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Investigation of The Prevalence of Secondary Bacterial Infection Associated with COVID-19 In Baghdad and Diyala Province

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Abstract

Background Coronavirus disease 2019 (COVID-19) is an epidemic disease produced via the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) beta coronavirus, which affects the lower respiratory tract. Secondary bacterial infection (SBI) is a serious and public problem in patients hospitalized with COVID-19 and caused 50% of deaths. Type A and O blood groups are more susceptible to infection with SBIs.

Objective To examine the relationship between SBI and ABO blood group with COVID-19 hospitalized patients in Baghdad and Diyala province.

Methods Three hundred and forty-two patients with COVID-19 were collected from several sources (nasal swab, pharyngeal swab, sputum, blood, and urine) of patients of different ages for the period between September to November 2021. Real-time reverse-transcription polymerase chain reaction technique was used to diagnose COVID-19 in the patients as well as selective and differential media, biochemical tests BACT/ALERT system, and VITEK 2 compact system were used to diagnose the isolates of SBIs. The disk diffusion technique was used to assess the susceptibility test of all isolates. ABO group analysis was done for totally patients with COVID-19 under the study.

Results Antimicrobial sensitivity test showed all SBIs were highly strong resistant to antibiotics. Fifty-seven isolates of bacteria were diagnosed including *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumonia*e, and *Escherichia coli*. Group A and O showed a higher rate of acquired SBIs. Duration of infection with COVID-19 showed 61% for 10 days and 30% for one month while 9% in the patients infected with SBI for more than one month. The result appeared that SBIs infection at were very high rate in COVID-19 patients who had untreated antibiotics compared with the patient treated with antibiotics through the duration of infection.

Conclusion The study revealed that many COVID-19 patients were more susceptible to infection with SBIs, especially in the early days of infection as well as there was a correlation between ABO blood groups and SBIs of COVID-19 patients.

Keywords COVID-19; SBIs, AST, ABO blood group

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List of abbreviations: AST = Antibiotics susceptibility test, COVID-19 = Coronavirus disease 2019, MDR = Multiple drug resistance, RT-PCR = Real-time reverse-transcription polymerase chain reaction, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, SBI = Secondary bacterial infection

Introduction

A new strain of coronavirus triggered a pneumonia outbreak in Wuhan, Hubei Province, China, in December 2019. The new virus, now known as severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has quickly spread in China and additional

parts of the world owing to its ability to successfully transmit among humans [1]. Corona virus disease 2019 (COVID-19) had been confirmed in over 12 million people worldwide as of July 15th, 2020, with over 570 thousand deaths [2]. Bacterial co-pathogens are frequently seen in viral respiratory tract illnesses like influenza, and they stay a major source of morbidity and mortality, needing prompt identification and antibacterial treatment [3]. Secondary bacterial infection (SBI) is a serious and common complication in patients hospitalized with COVID-19. It occurs at an estimated rate of 10-15% [4].

According to prior investigations, SBIs caused 50% of COVID-19 deaths; hence, patients with SBIs had a higher risk of death [5]. Because there are no randomized clinical trials on the use of empiric antibiotic medicines in COVID-19 patients, the present guidelines are based on the extrapolation of data from other viral pneumonia patients [6].

The ABO blood group might have a role in the immune pathogenesis of COVID-19 infection, according to increasing data [7]; A and B antigens are originating in the external the red blood cells and their presence or absence determines the ABO and Rh blood grouping of an individual.

The blood group antigens, glycolipids, and glycoproteins are hereditarily controlled and inherited in variable occurrences across human peoples [8]. However, studies are presently ongoing to identify biological signs that can forecast an individual's susceptibility to SARS-CoV-2; severity and COVID-19 clinical result have been related to serum levels of some laboratory factors [9]. The ABO blood group has been connected to diseases such as Influenza and Norovirus [10]. Now there is rising evidence that indicated the susceptibility to SARS-CoV-2 is linked to the ABO blood group of an individual. Also, conflicting evidence occurs on the relationship between the ABO blood group and the severity and the clinical result of COVID-19 disease [11].

The present study aimed to examine the relationship between SBI and ABO blood group with COVID-19 hospitalized patients in Baghdad and Diyala province.

