

Provenience of bacterial co- infection for SARS-CoV-2 virus in Iraqi patients

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Abstract

The SARS-CoV2 virus outbreak, which is what is causing COVID-19, has had a significant impact on people all over the world. It is noticeable that the emergence of bacterial growth or mutated species of bacteria is also a cause for concern. A substantial amount of literature indicates that the presence of multiple microbial infections in humans is associated with an elevated likelihood of experiencing more severe disease consequences. Limited research has been conducted on the co-infection of SARS-CoV-2 with other pathogens. This study is an examination of 300 patients in Iraq who were diagnosed with COVID-19 in the period from February to May 2022 and whose diagnoses were confirmed by laboratory testing. The objective of this research is to elucidate the various types of bacterial strains that co-occur in individuals from the Iraqi population who have contracted SARS-CoV2, with consideration given to their medical condition (severe, acute, or mild), gender, and the presence of the patient. The respiratory tract of the patient was sampled for sputum, which was subsequently cultured on various media. The antibiogram pattern and 16S gene were utilised via RT-PCR for identification purposes. The results of the study indicate that *Klebsiella pneumoniae* was the most prevalent bacterial strain, accounting for 21.21% of the total isolates. This was followed by *Pantoea agglomerans* at 16.3% and *Staphylococcus aureus* at 15.2%. Other bacterial strains identified in the study included *Serratia ficaria* at 10.9%, *Streptococcus thoraltensis* at 5.5%, *Acinetobacter baumannii* at 5.5%, and *Staphylococcus cohnii* at 5.5%. Additionally, 20% of the total bacterial isolates were unidentified by antibiogram pattern or 16S gene. The aforementioned findings are expected to serve as a valuable point of reference for the purpose of diagnosing and administering clinical treatment to individuals afflicted with COVID-19 in Iraq.

Keywords: COVID-19, SARS-CoV2, Sputum sample, Bacterial co-infection, VITEK2 system, and Novel bacteria.

1. Introduction

The emergence of COVID-19 in 2020 resulted in significant global disruption and widespread modifications[1]. The global pandemic has impacted the field of research regarding the impact of bacteria and viruses on various aspects of the world. Numerous research methodologies can be employed to investigate the nature of COVID-19 and its impact on the historical parasitic ailment, Bactria. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus, commonly referred to as COVID-19, has caused widespread global transmission and has resulted in substantial changes to our accustomed way of life since its identification in late 2019[2]. The SARS-CoV-2 virus bears resemblance to both the SARS-CoV and the MERS-CoV viruses. The genetic similarity between SARS-CoV-2 and SARS, which is estimated to be 79%, establishes a more direct association with SARS-like bat viral infections (MG772933) [3]. According to[4], both the SARS-CoV-2 and the SARS-CoV viruses employ the angiotensin-converting enzyme 2 (ACE2) receptor. SARS-CoV-2 predominantly targets the lungs as its primary site of infection, and unfortunately, there is currently no known cure for the virus. Conversely, individuals who are in a critical state of health and have multiple co-existing medical conditions might require admission to an intensive care unit, thereby increasing their susceptibility to contracting secondary or opportunistic infections. A significant proportion of individuals diagnosed with COVID-19 exhibit mild-to-moderate symptoms[5].

It is frequently observed that bacterial co-pathogens are present in viral infections of the airways, such as influenza, and are a significant contributor to both morbidity and mortality. As a result, it is crucial to promptly diagnose and administer antibacterial therapy[6]. The lack of comprehensive understanding regarding the prevalence, incidence, and attributes of bacterial infection among individuals afflicted with SARS-CoV-2 has been identified as a significant gap in knowledge[7]. This issue has been highlighted in recent literature. It is imperative to comprehend the percentage of COVID-19 patients who have acute respiratory bacterial co-infections and the responsible pathogens to effectively manage COVID-19 patients. This understanding is also essential to promoting the judicious use of antibiotics and reducing the adverse effects of their overuse[8]. The majority of research endeavors have primarily focused on SARS-CoV-2, with comparatively less attention given to the co-occurrence of SARS-CoV-2 with other infections. The presence of multiple pathogens may impede a precise diagnosis of the disease. The authors Wang et al. provided an update on the current state of SARS-CoV-2 co-infection in China, including information on concurrent bacterial and fungal infections[9]. The specific varieties of co-occurring pathogens and the relative prevalence of co-infection among individuals who have tested positive for SARS-CoV-2 remain uncertain. The present investigation involved an analysis of the clinical

