Development of a DNA microarray assay for rapid detection of fifteen bacterial pathogens in pneumonia

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Abstract

Background: Rapid identification of pathogenic bacteria is important for appropriate antimicrobial therapy of pneumonia, but traditional bacteria culture is time-consuming and laborious. The aim of this study was to develop and evaluate a DNA microarray assay for the simultaneously detection of fifteen bacteria species directly from respiratory tract specimens in patients with pneumonia. These species included Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Mycoplasma pneumoniae, Enterococcus faecalis, Enterococcus faecium, Enterobacter cloacae, Stenotrophomonas maltophilia, Burkholderia cepacia, Legionella pneumophila and Chlamydia pneumoniae. The 16S rDNA and specific genes of each pathogen were chosen as the amplification target, amplified with multiplex polymerase chain reaction (PCR), and hybridized to the oligonucleotide probes on the microarray.

Results: The DNA microarray can reach a detection limit of 10 3 copies/μL. Nineteen standard strains and 119 clinical isolates were correctly detected with our microarray and 3 non-target species from 4 clinical isolates were not detected. Meanwhile, bacterial pathogens were accurately identified when two or three bacterial targets were mixed together. Furthermore, the results of 99.4% (156/157) clinical specimens were the same to that from the conventional assay.

Conclusions: we developed a DNA microarray that could simultaneously detect various bacterial pathogens in pneumonia. The method described here has the potential to provide considerable labor and time savings due to its ability to screen for 15 bacterial pathogens simultaneously.

Background

Rapid identification of pathogenic bacteria is important for appropriate antimicrobial therapy of pneumonia [1]. However, current standard microbiological culture-based tests are laborious and time-consuming [2]. Patients often receive empirical broad-spectrum antimicrobial treatment while waiting for microbiology results. Hence, novel diagnostic approaches are urgently needed to improve early antimicrobial therapy of pneumonia.

Standard European guidelines for diagnosis and management of pneumonia state that molecular diagnosis is a promising method in rapid detecting pathogens [3]. Several molecular methods based on polymerase chain reaction (PCR) have been developed to detect species-specific genes. For example, the identification of *Pseudomonas aeruginosa* by amplification of the specific gene exotoxin A [4], the identification of *Mycoplasma pneumoniae* using a fragment of gene encoding for P1 cytadhesin protein [5], the identification of *Haemophilus infuenzae* by amplifying a fragment of gene encoding for P6 outer membrane protein [6], and many others [7]. However, these methods have a narrow diagnostic spectrum.

To solute this problem, multiplex PCR or ribosomal DNA (rDNA) were used [8-10]. Although multiplex PCR can simultaneously detect several bacteria, the number of bacteria is still limited in one test. 16S rDNA sequence exists universally within bacteria and includes conserved regions and species-specific regions

[11]. The most common method is use one universal primer pair to amplify the species-specific fragments of 16S rDNA. Even so, it is not possible to obtain a complete discrimination among some genera, like *Enterobacteriaceae*, the 16S rDNA sequences of *Klebsiella pneumoniae, Enterobacter cloacae* and *Escherichia coli* are very close [12].

To extend the detection spectrum and shorten the detection time, we develop a DNA microarray assay that can detect 15 respiratory bacterial pathogens in pneumonia including *Streptococcus pneumoniae, Staphylococcus aureus, Haemophilus influenzae, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Mycoplasma pneumoniae, Enterococcus faecalis, Enterococcus faecium, Enterobacter cloacae, Stenotrophomonas maltophilia, Burkholderia cepacia, Legionella pneumophila* and *Chlamydia pneumoniae*. In order to identify bacteria at species level we choose 16S rDNA probe combining with species–specific probe to detect each bacterium. The sequences of species–specific probes come from 15 species–specific genes.

