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HIGH-LEVEL AMINOGLYCOSIDE RESISTANCE *ENTEROCOCCUS* SPP IN A TERTIARY CARE HOSPITAL IN MEXICO.

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ABSTRACT

Enterococcus is one important cause hospital-acquired infections. High levels of resistance for aminoglycosides (HLAR) as gentamicin (HLGR) and streptomycin (HLSR) in *Enterococcus* isolates in a tertiary clinical care in Mexico City were studied.

Identified using Microscan® system. Resistance to ampicillin, streptomycin, gentamicin and vancomycin according to NCCLS. HLGR and HLSR were confirmed using disks. 91 strains were isolated and identified from clinical samples from January 1998 to January 1999. Two species were identified. 83 (91.2%) *E. faecalis* and 8/91 (8.8%) were *E. faecium*. *E. faecalis* in urine samples were 67/91 (73.6%). Neither showed vancomycin or ampicillin resistance; 1/8 *E. faecium* was ampicillin resistant. 30/83 (36%) *E. faecalis* and 3/8 *E. faecium* were gentamicin resistant; while 39/83 (47.0%) *E. faecalis* and 4/8(50%) *E. faecium* were streptomycin resistant. 14/83 (16%) *E. faecalis*, 3/8 *E. faecium* showed sensitive pattern for

gentamicin and streptomycin. None strains were β -lactamases producer. *E. faecalis* 12/83 (14.4%) were HLGR and 28/83 (33.7%) were HLSR. *E. faecium*. 2/8 were HLGR and 2/8 were HLSR. HLAR 33/83 (39.7%) were *E. faecalis* and 3/8(37.5%) were *E. faecium* isolated from urine. *E. faecalis* was more frequent than *E. faecium* and show that HLAR in Enterococci is high and could be a serious problem if spread as nosocomial infection.

Keywords: Enterococcus, High level aminoglycoside resistance, HLAR, HLGR, HLSR.

RESUMEN:

Enterococcus es una causa importante de infección intrahospitalaria. Se determinaron los niveles altos de resistencia para aminoglucósidos(HLAR), gentamicina (HLGR) y estreptomina (HLSR) en *Enterococcus* aislados de diversos casos clínicos en un hospital de tercer nivel en México, D.F.

La identificación se realizó usando el sistema de Microscan® y la resistencia a ampicilina, estreptomina, gentamicina, vancomicina, HLGR y HLSR de acuerdo a la NCCLS. 91 cepas fueron aisladas de muestras clínicas de Enero de 1998 a Enero 1999, se identificaron dos especies. 83 (91.2%) *E. faecalis* y 8/91 (8.8%) fueron *E. faecium*. 67/91 (73.6%) *E. faecalis* se aislaron de muestras de orina. Ninguna cepa mostró resistencia a vancomicina; 1/8 *E. faecium* fue ampicilina resistente. 30/83 (36%) *E. faecalis* y 3/8 *E. faecium* fueron resistentes a gentamicina, mientras que 39/83 (47.0%) *E. faecalis* y 4/8(50%) *E. faecium* fueron resistentes a estreptomina. Además 14/83(16%) *E. faecalis* y 3/8 *E. faecium* fueron sensibles para

ambos aminoglucósidos (gentamicina y estreptomina). Ninguna cepa fue productora de β -lactamasas. 12/83 (14.4%) *E. faecalis* mostraron HLGR y 28/83 (33.7%) HLSR. 2/8 *E. faecium* fueron HLGR y 2/8 fueron HLSR. De los aislamientos de orina 33/83 (39.7%) *E. faecalis* fueron HLAR y 3/8(37.5%) *E. faecium*. *E. faecalis* fue más frecuente que *E. faecium*. Este trabajo muestra que *Enterococcus* ssp. HLAR se aísla y podría ser un problema en brotes de infección nosocomial. .

