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Widespread Transfer of Resistance Genes between Bacterial Species in an Intensive Care Unit: Implications for Hospital Epidemiology

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A transferable plasmid encoding SHV-12 extended-spectrum β-lactamase, TEM-116, and aminoglycoside resistance was responsible for two sequential clonal outbreaks of *Enterobacter cloacae* and *Acinetobacter baumannii* bacteria. A similar plasmid was present among isolates of four different bacterial species. Recognition of plasmid transfer is crucial for control of outbreaks of multidrug-resistant nosocomial pathogens.

Antibiotic resistance genes are often carried in mobile genetic elements (1, 3, 6, 8, 16). The transfer of resistance genes between different bacterial species may go unnoticed by traditional infection control and epidemiological methods, thereby undermining hospital infection control policies (4, 5, 11). We describe the occurrence of two sequential outbreaks of multidrug resistant (MDR) *Enterobacter cloacae* and *Acinetobacter baumannii* bacteria at our Intensive Care Department (ICU) and provide evidence that both clones and other *Enterobacteriaceae* at the ICU harbored similar plasmids.

Between June and November 2000, aminoglycoside-resistant and extended-spectrum β-lactamase (ESBL)-producing *E. cloacae* isolates were isolated from 10 patients. In November 2000, this was followed by the isolation of *A. baumannii* strains with similar resistance patterns from three patients. Aminoglycoside resistance was determined by the disk diffusion test. For

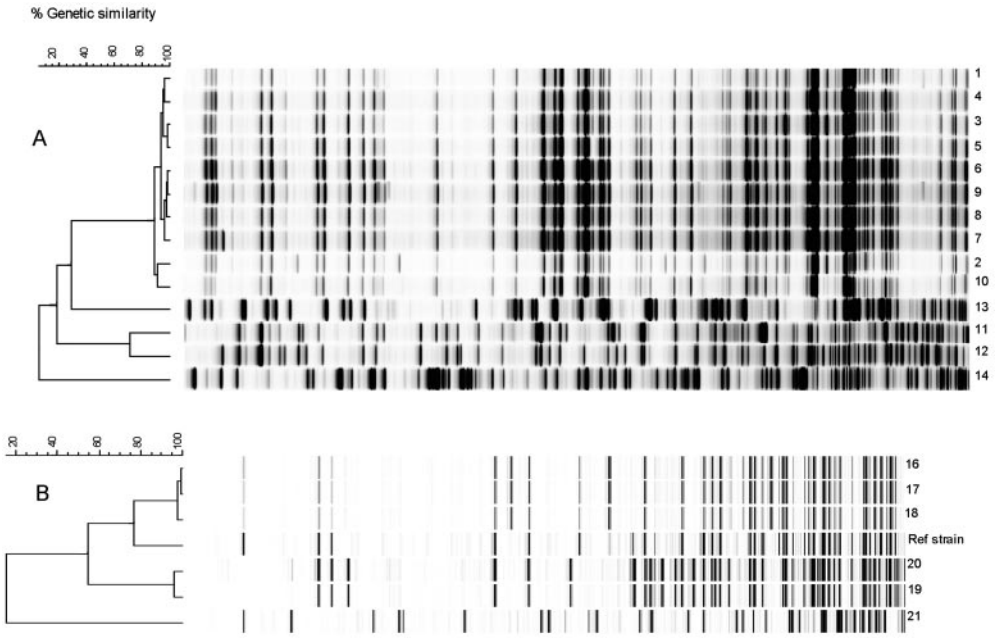

FIG. 1. Dendrogram showing the relatedness of AFLP patterns. AFLP patterns of MDR *E. cloacae* isolates (A) and MDR *A. baumannii* isolates (B) are shown. The numbers denote the patient numbers as given in Table 1.

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TABLE 1. Characteristics of isolated strains

