JOURNAL OF CLINICAL MICROBIOLOGY, Sept. 1996, p. 2129–2132 Vol. 34, No. 9
0095-1137/96/$04.00+0

Use of In-House Studies of Molecular Epidemiology and Full Species Identification for Controlling Spread of Vancomycin-Resistant *Enterococcus faecalis* Isolates

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Received 12 February 1996/Returned for modification 11 April 1996/Accepted 23 May 1996

Infection with multidrug-resistant (MDR) organisms is a major clinical challenge, and few, if any, therapeutic options remain available. Increasingly, infection control measures have taken on greater importance in preventing the nosocomial transmission of MDR organisms. During December 1994 and January 1995, we identified a cluster of vancomycin-resistant *Enterococcus faecalis* isolates involving 16 patients situated in different areas of our university-affiliated teaching hospital. Initial review of laboratory requisition forms for the patients' locations revealed no common association, suggesting that the occurrence was not due to horizontal spread. However, using genomic DNA extraction, restriction enzyme analysis, and gel electrophoresis, we found that 12 patients were infected with isolates originating from a single clone, 2 other patients were infected with isolates from a different clone, and the remaining 2 patients were infected with unique strains. Because the typing data suggested nosocomial spread, chart review was undertaken to determine a possible common exposure source. With three exceptions, clonal isolates were linked to patient movement between surgical floors, intensive care units, and a rehabilitation unit. A detailed review of patient records revealing the association would not have been performed without realization of clonality. Thus, the data demonstrate the utility of genomic typing for epidemiological purposes. In turn, targeted infection control measures that halted the spread of the potentially lethal MDR pathogen were instituted.

Infections caused by multidrug-resistant organisms present the medical community with increasing therapeutic dilemmas because antimicrobial agent treatment options are either limited or nonexistent. Of these organisms, particular attention has been paid to the vancomycin-resistant enterococci (VRE). Clinical VRE isolates were first recognized in 1986 (20), and their incidence has increased dramatically, becoming endemic in many institutions (4, 10). The frequency of nosocomial infections with VRE in U.S. hospitals increased from 0.3% in 1989 to 7.9% in 1993 (1).

The number of studies on the epidemiology of nosocomial enterococcal infections have increased correspondingly with the VRE epidemic. Of initial concern was the rapid emergence of strains with high-level aminoglycoside resistance. This is now accompanied by resistance to both vancomycin and ampicillin. As a result, infection control and molecular typing methods have taken on greater significance. Identifying clonal isolates and stopping the person-to-person transmission of organisms may be the only way to halt epidemic intrahospital spread. Typing rapidly determines whether an increase in enterococcal isolates at a given institution is due to one or multiple strains and whether the clonal (horizontal) dissemination of one strain is occurring or a specific resistance determinant is present (21). Once investigated, appropriate infection control measures along with hospital staff education can be implemented to interrupt the spread of VRE infection or colonization (6).

We describe our investigation of a 2-month vancomycin-resistant *Enterococcus faecalis* outbreak that demonstrates the usefulness of bacterial species identification and in-house typing of isolates for epidemiological purposes.

(This study was presented in part at the 33rd Annual Meeting of the Infectious Diseases Society of America, San Francisco, Calif., 16 to 18 September 1995 [poster 94].)

MATERIALS AND METHODS

Collection and identification of isolates. Isolates were collected during December 1994 and January 1995 from clinical specimens submitted to the Clinical Microbiology Laboratory at the Northwestern Memorial Hospital (Chicago, Ill.). Identification and antimicrobial agent susceptibility testing were performed by the Clinical Microbiology Laboratory using standard methods (3, 13).

Bacterial DNA preparation. A method adapted from the method of Pitcher et al. (16) was used to prepare bacterial DNA. Enterococcal colonies from a 24-h blood agar plate were suspended in TE (Tris-EDTA) buffer so that the turbidity was equal to that of a 2.0 McFarland nephelometer standard. Then the cells were treated with lysozyme and were incubated at 37°C for 30 min to weaken the cell walls. With the addition of guanidinium thiocyanate, the cells were lysed. Next, ammonium acetate was added to the suspension and DNA was extracted with chloroform-isoamyl alcohol. Lastly, the DNA was precipitated with isopropyl alcohol, washed with ethanol, and suspended and stored in TE buffer at 4°C.