characteristics exhibited by patients with COVID-19. Subsequently, a specific real-time RT-PCR technique was employed to detect 300 sputum respiratory samples. The present study aims to serve as a point of reference for epidemic prevention and clinical treatment in Iraq and other regions that are grappling with this epidemic.

2. Material and Methods

2.1. Clinical data and specimen collection

Between February 2nd and May 10th of 2022, a total of 300 samples were gathered from various local hospitals (namely Al-Numan Hospital, AL-Kindy Hospital, and AL-Shiffaa Centre) as well as several private laboratory centres. These samples had initially tested positive for SARS-CoV-2 via real-time RT-PCR. Upon admission, samples were promptly collected from each patient diagnosed with COVID-19. The medical records were the source of the clinical, laboratory, and outcome data. The recorded parameters included the patient's gender, medical condition, identification of bacterial growth, and location. The Control Ethics Committee granted approval for all procedures involving human materials in this study.

2.2. Manual and automated detection of bacterial isolates

Standard microbiological and automated techniques were employed to demonstrate antibiogram patterns of bacterial isolates for the purpose of identifying bacterial strains. The collected sputum was distributed onto various media, such as blood agar, Macconkey agar, brain-heart infusion agar, and broth. The specimens were subjected to incubation at a temperature of 37 degrees Celsius for a duration of 24 hours. Following this, colonies exhibiting suspicious characteristics were identified and selected for additional diagnostic evaluation based on their morphological justifications[10]. The selected strains were subsequently confirmed through the use of VITEK 2: Healthcare, leading to the diagnosis of bacterial ailments[11].

2.3. Molecular Identification of the 16S Gene

The fungal, bacterial, and yeast DNA MiniPrep genomic DNA reagent provided by ZYMO (USA) was employed for DNA extraction in the context of molecular diagnosis. The genomic DNA of all bacterial strains was obtained in accordance with the manufacturer's guidelines. The amplification of RT-PCR was conducted using the Maxime PCR PreMix kit (i-Taq). The polymerase chain reaction for screening 16S genes was conducted using a primer pair specifically designed for the 16S rRNA region. The forward primer sequence is 5'-AGAGTTTGATCCTGGCTCAG-3', and the reverse primer sequence is 5'-GGTTACCTTGTTACGACTT-3'. This primer pair was constructed in accordance with the methodology outlined by[12]. Every trial is comprised of a positive and negative

control. The confirmation of the amplification products was carried out through the analysis of 1.5% agarose gel electrophoresis, and their visualization was achieved by using Red Safe Nucleic Acid Staining Solution (0.13 g/ml).

2.4. Statistical analysis

The data was subjected to statistical evaluation utilizing the SPSS-25 software package, which stands for Statistical Packages for Social Sciences, version 25. The statistical information was conveyed through the use of fundamental measures of frequency, including means, standard deviations, and percentages. The one-way ANOVA test and post hoc test were employed as the statistical methodology to assess the statistical significance of variations among means in distinct groups with quantitative data.

3. Result:

3.1. Characteristics of patients

Table 1 presents the baseline characteristics of all cases and various groups. A group of 300 individuals received a diagnosis of SARS-CoV-2 infection and was subsequently categorized into three distinct clinical severity groups: severe, acute, and mild. The study reveals that the highest percentage of cases, amounting to 61.3%, pertain to participants who were hospitalized. The observations indicate that a higher proportion of men (54.3%) were present compared to women (45.7%). A study was conducted and illustrated to examine the correlation between the gender (figer1.a), the location of the patient (figer1.b), and the patient's medical status (figer1.c) with the presence of bacterial growth.

