JOURNAL OF CLINICAL MICROBIOLOGY, Oct. 2011, p. 3616–3620 Vol. 49, No. 10
0095-1137/11/$12.00 doi:10.1128/JCM.00528-11

Controlled Performance Evaluation of the DiversiLab Repetitive-Sequence-Based Genotyping System for Typing Multidrug-Resistant Health Care-Associated Bacterial Pathogens▽

Ariane Deplano,* Olivier Denis, Hector Rodriguez-Villalobos,† Raf De Ryck, Marc J. Struelens,‡ and Marie Hallin

Microbiology Laboratory, Hôpital Erasme—Université Libre de Bruxelles (ULB), Brussels, Belgium

Received 16 March 2011/Returned for modification 26 May 2011/Accepted 26 July 2011

Fast, reliable, and versatile typing tools are essential to differentiate among related bacterial strains for epidemiological investigation and surveillance of health care-associated infection with multidrug-resistant (MDR) pathogens. The DiversiLab (DL) system is a semiautomated repetitive-sequence-based PCR system designed for rapid genotyping. The DL system performance was assessed by comparing its reproducibility, typeability, discriminatory power, and concordance with those of pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) and by assessing its epidemiological concordance on well-characterized MDR bacterial strains ($n = 165$). These included *vanA Enterococcus faecium*, extended-spectrum β-lactamase (ESBL)-producing strains of *Klebsiella pneumoniae*, *Escherichia coli*, and *Acinetobacter baumannii*, and ESBL- or metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa* strains. The DL system showed very good performance for *E. faecium* and *K. pneumoniae* and good performance for other species, except for a discrimination index of <95% for *A. baumannii* and *E. coli* (93.9% and 93.5%, respectively) and incomplete concordance with MLST for *P. aeruginosa* (78.6%) and *E. coli* (97.0%). Occasional violations of MLST assignment by DL types were noted for *E. coli*. Complete epidemiological concordance was observed for all pathogens, as all outbreak-associated strains clustered in identical DL types that were distinct from those of unrelated strains. In conclusion, the DL system showed good to excellent performance, making it a reliable typing tool for investigation of outbreaks caused by study pathogens, even though it was generally less discriminating than PFGE analysis. For *E. coli* and *P. aeruginosa*, MLST cannot be reliably inferred from DL type due to phylogenetic group violation or discordance.

Rapid assessment of microbial clonal relationships enables the tracking of the spread of multidrug-resistant (MDR) pathogens and guides transmission control measures in the hospital setting. The ideal typing method should be rapid, easy to perform, high throughput, and applicable to a wide range of microorganisms and should meet performance criteria, such as full typeability, reproducibility, high discriminatory power, and concordance with validated typing methods as well as consistency with underlying subspecies genetic population structures (29, 31). In the hospital setting, identification of epidemic strains is usually based on fingerprinting methods, such as pulsed-field gel electrophoresis (PFGE), a labor-intensive method that requires 3 to 4 days to produce results. The DiversiLab (DL) system (bioMérieux, Marcy l'Etoile, France) is a repetitive-sequence-based PCR (rep-PCR) technology which offers semiautomated, easy-to-use, high-throughput, and rapid bacterial strain typing. It could be a suitable alternative to PFGE analysis for outbreak investigation of health care-associated pathogens. This method was recently validated for typing of *Acinetobacter* spp., *Escherichia coli*, and *Klebsiella* spp., but it was found insufficiently discriminative for typing of *Staphylococcus aureus* (12).

The purpose of this study was to assess the potential usefulness of the DL system for typing the most recovered bacterial pathogens causing hospital-associated outbreaks around the world. This was achieved by evaluating and comparing its performances with current standard typing methods, including PFGE and multilocus sequence typing (MLST), in terms of epidemiological concordance using a collection of well-characterized outbreak-related and epidemiologically unrelated MDR strains of *Enterococcus faecium*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *E. coli.*

MATERIALS AND METHODS

Bacterial strains. A total of 165 strains of MDR *E. faecium*, *A. baumannii*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli* of clinical origin were selected from national or local epidemiological surveys and outbreak investigations (Table 1). Each species set included epidemiologically unrelated strains showing distinct PFGE profiles and sets of well-documented outbreak-related strains showing similar/identical PFGE profiles. All strains were code-labeled before being tested and analyzed by observers who were blind to their reference type characteristics and epidemiological origins.