Results

Primers design and evaluation

Specific genes that target the 15 different bacterial species were selected based on thorough literature search for particular bacterial housekeeping genes. The 15 bacterial specific genes are *lytA* of *Streptococcus pneumoniae* [8], *nuc* of *Staphylococcus aureus* [8], *P6* of *Haemophilus influenzae* [32], *phoA* of *Escherichia coli* [13], *mdh* of *Klebsiella pneumoniae* [13], *toxA* of *Pseudomonas aeruginosa* [4], *gltA* of *Acinetobacter baumannii* [13], *P1* of *Mycoplasma pneumoniae* [5], *ddl* of *Enterococcus faecalis* and *Enterococcus faecium* [14], *dnaJ* of *Enterobacter cloacae* [15], *chitA* of *Stenotrophomonas maltophilia* [16], *recA* of *Burkholderia cepacia* [17], *mip* of *Legionella pneumophila* and *ompA* of *Chlamydia pneumoniae* [5,18]. All primers were designed by ourselves. Three pairs of primers were initially designed for each specific gene and the primer pairs were checked by BLAST search (http://www.ncbi.nih.gov). If all the 3 pairs of primers were not successfully amplified, we would design other 3 pairs of primers. After repeated screening, 16 paired primers, including a pair of 16S rDNA universal primer and 15 pairs of bacterial specific genes primers, were selected and successfully amplified (Table 1). All primers in one group for the multiplex asymmetric PCR have a similar melting temperature. The specificity of 16-paired primers was preliminarily tested by PCR and the PCR products examined by 2% agarose gel electrophoresis (Figure S1). All primers and probes were finally confirmed by sequence analysis of PCR products from the reference plasmids.

The limit of detection and accuracy of the microarray

The microarray layout was shown in Figure 1a. The detection limit of each probe can reach the level of 10^3copies/μL (Figure 2). A positive diagnostic hybridization was conferred only when three probes gave signals at the same time. The three probes were the positive control probe from 16S rDNA conserved

sequence, the specific probe from 16S rDNA specific sequence of each target bacterium and the other specific probe from specific genes of each target bacterium. 138 strains, including 19 standard strains and 119 clinical isolates (Table 2), were correctly detected with our microarray (Figure 1b). Three non-target bacterial species from 4 collection isolates were not detected (Figure 1b). The hybridization signals emerged orderly at the position corresponding to each target genus or species from bacterial cultures and all probes were not cross-hybridization with each target pathogen. For the 2 *Streptococcus viridans* isolates, we observed that only 16S rDNA specific probe of *Streptococcus spp.* and the universal 16S rDNA probe emerged signals. For one *Moraxella catarrhalis* isolates and one *Neisseria mucosa* isolates, hybridization reaction only appeared at the position of the universal 16S rDNA probe. Furthermore, water was processed in parallel with clinical samples was used as a negative PCR control and the hybridization results had no signals (Figure 1b). In addition, all components within a mock specimen, which consisted of two or three target bacteria, could be accurately identified despite the presence of other components (Figure 3a).

Detection of clinical specimens

Among the 157 clinical specimens, 105 specimens had only one pathogen, 36 specimens had two pathogens, 5 specimens had three pathogens, 11 specimens had no pathogens (Table 3). Firstly, 151 bacterial pathogens belonging to 10 target species in clinical samples were correctly identified by microarrays according to the results of bacterial culture. Secondly, one specimen identified by microarray was not the same as that of bacterial culture. In scanning images of this specimen from twice assays only probes for *Acinetobacter baumannii* and the universal 16S rDNA probe had signal, therefore we deduced that the specimen contains *Acinetobacter baumannii*. Meanwhile the results of three replicates PCR for the specimen based on the specific gene *nuc* of *Staphylococcus aureus* was negative. Finally, the microarray results of 40 bacterial pathogens belonged to 8 non-target species in clinical samples were negative (Table 3). However, for *Streptococcus viridans, Staphylococcus hominis, Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, 16S rDNA specific probes of these bacteria and the universal 16S rDNA probe had signals, which indicated that this microarray could identify some non-target bacterial at genus level. And for *Neisseria mucosa, Chryseobacterium indologenes, Ralstonia mannitolilytica,* and *Citrobacter freundii*, only the universal 16S rDNA probe had signals, which demonstrated that this microarray could identify whether specimens contained bacteria. The hybridization results of clinical samples which contains two or more target pathogens were shown in Figure 3b.

Discussion

We reported the development of a novel DNA microarray for 15 important respiratory bacterial pathogens and evaluated its potential as a promising diagnostic tool in pneumonia. We employed two probes, one from 16S rDNA specific sequence and the other from the specific gene sequence, to identify each target

bacterium. The detection limit of each probe can reach 10^3copies/μL. The detection accuracy of this microarray for clinical isolates and specimens are 100% and 99.4%, respectively.