Isolate[b]	Species	Mo of isolation	Patient (identification no.)	Site or source of isolation	Presence of[a]:					
					ESBL phenotype	Beta-lactamase gene encoding indicated enzyme				Similar plasmid with five PCR reactions
						SHV-2	SHV-12	TEM-1	TEM-116	
1	*E. cloacae*	April	13	Rectum	+	−	+	−	+	+
2	*E. cloacae*	May	12	Rectum	−	−	+	−	+	−
3	*E. cloacae**	June	1	Sputum	+	−	+	−	+	+
4	*E. cloacae*	June	11	Throat	+	−	+	−	+	−
5	*Enterobacter agglomerans*	June	15	Sputum	+	−	+	−	+	−
6	*Citrobacter freundii*	June	11	Sputum	+	−	+	−	+	−
7	*E. coli*	June	22	Sputum	−	−	+	−	+	−
8	*E. coli*	June	23	Sputum	−	−	+	−	+	−
9	*A. baumannii*	June	21	Throat	+	−	+	−	+	−
10	*E. cloacae**	July	2	Sputum	+	−	+	−	+	+
11	*E. coli*	July	24	Sputum	+	−	+	−	+	+
12	*Pseudomonas aeruginosa*	July	34	Sputum	−	−	+	−	+	−
13	*Klebsiella oxytoca*	July	36	Throat	+	−	+	−	+	+
14	*E. cloacae**	August	3	Sputum	+	−	+	−	+	+
15	*E. cloacae**	August	8	Rectum	+	−	+	−	+	+
16	*E. cloacae**	August	5	Throat	+	−	+	−	+	+
17	*E. cloacae**	August	7	Sputum	+	−	+	−	+	+
18	*E. cloacae*	August	14	Throat	+	−	+	−	+	−
19	*E. coli*	August	3	Rectum	+	−	+	−	+	+
20	*E. coli*	August	25	Throat	+	−	+	−	+	+
21	*E. cloacae**	September	9	Sputum	+	−	+	−	+	+
22	*E. cloacae**	September	4	Sputum	+	−	+	−	+	+
23	*E. cloacae**	September	6	Sputum	+	−	+	−	+	+
24	*E. coli*	September	26	Throat	+	−	+	−	+	−
25	*E. coli*	September	32	Sputum	+	−	+	−	+	−
26	*E. cloacae**	October	10	Sputum	+	−	+	−	+	+
27	*E. coli*	October	33	Rectum	+	−	+	−	+	−
28	*A. baumannii**	November	16	Sputum	+	−	+	−	+	+
29	*A. baumannii**	November	17	Throat	+	−	+	−	+	+
30	*A. baumannii**	November	18	Throat	+	−	+	−	+	+
31	*P. aeruginosa*	June	11	Sputum	+	+	+	−	−	−
32	*E. coli*	October	30	Rectum	−	+	+	−	−	−
33	*C. freundii*	July	6	Sputum	+	+	−	−	−	−
34	*A. baumannii*	August	20	Sputum	+	+	−	−	−	−
35	*P. aeruginosa*	August	35	Sputum	+	+	−	−	−	−
36	*E. coli*	September	27	Rectum	−	+	−	−	−	−
37	*E. coli*	September	28	Rectum	−	+	−	−	−	−
38	*P. aeruginosa*	September	14	Rectum	+	+	−	−	−	−
39	*E. coli*	October	31	Rectum	−	+	−	−	−	−
40	*Aeromonas* spp.	October	37	Sputum	−	+	−	−	−	−
41	*A. baumannii*	August	19	Sputum	+	+	−	+	−	−
42	*E. coli*	October	29	Rectum	−	+	−	+	−	−

[a] +, present; −, absent.
[b] All strains are gentamicin resistant. *, clonal outbreak strains.

ESBL production the double-disk synergy test was used, with disks containing cefotaxime, ceftazidime, cefpodoxime, and cefepime at distances of 30 and 20 mm (center to center) from a disk with amoxicillin and clavulanate (7, 9). AFLP analysis (15) confirmed the clonality of the *E. cloacae* strains and the clonality of the *A. baumannii* strains (Fig. 1).

The outbreak of *E. cloacae* bacteria had been noticed in August 2000 and was managed by standard infection control measures. During the period from April until November 2000, an increase of aminoglycoside-resistant gram-negative isolates in surveillance cultures from patients admitted to the ICU was observed. This ICU served as a control unit in an ongoing study to evaluate the effectiveness of selective decontamination of the digestive tract (2). Twenty-nine strains of eight different bacterial species were obtained from 26 patients admitted to the ICU. Nineteen isolates exhibited phenotypic evidence of ESBL production and aminoglycoside resistance, similar to the outbreak strains (Table 1).

