Evaluation Of Bacteria Staphylococcus Aureus Co-Infections Of The Respiratory Tract In Covid-19 Patients Admitted To Icu

Russell Aziz Nasser 1*, Muntadher Ali Alsaidi 2, Adil ALshamery 3

1, 2, 3 College of Medicine, University of Wasit, Wasit, Iraq
Emails: ta1997907@gmail.com 1, malsaidi@uowasit.edu.iq 2, adilalshamery@gemail.com 3

* Corresponding author: Russell Aziz Nasser
DOI: 10.47750/pnr.2022.13.S08.41

Abstract

This study is conducted on patients infected with COVID19 Those who are hospitalized in intensive care units in to AL-Shifa Center at AL-Zahraa Teaching Hospital for the period from December 2021 to March 2021, for age groups (41-80 years) and of both sexes. As 50 swabs samples are collected two swabs for each patient; one for culture and other for PCR after direct extraction for DNA, in order to investigate S. aureus infections associated with the emerging corona virus. During the laboratory diagnosis, in culturing obtained 24 (48%) positive samples for S. aureus. The extent of resistance of some commonly used antibiotics against S. aureus are more resistant to antibiotics and by 100% to isolates a higher sensitivity to 12 antibiotics including rifampicin, vancomycin resist, methicillin and tetracyclin. PCR detection of bacterial pathogens 50 (100%) for 16s RNA, while the mecA genes was 38 (76%), and showed no correlation with ICU admission, mortality, and inflammation markers. Although patients who received antimicrobial treatment were more often admitted to the ICU and had a higher mortality rate, PCR pathogen detection was not significantly related to antimicrobial treatment.

Keywords: COVID-19, S. aureus, Bacterial pathogens, Rapid multiplex PCR diagnostic, Antibiotic stewardship

Introduction

Respiratory illness caused by a novel coronavirus was first noted in December of 2019 in Wuhan, Hubei Province, China (Zhu et al., 2020). The novel coronavirus is now referred to as severe and critical acute respiratory syndrome coronavirus-2 (SARS-CoV-2) by the International Committee on Taxonomy of Viruses (ICTV). The SARS-CoV-2 was transmitted through respiratory tract and could induce pneumonia (Chen et al., 2020; Chu et al., 2020). By 6 April, WHO has reported of 1, 210, 956 laboratory-confirmed cases of SARS-CoV-2 infection and 67,594 deaths worldwide (WHO, 2020). The current outbreaks of coronavirus infection remind us that CoVs are still severe and critical threats to global public health. Co-infection with certain pathogens may also hinder accurate disease diagnosis. Wang et al. presented the latest status of the SARS-CoV-2 co-infection in China and added details on combined bacterial and fungal infections (Wang et al., 2020).
Staphylococcus aureus bacteraemia is an important cause of morbidity and mortality in both healthcare-associated (HA) and community-associated (CA) infections world- wide (Bassetti et al., 2012; Singh et al., 2018).
Staphylococcus aureus is responsible for an extensive range of human diseases, including bloodstream infections, pneumonia, endocarditis, food poisoning, toxic shock syndrome, skin and soft tissue infections, and bone and joint infections (Harmsen et al., 2003; Koreen et al., 2004). The prevalence of S. aureus varies depending on the healthcare facility, region and country. Furthermore, the prevalence of methicillin-susceptible S. aureus (MSSA) and methicillin-resistant S. aureus (MRSA) may also differ.

Previous reports have identified high rates of empirical antibiotic use, contrasting the low rates of laboratory-confirmed co-infections in COVID-19. For example, in one study, confirmed community-onset bacterial co-infection was reported in only 3.5% of the cases (of which 1.8% were bloodstream infections and 1.7% involved respiratory pathogens), although more than 50% of the patients were treated with empirical antibiotic therapy (Vaughn et al., 2020). Overuse of antimicrobials increases the risk of adverse effects such as multidrug-resistant nosocomial secondary infections (Sticchi et al., 2018).

Multiplex polymerase chain reaction (PCR) panels can rapidly identify the presence of relevant respiratory pathogens and may help clarify the indications for antimicrobial use as well as the choice of drugs. Therefore, this systematic prospective observational multicenter study aimed to determine whether general multiplex PCR-based screening for community-acquired bacterial pathogens (CABPs) at hospital admission could help physicians identify bacterial co-infections and predict the clinical course of COVID-19.

