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Evaluation of the DiversiLab Typing Method in a Multicenter Study Assessing Horizontal Spread of Highly Resistant Gram-Negative Rods[▽]

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The worldwide prevalence of highly resistant Gram-negative rods (HR-GNR) is increasing rapidly. Reliable typing methods are needed to detect and control outbreaks and to monitor the effectiveness of infection control programs in endemic situations. In this study, we investigated the performance of the DiversiLab typing method in comparison with the amplified fragment length polymorphism (AFLP) typing method. Six hundred fifty-three HR-GNR isolates, which were obtained during a 6-month prospective survey in 18 Dutch hospitals, were typed by AFLP and DiversiLab. Subsequently, the sensitivity and specificity of DiversiLab were calculated, using AFLP as the reference method. In addition, results were compared by means of epidemiological linkage, and Cohen's kappa for agreement was calculated. DiversiLab considered significantly more isolates (275) to belong to a cluster than AFLP (198) ($P < 0.001$). In direct comparison, the sensitivity was 83.8%, and the specificity was 78.6%. When epidemiological linkage was included in the analysis, DiversiLab considered eight isolates as secondary cases, which were considered unique in AFLP. Only two secondary cases, according to AFLP, were missed by DiversiLab. This results in a kappa for agreement of 0.985. In daily practice, a typing method has to be used in combination with epidemiological information. When this was done, DiversiLab was shown to be a reliable method for the typing of HR-GNR. This, in combination with the ease of use and the speed, makes DiversiLab an appropriate method for screening in routine clinical practice. When a cluster is suspected and the consequences of these findings are substantial, a confirmatory analysis should be performed.

Worldwide, the prevalence of highly resistant Gram-negative rods (HR-GNR) is increasing. In the annual report of 2008, the European Antimicrobial Resistance Surveillance System concluded that resistance of *Escherichia coli* to four antimicrobial classes, including broad-spectrum cephalosporins, is already among the four most common resistance patterns encountered in Europe (http://www.rivm.nl/earss/result/Monitoring_reports/Annual_reports.jsp). In *Klebsiella* spp., even 14% of invasive isolates are resistant to three classes of antimicrobial drugs, including broad-spectrum cephalosporins.

This increase can be caused by patients carrying resistant pathogens on admission, by horizontal transfer between patients, by selection of resistance caused by antimicrobial use, by transfer of resistance genes between microorganisms, or by combination of two or more of these mechanisms (1, 6, 8).

In The Netherlands, national guidelines have been defined that describe control measures to avoid the spread of resistant bacteria between patients (5). In case of a suspected outbreak, typing methods are essential to assess the presence and scale of the outbreak. The most commonly used typing methods for aerobic Gram-negative rods are amplified fragment length polymorphism (AFLP) and pulsed-field gel electrophoresis (PFGE). These methods are very reliable but also relatively expensive and time-consuming, and they require a high level of technical skill. Therefore, they cannot be used in most clinical laboratories. We tested a commercially available typing method, DiversiLab, on a well-defined collection of HR-GNR from a recent study in Dutch hospitals (9). The objective of this study was to determine the reliability of DiversiLab in comparison with the AFLP typing method for the typing of HR-GNR in a hospital setting.

MATERIALS AND METHODS

Strain collection. The HR-GNR isolates were part of a collection from a multicenter study performed in five university hospitals, eight teaching hospitals, and five general hospitals during a 6-month study period in 2007. All isolated strains, whether from clinical cultures or from screening cultures of clinical patients, were included (9). In total, 653 HR-GNR isolates were available and were included in this analysis. All isolates were sent to a central laboratory, where they were stored, using Microbank vials, at −70°C until further testing. Identification (GN) and antimicrobial susceptibility testing (AST-NO48) were performed by using the VITEK2 system (bioMérieux, Marcy l'Etoile, France). If the identification was considered "unacceptable" (probability below 85%) or if the result differed from the identification from the participating hospital, API 20E/20NE (bioMérieux, Marcy l'Etoile, France) was performed as a confirmation test, according to the manufacturer's recommendations. The indole spot test was used as a complementary test if recommended by the VITEK2 expert system.

