8 (6.8)	
***Serratia* spp.**				**<0.001**
No	368 (96.8)	262 (100.0)	106 (89.8)	
Yes	12 (3.2)	0 (0.0)	12 (10.2)	
Stenotrophomonas maltophilia				**0.017**
No	375 (98.7)	261 (99.6)	114 (96.6)	
Yes	5 (1.3)	1 (0.4)	4 (3.4)	
Streptococcus pneumoniae				0.502
No	379 (99.7)	261 (99.6)	118 (100.0)	
Yes	1 (0.3)	1 (0.4)	0 (0.0)	

3.4. Sputum Culture and Microbial Isolates as Potential Risk Factors of SARS-CoV-2 Infection

Of the 380 participants, 187 (49.2%) were positive for bacteria in sputum culture, whilst 193 (50.8%) were negative. Of the 193 sputum culture-negative subjects, 51 (26.4%) tested positive for SARS-CoV-2. In a logistic regression model, having *Moraxella catarrhalis* was significantly associated with decreased odds of SARS-CoV-2 infection [cOR = 0.14, 95% CI (0.03–0.62, p = 0.009]. On the other hand, *Stenotrophomonas maltophilia* was marginally associated with increased odds of SARS-CoV-2 infection [cOR = 9.16 (1.01–82.84), p = 0.049 (Table 4).

Table 4. Microbial risk factors associated with COVID-19 infection.

Microbial Isolate	cOR (95% CI)	p-Value	aOR (95% CI)	p-Value
Moraxella catarrhalis	0.14 (0.03–0.62)	**0.009**	0.15 (0.04–0.64)	**0.010**
Stenotrophomonas maltophilia	9.16 (1.01–82.84)	**0.049**	8.32 (0.92–75.32)	0.059

3.5. Distribution of Microbial Isolates According to the Disease Severity among COVID-19 Patients

Participants who tested positive for SARS-CoV-2 were further categorized according to disease severity. Although a higher proportion of Candida spp. and Serratia spp. were found among participants with severe SARS-CoV-2 disease whilst *Acinetobacter baumannii*, *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., *Stenotrophomonas maltophilia*, and *Proteus* spp. were common in those with mild or moderate disease, there was no significant difference in the proportions of microbes isolated between mild/moderate and those with the severe disease (p > 0.05) (Figure 1).

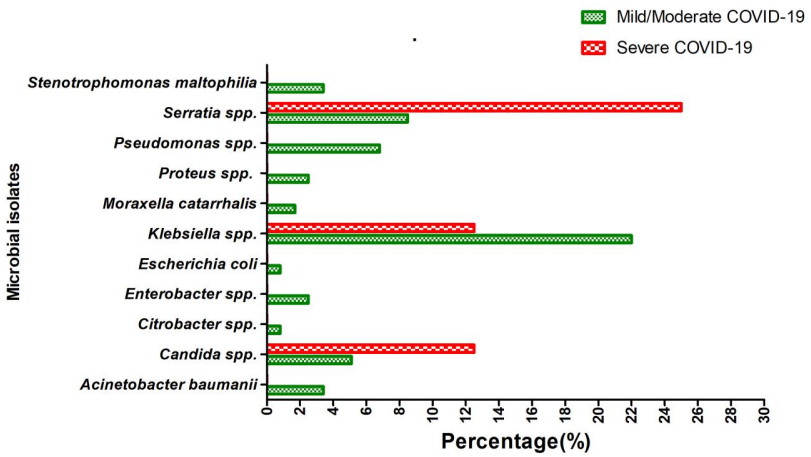

Figure 1. Microbial isolates and disease severity among SARS-CoV-2-infected patients.

