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Full Length Research Paper

Analysis of an outbreak of *Klebsiella pneumoniae* by the DiversiLab system and pulsed-field gel electrophoresis

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The aim of this study was to analyse the reason that five patients associated with bloodstream infections by *K. pneumoniae* in a surgical ward using Pulse Field Gel Electrophoresis (PFGE) and the repetitive-sequence-based PCR (rep-PCR) employing the DiversiLab system. In July 2010, eight isolates were collected from the same surgical ward of a hospital, and the specimens from sputum, blood and abdominal drainage fluid, respectively. The PFGE patterns after XbaI digestion and rep-PCR profiles produced by the DiversiLab system were determined for eight isolates. The Rep-PCR profiles produced by using the DiversiLab system showed that the eight isolates can be divided into two groups; the K8-02 as a group and the other seven strains is the other group. The eight strains of *K. pneumoniae* can be divided into two groups (A and B), seven strains were type A which the subtype A1 is the main-type (K8-01, K8-03, K8-04, K8-06, K8-08), and one is type B (K8-02). The PFGE and rep-PCR interpretations were concordant for the eight strains of *K. pneumoniae*. This data suggest that the DiversiLab system may be a reasonable alternative to PFGE for investigation and control of nosocomial infection outbreaks caused by *K. pneumoniae*, since it is easy to use, rapid and does not require highly skilled operators (Mazzariol et al., 2012).

Key words: *Klebsiella pneumoniae*, outbreak, pulse field gel electrophoresis (PFGE), rep-PCR, DiversiLab system.

INTRODUCTION

Klebsiella pneumoniae strain is one of the most important gram-negative pathogens causing hospital-acquired infection and community-acquired infection (Chen et al., 2013). In recent years, the infection and drug resistance of *K. pneumoniae* strain have been significantly increased; especially the growing in number of ESBLs-producing strains has become a major cause of death in patients with pneumonia. Therefore, rapid and accurate pathogen traceable epidemiological method is extremely important for the blocking and control of hospital infection epidemic spread. Now the major genotyping method for *K. pneumoniae* strain is PFGE, which is recognized as the gold standard of bacteria typing method with the highest discriminatory power (Han et al., 2013). However, PFGE requires specialized equipment and is technically demanding, labor intensive, and relatively slow, as it may take 2

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Abbreviations: ESBLs, Extended spectrum beta-lactamase; **CLSI**, clinical and laboratory standards institute; **PFGE**, pulse field gel electrophoresis; **rep-PCR**, repetitive-sequence-based PCR; ***K. pneumoniae***, *Klebsiella pneumoniae*.

Table 1. The clinical data of 8 *K. pneumoniae* strains.

Strain	Ward code	Patient code	Specimens type	Collection time
K8-01	S1	W	Sputum	July 17, 2010
K8-02	S1	W	Abdominal cavity drainage liquid	July 17, 2010
K8-03	S1	W	Blood	July 29, 2010
K8-04	S1	W	Blood	July 18, 2010
K8-05	S1	L	Blood	July 20, 2010
K8-06	S1	H	Blood	July 21, 2010
K8-07	S1	Y	Blood	July 29, 2010
K8-08	S1	J	Catheter	July 29, 2010

to 5 days to obtain results, depending on the organism and the methods utilized (Ligozzi et al., 2010). DiversiLab system is a typing method based the principle of rep-PCR, with the use of standardized operating, the high-resolution microfluidic chip and fluorescence detection system and an unified data processing software, has good repeatability, and can be used in the laboratory long-term epidemiological typing methods. It has been applied in *Acinetobacter baumannii* (Fontana et al., 2008), *Staphylococcus aureus* (Ross et al., 2005), *Pseudomonas aeruginosa* (Doléans-Jordheim et al., 2009), *Mycobacterium* (Cangelosi et al., 2004), and other molecular epidemiological studies. At present, reports on the DiversiLab system are rarely seen in China. In this study, we use the DiversiLab system to analyse genotypes of 8 *K. pneumoniae* strains, and compare the consistency between the DiversiLab system and Pulse Field Gel Electrophoresis (PFGE).

MATERIALS AND METHODS

Bacterial

The eight *K. pneumoniae* strains were collected from one surgical ward of a hospital in July 2010. All the strains were identified by using VITEK-2 compact automatic system (biomérieux, Marcy l' Etoile, France). The clinical datas are shown in Table 1.

