**CONCISE COMMUNICATION**

*Enterobacter* Species in a Pediatric Hospital: Horizontal Transfer or Selection in Individual Patients?

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*Enterobacter* species were studied longitudinally in a children’s hospital. In total, 287 *Enterobacter* isolates were obtained from 171 children in 15 different wards (from March 1995 through April 1997). Strains were typed by random amplified polymorphic DNA and pulsed-field gel electrophoresis, which were concordant in outcome. In total, 97 DNA types and 199 colonization events were identified. A predominant clone was isolated 111 times from 62 children; another clone was isolated 19 times from 10 patients. These clones caused 36% of all colonizations. In 34% of the children, *Enterobacter* clones were found in 2–4 patients. The remaining colonizations were due to unique *Enterobacter* isolates. A large proportion of the *Enterobacter* strains was acquired through cross-transmission. This finding contrasts with the prevailing opinion that resistant *Enterobacter* strains are selected primarily from the patient’s own gut flora.

*Enterobacter* species cause nosocomial infections and more frequently colonize hospitalized patients [1]. In addition, the frequent use of β-lactam antibiotics exerts a selective pressure toward β-lactamase–producing *Enterobacter*, resulting in an overgrowth of the patient’s resident flora [2]. Thus, resistant *Enterobacter* species appear in cultures derived from clinical specimens, even without the necessity of patient-to-patient transmission. However, outbreak analyses have revealed clonal relatedness of bacterial strains from different patients. This implicates cross-transmission, sometimes even nationwide [3]. Preventive measures to stop the spread of these potential pathogens clearly are warranted [4].

Children in pediatric hospitals often have an enhanced susceptibility to infection with *Enterobacter* species, which cause a variety of infections [5]. The capacity of *Enterobacter* species to spontaneously derepress antibiotic resistance genes and the resulting resistance toward many β-lactam antibiotics complicates the treatment of such infections [6]. Within the hospital setting, there is a need to prevent colonization with these potentially pathogenic bacteria. This requires knowledge of the mechanism(s) of emergence of *Enterobacter* species in the hospital. The proportion of *Enterobacter* infections due to spread of hospital clones versus those due to strains selected from the intestinal flora of individual patients has yet to be determined. Our prospective hospital-based study was initiated to estimate the contribution of both mechanisms in a pediatric hospital. The study was undertaken at a time when infections caused by *Enterobacter* species were occurring in epidemic levels.

**Materials and Methods**

**Clinical setting.** This study was performed in the 310-bed Sophia Pediatric Hospital of the Erasmus University Medical Center Rotterdam (Rotterdam, The Netherlands). Fifteen different wards participated, but the most important (with respect to the number of patients involved and the number of positive cultures obtained) were general pediatrics, surgery, and the intensive care units (ICUs) of the Departments of Neonatology, Pediatrics, and Pediatric Surgery.

**Bacterial isolates.** Surveillance samples were obtained from various body sites, although ~75% were rectal swabs. The other 25% were derived from presumed infectious foci. In total, 287 *Enterobacter* strains were collected from 171 patients from 6 March 1995 through 25 March 1997. All strains cultured were included, and a wide variety of clinical and surveillance samples was investigated. Bacteria were isolated by standard procedures and were identified to the species level by use of MicroScan technology (Dade International). For all patients, except those in ICUs, only the first isolate was included for further analysis. For patients in ICUs, the last isolate also was analyzed.

**DNA isolation.** Strains were grown overnight on Brucella blood agar. Three to 5 colonies were suspended in 150 μL of 25 mM Tris-HCl (pH 8.0), 10 mM EDTA, and 50 mM glucose and were treated...
with lysozyme. DNA was purified, according to the method of Boom et al. [7], and was stored for various periods at −20°C.