PCR and sequence analysis of ESBL-encoding genes was performed. An 827-bp gene fragment of the bla_{SHV} gene was amplified with primers SHV-F1 (5′-CTT TAC TCG CCT TTA TCG-3′) and SHV-R1 (5′-TCC CGC AGA TAA ATC ACC A-3′) and sequenced with amplification primers and primers bla_{SHV}-F2 (5′-ACT GCC TTT TTG CGC CAG AT-3′) and bla_{SHV}-R2 (5′-CAG TTC CGT TTC CCA GCG GT-3′). An 862-bp gene fragment of bla_{TEM} was amplified with primers TEM-F1 (5′-ATG AGT ATT CAA CAT TTC CG-3′) (14) and TEM-R1 (5′-GAC AGT TAC CAA TGC TTA ATC A-3′) (10) and sequenced with amplification primers and primers bla_{TEM}-F2 (5′-TAA CCA TGA GTG ATA ACA CT-3′) and

bla$_{TEM}$-R2 (5′-CCG ATC GTT GTC AGA AGT AA-3′). To prevent carryover contamination, the Uracil system (Roche) was applied. Both outbreak strains (*E. cloacae* and *A. baumannii*) proved to harbor the SHV-12 ESBL gene (12) and the TEM-116 beta-lactamase gene (http://www.lahey.org/studies/webt.htm). These two genes were also present in 18 of the 29 nonoutbreak gram-negative bacteria (Table 1).

Plasmids were isolated with the QIAgen Plasmid Midi kit (QIAGEN, Westburg B.V., The Netherlands) and were analyzed by 0.7% agarose Tris-borate-EDTA gel electrophoresis. Most isolates contained multiple plasmids, but a plasmid of approximately 65 kb in size was observed in the outbreak strains and in 18 nonoutbreak gram-negative bacteria. Transformation of plasmids from both outbreak strains and two other MDR gram-negative bacteria to *Escherichia coli* K-12 strains conveyed resistance to aminoglycosides and cephalosporins. All transformants shared a 65-kb plasmid, indicating that this plasmid contained genes for aminoglycoside resistance and for ESBL production. The 65-kb plasmid of a recombinant strain was digested with EcoRV and fragments were subcloned in pUC19. Selection for ceftazidime resistance yielded a clone with an insert of approximately 20 kb. The nucleotide sequences of both ends of the insert were determined with M13 sequencing primers specific for the cloning vector. One end of the insert contained the 5′ end of the *aac(3)-II* gene, which confers aminoglycoside resistance, followed by the 3′ end of the *bla*$_{TEM-116}$ gene. The opposite end of the insert contained two putative open reading frames, ORF20 and ORF21, which are present in the known antibiotic resistance plasmids pEL60 and pCTX-M13 (GenBank accession numbers AY422214 and AF550415, respectively). Based on these sequences, five new primers specific for the insert were developed (ABNN01 [5′-GAT ATC TCC TCT AAA CTG CAA A], ABNN02 [5′-GTT TAC TCA TAT ATA CTT TAG ATT], ABNN03 [5′-CGT AAC GCG GCA ACG ACC GTC T], ABNN04 [5′-CTG ATA GCC GGG CGA AAT CA], and ABNN05 [5′-ATG ATT AAG ATG GTT AGC GTC GTT]) and used for PCR on isolates. By using combinations of these and *bla*$_{TEM}$-F2 primers in independent PCRs, we determined that similar plasmids with identical organizations of *bla*$_{TEM}$ and *aaC(3)-II* and of ORF20 and ORF21, respectively, were present in the outbreak strains and in five other gram-negative isolates of four different species (Table 1). The data suggest that widespread ongoing dissemination of a similar plasmid among strains in the ICU ward occurred and supports previous published reports on (interspecies) plasmid transfer (13, 17).

The infection control measures taken after the *E. cloacae* outbreak, including extra attention for hand hygiene, wearing gloves and gowns during patient care activities, and isolation of patients who had MDR *E. cloacae* infections in private rooms, seem to have failed to prevent the clonal outbreak of MDR *A. baumannii* bacteria. Other MDR gram-negative bacilli may have acted as a reservoir for the resistance plasmid. Since the infection control measures were directed only at preventing the spread of the resistant *E. cloacae* strain, cross-infection with other strains was still possible. The subsequent outbreak of MDR *A. baumannii* bacteria led to stricter infection control measures: (i) control measures which were extended to all patients in the ICU, (ii) closure of the ICU to new admissions, and (iii) a dedicated nursing team for the patients colonized with the resistant strain. These measures were successful in halting the *A. baumannii* outbreak. A significant decrease in the prevalence of all MDR gram-negative bacilli was noted at the same time.

In conclusion, our study shows not only that plasmid outbreaks may go unrecognized but also that stringent measures directed against all MDR gram-negative bacilli can be effective in controlling such outbreaks.

