Could the Diversilab® semi-automated repetitive-sequence-based PCR be an acceptable technique for typing isolates of *Pseudomonas aeruginosa*? An answer from our experience and a review of the literature

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Could the Diversilab® semi-automated repetitive-sequence-based PCR be an acceptable technique for typing isolates of *Pseudomonas aeruginosa*? An answer from our experience and a review of the literature

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e-mail: florence.brossier@psl.aphp.fr
ABSTRACT

Recently the DiversiLab® (DL) system (bioMérieux) was developed as an automated platform that uses rep-PCR technology for standardized, reproducible DNA fingerprinting of bacteria. The purpose of this study was to evaluate the usefulness of DL rep-PCR for typing of *Pseudomonas aeruginosa* isolates. The performance of DL rep-PCR was compared with that of pulsed-field gel electrophoresis (PFGE) in a prospective multicenter study of patients with ventilator-associated pneumonia due to *P. aeruginosa* conducted in 3 intensive care units during a 31-month period. In total, 203 *P. aeruginosa* isolates from 66 patients, from whom at least 2 consecutive respiratory samples each were collected more than 48 h apart, were typed using DL rep-PCR; 40 isolates (corresponding to 20 patients) were also typed using PFGE of *Spe*I-digested DNA. The typeability was 100% with DL rep-PCR and 95% with PFGE. The discriminatory power was close for DL rep-PCR and for PFGE (Simpson’s index of diversity of 0.901 and 0.947, respectively). An insufficient agreement between DL rep-PCR and PFGE typing results was observed for the 40 selected isolates (adjusted Rand coefficient of 0.419), due mostly to isolates of the same DL rep-PCR type but of different PFGE types (adjusted Wallace coefficients of DL rep-PCR with PFGE of 0.306, and of PFGE with DL rep-PCR of 0.667). Considered together with published data, DL rep-PCR results should be interpreted with caution for the investigation of outbreaks caused by *P. aeruginosa*, and evaluated in conjunction with epidemiological data.

KEYWORDS

*Pseudomonas aeruginosa*, bacterial typing, rep-PCR, pulsed-field gel electrophoresis
INTRODUCTION

Among nosocomial infections, ventilator-associated pneumonia (VAP) is the most common complication in patients admitted to intensive care units (ICU) (Bergmans et al. 1998, Chastre 2002, Lambiase et al. 2009). Genotyping has been used to demonstrate that *P. aeruginosa* strains infecting ICU patients may originate from endogenous or exogenous sources (Bergmans et al. 1998, Blanc et al. 2007, Bonten et al. 1999, Cuttellod et al. 2011, Deplano et al. 2005, Fujitani et al. 2011, Hota et al. 2009, Lambiase et al. 2009, Pujana et al. 2000, Speijer et al. 1999, Thuong et al. 2003, Trautmann et al. 2005). The intestines are considered the most important endogenous source of microorganisms reaching the respiratory tract via the gastro-pulmonary route, via colonization of the skin or via transiently colonized hands of health-care workers (Bonten et al. 1999), while the relevance of exogenous reservoirs (e.g. contaminated equipment) and the importance of cross-transmission has been convincingly documented during outbreaks (Bonten et al. 1999, Hota et al. 2009).