Methods

To investigate the prevalence of infectious bacteria associated with COVID-19 patients, 342 specimens were collected from patients of various ages and sources, including nasal swabs, pharyngeal swabs, sputum, blood, and urine, after confirming that they were infected with COVID-19 using real-time reverse-transcription polymerase chain reaction (RT-PCR) technique; these samples were collected during the period from September 2021 to November 2021 at Medical City, Al-Shifa Hospital, Imam Ali Hospital and Al-Imamein Al-Kadhimein Medical city – Baghdad as well as from the hospitals in Baquba City. Officials from the hospitals gave their consent. All participants and healthcare professionals were informed about the study's goal. The advantages are explored, and the patients have been informed. There are no hazards to their health as a result of the study.

Patient information was including age, gender, use of artificial respiration, duration of infection, and receiving antibiotics or not. All the specimens (324 were cultured on differential and selective media (MacConkey agar, Blood agar, Mannitol salt agar, Pseudomonas agar, and Eosin methylene blue), also for the isolation; used BACT/ALERT system to detect an early bacterial growth in a special blood culture bottle. Colony characteristics, microscopic examination, and biochemical tests (Catalase test, Oxidase test and IMViC) were used to diagnose the isolates, VITEK 2 compact system was used to confirm the identification of the isolates. The disk diffusion method on Mueller-Hinton agar was used to assess the susceptibility of all isolates to different types of antibiotics, as recommended by the Clinical and Laboratory Standards Institute [12]. Blood group (ABO) analysis was performed for all patients.

Results

Bacterial isolation and identification

This study uses traditional methods for identification of the bacteria, which have relied on determining the phenotype of the causative organism using bacteriological methods including selective media cultivation, colonial morphology, and microscopic characteristics. From the total 342 samples; 57 isolates were obtained from patients with COVID-19 and distributed between Gram-positive and Gram-negative bacteria as shown in table 1.

Table 1. Isolation of Secondary bacterial infections according to sample sources

Types of Bacteria	Sources				
	Nasal Swab N (%)	Pharyngeal swab N (%)	Sputum N (%)	Blood N (%)	Urine N (%)
Staphylococcus aureus	11 (100)	3 (15)	0 (0.0)	0 (0.0)	2 (25)
Pseudomonas aeruginosa	0 (0.0)	0 (0.0)	5 (33.3)	1 (33.3)	0 (0.0)
Acinetobacter baumannii	0 (0.0)	6 (30)	3 (20)	0 (0.0)	0 (0.0)
Klebsiella pneumoniae	0 (0.0)	11 (55)	7 (46.6)	2 (66.6)	0 (0.0)
Escherichia coli	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	6 (75)
Total	11	20	15	3	8

Antimicrobial susceptibility test

All bacterial species were evaluated against 12 antimicrobial compounds of each bacteria using the disk diffusion method. The results in the figures (1,2,3) were evaluated according to (12) standards and revealed that there were numerous bacterium isolates with multiple drug resistance (MDR) from distinct types of bacteria.

Blood group associated with Secondary bacterial infection

Three hundred and forty-two patients with COVID-19, 57 patients were identified with SBIs. The current study showed that blood groups of COVID-19 patients associated with SBIs were graduated the risk of the infection at 42%, 35%, 20% and 3% for the O and A, B and AB respectively as appeared in figure 4.

Relationship between SBIs in COVID-19 patients and antibiotics usage

The current results established that COVID-19 patients who were not given or treated with classical antibiotics were more likely to develop a secondary bacterial infection (SBIs), as the percentage of patients who were untreated with antibiotics was (70%) and the percentage of those treated was (30%) as showed in figure 5.

