

MOLECULAR CHARACTERIZATION OF GLYCOPEPTIDE-RESISTANT *Enterococcus faecium* (VREfm) FROM 30 HOSPITALS IN ARGENTINA

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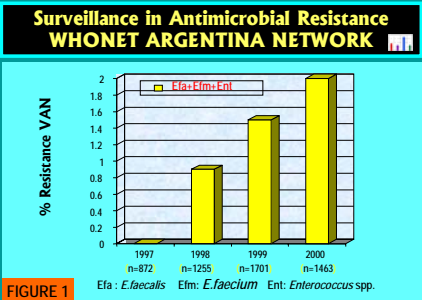


FIGURE 1 Efa: *E. faecalis* Efm: *E. faecium* Ent: *Enterococcus* spp.

OBJECTIVES

- TO DETERMINE THE RESISTANCE PATTERNS TO DIFFERENT ANTIMICROBIAL AGENTS.
- TO CHARACTERIZE THE MECHANISM OF GLYCOPEPTIDE RESISTANCE.
- TO DETERMINE THE CLONAL RELATIONSHIP BETWEEN ISOLATES BY PFGE.

Enterococci (Ent) as a cause of nosocomial infection have become more prevalent over the last 20 years, both in US and in western European countries. Moreover, strains of Ent have acquired resistance to almost all antimicrobial agents, including vancomycin (VAN). The first *Enterococcus faecium* vancomycin resistant (VREfm) clinical isolate in Argentina was detected during 1997. Since then, VREfm have emerged in our country as colonizing or infecting strains in many hospitals (Htals). Through the surveillance on antimicrobial resistance, conducted by the WHONET Argentina Network (37 Htals), we noted in the last three years, an increase in the prevalence of vancomycin resistance among non-mobile *Enterococcus* spp. (species other than *E. gallinarum* and *E. casseliflavus/flavescens*) from infecting samples (FIG. 1). From Jan. 1997 to Dec. 2000, we received at the Antimicrobial Division of the National Institute on Infectious Diseases, a total of 189 VREfm isolates from 30 Argentine hospitals (FIG. 2). Names and locations of the Htals., and number of strains recovered from the particular Htal. are listed in TABLE.

TABLE VRE Argentinian Collaborative Group: No. Isolates studied by Hospital						
HOSPITAL CODE	HOSPITAL	CITY	No. ISOLATES	PUBLIC/PRIVATE	MICROBIOLOGY / INFECTION TOLOGY / EPIDEMIOLOGY	
AEK	Htal. Aeronautico	Capital Federal	4	Private	Liliana Targa	Edith Carbono
ANT	Sanatorio Antartida	Capital Federal	1	Private	Adriana Fano	Javier Alicias
BAN	Policlinico Bancario	Capital Federal	2	Private	Graciela Miranda	Lopez Graciela
BAZ	Clin Baetnerica	Capital Federal	2	Private	Julio Pico	Marina Pantagua
CML	Clinica Modelo de Lanus	Buenos Aires	11	Private	Karina Menzior	Adriana Romani
COS	Htal. Cosme Argento	Capital Federal	18	Public	Laura Lopez Moral	Claudia Rodriguez
DUR	Htal. Durand	Capital Federal	20	Public	Rosa Fabre	R. Rovinsky / G. Levy Hara
EM	Htals "Evita"	Buenos Aires	18	Public	Ana Maria Taggeri	Edith Dimerstein
FAV	Fund. Favalaro	Capital Federal	5	Private	Marta Tokumoto	Claudia Nagel
FER	Htal. Fernandez	Capital Federal	18	Public	Sara Kaufman	
FLE	FLEB	Capital Federal	2	Private	L. Guillard / S. Manzanero	Marcelo Del Castillo
GAR	Htal. Garriban	Capital Federal	1	Public	Horacio Logarido	Carlos Bergallo
HCC	Htal. Cordoba	Cordoba	2	Public	Olego Perlo	
HRE	Htals Dr. V. Iturrumendi	Buenos Aires	1	Public	Victoria Monzani / Patricia Sosa	
IPK	Sanatorio Centro Medico IPAM	Buenos Aires	2	Private	Elena Sutil / Rodolfo Nekraso	Maria Caselli
LAE	Laboratorio Especializado	Capital Federal	1	Private	Claudia Ebi	
MIT	Sanatorio Mite	Capital Federal	1	Private	Ana Dimartino	Javier Alicias
MUN	Htal. Munio	Capital Federal	9	Public	Raquel Callejas / Elsa Cosio	
PER	Htal. Julio C. Perando	Chaco	1	Public	Maria Cristina Redondas	Emilio Ibarchi
PIK	Htal. Piron	Capital Federal	15	Public	L. Luardo IM. Pinto IL. Riera	Oscar Garcia Medina
PIR	Htal. Pirizano	Capital Federal	6	Public	Maria Julia	
POS	Htal. Dr. A. Posadas	Buenos Aires	11	Public	A. Di Bello / Adriana F. Lausi	Hector L. Lagune
QAL	Htal. de Quaberos	Capital Federal	12	Public	Jaime Kovensky	Nora Goldberg
QSD	Hospital Quila Fabris	Cordoba	1	Private	Hilma Bolliger	Paula Budini
ROY	Htal. Rivadavia	Capital Federal	1	Public	Alicia Luaso	
SMP	Htal. San Martin La Plata	Buenos Aires	20	Public	Carmen Lopez / Jose Castano	Cecilia Camerano
STJ	Htal. Francisco Santojanni	Capital Federal	2	Public	Maria Teresa Lopez Reyes	Gabriela Vidal / Pablo Scapellato
TOR	Htal. Torre	Capital Federal	1	Public	Maria Hoffman / Hugo Villar	Miriam Burgos
VLK	Htals Vicente Lopez y Planes	Buenos Aires	1	Public	Hebe Gallo	
ZUB	Hospital Zubizarreta	Capital Federal	1	Public	Rosa Mondino	