Antibiotic susceptibility tests

Antibiotic susceptibility test profiles for 11 antimicrobial agents including amoxicillin/clavulanic acid (AMC), aztreonam (ATM), ciprofloxacin (CIP), ceftriaxone (CRO), cefazolin (CFZ), ampicillin (AMP), meropenem (MEM), cefoperazone/sulbactam (CPZ/SU), cotrimoxazole (SXT), piperacillin/tazobactam (PIP/TA), gentamicin (GEN) were determined by using a broth microdilution method. Standards for antimicrobial susceptibility testing and interpretation were based on the CLSI 2011 guidelines (CLSI, 2011). *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as reference strains for susceptibility testing.

Pulse field gel electrophoresis method

Genomic DNA was prepared as described previously (Gori et al., 1996; Pang et al., 2002), but with some modifications. *K. pneumoniae* isolates were grown overnight on Mueller-Hinton plate. Takings a few pure colonies with a sterile cotton swab and dissolving with 2.5 ml TE buffer, then making 4.0 McFarland units, and incubating at 37°C. 150 µl prepared specimens were removed, 20 µl proteinase K and 30 µl 10% SDS (final concentration is 1%) were added, and mixed to warm-up at 56°C. Taking 2% low melting point gel and the prepared bacteria to fill the mold with the proportion of 1:1, then it was solidified at room temperature for 30 min. Then adding the molding of the plastic block to 1 ml lysis buffer which contains 5 µl proteinase K, and incubating for 2 h at 54°C shaking-bed. Then washed 2 times with 50°C preheated sterile distilled water, 10 min for each time, and washed 3 times with 50°C preheated TE buffer, 10 min for each time. Remove sample plastic block and cut down about 2 mm gel, then immersed it in digestion system which contains 200 µl restriction endonuclease enzyme Xba I, then 37°C digested overnight. Attach the digested gel block to the end of the glue comb, then place the comb at horizontally inverted plastic mold. The melted 1% electrophoresis agarose which had balanced at 50°C water to the mold was added, and then solidi-ficated at room temperature for 30 min. Setting the electrophoresis conditions as follows: the electrophoresis buffer 0.5 × TBE, switching time of 4 ~ 30 s, the field strength is 6 V/cm, temperature at 14°C, the electric field angle of 120°, and the electrophoresis time of 18.5 h, then view the result with ethidium bromide staining under ultraviolet light observations, pulsed-field gel electrophoresis (Tenover et al.,1995).

To determine the relationship between the band patterns as the following principles by visual inspection: 1) the same strains: the restriction map with same size and number is considered to be the one strain (main stream type); 2) closely related types: the electrophoretic bands have 3 or less different bands with the main phenol-type isolates due to mutation, insertion, deletion or inversion are considered to be the subtype of the main type; 3) may related types: type with 4 to 6 bands different is considered to be of different type; 4) not relevant: has more than 7 different bands with the main type is considered no correlation in epidemiology.

Rep-PCR using DiversiLab system

Using MOBIO UltraClean (TM) microbial DNA extraction kit to extract bacterial DNA and adjusting the DNA concentration to 25 ~ 50 mg/L with visible spectrophotometer (NanoDrop ND-1000). Rep-PCR was performed by using the DiversiLab *K. pneumoniae* kit for DNA fingerprinting (bioMerieux, Marcyl'Etoile, France) according to the manufacturer's instructions. Preparing the 25 µl PCR amplification system: the 18 µl rep-PCRMM1, 2.5 µl GeneAmp10 × PCR buffer, 2.0 µl Primer Mix, 0.5 µl AmpliTaq DNA polymerase and 2 µl template. Thermal cycling parameters were as follows: initial denaturation of 94°C for 30 s, annealing at 50°C for 30 s, extension at 70°C for 90 s, as a cycle, a total of 35 cycles, and a final extension at 70°C for 3 min. Analysis of PCR products were implemented by using DiversiLab System (bioMerieux) in which the amplified fragment of various size and fluorescent intensities were separated

Table 2. MIC values (µg/ml) of 8 *K. pneumoniae* strains.

