Construction of Genomic Library and High-Throughput Screening of Pseudomonas aeruginosa Novel Antigens for Potential Vaccines

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Hospital-acquired infections with Pseudomonas aeruginosa have become a great challenge in caring for critically ill and immunocompromised patients. The cause of high mortality is the presence of multi-drug resistant (MDR) strains, which confers a pressing need for vaccines. Although vaccines against P. aeruginosa have been in development for more than several decades, there is no vaccine for patients at present. In this study, we purified genomic DNA of P. aeruginosa from sera of patients affected, constructed genome-wide library with random recombinants, and screened candidate protein antigens by evaluating their protective effects in vivo. After 13-round of screening, 115 reactive recombinants were obtained, among which 13 antigens showed strong immunoreactivity (more than 10% reaction to PcrV, a well-characterized V-antigen of P. aeruginosa). These 13 antigens were: PpiA, PtsP, OprP, CAZ10_34235, HmuU_2, PcaK, CarAd, RecG, YjiR_5, LigD, KinB, RtcA, and PscF. In vivo studies showed that vaccination with PscF protected against lethal P. aeruginosa challenge, and decreased lung inflammation and injury. A genomic library of P. aeruginosa could be constructed in this way for the first time, which could not only screen candidate antigens but also in a high-throughput way. PscF was considered as an ideal promising vaccine candidate for combating P. aeruginosa infection and was supported for further evaluation of its safety and efficacy.

Key words genome-wide library; antigen screening; Pseudomonas aeruginosa

INTRODUCTION

Pseudomonas aeruginosa (PA) is a kind of Gram-negative bacteria, which is widely existed in a natural environment and is one of the most formidable opportunistic pathogens in the clinic.1) The prevalence of P. aeruginosa carriage is around 15% in hospitalized patients in the Intensive Care Unit (ICU).2) In the Pediatric Intensive Care Unit (PICU), P. aeruginosa infections even account for 55% bacterial infection in children.3) Furthermore, over the last 15 years, the nosocomial infection caused by P. aeruginosa is associated with prolonging hospital stay, high expense as well as complications.4–7) Anti-Pseudomonas aeruginosa agents that can be used to control P. aeruginosa infections at present are extremely limited in clinical practice because P. aeruginosa is known to utilize their high levels of intrinsic and acquired resistance mechanisms to counter most antibiotics.8–10) The multi-drug resistant (MDR) phenotype could be mediated by a wide array of mechanisms include multidrug efflux systems, enzyme production, outer membrane protein (porin) loss, and target mutations, as well as the formation of biofilms.11,12) The discovery and development of novel therapeutic strategies against P. aeruginosa infections are urgently demanded and gained more and more attention.

Our historical experience fighting against pathogenic microorganisms indicates that vaccines are one of the effective weapons to prevent and control them.13–15) Hence, the successful development of the P. aeruginosa vaccine will not only reduce the incidence of infectious diseases, lessen the indiscriminately use of antibiotics, but also reduce the severity of antibiotic resistance. In the past 40 years, numerous vaccines have been developed against P. aeruginosa infection, and protective antigens used in these studies included lipopolysaccharide (LPS), polysaccharide, polysaccharide conjugates, extracellular protein, outer membrane protein (OMP), flagella, type 3 secretion system (T3SS), IC 43,17) as well as pili.18) Several vaccines have entered phase II and III clinical trials, but there is no vaccine against P. aeruginosa authorized for immunization in humans so far.19,20) Failure of these antigens was mainly attributed to multiple pathways utilized by P. aeruginosa to cause infection and frequent variations in its genome. In contrast to some other bacterial genomes, whose size reflects gene duplication rather than genetic diversity, the P. aeruginosa genome has a large size of encoding almost 6000 genes and contains numerous and distinct gene families, which are predicted to encode outer membrane proteins, transport systems and enzymes.21,22) The diversity of P. aeruginosa strains and genome makes it extraordinarily difficult for selecting conversed antigens. As a consequence, the complexity and diversity of the genetic components lead to the difficulty of antigen screening by the evaluation of an individual protein.

