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Detection of pspA Gene (Pneumococcal Surface Protein A) in Streptococcus pneumoniae Isolated from COVID-19 Patients

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Abstract

Coronavirus disease 2019 (COVID-19) is a severe worldwide health issue. For a long time, it has been known that viral respiratory infections predispose people to bacterial infections. This study is conducted on patients infected with COVID19 who are hospitalized at Baqubah teaching hospital, for the period from October - 2021 to January -2022 December, for age groups (15-80 years) and of both sexes. As 100 sputum samples are collected, in order to investigate secondary bacterial infections associated with the emerging corona virus, all samples are growth-positive (100%). During the laboratory diagnosis 64 Streptococcus pneumoniae isolates are obtained from the positive samples. The gene that encodes to Pneumococcal surface protein A (PspA) of Streptococcus pneumoniae was investigated in 10 clinical isolates using conventional PCR technique. The results showed that all selected isolates (100%) have pspA gene.

Keywords: *Streptococcus pneumonia*e, COVID-19, *psp*A gene.

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الكشف عن جين pspA (بروتين سطح المكورات الرئويةA) في العقدية الرئوي المعزولة من مرضى COVID-19

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الخلاصة

يعد مرض فيروس كورونا 2019 (COVID-19) مشكلة صحية خطيرة في جميع أنحاء العالم. فمن المعروف منذ فترة طويلة أن التهابات الجهاز التنفسي الفيروسية تعطي الفرصة للعدوى البكتيرية. أجريت هذه الدراسة على مرضى مصابين بفيروس كورونا المستجد (COVID19) الذين تم نقلهم إلى مستشفى بعقوبة التعليمي، للفترة من أكتوبر - 2021 إلى يناير - 2022 ديسمبر، للفئات العمرية (15-80 سنة) ومن كلا الجنسين. تم جمع 100 عينة من البلغم، من أجل فحص العدوى البكتيرية الثانوية المرتبطة بفيروس كورونا المستجد، اظهرت جميع العينات إيجابية النمو (100٪). ومن خلال التشخيص المختبري، تم الحصول على 64 عزلة من المكورات العقدية ذات الرئة من العينات الموجبة، وتم فحص الجين المشفر للبروتين السطحي للمكورات الرئوية A (PspA) الخاص بالمكورات العقدية الرئوية في 10 عزلات سريرية. تم فحص جين *pspA* في عشر عزلات من *Streptococcus pneumoniae* باستخدام تقنية تفاعل البوليميراز المتسلسل التقليدية. تحتوي جميع المكورات العقدية ذات الرئة المختارة 10 (100٪) على جين *pspA*.

الكلمات المفتاحية: العقدية الرئوي، جين pspA، COVID-19

Introduction

Coronavirus is one of the most common pathogens that mostly targets the human respiratory system SARS-CoV-2 infection. Coronavirus disease has emerged as a new public health danger to humans. The new beta-coronavirus is to blame for this epidemic. COVID-19 has a number of key clinical characteristics, including high transmission rates, mild to severe clinical manifestations, and more serious radiological in the elderly. Coronaviruses enter cells through the ACE-2 receptor, which employ the ACE-2 receptor to enter cells [1]. To infiltrate human

cells, sin-converting enzyme 2 [ACE2) receptors are required, and these receptors are abundant in the intestinal epithelium [2].

A range of respiratory symptoms, including fever, dry cough, and dyspnea, as well as pneumonia, pulmonary edema, acute respiratory distress syndrome, and multiple organ failures, necessitate hospitalization in an intensive care unit and, in severe cases, death, are asymptomatic with coronavirus disease. [3]. Streptococcus pneumoniae is a common cause of pneumonia. Community-acquired pneumonia (CAP), bacteremia, otitis media, bacteremia , sinusitis, meningitis, and coinfection with other respiratory pathogens are all caused by Streptococcus pneumoniae, a gram-positive bacteria. [4] It is also a major cause of pneumonia , otitis media as well as invasive infections such as bacteremia and meningitis [5]. S. pneumoniae is a encapsulate diplococcus [6]. It often transmits to the upper respiratory tract in the form of aerosol droplets and colonizes the mucosal surface of the host nasopharynx and upper airway without causing significant clinical symptoms [7]

Pneumococcal surface protein A (PspA)

Pneumococcal surface protein A (PspA) is one of the most abundant cell surface protein of S. pneumoniae and a major determinant of protective immunity. PspA attributed virulence to the S. pneumoniae is essential for nasopharynx colonization, and in causing lung infection and bacteremia [8]. PspA is a vital component of the pneumococcal virulence arsenal – therefore, understanding the molecular aspects of this protein is essential in understanding pneumococcal pathogenesis and utilizing PspA as a target for treating or preventing pneumococcal pneumonia [14).