Random amplification of polymorphic DNA (RAPD). For each test, we combined 50 ng of bacterial DNA with a buffer containing 10 mM Tris·HCl (pH 9.0), 50 mM KCl, 2.5 mM MgCl₂, 0.01% gelatin, 0.1% Triton X-100, 0.2 mM dNTPs, and 0.2 U Taq polymerase (HT Biotechnology). Finally, 50 pmol of RAPD1 (5′-GGTT-GGCGAGGACG-3′) or enterobacterial repetitive intergenic consensus sequence 2 (5′-AAGTAAGTGACTGGGTTGAGAATTGCACG-3′) was included [8]. Amplifications were performed in a BioMed thermocycler (model 60) with predenaturation at 94°C, followed by 40 1-min cycles at 94°C, 1 min at 25°C, and 2 min at 74°C. Amplicons were size separated on 1% agarose gels in 0.5× Tris-borate-EDTA (TBE) buffer. After ethidium-bromide staining and photography (Mitsubishi p67E copy processor; Progress Control), digital images were manipulated by ColorVision software (Cocoon Software). DNA patterns were compared visually for similarity by 2 different persons, and a single genetic code was established for all strains. Differences in a single band position in either of the 2 patterns led to a novel combined RAPD type. RAPD data were interpreted without the knowledge of the origin of the strains or of the pulsed-field gel electrophoresis (PFGE) type (see below).

PFGE. Strains were grown on Brucella blood agar. A colony was suspended in 100 µL of 10 mM Tris·HCl (pH 8.0), 100 mM EDTA, and 10 mM EGTA and was mixed with an equal volume of 1% low-melting-point agarose (FMC Bioproducts) in 0.5× TBE. Plugs were treated with SDS and proteinase K [9]. After equilibration in restriction buffer, plugs were incubated for 24 h at 37°C in the presence of 50 U of XbaI (Boehringer Mannheim). Macrorestriction fragments were separated at 14°C with a contour-clamped homogeneous electric field (CHEF) mapper (BioRad) programmed in the auto-algorithm mode. Field strength was 6 V/cm, and switch time linearly increased from 5 to 35 s during 20 h. Gels were stained and photographed with the Mitsubishi p67E copy processor. Banding patterns were interpreted without prior knowledge of the origin of the strains.

Results

The first _Enterobacter_ isolate from a given patient was obtained 11.3 days (SD, 15.7 days; median, 5 days) after admission, which is suggestive of frequent nosocomial colonization. We collected 287 _Enterobacter_ isolates from 171 patients. Of these, 196 appeared to be _E. cloacae_, 16 were _E. agglomerans_, and 7 were _E. sakazakii_. For 68 strains, identification to the species level with the MicroScan system was reliable, but not to the genus level. In all, 50% of the strains were resistant toward third-generation cephalosporins, including cefotaxime (data not shown). Cumulative results synthesized from the multiperson interpretation of the analysis by use of the 2 RAPD primers resulted in ~100 RAPD types. PFGE analysis recorded 79 different types; 2 strains were not typeable. When both types of restriction fragment length polymorphism patterns were evaluated simultaneously, 81 RAPD types and 74 PFGE types remained. Inclusion of both the PFGE and the RAPD data into a single character resulted in 97 distinct DNA types.

Some of the 171 patients were colonized with ≥1 bacterial genotype, resulting in 199 colonizations. A predominant clone was isolated 111 times from 62 different children. Another major clone was isolated 19 times from 10 patients. Four other strains colonized 4 patients each. Another 5 clones colonized

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<th>RAPD1 type</th>
<th>ERIC-2 type</th>
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Note. In total, 199 colonization events were defined. Minor clone g is similar to major clone I and could possibly be considered to be a subtype of this clone. Occurrence of various strains on the basis of colonization incidence are shown in “Colonization” column. Because multiple colonizations were observed, the figures are smaller than those for individual patients. ERIC-2, enterobacterial repetitive intergenic consensus sequence 2; PFGE, pulsed-field gel electrophoresis; RAPD, random amplified polymorphic DNA; ■, clone that colonized 62 children; ▲, clone that colonized 10 children; □, unique isolates in 73 children.

a No. per clone.
3 patients each; 11 types were found in 2 patients. Seventy-four children were colonized by *Enterobacter* species not found in other patients (for unique isolates, see table 1 and figure 1). The first isolation of the dominant clone occurred in week 16, but the massive spread of that clone started at week 30 and seemed to wane toward the end of the study. The prevalent clone that colonized 10 patients first occurred at week 30 and was last seen at week 51. The smaller sets of genetically related *Enterobacter* species also were clustered by time and ward. This corroborates the reliability of the results of both independent DNA typing strategies. The *Enterobacter* strains were isolated most frequently from the neonatal ICU and from the general and surgical ICUs. Also, the spread of the dominant clones was most prominent in these units, which had only 60 of the 310 hospital beds. When the 2 most dominant strains were subtracted, the incidence of *Enterobacter* colonization was at a constant level.