Table 1. Characteristics of patients infected with SARS-CoV-2.

Characteristics		Frequency	Percent %
Gender	Female	137	45.7
	Male	163	54.3
Location of patient	Non-Hospitalizes	116	38.7
	Hospitalizes	184	61.3
Medical status	Sever	75	25.0
	Acute	75	25.0
	Mild	75	25.0
	Healthy Control	75	25.0
Bacterial Growth	Growth	190	63.3
	No Growth	110	36.7

Figure 1-a. Clarify the relationship between gender of SARS-CoV-2 patients and bacterial growth

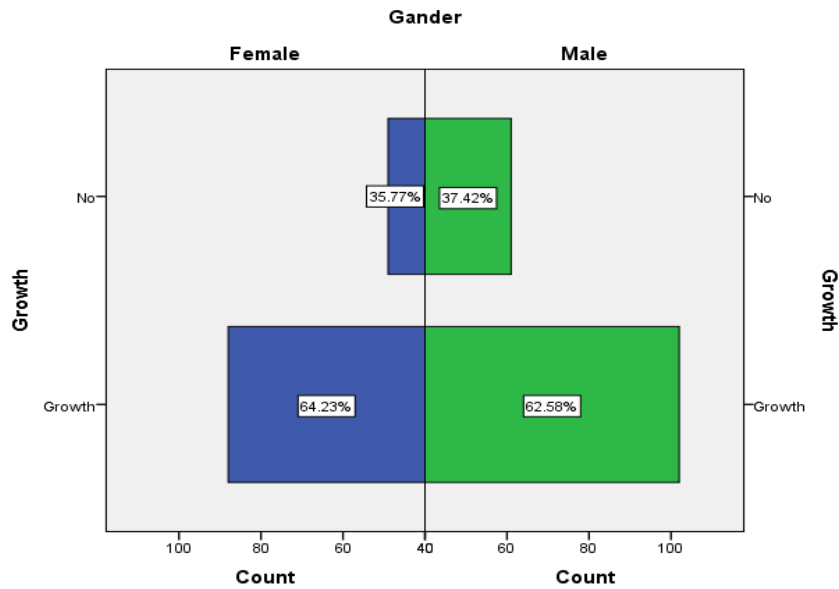


Figure 1-b. Clarify the relationship location of SARS-CoV-2 patients and bacterial growth

