Vancomycin Resistant Enterococci (VRE) among Non –Hospitalized Individuals in Gaza City, Palestine

Abdelraouf A. Elmanama

Medical Technology Department, Islamic University-Gaza, P.O Box 108, Gaza, Palestine

E-mail: Elmanama_144@yahoo.com

Received 14/07/2008 *Accepted* 01/12/2008

Abstract

Objectives: *This study was conducted to investigate the occurrence of VRE among non–hospitalized individuals and to evaluate the public awareness of the globally escalating issue of antibiotics resistance.*

Methods: *One hundred non-hospitalized individuals were interviewed and stool sample was collected from each during the period between January and June, 2006. Isolation of Enterococci was performed according to standard method. Each Enterococcus isolate was subjected to vancomycin susceptibility test. Results were statistically analyzed using SPSS software*

Results: *We obtained 84 enterococcal isolates out of one hundred stool sample, 32.1% of them were resistant to vancomycin. No significant difference between male and female, level of education, hospitalization, age and Enterococcus carriage or VRE. Higher percentage of VRE was observed among those who were previously admitted to hospitals.*

Conclusions: *A high percentage of Vancomycin resistance was demonstrated among Enterococcus spp. isolated from non-hospitalized individuals. No single risk factor could be attributed to acquiring VRE. Therefore, we may conclude that the general population is equally exposed.*

Keywords; *VRE, Enterococci, Gaza, antimicrobial resistance*

المكورات المعوية المقاومة للفانكوميسين في الأشخاص غير المقيمين في المستشفى في مدينة غزة, فلسطين

ملخص

الأهداف: أجريت هذه الدراسة بهدف التحقق من نسبة وجود المكورات المعوية المقاومة للفانكوميسين بين الأشخاص غير المقيمين في المشافي. و تهدف الدراسة الى معرفة مدى الوعي لدى عينة الدراسة بالنسبة لمشكلة مقاومة البكتيريا للمضادات الحيوية.

الطريقة: تم إجراء مقابلة مع مائة شخص من المجتمع غير مقيمين في المشافي في الفترة ما بين يناير إلى يونيو عام 2006 وجمعت عينات براز منهم. تم زراعة العينات و عزل المكورات المعوية بالطرق القياسية و تم فحص العزلات لدراسة مدى حساسيتها للفانكوميسين. و تم تحليل النتائج إحصائيا باستخدام برنامج SPSS.

النتائج: تم الحصول على 84 عزلة من 100 عينة براز شكلت المكورات المعوية المقاومة للفانكوميسين ما نسبته 32.1%. لم تظهر التحليلات الإحصائية فروق ذات دلالة معنوية بين نسبة حمل الميكروب بين الذكور و الإناث أو مستوى التعليم, الإقامة في المشافي و كذلك الأمر بالنسبة للعمر.

على الرغم من ذلك فقد أظهرت الدراسة نسبة اعلي من حمل الميكروب بين الذين أقاموا في المشافي من الذين لم يقيموا فيها.
الاستنتاج: أظهرت الدراسة نسبة عالية من المقاومة للفانكوميسين بين عزلات المكورات المعوية المعزولة من أشخاص غير مقيمين في المشافي. لم تستطع الدراسة ربط اكتساب المكورات المعوية المقاومة للفانكوميسين بأي عامل خطر محدد و عليه يمكن الخلوص إلى أن مجمل السكان ربما يكونوا معرضين بنفس الدرجة.
كلمات مفتاحية: المكورات المعوية المقاومة للفانكوميسين, مقاومة المضادات الحيوية, غزة

Introduction

Species of the genus Enterococcus are the most common Gram-positive cocci in the intestinal tract and the 4th most common nosocomial source of bacteremia in the United States[1]**.** Two clinically relevant species, *Enterococcus faecalis* and *Enterococcus faecium*, have been shown to acquire resistance to glycopeptides and account for a majority of enterococcal infections[2]**.** Enterococci occur almost everywhere, including soil, food, water, plants, animals, birds, and insects. They inhabit humans and other animal's gastrointestinal tract and the female genital tract [3]**.**

Detection of Vancomycin Resistant Enterococci (VRE) colonization can be accomplished by culturing the organism on a variety of selective and differential media, most commonly, bile esculin azide with 6 or 8 μg/mL of vancomycin (BEAV) agar[4]**.**

Enterococcus species are aerobic gram positive cocci that are found commonly as normal flora in the gastrointestinal tract. Enterococci have been shown to colonize on skin surfaces and in the genitourinary tract as well. Although there are at least 17 species of the genus Enterococcus, the most common enterococcal species related to infection and bacteremia are *E. faecium* (85%–95%) and *E. faecalis* (5%– 10%)**[5].**