The vancomycin-resistant *E. faecium* (VREF) collection included 2 sets of

* Corresponding author. Mailing address: Microbiology Laboratory, Hôpital Erasme—Université Libre de Bruxelles (ULB), Route de Lennik 808, 1070 Brussels, Belgium. Phone: 32 2 555 69 71. Fax: 32 2 555 31 10. E-mail: ariane.deplano@ulb.ac.be.

† Present address: Microbiology Laboratory, Cliniques Universitaires Saint Luc—Université Catholique de Louvain (UCL), Brussels, Belgium.

‡ Present address: Scientific Advice Unit, European Centre for Disease Prevention and Control, Stockholm, Sweden.

▽ Published ahead of print on 3 August 2011.

TABLE 1. Origins and characteristics of MDR bacterial collections[a]

Species	Study reference no.	Origin/resistance mechanism	No. of strains	PFGE type	MLST type (no. of strains)	DL type(s) (no. of strains)
E. faecium	8	Outbreak related/*van*A	8	A	ST16 (1)	1 (8)
	8	Unrelated/*van*A	5	Singleton	ND	3 (1), singleton (4)
	This study	Unrelated/*van*B	4	Singleton	ND	3 (1), singleton (3)
K. pneumoniae	20	Outbreak related/CTX-M-15	6	A	ST15 (1)	9 (6)
	20	Outbreak related/CTX-M-15	12	B	ST11 (1)	1 (12)
	27	National survey/ESBL	2	A	ND	9 (2)
	27	National survey/ESBL	2	C	ND	10 (2)
	27	National survey/ESBL	2	O	ND	6 (1), 7 (1)
	27	National survey/ESBL	2	ZB	ND	7 (2)
	27	National survey/ESBL	8	Singleton	ND	6 (1), singleton (7)
A baumannii	3	Outbreak related/OXA-23	4	39	ST109 (1)	3 (4)
	1	Belgian hospitals/OXA-23 and OXA-58	5	10	ST98 (1)	5 (1)
	1	Belgian hospitals/OXA-23 and OXA-58	3	12	ND	6 (3), 8 (1)
	1	Belgian hospitals/OXA-23 and OXA-58	4	42	ST109 (1)	3 (3)
	1	Belgian hospitals/OXA-23 and OXA-58	6	44	ST20 (1)	1 (4)
	1	Belgian hospitals/OXA-23 and OXA-58	4	Singleton	ND	5 (6)
	1	Belgian hospitals/OXA-23 and OXA-58			ST75 (1)	Singleton (1)
	1	Belgian hospitals/OXA-23 and OXA-58			ND	8 (1), singleton (2)
	4	Belgian hospitals/GES 11 and GES 12	4	49	ND	3 (4)
	4	Belgian hospitals/GES 11 and GES 12	1	39	ND	3 (1)
	4	Belgian hospitals/GES 11 and GES 12	2	Singleton	ND	Singleton (2)
E. coli	28	University hospital/CTX-M	2	6	ND	6 (2)
	28	University hospital/CTX-M	2	20	ND	Singleton (2)
	28	University hospital/CTX-M	2	21	ST34 (1)	10 (1)
	28	University hospital/CTX-M	2	23	ND	Singleton (1)
	28	University hospital/CTX-M	1	46	ST117 (1)	Singleton (1)
	28	University hospital/CTX-M	11	Singleton	ND	10 (1)
	28	University hospital/CTX-M			ND	10 (1)
	28	University hospital/CTX-M			ST2028 (1)	8 (1)
	28	University hospital/CTX-M			ST448 (1)	8 (1)
	28	University hospital/CTX-M			ST88 (1)	12 (1)
	28	University hospital/CTX-M			ND	10 (2), 12 (2), 6 (1), singleton (3)
	26	National survey/ESBL	13	6	ST131 (2)	6 (2)
	26	National survey/ESBL	1	46	ND	6 (9), 3 (1), 14 (1)
	26	National survey/ESBL	6	Singleton	ST167 (1)	10 (1)
	26	National survey/ESBL			ST88 (2)	12 (2)
	26	National survey/ESBL			ST540 (1)	Singleton (1)
	26	National survey/ESBL			ST501 (1)	3 (1)
	26	National survey/ESBL			ND	14 (1), singleton (1)
P. aeruginosa	9	Outbreak related/ MexXY efflux, decrease of OprD, overexpression of AmpC	4	A	ST235 (1)	1 (4)
	10	Belgian hospitals/VIM	5	Q	ST111 (1)	3 (5)
	10	Belgian hospitals/VIM	6	X	ST111 (1)	3 (6)
	10	Belgian hospitals/VIM	2	WW	ND	5 (2)
	10	Belgian hospitals/VIM	2	ZP	ST111 (1)	3 (2)
	10	Belgian hospitals/VIM	12	Singleton	ST234 (1)	Singleton (10), 4 (2)
	2, 15	Belgian hospitals/BEL and/or PER	10	ZT	ST235 (3)	2 (1), 6 (1), 9 (1)
	2, 15	Belgian hospitals/BEL and/or PER			ND	1 (1), 2 (1), 6 (2), 9 (1), singleton (2)