3.6. Antimicrobial Resistance Pattern amongst Isolated Pathogens in SARS-CoV-2 Infections

Acinetobacter baumannii showed the highest resistance to amoxiclav, ceftriaxone, and cefotaxime (100% each), followed by ceftazidime (50%) and gentamicin (25%). *Klebsiella* spp. showed high resistance to amoxicillin–clavulanic acid (65.2%) and ceftriaxone (52.2%), followed by cefotaxime and ceftazidime, each recording a resistance of 39.1%. *Klebsiella* spp. were, however, susceptible to amikacin. *Moraxella catarrhalis* showed 50% resistance to ciprofloxacin and no resistance to gentamicin, amikacin, ceftazidime, and meropenem. Generally, all the isolates but *Pseudomonas* spp. (37.5%) showed no resistance to amikacin. Most of the isolates, with the exception of *Enterobacter* spp., *Klebsiella* spp., *Proteus* spp., and *Serratia* spp., showed 100% resistance to the third-generation cephalosporins (ceftriaxone and cefotaxime). Additionally, all isolates but *Klebsiella* spp. (62.5%) showed 100% resistance to amoxicillin–clavulanic acid. With the exception of *Klebsiella* spp. (12.5%), *Moraxella catarrhalis* (50%), *Proteus* spp. (33.3%), *Serratia* spp. (16.7%), and *Stenotrophomonas maltophilia* (25%), all other isolates were susceptible to ciprofloxacin (Table 5).

Table 5. Antimicrobial resistance patterns amongst commonly isolated pathogens in COVID-19 Patients.

Antibiotic	*Acinetobacter baumannii* (*n* = 4)	*Enterobacter* spp. (*n* = 3)	*Escherichia coli* (*n* = 1)	*Klebsiella* spp. (*n* = 23)	*Moraxella catarrhalis* (*n* = 2)	*Proteus* spp. (*n* = 3)	*Pseudomonas* spp. (*n* = 8)	*Serratia* spp. (*n* = 12)	*Stenotrophomonas maltophilia* (*n* = 4)
CRO	4 (100.0)	1 (33.3)	1 (100.0)	12 (52.2)	-	1 (33.3)	-	3 (25.0)	4 (100.0)
CTX	4 (100.0)	1 (33.3)	1 (100.0)	9 (39.1)	-	1 (33.3)	-	2 (16.7)	4 (100.0)
AZM	0 (0.0)	1 (33.3)	1(100.0)	7 (30.4)	-	2 (66.7)	-	5 (41.7)	0 (0.0)
AMC	4 (100.0)	3 (100.0)	1 (100.0)	15 (65.2)	-	3 (100.0)	-	12 (100.0)	4 (100.0)
CIP	0 (0.0)	0 (0.0)	0 (0.0)	3 (13.0)	1 (50.0)	1 (33.3)	0 (0.0)	2 (16.7)	1 (25.0)
GEN	1 (25.0)	0 (0.0)	1 (100.0)	2 (8.7)	0 (0.0)	1 (33.3)	3 (37.5)	0 (0.0)	1(25.0)
CAZ	2 (50.0)	0 (0.0)	1 (100.0)	9 (39.1)	0 (0.0)	0 (0.0)	3(37.5)	1 (8.3)	2 (25.0)
AK	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (37.5)	0 (0.0)	0 (0.0)
MEM	-	-	-	-	0 (0.0)	-	4 (50.0)	-	-

Frequencies and percentages were recorded for resistance. CTX—cefotaxime, CAZ—ceftazidime, CRO—ceftrixone, AK—amikacin, GEN—gentamicin, CIP—ciprofloxacin, AMC—amoxicillin-clavulanic acid, MEM—meropenem, AZM—azithromycin.

4. Discussion

Microbial co-infection may play an important role in the occurrence and development of SARS-CoV-2 infection. Viral–bacterial co-infection cannot be ruled out in increased mortality rates in COVID-19 [15]. In this study, we investigated microbial organisms in the lower respiratory tract associated with SARS-CoV-2 infection.

We observed more males testing positive for SARS-CoV-2 infection; however, there was no significant association between gender and SARS-CoV-2 infection. Male predisposition to SARS-CoV-2 infection has been well described [16,17], as opposed to their female counterparts whose mast cells, together with estrogen protection and their X-chromosomes, enable them to stimulate more immune responses, which may provide some level of protection against SARS-CoV-2 infection. Although a recent study in Ghana found an association between age category and SARS-CoV-2 infection [14], this particular occurrence was not observed in our study.