Genotyping methods for reliable distinction between bacterial strains or clones comprise pulsed-field gel electrophoresis (PFGE), PCR assays including repetitive-sequence-based PCR (rep-PCR), restriction endonuclease analysis, multilocus sequence typing (MLST), multiple-locus variable-number tandem-repeat analysis (MLVA), DNA sequencing, PCR ribotyping and randomly amplified polymorphic DNA analysis (Fluit et al. 2010, Healy et al. 2005, van Belkum et al. 2007). PFGE of restriction-digested genomic DNA is considered to be the “gold standard” method for typing of many bacterial species, including *P. aeruginosa* (van Belkum et al. 2007). It is a highly discriminative procedure and interpretative guidelines have been established, but it is labor-intensive and skill-dependent. In light of these disadvantages, faster and easier methods have been employed. The rep-PCR methods allow rapid typing of bacteria by amplifying regions between noncoding repetitive sequences in their genomes and generating strain-specific band patterns; however, they are notorious for their susceptibility to minor variations in experimental conditions and reagents, resulting in poor reproducibility (Healy et al. 2005, van Belkum et al. 2007). Recently, bioMérieux (Marcy l’Etoile, France) has developed the semi-automated DiversiLab® system (DL) for standardized, reproducible DNA fingerprinting of bacteria using rep-PCR (Deplano et al. 2011, Doléans-Jordheim et al. 2009, Fluit et al. 2010, Grisold et al. 2010, Healy et al. 2005, Ratkai et al. 2010) without the main disadvantages of previous PCR-based assays. It uses microfluidics, DL reagent-specific rep-PCR amplification kits and software for data processing as well as fully automated data analysis allowing determination of genetic relatedness among isolates.
Here, we intended to evaluate the performance of the DL rep-PCR system for *P. aeruginosa* typing (comparatively with PFGE) since only few reports addressing this specific aim have been published so far and more data are required to reach a consensus regarding the performance and usefulness of this approach (Deplano et al. 2011, Doléans-Jordheim et al. 2009, Fluit et al. 2010, Maâtallah et al. 2013, Ratkai et al. 2010).

**MATERIALS AND METHODS**

**Study population**

A prospective multicenter study of patients with *P. aeruginosa* VAP was conducted in 3 intensive care units during a 31-month period as described previously (Luyt et al. 2014).

**Bacterial strains**

*P. aeruginosa* isolates included in the study were consecutively collected from patients in 3 intensive care units who contracted VAP or experienced recurrence or microbiological relapse during a 31-month period as described previously (Luyt et al. 2014). Recurrence was defined as the reappearance of clinical and pathological signs of infection with significant concentrations of persistent *P. aeruginosa* in lower respiratory tract specimens at least 2 days (but no more than 28 days) after completion of antibiotic therapy during the first episode, whereas microbiological relapse was defined as significant concentrations of *P. aeruginosa* in lower respiratory tract specimens at least 2 days (but no more than 28 days) after completion of antibiotic therapy during the first episode without clinical and pathological signs of infection. These strains collected from pure, fresh cultures were stored at -80°C on cryobeads.

**Genotypic methods**

To compare the *Pseudomonas aeruginosa* isolates responsible for the first and second episodes in the patients with a VAP recurrence or microbiological relapse, DNA of all *P. aeruginosa* isolates included in this study (n=203; from 66 patients) were subjected to DL rep-PCR, and DNA of 40 randomly selected of these isolates (from 20 patients) were also subjected to PFGE-*SpeI* (Fig. 1). The results obtained with DL rep-PCR and PFGE of *SpeI*-restricted genomic DNA were compared.

Reproducibility of DL was evaluated with the 40 isolates typed by PFGE and DL rep-PCR. Duplicate DNA extracts (A and B) of each isolate were amplified in 2 PCR runs (P1 and P2) and analyzed on 2 chips (C1 and C2). In total, 3 DL rep-PCR patterns (A-P1-C1, A-P2-C1, and B-P2-C2) were evaluated per isolate.

To avoid misinterpretation due to PCR amplification errors in rep-PCR, duplicate DNA extracts (A and B) were performed for 40 isolates.
Semi-automated DNA fingerprinting using DL rep-PCR