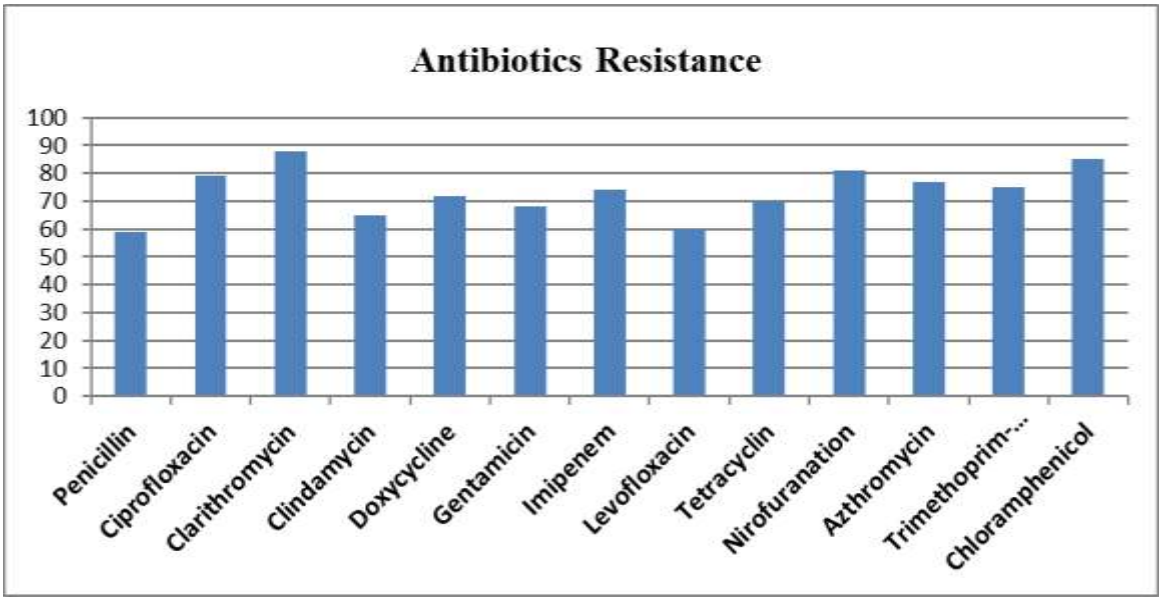

Figure 1. ***Staphylococcus aureus*** **resistance of antibiotics**

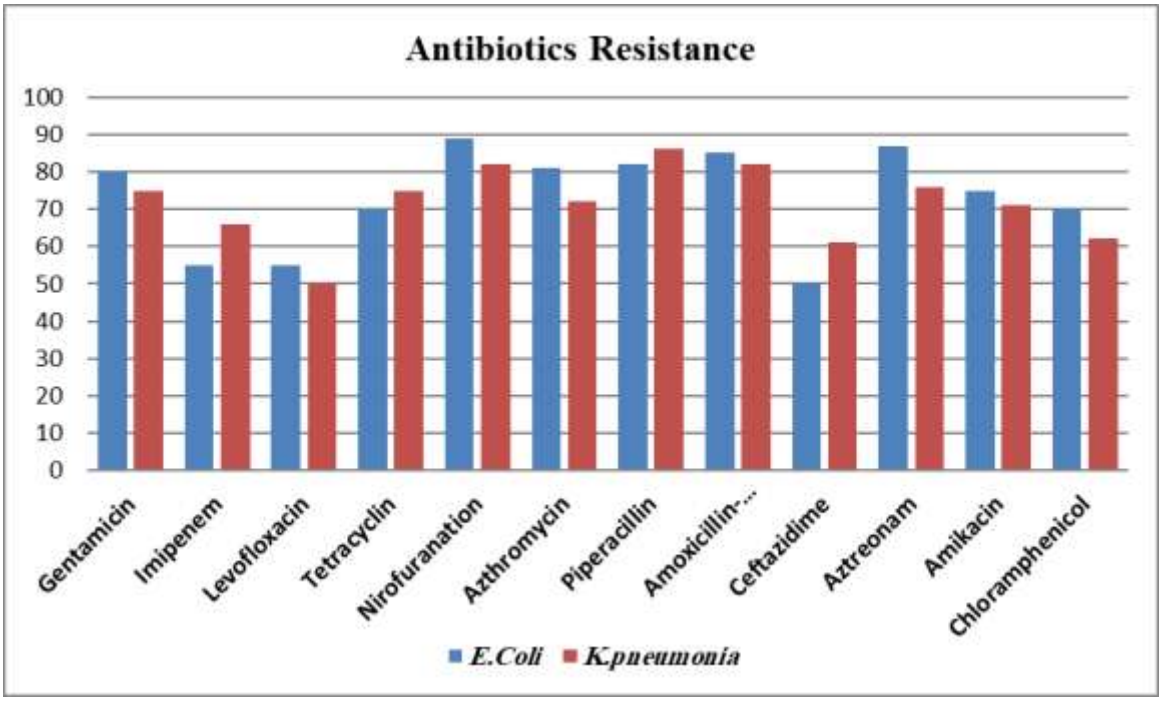

Figure 2. ***Escherichia coli*** **and** ***Klebsiella pneumoniae*** **resistance to antibiotics**

