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Epidemiological Fingerprinting of *Enterobacter cloacae* by Small-Fragment Restriction Endonuclease Analysis and Pulsed-Field Gel Electrophoresis of Genomic Restriction Fragments

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A cluster of infections caused by *Enterobacter cloacae* was observed among preterm neonates in a neonatal intensive care unit (NICU) of a pediatric hospital in Osnabrück, Germany. The presence of similar antimicrobial susceptibility patterns among the bacterial isolates prompted an investigation to determine whether a limited spread of a single strain existed. All 12 *E. cloacae* isolates from the NICU and 50 nonrelated strains were fingerprinted by small-fragment restriction endonuclease analysis (SF-REA) of *Eco*RI DNA digests. Selected isolates were further characterized by pulsed-field gel electrophoresis (PFGE) of *Not*I- or *Xba*I-generated genomic restriction fragments. Epidemiologically unrelated strains were clearly discriminated by both methods. Results achieved by SF-REA and PFGE revealed that of the 12 isolates from the NICU, 11 belonged to the same genotypic cluster. Since all reagents and equipment for both techniques are commercially available, DNA fingerprinting by SF-REA or PFGE is proposed as a useful tool in the microbiology laboratory for investigating the epidemiological relatedness of *E. cloacae* strains of clinical and environmental origin.

In recent years *Enterobacter cloacae* has been increasingly recognized as a nosocomial pathogen (10, 14). Outbreaks of infection have been reported in neonatal intensive care units (NICUs) (27, 32), surgical wards (6), and burn units (22, 25), associated with contaminated pharmaceutical products (24) or caused by cross-infection (28). Enterobacter was found to account for 4 to 12% of sepsis caused by gram-negative organisms (22) and accompanied by a relatively high mortality rate (5).

Tracking the causative agent in outbreak situations requires the precise identification of individual isolates beyond the species level. Traditional techniques used for typing *E. cloacae* are often based on phenotypic characteristics, including biotyping (29), antibiogram analysis (27), bacteriocin sensitivity or production (2, 8), O serotyping (15), and phage typing (13). Some of these techniques are not sufficiently sensitive to distinguish between different strains or are affected by physiological factors. Others depend on reagents that are not commercially available, thus limiting typing *E. cloacae* to a few specialized centers.

In more recent reports genotypic typing methods, such as plasmid profile analysis (25, 32) and ribotyping (3, 12), have been employed for the strain delineation of *E. cloacae*. The latter technique seems especially promising, since it takes recourse to stable strain characteristics and its results are clear and easy to interpret. Unfortunately, it is rather time-consuming and labor intensive, since Southern blotting is employed as an essential part of the procedure. In nosocomial investigations, typing methods that can be performed by the microbiology laboratory that serves the affected ward or hospital are needed because the rapid instigation of control measures depends on reliable typing data achieved in an appropriate period of time.

In this study we report on the use of small-fragment restriction endonuclease analysis (SF-REA) and pulsed-field gel electrophoresis (PFGE) as typing tools for *E. cloacae*. The results are compared with those achieved by traditional methods available in a routine microbiology laboratory and are evaluated with respect to typeability, reproducibility, discriminatory power, and ease of use.

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MATERIALS AND METHODS

Bacterial strains. A total of 62 *E. cloacae* strains from clinical specimens, catheters, and perfusor lines submitted to our diagnostic laboratory were included in this study. Bacteria were isolated and identified biochemically by standard procedures (11). Twelve isolates were from nine premature infants in an NICU of a pediatric hospital in Osnabrück, Germany. Information in detail is given in Table 1. Fifty epidemiologically unrelated strains were collected over a period of 3 months from patients in 15 hospitals of diverse geographic locations in Lower Saxony, Germany.

Biotyping. Bioprofiles were determined by the API 20E system (Api-bioMérieux) according to the manufacturer's instructions.

Antibiograms. Susceptibility to 14 antimicrobial agents was determined by the disk diffusion method on Mueller-Hinton agar (9) using the following substances (per disk): cefazolin (30 μg), cefotaxime (30 μg), ampicillin (10 μg), mezlocillin (30 μg), imipenem (10 μg), gentamicin (10 μg), ofloxacin (5 μg), chloramphenicol (30 μg), fosfomycin (50 μg), tetracycline (30 μg), co-trimoxazole (25 μg), nalidixic acid (30 μg), nitrofurantoin (300 μg), and colistin (10 μg).

