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Molecular Epidemiology of Acquisition of Ceftazidime-Resistant Gram-Negative Bacilli in a Nonoutbreak Setting

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We prospectively studied the acquisition of ceftazidime-resistant gram-negative bacilli (CAZ-RGN) in two surgical intensive care units (SICU) during a nonoutbreak period. Surveillance cultures were obtained from patients at the time of admission and serially thereafter. CAZ-RGN isolates were typed by pulsed-field gel electrophoresis (PFGE). Three hundred and forty-three patients were enrolled from whom 1,621 baseline and follow-up cultures were obtained. The most common species isolated from patients were *Pseudomonas aeruginosa* (22), *Enterobacter cloacae* (21), *Acinetobacter* spp. (13), *Enterobacter aerogenes* (11), *Citrobacter* spp. (10), *Pseudomonas* spp. (non *P. aeruginosa*) (9), and *Stenotrophomonas* spp. (7). For each species, PFGE strain types were highly diverse; no single type was recovered from more than four patients. Twenty-eight patients acquired a CAZ-RGN during the SICU stay; in six (21%), emergence of resistance from a previously susceptible strain was documented on the basis of matching serial strain types. Transmission of CAZ-RGN between patients occurred but was infrequent, as judged by analyzing strain types of epidemiologically linked patients. In conclusion, colonization with CAZ-RGN in SICU was associated with diverse species and strains, as determined by molecular typing. Emergence of resistance from previously susceptible strains appeared to be more important than horizontal transmission in acquisition of CAZ-RGN in a nonoutbreak period.

Since the introduction of broad-spectrum cephalosporins, the prevalence of gram-negative pathogens resistant to third-generation cephalosporins has been increasing (1). Infections due to these resistant pathogens cause considerable morbidity and mortality, and treatment options are limited (5).

Colonization with nosocomially acquired resistant organisms can occur via transmission from other infected or colonized patients or from exposure to environmental and personnel reservoirs. Alternatively, colonization with resistant gram-negative bacilli (RGN) can arise endogenously; previously susceptible gram-negative organisms in patients' gastrointestinal flora can develop resistance through the induction or stable derepression of group 1 chromosomal beta-lactamases upon exposure to broad-spectrum cephalosporins (11). The contribution of in vivo emergence of resistance to the overall prevalence of RGN in a nonoutbreak setting is not known. Studies of RGN outbreaks using molecular typing techniques have demonstrated that colonization and/or infection during an outbreak occurs through the dissemination of a single strain (4, 7, 14). In contrast, the molecular epidemiology of RGN during periods of endemicity has not been clearly defined.

To address these issues, we conducted a prospective cohort study of colonization with ceftazidime (CAZ)-RGN among patients in surgical intensive care units (SICU). The goals of this study were to examine modes of transmission of CAZ-RGN and to assess the frequency of exogenous acquisition versus emergence of resistance from endogenous flora as a mechanism of colonization with CAZ-RGN. Strain typing was performed by a highly discriminatory molecular typing method, pulsed-field gel electrophoresis (PFGE).

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MATERIALS AND METHODS

Patients admitted to two SICU at the Deaconess Hospital, a 431-bed tertiary referral hospital, between 15 January and 15 June 1995 were enrolled into the study. By using Starplex swabs (Etobicoke, Ontario, Canada), baseline surveillance cultures were collected from patients within 24 h of admission to the SICU. Specimens were obtained at the following sites: throat, rectum, inguinal fold, and nasogastric fluid, if a nasogastric tube was present. Follow-up surveillance cultures were collected at regular intervals for the duration of the patient's SICU admission (3 to 4 days, 7 to 8 days, 15 to 16 days, and every 2 weeks thereafter). Verbal consent was obtained from all patients or their relatives prior to specimen collection. A patient was classified as having acquired a CAZ-RGN if a CAZ-RGN was not isolated on baseline cultures but was detected from any of the follow-up cultures or if a different CAZ-RGN species was isolated from follow-up cultures than one isolated from baseline cultures. Patients were considered to be epidemiologically linked if they were admitted to the same SICU within a 14-day period.