Strain	(AMC)	(ATM)	(CIP)	(CRO)	(CFZ)	(AMP)	(MEM)	(CPZ/SU)	(SXT)	(PIP/TA)	(GEN)
K8-01	8	<=1	1	>=64	>=64	>=32	<=4	<=16	>=320	<=4	>=16
K8-02	>=32	<=1	>=4	<=1	8	>=32	<=4	<=16	<=20	>=128	<=1
K8-03	4	<=1	0.5	>=64	>=64	>=32	<=4	<=16	>=320	<=4	>=16
K8-04	4	<=1	0.5	>=64	>=64	>=32	<=4	<=16	>=320	<=4	>=16
K8-05	8	<=1	0.5	>=64	>=64	>=32	<=4	<=16	>=320	<=4	>=16
K8-06	4	<=1	0.5	>=64	>=64	>=32	<=4	<=16	>=320	<=4	>=16
K8-07	4	<=1	0.5	>=64	>=64	>=32	<=4	<=16	>=320	<=4	>=16
K8-08	4	<=1	1	>=64	>=64	>=32	<=4	<=16	>=320	<=4	>=16

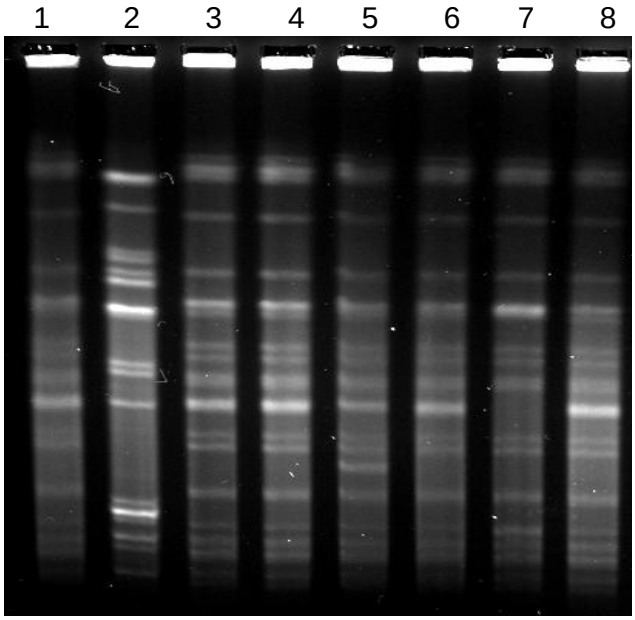

Figure 1. The PFGE typing results of eight *K. pneumoniae* strains. Note: 1 = K8-01, 2 = K8-02, 3 = K8-03, 4 = K8-04, 5 = K8-05, 6 = K8-06, 7 = K8-07, 8 = K8-08.

and detected using a microfluidics chip with the Aligent 2100 Bioanalyzer (Aligent Technologies, Santa Clara, CA, USA). Further analysis was performed with the web-based DiversiLab software version 3.3 with the Pearson correlation coefficient to determine distance matrices and the un-weighted-pair group method with arithmetic mean to create dendrograms.

The resulting DNA fingerprinting patterns were viewed as electropherograms, and the report included a dendrogram constructed from a similarity matrix and a virtual gel image of the fingerprint for each DNA sample. The criteria references of virtual gel image analysis are divided into three kinds: similar is greater than 97%, and no differences between bands; indistinguishable is greater than 95%, and has 1 to 2 different bands; different is less than 95%, and more than 2 different bands (Casolari et al., 2005).

RESULTS

Antibiotics susceptibility test

In 8 *K. pneumoniae* strains, the KB-02 was different with other isolates. The KB-02 was sensitive to SXT, GEN, MEM, CPZ/SU, CRO and ATM, and were resistant to AMC, CIP, CFZ, AMP and PIP/TA. The other 7 isolates were multiple drug-resistant *K. pneumonia*, which were sensitive to AMC, ATM, CIP, MEM, CPZ/SU and PIP/TA, and were resistant to CRO, CFZ, AMP, SXT and GEN. The results were in Table 2.

PFGE and rep-PCR using DiversiLab

The PFGE patterns after digestion with XbaI, and Rep-PCR using the DiversiLab system were performed for eight isolates. The eight isolates can be divided into two groups (A and B), seven strains were type A (K8-01, K8-03, K8-04, K8-05, K8-06, K8-07, K8-08), and one is type B (K8-02). Results are shown in Figure 1. The DiversiLab system showed the same results (Figures 2 and 3). The PFGE and the DiversiLab system interpretations were concordant for *K. pneumoniae* isolates. The DiversiLab system allowed a complete microbial typing analysis in approximately 4 h compared to 3 days for PFGE in our study.