Plasmid profile analysis. Plasmid DNA was isolated by an alkaline lysis method (23), electrophoresed on 0.8% agarose gels in TBE running buffer (89 mM Tris, 89 mM boric acid, 2 mM Na_2-EDTA, pH 8.3), and visualized by ethidium bromide staining (1 μg/ml, 30 min). Molecular weights were estimated by comparison with plasmids of known molecular size.

Chromosomal analysis by SF-REA. Whole-cell DNA for SF-REA was prepared by a modified guanidine thiocyanate–

TABLE 1. Phenotypic and genotypic characteristics of the *E. cloacae* isolates from the NICU used in this study[a]

Isolate no.	Date of isolation	Patient	Source	API 20E profile	Antibiogram												Plasmid profile (MDa)	SF-REA profile		PFGE profile	
					CZ	CTX	AM	MZ	IMP	GM	OFX	C	FF	TE	SXT	NA	F/M	CL		*EcoR*I *Hin*dIII	*Xba*I	*Not*I
1	22 Aug 1991	A	Blood culture	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
2	23 Aug 1991	B	Blood culture	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
3	2 Sep 1991	B	Venous catheter	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
4	23 Aug 1991	C	Blood culture	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
5	2 Sep 1991	C	Blood culture	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
6	27 Aug 1991	D	Blood culture	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
7	29 Aug 1991	E	Blood culture	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
8	1 Sep 1991	F	Blood culture	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
9	4 Sep 1991	F	Tracheal catheter	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	18, 21	a1 a2	a3	a4
10	2 Sep 1991	G	Perfusor line	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
11	2 Sep 1991	H	Perfusor line	1305573	R	S	R	R	S	R	S	S	R	S	S	S	S	S	21	a1 a2	a3	a4
12	11 Sep 1991	I	Ear swab	3305573	S	S	R	S	S	S	S	S	R	S	S	S	S	S	None	b1 b2	b3	b4

[a] Abbreviations: Aug, August; Sep, September; CZ, cefazolin; CTX, cefotaxime; AM, ampicillin; MZ, mezlocillin; IMP, imipenem; GM, gentamicin; OFX, ofloxacin; C, chloramphenicol; FF, fosfomycin; TE, tetracycline; SXT, co-trimoxazole; NA, nalidixic acid; F/M, nitrofurantoin; CL, colistin; R, resistant; S, sensitive.

Triton X-100 lysis procedure (4) followed by phenol-chloroform extraction and isopropanol precipitation as previously described (19). Restriction endonucleases were used in the appropriate buffers under the conditions recommended by the manufacturer (New England Biolabs).

Electrophoresis was carried out in TBE buffer on 6% polyacrylamide gels (0.75 mm thick) in an SE 250 Mighty Small 7-cm Vertical Dual Slab Unit (Hoefer Scientific Instruments) with 14-mA constant current for 90 min.

Silver staining of polyacrylamide gels was done as described by Bassam et al. (1) except for the incubation times of fixing and impregnating, which were both shortened to 15 min.

Chromosomal analysis by PFGE. Unsheared DNA from *E. cloacae* was prepared as described by Smith and Cantor (31), with modification. In brief, *E. cloacae* was grown on sheep blood agar, harvested by a polyester fiber-tipped applicator, suspended in 10 ml of suspension buffer (100 mM Na_2-EDTA, 20 mM NaCl, 10 mM Tris-HCl, pH 7.2) at a density of 3×10^8 cells per ml, and collected by centrifugation. The cell pellet was thoroughly resuspended in 0.5 ml of the same buffer, warmed to 42°C, mixed with an equal volume of preheated 1% low-melting-point agarose, and drawn into a tuberculin syringe. Agarose was allowed to solidify at ambient temperature for 30 min and was then extruded and placed in 1 ml of lysozyme solution (2 mg of lysozyme per ml in 100 mM Na_2-EDTA–50 mM NaCl–10 mM Tris-HCl–0.2% Na-deoxycholate–0.5% *N*-laurylsarcosine, pH 7.2). The samples were incubated for 2 h at 36°C with gentle shaking followed by a short rinse with washing solution (50 mM Na_2-EDTA, 20 mM Tris-HCl, pH 8.0). This was then changed to proteinase K solution (1 mg of proteinase K per ml in 100 mM Na_2-EDTA–1% *N*-laurylsarcosine–0.2% Na-deoxycholate, pH 8.0), and the agarose columns were incubated for 14 h at 42°C with gentle shaking. The blocks were then washed twice in washing solution, incubated for 15 min in washing solution supplemented with 1 mM phenylmethylsulfonyl fluoride in order to inactivate proteinase K, washed twice in washing solution again, and stored at 4°C.

