Identification of Genetically Unique Vancomycin Resistant Enterococcal Clusters in a New York City Medical Center

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ABSTRACT

Vancomycin resistant *enterococci* (VRE), an increasingly significant nosocomial pathogen in New York City hospitals, is capable of causing serious infections and is resistant to most antibiotics. High mortality rate is due to the inability to treat infected patients. At a local tertiary care medical center, 22 isolates from 14 patients were evaluated. The rapid increase of VRE over a short period of time suggested an outbreak caused by a single strain that had survived the intensely selective environment. The Vitek Microbiology System and agar dilution susceptibility test were used to phenotype the isolates. Plasmid profile, restriction enzyme analysis and Southern hybridization with *vanA* gene probe were performed in gathering DNA fingerprinting data.

Three dominant yet genetically unique strains were generated, which suggested that each strain arose independently and polyclonal dissemination was involved. The transfer of resistance between two species was also evident. Previous research describing such transfer emphasizes the magnitude of this problem. Moreover, the sudden rise and continual increase of VRE within the medical center and city hospitals in general, signify only the beginning of an extensive nosocomial outbreak.

LITERATURE RESEARCH

Enterococci are part of the normal flora of the human gastro-intestinal and lower genital tracts. These opportunistic bacterial pathogens cause infections that include bacteremia, wound sepsis, urinary tract and biliary tract infections (Murray, 1990). Not only are they highly resistant to ß-lactams and aminoglycosides, they are also capable of transferring and/or acquiring resistance. The New York City Department of Health conducted a study involving hospitals which reported VRE since 1989. By late 1991, 47% of the city's hospitals had reported VRE. No cross-hospital spread was shown, though intra-hospital spread was strongly suggested. One of the reasons attributed to this rapid increase of VRE was cross-infection in exposed wounds and catheters. Community-acquired VRE was also reported, suggesting that the organism had spread to non-hospital flora (Frieden, et al., 1992).

VRE is caused by two genes, *vanA* and *vanB*. The proteins responsible for *vanA* resistance are VanA and VanH, which can lead to the formation of a pentapeptide with an altered C-terminus which lacks affinity for vancomycin. *VanB* is believed to carry some structural and functional similarity to *vanA*. A recent discovery made regarding the transfer of resistance from *enterococci* to bacteria of another genus illustrates the promiscuity and adaptability of vancomycin resistance. Noble (1992) showed that *Enterococcus faecalis* is capable of transferring its high-level vancomycin resistance to *Staphylococcus aureus*.

Epidemiologic studies of VRE have been hindered by the lack of a convenient and accessible method. Formerly, biochemical and differences were used to judge whether two isolates are of the same strain. However, they are often unreliable and show little variation within a specie. In 1990, Murray et al. compared patterns generated by restriction endonuclease digestion (RED). The demonstration that different persons are infected with a single strain would suggest that an outbreak has occurred, while the presence of different strains would point away from an outbreak. Molecular epidemiology, or DNA fingerprinting, was further

enhanced by the usage of pulsed-field gel electrophoresis (PFGE). The combined use of whole-plasmid analysis, restriction enzyme analysis of plasmid DNA with two enzymes, and PFGE was suggested as a typing scheme for epidemiologic investigation of VRE (Donabedian, et al., 1992).

HYPOTHESIS

By means of subspecies typing schemes, it can be shown that a single strain responsible for the outbreak had survived the highly selective environment. Provided that the increase of clinical isolates occurred over a short period of time and plasmid-mediated resistance occurs over a relatively long period of time, it may be that the strain itself is moving from patient to patient.

METHODS AND PROCEDURES

A hundred and fifty-four VRE isolates were obtained from 88 patients during January 1991 to June 1992. Of the 1991 isolates, 22 were randomly selected for this epidemiologic study. The Vitek Microbiology System and agar dilution susceptibility testing (MICs) were used to phenotype the isolates. Plasmid profile, restriction enzyme analysis and Southern hybridization with *vanA* gene probe were performed in gathering DNA fingerprinting data.