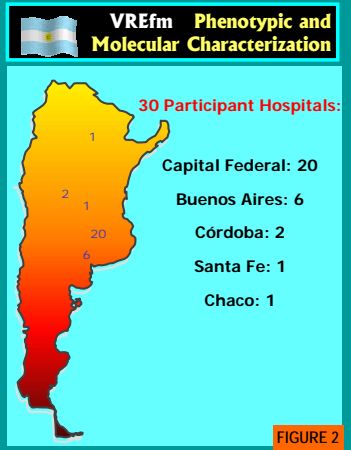


FIGURE 2

MATERIALS AND METHODS

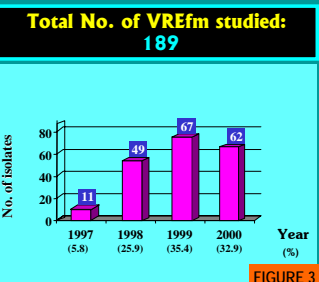


FIGURE 3

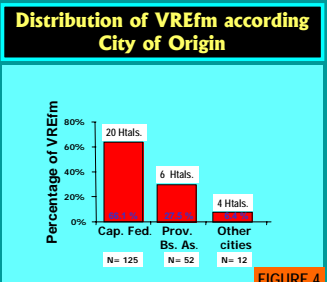


FIGURE 4

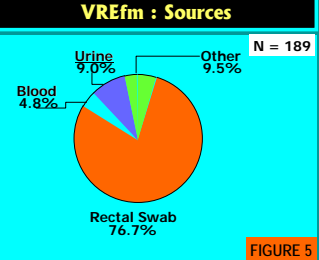


FIGURE 5

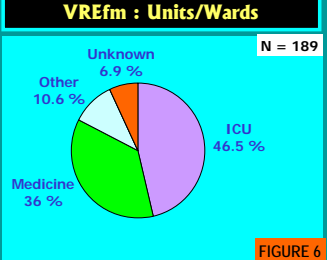


FIGURE 6

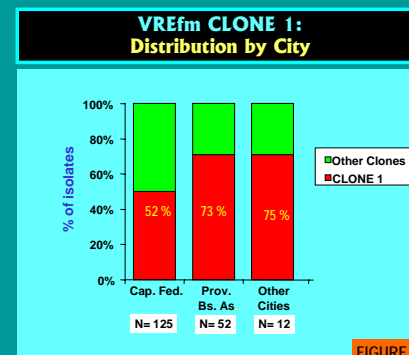
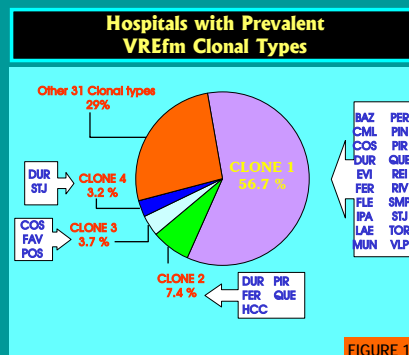
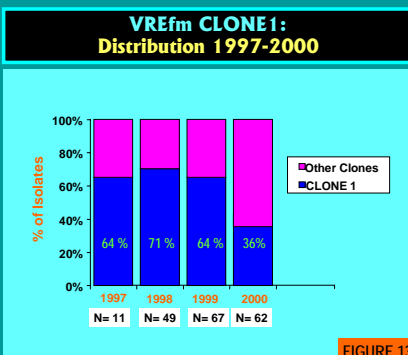
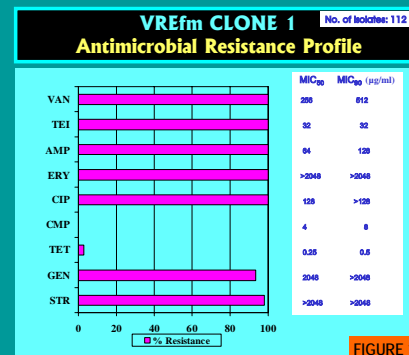
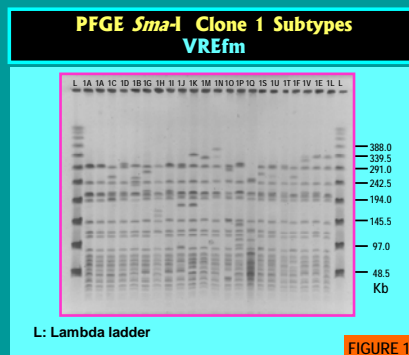
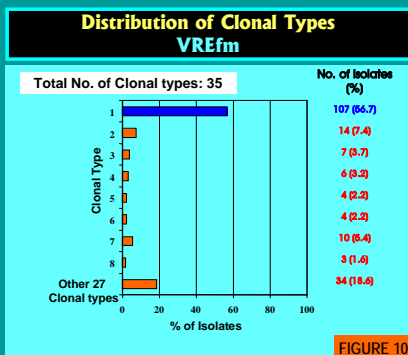
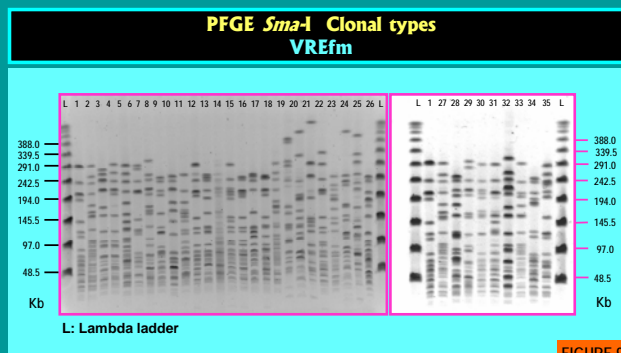
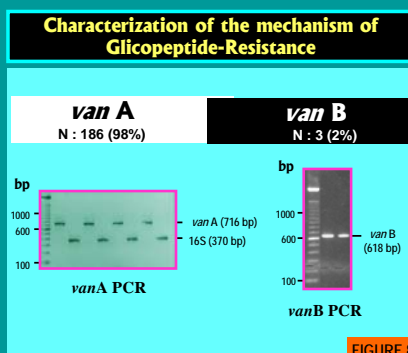
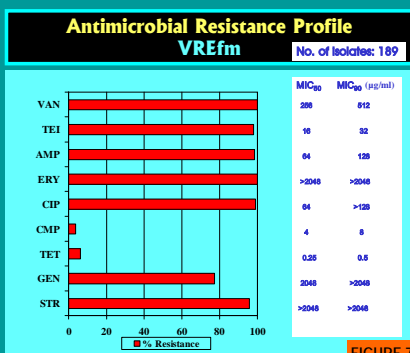
STRAINS: From Jan.1997 to Dec. 2000, a total of 189 VREfm were isolated from 30 Argentinean hospitals (Htals.) (FIG. 3). 125 (66.1%) VREfm were collected from 20 Htals. in Cap. Fed., 52 (27.5%) from 6 Htals. in Buenos Aires and 12 (6.4%) from 4 Htals. in Cordoba, Santa Fe and Chaco (FIG. 4). Collected strains were identified in each Htal. to species level by biochemical characterization using Facklam's recommendations (1998. *J. Clin. Microbiol.* 36:1584-1587). For each patient, only one isolate and one infection/colonization site was considered. Strains came from different clinical sources, but principally were from rectal swab (n/%) (145/76.7) (FIG. 5). Most of the VREfm were isolated from ICU (46.5%) and Medicine (36%) (FIG. 6). 80.5% patients from whom VREfm were recovered were colonized, 14.8% were infected and 4.7% was not possible to assess the clinical significance.