A particular strength of our study was that this microarray simultaneously applied genus-specific probe and species-specific probe to detect targeted bacterium. In recent years, DNA microarray have been developed to identify bacteria in lung diseases, but their target genes no more than two: one is species-specific genes [19], the other one is conserved genes, including rDNA genes and several phylogenetically conserved genes [11,12,20]. For the former, the number of detected bacteria is limited in one test. For the latter, one single marker could not give a no ambiguous detection of closely related or distant species [21]. Therefore, bacterial conserved genes combined with species-specific genes is necessary for accurate diagnosis of bacteria. To the best of our knowledge, there are no other assays simultaneously using 16S rDNA and bacterial species-specific gene for bacteria identification. Moreover, in this study, even if samples contained bacteria not belong to the fifteen bacteria, it also could be identified at genus level. This method might be a useful addition to the microarray technique.

Furthermore, this microarray could provide rapid bacteria identification directly from patient samples. Firstly, the entire experiment of this assay, from sample receipt to results dissemination, could be completed within 6h. It is much faster than current methods, because most methods require additional 18-24h for the growth of bacteria in clinic practice. Secondly, these fifteen target bacteria covered the most common bacterial causes of community acquired pneumonia (CAP) and hospital acquired pneumonia (HAP) [22,23], especially atypical pathogens which are difficult to identify because of lengthy and complicated cultural methods [24,25]. Finally, due to the high-throughput characteristic of microarray, our microarray can simultaneously detect 15 pathogen bacteria in one test. The timely and abundant identification results can help the early antimicrobial therapy of pneumonia and prevent the bacterial resistance caused by empirical antibiotic therapy. This microarray is worthy of being recommended in clinical application.

This assay was validated with 19 type strains, 119 clinical isolates belonging to 15 target species, 4 clinical isolates belonging to 3 non-target species and 8 mixed mock specimens. Bacterial strains were cultured overnight in 5ml of species-specific culture medium and growing temperature. All cells were collected for DNA extraction and 2.5μL of DNA template was used for PCR in microarray validation. This number has to be translated into number of bacteria since a correction factor has to be introduced due to the extraction efficiency and sample dilution [20]. However, based on the correctly identification of 19 type strains, 119 clinical isolates belonging to 15 target species, 8 mixed mock specimens and 4 clinical isolates belonging to 3 non-target species, the sensitivity and specificity are all 100% and the microarray was an efficient diagnostic method on clinical isolates. The criteria for selection of clinical isolates belonging to non-target species in this study was that they were often detected in respiratory tract specimens, but in most cases, they were not the main pathogenic bacteria. We used only 4 clinical isolates belonging to 3 non-target species and the number is small. Nevertheless, the detection was found specific for the 19 type strains, 119 clinical isolates belonging to 15 target species, 151 bacterial pathogens belonging to 10 target species in clinical samples and this assay did not detect any of the 4

clinical isolates belonging to non-target species and 40 bacterial pathogens belonging to 8 non-target species in clinical samples. We cannot exclude that other bacteria species in respiratory tract specimens would react on the selected probes, thus interfering with the detection. This probability is low given the very few cross-reactions observed on the 19 type strains, 123 clinical isolates and 191 bacterial pathogens in clinical samples tested here.

In this study, microarray results were compared with culture results, when microarray effectiveness was assessed on clinical specimens. Firstly, culture is still the most popular method and the gold standard for the identification of bacteria in clinical practice, even if its results can be both false negative and false positive. Secondly, in our study, 157 clinic specimens were collected before antibiotic therapy. Antibiotic therapy could reduce bacterial burden and viability which lead to culture negative [26]. Moreover, 121 out of 157 specimens were endotracheal aspirates and BALF which are often of better quality than expectorated sputum [27,28]. Therefore, to a certain degree, these operations prevented the occurrence of false negative and false positive during bacterial culture. Thirdly, sequencing method was used to confirm results when the results of culture and microarray were discordant. In this study, the results of culture and microarray were different in only one sputum sample. The culture result of this sample was *Staphylococcus aureus* and *Acinetobacter baumannii*, the microarray result was only *Acinetobacter baumannii*, and the results of three replicates PCR based on the specific gene *nuc* of *Staphylococcus aureus* was negative. Thus, no specimens were sequenced. Finally, of the 15 bacteria species present on the microarray, 10 different ones were found in the clinical samples and they are relatively easy to identify by culture. Hence, at last, this microarray method was compared with conventional culture method.