DISCUSSION

There are many bacteria genotyping methods, such as DiversiLab system, MLST, PFGE and ERIC. Among them, PFGE is recognized as the gold standard of bacteria typing method. Witt (Te et al., 2009) applied the DiversiLab system, MLST and PFGE to study 93 MRSA strains, and the Simpson coefficient among three methods were 0.860, 0.877 and 0.905, respectively. Although, the resolution is different, the three kinds of methods are commonly used in genotyping of MRSA. Qu et al. (2010) used the DiversiLab system for genotyping of *A. baumannii*, which got good results. The DiversiLab system is fast and simple, besides its commercial kits, which is available for laboratory epidemiological typing methods. The eight *K. pneumoniae* strains were isolated from the same surgical ward of a hospital during July 2010, and the specimens from sputum, blood and abdominal drainage fluid, respectively. Antibiotics susceptibility test showed that among the eight strains, seven strains

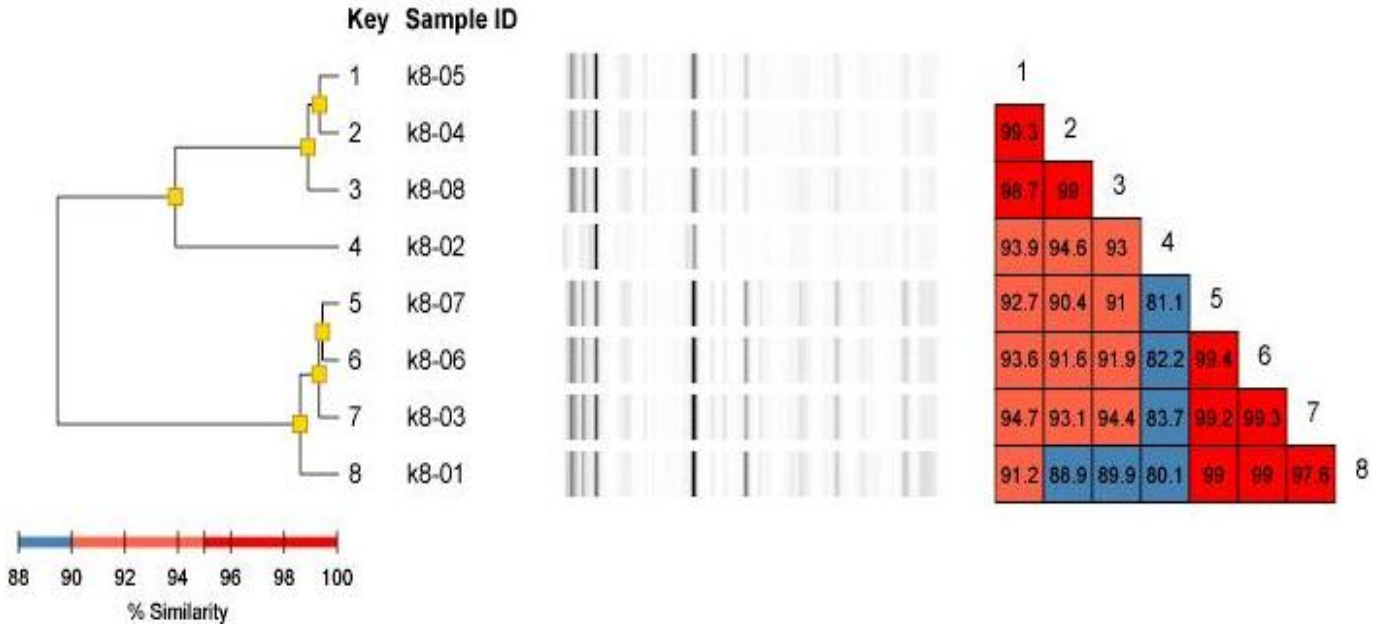

Figure 2. DiversiLab system typing results for *K. pneumoniae* strains; Note: The tree on the left side is the typing results of the DiversiLab system; figures in the right side of the matrix represent the similarity between the two strains, number on the right of the matrix represent strains, the Sample ID is the strain number.

