Infection, Genetics and Evolution 57 (2018) 82–87

Contents lists available at ScienceDirect

Infection, Genetics and Evolution

journal homepage: www.elsevier.com/locate/meegid

Research paper

Risk assessment of infected children with *Pseudomonas aeruginosa* pneumonia by combining host and pathogen predictors

Jiayi Chen[a], Yuhang Chen[a], Pengwei Hu[a,b,*], Tao Zhou[a], Xin Xu[a], Xiaofang Pei[a,*]

[a] Department of Public Health Laboratory Sciences, West China School of Public Health, Sichuan University, 16#, Section 3, Renmin South Road, Chengdu, Sichuan 610041, China

[b] Shenzhen Nanshan Center for Disease Control and Prevention, 95#, Nanshang Road, Shenzhen, Guangdong 518054, China

ARTICLE INFO

Keywords:
Risk assessment
Childhood pneumonia
Host response
Pseudomonas aeruginosa
Virulence factor
Antibiotic resistance

ABSTRACT

The current criteria of pneumonia severity, which mainly depend on clinical manifestations and laboratory findings from blood routine tests and X-ray examination, are still of great significance in preliminary diagnosis. However, the utility of traditional severe pneumonia indexes (SPI) without considering high virulence and multidrug resistance of *Pseudomonas aeruginosa* has limitations. Thus, it is of great value to make a risk assessment, which can serve as a complementary option for incomplete clinical diagnosis. This study aims to determine risk factors related to severe pneumonia and to comprehensively evaluate the risk conditions of infected children with *P. aeruginosa* pneumonia. We collected the clinical information of 184 hospitalized children with *P. aeruginosa* pneumonia and measured pathogen data on virulence factors and drug resistance. The risk assessment matrix was formed from the significant host and pathogen predictors, and the risk score was determined by the clinical references and the optimal critical values (OCV) of the receiver operator characteristic (ROC) curves. There were 103 (56%) and 81 (44%) infected children diagnosed as mild and severe pneumonia by SPI, respectively. Seven risk factors were significantly associated with severe pneumonia, including body temperature, respiratory rate, C-reactive protein, elastase, exotoxin-A, exoenzyme-U and multidrug resistances. Among 184 infected children, the risk assessment matrix displayed 62 cases (34%) at high risk, 51 cases (28%) at medium risk, and 71 cases (38%) at low risk in terms of pneumonia severity. On the basis of the SPI preliminary diagnosis, the risk assessment prompted that 31% (32/103) mild patients would be faced with a poorer outcome and 23% (19/81) severe patients might get a better prognosis. Therefore, the well-established assessment indicates that the interplay between host response, antibiotic resistance, and virulence may modulate the severity of *P. aeruginosa* pneumonia in infected children.

1. Introduction

Risk assessment is a systematic and structured procedure that depends on the identification of hazards and an adequate estimation of risks in an event with the purpose of making comparisons between risks so as to control or avoid them (Tziaferi et al., 2011). The risk assessment of infectious diseases aims to promote valid decision making in taking clinical measures that will control or remove the exposure to risk factors and reduce the harm to host in the course of infection. Bacterial infection damages, which pose a great threat to individual health and life, mainly include two categories: pathogen attacks (e.g., antibiotic resistance and virulence) and host responses (Juan et al., 2017). Moreover, the combination of both damages may result in severe acute diseases and poor outcomes, such as serious pneumonia.

Pseudomonas aeruginosa is one of the most important pathogens that often cause pneumonia to children under 12 years old. Regarding childhood pneumonia, the severity criteria are generally dependent on clinical manifestations and laboratory findings from blood routine tests and X-ray examination so far. Nevertheless, it is ill-prepared when highly virulent and/or multidrug resistant *P. aeruginosa* strains occur in susceptible populations (Gellatly and Hancock, 2013). Furthermore, such life-threatening strains can produce specific factors that lead to poor outcomes in vulnerable individuals, especially infected children (Jeong et al., 2014). Though the drug resistance of *P. aeruginosa* can be tested in the hospital, there is a lack of appropriate detection means for the virulence. Accordingly, the challenge is getting the presence or expression of significant virulence factors related to the severity of *P. aeruginosa* pneumonia.