The array was further assessed for its effectiveness on 157 clinical specimens from different patients. Polybacterial infections were well detected in 41 samples. Compared with culture results, the specificity and sensitivity of microarray were 100% and 99.4%, respectively. An increased sensitivity of molecular methods based on PCR were reported [29-32]. In this study, only *Staphylococcus aureus* in one sample was not detected by microarray. The first reason for the lower sensitivity might be attributable to DNA extraction or erroneous culture identification. In a lately study, except standard automated extraction protocol, an additional proteinase K and lysostaphin was necessary for efficient extraction of *Staphylococcus aureus* DNA from sputum sample, particularly mucopurulent sample [8, 33]. Unfortunately, no stored specimens could be re-extracted or re-cultured, because all the specimens were used for molecular work. The second reason for the lower sensitivity might be that the number of *Staphylococcus aureus* was enough to culture, but it was too small to detected by microarray. The last reason was that clinic specimens did not cover all fifteen target bacteria, especially atypical pathogens which are difficult to culture. Maybe it is because 157 clinical specimens were from intensive care unit of Pulmonary and Critical Care Medicine that 5 bacteria species were not found in these specimens. They are *Mycoplasma pneumoniae, Haemophilus infuenzae, Enterobacter cloacae, Legionella pneumophila,* and *Chlamydia pneumoniae,* and most of them are difficult to culture. Our DNA microarray would have obvious advantages in detecting these bacteria.

One of the weaknesses of this microarray was that it could not differentiate between colonization and infection, like many other molecular amplification tests. Although some report indicated that quantitative detection of pathogen bacteria could help for distinguishing colonization from infection [8, 34], meta-analysis showed that clinical outcomes were similar regardless of whether cultures were performed quantitatively or semiquantitatively [35]. Therefore, identifying the causative agent of infection in patients with pneumonia is still a long challenge for the clinical microbiology laboratory. Nevertheless, taking the shorten turn-around time and the high throughput into account, this assay is superior to culture methods.

In conclusion, this DNA microarray for the important bacterial causes of pneumonia has potential to provide a faster diagnosis tool than current standard methods. The accurate and timely identification directly from clinical specimens should improve patient management and prevent the inappropriate antibiotic therapy.

Materials And Methods

Study design

Firstly, we designed and evaluated the primers and probes of target genes and fabricated microarray. Secondly, the detection limit of this microarray was evaluated by using a series of 10-fold dilution (10^1 copies/μL to10^6 copies/μL) from recombinant plasmids. Thirdly, the accuracy of this microarray was evaluated by the genomic DNAs from 19 standard strains, 123 clinical isolates (Table 2). Subsequently, 8 mixtures with two or three of these genomic DNAs mixed randomly and were used as templates to assess the ability of this microarray to distinguish mixed pathogens. Finally, the sensitivity and specificity of this microarray was evaluated by clinical samples. Spontaneous sputum specimens, endotracheal sputum aspirate specimens and bronchoalveolar lavage fluid (BALF) specimens were collected in our department of Pulmonary and Critical Care Medicine. At the same time, culture and identification of pathogens were performed blindly at the Department of Microbiology in our Hospital. DNA direct sequencing was used to confirm results when the results were discordant.

Specimen collection and processing

The 19 standard strain DNAs, 123clinical isolates used in this study were obtained from Beijing Institute of Radiation Medicine and Chinese PLA General Hospital (Table 2). All 142 bacterial strains were cultured overnight in 5ml of species-specific culture medium and growing temperature. Genomic DNA of the cells were extracted by boiling with the same volume of lysate buffer (25mmol/L NaOH、0.1nmol/L EDTA、10mmol/L Tris-HCl、1%NP40、2%Chelex-100, 1%Triton X-100) for 10min, centrifuging for 2min at 12000rpm, absorbing the supernatant and storing at -70°C for testing [36]. 16S rDNA in multiple PCR was used as a control to ensure the standardization and adequacy of DNA templates from bacteria.