Bacterial isolates were cultured on Mueller Hinton agar plates for 24 h at 37°C. Total bacterial DNA was extracted using the UltraClean™ Microbial DNA Isolation Kit (MO Bio Laboratories, Carlsbad, CA, USA) following the manufacturer’s instructions. DNA yields were estimated by NanoDrop™ (Thermo Fisher Scientific) quantification. Rep-PCR was performed using the Pseudomonas DNA fingerprinting kit (Bacterial Barcodes, bioMérieux, Athens, GA, USA). Briefly, 50 ng of genomic DNA, 2.5 U of AmpliTaq DNA polymerase, and 1.5 µl of 10× PCR buffer (Applied Biosystems, Foster City, Calif.) were added to the rep-PCR master mix to achieve a total of 25 µl. Thermal cycles included an initial denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s and extension at 70°C for 90 s, and a final extension at 70°C for 3 min. The DNA amplicons were separated using microfluidics chips (Bacterial Barcodes) and the products were detected with the Agilent 2100 BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Rep-PCR fingerprint profiles were compared using DiversiLab® (version 3.4) software and the Pearson correlation coefficient (Bacterial Barcodes). Automated reports included dendrograms, virtual gel images, similarity matrices and scatter plots. The cut-off value for similarity to establish strain identity was 95% for DL rep-PCR (Deplano et al. 2011, Doléans-Jordheim et al. 2009, Ratkai et al. 2010).

Pulsed-field gel electrophoresis of SpeI-digested DNA

PFGE was performed according to Deplano et al. (Deplano et al. 2005). The Dice correlation coefficient was used to assess the similarity of the banding patterns of SpeI-digested DNA. Clustering was based on the unweighed pair-group method with arithmetic averages (UPGMA). PFGE patterns were analyzed with the software package GelCompar (Applied Maths) and interpreted using the criteria of Tenover et al. (Tenover et al. 1995) as adapted by van Belkum et al. (van Belkum et al. 2007). Isolates were considered to be indistinguishable, closely related, possibly related, or unrelated by PFGE. “Indistinguishable” was defined as absence of band differences, “closely related” as 1-3 band differences, “possibly related” as 4-6 band differences, and “unrelated” as more than 6 band differences (Tenover et al. 1995). Clonally derived strains from a single host cluster at similarity levels above 80% (van Belkum et al. 2007).

Determination of typeability, reproducibility, discriminatory power and typing system concordance

Typeability, reproducibility, discriminatory power (evaluated using the Simpson’s index of diversity) and typing system concordance (evaluated using the adjusted Rand and Wallace coefficients) (Maâtallah et al. 2013) were calculated using software available online (http://www.comparingpartitions.info).

Compliance with ethical standards
No conflict of interest exists for any of the authors.

This study was conducted in accordance with the ethical standards of our institution’s Committee for the Protection of Human Research Subjects (CCP Île-de-France VI, Groupe Hospitalier Pitié-Salpêtrière, Paris). This Committee approved this study. In accordance with French law, no informed consent was obtained because this study did not modify existing diagnostic or therapeutic strategies. However, the patients or their relatives received an information sheet describing the study and explaining that they were free to withdraw from it at any time. Patient information was anonymized and de-identified prior to analysis.

**RESULTS**

**Comparative genotypic analysis of all isolates using Diversilab® technology**

From the 208 *P. aeruginosa* isolates included in our study, five were excluded since they could not be recovered from storage. The remaining 203 isolates from 66 patients (181 isolates from 56 patients in the “recurrence” group and 22 isolates from 10 patients in the “microbiological relapse” group) were included in this study and subjected to DL rep-PCR (Fig. 1). The average number of isolates was 3.1 (203/66) per patient.

Using a similarity cut-off of 95% (Deplano et al. 2011, Doléans-Jordheim et al. 2009, Fluit et al. 2010, Maâtallah et al. 2013), DL rep-PCR typing allowed differentiation of the 203 isolates into 59 DL types of which 18 were singletons or had unique patterns and 41 were clonally related with several isolates (Fig. 1). Among the latter, 11 DL profiles were associated with isolates from different patients, and 30 DL profiles with isolates from single patients (Fig. 1). DL rep-PCR typing showed that among 66 patients, 45 patients were infected with a single clone represented by 129 isolates (29, 8, 1, 3, 2, 1 and 1 patient(s) with respectively 2, 3, 4, 5, 6, 7 and 9 isolates; 24/45 patients with isolates of the same DL rep-PCR type as isolates from other patients), 9 patients (19 corresponding isolates) were infected with different isolates (8 patients with 2 isolates and 1 patient with 3 isolates), and 12 patients (55 corresponding isolates) were infected with a mixture of at least 2 isolates of different DL types (6, 1, 2, 1 and 2 patients with respectively 3, 4, 5, 7 and 8 isolates).