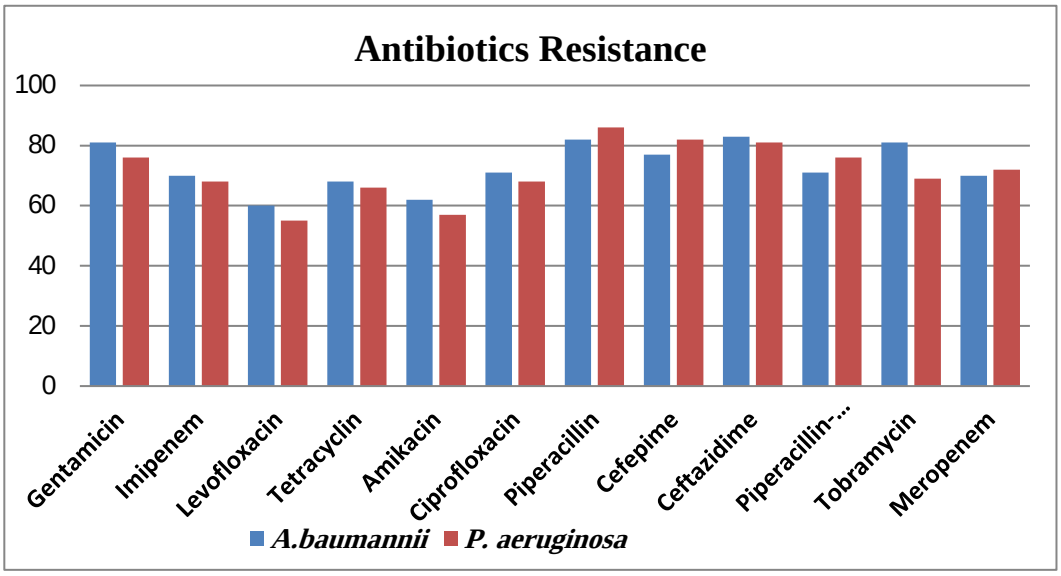

Figure 3. ***Acinetobacter baumannii and Pseudomonas aeruginosa*** **resistance to antibiotics**