The 157 participating patients with clinically and radiologically confirmed pneumonia were from intensive care unit of Pulmonary and Critical Care Medicine. All 36 spontaneous sputum specimens, 98 endotracheal sputum aspirate specimens, and 23 bronchoalveolar lavage fluid (BALF) specimens were collected between July 2013 and October 2014. All the specimens were immediately stored at -70°C for DNA extraction. At the same time, culture and identification of pathogens were performed blindly at the Department of Microbiology in our Hospital. Sputum samples were inoculated onto blood agar plates, chocolate agar plates and Macconkey agar plates using standard techniques, and incubated at 37℃ in 5% carbon dioxide in air for 18–24 h. Then the isolates were identified by colonial morphology, standard biochemical methods, VITEK-2 (bioMérieux), or matrix-assisted laser desorption ionization-time of flight mass spectrometry. All sera samples were collected and immediately refrigerated at 4°C for the immunoglobulin M antibodies assays of *Mycoplasma pneumoniae, Legionella pneumophila, Chlamydia pneumoniae*. The immunoglobulin M antibody detection kit (VIRCELL, Granada, Granada, Spain) was used according to the manufacturer's instructions, and the results were read on a EUROStar II immunofluorescence microscope (EUROIMMUN, Hanseatic City of Lubeck, Schleswig-Holstein, Germany).

The genomic DNAs of 157 clinical specimens were extracted by the following protocol: 30 min of liquefaction with 4%NaOH, 10 min of boiling for 50μl liquefied specimens and 50μl lysate buffer (25mmol/L NaOH、0.1nmol/L EDTA、10mmol/L Tris-HCl、1%NP40、2%Chelex-100, 1%Triton X-100), 2 min of waiting after absorbing in DNA adsorption column, 1 min of centrifuging at 12000rpm, 2 times of washing by 600μl 75% alcohol, and eluting in 50μl ddH_2O [36]. All the Genomic DNAs stored at -70℃ until use. We used 10ng of DNA template in multiplex PCR to ensure the adequacy of DNA templates. 16S rDNA in multiple PCR was also used as a control to ensure the standardization and adequacy of DNA templates.

Construction of reference plasmids

The standard strain DNAs in table 1 was used to construct the reference plasmids. Plasmids containing target genes were generated by cloning PCR products with the pMD18^{TM}-T vector system (TaKaRa, Shiga, Japan). All plasmids were defined by sequencing. Plasmid extracts were diluted in ddH_2O at 10^6 copies/μL in tenfold dilution series for using in microarray optimization.

Primers and probes design and evaluation

We selected both 16S rDNA and 15 bacterial specific genes as target genes to identify bacterial from species level. The 15 bacterial specific genes are *lytA* of *Streptococcus pneumoniae, nuc* of *Staphylococcus aureus, P6* of *Haemophilus influenzae, phoA* of *Escherichia coli, mdh* of *Klebsiella pneumoniae, toxA* of *Pseudomonas aeruginosa, gltA* of *Acinetobacter baumannii, P1* of *Mycoplasma pneumoniae, ddl* of *Enterococcus faecalis* and *Enterococcus faecium, dnaJ* of *Enterobacter cloacae,*

chitA of *Stenotrophomonas maltophilia, recA* of *Burkholderia cepacia, mip* of *Legionella pneumophila* and *ompA* of *Chlamydia pneumoniae.* All gene sequences were downloaded from NCBI (http://www.ncbi.nlm.nih.gov/genomes). A pair of universal primers was designed to amplify specific sequences in conserved upstream and downstream regions of 16S rDNA. In variable regions between universal primers, specific probes and a positive control probe were designed. *Klebsiella pneumoniae*, *Enterobacter cloacae* and *Escherichia coli* posse the same specific probe because of their highly similar sequences. For 15 bacterial specific genes, we designed the primers and probes using DNAMAN 6 and Oligo 7 software, respectively. Primers were picked in the conserved upstream or downstream regions and probes were designed at the variable part of the sequences. All primers and probes sequences were aligned using BLAST (http://blast.ncbi.nlm.nih.gov/) to compare the homology between potential targets belong to the same genus. To evaluate the efficiency of all primers, reference genomic DNAs of 15 bacteria were amplified and examined by 2% agarose gel electrophoresis. All primers and probes were finally confirmed by sequence analysis of PCR products from the reference plasmids.