Isolates. All swabs were streaked onto MacConkey medium supplemented with 1 μg of CAZ (Difco, Detroit, Mich.)/ml. A low concentration of CAZ was used to inhibit the growth of susceptible gram-negative pathogens yet still allow the growth of extended-spectrum beta-lactamase-producing gram-negative pathogens required for a parallel study. Swabs were frozen and stored at −70°C for future recovery of sensitive isolates. After 24 to 48 h of incubation at 35°C representative colonies with a morphology consistent with gram-negative organisms were subcultured onto MacConkey agar (Remel, Lenexa, Kans.). Species identification and MICs were determined by using Microscan (types 2 and 8, respectively) (Baxter, Parsippany, N.J.). All isolates were frozen and stored at −70°C for future strain typing. CAZ resistance was defined as an MIC of ≥16 μg/ml.

Among patients who acquired a CAZ-RGN, specimens obtained prior to the first positive culture for CAZ-RGN were thawed and streaked onto unsupplemented MacConkey agar in order to isolate susceptible gram-negative organisms. After overnight incubation at 35°C, species identification and susceptibility testing were conducted on representative colonies. CAZ-susceptible and CAZ-resistant isolates of the same species recovered from the same patient were typed by PFGE (see below). Antibiotic exposures for these patients were obtained by using computerized pharmacy databases.

PFGE. All isolates were grown overnight on blood agar plates at 35°C. Single colonies were inoculated into 5 ml of brain heart infusion broth (Difco) and incubated overnight at 35°C. Agarose plugs were prepared as described by Maslow et al. (9).

Genomic DNA was digested with 50 U of *Apa*I for *Acinetobacter* spp., 20 U of *Spe*I for *Pseudomonas* spp. and *Serratia* spp., and 20 U of *Xba*I for *Enterobacter*

spp., *Stenotrophomonas* spp., and *Citrobacter* spp., according to the manufacturer's recommendations (New England Biolabs, Beverely, Mass.). Fragments of DNA were separated in a 1% agarose gel and run in 0.5 M Tris-EDTA at 6 V/cm on a contour-clamped homogeneous electric field apparatus (CHEF DRIII; Bio-Rad Laboratories, Richmond, Calif.). Pulse times were from 5 to 8 s for 24 h for *Acinetobacter* spp., 0.5 to 35 s for 22 h for *Pseudomonas* spp., 5 to 35 s for 20 h for *Stenotrophomonas* spp., 5 to 50 s for 20 h for *Enterobacter* spp. and *Citrobacter* spp., and 5 to 60 s for 22 h for *Serratia* spp. (3, 8, 9, 13). The gels were stained with ethidium bromide and photographed under UV light.

Isolates were assigned to the same group (type) if their restriction patterns were indistinguishable or differed by only two or three bands, consistent with a single genetic event. Isolates with restriction profiles differing by more than three bands were assigned to distinct groups (12).

Distinct species of CAZ-RGN from each patient were analyzed by PFGE. The majority of CAZ-RGN species recovered from fewer than five patients were not typed with the exception of serial susceptible and resistant isolates.

RESULTS

A total of 416 patients were admitted to the SICU. Seventy-three patients were excluded due to patient refusal to participate or death or discharge from the SICU within 24 h of admission. Three hundred and forty-three patients had baseline cultures. Of these 343 patients, 100 remained in the SICU for ≥3 days and had follow-up cultures for the duration of their SICU stay (62 patients at day 3 to 4, 23 at day 7 to 8, 14 at day 15 to 16, and 1 at 30 days).

Patient isolates. During the 5-month study period, a total of 1,621 baseline and follow-up cultures were collected. Eighty-six patients had at least one positive culture; the first positive cultures were at baseline for 64 patients and on follow-up cultures for 22 patients. Patients colonized at baseline were usually persistently colonized with the same organism species; of 23 patients colonized at baseline that had follow-up cultures, 19 were colonized with the same species on follow-up. Including both baseline and follow-up cultures, 19 patients had more than one CAZ-RGN species isolated (four species in 2 patients, three species in 3 patients, and two species in 14 patients). Thus, 118 CAZ-RGN species were recovered from 86 patients. The set of CAZ-RGN included the following species, counting each species per patient once: *Pseudomonas aeruginosa* (22), *Enterobacter cloacae* (21), *Acinetobacter* spp. (13), *Enterobacter aerogenes* (11), *Citrobacter* spp. (10), non-*P. aeruginosa* pseudomonads (9), *Stenotrophomonas* spp. (7), *Klebsiella* spp. (4), *Hafnia* spp. (4), *Serratia* spp. (3), *Escherichia coli* (3), *Alcaligenes* spp. (3), *Morganella* spp. (2), *Enterobacter agglomerans* (1), *Proteus* sp. (1), CDC group 4C2 (1), and gram-negative non-lactose fermenters (3), the species of which could not be determined by Microscan.

