Association between *Streptococcus pneumoniae* And COVID-19 Co-infection in Patients with Severe Respiratory Diseases

Suhaib K. Ibrahim

Anesthesia Department, College of Health and medical techniques, Middle Technical University, Baghdad,

Iraq.

Correspondence email: suhaib.khalid@mtu.edu.iq

ABSTRACT

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Background: *Streptococcus pneumoniae* bacteria are Gram-positive, round to lancelet in morphology. Arranged in pairs (diplococci), they cause opportunistic infections when spread from the nasopharynx to other body locations, like typical pneumonia, septicemia, meningitis, and otitis media. **Methodology**: In this cross-sectional study, A hundred samples of sputum were taken at Baghdad teaching hospitals and other hospitals of the medical city from individuals suffering from respiratory tract infections. 50% of patients were diagnosed with pneumonia had COVID-19 positive. These samples were analyzed by several techniques (Gram stain, optochin sensitivity, and catalase, biofilm, and HiCrome agar as selective media), then cultured on Mueller-Hinton agar (M.H.) for antibiotic sensitivity. **Conclusion**: This study indicates the presence of highly resistant pneumococcus with multiple drug resistance (MDR) ability; the most effective antibiotic in this study was meropenem, which inhibits these bacterial pathogens.

Keywords: *Streptococcus pneumoniae*, Coronavirus -19, multiple drug resistance, severe respiratory distress syndrome. https://doi.org/10.24126/jobrc.2025.19.2.826

INTRODUCTION

Streptococcus pneumoniae, also named pneumococcus, can cause severe bacterial infections in the upper and lower respiratory tract; it was first discovered in 1881 by Pasteur from patients' saliva open-access suffered from rabies (1-4). Streptococcus pneumoniae (S. pneumoniae) bacteria are Gram-positive, round to lancelet in morphology, and non-obligate anaerobic microorganisms. Arranged in pairs (diplococci), considered characteristic features of these bacteria, they may also appear under the microscope as singles or cocci in chains. This pathogen is specified by a large capsule surrounding the rigid cell wall; these capsules contain polysaccharide material that is considered an essential pathogenic antigenic determinant of this organism (5). The human nasopharynx is the main reservoir of pneumococcus at a different level from one individual to another, causing opportunistic infections when spread to other body locations like typical pneumonia, septicemia, meningitis, and otitis media (6,7). Morbidity and mortality from pneumonia infections caused by Streptococcus pneumoniae are essential chronic problems among children < 5 years old and the elderly. Community-acquired pneumonia is a persistent case in adults, and troubleshooting causes multiple drug resistances (8-10). Pneumococci in children are a predominant cause of bacterial typical pneumonia, meningitis, otitis media, and sinusitis. Pneumococcal meningitis incidence has been very high in recent years, with an increase in mortality to about 40 % in the United States (11,12). The human nasopharynx is the main reservoir of pneumococcus at a different level from one individual to another, causing opportunistic infections when spread to other body locations like typical pneumonia, septicemia, meningitis, and otitis media. Transmission of this bacterial pathogen from one person to another can occur via direct contact, respiratory aerosols, and infected droplets. It can easily lead to the colonization of pathogens and adhering to nasal mucosa that reaches finally to nasopharyngeal epithelial cells, which is the first line of pneumococcal pathogenesis (13-15). The most common clinical feature of *Streptococcus pneumoniae* is the typical pneumonia in adults; it is characterized by a very short incubation period of about less than three days. Frequent and predominant symptoms include recurrent fever onset, unrepeated chilling, and chest pain, mucopurulent cough with sputum, vomiting, tachycardia, hypoxia, fatigue, and weakness. Very complicated cases share bacteremia, pericarditis, endobronchial obstruction, and abscess formation in the lung (16-18). Children up to 2 years old or younger are characterized by bacteremia and pneumococcal pneumonia but without typical lung infection, which is considered the most common clinical findings of this age group, in addition to about 40% of invasive illnesses in this age group with bacteremia in about 25-30% of invasive pneumococcal disease in the same children age. This pathogen is considered the first causative agent of meningitis in children above five years of age in the United States. Before the classical use of the pneumococcal vaccine, children about one year old had a high incidence of meningitis caused by pneumococcal diplococci (19-21).

