Original Article

Investigate The Incidence Of Bacterial Streptococcus Pyogenes And Klebsiellae Pneumoniae In COVID-19 Patients By Direct Detection

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
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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. pyogen and K. pneumoniae infections associated with the emerging corona virus. During the laboratory diagnosis, culturing was obtained 21 (42%) positive samples for S. pyogen and 5 (10%) for K. pneumoniae. The extent of resistance of some commonly used antibiotics against S. pyogen and K. pneumoniae was 8 antibiotic disk for each all.PCR detection for 50 patients after extraction for swab gave positive result for S. pyogen 5 (10%) and K. pnemonaie 7(14%). Compared to those without additional pathogens, patients with co-infections and/or secondary infections were more likely to receive antibiotics.

Keywords: Co-infection, COVID- 19, Streptococcus pyogen, Klebsiella pneumoniae, Secondary infection

Introduction

Coronavirus disease 2019 (COVID- 19) caused by severe acute respiratory syndrome coronavirus 2 (SARS- CoV-2) is an on-going pandemic (Huang et al., 2019). Most COVID- 19 cases are asymptomatic or have mild symptoms similar with other respiratory viral infections (Iuliano et al., 2018). Co-infections, especially secondary streptococcal or fungal infections, are important risk factors for poor outcomes of influenza pneumonias (Metzger and Sun, 2013). However, the role of co-infections and secondary infections in COVID-19 patients is not well described. Although antibiotics are not effective for SARS-CoV-2, which are empirically used in COVID- 19 patients suspected with co-infection or secondary infection. Therefore, understanding the epidemiological patterns of COVID- 19 with co-infection and secondary infection is crucial for clinical treatment and to help ensure rational use of antibiotics. Co-infections and super-infections are common in respiratory viral infections (McArdle et al., 2018; Paget and Trottein, 2019).According to the laboratory, clinical, and epidemiological studies, secondary or bacterial co-infections with other viruses can significantly increase the mortality rate in patients infected with viral infections (Beadling and

Slifka, 2004; Metzger and Sun, 2013). It has previously documented that the mortality rate of viral infections can be influenced by different factors, such as bacterial co-infection (Jia L. et al., 2017; Katsurada et al., 2017; Quah et al., 2018). The mechanisms of severe complications caused by influenza-bacterial co-infections mainly include a lack of effective immune response as well as pathogenic synergy (Paget and Trottein, 2019). Although multiple microbial agents can cause acute lower respiratory tract infections, in most cases, the disease is caused by viruses and bacteria at the same time (Dasaraju and Liu, 1996). Secondary and bacterial co-infections with pandemics and viral epidemics have irreversible consequences, especially in high-risk groups, including those with immunodeficiency or immunosuppression (MacIntyre and Bui, 2017)

Emerging evidence suggests that the number of patients with COVID-19 diagnosed with bacterial co-infections during hospitalization periods is increasingly raised (Bengoechea and Bamford, 2020; Hendaus and Jomha,2 020; Rawson et al., 2020). The source and specific nature of these infections are yet to be fully explored, but there is some evidence suggesting that multidrug-resistant bacteria are among the pathogens that are thought to be responsible for the development of these infections (Bengoechea and Bamford, 2020; Hendaus and Jomha, 2020; Rawson et al., 2020). It is now known that viral infections can weaken the host immunity, paving the way for the development of viral-bacterial co-infection (Smith and Sweet, 2002; Almand et al., 2017). The new coronavirus, COVID-19, is another example of this fact as most of the hospitalized patients with COVID-19 acquired a secondary bacterial infection (Rasmussen et al., 2020; Ritchie and Singanayagam, 2020; Tetro, 2020).

