Bacteriological Study of the lower Respiratory Tract in Adult Patients with COVID-19

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Abstract
Background: Due to the secondary bacterial and fungal infections in COVID-19 patients, the impact of the COVID-19 pandemic in Iraq was significant which led to high morbidity and mortality rates.one the main complication secondary bacterial and fungal infection post the viral infection wich led to damage of the respiratory tract mild to sever infection . Aim of the study : Amied To evaluate the type of bacterial infections that are concomitant with COVID-19 patients, to identify the antibiotics used to reduce mortality and morbidity in the selected group of patients. Patients and methods: Covid-19 patients at multi-isolation hospitals in Iraq (Baghdad and Al-Anbar provinces) were included in this study. Sputum specimens were studied by sputum direct smear and culture. Multiple blood parameters were studied as part of this research including white cells counts and their differential counts, C-reactive protein titer, procalcitonin titer and D-dimer titer Ethical approval was granted by the Ethical Approval Committee/the University of Anbar. Results: A total of Seventy-four (74) patients both male and female were showing symptoms of lymphopenia with include increased neutrophile count and other parameters. The number of Gram-positive bacterial isolates was (49), while Gram-negative bacterial isolates were (22). The number of Fungal isolates was(15) Mycoplasma pneumonia was (8), and a negative isolation result was found in one patient. Conclusion: Severe bacterial and fungal infections were associated with sepsis syndromes.The antibiotics used according to the result of the sputum investigation are to cover Gram-positive, Gram-negative, and Mycoplasma. Similarly, the treatment of fungal infection.
Keywords: COVID-19, sputum,bacterial &fungal infections, lymphopenia ,sepsis

1. Introduction

Since December 2019, SARS-CoV-2 started in Wuhan-China and spread out to the world causing more than 528,275,339 million confirmed cases that have been detected in 216 countries, with 6,293,414 fatalities reported to the date of this research [1]COVID-19 causes respiratory viral infections, however, the secondary bacterial infection is one of the main causes of its complications, particularly for high-risk patients such as those with immunodeficiency, immunosuppression, severe lung damage, oxygen therapy, on ventilators -whether invasive or non-invasive-, and hospital-acquired infections [2]. A high mortality rate among COVID-19 patients receiving convalescent plasma, particularly among those over 60 years old or with comorbidities[3].

According to recent data, the percentage of COVID-19 patients diagnosed with bacterial co-infections during hospitalization is growing. The multidrug-resistant bacteria have significant numbers among the microorganisms developed especially in ICUs. Many studies regarding hospitalized patients showed initial co-infection or subsequent bacterial pneumonia (11- 35% of cases) caused primarily by Streptococcus pneumonia and Staphylococcus aureus [4]. Co-infection rates have grown in critically ill patients admitted to ICUs around the world [5]. Co-infections with multidrug-resistant ESKAPE pathogens in COVID-19 patients are more possible due to contamination of surgical equipment, ventilators, and medical devices. ESKAPE pathogens such as Acinetobacter baumannii [6], Enterococcus faecium [7], Escherichia coli [8], Kliebsella pneumonia [9], Pseudomonas aeruginosa [10], and Staphylococcus aureus [11] have been observed, co-infecting COVID-19 patients. Antimicrobial resistance (AMR) is a prevalent feature of ESKAPE pathogens.

As a result, co-infections with the ESKAPE pathogens can obstruct COVID-19 patients' therapy and raise health risks. According to Mahmoudi's research [12], in a cross-sectional study of 340 patients using blood culture and endotracheal aspirate, 43 patients (12.46%) were found positive for ESKAPE pathogens, including 11 positive Klebsiella species, 9 positive methicillin-sensitive Staphylococcus aureus (MSSA), 6 positive methicillin-resistant Staphylococcus aureus (MRSA), 7 positive Escherichia coli coinfection, and 5 positive Enterobacter species. Another study by Hawazeen et al from ICU patients in Kirkuk, Iraq found secondary bacterial lung infection associated with COVID-19 as well. Out of 170 sputum specimens from COVID-19 patients, 119 samples were positive for bacterial isolates which represent 70% of total samples. Staphylococcus aureus ranked as the first isolation with 31.3% of total cases [13].