Bacterial pneumonia co-infection with coronavirus respiratory diseases is essential in exacerbating patient infections; the continuous pandemic coronavirus respiratory infection overrides host health immunity (22). COVID-19 spreads to the host's upper respiratory tract (URT). Lung colonization occurs in high percentages, causing severe respiratory distress syndrome (SARS) and contributing to a wide range of severity and mortality, with 20% of cases showing severe pneumonia in the lower respiratory tract. It also infects many systems and causes multi-organ failure with 5% mortality death (23-26). *Streptococcus pneumoniae* is a predominant bacterial pathogen that causes severe complications, reaching morbidity & mortality in corporations with respiratory virus-linked pneumonia in the infected lung. In previous studies, there is a fact that specific influenza and coronaviruses, when introduced to the lung, cause alteration in the respiratory tract entrance that promotes colonization, adhesion and triggering invasion of bacterial pneumococcus by lowering the immune system response to infection and inhibiting lung macrophage phagocytosis that leads to promote bacterial/ viral co-infection (27-31).

METHODOLOGY

1. Diagnosis of Patients with Covid-19

In this cross-sectional study, 100 sputum samples were collected from children and adult patients at Baghdad teaching hospitals and other hospitals of the medical city from November 2022 to January 2023, who suffered from severe acute respiratory pneumonia with complications and were directly diagnosed with COVID-19 in real-time (RT-PCR). Fifty patients had positive results, and the other 50 samples had negative results for the covid-19 investigation. Thirty out of 50 patients with COVID-19 were positive for *Streptococcus pneumoniae*. Of the other 50 patients who gave negative for COVID-19, 20 patients were infected with *Streptococcus pneumoniae*, and 30 were infected with other respiratory pathogens.

2. Samples collection and storage

All 100 sputum samples were collected from patients with respiratory tract infection from November 2022 to January 2023 ; the collection was done by particular screw-cupped container and sterile swab with enough volume; handling and labeling were done according to standard operating procedures (SOP), and all samples were stored at room temperature for 2 hours, refrigerated at four °C for no longer than 4 hours and for suitable identification stored at -20° C to -70° C for 24-48 hours (32).

3. Culture methods

I- Blood agar: colonies of *Streptococcus pneumoniae* were viewed on blood agar media after cultivation and incubation for 24 h at 37 °C(33) as in "Figure 1".



Figure 1: Streptococcus pneumoniae colonies on blood agar

II- HiCrome agar

The essential medium is selective and unique for Streptococci species; mainly group A ß-hemolytic streptococci from isolated specimens. Tryptophan salts and dextrose supplements were added to the media for the selective growth of streptococcus species and to inhibit Gram-negative bacteria by adding sodium derivatives like sulfate. Staphylococcus species were inhibited by the addition of crystal violet to the medium (34).

4. Biochemical tests

I. Catalase test

Certain Gram-positive cocci, like Staphylococcus species, produce catalase enzyme, so they are considered positive, while *Streptococcus* species are can't make this enzyme and considered negative; this test *in-vitro* is used to differentiate between two groups of Gram-positive cocci when the addition of a few drops of hydrogen peroxide (H_2O_2) on culture isolated on a glass slide it forms a bubble of oxygen by this enzyme that convert hydrogen peroxide to hydrogen & water (Table 1).

II. Optochin test

Specific tests are made to differentiate between two species of alpha-hemolytic non-groupable streptococci, so *Streptococcus pneumoniae* strains are susceptible to the chemical optochin test. In contrast, other alpha-hemolytics are resistant to this test (31).

5. Biofilm assay

A bacterial biofilm assay for all *Streptococcus pneumoniae* isolates was diagnosed using the microtiter plate method according to the crystal violet method. A 100µl of bacterial isolates in nutrient broth turbidity equal to 0.5 McFarland were added to each well with an equall volume of Tryptic soy broth (TSB), a specific broth medium that contains 0.2% glucose to enhance biofilm formation. The plates were then incubated at 37°C for an entire night. Incubated bacteria washed three times with phosphate-buffered saline (PBS) to remove non-adherent cells, and the cells were fixed for 10 minutes with 200 µl of 99% methanol. After applying 200 µl of 1% crystal violet dye to the plate, it was left for 10 mins. After washing with distilled water (D.W.) to get rid of an extra stain, 250 µl of 100% ethanol was added, an ELISA reader was used to detect the optical density (O.D.) at (570–600) nm. There was only one well-remaining, serving as a negative control with TSB medium, and one well serving as a positive control with broth and culture (35, 36) "Figure 2".