When comparing *P. aeruginosa* isolates responsible for the first and the second episode in the 66 patients who experienced recurrence or microbiological relapse, DL rep-PCR typing revealed that 68% (45/66) of the isolates were the same in both episodes, thereby establishing that, in one-third of these cases (21/66), recurrences or microbiological relapses were re-infections rather than relapses.

**Comparative genotypic analysis of 40 isolates using PFGE**

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179 **Comparative genotypic analysis of 40 isolates using PFGE**
The 40 isolates subjected to PFGE typing comprised 14 isolates of the same DL rep-PCR type (similarity index >95%) collected from a single patient, 5 isolates of different DL types (similarity index <95%) collected from a single patient and 21 isolates of identical DL type collected from several patients.

The typeability by PFGE was 95%. Two isolates from one patient (patient 15) were nontypeable by PFGE due to DNA degradation (despite addition of thiourea during electrophoresis); they could however be typed by DL rep-PCR (profile 8 in Fig. 2).

The PFGE-based dendrogram of the 38 isolates (collected from 19 patients) (Fig. 3) shows 16 different PFGE profiles, including 3 (D, F, O) that are subdivided into 2 subtypes (D1 and D2, F1 and F2 and O1 and O2: 2 subtypes of the same clone).

**Concordance between DL rep-PCR and PFGE**

Simpson’s index of diversity was close for DL rep-PCR and for PFGE (0.901 [95% CI: 0.846-0.963] and 0.947 [95% CI: 0.926-0.969], respectively). An index greater than 0.90 is considered desirable if the typing results are to be interpreted with confidence (Maâtallah et al. 2013).

Among the 38 isolates typeable by PFGE, 21 belonged to a DL type concordant with the PFGE type, 11 isolates belonged to the same DL type but had distinct PFGE profiles and 6 isolates belonged to different DL types but the same PFGE type. Among the 21 isolates of a DL type concordant with the PFGE type, isolates from patients 1, 2, 4, 5, 12, 17, 18, 19, 20, and 1 isolate from patient 16 had DL profiles with similarity >95% (Fig. 2) and related PFGE profiles (Fig. 3) (indistinguishable PFGE profiles were found for isolates from, respectively, patients 4 and 5, and patients 12 18 19 and 20; possibly related PFGE profiles for isolates from patients 1 and 2, with 4 band differences and similarity of 93%; closely related PFGE profiles for isolates from patients 16 and 17, with 2 band differences and similarity of 93%, the latter isolates belonging to subtypes of a single clone); the 2 isolates from patient 3 had different DL profiles (similarity, 75%) and different PFGE profiles (≥7 band differences; similarity, 42%; Fig. 3). Among the 11 isolates of the same DL type but with different PFGE profiles, isolates from patients 6, 7, 8, 9, 10 and 11 were of a unique DL type (Fig. 2) whereas their PFGE types were different (≥7 band difference, and from 38 to 62% of similarity, Fig. 3). Among the 6 isolates of different DL types but identical PFGE type, the 2 isolates from patient 13 and the 2 isolates from patient 14 showed similarity of 86% and 2 PFGE band differences (i.e. were closely related), and the 3 isolates from patient 16 had 3 distinct DL profiles (similarity, 90–93%; Fig. 2) but indistinguishable PFGE profiles (Fig. 3).