The use of rapid diagnostic tools and methods promoting the prescription of effective narrow-spectrum antibiotics should be taken into account. The outcome of viral pneumonia may be complicated by coinfections with other microbial agents. Studies have shown that patients with influenza respiratory tract infections had poorer clinical outcome when bacterial agents such as Staphylococcus aureus (S. aureus), Streptococcus pneumoniae (S. pneumoniae), Haemophilus influenzae (H. influenzae), Pseudomonas aeruginosa (P. aeruginosa) and Streptococcus pyogenes (S. pyogenes) complicated the primary state (Joseph et al., 2013; Chertow and Memoli, 2013; Golda et al., 2011; MacIntyre et al., 2018).

In another cohort of 3834 COVID-19 patients from 30 studies from China, United States and Spain reviewed by Lansbury et al, Mycoplasma pneumoniae, P. aeruginosa, H. influenzae, Klebsiella pneumoniae, Acinetobacter baumannii, Chlamydia spp., Enterococcus faecium, methicillin-resistant Staphylococcus aureus and Serratia marscencens were common bacterial agents associated with co-infection (Lansbury et al., 2020)

Material and Method

50swabs 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 Sterile swab with transport media directly and was swapped at least 1-3 times in site of nasopharyngeal and put in transport media of swab; directly inoculated on blood agar medium and MacConkey agar and incubated at 37ºC for 18-24 hour, same this swab inoculated on chocolate agar base and put in candle jar and incubated at 37ºC for 24-48 hours. All colonies from primary cultures were purified by sub culturing on blood agar and then re-incubated into as a selective medium for these bacteria and incubated at 37 ºC for 24 hours of blood hemolysis. Colonies were phenotypically diagnosed and the mucosal lactose-fermented colonies were focused on MacConkey medium and then transferred to solid EMB medium to separate Klebsiella from E. coli with metallic luster. Diffusion Method and based on (Kirby and Bauer 1966), included Ceftriaxone, Tetracycline, Amoxicillin / Clavulanic acid, Oxacillin, Erythromycin, Ceprofloxacin, meropenem and Azithromycin.

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 for S. pyogen tuf and mefA gene, while for K. pneumoniae rec A and wca G gene (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

Species	Gene	Primer Sequence 5'-3'	Amplicon size	Reference
S. pyogenes	Tuf	F:GTACAGTTGCTTCAGGACGTATC	195 bp	Picard et al., 2004
		R:GTTCGATTTCATCACGTTG		
	mef A	F:CTGTATGGAGCTACCTGTCTGG	294 bp	Nagai et al., 2001
		R:CCCAGCTTAGGTATACGTAC		
K. peumoniae	KP-27F3	F:GGATATCTGACCAGTCGG	176 bp	Dong et al., 2015
		R:GGGTTTTGCGTAATGATCTG		
	wca G	F:GGTTGGTCAGCAATCGTA	169 bp	Turton et al., 2010
		R:ACTATTCCGCCAACTTTTGC		

Results

A total of 50 patients with confirmed SARS-CoV-2 infection were 21(42%) positive for S. pyogen and 5 (10%) positive for K. pneumoniae. These isolates were identified according to the traditional technique, such as culture and microscopic examination, biochemical tests. But another swab when detected by PCR were obtained 5(10%) S. pyogen for tuf gene and 7 (14%) K. pneumoniae for rcsA gene isolates from 50 clinical sample secondary swabs were identified according to the molecular technique.

It indicated that S. pyogen bacteria possess many virulence factors that help it spread by breaking down connective tissues. Where Pfeiffer discovered during the outbreak of influenza in 1918 the bacterium Streptococcus pyogenes as a prominent bacterial organism in influenza, and these results also agreed with what is reached (Shakoor et al., 2019), where the study he concluded showed that the possession of these bacteria in the capsule is of great importance in the pathogenesis of bacteria.

Table (2): Distribution of gene types for S. pyogen bacteria

Types of gene	**Outcome**		**Total (%)**	**T-test**	**P-Value**
	Positive No. (%)	**Negative No. (%)**			
Tuf	5 (10%)	45 (90%)	50 (100%)	44.333	0.000
Mef A	3 (6%)	47 (94%)	50 (100%)	57.182	0.000

In the present study, a total of 5 (10%) specimens were detected with tuf gene by PCR technique, and 45 (90%) negative results for rcsA. while gave for mefA gene 3(6%) positive sample (Table 2, Figure 1), while for identification of K. pneumoniae by rcaA and wcaG gene (Table 3).