REA. Whole-cell DNA was digested with *Hin*dIII and *Hae*III according to the conditions recommended by the manufacturer (GIBCO BRL, Gaithersburg, Md.). Both restriction enzymes were used in our analysis. This ensured the reproducibility of clonality determination of restriction enzyme analysis (REA) results.

Agarose gel electrophoresis. Restriction endonuclease digestion of whole bacterial DNA was analyzed by electrophoresis on 0.6% agarose at 44 V for 16.5 h. The gel was stained with SYBR Green I (200 μg/ml) for at least 2 h. Photographs of the gel were taken with a UV transilluminator.

REA profile interpretation. All *Hin*dIII- and *Hae*III-restricted DNAs were compared with the DNAs from the other strains cut with the same enzyme. The similarity between isolates was determined by visual comparison of individual banding patterns by using the top 60 mm of the DNA band patterns on the gel. A control isolate and a 1-kb ladder were included on all gels for standardization.

Isolates with identical banding patterns were considered to be the same type (clone) and were given the same main "type letter" designation. Isolates with ≥90% similarity (or five or fewer band differences) were considered to be

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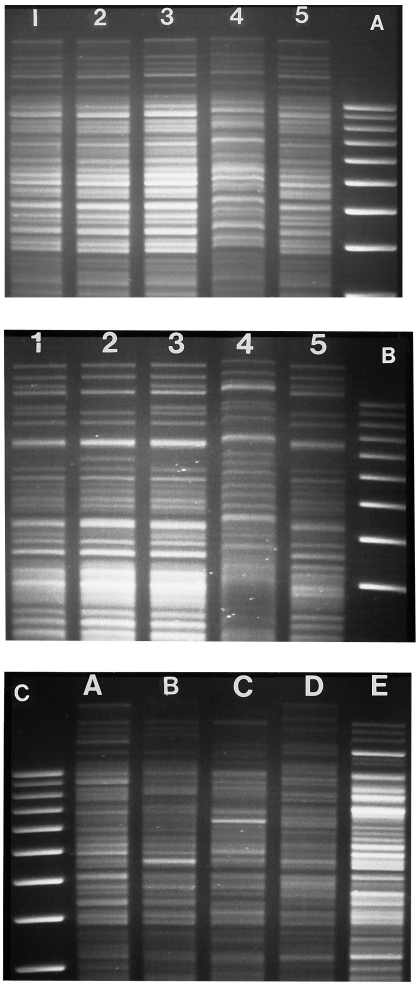

FIG. 1. Magnified views of restriction band patterns of the *E. faecalis* isolates involved in the outbreak comparing the *Hin*dIII REA (A) and the *Hae*III REA (B) patterns. (C) Differences between type A (current outbreak type) through type E (types A_0 through E_0) isolates determined with the *Hin*dIII enzyme. A similar banding pattern for isolates from different patients demonstrates that the same type (clone A_0) is present in the gels in panels A and B. Lanes 1 through 5, isolates designated in Table 1. While the DNA bands in lane 4 are somewhat shifted upward from the bands in the other lanes of the gels in panels A and B, it appears similar to the patterns in lanes 1, 2, 3, and 5 and distinct from the patterns of other types in the gel in panel C. A molecular mass standard is provided with each gel (unmarked lanes in each panel). Type A and four other types, types B through E, are indicated in panel C; the letter at the top of each lane indicates the specific type.

subtypes within the same type letter designation. Isolates with more than five band differences were considered to be sufficiently different to represent a new type letter designation (2). Determination of relatedness had to be the same by both enzyme analyses in order for isolates to be identified as clonal.

Two types of controls were run with each electrophoresis gel. Vancomycin-susceptible enterococci were run with each gel as a standard for a methodology control. Specific type letter strains of vancomycin-resistant *E. faecalis* were also run as controls to make an accurate determination of the type designation to assess clonality. Runs with all controls were repeated more than 50 times, with no deviation from the reference pattern.

Patient chart review. Data were collected from all charts by using a standardized form that included demographic information, admission location, subsequent transfers within the hospital, the consulting services evaluating the patients and the dates that the patients were seen by the consulting services, and any diagnostic or therapeutic procedures performed, along with their locations and dates.

