SHORT REPORT

Equal adhesion to pneumocytes by pneumococci inducing bacteraemia and pneumonia

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Abstract

Streptococcus pneumoniae (S. pneumoniae) is a leading agent worldwide, which could cause community-acquired pneumonia, bacteraemia, and meningitis. However, the pathogeneses remain unclear. This study was conducted to investigate gene pneumococcal surface antigen A (*psaA*) expression and the adhesion differences of various *S. pneumoniae* strains. A total of 24 (N) *S. pneumoniae* strains were collected: 11 from blood (bd-SP), 12 from sputum (sd-SP) and one was ATCC49619. One millilitre of A549 pneumocytes (3.3×10^8/L) and 100 µl of each *S. pneumoniae* strain at 1.0 McFarland were mixed and incubated under 37°C and 5% CO_2 for three hours. The cells were centrifuged and extracted for *psaA* mRNA analysis. The former experiment was redone. After culture, the adherent cells were collected and cultured on blood agar plates. The △ CT values of *psaA* were 18.9, 29.9±2.5, 29.6±2.0 and 16.0, 17.0±3.3, 18.6±3.8 for ATCC49619, bd-SP and sd-SP before and after stimulation respectively, with the colony units of 23, 68.4±6.7 and 59.1±7.7, which showed equal adhesion between bd-SP and sd-SP. Moderate *psaA* expression and adhesion of *S. pneumoniae* might facilitate its pathogenesis, excess of which induces faster S. *pneumoniae* clearance.

Keywords: *Streptococcus pneumoniae*; Alveolar epithelial cells; Virulence; Adhesion; pneumonia.

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Introduction

Streptococcus pneumoniae (S. pneumoniae) is the leading agent causing community-acquired pneumonia.[1] *S. pneumoniae* harbours multiple virulence factors including capsular polysaccharide (Cps) and pneumococcal surface antigen A (PsaA) and so on. The synergistic action of all the virulence factors plays a primary role in the progress of *S. pneumoniae* infections,[2] although pneumolysin is a premise for *S. pneumoniae*'s invasiveness. PsaA is a kind of hereditarily conserved and species-specific surface-binding protein weighting 37 KD and could serve as an adhesion molecule to mucosal and other cells, which is vital for *S. pneumoniae* to adhere to respiratory tract and its invasion and virulence.[3] There has been an increasing focus on PsaA since it was first reported in 1990. With proper immunogenicity, PsaA could stimulate the host to produce protective antibodies so as to hamper fatal *S. pneumoniae* infections.[4] To date, the mechanisms of pneumonia and bacteraemia induced by *S. pneumoniae* remain unclear. This study used A549 pneumocytes and 24 *S. pneumoniae* strains isolated from various sources to demonstrate the difference of *S. pneumoniae* adhesion and *psaA* expression.

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Methods and Results

Twenty-four *S. pneumoniae* strains were included in this study. One strain was the standard ATCC49619, 12 inducing pneumonia were from sputum samples and the other 11 causing bacteraemia were from blood samples. All the clinical strains were non-repetitive and identified using VITEK-2 compact analyser (bioMérieux Co., Marcy-Etoile, France). This study was approved by the Ethics Committee of Taizhou Municipal Hospital (approval number 2018/04/29). One millilitre of A549 cells at 3.3×10^8/L was inoculated into each well of a 24-well culture plate, among which one well was used as blank control and the other 23 were for stimulation. The plate was kept under 37°C and 5% CO_2 overnight. 100 µl of normal saline and *S. pneumoniae* at 1.0 Mcfarland were then added into the blank and sample wells respectively. The plate was again cultured at 37°C in a 5% CO_2 incubator. After three-hour culture, the plate was taken out and all the suspensions were transferred into 24 new 1.5 ml centrifuge tubes and centrifuged at 3000 g for 10 min. The sediments were for the extraction of total RNA following the manufacture's protocol (Takara Biological Engineering Co., Ltd. Dalian, China). RNA reverse transcription and real-time RT-PCR

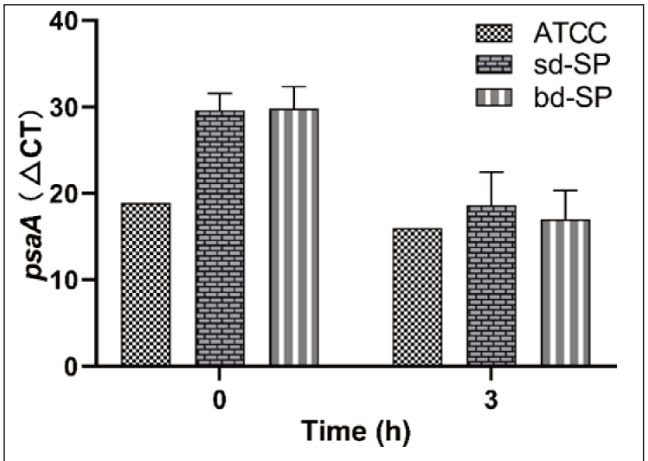

Figure-3: △CT values of *psaA* for the 3 *S. pneumoniae* groups.