* Corresponding authors at: Department of Public Health Laboratory Sciences, West China School of Public Health, Sichuan University, 16#, Section 3, Renmin Nan Lu, Chengdu, Sichuan 610041, China.

E-mail addresses: hpwvv@126.com (P. Hu), xxpei@scu.edu.cn (X. Pei).

https://doi.org/10.1016/j.meegid.2017.11.015
Received 2 October 2017; Received in revised form 6 November 2017; Accepted 12 November 2017
Available online 13 November 2017

P. aeruginosa often brings a higher mortality due to pneumonia in infected children than do other bacteria because of the former multi-drug resistances (MDR), multifarious virulence factors, and overreacted host responses (Juan et al., 2017; Livermore, 2002; Sullivan et al., 2014). Hence, *P. aeruginosa* pneumonia has become the second leading burden of bacterial pneumonia after *Staphylococcus aureus* pneumonia since 2003 (Vincent, 2003). Among pathogen-related factors affecting the outcome of *P. aeruginosa* pneumonia, the antibiotic resistance, particularly MDR profile, is certainly of paramount relevance. However, the real effect of drug resistance in the severity of *P. aeruginosa* pneumonia, besides the work on appropriate therapy chances, is not so well established (Juan et al., 2017). In addition to antimicrobial resistance, *P. aeruginosa* virulence is definitely a major driver of disease severity and outcome. A number of previous studies have demonstrated that the Exoenzyme U (ExoU) in the type III secretion system (T3SS) is clearly confirmed to determine a worse outcome in respiratory infections (Engel and Balachandran, 2009). In addition, early work has suggested that elastase, pyocyanin, alginate, hemolytic phospholipase C (PlcH), and exotoxin A (ToxA) might be the risk markers of disease severity among individuals with *P. aeruginosa* infection (Ball et al., 2002; Bleves et al., 2010; Engel and Balachandran, 2009). The severe presentation of acute pneumonia is associated with a worse outcome (Kang et al., 2003), and might be determined not only by *P. aeruginosa* virulence, but also by the noteworthy interaction between the pathogen and the host.

As commented above, therefore, here we characterize the potential factors impacting the severity and outcome of *P. aeruginosa* pneumonia in hospitalized children, including the aspects related to the host and the pathogen. On the basis of preliminary diagnosis by the severe pneumonia indexes (SPI) (The Subspecialty Group of Respiratory Diseases, 2013), we also make a retrospective risk assessment to the disease condition of infected children, in order to explore a complementary option with considering high virulence and multidrug resistance of *P. aeruginosa*.

2. Methods

2.1. Collection of clinical cases and data

A total of 259 infected children with *P. aeruginosa* pneumonia were hospitalized in the pediatric respiratory department of a local tertiary care hospital during 2013 to 2016. The 75 patients would be excluded from the study by reason that 3 patients had single congenital disease and 64 patients had single coinfection with other pathogens, as well as 8 patients had both congenital disease and coinfection. After the screening, the rest of 184 patients were considered the participants of risk assessment. The study was approved by the institutional review board, and the informed consent was given by the patients or the guardians. The blinded method was adopted for pediatricians, experimenters, and statistic assessors in this study. The case records are extracted from the hospital databases, including demographic characteristics, clinical manifestations and laboratory examinations. The clinical diagnosis of pneumonia that is classified into mild pneumonia and severe pneumonia is determined by chief pediatricians using the SPI (The Subspecialty Group of Respiratory Diseases, 2013).