Ninety-one isolates were examined by PFGE (53 from baseline cultures and 38 from follow-up cultures). Twenty-three isolates representing species recovered from fewer than five patients were not typed. Four other isolates could not be adequately digested (*E. aerogens* [1], *Citrobacter* sp. [1], and non-*P. aeruginosa* pseudomonads [2]). Types were highly diverse (number of strain types/number of isolates): *P. aeruginosa*, 22/22; *E. cloacae*, 21/21; *Acinetobacter* spp., 10/13; *E. aerogenes*, 9/10; *Citrobacter* spp., 9/9; non-*P. aeruginosa* pseudomonads, 7/7; *Stenotrophomonas* spp., 6/7; and *Serratia* spp., 2/2. All isolates recovered from baseline cultures were unique types (Fig. 1 to 3; Table 1).

Acquisition. Thirty-one isolates were acquired by 28 patients during the SICU admission. Six of these patients were colonized with a CAZ-RGN on baseline cultures but acquired a different species on follow-up. From these 28 patients who acquired a CAZ-RGN, stored baseline and follow-up specimens collected prior to acquisition were recultured in order to identify CAZ-susceptible gram-negative bacilli. One hundred and eighty-eight of the 236 stored swabs were positive for susceptible organisms. Of these, seven CAZ-susceptible isolates from seven patients (27%) were of the same species as the acquired CAZ-resistant isolate. Six of seven susceptible and resistant isolate pairs were indistinguishable by PFGE. The species were as follows: *Serratia* spp. (2), *P. aeruginosa* (2), *E. cloacae* (1), and *E. aerogenes* (1) (Fig. 4). Strains were recovered from the inguinal fold (2), nasogastric fluid (2), rectum (1), and throat (1). All six patients received beta-lactam antibiotics during the interval between SICU admission and acquisition. The antibiotics received were as follows (number of patients): CAZ (1), cefuroxime (1), ceftriaxone (1), imipenem (1), and cefazolin (2). The duration of exposure ranged from 1 to 15 days. These six patients were not epidemiologically linked

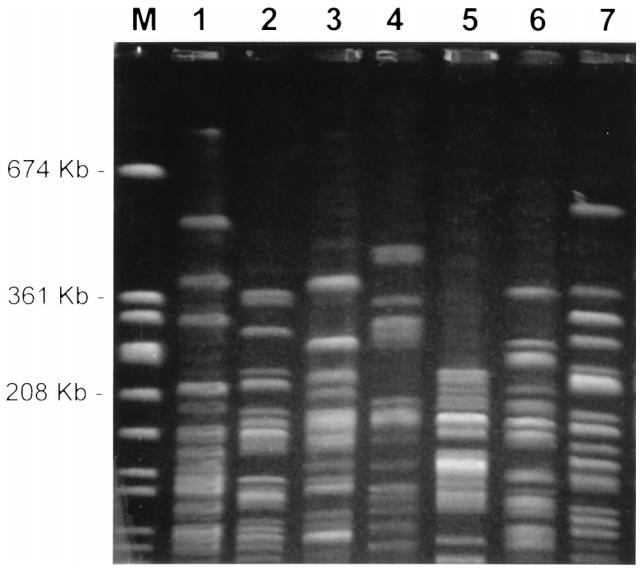

FIG. 1. PFGE types of CAZ-resistant *Citrobacter* sp. isolates. Lane M, *Staphylococcus aureus* ATCC 8325 size marker; lanes 1 to 7, isolates from different patients illustrating diversity of strains.