If more than one HR-GNR was recovered from one patient, the second strain was only included if this was another species or the same species with a different susceptibility pattern. The criteria for HR-GNR used are described in the Dutch national guideline for the control of highly resistant microorganisms (5). Table 1 shows a summary of these criteria.

AFLP typing method. For molecular typing, chromosomal DNA was isolated using the easyMag system (bioMérieux, Marcy l'Etoile, France). Amplified frag-

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TABLE 1. Definition of HR-GNR

Species	Type of resistance to[a]:						
	ESBL	CTZ	QUI	AMG	CAR	PIP	COT
Enterobacteriaceae							
Escherichia coli	A		B	B	A		
Klebsiella	A		B	B	A		
Other *Enterobacteriaceae*	A		B	B	A		B
Nonfermenters							
Acinetobacter		B	B	B	A		
Stenotrophomonas maltophilia							A
Other nonfermenters		C	C	C	C	C	C

[a] ESBL, extended-spectrum β-lactamase production; CTZ, ceftazidime; CAR, carbapenems; QUI, fluoroquinolones; AMG, aminoglycosides; PIP, piperacillin; COT, co-trimoxazole. In resistance type A, resistance against an antibacterial agent of the carbapenem group, and/or the presence of ESBL production, and/or resistance against cotrimoxazole is sufficient to define the microorganism as highly resistant. In resistance type B, resistance against antibacterial agents from at least two of the indicated groups or the specified antibacterial agent is required to define the microorganism as highly resistant. In resistance type C, resistance against antibacterial agents from at least three of the indicated groups or specified antibacterial agent is required to define the microorganism as highly resistant.

ment length polymorphism (AFLP) was performed as described by Savelkoul et al. (7). Subsequent to restriction, ligation, and amplification, the DNA fragments were separated on an ABI Prism 3130XL genetic analyzer (Applied Biosystems). Genetic relatedness was determined on basis of both visual and computerized interpretation of AFLP patterns.

DiversiLab typing method. The DiversiLab Microbial Typing System (bioMérieux, Marcy l'Etoile, France) is based on repetitive sequence-based PCR (rep-PCR), which amplifies the regions between the noncoding repetitive sequences in bacterial genomes.

The DNA was isolated by the DiversiLab Mo Bio UltraClean microbial DNA isolation kit, as recommended by the manufacturer. The DNA concentration was measured and set between 25 ng/μl and 30 ng/μl. Subsequently, the DNA was amplified using the DiversiLab fingerprinting kit for *Escherichia* spp., *Klebsiella* spp., and other species, according to the manufacturer's instructions. PCR was performed using the following parameters: initial denaturation (94°C) for 2 min, and then 35 cycles of 30 s of denaturation (94°C), 30 s of annealing (50 to 55°C depending on the species), and 90 s of extension (70°C), followed by 3 min of final extension (70°C) and ending at 4°C. The amplification products were separated with the Agilent B2100 bioanalyzer. Five microliters of DNA standard markers (used for normalization of sample runs) and 1 μl of the DNA product were used. All data were entered in the DiversiLab software system.

Definition of epidemiological linkage. HR-GNR isolates recovered from a specimen obtained from a patient more than 48 h after admission were classified as hospital-associated HR-GNR; HR-GNR strains recovered less than 48 h after admission were potential index cases. Nosocomial transmission was considered present if genotypically related strains were detected in two or more patients who had been in the same hospital ward within a maximum time window of 4 weeks before cultures turned HR-GNR positive. The isolates from patients with hospital-associated HR-GNR were considered secondary cases.

Statistical analysis. All data generated by AFLP were analyzed with Pearson's correlation coefficient and clustered by unweighted-pair group matrix analyses using BioNumerics software, v5.10 (Applied-Maths, Sint-Martens-Latum, Belgium). Clusters were formed according to the biological similarity of the strains (7).

Results of the DiversiLab typing method were analyzed with the DiversiLab software (version 3.4), which uses the Pearson correlation coefficient and the unweighted-pair group method with arithmetic averages to determine distance matrices and to create dendrograms. Reports were automatically generated. Isolates with a similarity of at least 95% were considered a cluster.