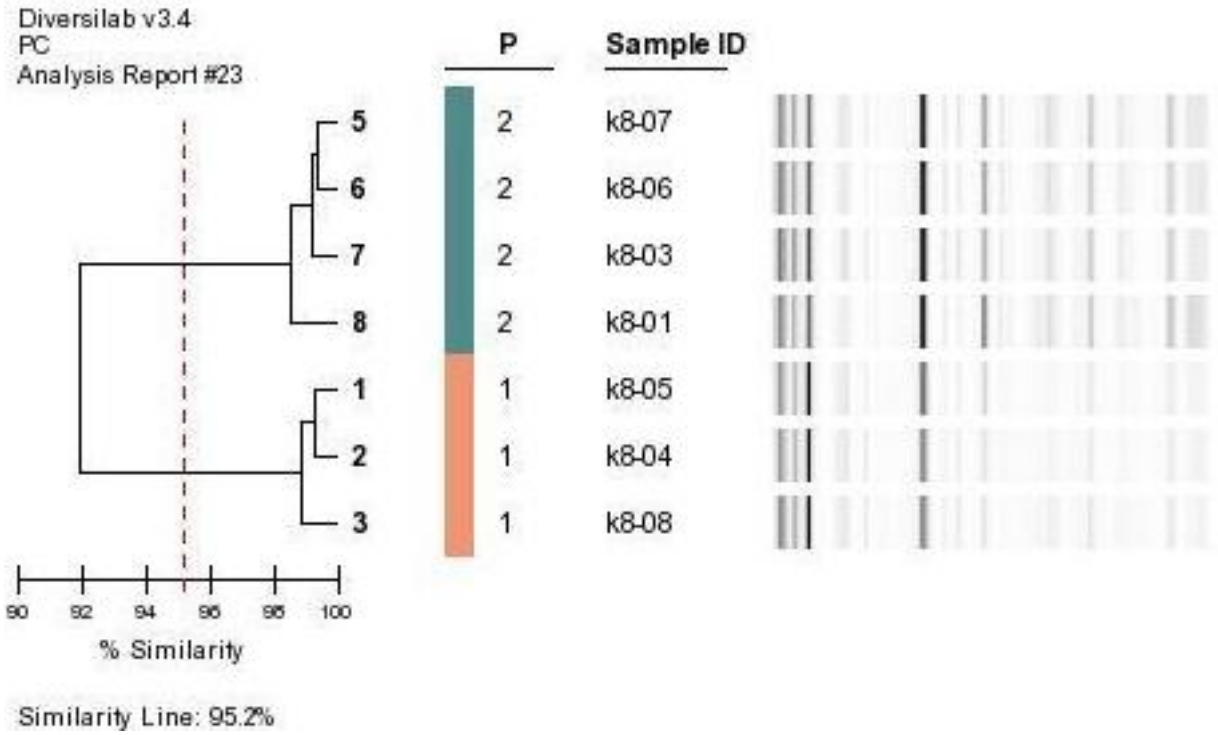

Figure 3. DiversiLab results of 7 *K. pneumoniae* strains except for K8-02 specimen.

have the same antimicrobial resistance pattern, except K8-2. The DiversiLab system and PFGE have proved these seven isolates belonging to the same type, so that the infection is likely an outbreak of hospital. The susceptibility pattern of the isolate K8-02 from abdominal cavity drainage fluid was different from three other strains

that came from blood and sputum samples, which were all from the patients W. And PFGE patterns and the DiversiLab system showed no correlation among these four *K. pneumoniae* strains.

According to the time of the five patients infected, the DiversiLab system analysis found that W was the first infection patient, and subsequently infected with the L, H, Y and J. Because these four *K. pneumoniae* strains were the same type, in addition to, the PFGE map also showed the same result. Therefore, the patient W was most likely the source of this outbreak of nosocomial infection, and spreaded to the patients L, H, Y and J through exogenous way. At present, the most common typing method for *K. pneumoniae* is PFGE, which is the gold standard of bacterial genotyping methods and homology analysis (Wise et al., 2009). But the PFGE results usually can be read with naked eye, and different operators will influence the result. Furthermore, the technology is time-consuming, because it normally takes 3~5 days to get the final results. On the other hand, DiversiLab system that based REP-PCR is a standardized and automated systems, and the result collection and analysis process has no subjective restrictions. It can analyse 12 specimens in 40 min and the total process only need 6~8 h. The DiversiLab system has high resolution, good repeatability and simple operation, is a real-time quantitative detection. But the system also has shortcomings.

The analysis is race operation that needs only the 1 µl each time, so that sometimes a bubble may need re-analysis of the amplified products and increases detection period and the cost. When using chip detection, even in the case of only several samples, the 12 sample holes and the standard hole must be added to the markers and gel fluorescent dye mixture, and result in reagent waste and cost. This study shows that the DiversiLab system is simple, fast, has high repeatability and high resolution, the results of quantitative processing, and various forms of output can be used as preferred genotyping method, especially on the short-term outbreak of a large number of specimens.

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