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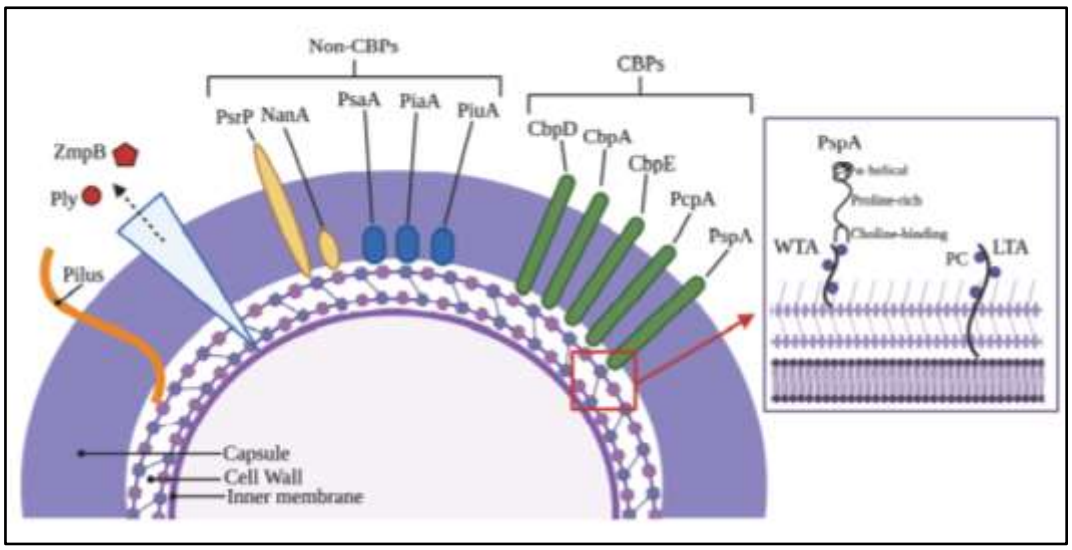

Figure 1: Major virulence factors of *Streptococcus pneumoniae* including Pneumococcal surface adhesin A (PsaA) (14).

Method Samples collection

Sputum samples were collected from 100 COVID -19 patients. from October- 2021 to January -2022, COVID -19 patients which hospitalized at Baquba teaching hospital were selected including both sex with age range (15–80) years Samples collection, isolation, identification, were all part of the first stage. Second stage includes genetic analysis using PCR to detect pneumococcal surface protein A (PspA) to *Streptococcus pneumoniae*.

Capsule Formation

The Indian ink method also known as the negative stain method was used to detect the susceptibility of bacterial isolates to capsular formation. A drop of the bacterial suspension was taken for each isolate and placed on the tip of a glass slide, and then a drop of Indian ink was added to it and mixed by a ring. The implant, another glass slide was placed on the tip of the first slide at an oblique and its components were withdrawn quietly for the purpose of spreading

the mixture along the surface of the slide, the slide was left to dry in the air, and then examined using an oil lens[9].

Optochin Sensitivity test

The test was used to distinguish between *Streptococcus pneumoniae* and other types of streptococci alpha hemolytic [10].

Chocolate agar plates were inoculated with alpha hemolytic bacterial colonies a schematic method distributed Optochin tablets at a concentration of 25 mg using a sterile forceps and then, incubating the dishes. A region of inhibition zone was formed around the disc indicating a positive test at temperature of 37 ºC for a period of 24 hour.

Bile Solubility test

This test was carried out by activating bacteria in the Brain heart infusion for 24 hours at a temperature of 37 °C. Then (0.5ml) a solution of sodium deoxycholate was added, and then the bacteria were incubated for 15 minutes. This test was used for the purpose of detecting the susceptibility of bacteria to the solubility of bile salts. Where *Streptococcus pneumoniae* bacteria dissolve in bile salts, while other types *Streptococcus* do not dissolve [11].