Numerous infections have been attributed to enterococci, including urinary tract infections, intra-abdominal abscesses, bacteremia, and endocarditis. Less commonly, meningitis and osteoarticular infections are attributed to enterococci**[6].**

Vancomycin resistance in enterococci has been superimposed on the increasing prevalence of high-level enterococcal resistance to penicillin and aminoglycosides, making it difficult to treat patients with infections caused by these microorganisms. The potential also exists for VRE to transfer vancomycin resistance genes to other gram-positive organisms, including Staphylococci. There have been a few documented cases of intermediate resistance to vancomycin in

methicillin-resistant *Staphylococcus aureus* (MRSA). With MRSA already a substantial problem for long-term care facilities (LTCFs), MRSA with vancomycin resistance would be devastating[7]**.**This study is considered as the first study in Gaza strip to investigate the occurrence of VRE among non–hospitalized individuals. We also attempted to assess the public awareness on antibiotic resistance issue and to examine the possible risk factors of acquiring VRE.

Material and Methods

One hundred, randomly selected healthy subjects (36 females and 64 males) were interviewed and either stool or rectal swab samples were collected. The objective of the research was explained and an informed consent was taken from all subjects. Subjects were asked to fill out a brief questionnaire and submit a stool sample or rectal swab, which was either collected by the participants themselves or by the investigator. The questionnaire consisted of both open and close ended questions dealing with personal, socioeconomic data, and attitude toward self-diagnosis and treatment of illness with antibiotics. The study started Jan 15 and lasted till June 6, 2006**.**

Rectal swabs or 1 g of stool specimen were placed in tubes containing 5 ml of Brain Heart Infusion Broth (BHIB) and transported to the laboratory. Subcultures were made onto the surface of freshly prepared Slanetz & Bartley Agar (oxoid) and Bile Esculine Agar (HiMedia) with no antibiotics. Plates were incubated at 37 ˚C for 48-72 hours. Colonies growing on agar with morphologically resembling enterococci were primarily identified by Gram staining, catalase test and by growth in 6.5% NaCl broth[8].

Resistance to vancomycin was screened by the modified Kirby-Bauer disk diffusion assay. An inoculum with a turbidity equivalent to that of a no. 1 McFarland standard and Mueller-Hinton agar (HiMedia, India) were used. Vancomycin disks (30 µg) were placed on the surface of the plate. The plates were read after incubation at 37 °C for 24 h. Zones of inhibition were interpreted according to the standard charts supplied with Vancomycin antibiotic[9].

Data obtained from microbiological investigation and from the questionnaire survey were uploaded to (SPSS version 11) software and analyzed using cross tabulating & chi square test.

Results

During the study period, 100 stool/rectal swab samples were collected from non-hospitalized individuals in Gaza city, 84 of them exhibited positive culture for Enterococci (table 1). No statistically significance difference between male and female enterococcal carriage. The same finding was observed with age.

Table 1: Distribution of Enterococcus positive stool culture according to age and sex

Stool culture for Enterococcus	Age in years						Total
	1-16		17- 40		Over 40		
	+ve	-ve	+ve	-ve	+ve	-ve	
Male	19	3	7	1	6	0	36
Female	21	2	20	6	11	4	64
Total	40	5	27	7	17	4	100
Total positive	40		27		17		84

The 84 enterococcal isolates were tested against vancomycin and as shown in table 2, 32.1% of the total isolates proved to be resistant to vancomycin. Neither age (table 2) nor sex (figure 1) could be considered as a risk factor.

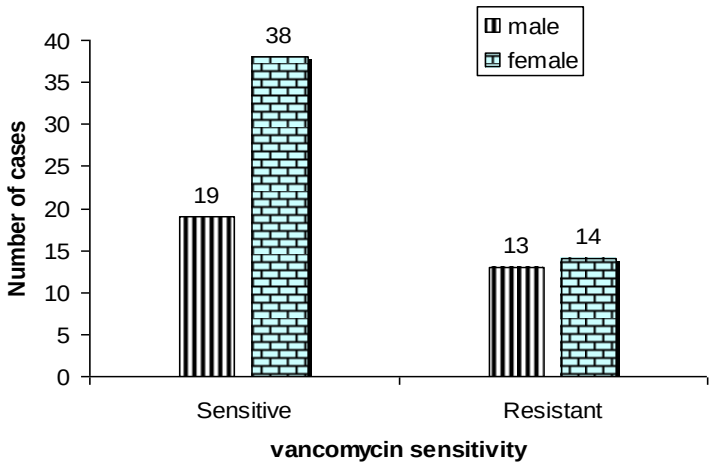

Figure 1: Distribution of VRE according to sex

Table 2: Distribution of VRE according to age

Age in years	Enterococcus sensitivity to vancomycin		Total
	Sensitive	Resistant	
1-16	25 62.5%	15 37.5%	40 100%
17-40	19 70.4%	8 29.6%	27 100%
Over 40	13 76.5%	4 23.7%	17 100%
Total	57 67.9%	27 32.1%	84 100%

Several possible risk factors for acquiring VRE were investigated, however, none of them showed statistically significant correlation with VRE carriage. Table 3 summarizes the investigated risk factors and their corresponding P value as calculated by Chi square test.