We compared the results of both typing methods in two distinctive ways. First, we compared the typing methods directly, with AFLP as the gold standard. If DiversiLab was in agreement with the gold standard and considered an isolate to be part of a cluster that was also found using AFLP, the result was scored as true positive. If DiversiLab and AFLP both considered an isolate to be unique, the result was scored as true negative. If the gold standard considered an isolate to be unique and DiversiLab considered it as part of a cluster, the result was scored as false positive. On the contrary, if the gold standard considered an isolate to be part of a cluster and DiversiLab did not or clustered it with other isolates not belonging to the AFLP-cluster, the result was considered false negative. Sensitivity was calculated by dividing the number of true positives by the sum of true positives and false negatives. Specificity was calculated by dividing the number of true negatives by the sum of true negatives and false positives. This calculation was done for each participating hospital separately.

Second, we included epidemiological linkage in the analysis and compared DiversiLab and AFLP with each other. This was done by calculation of the transmission index (TI). The TI was defined as the number of patients with a nosocomial transmission (secondary cases) divided by the number of patients with an HR-GNR not acquired by nosocomial transmission (potential index cases) (9). This was calculated for both AFLP and DiversiLab and for each hospital separately. Also, a Cohen's kappa for agreement was calculated by dividing the number of strains where AFLP and DiversiLab were in agreement by the total number of strains. This was done for each hospital separately.

RESULTS

Strain collection. Of a total of 892 HR-GNR included in the initial study (9), 653 isolates (559 *Enterobacteriaceae* and 94 nonfermentative Gram-negative rods) were available for further typing. As shown in Table 2, *E. coli* was the most prevalent species included in this study (296 isolates [53.0%]). Of the

TABLE 2. Distribution of species

Species	No. of isolates	AFLP		DiversiLab	
		No. (%) in cluster	No. (%) of secondary cases	No. (%) in cluster	No. (%) of secondary cases
Enterobacteriaceae[a]					
Citrobacter	28	0 (0.0)	0 (0.0)	6 (21.4)	0 (0.0)
Enterobacter	68	24 (37.5)	8 (33.3)	41 (59.4)	11 (26.8)
Escherichia coli	296	119 (40.2)	11 (9.2)	158 (53.4)	13 (8.2)
Klebsiella	110	34 (30.9)	13 (38.2)	40 (36.4)	13 (32.5)
Proteus	37	11 (29.7)	2 (18.2)	14 (37.8)	2 (14.3)
Nonfermentative GNR[b]					
Acinetobacter	15	2 (13.3)	0 (0.0)	0 (0.0)	0 (0.0)
Chryseobacterium	8	2 (25.0)	1 (50.0)	4 (50.0)	1 (25.0)
Pseudomonas	38	6 (15.8)	0 (0.0)	9 (23.7)	1 (11.1)
Stenotrophomonas	26	0 (0.0)	0 (0.0)	3 (11.5)	1 (33.3)

[a] Of 20 other *Enterobacteriaceae*, no clusters were found. This group consists of nine *Morganella morganii* isolates, two *Providencia* isolates, two *Salmonella* isolates, five *Serratia* isolates, and two *Shigella* isolates.

[b] Of seven other nonfermentative GNR, no clusters were found. This group consists of five *Achromobacter* isolates, one *Ochrobactrum anthropi* isolate, and one *Sphingomonas paucimobilis* isolate.

TABLE 3. Direct comparison of AFLP and DiversiLab

Hospital	AFLP		DiversiLab		Sensitivity (%)	Specificity (%)
	No. of clusters	No. (%) of isolates in clusters	No. of clusters	No. (%) of isolates in clusters		
1	8	27 (31.0)	13	44 (50.6)	100.0	71.7
2	2	6 (16.2)	4	11 (29.7)	100.0	86.7
3	7	19 (47.5)	8	21 (52.5)	94.7	85.7
4	0	0 (0.0)	0	0 (0.0)	NA[a]	100.0
5	6	17 (39.5)	7	26 (60.5)	94.7	66.7
6	2	4 (33.3)	2	4 (33.3)	100.0	100.0
7	8	20 (31.3)	8	25 (39.1)	75.0	79.6
8	0	0 (0.0)	0	0 (0.0)	NA	100.0
9	0	0 (0.0)	0	0 (0.0)	NA	100.0
10	0	0 (0.0)	0	0 (0.0)	NA	100.0
11	0	0 (0.0)	0	0 (0.0)	NA	100.0
12	7	18 (43.9)	7	20 (48.8)	83.0	82.6
13	2	7 (23.3)	2	7 (23.3)	57.1	87.0
14	1	2 (66.7)	1	2 (66.7)	100.0	100.0
15	2	4 (30.8)	2	4 (30.8)	100.0	100.0
16	6	12 (23.5)	10	21 (41.2)	91.7	76.9
17	11	38 (31.4)	16	57 (48.3)	76.9	69.6
18	9	26 (28.6)	11	33 (36.3)	61.5	76.9
Overall	71	201 (30.7)	91	276 (42.1)	83.8	78.6