INTRODUCTION

Enterococci are normal inhabitants of intestinal tract of human and colonize oral and vaginal cavity¹. *Enterococcus* can be found in soil, food, water, plants animals, birds and insects^{2, 3}.

Infections can be found in elderly patients and immunocompromised hospitalized patients that had received antimicrobial therapy and others risk factors as the administration of immunosuppressive drugs, circulatory failure or clinical invasive procedures⁴⁻⁶.

β -lactamic or aminoglycoside monotherapy often results in clinical failure because Enterococci strains show intrinsic low-levels of resistance⁶.

Therefore the treatment in a combination between β -lactamics and aminoglycosides is often used, but this combination can select high levels resistance aminoglycosides (HLRA) strains^{6, 7}. Enterococci β -lactamases producer is not observed but laboratory β -lactamic pattern needs to be made on clinical samples⁸, synergy between β -lactamics and aminoglycosides must be predicted for enterococci by screening test specially streptomycin and gentamicin in order to predict high levels aminoglycosides resistance (HLAR) in Enterococcus strains⁹⁻¹².

The increasing incidence of vancomycin resistance in enterococci strains (VRE) is necessary to report because the epidemiological impact at the hospital. VRE has been related with outbreaks in many centers in several countries¹³⁻¹⁷. High risk of vancomycin resistance has been observed in HLAR strains¹⁸.

HLAR and VRE had been report in Mexico for enterococci in hospitals where the use of these antibiotics are frequently^{19, 20}. In this report we showed an analysis of the high levels of resistance for aminoglycosides and vancomycin resistance in *Enterococcus* isolates in a tertiary adults care in Mexico City.

MATERIAL AND METHODS

Clinical samples and control strains. Throughout a year, we obtained strains of Enterococci from several clinical samples of hospitalized and outpatients of Hospital Central Militar in Mexico City in 1998, strains were isolated and identified using Microscan® system and species identification was carry out as Faklam's criteria²¹. Controls strains were *E. faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* 27853, *E. coli* 25922, *Haemophilus influenzae* ATCC 35540 and *E. faecalis* ATCC 51299.

Antimicrobial susceptibility test. All strains were tested with Kirby-Bauer disk diffusion method on Mueller-Hinton agar according to NCCLS using BBL® disks of 10 µg for ampicillin, streptomycin, gentamicin and 6 µg for vancomycin. Results were observed after incubation period at 35°C for 24-48 h²².

β -lactamases detection. The β -lactamases producer strains were tested using cefinase disks (BBL®), according to the manufacturer.

High levels aminoglycosides resistance test. HLGR and HLSR were screening for gentamicin and streptomycin using susceptibility test Microscan® with pos combo 4 l microplate dehydrated panels according to the manufacturer's and were confirmed using disks of HLGR and HLRS as describing by NCCLS²². Briefly, two or three colonies from a blood agar base (BAB) plus 5% sheep blood plate culture of 24-48 h at 35°C, were inoculated on brain heart infusion (BHI) and incubated 35°C until reach the 0.5 tube of Mac Farland and 10 ml from this suspension, Mueller-Hinton plates were inoculated²². BBL® disks were use to select high level resistance criteria, growth selection was observed with HLGR disk with 120 µg of gentamicin and 300 µg HLSR disk for streptomycin. The plates were incubated at 35°C 24 h and the size in mm of inhibition was measured. The results were evaluated according to NCCLS²².

RESULTS

Clinical Isolation. A total of 91 strains were isolated and identified as *Enterococcus* spp, from several clinical samples from January 1998 to January 1999, at Clinical Pathology laboratory, Microbiology Department in Hospital Central Militar in Mexico City. Two species were identified, 83 (91.2%) strains were *E. faecalis* and 8 (8.8%) were *E. faecium*. Most of them, *E. faecalis* (67/91) and *E. faecium* (7/8), were isolated from urine samples (table 1).

Table 1. Enterococci species and samples where the strains were isolated.

