Detection of Staphylococcal Pantone-Valentine Leukocidin (PVL) Gene in COVID-19 Patients

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Abstract

Background: Millions of people throughout the world have been impacted by the 2019 pandemic coronavirus disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (COVID-19). Critical risk factors for the severity and fatality rates of COVID-19 are bacterial co-infection and subsequent bacterial infection. It is also necessary to take into account the antibiotic resistant as a result of overuse. We will review the secondary bacterial infections and bacterial co-infections in COVID-19 patients in this study. we aimed To isolate Staphylococcus aureus and investigate the role of Panton-Valentine Leukocidin (PVL) toxin in severity of infection in COVID -19 patients. Study the antibiogram of Staph. aureus. **Methods:** This study was conducted with 100 patients of Iraqi-Kut city in both sexes, aged 18–70 years, in cooperation with AL-Zahraa Teaching Hospital in day 10 of COVID-19 infection. Collected 100 sputum samples (50 mild and 50 as severe), during period from December 2020 to June 2021, It was looked at whether individuals with the coronavirus disease of 2019 (COVID-19) had additional respiratory pathogens coinfected with them. Detection of bacterial strains by use VITEK2 system. detection of PVL gene and mec A gene by real time pcr technique. **Results:** In this study, collected 100 sputum samples from COVID-19 patients. the results showed that the percentage of bacterial infection (63%), fungal infection (16%), no growth (21 %). percentage of staphylococcus aureus was (17%); detection of PVL gene as positive results (29.4 %). **Conclusions:** simultaneous PVL gene and VITEK2 system identification of respiratory infections in SARS-CoV-2 patients, mec In particular during the COVID-19 pandemic, a gene by molecular diagnosis assays is required for determining the causal agents of coinfection.

Keywords: COVID-19, Bacterial Co infection, MeC A gene, PVL gene, Real-Time PCR, Staphylococcus aureus.

Introduction

Coronavirus disease 2019 (COVID-19) is an infectious illness brought on by coronavirus 2 that causes severe acute respiratory syndrome (SARS-CoV-2). Wuhan, China, saw the discovery of the first recorded case in December 2019 [1]. Since then, the illness has spread globally, causing an ongoing pandemic [2].

In viral respiratory tract illnesses like the flu, bacterial co-pathogens are frequently found and are a significant source of morbidity and mortality, necessitating prompt identification and antibacterial treatment [3,4,5].

It is essential to treat COVID-19 patients, ensure responsible use of antibiotics, and reduce the detrimental effects of overuse by being aware of how many COVID-19 patients have acute respiratory bacterial co-infection and the causative pathogens. Additionally, this information may significantly influence how empirical antibiotic therapy recommendations for COVID-19 patients are improved. We conducted a quick review to ascertain the frequency of bacterial infection in COVID-19 patients and to pinpoint the most prevalent co-infecting respiratory pathogens in these people[6].

Respiratory viruses impair the host's immunological response, which increases bacterial adherence to virus-infected cells. Pneumonia was the most frequent source (19%) in patients with COVID-19 who had S. aureus bacteraemia with an identifiable cause. Staphylococcus aureus coinfection causes bacteraemia and pneumonia in individuals with COVID-19. COVID-19 has been described as being complicated by PVL-producing strains causing necrotizing pneumonia [8]. During the SARS outbreak of 2004 [9], methicillin-resistant Staphylococcus aureus (MRSA) infections obtained in ICUs were more common.

Some strains of Staphylococcus aureus produce a cytotoxin called Panton-Valentine leukocidin (PVL). Primary skin infections and necrotizing pneumonia are caused by these strains [10,11.[

The development of lung bacterial coinfection or superinfection in some COVID-19 patients may have a negative prognostic impact [12,13,14].

In this large cohort, S. aureus was the most common cause of respiratory infections (early and late infections were combined for this estimate) [15]. S. aureus was the most frequently isolated pathogen from sputum and was found in 11 out of 93 hospitalized COVID-19 patients in a prospective, single center research done in Peru [16].