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It is a global concern since it occurs in 20-25% of all ICU patients resulting in significant mortality (22-71%). It is obtained by spontaneous mutation and horizontal transfer of novel resistance genes from other species. Antibiotic usage affects the pattern of resistance, which varies from area to region [14]. Antibiotic policies prevent the overuse of broad-spectrum antibiotics, reducing the emergence of resistant microorganisms [15]. It is critical to begin appropriate empirical antibiotics before getting microbiological findings. However, the rise of several Expanded Spectrum Beta-Lactamase (ESBL) generating microorganisms and multidrug-resistant (MDR) pathogens, MRSA, and other types of Gram-negative bacilli (GNB) makes this issue more challenging. ESBL-producing E. Coli and Klebsiella, MDR Pseudomonas, and carbapenem-resistant acinetobactor spp. are priority pathogens causing severity in ICUs. There are specific markers suggesting bacterial co-infection as a result of the immune system's regulation. Telemedicine in its most basic form has proven to be effective in the management of COVID-19 patients in places with limited resources. It offered necessary medical care while reducing the danger of disease transmission among healthcare personnel, patients, and their families [16].

2. Patients and Methods

Seventy-four (74) adult patients from both genders with a suspected secondary bacterial infection in multi- isolation hospitals in Baghdad and Anbar province were included in this study. During the period from March 1st, 2021 to March 1st, 2022; Sputum specimens and blood samples were collected from each patient to test for Coronavirus infections using RT-PCR and for (PCT) procalcitonin levels in blood samples. Sputum samples will be submitted to bacteriological investigation through cultivation on Blood agar, Chocolate agar, and MacConkey agar aerobically to identify bacterial types of secondary infections by VITEK2. While Sputum specimens were investigated for Mycoplasma pneumonia using the RT-PCR test.
Data were analyzed and plotted using suitable statistical methods by (SPSS) using version 26.0 including Chi-square, correlation, t-tests, mean, Std. deviation, frequency percentage, pie chart, and bar chart.

3. Results

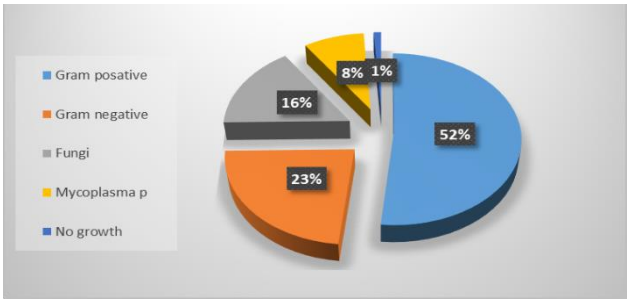

Figure 1 - Rate of bacteria isolation versus other types of Organisms

Gram-positive bacterial ranked the top isolation with (52%), followed by Gram-negative bacterial (23%) and Fungi (16%); While Mycoplasma pneumonia was (8%), and other specimens were showing a negative result of isolation (no growth) (1%).

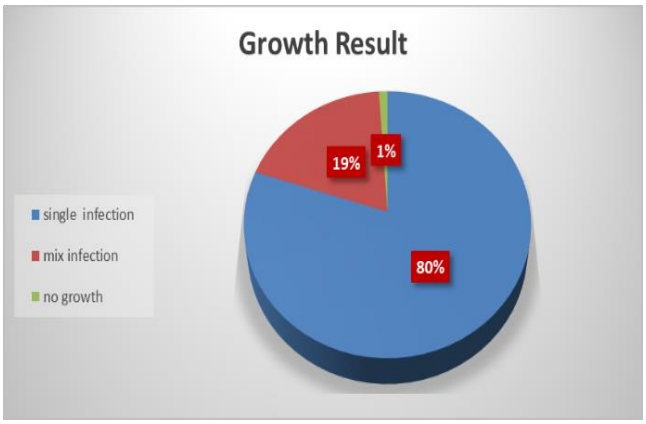

Figure 2 – Growth results representing the number of bacterial isolates

As for infections; out of (95) bacterial isolates, (80%) isolates were single bacterial types, while (19%) of isolates were mixed of two or more bacteria isolates, and no growth (1%).