RESULTS AND DISCUSSION

VRE have emerged rapidly in New York City since 1989. This rapid emergence over such a short time span suggests a multi-clonal origin. At the medical center from which the isolates for this study were obtained, the first allusion to an outbreak of VRE occurred in 1991. From data generated by DNA fingerprinting, there seemed to be three dominant strains, which suggests clonal dissemination involving more than one strain (table 1. *ie.* 80/A, 81/A, 100/L). The genetic diversity of the isolates indicates that genetically unique clusters developed independently from each other and then disseminated throughout the hospital. The 22 isolates evaluated were differentiated into 13 types by both the Vitek System and DNA fingerprinting. However, the groupings made by the two techniques were not consistent with each other. Some of the Vitek antibiograms contradicted MIC results. Also, the Vitek did not seem to do well in detecting *vanB* resistance (96/I, 99/K). These discrepancies put the Vitek as a reliable typing method into question.

According to genotypic data, a susceptible *E. faecalis* isolate (94/I) hybridized at the same spot a vancomycin resistant *E. faecium* (96/I) hybridized at. Both of these isolates were retrieved from the same patient. It is suggested that isolate 94/I acquired the gene from an *E. faecium* with a hybridization pattern similar to that of isolate 96/I. The gene probably mutated and became dysfunctional. Whatever the case may be, this example is significant in demonstrating that patients who become infected with VRE are capable of having resistance transferred to the susceptible *enterococci* which make up their normal flora. Isolate 83/B, which was identified as *E. avium* by Vitek, shared a similar RED pattern to that of isolates which were identified as *E. faecium* by Vitek. If isolate 83/B, in actuality, is a vancomycin susceptible *E. faecium*, then the Vitek has proven its unreliability, not only in detecting resistance, by also in species typing. However, it can also be said that isolate 83/B is an *E.*

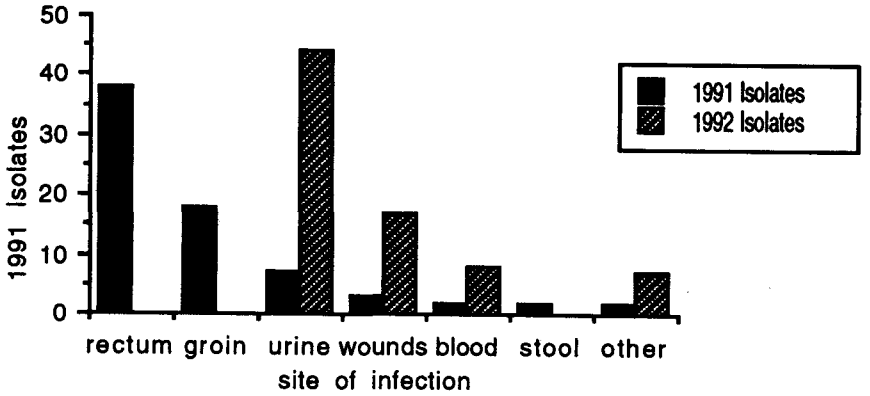
avium sharing the same RED pattern with a certain *E. faecium* strain. This case would suggest that RED alone would not suffice for species typing and can even pose an enormous problem for molecular epidemiologic typing.

Table 1. Characterization of twenty-two vancomycin resistant *enterococci* isolates recovered from patients during February 1991 to September 1991.