Overall, the adjusted Rand coefficient of 0.419 [95% CI: 0.096-0.700] revealed insufficient agreement between DL rep-PCR and PFGE typing. The adjusted Wallace coefficient for the concordance of DL rep-PCR with PFGE
of 0.306 [95% CI: 0.224-0.387] (i.e. the probability of 31% that a pair of isolates of the same DL rep-PCR type was also of the same PFGE type) and the adjusted Wallace coefficient for the concordance of PFGE with DL rep-PCR of 0.667 [95% CI: 0.515-0.818] (i.e. the probability of 67% that a pair of isolates of the same PFGE type was also of the same DL rep-PCR type) showed that PFGE was more predictive of DL rep-PCR results than was DL rep-PCR of PFGE results.

DISCUSSION

While whole-genome sequencing is performed increasingly in microbiological laboratories also for genotyping studies, many have opted for the DL system. One of the main problems in evaluating a genotyping method, including DL rep-PCR, is the definition of a suitable cut-off value. For PFGE, a cut-off may be defined (at least partially) by applying the so-called Tenover (1995) criteria as adapted by van Belkum et al. (2007). For DL rep-PCR, reported cut-off values vary from 93% to 99%, depending on the bacteria and on the authors (Deplano et al. 2011, Doléans-Jordheim et al. 2009, Fluit et al. 2010, Grisold et al. 2010, Healy et al. 2005, Maâtallah et al. 2013). In the present study, we chose a 95% cut-off since this is the most frequently used value (Deplano et al. 2011, Doléans-Jordheim et al. 2009, Fluit et al. 2010, Maâtallah et al. 2013). Overall, our results showed insufficient agreement between the DL rep-PCR and PFGE results (Table 1). The two methods tested here make use of different strategies. The rep repeats are more inclined to be directly involved in recombination events. However, SpeI analysis of restricted genomic DNA are also affected by recombinational events, but on a larger scale. So the disagreements between the PFGE and rep-PCR analyses might be the true situation. However insufficient agreement between the DL rep-PCR and PFGE results is partly due to the variability in DL rep-PCR reproducibility. Reproducibility was found to be higher when duplicate DNA extracts were amplified in the same PCR (100% (40/40); similarity > 98%), than when they were amplified in different PCRs (95% (38/40); similarity > 95%). On the other hand, the change in the chips used for analysis did not change DL rep-PCR reproducibility. Overall, DL rep-PCR reproducibility was 95% since the patterns produced by 2 isolate replicates showed similarity superior to the cut-off value determined for type assignment for 38/40 isolates. Although DL technology uses a semi-automated and highly standardized procedure that reduces variations due to technical problems, optimal reproducibility was probably not obtained because PCRs were not performed simultaneously. The discrepancies between DL rep-PCR and PFGE results were observed among isolates with identical DL profiles but different PFGE profiles (n=11), or, conversely, among isolates with different DL profiles but same PFGE profiles (n=6). The 11 P. aeruginosa isolates of the first category were collected from patients 6, 7, 8, 9, 10
and 11 who were hospitalized in 3 different ICUs at different times. The PFGE profiles were identical when the
isolates were collected from the same patient and different when collected from different patients. It is very
unlikely that these isolates belonged to the same clone, so large DL rep-PCR clusters do not necessarily imply
large outbreaks or closely related isolates, as shown by PFGE analysis. The limited variation in the DL patterns
obtained, due to the limited number of sites amplified, can be responsible of the poor performance of DL for these
isolates. Therefore, DL rep-PCR results should be analyzed taking into account epidemiological data and identical
DL types should be confirmed by PFGE analysis. The 6 isolates corresponding to the second category with
different DL but identical PFGE profiles (4 isolates from patients 13 and 14 and 2 isolates from patient 16; Figs. 2
and 3) were retested using DL rep-PCR. The 3 isolates from patient 16 (including 1 that was part of the 21
isolates whose DL type was concordant with the PFGE type) which initially showed 3 different DL profiles (with
similarities between 90 and 93%; Fig. 2,) were assigned the same DL profile after DL was performed a second
time. These results are in agreement with clinical observations, i.e. recurrence of VAP. On the other hand, the
isolates from patients 13 and 14 with closely related PFGE profiles (classified as subtypes of the same clone with
86% similarity and a 2 band difference) had different DL types as verified by repeat DL rep-PCR. This is possibly
because PFGE fragments are cut by a rare base cutter, so if there are multiple base deletions/additions in a highly
variable region it may lead to a fragment being cut again, or being longer or shorter by enough bases to affect the
position on the gel and thus its profile. Since these patients were hospitalized one year apart in different ICUs, the
DL results seemed more plausible than the PFGE results. Superiority of DL rep-PCR over PFGE has been
reported once, the authors arguing that these differences may reflect genetic drift among closely related isolates
(Doléans-Jordheim et al. 2009). Altogether, when the DL rep-PCR and PFGE profiles were analyzed taking into
account, epidemiological data such as type of hospital ward and date of hospitalization, DL appeared to be a
moderately reliable tool for investigating a hospital outbreak. We did not extend the scope of our work to study
where the DL rep-PCR method was failing to be predictive of the PFGE result, therefore none of the isolates
included in our study were sequenced.