We also observed that a significantly higher proportion of participants who tested negative for SARS-CoV-2 were symptomatic compared to those who tested positive for SARS-CoV-2. The symptoms observed could be as a result of respiratory tract pathogens other than SARS-CoV-2. This indicates the need to test such samples for a wider range of other pathogens. Again, our observation could also be due to emerging variants of SARS-CoV-2 with changing symptom profiles, as reported by Whitaker et al. (2022) [18]. Cough was the most common symptom observed in our study. This was similar to findings by Lapostolle et al. (2020) and Abayomi et al. (2021) in France and Nigeria, respectively, where cough, fever, and difficulty breathing were found to be the most common symptoms among COVID-19 patients [19,20]. Headache was found in this study to be an independent clinical risk factor associated with SARS-CoV-2 infection. This finding is in line with a study

conducted by Uygun et al. (2020) in Turkey [21]. Fernández et al. (2023) also observed a significantly higher proportion of the delta variant of SARS-CoV-2 to be associated with headache than the other SARS-CoV-2 variants [22].

Sputum cultures from this study revealed 56.8% co-infection of different bacteria with SARS-CoV-2. We also found that testing positive for bacteria in sputum culture was associated with a marginal risk for SARS-CoV-2 infection. This is consistent with findings by Langford et al. (2020), who reported a significant bacterial co-infection with SARS-CoV-2 in a meta-analysis of 24 cohort studies [23]. This could be due to the fact that bacterial infection causes death and inhibition of the activity of ciliated cells in the respiratory tract leading to decreased mucociliary clearance. Decreased mucociliary clearance may then enhance or facilitate the colonization of SARS-CoV-2 in the respiratory tract. Smoking may also cause damage to ciliated cells in the respiratory tract [10]; however, smoking was not included as a potential risk factor in the current study since we adopted the Ghana Health Service national questionnaire for COVID-19 data collection which did not consider smokers. Our findings of 56.8% bacterial co-infection with SARS-CoV-2 are higher when compared with Antinori et al. (2020) in Italy, who identified 7.7% co-infection [24], and Contou et al. (2020), who identified 28% co-infection in France [25]. The weather conditions in the Northern part of Ghana are predominantly dry and mostly associated with dust which may facilitate the passage of microbes to the respiratory tract compared to that of France and Italy. On the contrary, studies conducted in Iran reported a 100% co-infection of bacterial and SARS-CoV-2 [26] among patients on mechanical ventilation.

Klebsiella spp., *Moraxella catarrhalis,* and *Pseudomonas* spp. were the most commonly isolated bacteria in this study. This is consistent with previous studies that reported *P. aeruginosa* and *Klebsiella* spp. as the most commonly isolated bacteria among COVID-19 patients [23,27,28]. Among the microbial isolates, it was observed that *Serratia* spp. and *S. maltophilia* were more prevalent among SARS-CoV-2-positive individuals compared to SARS-CoV-2-negative individuals. This is consistent with findings from Lu et al. (2021), who found a relatively high abundance of *Serratia* spp. and *Stenotrophomonas maltophilia* in SARS-CoV-2-positive individuals compared to those who were negative [29]. Feehan et al. (2021) similarly found a significant association between *Serratia* spp. and SARS-CoV-2-positive individuals compared to SARS-CoV-2-negative individuals [30]. This association could be due to the enhancement of efficient replication of SARS-CoV-2 in the respiratory epithelial cells causing inflammation and cell damage leading to barrier deterioration and leaving the airways more susceptible to this bacterial infection. This mechanism was clearly demonstrated by Deinhardt-Emmer et al. [31]. On the other hand, *M. catarrhalis* was more prevalent among SARS-CoV-2-negative individuals than SARS-CoV-2-positive individuals. Subjects with *M. catarrhalis* were found to have decreased odds of acquiring SARS-CoV-2 infection. Although other studies found *M. catarrhalis* in SARS-CoV-2-positive individuals [32,33], they could not associate it with SARS-CoV-2 infection. Nevertheless, our findings agree with Goel et al. (2021), who also found *M. catarrhalis* in only SARS-CoV-2-negative individuals [34]. Similarly, Bolorunduro et al. (2022) reported significantly high *Moraxella catarrhalis* prevalence among SARS-CoV-2-negative individuals as compared to the SARS-CoV-2-positive individuals [35]. Although *M. catarrhalis* can cause disease in immunocompromised patients, it is still known to be part of the normal flora in the respiratory tract and may have a competitive mechanism with SARS-CoV-2 in the respiratory airways hence making it difficult for SARS-CoV-2 to thrive in the respiratory airways of people with *Moraxella catarrhalis.* Additionally, Chu et al. (2021) reported that SARS-CoV-2 infection causes upregulation of proinflammatory cytokines (immune activation) which may inhibit the growth of *Moraxella catarrhalis* in the respiratory tract of SARS-CoV-2-infected individuals [36]. Although *Serratia* spp., *Candida* spp., and *Klebsiella* spp. were found in COVID-19 patients with severe disease in this study, there was no significant difference in the proportions of microbial isolates. This was consistent with findings by Bolorunduro et al. (2022), who also reported no significant association between mild COVID-19 cases and bacterial co-infections [36]. Other authors have, however, recorded the presence of *Serratia* spp. and