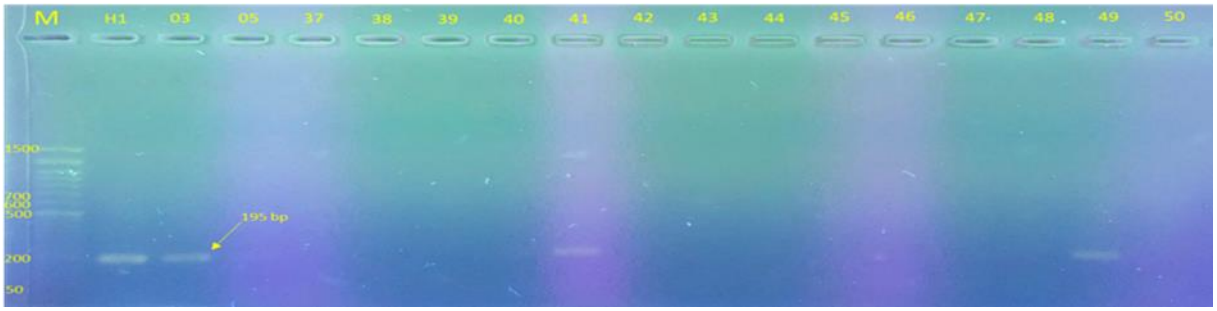

Figure (1): PCR products of the amplification of partial region of gene Tuf of Streptococcus pyogens The size of the PCR product is 195 bp. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder

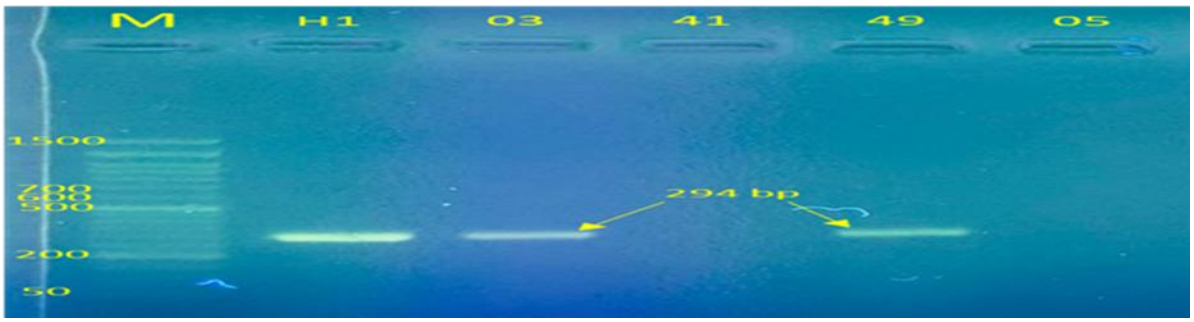

Figure (2): PCR products of the amplification of partial region of gene mefA of Streptococcus pyogensThe size of the PCR product is 294 bp. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder

Table (3): Distribution of gene types for K. pneumoniae bacteria

Types of gene	Outcome		Total (%)	T-test	P-Value
	Positive No. (%)	Negative No. (%)			
Rcs A	7 (14%)	43 (86%)	50 (100%)	37.523	0.000
Wca G	0 (0%)	50 (100%)	50 (100%)	0.000	1.000

In the present study, a total of 7 (14%) specimens were detected with rcsA by PCR technique, and 43 (86%) negative results for rcsA. while gave negative for all wcaG gene (Table 2, Figure 3)