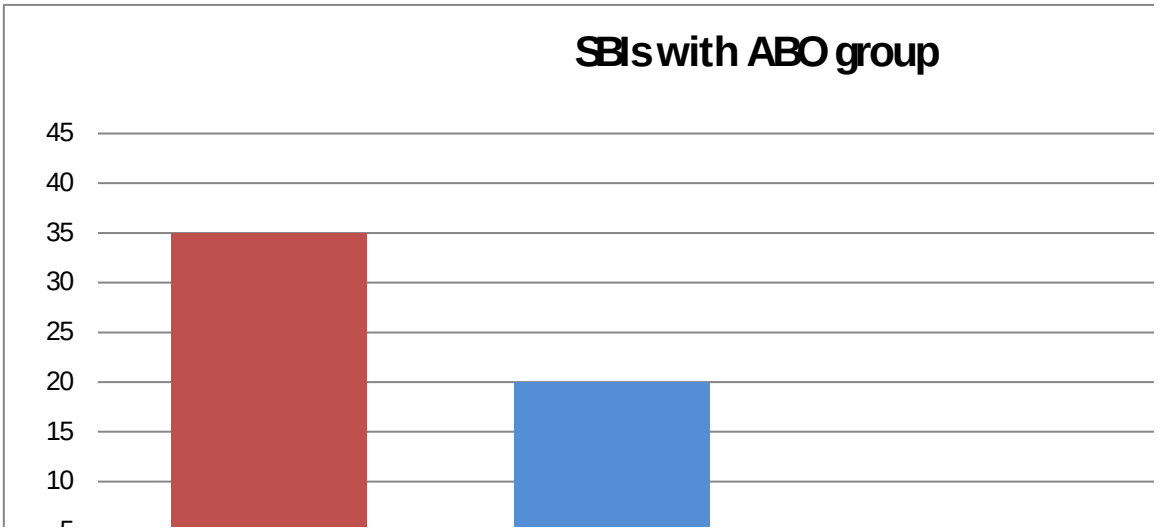

Figure 4. The relationship between the secondary bacterial infection and ABO group

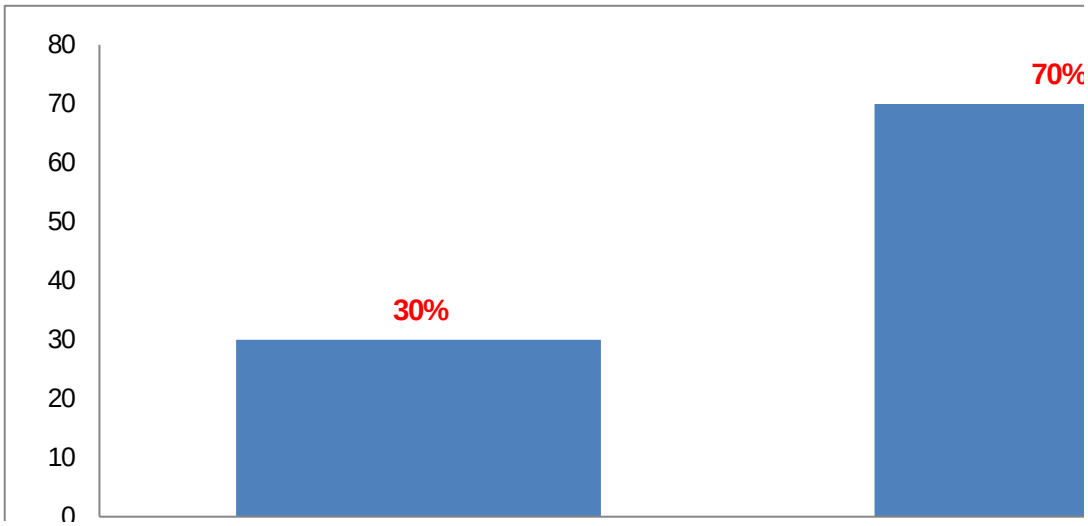

Figure 5. The percentage of treated and untreated antibiotics in COVID-19 patients associated with secondary bacterial infections

Relationship of SBIs with a duration of COVID-19 patients

The duration of infection with COVID-19 and SBIs included three parts, which included (<10 days, ١١-٣٠ days, and more than 1 month); the results shown in figure 6 showed that the high level of infection with SBIs was 61% during 10 days, followed by 30% in the period ١١-٣٠ days and 9% in more than 1-month, high levels of microorganisms will invade patients during the first month of infection with COVID-19 because of low immune system.