For restriction endonuclease digestion thin slices were cut off the agarose plugs, equilibrated in the appropriate nuclease buffer for 30 min, and then digested overnight with 0.3 to 1.0 U of enzyme per μl at 36°C. After equilibration in running buffer (0.5× TBE) the samples were then either loaded as solid plugs or heated for 2 min at 75°C and loaded as a molten mixture into the wells of a 1.0% agarose gel (SeaKem Gold; FMC) prepared in running buffer. Lambda concatemers (New England Biolabs) were used as the size standard.

PFGE was carried out with a contour-clamped homogeneous electric field apparatus (CHEF-DR II; Bio-Rad) at 14°C for 20 h at 200 V. Pulse time was linearly ramped from 3 to 15 s following *Not*I digestion and from 5 to 50 s following *Xba*I digestion.

RESULTS

Multiply resistant *E. cloacae* was isolated from blood cultures of six neonates in an NICU of a pediatric hospital in Osnabrück during a 3-week period in 1991. Additional isolates were from intravenous and tracheal catheter tips and from perfusor lines of two neonates who did not exhibit clinical symptoms of bacteremia.

Cultures of environmental samples obtained from many areas of the unit and from hospital personnel during this

period failed to reveal *E. cloacae*. No *E. cloacae* was detected in freshly prepared intravenous solutions, unused intravenous administration sets, fluids, medications, and additives. All clinical isolates were characterized by conventional methods available in a routine microbiology laboratory. They were related by identical biotypes and similar plasmid profiles and identified by a characteristic antibiogram (resistance to cefazolin, ampicillin, mezlocillin, gentamicin, and fosfomycin). One *E. cloacae* strain from an ear swab of another baby isolated 2 weeks after the cluster of infections was observed to be clearly different from the assumed epidemic strain in antibiogram and biotype and did not harbor any detectable plasmid DNA.

A plasmid of 21 MDa was found in all 11 plasmid-carrying isolates. In 10 of these the 21-MDa plasmid was the only plasmid species identified, while in one strain (no. 9) that was isolated from a tracheal catheter tip the 21-MDa plasmid was found together with a plasmid of 18 MDa. This additional plasmid in isolate no. 9 did not alter its biochemical and resistance properties.

In the present work we have applied two chromosome-based typing systems, namely, SF-REA and PFGE, in order to characterize *E. cloacae* strains.

Preliminary studies for SF-REA were done with restriction endonucleases *Bgl*I, *Bsa*AI, *Dra*I, *Eco*RI, *Hin*dIII, *Hin*fI, *Pst*I, *Pvu*II, *Sau*96I, and *Ssp*I. Of these, *Eco*RI yielded restriction fragment patterns that were most easily and best distinguished. Therefore, all primary digestions were done with *Eco*RI. Secondary studies were performed with restriction endonuclease *Hin*dIII. Both enzymes produced clear patterns on polyacrylamide gels in the size range of 0.6 to 3.0 kb.

By running various combinations of *Eco*RI-generated DNA digests on several polyacrylamide gels, it was demonstrated that of 50 nonrelated *E. cloacae* strains, representing four API 20E bioprofiles and seven antibiogram types, each isolate produced a unique fingerprint pattern. From these results Simpson's numerical index of diversity was calculated as 0.9996. This indicates a high degree of confidence when typing results are to be interpreted (20). Typeability of the isolates under investigation was 100%.