[a] Singleton, type harbored by a single strain; ND, not done.

strains: (i) pan-resistant *vanA*-producing strains ($n = 8$) belonging to the so-called clonal complex 17 from an outbreak in the hematology unit of a university hospital (8) and (ii) *vanA*- or *vanB*-producing strains ($n = 9$) showing sporadic PFGE types and recovered from unrelated cases of colonization in different units and at different periods of time at the same university hospital. The extended-spectrum β-lactamase (ESBL)-producing *K. pneumoniae* collection included 2 sets of strains: (i) CTX-M-15-producing *K. pneumoniae* strains ($n = 18$) from an outbreak in the intensive care unit (ICU) of a university hospital (20) and (ii) strains collected in different hospitals in a national survey conducted in Belgium in 2006 ($n = 16$) (14). The *A. baumannii* collection included three sets of ESBL- or carbapenemase-producing strains: (i) outbreak-related OXA-23-producing strains ($n = 4$) (3), (ii) OXA-23- and OXA-58-producing strains collected in Belgian hospitals in 2007 and 2008 ($n = 22$) (1), and (iii) GES-producing strains collected in Belgian hospitals in 2008 and 2009 ($n = 7$) (4). The *E. coli* collection included two sets of CTX-M-15-producing strains: (i) strains collected in a university hospital between 2000 and 2003 ($n = 20$) (28) and (ii) epidemiologically unrelated strains collected during a Belgian national survey conducted in 2006 ($n = 20$) (26). The *P. aeruginosa* collection included three sets of multidrug-resistant strains: (i) strains from a clonal outbreak in the ICU of a university hospital ($n = 4$) (9), (ii) VIM metallo-β-lactamase (MBL)-producing strains collected from several Belgian hospitals from 2004 to 2008, including epidemic ($n = 15$) and sporadic PFGE types ($n = 12$) (10), and (iii) BEL-1 and other ESBL-producing strains ($n = 10$) collected in Belgian hospitals from 2006 to 2008 (2, 15).

DiversiLab typing. All bacterial strains were subcultured from cultures cryopreserved at −80°C onto 5% sheep blood agar medium for 24 to 48 h of incubation. Cultures were checked for purity and code-labeled for blind testing. The coded agar plates were then processed for genomic DNA extraction and preparation using the UltraClean microbial DNA isolation kit (bioMérieux). DNA quantification was performed with the ND-1000 spectrophotometer (NanoDrop, Wilmington, DE). rep-PCR amplification was performed with the designated DL fingerprinting kit for each species according to the manufacturer's instructions. rep-PCR products were detected and sized using microfluidic LabChips placed on an Agilent 2100 bioanalyzer (Agilent Technologies, Diegem, Belgium) according to the manufacturer's instructions.