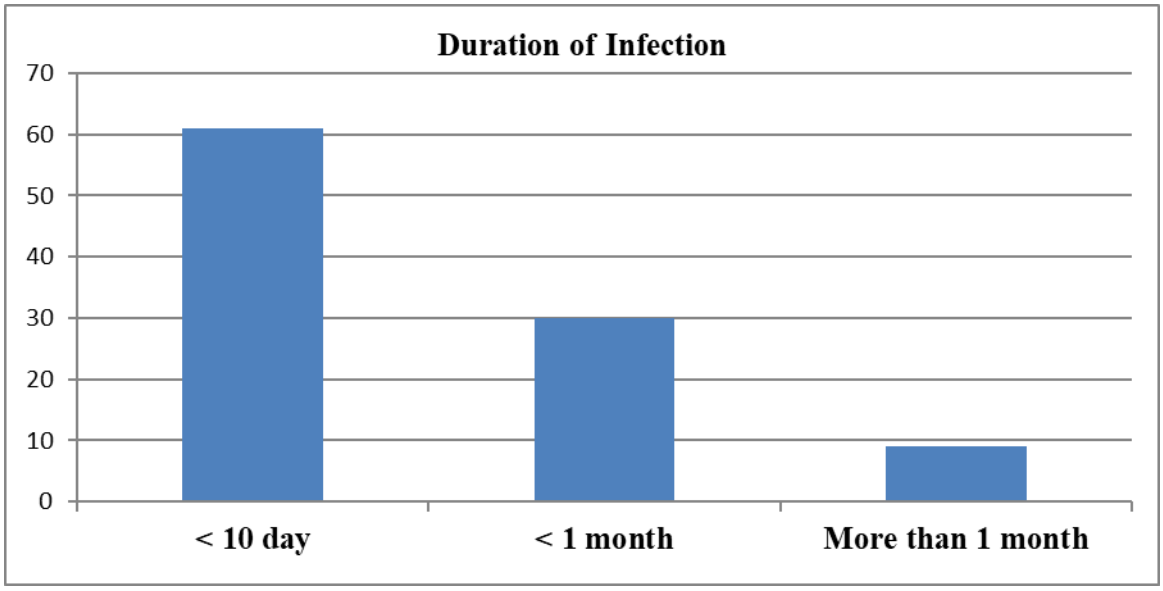

Figure 6. The percentage of treated and untreated antibiotics in COVID-19 patients associated with secondary bacterial infections

Discussion

Gram-positive bacteria *Staphylococcus aureus*, (16 isolates) were isolated from nasal swabs, pharyngeal swabs, and urine whereas the percentage was 100%, 15% and 25% respectively. Instead, four types of Gram-negative bacteria; *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, and *Escherichia coli* were isolated and distributed as follows: 6 (33.3%,) isolates of *Pseudomonas aeruginosa* from sputum and blood, 9 isolates of *Acinetobacter baumannii* from pharyngeal swab and sputum were 6 (30%) and 3 (20%) respectively, as well as 20 isolates of *Klebsiella pneumoniae* isolated from the pharyngeal swab (55%), sputum (46.6%) and blood (66.6%) and 6 (75%) of *Escherichia coli* were isolated from urine samples only (Table 1).

The current results are in agreement with several studies as; Fu et al. [13] who showed most patients with COVID-19 developed a SBI that included *Acinetobacter baumannii*, *Klebsiella pneumonia*e, and *Staphylococcus aureus*, which isolated from blood, pharyngeal, and sputum. Multiple organisms were found in patients with COVID-19, according to Chen et al [1]. Drug-resistant microorganisms, such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae* have been linked to infections in COVID-19 patients [14-16]. SBIs can develop in COVID-19 patients, resulting in a significant fatality rate. The severity of the illness at the time of admission was linked to the occurrence of SBIs and the resistance levels of the principal isolated bacteria were usually high.

Fifty-seven isolates have been tested for sensitivity to antibiotics [12] and the current results shown in the figures (1,2,3) showed that isolates (SBIs) of *Staphylococcus aureus*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and were highly resistant to antibiotics, the current results agree with [17] when mentioned in his paper the high occurrence of carbapenem-resistant *Klebsiella pneumonia*e and *Acinetobacter baumannii* long-term nosocomial infection at the main campus hospital, particularly among Covid-19 patients. The bacteria *Acinetobacter baumannii* and *Klebsiella pneumoniae* were shown to be the most public causes of bacterial infection, accounting for 90.6% of all cases, and were closely linked to death [17]. The contaminated environment was proven to be a substantial cause of nosocomial infection [1]. The antimicrobial resistance rates of the principal identified bacteria are commonly high, implying that more precise antibacterial agent administration for SBIs in COVID-19 patients is required [18].

Behind COVID-19, SBIs had evolved into a secret threat. One of the most important factors in the successful treatment of COVID-19 is the use of an efficient antibiotic regimen. In severe COVID-19 patients with SBIs, a brief guide recommends empiric antibiotic treatment for all potential microorganisms [19].

Antibacterial drugs may be used more widely, which could lead to changes in etiology and antimicrobial resistance. SBIs in COVID-19 patients should be treated based on additional microbiological information [20]. In studies on the clinical features of COVID-19, certain occurrences of bacterial infections were recorded; however, there were no methodical studies on the origin of SBIs, and the total of positive cultures was minimal [21].