No variation in the fingerprint pattern of a given strain was observed in DNAs prepared on different occasions and after 40 subcultures of the strain.

Representative gel patterns of 11 unrelated *E. cloacae* isolates are shown in Fig. 1. The broad band in the upper fourth of the gel consists of restriction fragments of >3.0 kb that cannot be separated on polyacrylamide gels.

As can be seen in Fig. 2, the *Eco*RI restriction patterns of all 11 NICU isolates with the epidemic antibiogram were identical. Isolate no. 12, which was isolated 1 week after the assumed epidemic was terminated, clearly differed from strains 1 to 11. These results were also confirmed by analyzing *Hin*dIII DNA digestion patterns.

Five restriction endonucleases with infrequent recognition sites (*Apa*I, *Nhe*I, *Not*I, *Spe*I, and *Xba*I) were used to establish the most discriminatory banding patterns in genome analysis by PFGE. *Not*I and *Xba*I gave the most useful restriction fragment patterns and were subsequently used for all further digestions. We found that in *E. cloacae* DNA *Xba*I sites were rarer than *Not*I sites. Fragment sizes in *Xba*I-digested DNAs ranged from approximately 30 to 700 kb, as determined by comparison of the mobilities of the fragments with those of lambda concatemers. *Not*I fragments ranged from 20 to 200 kb in size.

Epidemiologically independent isolates gave unique banding patterns that were easily distinguishable from one another and from those of the isolates from the NICU. Figure 3 demonstrates the marked degree of restriction fragment length polymorphism apparent in *Xba*I-digested *E. cloacae* DNA of unrelated strains.

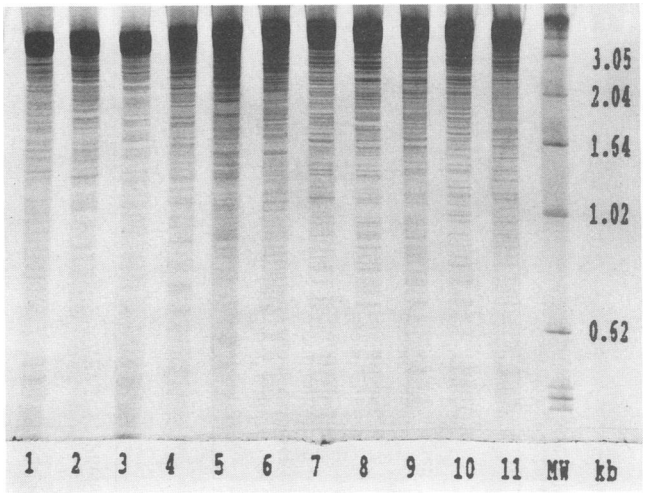

FIG. 1. *Eco*RI-cleaved *E. cloacae* DNA after electrophoresis in a 6% polyacrylamide gel and silver staining. Lane 1, epidemic strain from the NICU; lanes 2 to 11, unrelated strains from 10 different hospitals; lane MW, size standard (1-kb ladder mixed with pBR322 DNA *Msp*I digest).

In contrast, strains 1 to 11 from the NICU exhibited identical or very similar banding patterns but clearly differed from strain no. 12 (Fig. 4).

Since considerable restriction fragment length polymorphism was demonstrated in unrelated strains, the corresponding typing results achieved by both systems, SF-REA and PFGE, supported the conclusion that isolates 1 to 11 from eight patients in the NICU were very closely related or identical, respectively. Isolate no. 12 was clearly separated from the epidemic strain. The typing results are summarized in Table 1.