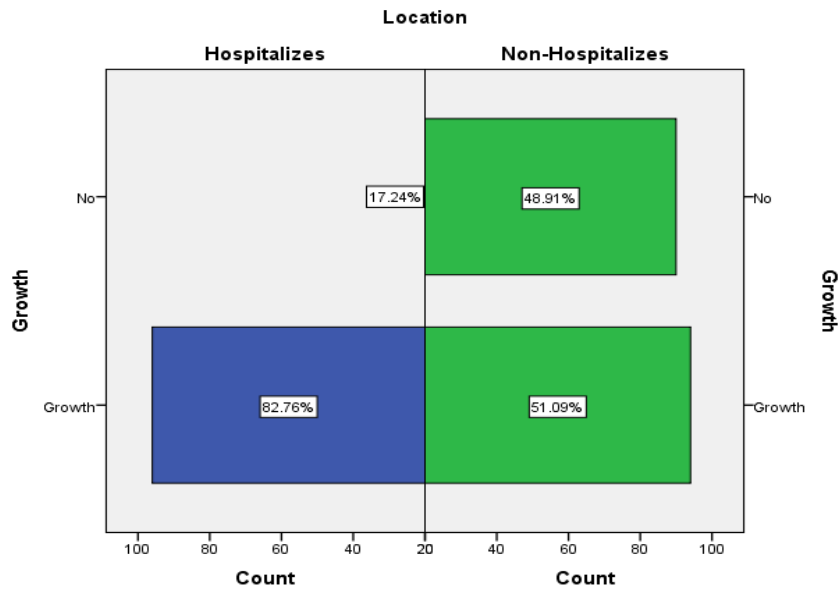
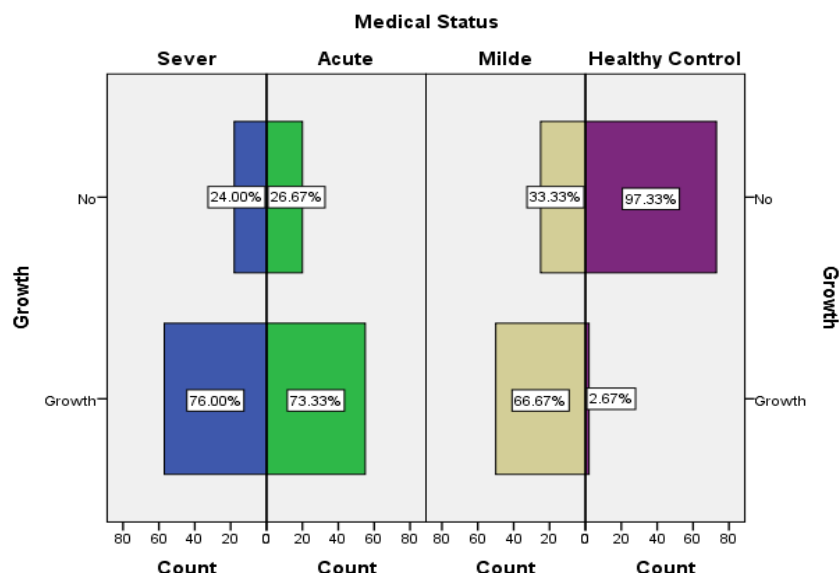


Figure 1-c. Clarify the relationship between Medical status of SARS-CoV-2 patients and bacterial growth



3.2. Detection on various types of media

The selection of colonies for supplementary diagnostic testing was predicated on their morphological characteristics. A variety of biochemical assays, comprising oxidase, catalase, methyl red, and coagulase, were executed. The bacterial isolates identified in various specimens were *Streptococcus thoraltensis*, *Staphylococcus cohnii*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pantoea agglomerans*, *Serratia ficaria*, and *Acinetobacter baumannii*, as determined by biochemical tests. as demonstrated in the subsequent table:

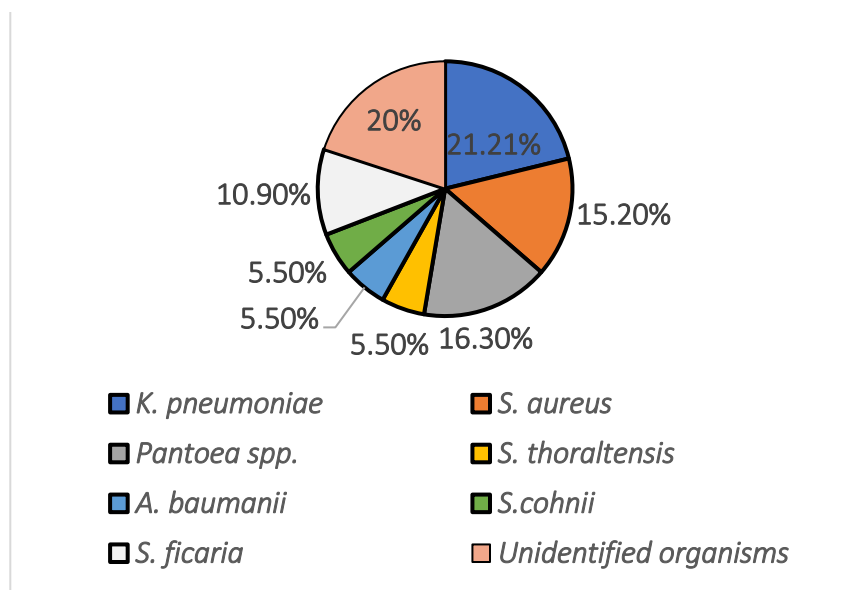
Table (2): Results of manual investigation and biochemical tests for bacterial isolates