Data analysis. After each run on the Agilent analyzer, DNA fragment patterns were automatically transferred to a secure DL website database and analyzed by the software using Pearson correlation coefficient pairwise pattern matching and

the unweighted pair group method with arithmetic mean (UPGMA) clustering algorithm.

Typing system evaluation. ESGEM (European Study Group on Epidemiological Markers) criteria for evaluating performance of the DiversiLab typing system per species were used as previously described (29, 31).

(i) Type assignment cutoff value. Type assignment cutoff values, defined as the number of band differences (similarity cutoff) admitted to consider the duplicate DL patterns as belonging to the same DL type, were determined for each species on duplicate DNA extracts of three strains tested in distinct PCR runs and chips. Other performance criteria were evaluated considering the identity cutoff and number of band differences determined during this step.

(ii) Typeability. Typeability (T) is defined as the proportion (%) of strains that are assigned a type by the typing system (29).

(iii) Reproducibility. Reproducibility (R), defined as the ability of a typing method to assign the same type to a strain tested on independent occasions, was evaluated for each species on one strain randomly selected among the collection. For each strain, duplicate DNA extracts (A and B) were amplified in 2 PCR runs (P1 and P2) and analyzed on 2 chips (C1 and C2). A total of 6 DL patterns (A-P1-C1, A-P1-C2, A-P2-C2, B-P1-C1, B-P1-C2, and B-P2-C2) were evaluated per strain.

(iv) Discriminatory power. The discriminatory power, defined as the average probability that the typing system will assign a different type to two unrelated strains, was evaluated on epidemiological unrelated strains and calculated using Simpson's index of diversity (D) (18). Ninety-five percent confidence intervals (CI) were determined for each species as described in reference 17.

(v) Concordance. Concordance between typing methods was calculated according to the method of Robinson et al. (25). Briefly, all possible pairs of strains are examined, and their types are classified as either identical or different by each of the two methods and then cross-classified in a two-by-two table. Concordance, expressed in percentage, corresponds to the proportion of pairs for which both methods are in agreement. Concordance with PFGE type and, when available, concordance with phylogenetic population structure as defined by MLST were calculated.

(vi) Epidemiological concordance. Epidemiological concordance was evaluated as the proportion of outbreak-related strains of each species correctly grouped in the same DiversiLab type (Table 1).

RESULTS

Type assignment. For all species, based on results of duplicate isolates, a cutoff band difference in DL patterns of ≤2 was set to consider them as belonging to the same DL type. According to species, this ≤2 band difference corresponded to a Pearson correlation coefficient of a DL pattern similarity cutoff value ranging between 94% for *E. faecium* and 97% for *A. baumannii*, with a 95% threshold for *K. pneumoniae*, *E. coli*, and *P. aeruginosa* (Table 2).

Typeability and reproducibility. A 100% typeability was observed for the entire bacterial collection (n = 165 samples). DL displayed patterns showing variable numbers of DNA fragments, ranging between 7 and 23, depending on the bacterial pathogen examined (Table 2). Complete (100%) reproducibility was observed for each species, as DL patterns produced by the 6 strain replicates showed similarity superior to the cutoff value determined for type assignment (Table 2). We observed that reproducibility was better for duplicate DNA extracts amplified into the same PCR run on the same or different chips than for those amplified in different PCR runs (data not shown).

Discriminatory power of DiversiLab. The D index, evaluated on the epidemiologically unrelated strain subsets within each species, was excellent (D > 95%; CI, 93 to 100%) for *E. faecium*, *K. pneumoniae*, and *P. aeruginosa* strains (Table 2). For *A. baumannii* strains, the D index was 93.9% (CI, 85.8 to 100%), due mainly to clustering of epidemiologically unrelated strains into the same DL type. Finally, a D index of 93.5% (CI,

TABLE 2. Performances of the DiversiLab typing system by bacterial species[a]