3-12 Molecular Detection

Pneumococcal surface protein A (*PspA*) were detected of *Streptococcus pneumoniae* using conventional PCR technique. Genomic DNA was extracted from bacterial growth according to the protocol of ZymoBIOMICS DNA Kits following the manufactures instructions DNA extracts were prepared from 10 *S. pneumonia*e isolates.DNA concentration was between 15 – 30ng/µL. Whereas the purity of DNA was found to between (1.6 – 1.8). Primer used in the study (Table 1).

Table 1: Primer used in the study

PRIMER NAME	SEQUENCES (5/ - 3/)		GENE	AMPLICON SIZE (BP)	REFERANCE
*psp*A	F	CATAGACTAGAACAAGAGCTCAAA	pspA	214	12
	R	CTACATTATTGTTTTCTTCAGCAG			

Polymerase Chain Reaction Technique (PCR)

Components of each PCR mixture were mixed together in Eppendorf tube by vortex before settings into thermocycler. The reaction was made in a PCR thermal cycler apparatus, and after several trials, and according to the manufacture's guide (Table 2and 3).

Table 2: Protocol of PCR reaction mixture volumes used in the current study

COMPONENTS	CONCENTRATION
Taq PCR PreMix	5µl
Forward primer	(1 µl) 10 picomols/µl
Reverse primer	(1 µl) 10 picomols/µl
DNA	1.5µl
Distill water	16.5 µl
Final volume	25µl

Table 3: The optimum condition of detection *pspA* gene

PHASE	TM (°C)	TIME	NO. OF CYCLE
Initial Denaturation	94°C	3 min.	1 cycle
Denaturation -2	94°C	45sec	35 cycle
Annealing	63°C	45sec	
Extension-1	72°C	45sec	
Extension -2	72°C	7 min.	1 cycle
Hold	10°C	10min'	1 cycle

Results and Discussion

Description of study samples

This study performed from October- 2021 to January -2022. It has carried out by using 100 sample COVID-19 patients that were Baequbah teaching hospital, from both sex with age range (15–80) years.

Bacterial Culture

Streptococcus pneumoniae

The Culturological examinations revealed. gram positive, short spheroids in the form chains or pairs (diplococci). The colonies were small circular, facultative anaerobic *streptococci* cause Alpha hemolysis (under anaerobic conditions). Pneumococci were sensitive to optochin disk and dissolved in bile salts.While these two characteristics are important to distinguish *S. pneumonia*e from the rest of the species, for type *S. viridan*

was resistant to optochin disk and insoluble in bile salts and all *Streptococcus pneumoniae* had capsule.The results culture of sputum samples of COVID -19 patients showed *Streptococcus pneumonia* 64 (40%).Similarly, our results were close to Zhu *et al.*, (2020). He found that more than half patients were infected by *S. pneumoniae*, followed by *Klebsiella pneumoniae* and *Haemophilus influenzae*. Furthermore Whereas, Hedberg *et al.*, (2022) found that, *S. pneumoniae* for SARS-CoV-2 was only 28% [13].

Table 4: Results biochemical tests *Streptococcus pneumoniae*

Biochemical tests.	Gram stain	Catalase	Oxadiase	Hemolysis	Bile salt	Optochin disk	Capsule
S. pneumoniae	+	_	_	Alpha	+	S	+