[a] NA, not applicable.

nonfermentative Gram-negative rods, *Pseudomonas* spp. were the most prevalent (38 isolates [40.4%]).

Direct comparison of AFLP and DiversiLab. Using AFLP, 188 *Enterobacteriaceae* and 10 nonfermentative Gram-negative rods were considered to belong to a cluster. In total, 71 clusters were found, with a median cluster size of 2 isolates. The largest cluster contained 11 isolates. Using DiversiLab, 259 *Enterobacteriaceae* and 16 nonfermentative Gram-negative rods belonged to a cluster. These isolates are distributed over 91 clusters. The median cluster size was 2, and the largest cluster contained 13 isolates. The number of isolates belonging to a cluster was significantly higher for *Enterobacteriaceae* in DiversiLab compared with AFLP ($P < 0.001$). Using AFLP, the isolates of five hospitals were all unique. The other hospitals revealed 1 to 11 clusters. Using DiversiLab, the same five hospitals revealed no clusters. The other hospitals revealed between 1 and 16 clusters. In Table 3, the number of clusters and the number of isolates in the clusters of each hospital are shown. Also, the sensitivity and specificity, calculated by direct comparison of AFLP and DiversiLab, are calculated. The overall sensitivity was 83.8% and ranged from 57.1% to 100.0% in the various hospitals. The overall specificity was 78.6%, with a range from 66.7% to 100%.

Comparison of transmission indices. In Table 4, the results with the addition of epidemiological linkage are shown. The numbers of primary and secondary cases per hospital are given, as well as the transmission index and the kappa value within each hospital.

The TI values in 14 out of 18 hospitals were identical for AFLP and DiversiLab. AFLP and DiversiLab were not in agreement for the epidemiological linkage of 10 isolates coming from four hospitals: 8 isolates were considered primary cases by AFLP and secondary cases by DiversiLab, and 2 isolates were considered primary cases in DiversiLab and secondary cases by AFLP. This results in a kappa of 0.985 (range, 0.884 to 1.000).

Organisms within a cluster did not always exhibit the same resistance phenotype, even if transmission was suspected. However, for all but 1 of the 10 isolates where AFLP and DiversiLab were not in agreement for the epidemiological linkage, the resistance patterns of the primary and secondary cases were identical.

DISCUSSION

In this study, we evaluated the performance of the DiversiLab typing method. This was done by using a well-described collection of HR-GNR. Our evaluation showed that DiversiLab considered significantly more isolates to be part of a cluster compared to AFLP ($P < 0.001$). In a direct comparison, the sensitivity was 83.8%, and the specificity was 78.6%. However, when epidemiological linkage was included in the analysis, the performance of DiversiLab was comparable to AFLP. In other words, the interrelationships between the isolates in the clusters found with DiversiLab were comparable to the interrelationships between the isolates in the clusters found with AFLP. In 14 hospitals, the results were in full agreement. As a result, the overall kappa was very high (0.985), which indicated a good concordance between the two tests.

A number of other studies evaluating DiversiLab have been published (2–4). Most evaluate DiversiLab for only one species, and a number of reference methods have been used. Fluit et al. evaluated DiversiLab with a number of well-typed species and showed that DiversiLab was a useful tool to help identify hospital outbreaks of *Acinetobacter* spp., *Stenotrophomonas maltophilia*, *Enterobacter cloacae*, *Klebsiella* spp., and *E. coli*, but was considered inadequate for *Pseudomonas aeruginosa* (3). Our results are in line with these findings, except for the

TABLE 4. Comparison of the numbers of primary and secondary cases and transmission index values