Biochemical test	Oxidase	Catalase	Methyl red	Coagulase
Gram-positive bacterial isolates				
<i>Streptococcus thoraltensis</i>	-	-	+	-
<i>Staphylococcus cohnii</i>	-	+	+	-
<i>Staphylococcus aureus</i>	-	+	+	+
Gram-negative bacterial isolates				
<i>Pantoea agglomerans</i>	-	+	-	-
<i>Serratia ficaria</i>	-	+	-	-
<i>Acinetobacter baumannii</i>	-	+	-	-

3.3. VITEK2 system identification of bacterial isolates

The VITEK2 platform was employed to locate a total of 190 bacterial isolates, and the results are presented in Figure 3. The results indicate that *Klebsiella pneumoniae* was the most frequently identified strain of bacteria, representing 21.21% of the total isolates. This was followed by *Pantoea* spp. at 16.3% and *Staphylococcus aureus* at 15.2%. Other identified strains included *Serratia ficaria* at 10.9%, *Streptococcus thoraltensis* at 5.5%, *Acinetobacter baumannii* at 5.5%, and *Staphylococcus cohnii* at 5.5%. Additionally, 20% of the total bacterial isolates were comprised of unidentified organisms.

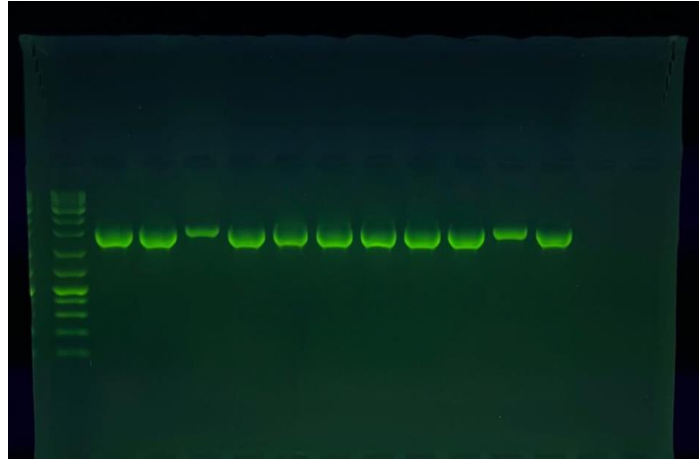
Figure (3): Results of VITEK2 system of bacterial isolates



3.4. Molecular identification of bacterial isolates

The present investigation involved the detection of the PCR product of individual bacterial isolates based on the 16S gene, as depicted in Figure 4. The DNA extracted from bacterial samples resulted in the amplification of a product of the anticipated size (620 bp), thereby validating the existence of DNA from *Klebsiella pneumoniae*, *Pantoea* spp., *Staphylococcus aureus*, *Serratia ficaria*, *Streptococcus thoraltensis*, *Acinetobacter baumannii*, and *Staphylococcus cohnii*. The band patterns of the samples were cross-referenced with the standard positive control of the test kit, resulting in the identification of all the aforementioned isolates. There was no evidence of contamination or inhibition observed.

Figure 4. PCR reaction for diagnosis of bacterial samples from SARS-CoV2 patients



Co-infections considering Gender and Medical status

Out of the total of 276 patients, 219 were identified as having a bacterial co-infection. Specifically, 106 (65%) of the male patients and 113 (61.3%) of the female patients were found to have co-infections. The study findings indicate that a total of eight pathogens were detected in co-infection cases among Iraqi patients with SARS-CoV-2. The data presented in Figure 5-a, b, and c indicate that there was a notable increase in the incidence of co-infection with *K. pneumonia* and *S. aureus* among individuals of both genders who were diagnosed with mild, acute, and severe cases of SARS-CoV-2. The co-infections of *Pantoea agglomerans* and *Serratia ficaria* were observed to be more prevalent in severe cases of both male and female patients with acute symptoms, as well as those with mild SARS-CoV-2 infection. The co-occurrence of *Streptococcus thoraltensis* and *Staphylococcus cohnii* was found to have the lowest incidence across all medical conditions. In contrast, the prevalence rate of *Acinetobacter baumannii* isolates is greater among patients with mild symptoms as opposed to those with severe and acute SARS-CoV-2 infections.