Figure 2: The final step of S. pneumoniae biofilm formation by crystal violet

6. Antibiotic Susceptibility Testing

This test was done by transferring colonies from an overnight nutrient agar plate culture to 3 ml of normal saline, the turbidity was reached to 0.5 McFarland equal to 1.5x10⁸ CFU/ml, the Kirby-Bauer disk diffusion technique was used to assess the antibiotic susceptibility of Streptococcus pneumoniae isolates. The bacterial solution was applied to a sterile cotton swab, and any extra fluid was squeezed out by pushing the swab up against the tube wall. Muller Hinton agar plates were streaked with the bacterial suspension and left to dry for ten minutes. Antibiotic discs were put on the surface of inoculated media, and then the plate was incubated for 24 hours at 35°C. Following the incubation, the inhibition zones were measured and compared to the Clinical Laboratory Standards Institute (CLSI) breakpoints (37). 7. Statistical analysis

The statistical data analysis approaches were used to analyze and assess the study's results using (SPSS) ver. (26.0) by Chi-square and t-test to examine the association between typical pneumonia caused by Streptococcus pneumonia and associated with COVID-19 co-infection. P values ≤ 0.05 were regarded as statistically significant (S), P values >0.05 were non-significant (NS), and P values ≤ 0.01 were considered statistically highly significant (HS).

RESULTS

Fifty Out of 100 sputum samples were collected diagnosed as Streptococcus positive (25 samples from adults and 25 from children). It was determined that the remaining 50 sputum samples had different bacterial illnesses. (Table 1).

Age	S.pneumonea isolates	Other G+ve & -ve bacteria	Total
Children	25	25	50
Adult	25	25	50
Total	50	50	100

Table 1: Distribution of collected sputum samples depending on age.

Fifty patients had positive results with COVID-19, including 60% diagnosed with pneumonia introduced by Streptococcus pneumoniae. Of the other 50 patients negative for COVID-19, 40% had respiratory infections with Streptococcus pneumoniae alone. This study revealed that pneumococcus bacteria are more frequent in COVID-19 patients co-infection than another group of patients not infected by coronavirus, at highly significant differences (p=0.01) (Table 2).

Table 2: Positive bacterial samples with Covid-19 co-infection							
Infection	S.pneumonea isolates	Other-bacterial infections	Total	p.value			
Co-infection with Covid-19	30(60%)	20(40%)	50	0.01*HS X ² =8			
No co-infection with Covid-19	20(40%)	30(60%)	50				
Total	50(100%)	50(100%)	100				

* *P-value* ≤0.01 considered highly significant (HS)

In the present study concerning biofilm formation ability, Streptococcus pneumoniae can be classified into two groups depending on their nature: co-infection and non-co-infection with COVID-19, as the biofilm was measured for each group. The co-infection group showed more frequent biofilm formation. It may cause severe lung damage through a thick layer of biofilm formation in about 70% of moderate-to-strong biofilm producers (Table 3).

Table 3: Bacterial biofilm formation between two study groups.						
S. pneumonia	No.	Non-coinfection	No.			
co-infection	(%)	S.pneumonia	(%)			
with covid-19						
Normal < 0.80	3(10)	Normal <0.50	5(25)			
Weak 0.80-0.150	6(20)	weak (0.50-0.100)	5(25)			
Moderate 0.150-0.300	12(40)	Moderate(0.100-0.200)	6(30)			
High > 0.300	9(30)	high > 0.200	4(20)			
Total	30(100%)	Total	20(100%)			

The antibiotic sensitivity tests were done for each group; the co-infection patients group showed a high resistance rate (56.25%) and low sensitivity (43.75%) for all antibiotics used compared to the non-co-infection group with coronavirus (100%) sensitivity of bacteria to meropenem for each group considered the most effective antibiotics for treatment of patients with bacterial pneumococcal infection. All other antibiotics show different levels of effect depending on the kind of group. The total percentage of resistance to 8 antibiotic discs was 56.25% in a COVID-19 co-infection and only 20% in a non-co-infection group at a highly statistically significant p. 0.00 (Table 4).