2.2. Assays for virulence factors of P. aeruginosa

A total of 184 clinical *P. aeruginosa* strains were isolated from the sputum cultures on blood agar plates and identified by using the API 20NE system (BioMérieux, Marcy l'Etoile, France). As previously described, the activity (unit: absorbance, Abs) of ToxA (Schultz et al., 1979), elastase (Karatuna and Yagci, 2010), pyocyanin (Karatuna and Yagci, 2010), and alginate (Yasuda et al., 1993) was measured by the spectrophotometry; the activity (unit: millimetre, mm) of PlcH (Gubash, 1991) was gauged by the diametric measurement to the blood-plate hemolysis circle; the presence of *exoU* gene (Ajayi et al., 2003) was identified by the polymerase chain reaction and by the agarose gel electrophoresis. Control strains were *P. aeruginosa* ATCC-27853 for ToxA, elastase, pyocyanin, alginate, and PlcH, and a clinically originated strain for *exoU*. All assays started with a population density of $OD_{600\ nm}$ equal to 0.1 in triplicate and were performed at 37 °C for 24 h.

2.3. Antimicrobial susceptibility assays

Antimicrobial susceptibility was detected by the disk diffusion method on Mueller-Hinton agar according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). The test was performed for 12 antimicrobial agents: Ceftazidime, Gentamicin, Piperacillin, Tobramycin, Aztreonam, Amikacin, Cefepime, Ciprofloxacin, Levofloxacin, Piperacillin/Tazobactam, Meropenem, and Imipenem. The reference strains *P. aeruginosa* ATCC-27853 and *E. coli* ATCC-25922 were used for quality control. An isolate was defined as multidrug-resistant (MDR) if it was resistant to ≥ 1 drug in ≥ 3 categories of drugs (Hu et al., 2017).

2.4. Data process and analysis

Data analysis was performed using the statistical software of SPSS 19.0 version. All measured values per variable were subjected to the exploratory analysis. Quantitative and qualitative variables were presented with the mean (or the median) and with the frequency, respectively. The *t*-test was used for the comparison of two means, and the Mann-Whitney *U* test was applied to the comparison of two medians. The Chi-square (χ^2) test was appropriate to the frequency comparison. The discriminatory power (sensitivity and specificity) of variables was estimated by calculating the area under the receiver operator characteristic (ROC) curves with 95% confidence intervals. The optimal critical value (OCV) was defined as the point on the ROC curve closest to the upper left corner. All tests were two-tailed, and *P* values ≤ 0.05 were considered statistically significant.

2.5. Risk assessment of childhood pneumonia

The risk assessment of pneumonia severity was made at the fourth day after hospitalization of infected children. The risk matrix was used as the method of risk assessment in this study. The matrix is formed by the risk zones which are divided into low-risk, medium-risk, and high-risk zones. As shown in Fig. 1, the low-risk zone contained 11 light grey blocks, including I-I to I-V, II-I to II-III, III-I, III-II, and IV-I; the medium-risk zone consisted of 8 medium grey blocks, including II-IV, II-V, III-III, III-IV, IV-II, IV-III, V-I, and V-II; the high-risk zone included 6 dark grey blocks of III-V, IV-IV, IV-V, V-III, V-IV, and V-V. The assessment results could be credibly informed in the corresponding risk blocks.

The individual pneumonia severity could be assessed according to the combination of the degrees (from 1 to 5) of risk consequences and the grades (from 1 to 5) of risk exposures. The risk consequences were determined by the total risk scores of host responses. According to clinical reference values and the OCV, each host response contained three or four levels: normal (A = 0: within the normal reference ranges), mild (B = 1: between the normal reference and the OCV), severe (C = 2: between the OCV and the severe reference), and critical (D = 3: above the severe reference). The risk exposures were dependent on the number of significant pathogen factors (virulence factors and drug resistance). The measured values of pathogen factors were divided into two exposure levels: low (A = 0: below the OCV of virulence factors, *exoU* negative, and non-MDR), and high (B = 1: above the OCV of virulence factors, *exoU* positive, and MDR).