In this study, we constructed a genome-wide library from a clinical strain P. aeruginosa strain XN-1 that was isolated from a severely infected patient in Southwest Hospital in China. And, this library was subjected to 13 rounds of screening by using an enzyme-linked immunosorbent assay (ELISA), in which serum of convalescent patients with P. aeruginosa infection was used as a primary antibody. In total, we obtained 115 reactive recombinants, among which...
13 antigens showed strong immunoreactivity (more than 10% reaction to \( \text{PcrV} \), a well-characterized V-antigen of PA), specifically \( \text{PpiA, Psp, OyrP, CAZ10_34235, HmuU_2, PcaK, CarAd, RecG, YjiR_5, LigD, KinB, RtcA, and PscF} \). Vaccination with \( \text{PcrV} \) effectively protected mice from \( \text{P. aeruginosa} \). To screen out strong reactive antigens, novel antigens’ immunogenicity and their effects of protective rates were evaluated by experiments on BALB/c mice. Novel candidate antigens of \( \text{P. aeruginosa} \) were screened out in this way, which would lay a firm basis for the development of its vaccine.

**MATERIALS AND METHODS**

**Ethics Statement** In this study, all animal care and use were performed according to the rules of Animal Ethics Procedures and regulations of the People’s Republic of China. All animal experiments in this study were approved by the Animal Ethical and Experimental Committee of Chongqing International Science and Technology Cooperation Center for Child Development and Disorders. All surgeries were conducted under the circumstance of sodium pentobarbital anesthesia, and all efforts were engaged to minimize suffering.

**Bacterial Strains** The \( \text{P. aeruginosa} \) strain named XN-1, strain number CCTCC M 2015730, was isolated from a severely infected patient in Southwest Hospital in China, whose serotype was detected by Mei serotyping kit (Mei assay, Meiji Seika, Japan).

**Animals** Eight–twelve week-old female BALB/c mice (weight at 18.0–22.0 g) were purchased from Experimental Animal Center of Chongqing Medical University, under the circumstances of specific pathogen-free (SPF) conditions. Female New Zealand white rabbits (weight at 2.0–2.2 kg) were provided by TenXin Company (Chongqing, China).

**High-Throughput Screening of \( \text{P. aeruginosa} \) Novel Antigens** The anti-maltose binding protein (anti-MBP) antibody was coated on ELISA plates to capture MBP fusion protein expressed by random recombinants. Moreover, the serum of patients who were infected with \( \text{P. aeruginosa} \) was used as a primary antibody to detect reactivity. After that, the encoding sequence of strong reactive antigens was tested by genetic sequencing, and their genetic information was obtained by sequence alignment on the BLAST website (Fig. 1). Moreover, the relative reactivities of antigens were ranked according to their titers of ELISA.

**Construction of the Genomic Library** Genomic DNA of \( \text{P. aeruginosa} \) XN-1 was extracted by using Wizard Genomic DNA Purification kit (Promega, U.S.A.) following the protocol and was digested with a restriction enzyme named \( \text{Sau3AI (\text{GATC})} \) to get random fragments with the size of between 100 and 1000bp. Then, these digested genomic DNA fragments were inserted to the plasmid of vector \( \text{pMal-c5x} \) digested by restriction enzyme named \( \text{BamHI (\text{G’GATCC})} \), followed by dephosphorylation with Shrimp Alkaline Phosphatase (TaKaRa Bio, China) (Supplementary Fig. 1), to construct randomly recombinant plasmids. After purification, the fragments of the XN-1 genome were ligated to the \( \text{pMal-c5x} \) vector. Moreover, the reaction mixture was transformed into \( \text{Escherichia coli X-Blue Competent cells} \) (Huayueyang Biotech Company, China), which were used to construct a genome-wide library by collecting colonies grown on antibiotic plates not only massively, but also simultaneously. And all these recombinant plasmids were confirmed respectively by restriction endonuclease digestion and DNA sequencing.

**Identification of Random Recombinants** To verify the success of the construction of \( \text{P. aeruginosa} \) antigen library, three clones were selected randomly from the same plate, which was named as clone A, B, and C. After digestion with enzyme \( \text{BamHI} \), both clone A and clone C released a fragment of 1000bp, indicating that genome fragments of \( \text{P. aeruginosa} \) XN-1 were successfully inserted into clone A and clone C, while clone B was considered as a negative (Supplementary Fig. 2).