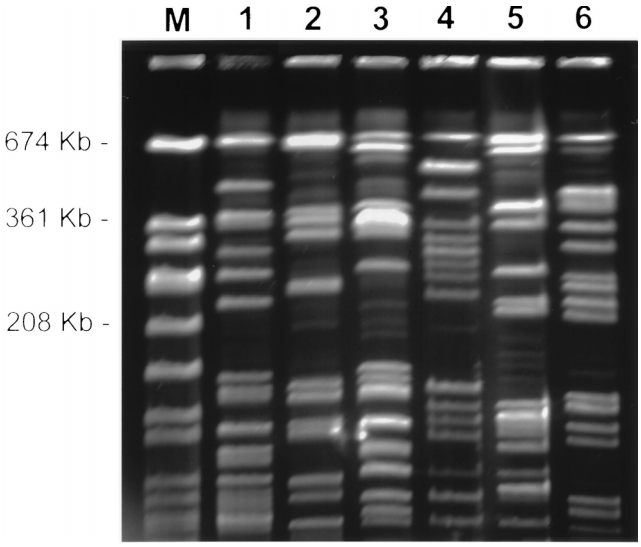

FIG. 2. PFGE types of CAZ-resistant *Stenotrophomonas* sp. isolates. Lane M, *S. aureus* ATCC 8325 size marker; lanes 1 to 6, isolates from different patients illustrating diversity of strains.

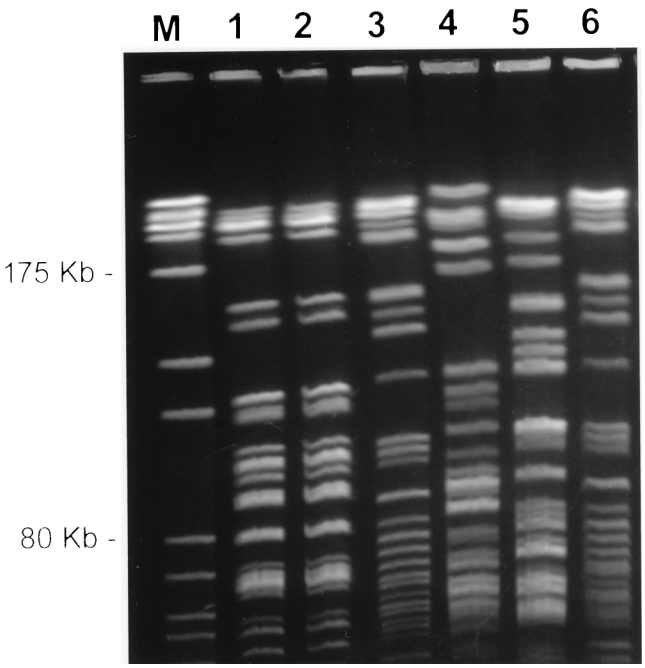

FIG. 3. PFGE types of CAZ-resistant *Acinetobacter* sp. isolates. Lane M, *S. aureus* ATCC 8325 size marker; lanes 1 and 2, isolates representing the same strain type from two epidemiologically linked patients; lanes 3 to 6, isolates from different patients illustrating diverse strain types.

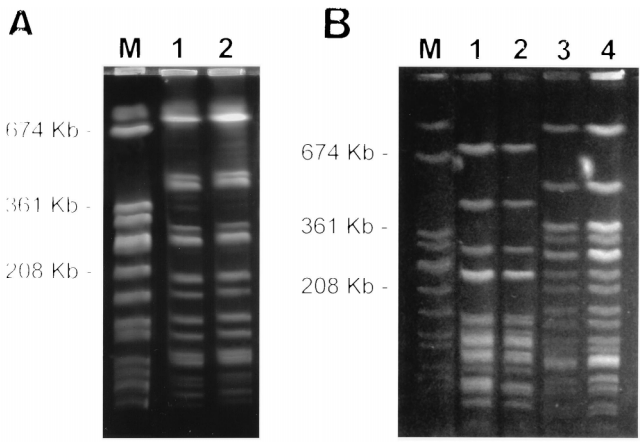

FIG. 4. (A) PFGE types of *E. cloacae* isolates. Lane M, *S. aureus* ATCC 8325 size marker; lanes 1 and 2, serial CAZ-susceptible and CAZ-resistant isolates, respectively, from an individual patient representing indistinguishable PFGE profiles. (B) PFGE types of *Serratia* sp. isolates. Lane M, size marker *S. aureus* ATCC 8325; lanes 1 and 2, serial CAZ-susceptible and CAZ-resistant isolates, respectively, belonging to the same strain type from a single patient; lanes 3 and 4, serial CAZ-susceptible and CAZ-resistant isolates, respectively, with indistinguishable PFGE profiles from another patient.

to other patients who were colonized with the same species of CAZ-RGN.