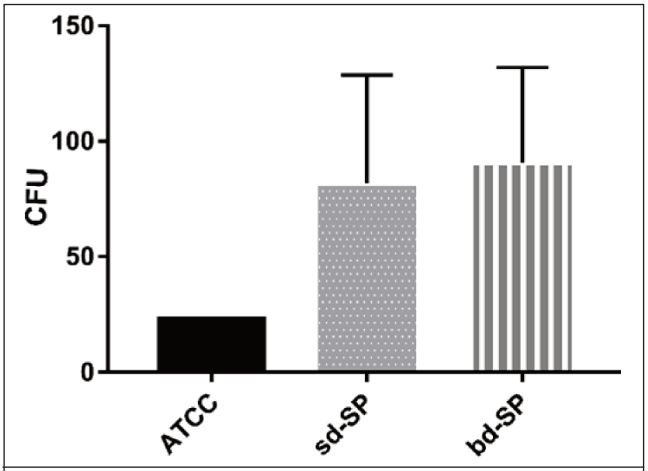

Figure-3: Colony-forming units for the 3 *S. pneumoniae* groups.

were done as reference.[5]

One ml of A549 cells at 3.3×10^8/L was inoculated into each well of a 24-well culture plate, in which one well was used as blank control and the others were for adhesion experiment. 100 µl of normal saline and *S. pneumoniae* at 1.0 Mcfarland were then added into blank and sample wells respectively. The plate was cultured at 37°C in a 5% CO_2 incubator. After three hours, the plate was taken out and all the suspensions were discarded. 100 µl of D-Hank's buffer was used to wash each well three times. 100 µl of 0.25% trypsin was added into each well to digest the adherent cells for 3 minutes. The residue was collected and diluted as $1:10^2$, $1:10^3$, $1:10^4$, $1:10^5$ and 1: 4×10^5 using normal saline. 100 µl of the dilution was then inoculated onto blood agar plates and spread by a spreader. Colony forming units (CFU) were counted after overnight culture at 37°C. All the experiments were done at Wenzhou Medical University, Wenzhou, Zhejiang Province, China from February to April 2019. Statistics were made using SPSS19.0 software (SPSS, Chicago, IL, USA).

The study demonstrated the different *psaA* expression and adhesion to A549 cells among ATCC49619, bd-SP and sd-SP. Figure-1 confirmed higher *psaA* expression in ATCC49619 than in both bd-SP and sd-SP before stimulation but after three-hour stimulation, *psaA* in bd-SP equalled ATCC49619 and that in sd-SP was lower than ATCC49619. It showed different virulence gene expression under survival pressure or not. Figure-2 showed weaker adhesion of ATCC49619 than both bd-SP and sd-SP. The contradiction between Figure-1 and Figure-2 may lie in other factors rather than *psaA* solely.

Discussion

Sufficient adhesion to dendritic cells is a vital step for the pathogenesis of *S. pneumoniae*, such as pneumonia and bacteraemia.[2] Furthermore, *S. pneumoniae* bacteraemia is usually via respiratory infection or colonisation. PsaA is a pivotal protein for *S. pneumoniae* to adhere to alveolar epithelial cells and also a premise for its colonisation and pathogenesis.[6] However, PsaA could also stimulate the host secreting protective antibodies, which are harmful to *S. pneumoniae*. Away from evolution, ATCC49619 shows weaker virulence. Weaker adhesion of ATCC49619 may result in quicker clearance by the host's respiratory mucosa and epithelial cells.[7] Figure 1 and 2 also show equal *psaA* expression and adhesion to A549 pneumocytes between bd-SP and sd-SP. And *psaA* expressed higher after stimulation than before stimulation. This study preliminarily confirms that the difference of psaA expression and adhesion to pneumocytes should not be the major factor for different types of *S. pneumoniae* infections, e.g. pneumonia and bacteraemia.

Conclusion

In conclusion, although clinical *S. pneumoniae* strains show different *psaA* expression and adhesion to pneumocytes with ATCC49619, differences of *psaA* expression and adhesion to pneumocytes should not be the pivotal factor for different types of *S. pneumoniae* infections, e.g. pneumonia and bacteraemia.

Disclaimer: None.

Conflict of interest: None.

Ethical approval: This work was approved by the Ethics Committee of Taizhou Municipal Hospital.

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