Comparison of PFGE and multilocus sequence typing for analysis of *Klebsiella pneumoniae* isolates

Klebsiella pneumoniae represents an important nosocomial pathogen causing urinary, respiratory and blood infections (Brisse *et al.*, 2006; Podschun & Ullmann, 1998). Hospital outbreaks due to *K. pneumoniae* are frequent and especially feared when caused by multidrug-resistant strains, such as extended-spectrum β -lactamase producers (Paterson & Bonomo, 2005). DNA-based strain typing methods are used to distinguish *K. pneumoniae* clinical isolates in order to understand transmission patterns and to help management of hospital infections. Molecular serotyping, based on PCR-RFLP of the *cps* operon responsible for capsular polysaccharide expression, has a higher discriminatory ability than traditional K typing (Brisse *et al.*, 2004), and ribotyping is also applicable to *K. pneumoniae* (Brisse & Verhoef, 2001). Nevertheless, the most commonly used method is PFGE analysis of macrorestriction fragments (Arlet *et al.*, 1994). The main advantage of PFGE lies in its high discriminatory power (Hansen *et al.*, 2002), but PFGE is technically demanding and requires a high level of coordination (e.g. <http://www.cdc.gov/pulsenet>) to achieve inter-laboratory reproducibility. In contrast, multilocus sequence typing (MLST) provides unambiguous data that are suitable for global epidemiology and evolutionary studies (Maiden *et al.*, 1998). A MLST method was previously developed for *K. pneumoniae*, and analysis of nosocomial isolates showed that MLST can discriminate among epidemiologically unrelated isolates (Diancourt *et al.*, 2005). However, the discriminatory power of MLST was not compared to that of PFGE. In our previous study (Diancourt *et al.*, 2005), 28 isolates belonged to 11 groups that were not distinguished by MLST nor by ribotyping. Among these 11 groups, 5 comprised isolates from distinct countries or separated by large sampling times. For these apparently unrelated cases the

isolates could be suspected as being genotypically undistinguishable due to an insufficient discriminatory power of MLST and ribotyping, rather than due to an undocumented epidemiological link. We report here on the comparison of the discriminatory power of PFGE with previously reported methods.

Sixty-three *K. pneumoniae* isolates from different European hospitals and clinical sources, which were previously analysed by MLST, ribotyping and molecular serotyping (Brisse *et al.*, 2004; Diancourt *et al.*, 2005), were included (Fig. 1). Allele sequences and profiles are available at <http://www.pasteur.fr/mlst>. Genome fingerprinting by PFGE using enzyme *Xba*I was performed as described by Arlet *et al.* (1994), with slight modifications. The banding patterns obtained were compared with a band tolerance parameter of 1% and a pattern optimization parameter of 1% using BioNumerics v4.6 software (Applied Maths). PFGE profiles were considered different when there were three or more band differences between them. Simpson's indices of diversity (Hunter & Gaston, 1988) were calculated using the online tool at <http://www.comparingpartitions.info>.

Of the 67 isolates analysed previously (Diancourt *et al.*, 2005), 63 were available for PFGE analysis; these strains belonged to 39 MLST sequence types (STs) and 45 ribotypes (RTs). Fifty-six distinct PFGE types (PTs) were recognized, with a Simpson's index of discrimination of 99.6%, compared to 97.5 and 98.5% for MLST and ribotyping, respectively. The 58 isolates for which molecular serotyping data were available (Fig. 1) were subdivided into 37 distinct C patterns (Simpson's index 98.2%). There were seven pairs of isolates with the same PT (PT3, PT9, PT10, PT13, PT31, PT45 and PT49). Isolates of each of these pairs except one (PT10) had the same ST and the same RT. The two strains of PT10 had the same

ST (ST29) but differed by their RT (RT28 or RT30). Hence, in no case did MLST subdivide PTs. There was a strong concordance between PTs and the source of the isolates. Indeed, all seven pairs of isolates with the same PT came from the same centre and were isolated within a few weeks of each other (Fig. 1); the only exception was PT10, consistent with RT data.

Conversely, several STs and RTs could be subdivided into distinct PTs. Indeed, among the 11 groups with the same ST and RT, only 3 pairs of isolates had the same PT; the remaining 8 groups (22 isolates) were subdivided into 19 PTs. For example, the five strains with ST15 and RT38 were subdivided into four PTs (Fig. 1); the only two strains sharing the same PT were those coming from Lille. Hence, strains coming from distinct centres had distinct PTs, even when they had the same ST or RT. These results show that PFGE provides a finer-grained image of relationships among strains, providing more precise information for epidemiological purposes. If one excludes isolates that are putatively epidemiologically related, as suggested by their shared PTs, the discrimination power of MLST and ribotyping among unrelated strains is 98.1 and 99.0%, respectively.

In conclusion, 63 *K. pneumoniae* isolates were characterized by PFGE, MLST, ribotyping and molecular serotyping. High concordance among methods was found, and discrimination among epidemiologically non-related strains was higher with PFGE, which thus appears more suitable for short-term epidemiology. Therefore, for *K. pneumoniae* isolates that are not distinguished by MLST, PFGE analysis is recommended to address fine-scale epidemiological questions. This result is consistent with the fact that PFGE can detect chromosomal rearrangements, which may be caused, for example, by mobile elements with rapid evolutionary

Fig. 1. UPGMA dendrogram based on MLST profiles and its correspondence with PFGE patterns. The columns to the right of the PFGE patterns correspond (from left to right) to the strain ID number in our laboratory collection (SB number), original strain name, ST, RT, molecular serotype (C pattern), PT, city of isolation and date of isolation. C patterns could not be obtained for one strain (indicated by 'failed' in the corresponding column) and were not analysed (NA) for four other strains.

rates. In contrast, MLST is more appropriate for strain phylogeny and large-scale epidemiology. Indeed, determination of phylogenetic relationships among strains with distinct PFGE patterns is imprecise. For example, strains belonging to the same ST did not always cluster in a single branch based on cluster analysis of PFGE patterns (not shown), and there was only a weak correlation ($r=0.21$) between the genetic distances calculated based on PFGE patterns and MLST data. Therefore, we see PFGE and MLST as complementary methods, which are appropriate for studies at distinct scales, i.e. local epidemiology versus global population structure, respectively.

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