From figure (4), blood types, A and O were crucial parameters to consider when assessing the prognosis of COVID-19 patients with SBIs, these results agree with Muñiz-Diaz et al. [22] when showing the rate of blood groups, A, O, B and AB were (47%, 41%, 7% and 3%) respectively. The current results were similar to another results study done by Zheng et al. [23] looked at the clinical features of 134 COVID-19 cases in China and reported that the males were more prevalent than females, and the percentage of older patients with the underlying disease was comparatively high. Importantly, the researcher discovered in their study that the ABO group related to COVID-19 patients were A: 43.82%, B: 26.91%, O: 19.21%, and AB: 10.1 %.

According to the study carried out by Ngassaki-Yoka et al. [24], SBI has been linked to the ABO

polymorphism; the presence or lack of A/B antigens, as well as the presence or absence of anti-A/B antibodies, provide strong or weak defense barriers against infection. The ABO gene is found in many vertebrate species, and it has so benefited them. However, possessing both functional A and B genes in a species may not be necessary because anti-A/B antibodies may be lost over time. However, frequent A/B specificity gene conversions that result in amino acid changes or recombination with nonfunctional incomplete genes may have conferred resistance to microbial attacks [25].

ABO blood group antigens can be discovered in the human respiratory, digestive, and reproductive systems [26]. As well as ABO blood groups have been connected to the development and transmission of numerous diseases in the past, probably because blood group antigens act as virus receptors [27].

In Figure 5, as shown above, the high rate of untreated antibiotics for patients with COVID-19 who acquired SBI was (70%) while the patients who were treated with antibiotics were (30%), our results were close to a study carried out by Langford et al. [25] when studying the effect of antibiotics on COVID-19 patients and those who acquired SBI, noted most of the patients who treated antibiotics were a lower rate to infect SBIs. The number of COVID-19 patients with SBIs varies substantially, from 0 to 100% in those who died, as does antimicrobial use, which ranges from 20% to 100 % depending on the severity of the illness [28].

Antibiotics are useless in the treatment of COVID-19, but they are administered for a variety of reasons in patients with suspected or confirmed COVID-19. This includes the difficulty in excluding bacterial co-infection at the time of presentation, as well as the risk of bacterial secondary infection later in the disease [2]. Several strategies suggest the use of experiential antibiotics for severe COVID-19 patients [6] based on concerns about an increase in mortality in patients with bacterial superinfection during influenza pandemics. This premise, however, raises concerns about antibiotic misuse and the ensuing harm caused by bacterial resistance.

The current study compared the period of infection from the first days to more than ten days, and more than a month of patients with COVID-19 and SBI, the results shown in figure 6 revealed that the early days (<10 days) from infection with COVID-19 which were more susceptible to SBIs. The early stages of COVID-19 infection have severe consequences for individuals, especially those with weak immune systems, the elderly and those with chronic diseases [29]. Since it is a new virus, the correct medication protocol for it was not known at the beginning of the infection, so the patient is at risk of contracting it SBI. Respiratory failure or failure of many organs in the early days is the direct cause of death in COVID-19 patients and SBIs it has an important role in the infection [18].

Worry can also be influenced by financial means with fewer financial means, getting quality medical treatment and COVID-19 preventative tools like face masks and cleaning equipment can be problematic. Age and ethnicity are two demographic factors that may be linked to increased anxiety as a result of inequities in job insecurity [30].

In conclusion, SBI are a common source of morbidity and mortality in viral respiratory tract infections like COVID-19. Antimicrobial resistance rates of the most often discovered bacteria are generally high, emphasizing that SBIs in COVID-19 patients hospitalized require more specific antibacterial drug treatment. Individuals in groups A and O had a higher risk of SBIs, but those in groups AB and B had a lower susceptibility. SBIs were more common in COVID-19 individuals who did not get antibiotics and were in the early stages of infection (less than 10 days).

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Author contribution

Both authors participated in concept and design, acquisition, analysis, interpretation of

data, statistical analysis, administrative, technical, and material support, and critical revision of the manuscript.

Conflict of interest

None.

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