Sample	<i>E. faecalis</i> (N=53)		<i>E. faecium</i> (N=6)	
	Hospitalized patients	Outpatients	Hospitalized patients	Outpatients
Urine	37	5	30	2
skin abcess	11	1	0	0
Blood	1	0	0	0
Bone	1	0	0	0
CSF	1	0	0	0
Hepatic abcess	1	0	0	0
Catheter	1	0	0	0

Antimicrobial resistance. None strains of *E. faecalis* showed vancomycin or ampicillin resistance, but only 1/8(12.5%) *E. faecium* strain, was ampicillin resistant. *E. faecalis* 30/83 (36%) were gentamicin resistant, which 21/30 (70%) from urine samples wich 16/30 (53.3%) of hospitalized patients and 5/30 (16.6%) were from outpatients. *E. faecium* gentamicin resistant were 2/8 from hospitalized patients and only 1 was from a outpatient disease 39/83 (47%) were streptomycin resistant, 31/39 (79.48%) were from urine. 28/39(70%) from hospitalized patients and 5/39(7%) from outpatients. *E. faecium* streptomycin resistant were 3/8 from hospitalized patients and only 1 was from a outpatient disease. (table 2a, 2b,2c).

Table 2a. Number of strains with resistance for each antibiotic by specie

Specie	Ampicillin	Gentamicin	Streptomycin
<i>E. faecalis</i>	0/83.	30/83(36%)	39/83(48%)
<i>E. faecium</i>	1/8.	28(12%)	4/8(37.5%)

Table 2b. samples were gentamicin resistant strains were isolated

	<i>E. faecalis</i>	<i>E. Faecium</i>	<i>E. faecalis</i>	<i>E. faecium</i>
Sample	Hospitalized patients		Outpatients	
Urine	16 (19.2%)	2(2%)	5(6%)	1
skin abcess	4(4.8%)	0	0	0
Blood	1	0	0	0
Bone	1	0	0	0
CSF	1	0	0	0
Liver CSF	1	0	0	0
Catheter	1	0	0	0

Table 2c. samples were streptomycin resistant strains were isolated

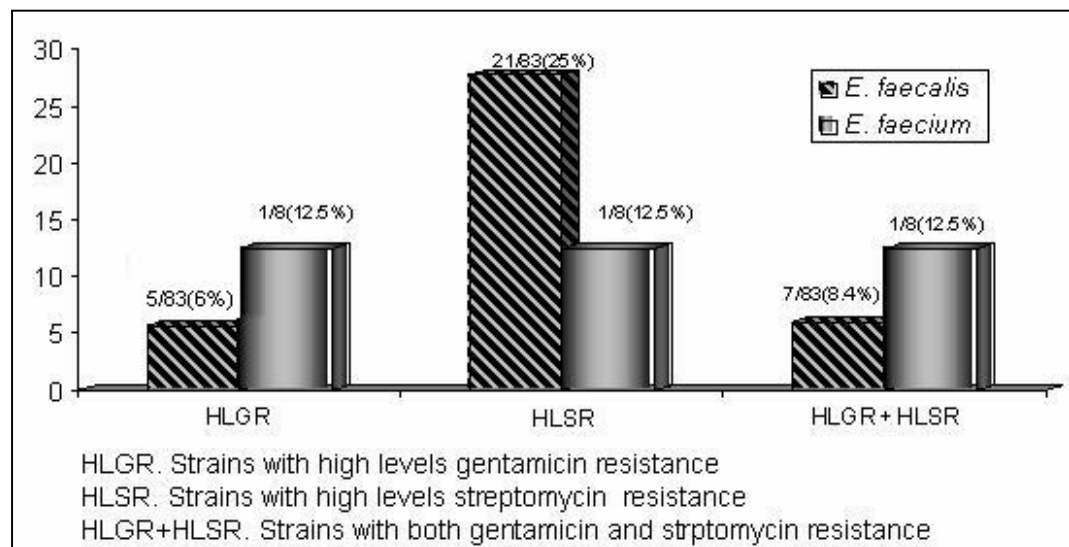
	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. faecalis</i>	<i>E. Faecium</i>
Sample	Hospitalized patients		Outpatients	
Urine	28(33%)	2(25%)	3(3.6%)	1(12.5%)
skin abcess	3(3.6%)	1	0	0
Blood	1	0	0	0
Bone	1	0	0	0
CSF	1	0	0	0
Liver abcess	1	0	0	0
Catheter	1	0	0	0

β -lactamases production. None enterococci spp. strains were β -lactamase producer.