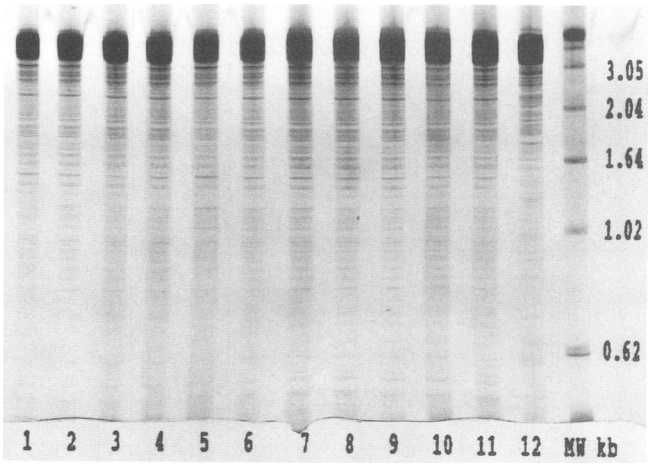

FIG. 2. *Eco*RI-generated DNA digest patterns of the 12 *E. cloacae* isolates from the NICU. Lane numbers correspond to isolate numbers in Table 1 and Fig. 4. Lane MW, size standard (1-kb-ladder mixed with pBR322 DNA *Msp*I digest).

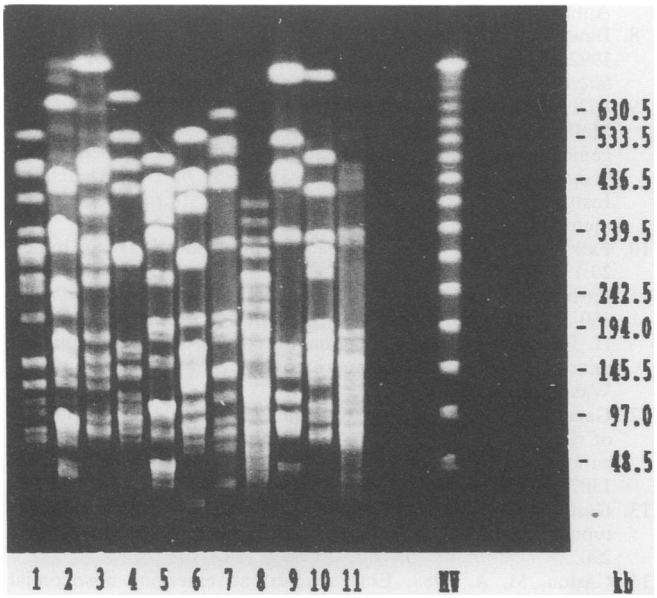

FIG. 3. *Xba*I-cleaved *E. cloacae* DNA after PFGE and ethidium bromide staining. Lane 1, epidemic strain from the NICU; lanes 2 to 11, unrelated strains from 10 different hospitals (strains correspond to those in Fig. 1); lane MW, size standard (phage lambda concatemers).

DISCUSSION

The purpose of epidemiological typing is to identify groups of infections coming from the same or different sources and to determine their route of transmission. To attain this goal the precise identification of bacterial isolates beyond the species level is essential.

Unfortunately, simple phenotypic typing methods often are not sufficiently sensitive to distinguish between strains or run the risk of rendering variable results when performed on different occasions (26). The combination of two or more of these techniques is still required for typing *E. cloacae* (8, 14).

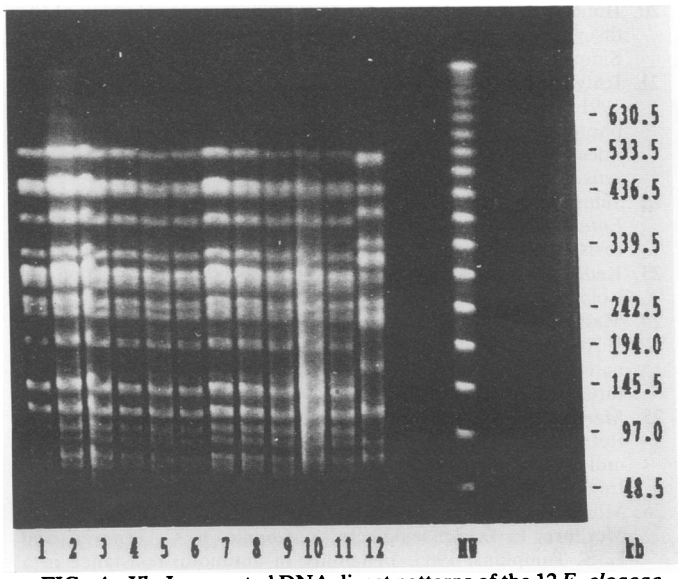

FIG. 4. *Xba*I-generated DNA digest patterns of the 12 *E. cloacae* isolates from the NICU. Lane numbers correspond to isolate numbers in Table 1 and Fig. 2. Lane MW, size standard (phage lambda concatemers).

