Multilocus sequence typing compared to PFGE for molecular typing of extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates

Comparaison de la technique multilocus sequence typing et l’électrophorèse en champ pulsé pour le typage moléculaire des isolats de *Klebsiella pneumoniae* producteurs de bêta-lactamases à spectre élargi

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Extended spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* is a multidrug resistant bacteria frequently involved in severe hospital-acquired infections and characterized by a great epidemic potential to disseminate [1, 2]. The production of ESBL in *K. pneumoniae* restricted considerably the available therapeutic arsenal in the treatment of these severe infections. Study for monitoring antimicrobial resistance trends (SMART) reported an increase in worldwide prevalence of ESBL-producing *K. pneumoniae*, between 2005 and 2007, from 19.2 to 30% [3]. Molecular strain typing procedures are becoming widely established as part of nosocomial infection control programs. These tools were used to recognize clusters and to establish clonal relationships among ESBL-producing *K. pneumoniae* isolates in order to clearly understand the local and global spread of this resistance. Pulsed field gel electrophoresis (PFGE) is the most widely used tool for molecular typing of bacterial strains [4]. New combined typing systems based on genomic sequencing such as multilocus sequence typing (MLST) can provide comprehensive and phylogenetically relevant information on multiresistant clones. In addition, the MLST analysis is highly discriminatory and universal method providing unambiguous and portable data between laboratories via the Internet [5].

The aim of this study was to compare the discriminatory ability and potential utility of PFGE and MLST for the genetic characterization of ESBL-producing *K. pneumoniae* isolates. For this purpose, the 23 isolates analyzed previously by MLST (figure 1) [6] and representing each specific clone were selected for PFGE analysis. In addition, five other isolates among the remaining isolates previously described [6] were selected for MLST and PFGE analysis because there was a point of similarity in the restriction plasmid profile or ESBL type or epidemiology (association with the other isolates in the paediatric unit or ICU) or to allow representation of isolates from all years. Genome fingerprinting by PFGE using enzyme XbaI was performed.
as previously described [7]. The banding patterns obtained were compared by Gel-Pro Analyzer version 3.1 and a dendrogram was constructed using the UPGMA algorithm and Dice similarity coefficient (XLSTAT, version 2011). PFGE profiles were compared using the criteria established by Tenover et al. [8] and pulsotypes were assigned to clusters of isolates with greater or equal to 85% similarity from the dendrogram. Among the 28 isolates studied here, 23 were subjected to MLST analysis in our previous study (figure 1) [6]. In this study, we performed MLST analysis for the remaining five isolates (figure 1) as described previously [5]. Allele sequences and profiles are available at http://www.pasteur.fr/mlst.

The XbaI digestion of the 28 ESBL-producing isolates identified 22 unique patterns (X1-X22). When comparing genetic relatedness, defined as isolates sharing greater or equal to 85% of the bands, PFGE classified 13 CTX-M-15-producing isolates into four clusters of two or more denoted A, B, E and F (figure 1), and two sporadic cases (isolates 46, 49). The 2 CTX-M-27-producing isolates were clustered in the same group G. The 3 SHV-2a-producing isolates were classified into a cluster of two-noted C and one sporadic case (isolate 30). PFGE showed diversity of the SHV-12 producing isolates (three clusters D, F, G and 10 sporadic cases).

The isolates 30, 32, 41, 45 and 48 analyzed by MLST were assigned respectively to sequence types ST36 (not detected in our previous study [6]), ST25, ST133 and ST147. MLST analysis showed the dissemination of a ST25 CTX-M-27-producing K. pneumoniae clone in Pediatric unit in 2002 and confirm a clonal relationship of isolates 31 and 32 which PstI digestion of plasmid DNA from transconjugants containing bla\text{CTX-M-27} yielded an identical restriction profile [6]. MLST analysis showed also the extraordinary capacity of ST147 CTX-M-15-producing K. pneumoniae clone to disseminate because it is detected not only in Pediatric and Intensive care Units but also in Surgery (isolate 48). Furthermore, this specific clone can persist for years in those units (figure 1).

Our study showed correlation between PFGE and MLST results. Indeed, isolates sharing greater or equal to 85% of the bands and classified in the same cluster were assigned to the same sequence type. However, PFGE showed a higher discriminatory ability than MLST when typing K. pneumoniae as described by other authors [9]. In all
cases but two, the same ST contained strains with distinct (albeit related banding) patterns. The specific CTX-M-15-producing clone ST147 showed two pulsotypes (X1a and X1b, cluster A), which were one band different. On the other hand, specific CTX-M-15-producing clone ST101 showed two pulsotypes (X15 and X16, cluster E), which were four bands different. The specific clone ST107, which co-produced CTX-M-15 and SHV-12 showed two PFGE types (X19 and X20, cluster F) which were four bands different. The specific clone ST25, which co-produced CTX-M-27 and SHV-12 showed two PFGE types (X20 and X21, cluster G) which were four bands different. On the other hand, the specific SHV-12-producing clone ST309 showed two pulsotypes (X13 and X14, cluster D), which were four bands different. The specific SHV-2a-producing clone ST322 showed two pulsotypes (X8a and X8b, cluster C), which were two bands different. Our PFGE data provides evidence of microevolution of the specific ESBL-producing clones ST147, ST101, ST107, ST25, ST309 and ST322 probably due to DNA rearrangements and the observation of multiple PFGE types within a ST suggests that insertions and deletions of large blocks of DNA are more common in ESBL-producing \textit{K. pneumoniae} isolates than either point mutations or homologous recombination in MLST genes. Some authors proposed including of antibiotic resistance genes such as SHV or TEM in MLST scheme that increased the discriminatory ability of MLST in comparing ESBL-producing isolates \cite{10}. This increased discrimination, as explained by those authors, may reflect horizontal transfer of plasmid-borne resistance genes within a conserved genetic background or more rapid change within TEM and SHV due to selective pressure.

In two cases, the same ST contained strains with unrelated PFGE types (< 85% similarity from the dendrogram): specific ESBL-producing clones ST133 (X2, X7) and ST321 (X4, X5 and X6). These results showed that MLST was better than PFGE for detecting genetic relatedness and suggested a great diversity within strains of clone ST133 and ST321 as previously described for \textit{Escherichia coli} strains belonging to ST131 clone \cite{11} and the introduction into our hospital of different \textit{K. pneumoniae} strains belonging to clones ST133 and ST321.

In summary, our study showed that PFGE has higher discriminatory ability than MLST but showed also that MLST is more efficient to detect the dissemination of the specific clones. MLST and PFGE proved to be complementary methods in analyzing epidemiology of CTX-M or SHV-producing \textit{K. pneumoniae} isolates. We suggest performing PFGE analysis to define clusters of ESBL-producing \textit{K. pneumoniae} isolates then performing MLST analysis for representative of each cluster to identify specific clones.

**Conflicts of interests:** none.

**References**