A quantitative analysis of the 199 *Enterobacter* colonizations showed that 72 (36%) were caused by the 2 major clones, and 34% of all colonizations were caused by clones detected in 2 of 4 patients. The 74 colonizations by unique isolates made up only 35% of all colonizations.

**Discussion**

Fundamental to *Enterobacter* colonization and infection is that strains readily generate novel resistance types, especially in the presence of selective antimicrobial agents [1]. Colonization or infection by multiresistant *Enterobacter* organisms might be further facilitated by low concentrations of antibiotics that induce elevated adhesion capacities [10]. *Enterobacter* colonization may initially seem to be insignificant, but it may be an omen of massive cross-transmission in a clinical setting, with possibly enhanced morbidity and mortality [11]. When antibiotic usage against gram-negative rods, such as *Enterobacter* species, is modulated, resistance development can be circumvented [4].

In this study we documented the spread of *Enterobacter* species in the intensive care wards of a pediatric university hospital involving many patients. To track bacterial spread, several molecular typing tools have been used previously for *Enterobacter* organisms in the recent past. Ribotyping [12], repetitive extragenic palindromic polymerase chain reaction [9], RAPD analysis [12], and PFGE [3] have been used frequently, either alone or in combination. This illustrates the robustness of these methods and suggests that the clusters of strains that we encountered have been identified adequately. It is alarming that clonal spread of *Enterobacter* species can occur throughout a hospital in a narrow time frame (figure 1). This suggests that, in children’s hospitals where patients are mobile, microbial barriers should be strictly enforced. We suggest that colonization pressure (i.e., the proportion of other patients colonized), as defined by Bonten et al. [13], played an important role in the clonal expansion documented in our hospital.

Some 35% of the colonizations were caused by bacterial genotypes that were not found in other patients, as defined by genetic typing. This scenario also has been documented during nonoutbreak periods in other institutions [14]. About 36% of all colonizations were caused by 2 major clones that colonized 62 and 10 patients, respectively. This suggests that these strains are highly transmissible. Unfortunately, in our setting, no com-

![Figure 1](image-url)

**Figure 1.** Colonization of individual pediatric patients by clonally related or independent *Enterobacter* isolates at Sophia Children’s Hospital (Rotterdam, The Netherlands). Horizontal bar indicates duration of screening period (weeks). Various departments (elaborated in Materials and Methods) are depicted separately. ic, Intensive care.
mon (environmental) source could be identified. Other investigations successfully have identified hand-to-hand contact [15], food [16], and various other sources of infection (summarized in [1]). Preliminary observations suggest that the major clone (type I; table 1) has a higher attachment affinity for various human cell surfaces (W. Gaastra, University Medical Center, Utrecht, The Netherlands, personal communication). Whether this is a sufficient explanation for the observed spread is subject to further investigations.

The remaining third of the colonizations occurred with DNA types of Enterobacter species that colonized small groups of patients (2–4 patients/clone). This type of small-scale dissemination of certain genotypes was detected adequately by both genotyping procedures. Prolongation of the epidemiology study in the neonatology ICU for 3 months after the study period showed persistence of the epidemic type. During these months, 78 additional isolates were obtained, of which 42 (54%) presented with the epidemic genotype (data not shown). This clearly shows that, in the neonatal ICU, there is frequent exchange and long-term persistence of strains.

Although 3 basic mechanisms of colonization were identified, there was primarily a massive spread of a limited number of major clonal types. There also was a limited spread of a larger number of genotypes. Finally, apparent selection of unique types from the endogenous flora of the patient accounted for one-third of all culture-positive colonizations. Thus, it appears as if stopping the clinical threat posed by multiresistant Enterobacter species requires a 2-fold preventive approach. Both the restricted use of antibiotics and the level of hospital hygiene need critical appraisal.Transient colonization of health care workers’ hands and inanimate surfaces because of lapses in infection control should be prevented. To achieve the ultimate goal, suppression of spread of multiresistant Enterobacter species, large typing studies such as ours, may offer insight into whether our observations are general or restricted to the Rotterdam hospital setting. Several smaller studies suggest the first option is the most likely (reviewed in [17]), but a recent detailed review came to the opposite conclusion, claiming that the overwhelming majority of strains are selected individually [1]. Our evidence strongly suggests that clonal spread may largely explain the incidence of multiresistant Enterobacter species in some clinical settings.

Acknowledgments

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References