Patients and Methods

Patients

In this study, were collected 100 sputum sample of

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patients who were admitted to the Alzahraa teaching Hospital and AL karama teaching hospital between December 2020 and June 2021. The SARS-CoV-2 nucleic acid real-time polymerase chain reaction (RT-PCR) analysis performed on samples obtained from nasal and oropharyngeal swabs resulted in the confirmation of 100 individuals as being positive for COVID-19 infection.

Inclusion criteria: Positive results for COVID-19, Ages (18-70 years) and both sexes. Exclusion criteria: Patients that received of dexamethasone drugs and aged below 18 years.

This study was subject to ethical concerns and carried out in accordance with the consent form provided by the Iraqi Ministry of Health for the purpose of collecting samples.

Conducted a rapid systematic review guided by recommendations to determine the prevalence of respiratory bacterial infections among individuals with confirmed active COVID-19 infection [17].

The consent form for the sample collection for this study was provided by the Iraqi Ministry of Health, and it was followed.

An expedited systematic review was conducted with the help of recommendations [17] to determine the prevalence of respiratory bacterial infections among individuals with confirmed active COVID-19 infection.

Sputum sampling

In this research the specimens collected of 100 sputum samples of COVID 19 patients and sent to the microbiology lab to confirm the diagnosis of bacterial Co-infection by use suitable culture media . Blood agar was used to perform a subculture at first. MacConky agar, biochemical tests, confirmed by VITEK-2 system for identification and antibiotic sensitivity tests.

Staphylococcus aureus is diagnosis and isolated by use Manitol salt agar, Catalase test, coagulase test, in accordance with the manufacturer's instructions and Clinical and Laboratory Standards Institute recommendations, utilizing the VITEK 2 automated platform (bioMérieux, France), colonies of staph. aureus kept in brain heart infusion with 20% of glycerol and stored in refrigerator.

DNA extraction:

Staphylococcus aureus colonies are dissolved and cultured on the activated medium; use Luria-Bertani medium before extraction. staphylococcus aureus colonies DNA was extraction using Bacterial Genomic DNA Extraction Kit (Solarbio, china) according to the manufacturer's instructions, with the addition of 20 lit of lysostaphin to the bacterial suspension.DNA Purity and concentration for all extracted DNA samples by using nanodrop system.

PVL gene and Mec A gene

Genotyping of staphylococcal *pvl* gene and *mec*A gene was performed using specific PCR. The following probe and primer sequences were used in this study:

mecA gene-fw; CAATGCCAAAATCTCAGGTAAAGTG, rev AACCATCGTTACGGATTGCTTC

Probe FAM- ATGAGCTATATGAGAACGG-MGBNFQ

pvl gene- fw; AAATGCTGGACAAAACTTCTTGG, rev TTTGCAGCGTTTTGTTTTCG

Probe VIC-AAATGCCAGTGTTATCC-MGBNFQ. TaqMan Universal PCR master mix (ABI, USA). The final volume of each PCR reaction was 25 µL , which was composed of 4 µL genomic DNA, 12.5 mL of PCR master mix (2X), 1.0 mM of each forward and reverse primer, and 25 mL of nuclease-free water.

The Perfect Start TM II Probe qPCR Super Mix is a complete, ready-to-use qPCR mixture that lacks only the probe, primer, and template. It includes dNTPs, PCR stabilizer, PCR enhancer, and Perfect Start TM Taq DNA Polymerase. A template, primer, probe, passive reference dye (optional), and nuclease-free water can be added to the provided 2 concentration of qPCR Super Mix to make it usable at a 1 concentration.

A PCR Thermocycler was used for all PCR experiments. The PCR was performed under the following conditions: initial denaturation at 95°C for 5 min; 40 cycles with each cycle containing denaturation at 94°C for 5 sec; annealing at 60°C for 25 sec; elongation at 72°C for 20 sec; and final cycle followed by extension at 72°C for 10 min.