RESULTS

A total of 16 patients (19 specimens) were infected with vancomycin-resistant *E. faecalis* isolates, and all isolates were susceptible to 10 μg of teicoplanin per ml, which was consistent with the definition of the VanB phenotype. *Hin*dIII and *Hae*III restriction patterns identified 12 patients (75%) infected with one identical clonal isolate type, designated type A_0. Two patients (13%) were infected with a different clonal isolate type, and the two isolates had different subtypes. The remaining two patients were infected with uniquely distinct strains (Fig. 1).

Five of 12 patients infected with clonal type A_0 isolates had been on the same surgical floor. Of these five patients, one patient had also been in the surgical intensive care unit. Another isolate was found in the medical intensive care unit, situated adjacent to the surgical intensive care unit. A seventh patient was on a different surgical floor, but after transfer to the surgical intensive care unit, that patient also developed infection with a vancomycin-resistant *E. faecalis* isolate. Two additional isolates were found in the rehabilitation unit where transplant patients from surgical floors commonly were sent prior to discharge home. The remaining three patients were not located on any of the surgical, intensive care, or rehabilitation units involved with the VRE outbreak. Interestingly, two of these patients were admitted with vancomycin-resistant *E. faecalis* infections. One individual, who had a history of frequent hospitalizations at different institutions, came from a nursing home that admitted patients from 20 surrounding hospitals. The second individual was admitted from home but also had a history of multiple recent hospitalizations. The last patient, an outpatient, had not been in a hospital within 2 years but had a history of fecal and urinary incontinence.

The two patients infected with the second clonal type isolate, designated type D, also were linked to the surgical floor and the rehabilitation unit, respectively. The two nonclonal isolates remained unassociated after review of their medical records (Table 1).

Retrospectively, we found that the clone involved in this outbreak investigation was first recovered at our medical center in May 1993 on a rectal surveillance culture. Initially, it was

TABLE 1. Patient location and characteristics of vancomycin-resistant *E. faecalis* isolates[a]

Lane in Fig. 1	Isolate no.	Phenotype	Type	Admission floor	Transfer floor
1	F80[b]	VanB	A_0	Surgical 15	
2	F62	VanB	A_0	Surgical 15	
3	F67	VanB	A_0	Medical 9	Surgical 15
4	F66	VanB	A_0	Surgical 15	
5	F69[c]	VanB	A_0	Medical 10	
Not pictured	F63	VanB	A_0	Rehabilitation	
	F65	VanB	A_0	Surgical 15	Surgical ICU[d]
	F70[c]	VanB	A_0	Medical 10	
	F71	VanB	A_0	Rehabilitation	
	F73[b]	VanB	A_0	Outpatient	
	F74	VanB	A_0	Outpatient	
	F76[b]	VanB	A_0	Surgical 15	
	F77	VanB	A_0	Surgical 15	
	F79	VanB	A_0	Medical ICU	Medical 14
	F81	VanB	A_0	Surgical 11	Surgical ICU
	F68	VanB	D_1	Rehabilitation	
	F72	VanB	D_0	Surgical 15	
	F75	VanB	M_0	Medical 9	
	F64	VanB	K_0	Medical 8	

[a] For the specific isolate types, admission floors and movement between units are shown, demonstrating the link that propagated the vancomycin-resistant *E. faecalis* outbreak.
[b] Patients admitted with vancomycin-resistant *E. faecalis* infections.
[c] Duplicate isolates.
[d] ICU, intensive care unit.

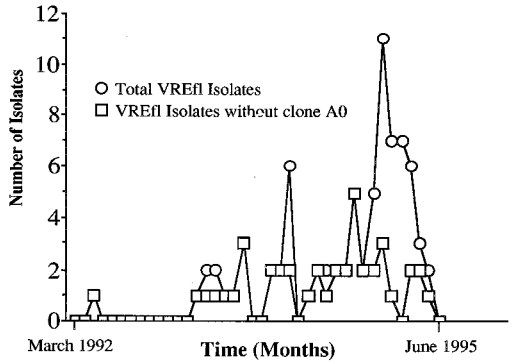

FIG. 2. Monthly trend of total and clonal vancomycin-resistant *E. faecalis* (VRE_{fl}) isolates. The numbers of unique patients per month from whom isolates of vancomycin-resistant *E. faecalis* were recovered are depicted. Isolates from two patients included in the January 1995 total count also were found in December 1994. These two duplicates account for the fact that there appeared to be 18 total patients during the November 1994 to January 1995 outbreak, when only 16 patients infected with vancomycin-resistant *E. faecalis* isolates are discussed in the text. Increased numbers of clonal vancomycin-resistant *E. faecalis* isolates at the time of the outbreak are illustrated. Also portrayed is the return of the numbers of vancomycin-resistant *E. faecalis* isolates to baseline levels after enhanced infection control measures were instituted during the third week of January 1995.