Although the agreement between both techniques was insufficient, our results showing that identical isolates were
recovered from different patients strongly suggest that infection with *P. aeruginosa* was caused by cross-
acquisition and/or due to a common exogenous source even in non-epidemic settings as reported previously
(Thuong et al. 2003).

We also confirmed that VAP recurrences were mostly (68%) due to the same strain that caused the first episode,
rather than to the acquisition of a new strain (Rello et al. 1998).
The DL system has been applied to the characterization of many bacterial species, it was evaluated for its performance and feasibility of identification of hospital outbreaks, and it has been shown to be useful for some species like *K. pneumoniae* but less so for others like *P. aeruginosa* (Deplano et al. 2011, Fluit et al. 2010). However, available data regarding DL applied to *P. aeruginosa* are contradictory (Table 1). Some authors concluded that rep-PCR DL is a powerful technique applicable to epidemics, yielding similar results as PFGE (Doléans-Jordheim et al. 2009, Ratkai et al. 2010), or less discriminating than PFGE for strains of *P. aeruginosa* (Deplano 2011), whereas others concluded that DL is inadequate for typing of *P. aeruginosa* (Fluit et al. 2010).

In the present study, DL rep-PCR had the advantage of allowing the typing of *P. aeruginosa* isolates nontypeable by PFGE and the disadvantage of yielding insufficient agreement with PFGE typing as shown by poor adjusted Rand and Wallace coefficients. Despite the high Simpson index of diversity, DL rep-PCR results should be interpreted with caution for the investigation of outbreaks caused by *P. aeruginosa*, and evaluated in conjunction with epidemiological data.

**ACKNOWLEDGMENTS**

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**REFERENCES**


Table 1 Diversilab® rep-PCR results obtained in the present study or published in the literature