Thus, 25 acquired resistant isolates from 22 patients could not be linked to a susceptible strain from prior cultures. The species of these isolates were as follows: *Acinetobacter* spp. (6), *P. aeruginosa* (8), *Stenotrophomonas* spp. (4), *E. cloacae* (2), *E. aerogenes* (1), *Citrobacter* sp. (1), non-*P. aeruginosa* pseudomonads (1), *Alcaligenes* sp. (1), and CDC group 4C2 (1). Acquisition was detected at the following sites: rectum (10), inguinal fold (8), throat (6), and nasogastric fluid (3). Two patients acquired a resistant organism at more than one site (rectum and inguinal fold for both). Of these 22 patients, 19 were epidemiologically linked to another patient colonized with the same species of CAZ-RGN. However, typing by PFGE revealed that only two (9%) linked pairs of isolates had an indistinguishable restriction profile (*Acinetobacter* sp. and *E. aerogenes*).

TABLE 1. CAZ-RGN isolated from five or more patients

Species	No. of CAZ-RGN	
	No. of isolates[a]	No. of strain types[a]
P. aeruginosa	14/22	14/22
E. cloacae	19/21	19/21
Acinetobacter spp.	7/13	7/10
E. aerogenes	9/10	9/9
Citrobacter spp.	8/9	8/9
Non-*P. aeruginosa* pseudomonads	6/7	6/7
Stenotrophomonas spp.	3/7	3/6

[a] Baseline species/baseline species plus exogenously acquired species.

DISCUSSION

We conducted a prospective surveillance study of CAZ-RGN colonization by collecting epidemiologically linked patient isolates from two SICU during a 5-month period. The purpose of the study was to gain a better understanding of the epidemiology of CAZ-RGN colonization in a nonoutbreak setting. A highly discriminatory typing method was used to assess transmission and sources of acquisition.

The results of this study highlight the complexity of the epidemiology of CAZ-RGN. First, CAZ-RGN from patients encompassed multiple species, each of which was found to be highly diverse by PFGE. Most patients with CAZ-RGN were already colonized at the time of admission to the SICU, and each of these patients was colonized with a unique strain type. This finding suggests that patients admitted to the SICU represent ongoing sources of new bacterial strains, presumably acquired in other wards or institutions. Second, for patients in whom a new CAZ-RGN organism was acquired during the SICU admission, both emergence of resistance from previously susceptible strains and cross-transmission between patients were identified as modes of acquisition. However, the former mechanism appeared to be more important.

Numerous clinical studies have identified exposure to third-generation cephalosporins as a risk factor for selection of multidrug-resistant gram-negative pathogens (1, 6). In this study, we quantified the frequency of this event by systematically reculturing stored specimens from patients who acquired CAZ-RGN and by comparing PFGE types of CAZ-susceptible and CAZ-resistant isolates. Twenty-one percent of acquisitions were attributable to this mechanism. The majority of these patients were exposed to cephalosporins during the interval between detection of the CAZ-susceptible and CAZ-resistant gram-negative species. This finding is consistent with the premise that the mechanism of resistance is through the induction or stable derepression of the group 1 chromosomal beta-lactamase (11). Two patients in whom emergence of CAZ resistance was detected were exposed to cefazolin alone. In these patients, cefazolin may have eradicated the susceptible

gram-negative flora, thereby allowing the growth of a subpopulation of CAZ-RGN or may have induced the group 1 chromosomal beta-lactamase (10).

Certain limitations in the methodology of this study are worth noting. The ability to detect susceptible isolates of the same species may have been diminished by reculturing frozen swabs, thereby underestimating the frequency of endogenous acquisition. It is also possible that some patients who acquired a CAZ-RGN were actually colonized on admission with rare subpopulations of organisms that were not detected on initial cultures.

Patient-to-patient transmission was identified as the likely mechanism of acquisition in two patients and accounted for only 6% of all the acquired strains. A study by Chetchotisakd et al. (2), examining clinical bacterial isolates from an ICU population, also found very few instances of transmission, as judged by the presence of matching PFGE types. This apparent low frequency of transmission between patients may be typical of periods of endemicity.

In conclusion, colonization with CAZ-RGN in SICU was common but was associated with diverse species and strains, as determined by molecular typing. For patients who acquired a CAZ-RGN in a nonoutbreak setting, endogenous acquisition appeared to be more important than horizontal transmission. This finding suggests that reducing the frequency of multidrug-resistant gram-negative pathogens in settings of endemicity may be accomplished more effectively through modification of antibiotic use than through infection control measures.

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