Results

The study population herein comprised total of 100 patients, including females and males, aged (18-70) years. who had received a diagnosis of COVID-19 infection. one hundred sputum samples were collected of COVID-19 patients; 50 samples were classified as mild and 50 samples as severe.

As shown in table (1), the percentage of bacterial infection in severe stage was 39 (78.0%); and in mild stage was 24 (48.0%). While the percentage of fungal infection in severe stage was 9 (18.0%); mild stage 7 (14%) and finally, there was no growth in severe stage in 2(4.0%) cases; and while 19 (38.0%) cases in mild stage, statistically, there is significant differences at p value <0.001 among disease stages with co- infections.

Table (1): Descriptive frequency of culture results in study groups.

		Stage		Total
		Severe	Mild	
Culture	No growth	2	19	21
		4.0%	38.0%	21.0%
	Bacteria	39	24	63
		78.0%	48.0%	63.0%
	Fungi	9	7	16
		18.0%	14.0%	16.0%
P value		<0.001		100.0%

The present study showed that the percentage of Staphylococcus aureus was 17 % (84.6% in severe stage, 24.0% in mild stage) and percentage of No growth was 21% (15.4% severe, 76.0% mild). Statistically, there were highly significant observed between the Severe and Mild stage among the staphylococcus aureus infection and No growth culture (P value<0.001) (Table-2).

Table (2). Descriptive frequency of *Staphylococcus aureus* isolates according to stage of disease groups.

		Stage		Total
		Severe	Mild	
	S. aureus	11	6	17
		84.6%	24.0%	44.7%
	No growth culture	2	19	21
		15.4%	76.0%	55.3%
Total		13	25	38
		100.0%	100.0%	100.0%
P value		<0.001**		

The VITEK 2 automated platform was used to identify S. aureus, confirm methicillin resistance, and test for antibiotic susceptibility (bioMerieux, France), the result showed that 17% of patients were positive for this bacteria and 83% were negative.

The present study showed that the qRT-PCR based PVL gene results showed that 29.4% was positive for this bacteria (36.4% in severe stage, 16.7% in mild stage) and 70.6% was negative (63.6% in severe stage, 83.3% in mild stage). There was no significant difference among stage of disease P value= 0.395 as shown in (table-3).

Table (3): The frequency of *Staphylococcus aureus pvl* gene according to stages of disease groups.

		Stage		Total
		Severe	**Mild**	
PVL	Positive	4	1	5
		36.4%	16.7%	29.4%
	Negative	7	5	12
		63.6%	83.3%	70.6%
Total		11	6	17
		100.0%	100.0%	100.0%
P value		0.395		

The present study showed that the percentage of Methicillin-resistantn Staphylococcus aureus by detection MecA gene in qRT-PCR was 7(54.5% in severe stage, 16.7% in mild stage), and percentage of Methicillin- Sensitive Staphylococcus aureus was 10 (45.5% in severe stage, 83.3% in mild stage). Statistically, there were no significant observed between the Severe and Mild stage among the MRSA) and (MSSA) (P value 0.160) (table-4).

Table (4): The frequency of *Staphylococcus aureus* methicillin resistance pattern according to stage of disease groups.

		Stage		Total
		Severe	**Mild**	
Methicillin	Resistant (MRSA)	6	1	7
		54.5%	16.7%	41.2%
	Sensitive (MSSA)	5	5	10
		45.5%	83.3%	58.8%
Total		11	6	17
		100.0%	100.0%	100.0%
P value		0.160		

Discussion

The global dissemination of COVID-19 has been incredibly quick. Secondary bacterial infections should be considered the most dangerous events among the disease's probable consequences [18]. Recent study suggested that S. aureus-associated infections are among the most significant bacterial etiologies. For instance, a COVID-19 hospitalized patient observational cohort research [12] looked into the prevalence of co-infections and superinfections.