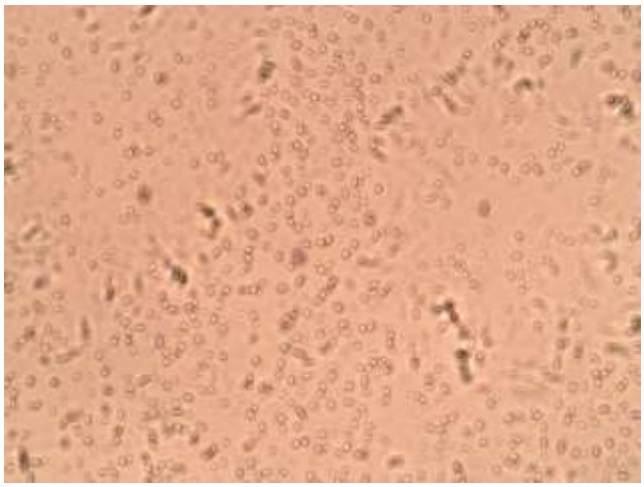

Figure 2: *Streptococcus pneumoniae* capsule

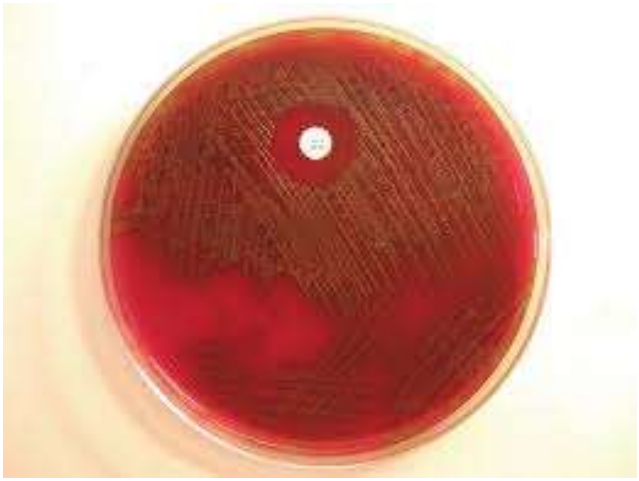

Figure 3: *Streptococcus pneumonia*e optochin sensitive

Molecular Detection

Molecular detection of virulence gene *psp*A

This study was carried out in order to detect pspA gene in 10 S.pneumoniae isolates. PspA gene was screened by PCR technique was used to detect the S. pneumoniae species (measuring 214 bp). Results showed that all the isolates had PspA (100%). The isolates (S3,S4, and S6)

showed duple band, one of the likely cases of multiple band in PCR is nonspecific primer annealing.To remedy this ,you can try increasing annealing temperature, increasing the concentration of MgCl2 ,or decreasing the concentration of primer. pneumococcal surface protein A (PspA) is virulence factor wide with range of serological variations. It's a major pneumococcal virulence factor that's been studied as a core component of a capsular serotype-independent pneumococcal vaccine. PspA impacted the bacterium's localization within the airway thereby enhancing pneumococcal virulence during pneumonia by uses GAPDH to adhere to dying lung cells during infection [14].

The co-reactive between SARS-CoV-2 proteins pspA can help to form PspA-GAPDH-mediated binding to lung cells increased S. pneumoniae localization in the lower airway, and this was enhanced by pneumolysin exposure or co-infection with viruses [15] pspA complex protein its ability to act as virulence factor in Streptococcus pneumoniae. pneumococcal surface protein A, protects the bacteria from Creactive protein-mediated activation of complement and from killing by lactoferricin, cationic antimicrobial peptide. two functions for pspA, as an adhesin and means to co-opt host metabolic enzymes for its benefit [12].

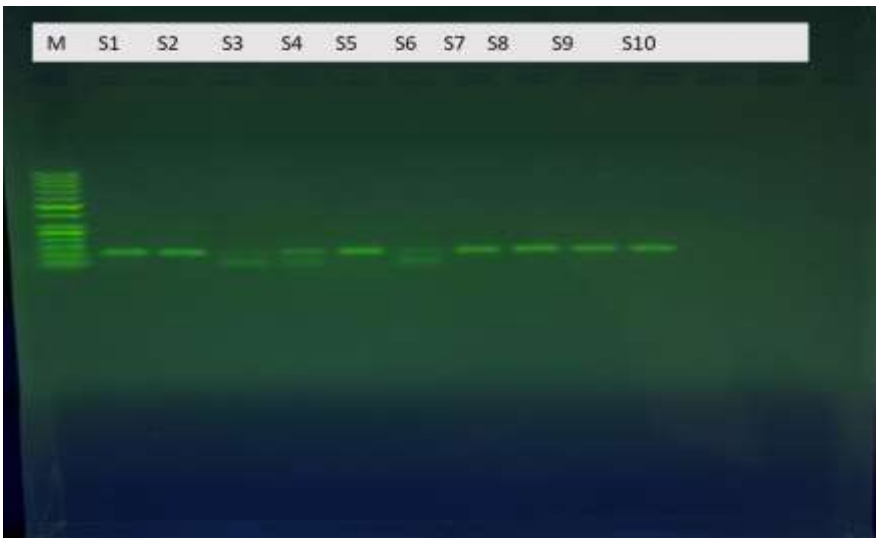

Figure 4: Amplification of *psp*A gene of *S. pneumonia*e samples fractionated on 1.5% agarose gel electrophoresis stained, M: 100bp ladder marker 214 bp PCR products

Conclusion

Streptococcus pneumoniae was the most abundance bacterial isolates. *pneumonia*e isolates selected had *psp*A (100%). PspA complex protein contribute in *S. pneumoniae* pathogenesis because it is an important virulence factor to develop of diseases.

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