Species (no. of strains)	No. of DNA bands in DL patterns (range)	Cutoff similarity (%) for type assignment	% reproducibility (similarity value for duplicates)	No. of PFGE types/no. of DL types	D index (%) (no. of unrelated strains)	Confidence interval of D index (%)	Concordance with PFGE type (%)	Concordance with MLST type (%) (no. of strains)	Epidemiologic concordance (%) (no. of strains by outbreak)
E. faecium (17)	12–16	>94	>97.2	10/9	97.8 (10)	92.7–100	100	NA	100 (8)
K. pneumoniae (34)	10–18	>95	>95.2	13/12	98.7 (13)	95.6–100	99.3	NA	100 (18)
A. baumannii (33)	9–13	>97	>98	11/10	93.9 (12)	85.8–100	88.4	100 (5)	100 (4)
E. coli (40)	15–23	>95	>98.3	22/15	93.5 (22)	87.4–99.6	91.0	97 (12)	NA
P. aeruginosa (41)	7–14	>95	>99	18/19	97.4 (18)	93.4–100	87.9	78.6 (8)	100 (4)

[a] NA, not analyzed.

87.4 to 99.6%) was observed for *E. coli* due to the lower number of DL types ($n = 15$) than PFGE types ($n = 22$) (Table 2).

Concordance of DiversiLab with PFGE and MLST. Excellent concordance was observed between DL, PFGE, and MLST for *E. faecium* and *K. pneumoniae* (Table 2). For *E. coli*, DL showed a lower concordance with PFGE. Discrepancies between the two methods were principally due to strains classified as singletons by one method but presenting the same type as other strains by the other method (Table 1). Discrepancies were also observed with strains belonging to two DL types (8 and 10) but to 4 different PFGE and MLST types (ST448, ST2028, ST167, and ST34). These violations of MLST assignment by DL led to a concordance of 97% between the two methods (Table 2).

Regarding *A. baumannii*, DL showed 88.4% concordance with PFGE. This was due to the fact that strains belonging to three PFGE types (12, 39, and 49) were indistinguishable by DL typing (Table 1). MLST analysis of representative strains belonging to PFGE types 12 and 39 grouped them in ST109, leading to a 100% concordance of DL with MLST for *A. baumannii* (Tables 1 and 2).

For *P. aeruginosa*, concordance between DL and PFGE was 87.9%, because (i) 10 strains belonging to the same PFGE type (ZT) were distributed into six DL types and (ii) 9 strains belonging to PFGE types Q, X, and ZP were classified into the same DL type (3) (Table 1). VIM-producing *P. aeruginosa* strains of PFGE types Q, X, and ZP were classified into ST111 by MLST (Table 1). MLST analysis of three strains belonging to PFGE type ZT but to three DL types revealed that they all belong to ST235. The classification of these three ST235 *P. aeruginosa* strains into distinct DL types reduced the concordance value of DL and MLST analysis to 78.6% (Table 2).

Epidemiological concordance. This evaluation was performed for each species, except *E. coli*, on sets of 4 to 18 outbreak-associated strains displaying identical PFGE types (Tables 1 and 2). Complete (100%) epidemiological concordance was observed for all species, as all outbreak-related strains displayed an identical DL type that was distinct from those observed for the majority (96.5%) of outbreak-unrelated strains (Tables 1 and 2).

DISCUSSION

Epidemiological (sub-) typing tools are essential to rapidly trace the spread of hospital-associated pathogens, especially MDR organisms that pose major therapeutic problems. Currently, PFGE analysis remains a widely used method thanks to its excellent discriminatory power, good reproducibility, and applicability to a wide range of bacterial pathogens. However, PFGE typing is time-consuming and requires advanced technical skills. Furthermore, it is not a definitive typing method that easily allows for the exchange of data. On the other hand, MLST analysis is an electronically portable, universal, and definitive bacterial typing method. Nevertheless, MLST is too labor-intensive, costly, and insufficiently discriminating for routine use in outbreak investigations and local surveillance. Another increasingly used method is multilocus variable-number tandem repeat analysis (MLVA). This PCR-based typing method amplifies variable-number tandem repeat (VNTR) regions from bacterial genomes. MLVA typing schemes have been developed for a number of health care-associated pathogens and, in general, correlate well with other genotypic methods (22, 30). Their major drawback is that the evolution of repeats may in some cases be too rapid, thereby compromising epidemiological concordance. In the present study, we evaluated another technology, the analysis of noncoding repeat DNA elements by rep-PCR genome fingerprinting. The DL system is a rapid and semiautomated rep-PCR commercial system that uses standard PCR kits and high-resolution amplicon detection by microfluidic capillary electrophoresis. Its discriminatory power and concordance with other typing methods have been previously evaluated for a number of bacterial pathogens (5, 7, 11, 13, 16, 19, 21, 23, 24). A recent study analyzed its usefulness in identifying hospital outbreaks caused by different bacterial pathogens (12). In these studies, the DL system has shown a variable reliability, depending on the species tested. Therefore, it requires a validation step for each bacterial species prior to its use in a routine clinical laboratory.