Antibiotics	Dose	Sensitivity in NO(%)		Resistant in NO(%)	
	micro	S.pneumoneae	Non	Co-infection	Non
	gram	Co-infection	Co-infection	S.pneumoneae	Co-infection
meropenem	10	30(100%)	20(100%)	0(0%)	0(0%)
Ceftriaxone	20	21(70%)	20(100%)	9(30%)	0(0%)
Gentamicin	10	3(10%)	10(50%)	27(90%)	10(50%)
Amoxicillin	30	9(30%)	16(80%)	21(70%)	4(20%)
Amikacin	30	9(30%)	16(80%)	21(70%)	4(20%)
Levofloxacin	10	21(70%)	20(100%)	9(30%)	0(0%)
Tetracycline	20	9(30%)	16(80%)	21(70%)	4(20%)
Penicillin	30	3(10%)	10(50%)	27(90%)	10(50%)
Total %		43.75 %	80%	56.25	20%
p-value	0.00	X ² =33.4	(% of R)		

Table 4: Antibiotics sensitivity tests of bacterial isolates.

* *P-value* ≤0.01 considered highly significant (HS)

The bacterial antibiotic resistance range is affected by biofilm production (strong biofilm enhances bacterial resistance to antibiotics); for a co-infection group with COVID-19, *Streptococcus pneumoniae* had a variable ability to biofilm formation that measured by ELISA reader and classified into (0%) normal or non-biofilm producers were < 0.80, which inhibited by all antibiotics (20%) weak biofilms producers (0.80-0.150) that resisted only gentamicin and penicillin (40%) moderate biofilms producers (0.150-0.300) were sensitive to imipenem, ceftriaxone and levofloxacin; high biofilms production > 0.300 was susceptible only to meropenem (100%); and resistant to all types of antibiotics in different rate (Table 5).

Table 5: Antibiotic resistance of <i>Streptococcus pneumoni</i>	e and	d its relation	biofilm	formation
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Biofilm range	Bacterial antibiotic resistance & biofilm formation in covid-19 group NO(%)							
Antibiotic R%	MERO	CEF	LEVO	AM	AK	TC	GE	PC
normal ≤ 0.80	0	0	0	0	0	0	0	0
Weak(0.80-0.150)	0	0	0	0	0	0	6(20)	6(20)
Moderate(0.150-0.300)	0	0	0	12(40)	12(40)	12(40)	12(40)	12(40)
$High \ge 0.300$	0	9(30)	9(30)	9(30)	9(30)	9(30)	9(30)	9(30)
Total	0(0)	9(30)	9(30)	21(70)	21(70)	21(70)	27(90)	27(90)

DISCUSSION

Streptococcus pneumoniae is linked with an increasing range of deaths in the world and causes millions of deaths among children worldwide in about 70% of developed countries. It is affected by severe respiratory pneumonia with typical complications (38). The current study results revealed that 50% of children & adult patients were affected by pneumococcal respiratory diseases. Fifty patients were diagnosed with pneumonia infected by Streptococcus pneumoniae, 25 samples were analyzed as children, and 25 samples were adults; this study's results are in agreement with the other study, whose results showed that 301 (45%) of patients were children from a total of 668 patients (39). The current study results revealed that 50 % of the patients had respiratory tract infections (pneumonia) caused by Streptococcus pneumoniae in both study groups; the current study results do not agree with another study, whose results showed that thousands of sputum samples were collected from both age groups and diagnosed with many Gram-positive and negative bacteria other than diplococci (40). The antibiotic sensitivity tests in the present study showed that meropenem was the most effective antibiotic. Ceftriaxone and levofloxacin had a good effect and inhibited about 70 % of bacteria. Regarding antibiotic results, the current study results were in agreement with other study, whose results revealed that 100% of Streptococcus pneumoniae isolates were sensitive to meropenem, 98% were susceptible to levofloxacin, and 91% exposed to ceftriaxone (41). Biofilm is an essential virulence factor of bacteria and is a multilayer polymer material of many substances like carbohydrates, lipids, and some proteins. A biofilm of pneumococcus in the current study formed a strong biofilm in about 70% of the COVID-19 co-infection group. These study results were in agreement with another study, whose results reported that 46.7% of pneumococci could produce strong biofilm, 43.3% of pneumococcus strains had moderate biofilm, and only 11.6% could weak biofilm production (42).