present infrequently, averaging one or two new isolates every 1 to 3 months. However, during the 3 months preceding the outbreak, one to four new isolates were found monthly. Two isolates of vancomycin-resistant *E. faecalis* were found on surveillance cultures in November 1994, but all other isolates, including those isolated during the outbreak, were from clinical specimens taken for the evaluation of possible infection. After recognition of this clone, targeted infection control measures were implemented during the third week of January 1995. These control measures required all workers to wash their hands and to wear a gown and gloves before entering the room of a patient known to be positive for VRE, whether or not any patient contact was anticipated. For the other patients on these units, all workers washed their hands and wore gloves before entering the patients' rooms. Following the institution of these practices the number of isolates of the A_0 clone, including isolates from both infected and colonized patients, progressively fell to zero. Also, the total number of vancomycin-resistant *E. faecalis* isolates at our institution returned to baseline levels (Fig. 2). In 1994, of a total of 1,177 *E. faecalis* isolates from the clinical laboratory, 59 (5%) were resistant to vancomycin.

DISCUSSION

The microbiology laboratory is the first line of defense when controlling the nosocomial spread of multidrug-resistant pathogens in a hospital (6). In order to establish the extent of a potential VRE problem, the laboratory must be able to determine reliably and rapidly both the genus and species and the resistance patterns of isolates from colonized or infected patients. Molecular typing techniques then can ascertain the presence or absence of different strains of vancomycin-resistant *E. faecalis* by determining clonality. If a problem is identified, infection control coordinators and hospital staff must be promptly notified (6, 19).

The laboratory's key importance is demonstrated by our outbreak investigation, which was undertaken because of an increase in the numbers of clonal vancomycin-resistant *E. faecalis* isolates at our medical center. While it appears that 2 months may be a long time to recognize a potential outbreak, we have seen VRE in Chicago since 1992 and have actively managed the problem with a primary focus on vancomycin-resistant *E. faecium* isolates. If species identification were not routinely performed in our laboratory, the increased numbers of vancomycin-resistant *E. faecalis* isolates would have been lost among the greater numbers of vancomycin-resistant *E. faecium* isolates that we recovered (Fig. 3). Through early 1996 we have recognized more than 43 distinct genomic types of vancomycin-resistant *E. faecium*, and since typing the *E. faecalis* strains, we have found 20 types of vancomycin-resistant *E. faecalis*. Our vancomycin-resistant *E. faecium* isolates are divided between 65% with a VanA phenotype and 35% with a VanB phenotype, as opposed to the *E. faecalis* isolates in the present study, which were all of the VanB phenotype. Typing was done because of the observed 2-month surge in the number of vancomycin-resistant *E. faecalis* isolates and as noted in Fig. 2, the entire increase in the numbers of vancomycin-resistant *E. faecalis* isolates at that time was due to the spread of the A_0 clone. Concurrent with the laboratory typing, a review of laboratory requisition forms containing each patient's location did not reveal a common association, thus suggesting that the occurrence of VRE isolates was not due to horizontal spread. However, genomic typing, which was done during the second week of January 1995, found that 75% of the patients were infected with isolates from a single clone, while two other patients were infected with isolates from another clone. The data strongly suggested nosocomial spread. Subsequent careful chart review revealed the epidemiologic unifying factor, namely, patient movement between the surgical, intensive care, and rehabilitation units. Without the realization of strain clonality, a review of patient records demonstrating the association would not have been done. As a result, appropriate infection control measures along with expanded staff education were implemented during the third week of January 1995 on selected units to control the spread of the vancomycin-resistant *E. faecalis* isolates.