**Recombinant Protein Expressions** There were 2392 colonies in all picked from a relatively fresh plate (<4 weeks) and grown at 37°C in 20mL Luria–Bertani medium (Leagene Biotechnologies, China) containing 100 \( \mu \text{g/mL} \) of Ampicillin antibiotics. The recombinant proteins were induced by adding 1.0 mM isopropyl \( \beta\text{-d-thiogalactoside (IPTG)} \) at 37°C for 15 h. Then, these proteins were purified by MBP resin using affinity chromatography, and bicinchoninic acid (BCA) from Applygen Technologies Inc. (Beijing, China) was used to measure their concentrations.
Evaluations of Genomic Libraries and Protein Expressions of Inserted Fragments A single colony PCR assay was used to initially determine the presence of DNA fragments and their size distribution in the genomic libraries, which were selected from the same plate after X-Blue competent cell transformation (Supplementary Fig. 3a). The inserted fragments of samples No. 1, No. 2, and No. 3 were about 1000, 250, and 100 bp, respectively, but samples No. 4 and No. 5 did not have any fragment inserted, while sample No. 6 was used as a positive control. Results of PCR indicated that inserted fragments had a broad size distribution, and P. aeruginosa genomic library libraries were constructed with acceptable complexity and representation. To evaluate their expressions, random recombinants were selected from different screening batches and numbered as 1.1, 4.2, 4.19, 5.13, 5.15, and 5.18 respectively. After induced with IPTG, sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was used to detect protein expressions. As the results indicated, pMal-c5x vector and pMal-c5x-PcrV expressed protein with expected sizes after IPTG induction. Moreover, samples No. 1.1, No. 5.18, No. 5.15, and No. 4.2 were observed to have protein bands at 55, 50, 45, and 50 kD respectively, which suggested that these recombinants could express MBP fusion proteins correctly. However, samples No. 5.13 and No. 4.19 did not, with no obvious protein band and not expressing MBP fusion protein (Supplementary Fig. 3b). Preparations of rabbit anti-MBP immune globulin G (IgG) antibody. The MBP were produced by pMAL-c5x/X-Blue transformed E. coli. After induced by 0.4 mM IPTG, MBP was purified by amylose affinity resin (NEB company, New England, U.S.A.). After mixing by 50 µL with Freund Adjuvant (Sigma), 200 µL of MBP-recombinant protein was injected with the final concentration of 0.5 mg/rabbit on days 1st, 14th, and 21st respectively. On the 28th day, blood was collected by cardiac puncture under anaesthesia on the rabbits. Serum was obtained by allowing blood to stand for 1 h at 37°C followed by centrifugation with 8000 rpm for 10 min at 4°C to remove the clotted material. Anti-MBP IgG antibody was obtained by affinity purification with Protein A.

Screening Strong Reactive Antigens The rabbit anti-MBP IgG antibody was diluted with coating buffer (50 mM of sodium carbonate/bicarbonate buffer, pH 9.6) to the final concentration of 10 µg/mL, and was coated on the wells of microwriter plates at 37°C for 4 h. After washing with sterile phosphate tween buffer (PBST) four times, 100 µL supernatant of recombinant proteins was added into each well and incubated for 1 h at 37°C. Then strips were washed with PBST for four times, a convalescent mixed serum of P. aeruginosa infection patients was used as the primary antibody, and goat anti-human IgG was used as a secondary antibody for ELISA detection. As a result, the relative reactivity of each random recombinant compared to PcrV was calculated by the formula, Relative Reactivity % = (ODx−ODprev)/(ODx−ODprev) × 100%. In this formula, ODx is the value of random recombinants in OD600nm while ODprev is a negative control. The mean of log2 titers were used to express antibody express (ns = no significance.) Besides, we also use one-way ANOVA to analyze multiple comparisons between different groups.