Hospital	AFLP			DiversiLab			Cohen's kappa for agreement
	No. of cases		Transmission index value	No. of cases		Transmission index value	
	Primary	Secondary		Primary	Secondary		
1	84	3	0.036	84	3	0.036	1.000
2	36	1	0.028	36	1	0.028	1.000
3	35	5	0.143	35	5	0.143	1.000
4	4	0	0.000	4	0	0.000	1.000
5	38	5	0.132	33	10	0.303	0.884
6	12	0	0.000	12	0	0.000	1.000
7	62	2	0.032	61	3	0.049	0.984
8	5	0	0.000	5	0	0.000	1.000
9	4	0	0.000	4	0	0.000	1.000
10	7	0	0.000	7	0	0.000	1.000
11	3	0	0.000	3	0	0.000	1.000
12	38	3	0.079	38	3	0.079	1.000
13	27	3	0.111	28	2	0.071	0.900
14	3	0	0.000	3	0	0.000	1.000
15	13	0	0.000	13	0	0.000	1.000
16	45	6	0.133	45	6	0.133	1.000
17	111	7	0.063	111	7	0.063	1.000
18	90	1	0.011	89	2	0.022	0.989
Overall	617	36	0.058	611	42	0.069	0.985

conclusions regarding *P. aeruginosa*. However, another recent study showed a good performance of DiversiLab with *P. aeruginosa* in comparison with PFGE (2). Our results were also in line with a study by Grisold and colleagues (4), who showed concordant results for identification of outbreak and non-outbreak-related *Acinetobacter baumannii* and extended-spectrum β-lactamase (ESBL)-producing *Klebsiella pneumoniae* strains for PFGE and DiversiLab.

Our study is, according to us, unique because we included all resistant Gram-negative species found in several hospitals in a specific period. In addition, our analysis includes epidemiological linkage, which is mandatory to evaluate the effect of using the typing method in daily practice. The main limitation of our study was that we only included highly resistant Gram-negative rods, whereas in daily practice, the method will also be used for outbreaks of highly resistant Gram-positive bacteria.

In conclusion, our study shows that the sensitivity and specificity of DiversiLab are lower than those of AFLP. However, when epidemiological linkage is included in the analysis, the performance is very good (kappa of 0.985), leading to similar conclusions about horizontal spread of HR-GNR. The good performance, in combination with the ease of use and the speed, makes DiversiLab an appropriate screening method in routine clinical practice. However, when the screening indicates a severe problem that would justify extensive control measures, a more discriminatory method (e.g., AFLP) should be used to determine the true extent of the outbreak.

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REFERENCES

1. **Austin, D. J., K. G. Kristinsson, and R. M. Anderson.** 1999. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc. Natl. Acad. Sci. U. S. A. **96:**1152–1156.
2. **Doléans-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.
3. **Fluit, A. C., et al.** 2010. Evaluation of the DiversiLab system for the detection of hospital outbreaks of different bacterial species. J. Clin. Microbiol. **48:** 3979–3989.
4. **Grisold, A. J., et al.** 2010. Use of automated repetitive-sequence-based PCR for rapid laboratory confirmation of nosocomial outbreaks. J. Infect. **60:** 40–51.
5. **Kluytmans-VandenBergh, M. F. Q., J. A. J. W. Kluytmans, and A. Vos.** 2005. Dutch guideline for preventing nosocomial transmission of highly resistant micro-organisms (HRMO). Infection **33:**309–313.
6. **Paterson, D. L.** 2006. Resistance in gram-negative bacteria: Enterobacteriaceae. Am. J. Infect. Control **34:**S64–S73.
7. **Savelkoul, P. H., et al.** 1999. Amplified-fragment length polymorphism analysis: the state of an art. J. Clin. Microbiol. **37:**3083–3091.
8. **Strausbaugh, L. J., J. D. Siegel, and R. A. Weinstein.** 2006. Preventing transmission of multidrug-resistant bacteria in health care settings: a tale of 2 guidelines. Clin. Infect. Dis. **42:**828–835.
9. **Willemsen, I., et al.** 2011. Highly resistant gram-negative micro-organisms: incidence density and occurrence of nosocomial transmission (TRIANGLe study). Infect. Control Hosp. Epidemiol. **32:**333–341.