SUSCEPTIBILITY TESTING: Minimal inhibitory concentrations (MICs) to ampicillin (AMP), vancomycin (VAN), teicoplanin (TEI), gentamicin (GEN), streptomycin (STR), tetracycline (TET), chloramphenicol (CMP), erythromycin (ERY), ciprofloxacin (CIP) were determined by agar dilution according NCCLS M7-A5.

PCR: The presence of van genes was investigated by PCR with a Biometra thermal cycler, using specific primers for *vanA* and *vanB* (Courvalain P. 1995. *J. Clin. Microbiol.* 33:24-27) in standard conditions. Specific primers for 16S gene were used as control of DNA extraction (Greisen, K.1994. *J. Clin. Microbiol.* 2:335-357). DNA template was prepared by boiling, and 5µl of the supernatant was used for the reaction mixture.

PFGE: Enterococcal genomic DNA was prepared and digested with *SmaI*, as previously described (De Lencastre. 1999. *Microb. Drug Resist.* 5:113-128). DNA fragments were separated in 0.8% agarose using a CHEF-DRIII (Bio-Rad Laboratories, CA), in same conditions that described by De Lencastre. Isolates were considered genetically indistinguishable and were assigned to the same strain type (e.g. type A) if their restriction patterns had the same number and size of bands. Isolates with 1-6 band differences in their restriction pattern were considered closely or possible related and were assigned to a subtype (e.g., subtype A1). Isolates whose restriction patterns differed by >6 bands were considered to be unrelated and were assigned to different strain types (e.g. A, B, C, etc.). The similarity between isolates was determined by visual comparison.

RESULTS



CONCLUDING REMARKS

✗ The percentage of resistance was high for VAN, TEI, AMP, STR, GEN, ERY and CIP, but relatively low for CMP and TET (3.7 and 6.3% respectively) (FIG.7).

✗ 98% of the isolates were genotype *vanA* and only 3 strains presented genotype *vanB* (FIG.8).

✗ A total of 35 different clonal types were identified (FIG.9).

✗ Almost 60% of the isolates belonged to VREfm CLONE 1 (FIG.10).

✗ VREfm CLONE 1 was classified in 24 clonal subtypes (FIG.11).

✗ VREfm CLONE 1 was susceptible to TET and CMP, resistant to ERY, CIP and highly resistant to STR, GEN and AMP, avoiding the possibility of synergistic activity with aminoglycosides (FIG.12).

✗ VREfm CLONE 1 was present during all the period in study (FIG.13)

✗ VREfm CLONE 1 was detected in 20/30 Htals (FIG.14).

✗ VREfm CLONE 1 was dominant in 10/20 Htals. from Capital Federal , 4/6 Htals. from Provincia de Buenos Aires and 3/4 from other cities (FIG. 15).

✗ The increase in the incidence of VREfm in Argentina was due, at least in part, to the Clonal Dissemination of VREfm 1 within the Htals. and between different Htals.