Preparations of Recombinant Strong Reactive Antigens We used PCR to amplify inserted DNA fragments of 13 antigens respectively, to obtain the full-length sequences of the inserted gene fragments by using respective primers shown in Supplementary Table S1. Meanwhile, all the amplified conditions of candidate antigens were shown in Supplementary Table S2, and the amplification system was in Supplementary Table S3. Then these genes were cloned into pMal-c5x vector to express MBP-antigen fusion protein. The full-length of DNA sequences were then ligated to vector pMal-c5x, and transformed into E. coli. After induction with 0.4 mM IPTG, 115 in all recombinants strong reactive antigens were obtained, and 13 strong reactive candidate antigens of P. aeruginosa were obtained by DNA sequencing and purified by amylose affinity resin. Then these 13 antigens plus MBP, Al(OH)3 control group consisted of fifteen groups for the first, second-round animal experiment.

Levels of Antibody IgG Detected in Mice Immunized with Strong Reactive Antigens One hundred and fifty female Balb/c mice in total were divided into 15 groups, which were matched for sex, age, and weight for the first round, and another 150 Balb/c mice were also matched for the second round under the same condition. Purified candidate antigens were mixed with Al(OH)3 adjuvant and emulsified at 4°C for 4 h. Each emulsified antigen was injected into 10 Balb/c mice intraperitoneally with the concentration of 50 µg/mouse on the 1st day, the 14th day, and the 21st day respectively, with MBP and Al(OH)3 injection on mice as a negative control and an adjuvant control. After the final injection on the 7th day, 500 µL tail venous blood was collected from mice for ELISA assay. In ELISA results, the cut-off value of specific IgG antibody was calculated by the formula, cut-off value% = mean of the control group [MBP, Al(OH)3] × 2.1 × 100. And in the second round, another 150 Balb/c mice in these 15 groups were also gone through the same immunity process and were calculated their cut-off value%. Thus candidate antigens were ranked according to their mean cut-off values.

Evaluations of the Protective Effect of Strong Reactive Antigens On the 10th day after the final immunization, all vaccine mice were challenged with 1% pentobarbital sodium as an anesthetic. After that, a volume of 20 µL P. aeruginosa XN-1, with a lethal dose of 2 × 10⁸ colony forming unit, was used for tracheal intubation on mice. Moreover, activities and survival rates of mice were carefully recorded every 12 h for seven consecutive days, and the protection rates of these reactive antigens were calculated, while the top five of them were listed.

Statistical Analysis The data was presented as mean ± standard deviation (S.D.) or mean ± standard error of mean (S.E.M.). The scores were recorded in a blind way. Kaplan–Meier survival curves were used to analyze survival data. To calculate p-values, non-parametric Mann–Whitney test, log-rank test, Student’s t test, one-way ANOVA with Bonferroni correction were used to depend on sample distribution and variation as mentioned in figure legends (SPSS statistics 18.0 and GraphPad Prism 6.0). Significant difference was accepted at p < 0.05.

RESULTS Screening of P. aeruginosa Strong Reactive Antigens Twelve convalescent sera of P. aeruginosa infected patients were collected from the Southwest Hospital and mixed in equal volumes. Through 13 cycles of screening, a total of
2392 random recombinants with DNA fragments were selected, 115 reactive recombinants were obtained by ELISA testing, among which 13 antigens displayed stronger reactivity than the control MBP group (Fig. 2). These reactive antigens were as follows, PpiA, PtsP, OprP, CAZ10_34235, HmuU_2, PcaK, CarAd, RecG, YjiR_5, LigD, KinB, RtcA, PscF, compared to MBP as control (p < 0.0001). Their bioinformatics information including sample numbers, gene, protein name, subcellular localization, amino acid sequence inserted, and length of amino acid insertion fragments was listed in Table 1.

**Evaluations of Protective Effects of Candidate Antigens**

After IPTG induction, MBP-antigen recombinant proteins were purified with MBP tag. Firstly, the purity of thirteen antigens was more than 80% detected by SDS-PAGE, which could be made use of the following animal experiments (Supplementary Figs. 4a, b). Further, titlers of antigen-specific IgG antibodies in rat serum were detected by ELISA (Fig. 2). Further, titlers of antigen-specific IgG antibodies in rat serum were detected by ELISA (Fig. 2). Among them, antigen PscF has the best protective rate which reaches 90%, and the second protective rate was 80% indicating antigens LigD and RecG, while the third protective rate was 60% with antigens OprP and PtsP respectively, compared to 40% with MBP on mice as a negative control, with 30% Al(OH)₃ as an adjuvant control alone. Moreover, the survival rates of PscF groups were significantly higher than that of Al(OH)₃ group (Pₚₛₛ₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅₋₅-
DISCUSSION