The aim of this study was to assess the reliability and usefulness of the DL system for typing pathogens commonly causing nosocomial outbreaks. Our major goal was to assess its epidemiological concordance using epidemiologically well-characterized strain collections of outbreak-related and unrelated MDR pathogens that are highly prevalent in our hospitals: VREF and ESBL- or carbapenemase-producing *K. pneumoniae*, *A. baumannii*, *E. coli*, and *P. aeruginosa* strains. The first step of this evaluation was to determine, for each species, the suitable cutoff similarity level to consider DL patterns as belonging to the same DL type. We observed a variation of up to 2 DNA bands between patterns of duplicate strains, corresponding to a cutoff similarity value range between 94 and 97% according to species. In a previous study, Fluit et al. (12) have set a higher similarity cutoff value (>98% for Gram-negative bacteria and >99% for Gram-positive bacteria). In our experience, these cutoff values were too high for the reproducibility observed with the DL system. These interlaboratory discrepancies in the levels of reproducibility, together with the lack of species-specific similarity cutoff values recommended by the manufacturer, underline the need for each user to validate the DL protocol for each species and to set its type similarity cutoff values prior to routine use.

In a second step, the DL typing system performance criteria of DL, including typeability, reproducibility, discriminatory power, and concordance with PFGE and MLST analysis, were measured for each pathogen. Excellent performance values were found with VREF strains in accordance with previous data showing that DL could effectively detect VREF outbreaks in spite of lower discrimination than PFGE analysis (7). However, other authors described limited usefulness of DL compared with MLVA to identify hospital outbreak-associated *E. faecium* strains (12). For *K. pneumoniae*, excellent performance was noted for all criteria, thereby supporting the conclusion of previous studies that DL is a useful tool for *K. pneumoniae* outbreak investigation (12, 16). For *A. baumannii* strains, DL was also found to perform equally well as PFGE analysis, confirming previous reports (13, 16). Regarding *E. coli*, DL presented good performance overall, except for occasional violations of MLST assignment. Our findings are in accordance with conclusions from a previous work that showed that DL is helpful for ruling out unrelated *E. coli* strains from

outbreak-related ones but needs confirmation by another technique (e.g., PFGE analysis) for reliable determination of outbreak-related strains (5). However, other authors found DL to be reliable for identification of uropathogenic O25:H4-ST131 or ST131-producing CTX-M-15 *E. coli* clones (19, 23). Regarding *P. aeruginosa*, DL was more discriminating than MLST by splitting ST235 strains into distinct DL types. However, the low concordance between DL and PFGE has to be pointed out, with PFGE distributing into several type strains belonging to the same DL type and vice versa. Other investigators have documented the usefulness of DL for outbreak investigation and detection of multidrug-resistant clone *P. aeruginosa* ST618 in hospital wards (11, 24).

Finally, DL demonstrated excellent epidemiological concordance by correctly linking all outbreak-related strains of VREF, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* in accordance with recent studies (12, 16, 24).

In conclusion, the DL system offers the following advantages: it is simple and easy to perform and has a rapid turnaround time of 1 to 2 days, which is substantially faster than PFGE and MLST analysis. Moreover, Web-based software analysis provides easy and standardized comparison and generates user-friendly reports. Among the major drawbacks of DL are the necessity to use different fingerprint kits for each bacterial species, the high cost of reagents and kits (acquisition cost per strain, 45 euros), and the lack of manufacturer instructions on similarity cutoff values for classifying closely related rep-PCR profiles as identical genotypes.