Table 1 - Type of bacterial isolates organisms

Type of Bacteria	Frequency	Percent
Staphylococcus aureus	24	25.5
Staphylococcus haemolyticus	4	3.2
Staphylococcus epidermidis	1	1.1
Staphylococcus lentus	1	1.1
Streptococcus pneumonia	14	14.9
Streptococcus salivarius	2	2.1
Streptococcus gordonii	1	1.1
Streptococcus sanguinis	1	1.1
Klebsiella pneumonia	15	16
Escherichia coli	2	2.1
Enterobacter aerogenes	1	1.1
Kocuria kristinae	1	1.1
Serratia fonticola	1	1.1
Acinetobacter baumannii	3	3.2
Mycoplasma pneumonia	8	8.5
Candida albicans	15	16
Total	94	100

Staphylococcus aureus ranked the top isolate (24, 25.5%) followed by Klebsiella pneumonia (15, 16%) and Streptococcus pneumonia (14, 14.9%), while Candida albicans had a high frequency of isolation (15, 16%), and mycoplasma pneumonia was isolates for (8, 8.5%) of specimens. Other organisms combined were insignificant isolates that range between (1-3.2%). See Table (1).

Table 2 - Total leucocytic and differential count as well as other parameters (CRP/D-DIMER /PCT) in patients with Gram-negative and positive bacterial isolate

Parameter	Culture Gram	Mean	Std. Deviation	T	Sig. (2-tailed)
WBC	+ve	14.0631	6.65	-.629	.531
	-ve	15.2675	7.05		
LYM %	+ve	14.9940	27.12	.068	.946
	-ve	14.4875	22.42		
NUT %	+ve	73.5396	28.98	.665	.508
	-ve	67.9563	31.60		
CRP/mg/L	+ve	75.7022	39.40	.196	.845
	-ve	73.5625	34.99		
PCT/ng/L	+ve	1.6673	5.85	-1.774	.080
	-ve	5.0014	8.83		
D-dimer ng/mL	+ve	2604.8615	3314.20	-.347	.729
	-ve	2948.4156	4033.91		
The parameters WBC/LYM/NUT/CRP/PCT/D-Dimer showed that there were no significant differences between Gram-positive and Gram-negative isolates at a level of significance less than (P <0.05).					

4. Discussion

In cases of COVID-19 with lung damage, significant hypoxemia occurs once bacterial infection causes rapid deterioration in form of hypoxia, tachypnea, and oxygen demand requiring assisted ventilation. The use of steroids increases the risk of secondary bacterial infection. In another way, a severe bacterial infection might be associated with sepsis syndromes or septic shock leading to multi-organ failure and impending high risk to patient life. The time factor is very important to save patients' life, thus, antibiotics should be administered immediately after collecting specimen samples for bacteriological study including culture and sensitivity which consume time. The antibiotic is the cornerstone of managing bacterial infection, thus, selecting the correct antibiotics is important for the patients'' treatment. Therefore, this study is defining the guidelines for antibiotics used in these conditions.

According to the results of this study, antibiotics should cover Gram-positive including staphylococci and MERSA (51.6%) and Gram-negative bacteria (23.4%), while mycoplasma pneumonia was (8.42%). However, bacterial type evidence should be identified to adjust antibiotics to specific bacteria in the case of an immunocompromised patient. In case of fungal infection evidence like oral thrush that does not respond to the antibiotic, it is recommended to add antifungal therapy until evidence of fungal investigations appears. All patients had significant lymphopenia despite COVID-19 management, and all of them got secondary pyogenic and fungal infections; which means the prediction of secondary pyogenic or fungal infection can be predicted in this specific criteria. (Persistent lymphopenia risk of secondary bacterial infection).

In this study, there was leukocytosis and neutrophilia with elevated CRP titer and PCT in gram-positive and negative but there was a non-significant difference regarding this parameter with the type of bacteria.

Regarding this parameter in correlation with fungal infection significant leukocytosis, neutrophilia elevated CRP, and normal PCT level this point strongly helps the clinician to predict the fungal infection and treatment.

All Mycoplasma isolates (8) Bactria infections were contaminated with secondary to Gram-positive and fungal infection no pure mycoplasma infection alone. According to the result of our bacterial study most bacterial Gram-positive were Staphylococci (26) cases out of 49 (Staph aureus 24 and staph epidermidis 1 and Staph lentus 1). While most common Gram-negative was Klebsiella pneumonia (15) Escherichia coli (2) Acinetobacter baumannii (3) out of (22) cases.

5. Conclusion

This study showed bacterial infection post-COVID-19 pneumonia with presumption antibiotics used prior results of bacteriological results.

Limitations :This study provides an overall picture of the bacterial type secondary to COVID-19 pneumonia. As its aerobic bacteria in lung infection, we did not study anaerobic bacteria.We recommend another study for anaerobic bacterial infection.

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