**Material and Method**

A total 50 swabs samples are collected from patients confirmed to have been infected with the emerging coronavirus, COVID-19, for both sexes (males - females) and for ages from 41-90 years and those in intensive care units (ICU) in to AL-Shifa Center at AL-Zahraa Teaching Hospital for the period from December 2021 to March, with the help of doctors Resident specialists, the samples are transferred directly to the microbiology laboratory at the Al-Kut Hospital for Women and Children. Samples are planted on blood agar medium and cultured on Manitol salt agar medium and incubated at 37°C for 18-24 hour, to investigate S. aureus bacterial. Associated with the emerging corona virus COVID-19, the bacteria were diagnosed through phenotypic and microscopic examination and through biochemical tests that included (oxidase, catalase, coagulase, a sensitivity test is conducted for bacteria under study and to determine their resistance against 12 antibiotics by Disks method. Diffusion Method and based on (Kirby and Bauer, 1966), which included, methicillin, rifampicin, vancomycin, Meropenem, Tetracyclin, Ceftriaxone, Ciprofloxacin, Azethromycin, Oxacillin, Erythromycin, penicillin G and tetracycline. Second swab sample was direct extraction for Genomic DNA Extraction Kit G-Spin Total DNA Extraction (As I mentioned in the instructions for the kit), and then in thermocycler multiplex PCR using 16s rRNA and mecA gene for S. aureus (Table 1). Statistically, the findings were analyzed by the t-test in the GraphPad Prism (6.0.1) Software, and differences were considered significant at P<0.05 (Gharban and Yousif, 2020; Gharban and Al-Shaeli, 2021).

**Table (1): Primers used in this study**

<table>
<thead>
<tr>
<th>Gene</th>
<th>5'-3'</th>
<th>Product size</th>
<th>Accession number</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16sRNA</td>
<td>F:GGTCTTGCTGTCACTTATAGATGG</td>
<td>164 bp</td>
<td>CP098686.1</td>
<td>Alves et al., 2018</td>
</tr>
<tr>
<td></td>
<td>R:CGGAAGATTCTCTACTGCTG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA</td>
<td>F:TGCTATCCACCCTCAAAACAG</td>
<td>286 bp</td>
<td>MW682923.1</td>
<td>Tsuchizaki et al., 2006</td>
</tr>
<tr>
<td></td>
<td>R:ACGTGTGTAACCACCCCCAAGA</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results**

A total of 50 patients with confirmed SARS-CoV-2 infection were 24 (48%) positive for Staphylococcus aureus. These isolates were identified according to the traditional technique, such as culture and microscopic examination, biochemical tests. And then obtained 50 isolates from 50 clinical sample secondary swabs were identified according
to the molecular technique confirmed by 16SrRNA percentage. Primary isolation indicates that these bacteria carries
the characteristics of S. aureus, thus the bacterial isolates were purified by sub culturing on the mannitol salt agar
(MSA). However, these results in the present study were agreed with study conducted by (Alaslawi and Corkery,
2016). Also, MSA is highly selective, and specimens from heavily contaminated sources may be streaked on this
medium without danger of overgrowth. Likewise, the appearance of colonies were yellow or golden, which was
consistent with Clauditz et al. (2006) who reported that a carotenoid pigment (staphyloxanthin) was responsible
for the characteristic golden color of S. aureus and plays a role in the ecological fitness of S. aureus. As well as, the
present study agreed with study achieved by Liu and Nizet (2009).
The bacterium of S. aureus was diagnosed in the laboratory by biochemical tests; these results were identical with
the results of the researcher Alaslawi and Corkery (2016), where the coagulase, catalase and staphylxanthin test. As
well as, nitrate reduction and acetone production gave a positive result, while both the oxidase test and the motility
test gave a negative result, as well as the emergence of colonies yellow on the medium of the facultative mannitol
and the appearance of violet clusters under the microscope. The sensitive and resistance of the isolates in the current
study of S. aureus was determined to about 11 different antibiotics and was based on measuring the diameter of the
inhibition zone and comparing it with what was reported in the 2022 CLSI.
The results in the current study were showed that MRSA was constituted 23(95%) out of 24 isolates for S. aureus.
These results in the present study were agreement with mohammed et al. (2018). One of the S. aureus isolates was
detected as methicillin-resistant Staphylococcus aureus (MRSA) and resistant to all other evaluated agents i.e.
penicillin, rifampicin, vancomycin, Meropenem, Tetracyclin, Ceftriaxone, Ciprofloxacin, Azethromycin, Oxacillin,
Erythromycin, Methicillin. While the molecular detection for another swab, a total of 50 specimens of S. aureus
were detected with 16SrRNA and 38 mecA genes (Table 2, Figures 1, 2).