Of the genotypic typing methods, plasmid profiling is favored as a simple and inexpensive technique (22, 25, 32). In our hands there was not a great deal of variation in the plasmid profiles of unrelated isolates, and most strains had only a few plasmids. However, the limited diversity of patterns in this method may be restrictive in large epidemiological studies, thus making further analysis by restriction endonuclease digestion necessary. Instability of profiles due to acquisition, loss, or transfer of plasmids may be another handicap of this technique, and identical plasmid profiles may not reflect the overall genetic relatedness of isolates (7, 19, 26).

The aim of this study was to evaluate the polymorphisms provided by two chromosome-based epidemiological marker systems, SF-REA and PFGE, which both offer the advantages of chromosomal stability and high typeability of *E. cloacae* isolates in a nonreference laboratory. SF-REA has previously successfully been applied to the epidemiological fingerprinting of *Staphylococcus aureus* (17), *Legionella pneumophila* (18), and *Klebsiella pneumoniae* (19), and PFGE analysis is accepted as a useful typing tool for several bacterial species (16, 21, 30).

Essential for understanding the molecular epidemiology of *E. cloacae* is the demonstration of diversity on the subspecies level. The presence of such diversity is the basis for the interpretation that isolates with identical or almost-identical patterns represent a single clone.

This study demonstrates that SF-REA as well as PFGE may be used to reproducibly fingerprint *E. cloacae* isolates. In all isolates banding patterns with differences in both band position and band intensity were demonstrated. Polymorphisms in whole-cell DNA were detected frequently in unrelated strains and revealed to be useful as genetic markers. The stability of the marker system was demonstrated by the identification of the same REA patterns before and after 40 subcultures of selected isolates.

The sensitivity of both methods is dependent on the restriction enzyme in use. In SF-REA it was highest for *Eco*RI, which separated 50 of 50 nonrelated strains. Of the several rare cutting enzymes employed in PFGE analysis of *E. cloacae* DNA, *Xba*I and *Not*I produced the most discriminative banding patterns.

In an outbreak of severe bacteremia due to *E. cloacae* that occurred in six patients in an NICU, evidence indicated that the infections were caused by a single strain. Seven blood culture isolates from six patients exhibited identical biochemical properties, antibiograms, and plasmid profiles. Four additional isolates from catheters and perfusor lines from patients in the same ward shared these characteristics, except for one tracheal isolate which exhibited a slightly different plasmid profile. All 11 isolates had identical restriction fragment patterns when analyzed by SF-REA, indicating that they were derivatives of a single strain. The possibility that unrelated strains of *E. cloacae* would share these characteristics is highly unlikely. The same correlation between isolates 1 to 11 could be drawn from *Not*I and *Xba*I digestion patterns separated by PFGE. Both techniques clearly separated an ear swab isolate derived from another baby in the same ward from the epidemic strain. Finding the same strain of *E. cloacae* in eight patients and their intravenous devices strongly suggested a common source. Infection appeared to occur during intravenous therapy. In baby C *E.*

cloacae was again detected in blood 10 days after primary isolation, even though she received adequate antibiotic therapy (ceftriaxone).

Although *E. cloacae* resembling the epidemic strain was not isolated from any of the (preselected) intravenous solution samples sent to our laboratory for microbiological examination, one of these was implicated as the probable source. The epidemic was controlled by discharging all intravenous fluids, medications, and additives in use and by reemphasizing aseptic techniques.