CONCLUSIONS

It is concluded that patients who have been previously diagnosed with COVID-19 are more likely to have virulent strains of diplococci causing severe respiratory complications like typical pneumonia. These isolates also exhibit significantly higher levels of antibiotic resistance and robust biofilm production compared to isolates from patients who do not have COVID-19 infection. The best medication for eliminating pneumococcus from such patients is meropenem.

Ethical Clearance

This study was approved by the Ethical Committee of the College of Health and Medical Techniques-Research Unit, Baghdad, Iraq.

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Conflicts of interests

The authors affirm that any conflicts of interest do not impact the publication of this paper.

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العلاقة بين بكتريا العقدية الرئوية والإصابة المرافقة بفيروس كورونا-19 لدى المرضى المصابين بالأمراض التنفسية الحادة

صهيب خالد إبراهيم

قسم التخدير ، كلية التقنيات الصحية والطبية ، الجامعة التقنية الوسطى ، بغداد ،العراق.

الخلاصة

الخلفية: البكتريا العقدية الرئوية Streptococcus pneumoniae هي بكتريا موجبة لصبغة كرام ، ذات شكل دائري الى رمحي تحت المجهر . مجهريا تترتب على شكل ازواج ثنائية المظهر ، تسبب العديد من الامراض الانتهازية في حال انتشار ها من تجويف الانف انتقالا الى امكن اخرى داخل جسم الانسان ومن بين هذه الامراض التي تسببها هي ذات الرئة المثالي ، تعفن الدم ، السحايا ، والتهاب الأذن الوسطى . الهدف : لهذه الدراسة هو لتحديد نسبة اصابات القناة التنفسية المتمثلة بذات الرئة المثالي ، تعفن الدم ، السحايا ، والتهاب الأذن الوسطى . الهدف : لهذه الدراسة هو لتحديد نسبة اصابات القناة التنفسية المتمثلة بذات الرئة المثالي ، تعفن الدم ، السحايا ، والتهاب الأذن الوسطى . الهدف : المراحفة لغير وس كورونا لهؤلاء المرضى وذلك لغرض القضاء على هذا النوع من اصابات الجهاز التنفسي الحاد طريقة العمل: وفي هذه الدراسة المستقطعة عرضيا ، تم جمع 100 عينة بلغم من المرضى الذين يعانون من امراض القناة التنفسية ، حيث ان 50 بالمئة من هؤلاء المرضى كانوا يعان بعانون من امراض القناة التنفسية ، حيث ان 50 بالمئة من هؤلاء المرضى كانوا يعانين معانون ألى يعانون من امراض القناة التنفسية ، حيث ان 50 بالمئة من هؤلاء المرضى كانوا يعانون ما مالم صنى الذين يعانون من امراض القناة التنفسية ، حيث ان 50 بالمئة من هؤلاء المرضى كانوا يعانون من النا ولي عائزي البلغم للكشف عن هذه البكتريا بفوصات مختبرية عديدة منها صبغة المستقطعة عرضيا ، تم حمع 100 عينة بلغم من المرضى الذين يعانون من امراض القناة التنفسية ، حيث ان 50 بالمئة من هؤلاء المرضى كانوا يعانون ما المناوز بالغم الكثري الغروبي الى روبي كانوا يعانون ما مراض القناء التنفسية من المرضى الكروم أكار يعانون ما مان مرافية من قبل هذه البكتريا الرئوية . تم تشخيص عنيات البلغم للكشف عن هذه البكتريا بفوصات مختبرية عديدة من الكروم أكار م ، فحص حسانية المنون من مارض قالة من موري افقة من المرضى المرضى الم مان الوسمى مالم من الكروم أكار م ، فحص حسانية الوبيتية المئوي النائين وبعن عان مرضى الكروم أكار أكاره ، فحص حسانية المضادات الحيوية التئاتي أله مال الكروم أكان أكان اكن مائم من مامم من موى من المان ما والمامن الن وما ما مالم ما مامم من مامم ما مومي معروبي ما مامم الكروم أول المان ما ماروى والمامن الكرم ما مامم الكان ما مارضى فالم مالم ما المرضى المرضى

ا**لكلمات المفتاحية** : البكتريا العقدية الرئوية ، فايروس كورونا -19 ، مقاومة متعددة للأدوية ، متلازمة الضائقة التنفسية الشديدة .