Microarray preparation

This DNA microarray was designed to have 32 probes, including 1 universal 16S rDNA probe, 3 negtive control probes into eight columns and eight rows. The universal 16S rDNA probe used for detect whether samples contain bacteria. Probes were synthesized by Sangon Biotech Co., Ltd. (Shanghai). Each probe, at 50μM final concentration, was spotted twice repeatedly by a noncontact inkjet Nanoplotter 2.1 (GeSim, Dresden, Germany) on the aldehyde-chip after mixing with uniform proportional printing buffer (5% glycerol, 0.1% sodium dodecyl sulfate (SDS), 6×salinesodium citrate buffer (SSC), and 2% (wt/vol) Ficoll 400). The microarray layout was shown in Figure 1a. Microarrays were prepared as our research group previously described [37].

Multiplex asymmetric PCR

Primers of 16S rDNA and 15 specific genes were divided into three groups for the multiplex asymmetric PCR. Reactions were carried out on a Veritil 96-well Thermal Cycler instrument (applied biosystems by life technologies, Singapore). Final reaction volume for each multiplex asymmetric PCR was 25μl with the same reagents of Multiplex PCR 5×Master Mix (5μl, New England Biolabs, UK) and DNA template (2.5μl). The forward and reverse primers concentrations of 16S rDNA, *P6* and *mip* were 0.08 μM and 0.4 μM, respectively. The others were 0.16 μM and 0.8 μM, respectively. Cycle parameters were optimized as follows: 10min at 95℃; 35 cycles of 30s at 95℃, 30s at 55℃, and 1min at 68℃; and a final extension of 5min at 68℃.

Hybridization and signal detection

Before hybridizing, PCR products were denatured at 98℃ for 5min and chilled on ice. 2.5μl of each amplification product from the three multiplex PCR reactions was mixed with 7.5μl of hybridization buffer (0.6% SDS, 10% formylamine, 8×SSC, and 10×Denhardt). A total of 15μl hybridization mixture was reacted with the probes at 45℃ for 1h. After that, the slide was washed once with washing buffer A (1×SSC and 0.2%SDS), washing buffer B (0.2×SSC), and washing buffer C (0.1×SSC) for 1min, then dried by centrifuging. Subsequently, 1:1500 diluted streptavidin-horseradish peroxidase (HRP) was incubated in each reaction chamber on the chip for 30min at 37℃, and the slide was washed once with PBST (0.05% Tween 20) 1min and dried by centrifuging. Finaly, the hybridization region on the slide was covered by 20μl phospho-tyrosine (Millipore, USA), and detected signal immediately by portable chemiluminescence biochip imager (Academy of Military Medical Sciences, China).

Abbreviations

PCR: polymerase chain reaction; rDNA: ribosomal DNA; ATCC: American Type Culture Collection; CMCC: National Center for Medical Culture Collections; CGMCC: China General Microbiological Culture Collection Center; CAP: community acquired pneumonia; HAP: hospital acquired pneumonia; BALF: bronchoalveolar lavage fluid; SDS: sodium dodecyl sulfate; SCC: salinesodium citrate buffer; HRP: streptavidin-horseradish peroxidase.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Chinese PLA General Hospital (No. S2014-049-01). All experiments were conducted in accordance with the relevant guidelines and regulations. All patients involved in the study provided informed consent and all personal information was kept confidential.

Consent for publication

Not applicable.

Availability of data and materials

The data used and analysed for the current study are available upon request from the first author Xiuqing Ma (E-mail: mxq820812@163.com)

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LAC and QQL designed the study, analyzed the data and proofread the manuscript. XQM was responsible for experiment performance and manuscript drafting. YQL helped with analyzed the data. YL, YL and LY collected samples. CSL provided part of experimental materials. All authors read and approved the final manuscript.