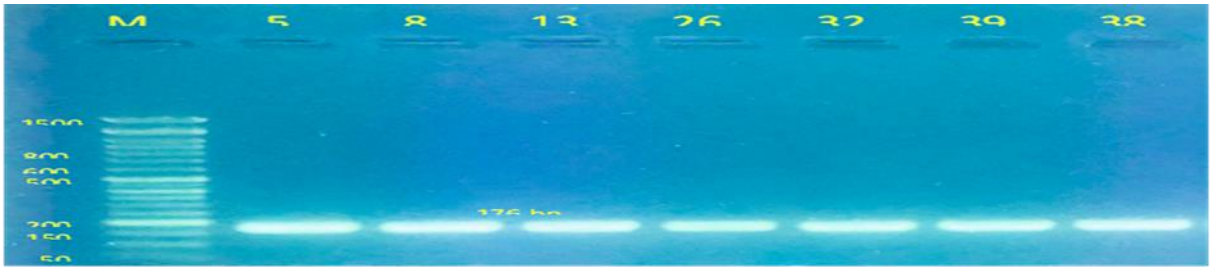

Figure (3): PCR products of the amplification of partial region of gene rcsA of Klebsiella pneumonia The size of the PCR product is 176 bp. The gel was 1.5% and the DNA dye is RedSafe (Intron, Korea). V: 90, Time: 45 minutes. M: DNA ladder

Klebsiella pneumoniae is a widespread nosocomial pathogen and the most significant member of the genus Klebsiella in the family Enterobacteriaceae. Researchers have confirmed that thebacteremia caused by K. pneumoniae can greatly increase in patient mortality (Zammit et al., 2014). Primary infections caused by classical K. pneumoniae strains are usually pneumonias or UTIs. Classical K. pneumoniae strains also cause very serious infections such as bacteremia, and these can be either primary bacteremia or secondary bacteremia that arises from secondary spread from a primary infection in the lungs or bladder (Kang et al., 2006; Qureshi et al., 2012; Zarkotou et al., 2011).

Klebsiella pneumoniae is the underlying cause of 11.8% of HAPs (Magill et al., 2014). Klebsiella pneumoniae HAP presents similarly to other nosocomial pneumonias, with respiratory symptoms that may include cough and unilateral pulmonary infiltrates and systemic symptoms that include fever and leukocytosis (Korvick et al., 1991). These HAPs occur in both ventilated and nonventilated patients, and K. pneumoniae is the causative agent in 8 to 12% and 7% of these cases, respectively (Restrepo et al., 2013; Lee et al., 2013; Jones, 2010).

Worryingly, but not unexpectedly, there is a significantly higher risk of K. pneumoniae being multidrug resistant in nosocomial infections than in community-acquired infections because many patients have been treated with antibiotics and are carrying antibiotic-resistant flora (Kang et al., 2006). While CAPs are fairly common, they are potentially serious infections that can progress rapidly and lead to hospitalization, intensive care unit (ICU) stays, and high rates of morbidity and mortality (Restrepo et al., 2013).

The regulation of the capsule synthesis A and B genes (rcsA and rcsB) can all increase capsule production. In addition, strains of the K1 and K2 serotypes are more resistant to phagocytosis and intracellular killing by alveolar macrophages and neutrophils than other strains, and this phenotype is independent of whether they are hypercapsule producers (Lee et al., 2014).

The wcaG gene is involved in this fucose synthesis and, not surprisingly, is associated with K. pneumoniae virulence (Yeh et al., 2010; Wu et al., 2008). One study found that this gene was present in 88% of clinical isolates with a range of serotypes and was found in both HV and classical strains (Turton et al., 2010). Furthermore, work with an HV K. pneumoniae strain noted that wcaG was necessary for hypercapsule production, but not LPS production, and virulence in a mouse i.p. injection model (Yeh et al., 2010).