High level aminoglycoside resistance. *E. faecalis* 33/83 were HLAR, 12/83 (14.4%) were HLGR, 28/83 (33.7%) were HLSR. While *E. faecium* 3/8 HLAR, 2/8 (25%) showed HLGR and 2/8 HLSR (table 3). With *E. faecalis* 5/83 (6.0%) were HLGR and 21/83 (25%) were HLSR alone, 7/83 (8.4%) showed resistance for both gentamicin and streptomycin resistance. While *E. faecium* 1/8 (12.5%) showed HLGR and 1/8 (12.5%) HLSR alone, 1/8 (12.5%) were for both gentamicin and streptomycin resistance (figure 1).

Table 3. Strains with HLGR and HLSR

Specie	HLGR	HLSR	Total HLAR strains
<i>E. faecalis</i>	12/83(14.4%)	28/83(33.7%)	33/83(48.1%)
<i>E. faecium</i>	2/8(25%)	2/8(12.5%)	3/8 (37.5%)

**Figure 1.** Percentage obtained of strains HLSR, HLGR or HLSR+HLGR isolated on a year in a Tertiary clinical care

DISCUSSION

Antimicrobial resistance in Enterococcus has been increasing prevalence mainly in hospitalized patients²³⁻²⁵. Few studies have been performed in Mexico about the species and antimicrobial resistance. In 1996, Sifuentes-Osornio et al.¹⁹, described the susceptibility in a tertiary-care adults center with a total of 407 enterococci strains identified. 162 from outpatients; 171 (42%) from urine as main isolate site. 325 (80%) were *E. faecalis*

and 61 (15%) were *E. faecium*. As us no β -lactamases production were detected and 12% HLGR, all were susceptible vancomycin¹⁹. In a tertiary-care pediatric hospital in Mexico City 289 isolates, 38% from urine, 30% from catheter tips, 13.8% from surgical wounds, 11.8% from blood, 3.1%

from peritoneal fluid, 2.1% from CSF and 3.3% from others showed *E. faecalis* 76.1%, *E. avium* 10%, *E. faecium* 5.2%, *E. hirae* 1.4%, *E. malodoratus* 1.4% and *E. casseliflavus* 0.6%. Ampicillin resistance were 29%, imipenem 17% and vancomycin 3%. None isolate had intermediate resistance to vancomycin. HLGR were 5.1% from 15 isolates, 6 were *E. faecalis*, 4 *E. avium*, 3 *E. faecium* and 2 *E. casseliflavus*. From 6 isolates HLSR none were resistant to vancomycin and one was β -hemolytic²⁰.

We detected in a year in a tertiary-care hospital 91 strains from different clinical samples from hospitalized 59/91 and outpatients 32/91, which were 83 (91.2%) *E. faecalis* and 8 (8.8%) *E. faecium*. The main site of isolation of strains was urine 67/83 *E. faecalis* and 7/8 *E. faecium*. These results were in accordance with others studies²³⁻²⁵. The emergence of enterococcus infection has related with urologic catheter in immunocompromised patients. Also *Enterococcus* have been implicated as nosocomial pathogen²⁶.