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Supplementary Figure Legend

Figure S1. PCR products examined by 2% agarose gel electrophoresis. **a**, Agarose gel electrophoresis of PCR products amplified using the universal 16S rDNA primer. DNA templates were extracted from: 1 *ddH_2O*; 2 *Haemophilus influenzae* (ATCC9007); 3 *Haemophilus influenzae* (ATCC33533); 4 *Staphylococcus aureus*; 5 *Acinetobacter baumannii*; 6 *Escherichia coli*; 7 *Streptococcus pneumoniae*; 8 *Pseudomonas aeruginosa*; 9 *Chlamydia pneumoniae*; 10 *Mycoplasma pneumoniae*; 11 *Legionella pneumophila*; 12 *Klebsiella pneumoniae*; 13 *Enterococcus faecalis*; 14 *Enterococcus faecium*; 15 *Stenotrophomonas maltophilia*; 16 *Burkholderia cepacia*; 17 *Enterobacter cloacae*; respectively. **b**, Agarose gel electrophoresis of PCR products amplified using 15 pairs of primers for the 15 bacterial specific genes. The 15 bacterial specific genes were 1 *P1*; 2 *ddl (for Enterococcus faecalis)*; 3 dnaJ; 4 *mdh*; 5 *chitA*; 6 *lytA*; 7 *recA*; 8 *phoA*; 9 *ddH_2O*; 10 *ddl (for Enterococcus faecium)*; 11 *gltA*; 12 *mip*; 13 *nuc*; 14 *toxA*; 15 *ompA*; 16 *P6*, respectively.

Tables

[Please see the supplementary files section to view the tables.]