Bacterial coinfection is also common in viral pneumonia especially in critically ill patients (Zhou et al., 2020). Among patients infected with respiratory viruses, the number of cases of primary coinfection or secondary bacterial pneumonia is between 11 and 35% (Klein et al. 2016). A totals of50 nasopharyngeal swab samples were analyzed in the present study. Result of culturing gave 21 (42%) positive sample for S.pyogen.As for the other antibiotics, it was resistant (100%) included all of Ceftriaxone, Tetracycline, Amoxicillin\Clavulanic acid, Oxacillin, Erythromycin, Ceprofloxacin. These results are in agreement with the findings (Kebede et al., 2021), where the results showed that high resist of S. pyogen for beta-lactam antibiotics. This is due to a lack of production beta-lactam by S. pyogen. However, the resistance of penicillins to pyogenes is due to escape from the entry of the epithelial cells which are penetrated by the penicillin (Kaplan et al., 2006), by transforming the biofilm (Ogawa et al., 2011).

These result showed decrease in S. pyogenes concomitantly to the large implementation of preventive measures to curb the COVID-19 pandemic. These findings are in line with several reports based on either the whole population with data issued from nationwide surveillance systems (Brueggemann et al., 2021; Steens et al., 2022). or on inpatients such as the pediatric population (McNeil et al., 2021). On the opposite, one study reported an increase of such infections during intervals when COVID-19 incidence slowed down, that is to date not clearly explained (Khongyot and Moriyasu, 2019).

Isolation of S. pyogens with a percentage of 42% is one of the bacterial causes associated with secondary infection, and these results are consistent with what is reached (Musher et al., 2021). It indicated that S. pyogen bacteria possess many virulence factors that help it spread by breaking down connective tissues. Where Pfeiffer discovered during the outbreak of influenza in 1918 the bacterium Streptococcus pyogenes as a prominent bacterial organism in

influenza, and these results also agreed with what is reached (Shakoor et al., 2019), where the study he concluded showed that the possession of these bacteria in the capsule is of great importance in the pathogenesis of bacteria.

Coinfection with bacteria has a great influence on the progression and prognosis of the disease, especially in severe patients, which can lead to increased needs for intensive care, antibiotic treatment, and increased deaths (Kiedrowski and Bomberger, 2018; Lim et al., 2019). SARS-CoV-2 infection can damage lymphocytes, especially B cells, T cells, and NK cells, which will lead to the immune system's impairment during the period of disease (Wang et al. 2020a). The decrease of lymphocytes and host immune function may be the main reason for the coinfection (Luo et al., 2019). The mortality is more significant in severe cases compared with the non severe group (Qin et al. 2020) due to the higher coinfection rate in severe patients.

The results of the study indicated that bacteria K. pneumoniae was more sensitive to antibiotics Meropenem 5 (100%). As for the antibiotics that the bacteria were resistant to Amoxicillin / clavulanic acid, Oxacillin, Erythromycin and by (100%) For both of them, followed by Tetracycline, Ceftriaxone, Ceprofloxacin, Azethromycin bacteria were resistant in the rate of 4(80%). The results of the current study showed that the antibiotic meropenem. It was the most anti-bacterial, was very sensitive to this antibiotic. Therefore, it is considered the best treatment for severe injuries, It also came in line with a study conducted by Chinese researchers (Xie et al., 2017)

The results of current study showed resistant for beta-lactame compatible with (Riwe et al., 2020), due to being they are negative bacilli, so Klebsiella pulmonary infection is the reason for many clinical infection in people. The Rcsphosphorelay system regulates capsule expression in E. coli and other Enterobacteriaciae and is induced by cues such as membrane stress (Wall et al., 2018; Majdalani and Gottesman, 2005). The RcsA is a component of the system which autoregulates and increases its own expression when activated. Note, however, that RcsA is not required for all permutations of Rcs signaling as RcsB can interact with a number of partner proteins to regulate transcription (Majdalani and Gottesman, 2005).

Conclusion

Co-infections and secondary infections existed in hospitalized COVID-19 patients and were relevant to the disease severity. Screening of common respiratory pathogens and hospital infection control should be strengthened.

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