Figure 5-a. Distribution of respiratory pathogens with the Mild SARS-CoV-2 co-infection in gender.

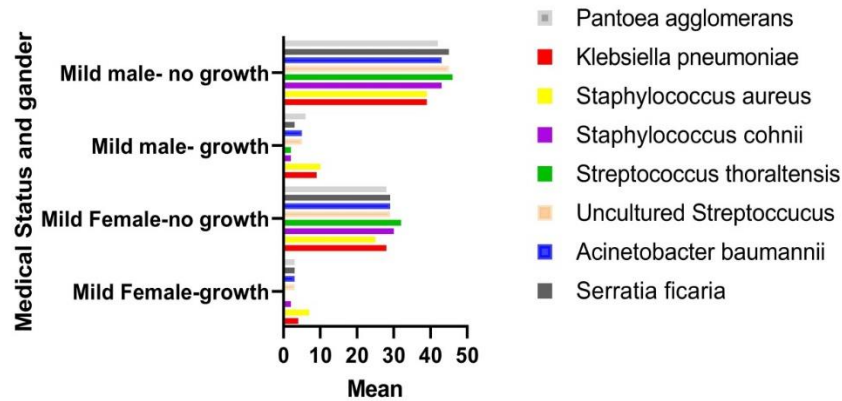


Figure 5-b. Distribution of respiratory pathogens with the Acute SARS-CoV-2 co-infection in gender.

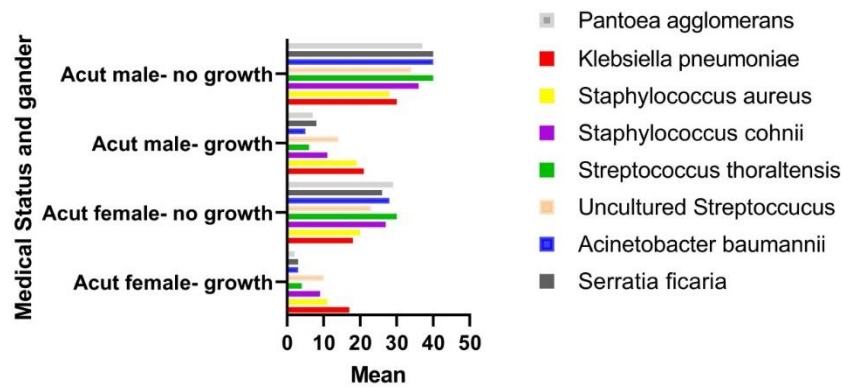
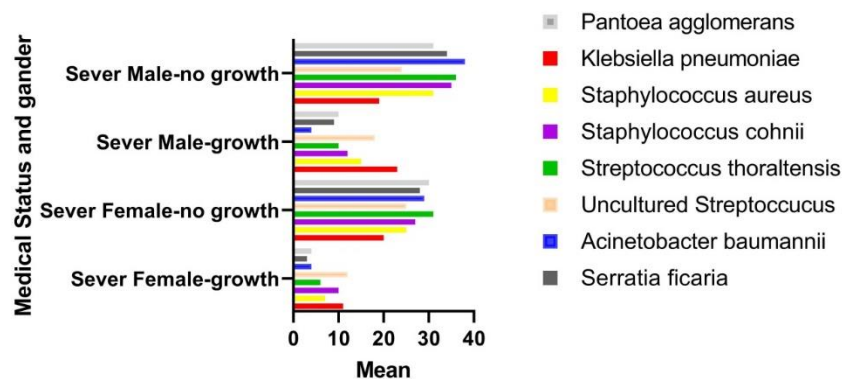


Figure 5-c. Distribution of respiratory pathogens with the Sever SARS-CoV-2 co-infection in gender.