Staff education was directed at physicians, nursing personnel, and environmental services personnel. Strict adherence to hand-washing procedures, compliance with glove and gown barrier precautions, and environmental surface cleaning were emphasized (6, 14). Further infection control measures included the use of larger, more noticeable isolation and precaution signs placed on the center of the door to each patient's

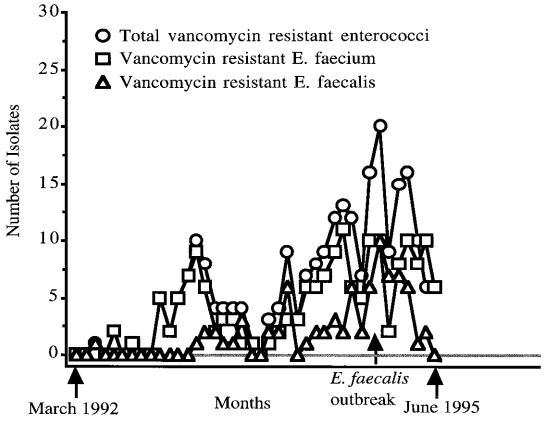

FIG. 3. Total monthly counts of VRE, vancomycin-resistant *E. faecalis*, and vancomycin-resistant *E. faecium* isolates. The figure depicts the numbers of patients infected per month and demonstrates how the *E. faecalis* isolates are easily lost among the larger numbers of resistant *E. faecium* isolates and the total numbers of isolates of vancomycin-resistant *Enterococcus* species.

room. Also, more focused screening of patients and the environment was initiated. Within 8 weeks these efforts were rewarded by the elimination of multiple infections with the A_0 clone and a return to baseline levels of vancomycin-resistant *E. faecalis* isolates (Fig. 2). While our outbreak investigation involved vancomycin-resistant *E. faecalis* isolates, vancomycin-resistant *E. faecium* isolates remain a greater problem at our hospital. In 1994, of a total of 265 isolates of *E. faecium*, 153 (58%) were vancomycin resistant. Thus, both organisms must be tracked and typed because each can cause nosocomial VRE colonization and infection.

Selection of an appropriate typing system is not readily apparent from a review of the literature. Useful methods appear to be restriction of genomic DNA followed by conventional electrophoresis (REA analysis, as in our laboratory), pulsed-field gel electrophoresis, and rRNA gene probing (ribotyping). All methods appear to be highly reproducible and to have been applied to bacterial outbreaks (5, 8, 18). REA analysis and pulsed-field gel electrophoresis are more discriminatory than ribotyping (5, 8, 18), and a few isolates are not typeable by pulsed-field gel electrophoresis (8). We have chosen REA analysis because of our familiarity with the method, its low cost, and the speed with which it can be applied by a clinical laboratory to the evaluation of a new potential outbreak of nosocomial infection (15).

VRE are important nosocomial pathogens that pose serious clinical challenges. Treatment options are confined to experimental or unstudied antibiotic regimens. The dramatic rise in nosocomial VRE infections in the United States, especially in the intensive care unit setting, which increased from a frequency of 0.4% in 1989 to one of 13.6% in 1993, further underscores the scope of the problem (1). As part of the normal human flora of the gastrointestinal and genitourinary tracts, enterococci cause endogenous infections. However, they also can be exogenously acquired. Enterococci are hardy organisms because they can persist in the environment for prolonged periods of time, despite adverse conditions (14). Ample evidence exists of strain spread between patients, either directly or indirectly, and between medical centers and nursing homes (7, 11, 12, 22, 23). Strains of enterococci causing nosocomial infection not only have been cultured from the hands and gastrointestinal tracts of medical personnel but also are frequently isolated from hospital environmental surfaces including rectal thermometer probes (9, 14, 17). The patients most at risk for VRE colonization and infection include those who are immunosuppressed or critically ill, those who had prior antimicrobial agent exposure, patients with long hospitalizations, and individuals undergoing intra-abdominal, pelvic, or cardiothoracic surgery.

In summary, our observations confirm that the nosocomial transmission of VRE occurs primarily among high-risk patients and that the clinical microbiology laboratory plays an important role in identifying such outbreaks. Control of our outbreak was accomplished by intensive staff education, focused and thorough environmental cleaning, and aggressive surveillance with isolation practices. Uniformly effective treatment for patients with VRE infections does not exist; therefore, efforts must be directed at preventing colonization and the nosocomial spread of the organism.

ACKNOWLEDGMENT

This work was supported by Northwestern Memorial Hospital and Northwestern University.

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