All *E. faecalis* were ampicillin susceptible and only 1 *E. faecium* strain showed ampicillin resistance. Actually vancomycin resistance to enterococci (VRE) is investigated in several countries¹³⁻¹⁷, but in this none vancomycin resistance were observe. We suspected that vancomycin is restricted to use in this hospital. We did not observe none strain β -lactamases producer neither *E. faecalis* nor *E. faecium* showed β -lactamases detection that it was included as surveillance program.

The rate of aminoglycosides resistance were gentamicin 21/83 (25%) *E. faecalis* and 3/8 (37.5%) *E. faecium*. Streptomycin 31/83 (37.3%) *E. faecalis* and 3/8 (37.5%) *E. faecium* were isolate from urine samples. Only 1/11 strains came from blood cultures; this was resistant for gentamicin and streptomycin (figure 1).

Overall *E. faecalis* HLGR were 12/30, HLSR was 28/40. *E. faecium* was in low frequency than *E. faecalis* but we can suppose more level of HLAR for *E. faecium* because despite the isolation were less than *E. faecalis* the HLAR percentage observed for *E. faecium* was higher (HLGR: 12/83, 14.45% vs 2/8, 25%; HLSR: 28/83, 33.73% vs 2/8, 25%).

Other authors has showed that HLAR can be two-folds higher in VRE isolates than in isolates of VSE¹⁸ but vancomycin resistance was not observe in these isolates, instead resistance for both aminoglycosides was observe. It is important to detect HLRG and HLRS because this would also help to limit the intrahospitalary dissemination of resistance and establish a surveillance program about the use of vancomycin and aminoglycosides for management of enterococci infections.

This study show that HLAR in Enterococci is high and could be a serious problem in enterococci spread as nosocomial infection and HLGR is a possible risk and must be examined in the antibiotic susceptibility pattern including HLAR detection and glycopeptide screening.

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enterococci are important nosocomial agents and serious infections caused by them are often treated with a combination of cell wall inhibitor and aminoglycoside. However, the presence of high level aminoglycoside resistance in these isolates makes this treatment combination ineffective. The prevalence of such isolates in a tertiary care set up has important diagnostic and therapeutic implications.

This study was conducted in a tertiary clinical care in Mexico City. The isolates were studied, identified using Microscan® system according to NCCLS, ampicillin, streptomycin, gentamicin and vancomycin. The number of clinical samples is low, nevertheless the authors emphasizes the importance to make routine testing of the enterococcal isolates for high level aminoglycoside resistance. Alternative treatment regimens need to be sought, besides prudent use of antibiotics.

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The role of *Enterococcus* spp. in acquiring antimicrobial resistance, particularly towards penicillins or vancomycin, as well as to aminoglycosides, is well established. Nosocomial infections caused by *Enterococcus* spp. are growing in importance and the frequency of nosocomial enterococcal bacteremia has risen continuously since 1990's worldwide according to National Nosocomial Infections System of the Centers for Disease Control and Prevention. Even though the basic molecular mechanisms concerning aminoglycoside resistance, such as reduced uptake, alterations at the ribosomal binding sites, or production of aminoglycoside modifying enzymes, have been proposed, more epidemiological data is needed in order to establish a strain-specific prognosis and management of patients.

Within this respect, it is important to evaluate the drug susceptibility of these bacterial strains isolated from hospitalized patients. Using Microscan® assay, which is a highly sensitive and preferred screening method, as approved by the National Committee for Clinical Laboratory Standards for detection of high-level aminoglycoside resistance in enterococci, the authors compare the epidemiological, microbiological, clinical, and prognostic characteristics of bacteremias caused by *Enterococcus* spp. with and without high-level antibiotic resistance, comprising an original study performed in a tertiary adults care facilities in Mexico City. An analysis of the high levels of aminoglycosides and vancomycin

resistance in *Enterococcus* isolates is clearly presented. The results are statistically significant and in agreement with other relevant studies. In conclusion, the experimental design undertaken for this study is highly recommended for following the bacterial multidrug-resistant profiles in medical centers and represents an important step in controlling the emergence and spread of vancomycin-resistant enterococci.

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