Tracking nosocomial Vancomycin-resistant *Enterococcus faecium* outbreak by clinical and molecular epidemiology in a teaching Hospital in Bogota, Colombia. 2016.

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ABSTRACT

VRE*fm* was first isolated in Europe and the United States by the end of the 1980s because of misuse and overuse of antibiotics in livestock and hospital settings. (1) *Enterococcus spp.* genus is ranked as the fifth most frequently identified pathogens among agents responsible for HAIs, according to the latest report of the Centers for Disease Control and Prevention (CDC) (2,3). It belongs to the *ESKAPE* bacteria which have the ability to "escape" from most of the currently available antibiotics; This is why, in March 2017, the WHO included Vancomycin-resistant *Enterococci* (VRE) as priority pathogen for research and development of new therapeutic options within the health-care setting, as an urgent measure of global public health. (4). This tendency is also observed in Colombia where *Enterococcus spp.* was classified amongst the five most common microorganisms related to HAIs during the past years. (5) The purpose of this study is to describe the spread of VRE*fm* occurred at a 802-bed hospital in Bogotá during 2016 using molecular and epidemiological approaches.

INTRODUCTION

The antimicrobial resistance (AMR) is a public health problem directly related with the attending medical practice and the abuse of prescribing antimicrobial drugs in situations they do not represent a health benefit. Microorganisms with extended antimicrobial resistance patterns are a threat to the current global epidemiological transition (communicable diseases to non-communicable diseases) and it is estimated for the year 2050 that infectious diseases could reemerge as main cause of mortality in the world due to the limited therapeutic spectrum (6,7).

In Colombia the first VRE*fm* isolation was found in 1998 from a hospital in Medellin, and from then, it has spread all around the country. (Figure 1). Within our Hospital, the first documented *Efm* was identified in January 2001 and it was followed by the first VR*Efm* in August of the same year. During May 2016, the first VRE*fm* outbreak was identified as consequence of an abrupt introduction of this microorganism as one of the main causes of HAIs in the Hospital. (Figure 2).

Historically in Colombia, strategies for identification and control of infectious diseases outbreaks has been focused on clinical and epidemiological perspectives due to the higher costs and low availability of molecular biology techniques (8-12). Translational medicine with its *"bench-to-bedside"* approach has been acquiring recent significance in the health-care setting, with main goal to contribute with strategies for disease treatment and prevention working through molecular biology techniques.(12) The present study aims at characterizing an epidemiological outbreak by Vancomycin-resistant *Enterococcus faecium* (VRE*fm*), inside the hospital with the largest installed bed capacity in Colombia during 2016 using the clonal analysis with Multiple locus Variable Number Tandem Repeats fingerprinting (MLVF) and an epidemiologic follow-up.

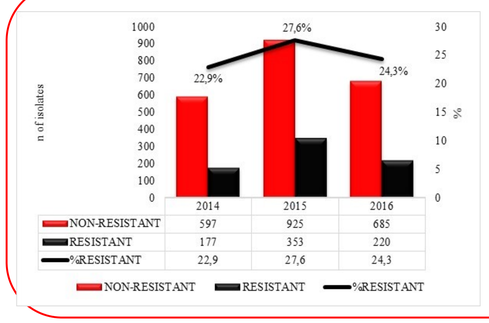


Figure 1. Source: *Instituto Nacional de Salud*, Colombia. Self-made structure. Number of VRE*fm* isolates reported to the National Institute of Health in Colombia between years 2014-2016, discriminated by Vancomycin resistance patterns.

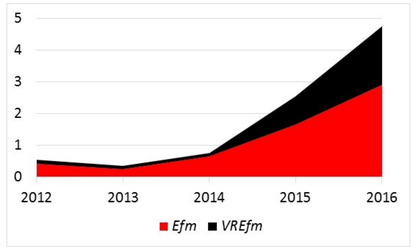


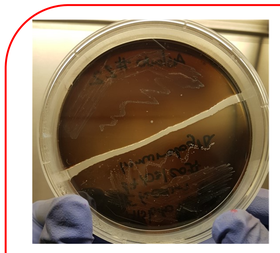
Figure 2. Annual incidence of *Enterococcus faecium* in the Hospital in Bogota, according to Vancomycin-resistant profile per 1000 patients during 2012 to 2016.

MATERIAL AND METHODS

The present study was conducted in an 802-bed, tertiary-care teaching Hospital, in Bogotá, Colombia.

For the microbiology study and molecular typing, a total of thirteen13 nonduplicated VRE*fm* isolates were recovered in a 2-month period from the 15 outbreak patients. Isolates from the first and third patients of the outbreak were not included in the molecular approach since it was not possible to recover at least one sample. Antimicrobial drugs sensibility was determined by two different techniques: automatized method with VITEK 2 system (software version 1.02 bioMérieux, USA), and manual microdilution methods for glycopeptide susceptibility of Vancomycin and Teicoplanin, based on Clinical Laboratory Standards Institute (CLSI) 2014 criteria. Multiple locus Variable Number Tandem Repeats fingerprinting (MLVF) was the typing method we finally adapted to determinate clonality, and presence or absence of *VanA* genes was evaluated by PCR.

