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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Isolate\textsuperscript{a} & Species & Mo of isolation & Patient (identification no.) & Site or source of isolation & Presence of\textsuperscript{b}: & Beta-lactamase gene encoding enzyme & Similar plasmid with five PCR reactions \\
& & & & & ESBL phenotype & 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 & June & 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 & -- & + & + & -- \\

\textsuperscript{a} +, present; --, absent.  
\textsuperscript{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 ceftifipime 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\textsubscript{TEM} gene was amplified with primers SHV-F1 (5\textsuperscript{'}-CTT TAC TCG CCT TTA TCG-3\textsuperscript{'})) and SHV-R1 (5\textsuperscript{'}-TCC CGC AGA TAA ATC ACC A-3\textsuperscript{'})) and sequenced with amplification primers and primers bla\textsubscript{SHV}-F2 (5\textsuperscript{'}-ACT GCC TTT TTG CGC AGA TAA ATC ACC A-3\textsuperscript{'})) and bla\textsubscript{SHV}-R2 (5\textsuperscript{'}-CAG TTC CTT TTC CCA GCG GT-3\textsuperscript{'})). An 862-bp gene fragment of bla\textsubscript{TEM} was amplified with primers TEM-F1 (5\textsuperscript{'}-ATG AGT ATG TAA CAT TTC CG-3\textsuperscript{'})) and TEM-R1 (5\textsuperscript{'}-GAC AGT TAC CAA TGC TTA ATC A-3\textsuperscript{'})) (14) and sequenced with amplification primers and primers bla\textsubscript{TEM}-F2 (5\textsuperscript{'}-TAA CCA TGA GTG ATA ACA CT-3\textsuperscript{'})) and
**blₐTEM-R** (5'-CCG ATC GTG 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 cefazidime 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 blaTEM-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 pELO0 and pCTX-M13 (GenBank accession numbers AY422214 and AF550415, respectively). Based on these sequences, five new primers specific for the insert were developed (ABNNO1 [5'-GAT ATC TCC TCT AAA CTG CAA A], ABNNO2 [5'-GTG TAC TCA TAT ATG GTT TAG ATT], ABNNO3 [5'-CGT AAG GCC GCA ACC AGC GTC T], ABNNO4 [5'-CTG ATC GTA GCC GGG CGA AAT CA], and ABNNO5 [5'-ATG ATT AAG ATG GTT AGC GTC GTT]) and used for PCR on isolates. By using combinations of these and blaTEM-F2 primers in independent PCRs, we determined that similar plasmids with identical organizations of blaTEM 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 unpublished 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**