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REFERENCES

1. **Bradford, P. A.** 2001. Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin. Microbiol. Rev. **14:**933–951.
2. **de Jonge, E., M. J. Schultz, L. Spanjaard, P. M. Bossuyt, M. B. Vroom, J. Dankert, and J. Kesecioglu.** 2003. Effects of selective decontamination of digestive tract on mortality and acquisition of resistant bacteria in intensive care: a randomised controlled trial. Lancet **362:**1011–1016.
3. **Dzidic, S., and V. Bedekovic.** 2003. Horizontal gene transfer-emerging multidrug resistance in hospital bacteria. Acta Pharmacol. Sin. **24:**519–526.
4. **Gniadkowski, M., A. Palucha, P. Grzesiowski, and W. Hryniewicz.** 1998. Outbreak of ceftazidime-resistant *Klebsiella pneumoniae* in a pediatric hospital in Warsaw, Poland: clonal spread of the TEM-47 extended-spectrum beta-lactamase (ESBL)-producing strain and transfer of a plasmid carrying the SHV-5-like ESBL-encoding gene. Antimicrob. Agents Chemother. **42:**3079–3085.
5. **Heritage, J., P. A. Chambers, C. Tyndall, and E. S. Buescher.** 2003. SHV-34: an extended-spectrum beta-lactamase encoded by an epidemic plasmid. J. Antimicrob. Chemother. **52:**1015–1017.
6. **Jacoby, G. A., and L. S. Munoz-Price.** 2005. The new beta-lactamases. N. Engl. J. Med. **352:**380–391.
7. **Jarlier, V., M. H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum beta-lactamases conferring transferable resistance to newer beta-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
8. **Livermore, D. M.** 2003. Bacterial resistance: origins, epidemiology, and impact. Clin. Infect. Dis. **36:**S11–S23.
9. **Livermore, D. M., and D. F. Brown.** 2001. Detection of beta-lactamase-mediated resistance. J. Antimicrob. Chemother. **48**(Suppl. 1)**:**59–64.
10. **Mabilat, C., and S. Goussard.** 1993. PCR detection and indentification of genes for extended-spectrum β-lactamases, p. 553–559. *In* D. H. Persing, T. F. Smith, F. C. Tenover, and T. J. White (ed.), Diagnostic molecular microbiology: principles and applications. American Society for Microbiology, Washington, D.C.
11. **Neuwirth, C., E. Siebor, J. Lopez, A. Pechinot, and A. Kazmierczak.** 1996. Outbreak of TEM-24-producing *Enterobacter aerogenes* in an intensive care unit and dissemination of the extended-spectrum β-lactamase to other members of the family *Enterobacteriaceae*. J. Clin. Microbiol. **34:**76–79.
12. **Nuesch-Inderbinen, M. T., F. H. Kayser, and H. Hachler.** 1997. Survey and molecular genetics of SHV beta-lactamases in *Enterobacteriaceae* in Switzerland: two novel enzymes, SHV-11 and SHV-12. Antimicrob. Agents Chemother. **41:**943–949.
13. **Palucha, A., B. Mikiewicz, W. Hryniewicz, and M. Gniadkowski.** 1999. Concurrent outbreaks of extended-spectrum beta-lactamase-producing organisms of the family Enterobacteriaceae in a Warsaw hospital. J. Antimicrob. Chemother. **44:**489–499.
14. **Rasheed, J. K., C. Jay, B. Metchock, F. Berkowitz, L. Weigel, J. Crellin, C. Steward, B. Hill, A. A. Medeiros, and F. C. Tenover.** 1997. Evolution of extended-spectrum beta-lactam resistance (SHV-8) in a strain of *Escherichia coli* during multiple episodes of bacteremia. Antimicrob. Agents Chemother. **41:**647–653.
15. **Savelkoul, P. H., H. J. Aarts, J. de Haas, L. Dijkshoorn, B. Duim, M. Otsen, J. L. Rademaker, L. Schouls, and J. A. Lenstra.** 1999. Amplified-fragment length polymorphism analysis: the state of an art. J. Clin. Microbiol. **37:**3083–3091.
16. **Sturenburg, E., and D. Mack.** 2003. Extended-spectrum beta-lactamases: implications for the clinical microbiology laboratory, therapy, and infection control. J. Infect. **47:**273–295.
17. **Wu, T. L., J. H. Chia, L. H. Su, A. J. Kuo, C. Chu, and C. H. Chiu.** 2003. Dissemination of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in pediatric intensive care units. J. Clin. Microbiol. **41:**4836–4838.