Klebsiella spp. in severely ill COVID-19 patients [35–39]. *Candida* spp. was also reported by Silva et al. (2021) in critically ill COVID-19 patients with increased odds of death [40]. These organisms (*Klebsiella* spp., *Serratia* spp., and *Candida* spp.) may have a role to play in COVID-19 severity and hence further studies are needed to properly ascertain this.

We also determined the antimicrobial susceptibility of isolates among SARS-CoV-2 infection and found that *A. baumannii* was completely resistant to amoxicillin–clavulanic acid, ceftriaxone, and cefotaxime, followed by ceftazidime (50%) and gentamicin (25%). This was consistent with findings by Sharifipour et al. (2020) who reported high-level resistance of *A. baumannii* isolates to all tested antibiotics in an evaluation of bacterial co-infections of the respiratory tract in COVID-19 patients in Iran [26]. Although *A. baumannii* is known to be a hospital-acquired infection with multiple antibiotic resistance, increased use of antibiotics in managing COVID-19 patients during the pandemic could be a contributory factor to the selection of antimicrobial resistance (AMR) among the bacteria. Additionally, safety measures kept in place to prevent SARS-CoV-2 infection such as regular washing of hands with soap and the use of hand sanitizers may also contribute to the selection of antimicrobial resistance. This was well explained by Egyir et al. (2020), who stated that soaps and sanitizers contain antibacterial activity and may become accumulated in waste, leading to the selection of AMR [41]. We also found that most of the isolates showed total resistance to amoxicillin–clavulanic acid, with the exception of *Klebsiella* spp. (65.2%). It is, however, not surprising in our findings, in which *E. coli*, *Proteus* spp., *Enterobacter* spp., and *Klebsiella* spp. showed 100%, 66.7%, 33.3%, and 30.4% resistance, respectively, to the broad-spectrum macrolide (azithromycin). This antibiotic was widely used for the treatment of presumptive secondary bacterial infection, and this might have increased the likelihood of selection for antimicrobial resistance. Despite the level of resistance demonstrated by bacteria towards the tested antibiotics, all the isolates but *Pseudomonas* spp. showed no resistance towards amikacin. This could be because amikacin is generally administered intravenously with no oral version currently available; hence, its route of administration has limited the frequency of use, preventing its resistance. Additionally, due to the nephrotoxicity nature of amikacin, clinicians are cautious in its administration to patients without prior knowledge of the status of their renal function [42].

Due to the design of our study, long-term follow-up of the patients to obtain disease outcome reports was not possible. Additionally, we were not able to capture data on underlying chronic diseases that are known to be associated with complications of COVID-19 and therefore could not associate underlying conditions and bacterial infections with COVID-19 deaths.

5. Conclusions

This study identified *Klebsiella* spp. and *Pseudomonas* spp. as the common bacterial pathogens in sputum of COVID-19 patients and also found cough, headache, and *Moraxella catarrhalis* as independent risks factors associated with SARS-CoV-2 infection in the Ghanaian population. Healthcare professionals should investigate possible bacterial co-infections and be guided by their antibiotic resistance patterns in the care of patients with COVID-19.

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