Figures

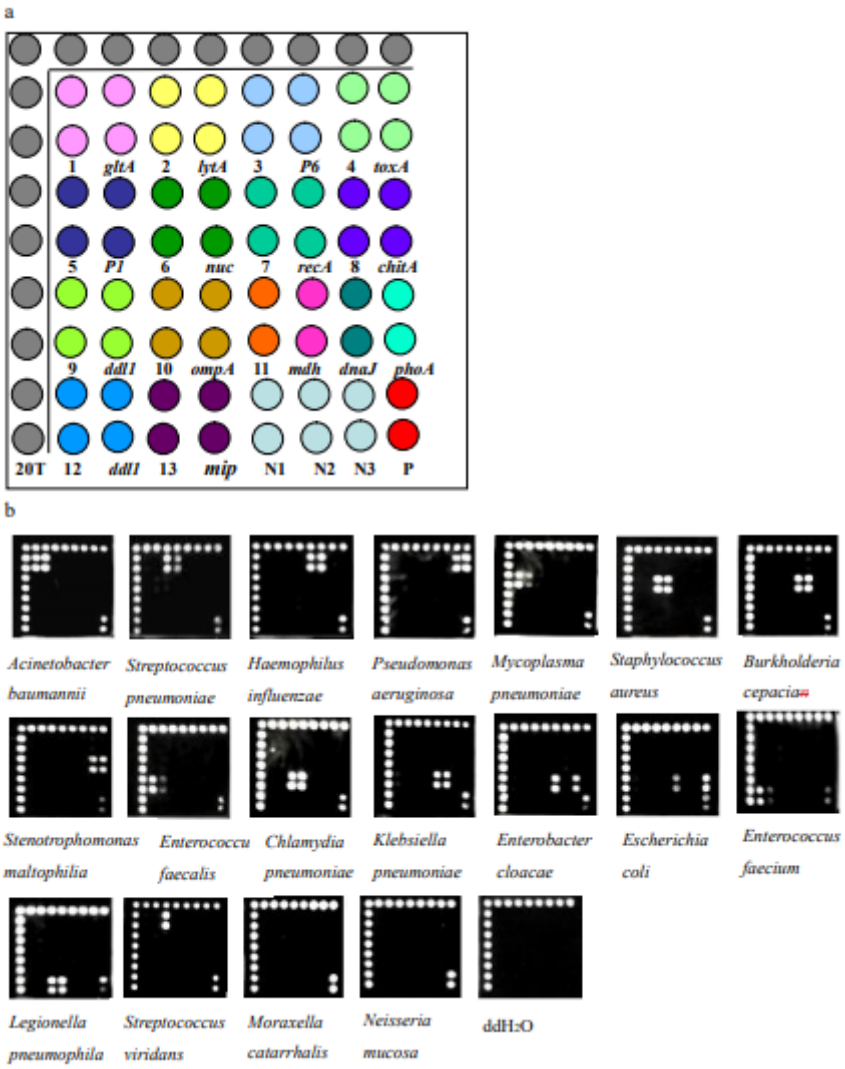

Figure 1

a. The layout of the hybridization capture-chip. The probe 20T is the QC probe. The probe N1, N2, N3 are the negative control probes. The probe P is the universal 16S rDNA probe. Each probe was spotted as two. The sequences of probe 1-13 all come from 16S rDNA and their corresponding target pathogen were: 1 Acinetobacter baumannii; 2 Streptococcus pneumoniae; 3 Haemophilus influenzae; 4 Pseudomonas aeruginosa; 5 Mycoplasma pneumoniae; 6 Staphylococcus aureus; 7 Burkholderia cepacia; 8 Stenotrophomonas maltophilia; 9 Enterococcus faecalis; 10 Chlamydia pneumoniae; 11 Klebsiella pneumoniae or Enterobacter cloacae or Escherichia coli; 12 Enterococcus faecium; 13 Legionella pneumophila, respectively. b. The typical hybridization results of fifteen species of bacterial pathogens in pneumonia, non-target bacteria from pure bacterial cultures and ddH2O.

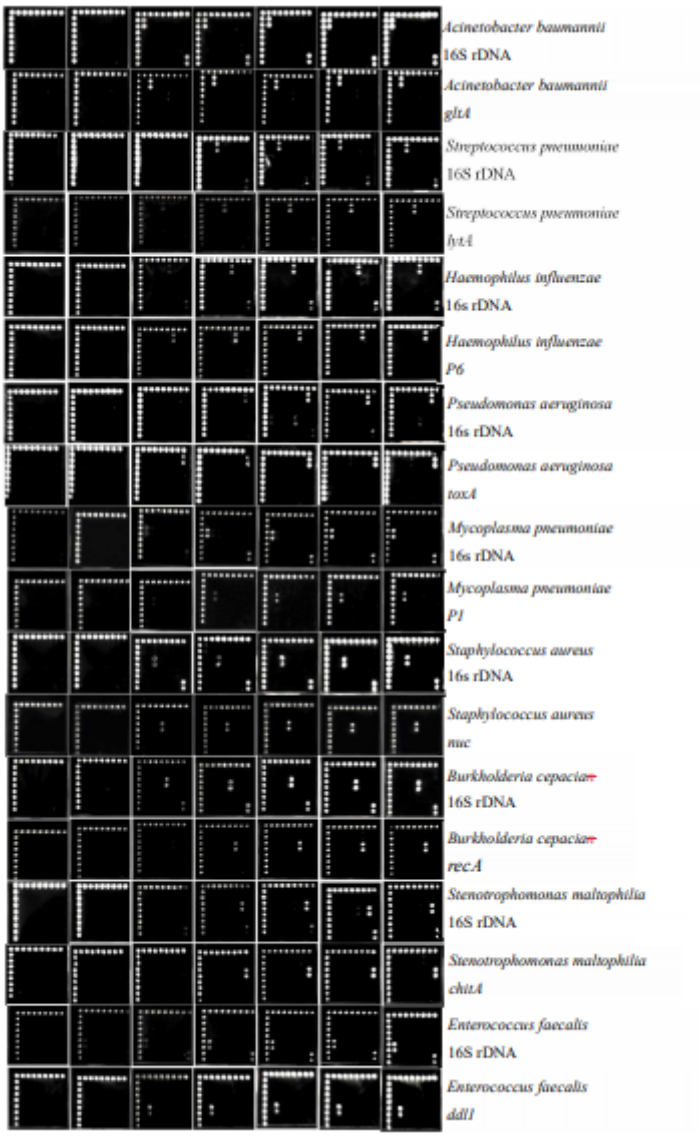

Figure 2

The sensitivity of the pathogen probes. Microarray hybridized with PCR products which diluted for concentration gradient. 10μL dilution used in each well, and the concentration of probes were 50μM.

Figure 3

The specificity of the pathogen probes. a Microarray hybridized with PCR products amplified from mixed plasmid DNAs. b The hybridization results of clinical samples which contains two or more target pathogens.

Supplementary Files

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- Table1.pdf
- FigureS1.pdf
- Table3.pdf
- Table2.pdf