Rapid supply of typing data to an infection control team may help avert infection-related costs and morbidity. The DL system can be considered for use in the routine clinical laboratory as a rapid, first-line screening typing tool for investigating potential outbreaks of health care-associated infections, as previously suggested (6, 19). In our experience, it was a reliable typing method for outbreak investigation, even though less discriminating than PFGE analysis. However, regarding *E. coli* isolates sharing the same DL type, results should be confirmed by additional markers, as occasional violations of MLST assignment by DL typing do occur.

ACKNOWLEDGMENT

We thank bioMérieux for the providing access to DiversiLab software during this evaluation.

REFERENCES

1. **Bauraing, C., et al.** 2008. Characterization of carbapenem-resistant *Acinetobacter baumannii* isolates recovered from 13 different Belgian hospitals between 2004 and 2008. 48th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.
2. **Bogaerts, P., C. Bauraing, A. Deplano, and Y. Glupczynski.** 2007. Emergence and dissemination of BEL-1-producing *Pseudomonas aeruginosa* in Belgium. Antimicrob. Agents Chemother. **51:**1584–1585.
3. **Bogaerts, P., et al.** 2008. Carbapenem-resistant *Acinetobacter baumannii* isolates expressing the *bla* (OXA-23) gene associated with ISAba4 in Belgium. Antimicrob. Agents Chemother. **52:**4205–4206.
4. **Bogaerts, P., et al.** 2010. GES extended-spectrum beta-lactamases in *Acinetobacter baumannii* isolates in Belgium. Antimicrob. Agents Chemother. **54:**4872–4878.
5. **Brolund, A., et al.** 2010. The DiversiLab system versus pulsed-field gel electrophoresis: characterisation of extended spectrum beta-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*. J. Microbiol. Methods **83:**224–230.
6. **Carretto, E., et al.** 2008. Use of the DiversiLab semiautomated repetitive-sequence-based polymerase chain reaction for epidemiologic analysis on *Acinetobacter baumannii* isolates in different Italian hospitals. Diagn. Microbiol. Infect. Dis. **60:**1–7.
7. **Chuang, Y. C., J. T. Wang, M. L. Chen, and Y. C. Chen.** 2010. Comparison of an automated repetitive-sequence-based PCR microbial typing system with pulsed-field gel electrophoresis for molecular typing of vancomycin-resistant *Enterococcus faecium*. J. Clin. Microbiol. **48:**2897–2901.
8. **Deplano, A., et al.** 2007. Outbreak of hospital-adapted clonal complex-17 vancomycin-resistant *Enterococcus faecium* strain in a haematology unit: role of rapid typing for early control. J. Antimicrob. Chemother. **60:**849–854.
9. **Deplano, A., et al.** 2005. Molecular characterization of an epidemic clone of panantibiotic-resistant *Pseudomonas aeruginosa*. J. Clin. Microbiol. **43:**1198–1204.
10. **Deplano, A., et al.** 2007. Emergence and dissemination of multidrug resistant clones of *Pseudomonas aeruginosa* producing VIM-2 metallo-beta-lactamase in Belgium. Euro. Surveill. **12:**e0701118.2.
11. **Doleans-Jordheim, A., et al.** 2009. Reliability of *Pseudomonas aeruginosa* semi-automated rep-PCR genotyping in various epidemiological situations. Eur. J. Clin. Microbiol. Infect. Dis. **28:**1105–1111.
12. **Fluit, A. C., et al.** 2010. Evaluation of the DiversiLab system for detection of hospital outbreaks of infections by different bacterial species. J. Clin. Microbiol. **48:**3979–3989.
13. **Fontana, C., et al.** 2008. *Acinetobacter baumannii* in intensive care unit: a novel system to study clonal relationship among the isolates. BMC Infect. Dis. **8:**79.
14. **Glupczynski, Y., C. Berhin, H. Rodriguez-Villalobos, M. J. Struelens, and B. Jans.** 2009. Epidemiology of extended spectrum beta-lactamase-producing *Enterobacteriaceae* in Belgium: preliminary results of a national survey in 2006, abstr. P1355. Abstr. 17th Euro. Cong. Clin. Microbiol. Infect. Dis., Munich, Germany, 31 March to 3 April 2007.