In conclusion, in typing *E. cloacae* both SF-REA and PFGE are useful variants of conventional gel electrophoresis, which in turn has previously been used to document the spread of epidemic plasmids or strains (25, 32). Resulting gel patterns are less complex than genomic fingerprints on conventional agarose gels, which sometimes consist of hundreds of bands. The advantage of PFGE is that it can be used to analyze a small number of large DNA fragments over a wide size range; this makes analysis of whole bacterial genomes on a single gel possible. In contrast, SF-REA achieves strain discrimination by focusing on the small and very small restriction fragments which represent only a minor part of the genome. Resulting gel patterns consist of 30 to 40 well-separated bands that form more complex patterns than PFGE fingerprints and are slightly more difficult to interpret. However, typing results are quicker to achieve by SF-REA, equipment costs come to only 1/10 of those of a PFGE apparatus, and the costs for reagents and enzymes are significantly lower. All in all, SF-REA and PFGE seem to be of the same value in typing *E. cloacae*, but in our laboratory we prefer SF-REA for economical considerations and because results are more rapidly achieved. An experienced worker can extract DNA from 30 isolates in 1 day and obtain results from electrophoresis on the second day.

Both techniques are reliable means for typing *E. cloacae* and should facilitate further epidemiologic investigations. They are especially promising for use in nonreference laboratories.

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REFERENCES

1. **Bassam, B. J., G. Caetano-Anollés, and P. M. Gresshoff.** 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal. Biochem. **196:**80–83.
2. **Bauernfeind, A., and C. Petermüller.** 1984. Typing of *Enterobacter* spp. by bacteriocin susceptibility and its use in epidemiological analysis. J. Clin. Microbiol. **20:**70–73.
3. **Bingen, E., E. Denamur, N. Lambert-Zechovsky, N. Brahimi, M. El Lakani, and J. Elion.** 1992. Rapid genotyping shows the absence of cross-contamination in *Enterobacter cloacae* nosocomial infections. J. Hosp. Infect. **21:**95–101.
4. **Boom, R., C. J. A. Sol, M. M. M. Salimans, C. L. Jansen, P. M. E. Wertheim-van-Dillen, and J. van der Noordaa.** 1990. Rapid and simple method for purification of nucleic acids. J. Clin. Microbiol. **28:**495–503.
5. **Bouza, E., M. G. de la Torre, A. Erice, E. Loza, J. M. Diaz-Borrego, and L. Buzón.** 1985. Enterobacter bacteremia: an analysis of 50 episodes. Arch. Intern. Med. **145:**1024–1027.
6. **Burchard, K. W., D. T. Barrall, M. Reed, and G. J. Slotman.** 1986. Enterobacter bacteremia in surgical patients. Surgery **100:**857–862.
7. **Courtney, M.-A., J. R. Miller, J. Summersgill, J. Melo, M. J. Raff, and U. N. Streips.** 1980. R-factor responsible for an outbreak of multiply antibiotic-resistant *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **18:**926–929.
8. **Daw, M. A., G. D. Corcoran, F. R. Falkiner, and C. T. Keane.** 1992. Application and assessment of cloacin typing of *Enterobacter cloacae*. J. Hosp. Infect. **20:**141–151.
9. **DIN Deutsches Institut für Normung e. V.** 1987. DIN 58940(3). Methods for the determination of the susceptibility of pathogenic bacteria (without mycobacteria) to chemotherapeutic substances; agar diffusion test, p. 193–196. *In* DIN Deutsches Institut für Normung e. V., Medizinische Mikrobiologie, Normen und weitere Auflagen. Beuth, Berlin.
10. **Falkiner, F. R.** 1992. Enterobacter in hospital. J. Hosp. Infect. **20:**137–140.
11. **Farmer, J. J., III, and M. T. Kelly.** 1991. *Enterobacteriaceae*, p. 360–383. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
12. **Garaizar, J., M. E. Kaufmann, and T. L. Pitt.** 1991. Comparison of ribotyping with conventional methods for the type identification of *Enterobacter cloacae*. J. Clin. Microbiol. **29:**1303–1307.