The 100 interviewed subjects answered several questions regarding their attitude to self diagnosis and self medication or malpractices. Although a high percentage (84%) was shown to carry Enterococcus in their intestinal tract and a considerable percentage (32.1%) were carrying the VRE, only few of them (10) admitted of not possessing any information about the risks of antibiotic misuse. All of the 10 are in the age group 1-16 (Table 4).

Discussion

Enterococci were found in 84% of intestinal tract of the study sample. This proportion of non hospitalized patients who carry enterococci is approximately similar to that found in previous studies, in which 80% of community carried these microorganisms[10].

VRE were isolated from 32.1% of the 100 individuals living in the community. Such high rates among non hospitalized individuals may be explained by the presence of risk factors for VRE acquisition, such as antimicrobial use, frequent and prolonged hospitalization and severity of underlying diseases. This finding is in disagreement with study in Hong Kong in which VRE were not isolated from either

healthy or hospitalized patients[11] and a very low parentage (2%) was reported in the Netherlands[10].

Table 3: Possible risk factors for acquiring VRE

Variables		VSE		VRE		P-value
		No.	%	No.	%	
Age	1-16	25	62.5	15	37.5	0.554
	17-40	19	70.4	8	29.6	
	Over 40	13	76.5	4	23.5	
Sex	Male	19	59.4	13	40.6	0.144
	Female	38	73.1	14	26.9	
Residence	City	55	67.9	26	32.1	0.693
	Village	2	66.7	1	33.3	
work	Student	23	65.7	12	34.3	0.975
	Housewife	7	77.8	2	22.2	
	Employee	4	66.7	2	33.3	
	Laborer	2	66.7	1	33.3	
	No work	21	67.7	10	32.3	
Level of education	Pre-school	12	70.6	5	29.4	0.863
	Un-educated	6	60.0	4	40.0	
	1-12 grade	33	70.2	14	29.8	
	University	6	60.0	4	40.0	
Admitted to hospital	yes	10	52.6	9	47.4	0.092
	no	47	72.3	18	27.7	
Antibiotic consumed within the last 2 years	yes	28	68.3	13	31.7	0.560
	no	29	67.4	14	32.6	
Compliance with physician instruction	yes	53	67.1	26	32.9	0.481
	no	4	80.0	1	20.0	
Pre-mature cessation of therapy	yes	4	80	1	20.0	0.481
	no	53	67.1	26	32.9	

P<0.05 is significant; P>0.05 is not significant

Table 4: Awareness of antimicrobial resistance among the study population

Age in years	Do you possess information about the risks of antibiotic misuse		Total
	Yes	No	
1-16	35 77.8%	10 22.2%	45 100%
17-40	34 100%	0 0%	34 100%
Over 40	21 100%	0 0%	21 100%
Total	90 90%	10 10%	100 100%

P= 0.01

Although outbreaks of VRE have been reported in Europe, carriage rates are generally lower, with most workers reporting rates between 0.8% and 2%**.** Even though many patients had received antibiotics, it did not result in carriage of VRE, although receipt of antibiotics has been shown to be a risk factor by other workers[11]**.**

In a Belgian study[12] , 11 (28%) of 40 volunteers living in the community who were healthy, who were not health care workers, and who had not received antibiotics for at least 1 year were colonized with VRE. The results of North American studies performed in the Houston, Texas, metropolitan area, however, contrast with the European data, since VRE appeared to be absent from healthy people in Houston[13]**.**

The presence of VRE in the stools of non-hospitalized individuals suggests that VRE form part of the normal human fecal flora or can be acquired in the community, as confirmed by several other studies[12]. A possible source of VRE could be the food chain, since VRE has been reported in the feces of farm animals and in animal product-based foodstuffs**.** The origin of the contamination of meat remains unknown, but it might occur during processing and packaging or through the intestinal flora of slaughtered animals[12]**.**

Some European investigators have raised the possibility that the glycopeptide avoparcin, which has been used as a food additive for growth enhancement in animals for nearly 20 years, might have selected VRE strains in animals[14]. The gastrointestinal tract is probably the major reservoir in humans, from which subsequent infection can eventually develop. This is in agreement with a report from New York City[15]. Food has been proposed as a source of transmission[16]. Others have put forward pets and other domestic animals[14]. Furthermore, the use of antibiotics as feed additives for growth enhancement in animals may be associated with the emergence of VRE[17].