15. **Glupczynski, Y., et al.** 2010. Detection and characterization of class A extended-spectrum-beta-lactamase-producing *Pseudomonas aeruginosa* isolates in Belgian hospitals. J. Antimicrob. Chemother. **65:**866–871.
16. **Grisold, A. J., et al.** 2009. Use of automated repetitive-sequence-based PCR for rapid laboratory confirmation of nosocomial outbreaks. J. Infect. **60:**44–51.
17. **Grundmann, H., S. Hori, and G. Tanner.** 2001. Determining confidence intervals when measuring genetic diversity and the discriminatory abilities of typing methods for microorganisms. J. Clin. Microbiol. **39:**4190–4192.
18. **Hunter, P. R.** 1990. Reproducibility and indices of discriminatory power of microbial typing methods. J. Clin. Microbiol. **28:**1903–1905.
19. **Lau, S. H., et al.** 2009. Rapid identification of uropathogenic *Escherichia coli* of the O25:H4-ST131 clonal lineage using the DiversiLab repetitive sequence-based PCR system. Clin. Microbiol. Infect. **16:**232–237.
20. **Laurent, C., et al.** 2008. Intensive care unit outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* controlled by cohorting patients and reinforcing infection control measures. Infect. Control Hosp. Epidemiol. **29:**517–524.
21. **Ligozzi, M., R. Fontana, M. Aldegheri, G. Scalet, and G. Lo Cascio.** 2010. Comparative evaluation of an automated repetitive-sequence-based PCR instrument versus pulsed-field gel electrophoresis in the setting of a *Serratia marcescens* nosocomial infection outbreak. J. Clin. Microbiol. **48:**1690–1695.
22. **Onteniente, L., S. Brisse, P. T. Tassios, and G. Vergnaud.** 2003. Evaluation of the polymorphisms associated with tandem repeats for *Pseudomonas aeruginosa* strain typing. J. Clin. Microbiol. **41:**4991–4997.
23. **Pitout, J. D., et al.** 2009. Using a commercial DiversiLab semiautomated repetitive sequence-based PCR typing technique for identification of *Escherichia coli* clone ST131 producing CTX-M-15. J. Clin. Microbiol. **47:**1212–1215.
24. **Ratkai, C., et al.** 2010. Successful application of the DiversiLab repetitive-sequence-based PCR typing system for confirmation of the circulation of a multiresistant *Pseudomonas aeruginosa* clone in different hospital wards. Diagn. Microbiol. Infect. Dis. **67:**202–206.
25. **Robinson, D. A., et al.** 1998. The IS1167 insertion sequence is a phylogenetically informative marker among isolates of serotype 6B *Streptococcus pneumoniae*. J. Mol. Evol. **47:**222–229.
26. **Rodriguez-Villalobos, H., et al.** 2009. Clonal dissemination of CTX-M-15 producing *E. coli* in Belgium: a multicentre surveillance study. 19th Euro. Congr. Clin. Microbiol. Infect. Dis., Helsinki, Finland, 16 to 19 May 2009.
27. **Rodriguez-Villalobos, H., et al.** 2010. Trends in production of extended-spectrum beta-lactamases among *Enterobacteriaceae* of clinical interest: results of a nationwide survey in Belgian hospitals. J. Antimicrob. Chemother. **66:**37–47.
28. **Rodriguez-Villalobos, H., et al.** 2005. Emergence of CTX-M extended spectrum beta-lactamase-producing *Escherichia coli* in Belgium. Euro. Surveill. **10:**e050224.
29. **Struelens, M. J.** 1996. Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clin. Microbiol. Infect. **2:**2–11.
30. **van Belkum, A.** 2007. Tracing isolates of bacterial species by multilocus variable number of tandem repeat analysis (MLVA). FEMS Immunol. Med. Microbiol. **49:**22–27.
31. **van Belkum, A., et al.** 2007. Guidelines for the validation and application of typing methods for use in bacterial epidemiology. Clin. Microbiol. Infect. **13:**1–46.