13. **Gaston, M. A.** 1987. Isolation and selection of a bacteriophage-typing set for *Enterobacter cloacae*. J. Med. Microbiol. **24:**285–290.
14. **Gaston, M. A.** 1988. Enterobacter: an emerging nosocomial pathogen. J. Hosp. Infect. **11:**197–208.
15. **Gaston, M. A., C. Bucher, and T. L. Pitt.** 1983. O serotyping scheme for *Enterobacter cloacae*. J. Clin. Microbiol. **18:**1079–1083.
16. **Grothues, D., U. Koopmann, H. von der Hardt, and B. Tümmler.** 1988. Genome fingerprinting of *Pseudomonas aeruginosa* indicates colonization of cystic fibrosis siblings with closely related strains. J. Clin. Microbiol. **26:**1973–1977.
17. **Haertl, R., and G. Bandlow.** 1990. Application of small fragment restriction endonuclease analysis (SF-REA) to the epidemiological fingerprinting of *Staphylococcus aureus*. J. Med. Microbiol. **33:**91–96.
18. **Haertl, R., and G. Bandlow.** 1991. Subtyping of *Legionella pneumophila* serogroup 1 isolates by small-fragment restriction endonuclease analysis. Eur. J. Clin. Microbiol. Infect. Dis. **10:**630–635.
19. **Haertl, R., R. Barten, and G. Bandlow.** 1991. Epidemiological fingerprinting of *Klebsiella pneumoniae* by small-fragment-restriction-endonuclease-analysis (SF-REA). Scand. J. Infect. Dis. **23:**737–743.
20. **Hunter, P. R., and M. A. Gaston.** 1988. Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. J. Clin. Microbiol. **26:**2465–2466.
21. **Ichiyama, S., M. Ohta, K. Shimokata, N. Kato, and J. Takeuchi.** 1991. Genomic DNA fingerprinting by pulsed-field gel electrophoresis as an epidemiological marker for study of nosocomial infections caused by methicillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol. **29:**2690–2695.
22. **John, J. F., Jr., R. J. Sharbaugh, and E. R. Bannister.** 1982. *Enterobacter cloacae*: bacteremia, epidemiology, and antibiotic resistance. Rev. Infect. Dis. **4:**13–28.
23. **Kado, C. I., and S. T. Liu.** 1981. Rapid procedure for detection and isolation of large and small plasmids. J. Bacteriol. **145:**1365–1373.
24. **Maki, D. G., F. S. Rhame, D. C. Mackel, and J. V. Bennett.** 1976. Nationwide epidemic of septicemia caused by contaminated intravenous products. I. Epidemiologic and clinical features. Am. J. Med. **60:**471–485.
25. **Markowitz, S. M., S. M. Smith, and D. S. Williams.** 1983. Retrospective analysis of plasmid patterns in a study of burn unit outbreaks of infection due to *Enterobacter cloacae*. J. Infect. Dis. **148:**18–23.
26. **Mickelson, P. A., J. J. Plorde, K. P. Gordon, C. Hargiss, J. McClure, F. D. Schoenknecht, F. Condie, F. C. Tenover, and L. S. Tompkins.** 1985. Instability of antibiotic resistance in a strain of *Staphylococcus epidermidis* isolated from an outbreak of prosthetic valve endocarditis. J. Infect. Dis. **152:**50–58.
27. **Modi, N., V. Damjanovic, and R. W. I. Cooke.** 1987. Outbreak of cephalosporin resistant *Enterobacter cloacae* infection in a

neonatal intensive care unit. Arch. Dis. Child. **62:**148–151.
28. **Mummery, R. V., B. Rowe, and R. J. Gross.** 1974. Urinary-tract infections due to atypical *Enterobacter cloacae*. Lancet **ii:**1333.
29. **Old, D. C.** 1982. Biotyping of *Enterobacter cloacae*. J. Clin. Pathol. **35:**875–878.
30. **Ott, M., L. Bender, R. Marre, and J. Hacker.** 1991. Pulsed field electrophoresis of genomic restriction fragments for the detection of nosocomial *Legionella pneumophila* in hospital water supplies. J. Clin. Microbiol. **29:**813–815.
31. **Smith, C. L., and C. R. Cantor.** 1987. Purification, specific fragmentation, and separation of large DNA molecules. Methods Enzymol. **155:**449–467.
32. **Wang, C. C., M. L. Chu, L. J. Ho, and R. C. Hwang.** 1991. Analysis of plasmid patterns in paediatric intensive care unit outbreaks of nosocomial infection due to *Enterobacter cloacae*. J. Hosp. Infect. **19:**33–40.