Isolate no.& Patient	Vitek Biotype	Antibiogram Va	Gm	St	Amp	MICS Van	Amp	Teico	HLGR/ HLSR	Plasmid Profile Pattern	REA Pattern	Southern Hybridization	Class	Infection Site	Species
80/A	77627230540	R	R	R	R	1024	256	>128	+/+	A	I	1	A	wound	*E. faecium*
81/A	77617230540	I	S	S	R	512	256	64	-/+	C	I	1	A	groin	*E. faecium*
82/A	77617230540	R	R	R	R	512	256	64	-/+	D	III	N	S	rectum	*E. faecium*
83/B	77627330340	R	R	R	R	2.0	2.0	<0.5	-/-	A	I	1	A	stool	*E. avium*
84/B	77737230140	R	R	R	R	512	256	>128	-/+	A	I	1	A	urine	*E. faecium*
85/C	77737230140	R	R	R	R	512	256	>128	+/+	B	I	1	A	groin	*E. faecium*
86/D	77637230540	R	S	R	R	512	128	>128	+/+	A	I	1	A	rectum	*E. faecium*
87/D	77627230540	R	R	R	R	1024	128	>128	+/+	A	I	1	A	groin	*E. faecium*
88/E	77737364340	R	R	R	MS	512	128	32	-/+	C	I	1	A	rectum	*E. faecalis*
89/E	77737230140	R	S	R	R	1024	512	>128	+/+	A	NA	1	A	groin	*E. faecium*
90/E	77637230540	R	S	R	R	512	256	32	-/+	D	NA	1	A	wound	*E. faecium*
91/F	77737374340	R	R	R	R	1024	512	>128	-/+	D	NA	1	A	groin	*E. faecalis*
92/G	77637270540	I	R	R	R	1024	512	>128	+/+	A	I	1	A	groin	*E. faecium*
93/H	77737230140	R	S	R	R	512	512	128	+/+	B	III	1	A	rectum	*E. faecium*
94/I	73735364340	S	R	R	MS	1.0	<0.5	<0.5	-/-	D	II	2	S	stool	*E. faecalis*
95/I	73735374340	S	S	R	MS	1.0	2.0	<0.5	+/+	D	II	N	S	axilla	*E. faecalis*
96/I	77637270540	I	R	R	R	64	128	<0.5	-/+	D	I	2	B	urine	*E. faecium*
97/J	77737364340	R	R	R	MS	512	<0.5	>128	-/+	E	IV	1	A	rectum	*E. faecalis*
99/K	77737270540	S	S	R	R	64	64	<0.5	+/+	D	I	3	B	blood	*E. faecium*
100/L	77737230140	R	R	R	R	512	512	>128	+/+	A	III	1	A	urine	*E. faecium*
102/M	77627270540	I	R	R	R	512	64	32	+/+	A	III	1	A	urine	*E. faecium*
103/N	77637230540	R	R	R	R	512	256	32	+/+	A	III	1	A	blood	*E. faecium*

The following abbreviations are used for the data shown above:
Va or Van - vancomycin Gm - gentamicin St - streptomycin Amp - ampicillin Teico - teicoplanin
R - resistant I - intermediate MS - moderately susceptible S - susceptible NA - not available
N - no homology HLGR - high level gentamicin resistance HLSR - high level streptomycin resistance
Class designates whether the isolate harbors vanA (A), vanB (B), or no (S) resistance genes.

Figure 1. Comparison of infection sites between 1991 and 1992 isolates.



Of the 80 isolates collected in 1991, 49% were from rectal infections. Another 23% came from the groin, whereas only 5% and 2% were isolated from wounds and blood, respectively (figure 1). However, preliminary data evaluating the 1992 isolates indicated that, out of 79 isolates, 22% were from wounds and 11% from blood. A dramatic increase was also seen in urine isolates, where in 1991 made up only 9% of isolates and jumped to 58% in 1992. Other infection sites that were reported in 1992 were peritoneal fluid and bile drainage. None of these isolates were reported in 1991. This observation is significant in that the virulence of the *enterococci* is no longer limited to its normal habitat, but has instead it moved on to invading other areas such as the bloodstream and wounds. This progression not only denotes that the *enterococcus* is a reservoir for resistance, but also the poly-clonal dissemination will continue to grow into an outbreak of greater magnitude, which would be much more difficult to eradicate.

FUTURE RESEARCH

Extensive molecular analysis has suggested that an enterococcal strain can harbor an inactive resistance gene, and its transfer to another strain would activate resistance. More research should be done to verify this conjecture. Moreover, due to the fact that chromosomal and plasmid analysis are subject to individual interpretation, a method should be developed to facilitate interpretation, and hence increase the reliability of molecular epidemiology.

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