For the transmission analysis, Hospital tracking information was obtained from medical records of all patients belonging the outbreak (15 patients). We adopted the time-location-molecular (TLM) algorithm, described and implemented by Willmann *et al* (34) in Germany in 2015.



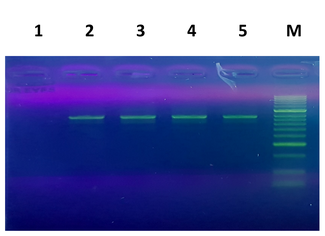


Figure 3. *VREfm* isolation in BD *Enterococcosel Agar* .

Figure 4. Validation of VNTR-1 by touchdown PCR screening. Fragments were visualized on 2% agarose gel and gelred nucleic acid gel stain. Lane 1, negative control (water); lane 2, *p2* sample; lane 3, *p4* sample; lane 4, *p5* sample; lane 5, *p6* sample; lane M, 1kb Hyperladder.

RESULTS

Results of clinical and molecular epidemiology approach are shown in Table 1.

ESTIMATED ROUTES OF TRANMISSION	PATIENT	CLONAL PROFILE	MORTALITY	SOURCE OF INFECTION	CLINICAL CONDITION	WARD	CLINICAL SAMPLE	*i and j gen*	MIC VAN	MIC TEC	PHENOTYPIC RESISTANCE PATTERN
	p1	–		H	IX	7	W	A	–	–	–
	p2	1		H	IX	3	B	A	512	32	VanA
	p3	–		H	CL	4	U	A	–	–	–
	p4	1		H	IX	2	W	A	256	64	VanA
	p5	1		H	IX	6	U	A	>512	128	VanA/VanM
	p6	2		H	IX	5	U	A	256	32	VanA
	p7	1		H	IX	5	W	A	>512	32	VanA
	p8	1		H	IX	4	W	A	>512	32	VanA
	p9	1		H	IX	2	U	A	256	32	VanA
	p10	4		EH	IX	ER	U	A	64	8	VanD
	p11	3		H	IX	6	U	A	256	64	VanA
	p12	1		EH	CL	7	U	A	>512	32	VanA
	p13	1		H	CL	6	U	A	256	16	VanA
	p14	2		H	IX	5	U	A	256	32	VanA
	p15	1		H	IX	2	OT	A	256	32	VanA

Table 1. Clonal profiles by MLVF related with epidemiological and microbiological data. 1. Estimated probability of transmission routes, according to Clonal profiles by MLVF and epidemiological data. 2. PATIENT, "p" refers to isolates obtained from outbreak patients (isolates from the first and third patients were not included because no samples were recovered from them). 3. CLONAL PROFILE according MLVF. We identified 4 different clonal profiles. 4. MORTALITY expresses the number of patient who died during the studied period of time studied. 5. SOURCE OF INFECTION refers the estimated location from where patient acquired infection; H: Intrahospital, EH: Outofhospital. 6. CLINICAL STATUS: IX: Clinical infection, CL: bacterial colonization. 7. WARD: Hospital floors where the isolate was obtained. ER: Emergency room. Second floor (2) refers to ICU. 8. CLINICAL SAMPLE from which isolate was recovered: surgical wound (W), urine (U), orotracheal sample (OT), blood (B). 9. *VanA* GEN: Identification of *VanA* gene. 10-11. MIC VAN/TEC: Vancomycin and Teicoplanin MICs according to microdilutions. 12. LEVEL OF VAN R- level of vancomycin- resistant according to MICs, classified as High (H) and Medium (M).

DISCUSSION

Molecular results obtained in this study allowed us to achieve our goal to describe the VRE*fm* spread; four (4) different clonalities were identified by MLVF, 69% (9 isolates) belonged to clone 1. These findings indicates a mixed spread of VRE*fm* clones across the hospital, with only one representing the main proportion of clones identified in isolates. When we associated this data to clinical epidemiology tracking (patient's location during hospitalization), mostly of patients were not directly exposed to each other, but remained hospitalized in same floor wards by at least 24 hours; Additionally, lack of spread in three and four clonal profiles suggests an adequate terminal room cleaning within the Hospital wards, leading to the reduction of these strains to keep on spreading. This suggests cross-contamination between patients via healthcare workers by a lack adherence to epidemiological sanitary measurements to prevent Health Care Associated Infections in Hospital settings, as hand washing and an appropriate using of medical devices could lead to the spread of VRE*fm* strains. Further reasons for this almost unique clone spread could be explained by different factors, as virulence capacities associated to this clonal profile; nevertheless, this approach also contributed us to an accurate evaluation of the effectiveness in the epidemiological measurements taken for the outbreak control and we plan to keep on using in next opportunities.