In keeping with a recent meta-analysis [19,20], males were more frequently infected than females in the current investigation. Sex hormones, which are crucial for both innate and adaptive immunity, may explain why females frequently exhibit lower susceptibility to viral infections and better immunological responses than males [21].

The current study shown the percentage of bacterial infection in severe stage was 39 (78.0%); and in mild stage was 24 (48.0%). While the percentage of fungal infection in severe stage was 9 (18.0%); mild stage 7 (14%) and finally, there was no growth in severe stage in 2(4.0%) cases; and while 19 (38.0%) cases in mild stage, statistically, there is significant differences at p value <0.001 among disease stages with co- infections.

According to another study [22], In COVID-19, bacterial coinfection is present anywhere between 3% and 30% of the time. In the current COVID-19 pandemic, Zhou et al. (2020) found that secondary bacterial infections were present in 50% of patients who died, while another investigation found both bacterial and fungal infections [23]. Some recommendations call for empirical antibiotics because COVID-19 illness and atypical bacterial pneumonia or nosocomial pneumonia have similar clinical phenotypes and are difficult to distinguish from one another [24]. 56.6% of patients in a study done at 38 hospitals in Michigan got empirical antibiotic therapy [25].

Patients with COVID-19 appear to be less susceptible to bacterial infection (7%–14%) than those who have pneumonia caused by PVL-producing strains, which has been described as aggravating COVID-19 [26]. During the SARS outbreak of 2004 [9], methicillin-resistant Staphylococcus aureus (MRSA) infections obtained in ICUs were more common.

The present study showed that, the percentage of Staphylococcus aureus was 17 % (84.6% in severe stage, 24.0% in mild stage) while there is no growth in 21% (15.4% severe, 76.0% mild). Statistically, there were highly significant differences observed between culture results (staphylococcus aureus and No growth) in study groups (Severe and Mild stage) (P value<0.001).

Most pathogens were Staphylococcus aureus. Particularly, 30% of the respiratory infections were caused by methicillin-sensitive S. aureus (MSSA), and this finding was consistent with other publications that claimed S. aureus to be the most common cause of co-infection or secondary superinfection with COVID-19 [28].

The present study showed that the qRT-PCR based PVL gene results showed that 29.4% was positive for this bacterium (36.4% in severe stage, 16.7% in mild stage) and 70.6% was negative (63.6% in severe stage, 83.3% in mild stage). There was non-significant difference among PVL gene and stage of disease (severe and mild) P value= 0.395.

According to a study carried out in Germany, hospital employees who carry the MRSA isolates had a prevalence of 11.3%, with 9.1% of the isolates being PVL(+) MRSA and 2.2% being PVL(-) MRSA [29].

An influenza-like illness usually precedes S. aureus necrotizing pneumonia with PVL secretion [30]. According to some research, the novel SARS-CoV-2 may operate as a catalyst for S. aureus necrotizing pneumonia that produces PVL.

The present study showed that the percentage of Methicillin-resistant Staphylococcus aureus by detection mecA gene in qRT-PCR was 7(54.5% in Severe stage, 16.7% in mild stage), and percentage of Methicillin- Sensitive Staphylococcus aureus was 10 (45.5% in severe stage, 83.3% in mild stage). Statistically, non- significant differences observed between methicillin results (Resistant, and Sensitive) and study groups (severe and mild) (P value 0.160).

S. aureus was found in 31/66 positive bronchoalveolar lavage fluid (BALF) samples, and in 27/86 positive sputum samples, of which 14/27 were mecA positive (52%), and 16/21 of which were mecA positive (76%). The most recent study was conducted by Fontana et al. [31].

According to several research, methicillin resistant While Staphylococcus aureus carriage dramatically fell during the pandemic period compared to pre-pandemic, Staphylococcus aureus rates more than tripled. The rise in methicillin-resistant Staphylococcus aureus rates may be related to the recent trend of the bacteria becoming more prevalent or it may be a result of the pandemic's response efforts [32].

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