*P. aeruginosa* is a Gram-negative bacterium that causes widely severe infection in critically ill patients.²⁸ For the moment, it is one of the highest infectious, opportunist pathogens in ICU ward and ventilator-associated pneumonia (VAP).²⁴,²⁵ In common VAP, *P. aeruginosa* attributes to lethality as 13.5% even with sufficient antibiotic treating. Nevertheless, in MDR strains combined VAP, even with adequate antibiotic treatment, *P. aeruginosa* inducing mortality has risen to 41.9%.²⁶ And this phenomenon may be closely related to the pathogenesis of it.²⁷ In this study, we have constructed a genome-wide library from a clinic strain *P. aeruginosa* XN-1 and acquired several reactive recombinants and candidate antigens. However, we do not cover the entire genome of *P. aeruginosa* for the present, which needs a larger size of the sample to get more recombinant plasmids by this high-throughput screening method.

As a key virulence factor, PcrV plays an important role in the pathogenesis of *P. aeruginosa* and is an excellent candidate antigen for the *P. aeruginosa* vaccine.²⁹–³³ For instance, the recombinant DNA vaccine pRES-toxAm-perV was a candidate vaccine for the prevention and control of *P. aeruginosa* infection in the treatment of PA-induced lung infections.³⁴ In the study conducted by Ali et al., MEDI3902 exhibited a desired antibacterial activity by inhibition of PcrV, promoting opsonophagocytic killing (OPK) activity, and targeting inhibiting *P. aeruginosa* host cell attachment.³⁵ In another study conducted by Milla et al., recombinant anti-PA-PcrV antibody Fab' fragment inhibited the function of TTSS, which at the same did not activate immune cells and inflammation.³⁶ Very recently, in yet another study (for Aguilera-Herce et al.), an attenuated strain of *S. enterica* was an eligible carrier for delivering *P. aeruginosa* antigen PcrV to design a vaccine to fight against its infection.³⁷ As a result, PcrV could be used as a diagnostic marker for *P. aeruginosa* infection in serological experiments. All factors considered, we have used PcrV as a positive control in the reactions of antigen evaluation in this study, with MBP as a negative control, Al(OH)₃ as an adjuvant control.

Moreover, PscF was considered as the candidate antigen of *P. aeruginosa* vaccine for the following reasons. First of all, PscF was located on the main component of the T3SS needle complex of *P. aeruginosa*³⁸ and played an important role in regulating nuclear factor-kappaB (NF-κB) and AP-1 pro-inflammatory cytokine pathway.³⁹ Secondly, PscF promoted mature immune response of caspase-1 and interleukin (IL)-1β and enabled PscG and PscE to exist stably in *P. aeruginosa* cells before polymerization,⁴⁰,⁴¹ which could be recognized as a target for *P. aeruginosa* vaccine. Our animal experiment results indicated that PscF had the highest IgG antibody titer and the best immuno-protective effects in vivo among 13 candidate antigens whose survival rate rose to 90%, compared to 40% in MBP negative control, 30% in Al(OH)₃ adjuvant control.

Above all, these results indicate that PscF might be an ideal antigen for *P. aeruginosa* vaccine. The whole genome-wide library from a clinical strain *P. aeruginosa* strain XN-1 was constructed successfully in this study. Based on this library, thirteen strong reactive antigens were successfully screened immunologically.

Finally, PscF was identified as a potential candidate protein antigen of *P. aeruginosa*, as a result of the best immunogenicity and the highest survival rate among animal experiments, which would lay a solid foundation for the development of novel protein vaccines of *P. aeruginose*.

However, our genomic library of *P. aeruginosa* did not list detailed statistical data of neo-antigens’ functions, which would be a limitation of our study. Furthermore, we would like to apply this high-throughput method for screening antigens among other *P. aeruginosa* clinical isolations, combined with HPLC to measure molecular weight and endotoxin levels before immunization for the next.

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**Conflict of Interest** The authors declare no conflict of interest.

**Supplementary Materials** The online version of this article contains supplementary materials.

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