Some investigators, however, have cautioned against comparing the results of similar studies, since differences in methodology could, at least in part, explain the observed differences in isolation rates[13].

Conclusions and recommendations

A high percentage of vancomycin resistant was demonstrated among *Enterococcus* spp. isolated from non-hospitalized individuals. No single risk factor could be attributed to acquiring VRE. Therefore, we may conclude that the general populations may be equally exposed. It is recommended that further research to pinpoint the source/s of acquiring VRE should be conducted. Meantime, local hospitals should adapt strict infection control measure to prevent nosocomial infection.

References

1. Biedenbach, D.; Moet, G.; and Jones R. Occurrence and antimicrobial resistance pattern comparisons among bloodstream infection isolates from the SENTRY Antimicrobial Surveillance Program (1997–2002). Diagn Microbiol Infect Dis 2004; 50:59–69.
2. Moellering, R. *Enterococcus* species, *Streptococcus bovis*, and *Leuconostoc* species. In Principles and Practices of Infectious Diseases, 6th ed. vol. 2. Eds, GL Mandell, JR Bennett, R Dolin. Philadelphia, PA: Elsevier Churchill Livingstone. 2005
3. Facklam, R.; and Sahm, D. Enterococcus. In: Murray PR, editor. Manual of Clinical Microbiology. 6th ed. Washington: American Society for Microbiology; 1995. p. 308-14.

4. Winn, W.; Koneman, E. Koneman's Color Atlas and Textbook of Diagnostic Microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins. 2006 p. 672–764.
5. DeLisle, S.; and Pel, T. Conundrums in the management of critically ill patients. Chest 2003;123(5):504S–18S.
6. Kauffman, C. Therapeutic and preventative options for the management of vancomycin-resistant enterococcal infections. J Antimicrob Chemother 2003; 51(Suppl S3): 23–30.
7. Massachusetts Department of Public Health Division of Epidemiology and Immunization. 2001; Infection Control Guidelines for Long-Term Care Facilities. (www.mass.gov/Eeohhs2/docs/dph/cdc/infection_control/gi_disease_guide.pdf) Last accessed on November 13, 2008.
8. Jones, R.; Sader, H.; Erwin, M.; and Anderson, S. Emerging multiply resistant enterococci among clinical isolates. Diagn Microbiol Infect. 1995; 21:85-93.
9. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial susceptibility tests, 4th ed. Approved standards. NCCLS document M7-A2. 1990; National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. Endtz, H.; van den Braak, N.; van Belkum, A.; Kluytmans, J.; Koeleman, J.; Spanjaard, L.; Voss, A.; Weersink, A., Vandenbroucke-Grauls, C.; Buiting, A.; van Duin, A.; and Verbrugh, H. Fecal carriage of vancomycin-resistant enterococci in hospitalized patients and those living in the community in The Netherlands J Clin Microbiol 1997 35: 3026-3031
11. Boost, M.; Lai, L.; and O'Donoghue M. Drug resistance in fecal enterococci in Hong Kong. J Infect Chemother. 2004;10(6):326-30.
12. Van Belkum, A.; Van den Braak, N.; Thomassen, R.; Verbrugh, H., and Endtz, H. Vancomycin-resistant enterococci in cats and dogs. Lancet 1996; 348:1038–1039.
13. Coque, T.; Tomakayo, J.; Ricke, S.; Okhuysen, P.; and Murray, B. Vancomycin-resistant enterococci from nosocomial, community, and animal sources in the United States. Antimicrob. Agents Chemother. 1996; 40:2605–2609.
14. Gambarotto, K.; Ploy, M.; Turlure, P.; Grelaud, C.; Martin, C.; Bordessoule, D.; and Denis, F. Prevalence of vancomycin-resistant enterococci in fecal samples from hospitalized patients and nonhospitalized controls in a cattle-rearing area of France. J. Clin. Microbiol. 2000; 38:620-624.
15. Papanicolaou, G.; Meyers, B.; Meyers, J.; Mendelson, M.; Lou, W.; Emre, S.; Sheiner, P.; and Miller, C. Nosocomial infections with

vancomycin-resistant *Enterococcus faecium* in liver transplant recipients: risk factors for acquisition and mortality. Clin. Infect. Dis. 1996; 23:760–766

16. Donnelly, J.; Voss, A.; Witte, W.; and Murray, B. Does the use in animals of antimicrobial agents, including glycopeptide antibiotics, influence the efficacy of antimicrobial therapy in humans? J. Antimicrob. Chemother. 1996; 37:389–390.
17. Klare, I.; Heier, H.; Claus, H.; Reissbrodt, R.; and Witte, W. *vanA* mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol. Lett. 1995; 125:165–172.

****