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<td>203</td>
<td>133</td>
<td>13</td>
<td>52</td>
<td>41</td>
<td>34</td>
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<td>100</td>
<td>100</td>
<td>100</td>
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<td>Na</td>
<td>Na</td>
<td>100</td>
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<td>nd</td>
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<td>nd</td>
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<tr>
<td>DL</td>
<td>0.901</td>
<td>0.961</td>
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<td>0.977(0.958-0.997)</td>
<td>0.974(0.934-0.100)</td>
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<td>0.989</td>
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<td>MLST&lt;sup&gt;a&lt;/sup&gt;</td>
<td>na</td>
<td>0.906</td>
<td>na</td>
<td>0.928(0.828-0.989)</td>
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<td>MLVA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>na</td>
<td>0.980</td>
<td>0.985(0.949-1.0)</td>
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<td>(Adjusted) Wallace index</td>
<td></td>
<td></td>
<td></td>
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<td>PFGE to predict DL</td>
<td>0.667(0.515-0.818)</td>
<td>0.617 (0.530-0.704)</td>
<td>0.333&lt;sup&gt;c&lt;/sup&gt;</td>
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<td>na</td>
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<tr>
<td>DL to predict PFGE</td>
<td>0.306(0.224-0.387)</td>
<td>0.166 (0.096-0.237)</td>
<td>1.0&lt;sup&gt;c&lt;/sup&gt;</td>
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<tr>
<td>DL to predict MLST</td>
<td>na</td>
<td>0.642(0.547-0.737)</td>
<td>na</td>
<td>0.182&lt;sup&gt;c&lt;/sup&gt;</td>
<td>na</td>
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<tr>
<td>MLST to predict DL</td>
<td>na</td>
<td>0.250(0.167-0.333)</td>
<td>na</td>
<td>0.333&lt;sup&gt;c&lt;/sup&gt;</td>
<td>na</td>
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<tr>
<td>DL to predict MLVA</td>
<td>na</td>
<td>0.242 (0.137-0.347)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
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<tr>
<td>MLVA to predict DL</td>
<td>na</td>
<td>0.486 (0.342-0.629)</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
<td>na</td>
</tr>
<tr>
<td>Adjusted Rand coefficient</td>
<td>0.419(0.096-0.700)</td>
<td>not done</td>
<td>0.488</td>
<td>0.212</td>
<td>0.879&lt;sup&gt;d&lt;/sup&gt; (DL-PFGE)</td>
<td>na</td>
<td>nd</td>
</tr>
</tbody>
</table>

<sup>a</sup> na: not applicable; nd: not done

<sup>b</sup> MLST: Multi Locus Sequence Typing; MLVA: Multiple-Locus Variable number tandem repeat Analysis

<sup>c</sup> Wallace index not adjusted

<sup>d</sup> Not the adjusted Rand coefficient, but proportion of pairs for which both methods are in agreement calculated according to another method
FIGURE LEGENDS

Fig. 1 Flow chart depicting the Diversilab® rep-PCR analysis of 208 *Pseudomonas aeruginosa* isolates

Fig. 2 Diversilab® rep-PCR-based dendrogram of the 40 *Pseudomonas aeruginosa* isolates from 20 patients also analyzed by PFGE. Produced by Pearson analysis of the rep-PCR patterns and unweighted pair-group method with arithmetic averages. Percent similarities between isolates are shown.

Fig. 3 PFGE-based dendrogram of the 40 *Pseudomonas aeruginosa* isolates from 20 patients also studied by Diversilab® rep-PCR. Produced by Dice analysis of the PFGE patterns and unweighted pair-group method with arithmetic averages. Percent similarities between isolates are shown.
208 isolates

5 strains not recovered

203 isolates included in the study

59 DL types

18 DL-types singletons or unique patterns (18 isolates corresponding to 13 patients)

41 DL-types containing ≥ 2 isolates (185 isolates corresponding to 62 patients)

11 DL-types containing isolates from different patients (107 isolates corresponding to 38 patients)

30 DL-types containing isolates from a single patient (78 isolates corresponding to 30 patients)

173x146mm (300 x 300 DPI)
172x230mm (300 x 300 DPI)
PFGE profile

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<td>A</td>
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<td>C</td>
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<td>D2</td>
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DL profile  ICU

4  CR  4  CR  4  HM  4  HM  4  HM  6  CR  6  CR  7  GC  7  GC  14  CR  14  CR  9  HM  9  HM  9  GC  10  GC  11  GC  11  GC  15  CR  3  GC  3  GC  3  GC  3  GC  5  GC  5  GC  4  CR  4  CR  4  CR  nontypeable  HM  nontypeable  HM  4  HM  13  HM  13  HM  7  CR  1  GC  1  GC  1  GC  1  GC  4  GC  4  GC

140x159mm (300 x 300 DPI)