4. Discussion:

The presence of co-infection has the potential to considerably impede the immune system of the host, heighten intolerance to antibacterial therapy, and adversely affect the prognosis of the disease[13]. The findings of our investigation reveal that a considerable proportion of COVID-19 patients, specifically 63.3%, exhibited the possibility of being co-infected with one or more additional pathogens, comprising eight distinct bacterial species. The findings indicate a lack of discernible correlation between co-infection and both admission to the intensive care unit and mortality. The co-occurrence of bacterial infection in the context of viral pneumonia has been identified as a significant contributor to mortality, as reported by[14]. The findings of our study indicate that bacterial co-infections were prevalent among the entire population of COVID-19 patients and indicate that *K. pneumonia* and *S. aureus* were the predominant bacterial co-infections observed. While certain microorganisms may coexist, they are classified as conditional pathogens. There is a possibility that they could become pathogenic in instances where the immune function of patients with COVID-19 is compromised. According to previous research studies conducted by[14], [15], it has been proposed that the utilisation of pneumococcal conjugate and polysaccharide vaccines could potentially serve as a viable strategy for mitigating the prevalence of the most prevalent co-infection during the ongoing COVID-19 pandemic. Certain pathogens have been identified to possess antibiotic resistance, which could potentially complicate the management of COVID-19 patients[9]. The present study revealed that the severe and acute categories exhibited the highest rates of co-infection with bacteria. The incidence of co-infection in females was comparatively lower than that in males, indicating that the male population is more vulnerable to other respiratory pathogens in comparison to females. The prevalence of co-infection among pathogenic species was found to be greater in hospitalised patients as compared to non-hospitalised individuals.

It has been discovered that exposure to COVID-19 can cause "L-form growth" in a number of different bacterial species[16]. The bacteria are able to survive and even reproduce in this environment because it is possible for them to do so. This might result in hazardous repercussions, such as antibiotic-resistant bacterial infections, which are far more difficult to treat than standard bacterial infections[17].

When a particular strain of bacteria has been exposed to a virus for a longer period of time, there is a greater possibility that the bacteria will develop immunity to the virus. Because of this, it is essential to put an end to outbreaks as fast as possible in order to restrict the spread of the virus and lessen the likelihood that bacteria may develop immunity to it[7].

5. Conclusion

In conclusion, the findings of this study suggest that further research is needed to fully understand the implications of the observed results. Additionally, it is recommended that future studies consider alternative methodologies and larger sample sizes to increase the generalizability of the findings. Overall, this study provides valuable insights into the topic at hand and serves as a foundation for future research in this area. The current investigation aimed to examine the existence of eight respiratory pathogens in a cohort of 300 individuals diagnosed with COVID-19. Currently, an escalating number of patients with infectious diseases are exhibiting an elevated incidence of multipathogen co-infection, thereby exacerbating the challenge of clinical diagnosis as well as therapy. Consequently, during the testing of SARS-CoV-2, it was necessary to simultaneously screen for other respiratory pathogens that were crucial for accurate diagnosis and treatment.

Ethical Clearance

The Research Ethical Committee at scientific research by ethical approval of environmental, health, higher education, and scientific research ministries in Iraq.

Conflict of interest:

The authors have no conflicts of interest regarding this investigation

Bibliography

- [1] E. C. Holmes et al., "The origins of SARS-CoV-2: A critical review," *Cell*, vol. 184, no. 19, Elsevier B.V., pp. 4848–4856, Sep. 16, 2021, doi: 10.1016/j.cell.2021.08.017.
- [2] K. G. Andersen, A. Rambaut, W. I. Lipkin, E. C. Holmes, and R. F. Garry, "The proximal origin of SARS-CoV-2," *Nature Medicine*, vol. 26, no. 4, Nature Research, pp. 450–452, Apr. 01, 2020, doi: 10.1038/s41591-020-0820-9.
- [3] I. Ali, J. I. Dasti, S. N. Khan, and A. Jabbar, "A Brief Overview of SARS-CoV-2," *Arch Clin Microbiol*, vol. 11, p. 118, 2020, doi: 10.36648/1989-8436.11.4.118.
- [4] M. Hoffmann, H. Kleine-Weber, N. Krüger, M. Müller, C. Drosten, and S. Pöhlmann, "The novel coronavirus 2019 (2019-nCoV) uses the SARS-coronavirus receptor ACE2 and the cellular protease TMPRSS2 for entry into target cells," *BioRxiv*, 2020.
- [5] V. Baskaran et al., "Co-infection in critically ill patients with COVID-19: An observational cohort study from England," *J. Med. Microbiol.*, vol. 70, no. 4, 2021, doi: 10.1099/JMM.0.001350.
- [6] S. M. Karaba et al., "Prevalence of co-infection at the time of hospital admission in COVID-19 Patients, A multicenter study," *Open Forum Infect. Dis.*, vol. 8, no. 1, pp. 1–7, 2021, doi: 10.1093/ofid/ofaa578.

- [7] X. Zhu et al., “Co-infection with respiratory pathogens among COVID-2019 cases,” *Virus Res.*, vol. 285, no. May, p. 198005, 2020, doi: 10.1016/j.virusres.2020.198005.
- [8] S. D. Gallacher and A. Seaton, “Meningococcal meningitis and COVID-19 co-infection,” *BMJ Case Rep.*, vol. 13, no. 8, 2020, doi: 10.1136/bcr-2020-237366.
- [9] L. Wang et al., “An observational cohort study of bacterial co-infection and implications for empirical antibiotic therapy in patients presenting with COVID-19 to hospitals in North West London,” *J. Antimicrob. Chemother.*, vol. 76, no. 3, pp. 796–803, 2021, doi: 10.1093/jac/dkaa475.
- [10] Madigan, Martinko, Bender, Buckley, and Stahl, “Brock: Biology of Microorganisms,” no. January 2010, pp. 95–119, 2015.
- [11] N. A. Nimer, R. J. Al-Saa’da, and O. Abuelaish, “Accuracy of the VITEK® 2 system for a rapid and direct identification and susceptibility testing of Gram-negative rods and Gram-positive cocci in blood samples,” *East. Mediterr. Heal. J.*, vol. 22, no. 3, pp. 193–200, 2016, doi: 10.26719/2016.22.3.193.
- [12] J. C. Martinati, F. T. H. Pacheco, V. F. O. De Miranda, and S. M. Tsai, “16S-23S rDNA: Polymorphisms and their use for detection and identification of *Xylella fastidiosa* strains,” *Brazilian J. Microbiol.*, vol. 38, no. 1, pp. 159–165, 2007, doi: 10.1590/S1517-83822007000100033.
- [13] B. Hu, H. Guo, P. Zhou, and Z. L. Shi, “Characteristics of SARS-CoV-2 and COVID-19,” *Nature Reviews Microbiology*, vol. 19, no. 3. Nature Research, pp. 141–154, Mar. 01, 2021, doi: 10.1038/s41579-020-00459-7.
- [14] F. He et al., “Respiratory bacterial pathogen spectrum among COVID-19 infected and non-COVID-19 virus infected pneumonia patients,” *Diagn. Microbiol. Infect. Dis.*, vol. 98, no. 4, p. 115199, 2020, doi: 10.1016/j.diagmicrobio.2020.115199.
- [15] N. Zhu et al., “A Novel Coronavirus from Patients with Pneumonia in China, 2019,” *N. Engl. J. Med.*, vol. 382, no. 8, pp. 727–733, 2020, doi: 10.1056/NEJMoa2001017.
- [16] B. J. Langford et al., “Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis,” *Clin. Microbiol. Infect.*, vol. 26, no. 12, pp. 1622–1629, 2020, doi: 10.1016/j.cmi.2020.07.016.
- [17] C. Garcia-Vidal et al., “Incidence of co-infections and superinfections in hospitalized patients with COVID-19: a retrospective cohort study,” *Clin. Microbiol. Infect.*, vol. 27, no. 1, pp. 83–88, 2021, doi: 10.1016/j.cmi.2020.07.041.