Table (2): Distribution of co infection of S. aureus in covid 19 patient detect by PCR

<table>
<thead>
<tr>
<th>Types of gene</th>
<th>Outcome</th>
<th>Total (%)</th>
<th>T-test</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive No. (%)</td>
<td>Negative No. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>16S rRNA</td>
<td>50 (100%)</td>
<td>0 (0)</td>
<td>50 (100%)</td>
<td>0.000</td>
</tr>
<tr>
<td>Mec A</td>
<td>38 (76%)</td>
<td>12 (24%)</td>
<td>50 (100%)</td>
<td>20.324</td>
</tr>
</tbody>
</table>

Figure (1): PCR products of S. aureus 16S rRNA gene. The size of the PCR products is 164 bp. The gel was
1.5% and the DNA dye is RedSafe (Intron, Korea). V: 95, Time: 45 minutes. M: DNA ladder
In both high and weak biofilm formation isolates, all pathogenic genes were found; these results in the current study were agreed with a study conducted by Bimanand et al. (2018). While the mecA gene were carried in 38 (76%) of sample carried positive, but 12 (24%) isolates were considered negative result for mecA gene, these results in the current study were agreed with a study conducted by Heizmann and Magiera (2017).

Discussion

COVID-19, a viral pneumonia with an unusual outbreak, is considered as a new public health concern threatening us worldwide. Recent studies show that 2019-nCoV or SARS-CoV-2 originated from an animal source and later adapted to other variants as it crossed the species barrier to ultimately infect humans (Li et al., 2020; Singhal, 2020). In recent months, less attention has been paid to hospital-acquired infections and opportunistic microorganisms, which could be due to the outbreak of COVID-19 and its consequent long-term hospitalization of patients, and high workload on the healthcare personnel. In this study, with a focus on secondary infection of the lower respiratory tract of patients, S. aureus was the most common organism. In recent years, emerging strains of both species that have acquired additional genetic features have shown to be commonly associated with hypervirulence and resistant to many types of antibiotics (Paczosa and Mecsas, 2016; Sampedro and Wardenburg, 2017). In addition, both A. baumannii and S. aureus were among the most isolated bacteria from non COVID-19 ICU patients in Iran and other countries (Papazian et al., 2020). This incidence rate is higher than similar recently published articles. In Fu et al. study, 13.9% (5 of 36) of the patients in the ICU were diagnosed with severe acute respiratory syndrome coronavirus 2 and secondary bacterial infection. In another report that was published from a UK secondary care setting, amongst 836 patients identified as SARS-CoV-2, 27 cases (3.2%) had early confirmed bacterial isolates identified (0–5 days post admission) rising to 51 cases (6.1%) during the admission (Fu et al., 2020; Hughes et al., 2020).

In our study, one of the strains of S. aureus was identified as MRSA. This organism plays an important role in the severe complication of infections in ICU environments. The probability of acquiring MRSA may increase (> 2.5–4 times) in patients with longer stays in the ward, i.e. more than one week (Hardy et al., 2004). Different studies have also shown that lower respiratory tract infections caused by MRSA can be associated with a significant level of mortality in the patients admitted to ICUs (Rello et al., 1994; Thompson, 2004).

The co-infection of the influenza virus with Staphylococcus aureus, especially MRSA, has been previously documented. In a study performed by Bhat et al. (2005) during the 2003–2004 influenza seasons, bacterial co-infections were identified in 24 of 102 cases. Accordingly, S. aureus was the most common etiology (11 cases); six of these 11 cases were detected as methicillin-resistant strains (Bhat et al., 2005). Randolph et al. (2011) reported that among 838 children with influenza A (H1N1) virus admitted to a pediatric intensive care unit during the 2009 influenza A (H1N1) pandemic, 71 (8.5%) had a presumed diagnosis of early S. aureus co-infection of the lung with
48% positive for MRSA. Moreover, project on mouse model findings also showed that secondary infection with methicillin-resistant Staphylococcus aureus after infection with influenza virus was associated with high mortality rates (Randolph et al., 2011)

Conclusion
50 swabs sample screening using multiplex PCR with nasopharyngeal swabs may facilitate prediction or identification of bacterial co-infections in the early phase of COVID-19-related hospitalization. Most patients with positive PCR results appear to be colonized rather than infected at that time, questioning the value of routine antibiotic treatment on admission in COVID-19 patients.

References