

VANCOMYCIN-RESISTANT ENTEROCOCCI (VRE) AS NORMAL FLORA OF THE INTESTINE IN PATIENTS AT A TERTIARY CARE HOSPITAL

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There has been a rapid increase in the incidence of infection and colonization with vancomycin-resistant enterococci (VRE) in American and European hospitals in the last six to seven years, with fecal carriage reported to be as high as 86% on some services. In order to determine the frequency of VRE as normal flora of the intestine in Saudi patients, microbiological analysis of stool specimens from 4276 patients from a tertiary care referral hospital was performed. VRE, identified as *Enterococcus faecium*, was found in six patients. None of the patients had any clinical disease associated with VRE. Five were hospitalized patients, four with severe underlying diseases; five had a history of prior antimicrobial therapy with broad-spectrum antibiotics and two of them were treated with vancomycin. *Ann Saudi Med* 1996;16(6):625-628.

Enterococci constitute normal flora of the human intestine. Although considered as bacteria of low virulence, they have been associated with urinary tract infections, intra-abdominal infections, bacteremia and endocarditis, especially in compromised patients. They account for 12% of all nosocomial infection reported in the USA, second only to *Escherichia coli* in incidence.^{1,2} During the last 15 years, these bacteria have become more resistant to ampicillin and have acquired a high level of resistance to aminoglycosides.³⁻⁵ The most disturbing trend to emerge is vancomycin resistance. Since the initial report of vancomycin-resistant enterococci (VRE) from France in 1988,⁶ their occurrence has been reported from the USA, the UK, Germany, the Netherlands, Spain and Saudi Arabia.⁷⁻¹⁴ A rapid increase in the incidence of infection and colonization with VRE has been seen in US hospitals in the last five years, with a fecal carriage rate of as high as 86% in Pittsburgh.¹⁵ Since no one has reported the colonization of Saudi patients with VRE, we did a prospective study to determine the prevalence of its fecal carriage in patients at a tertiary care center. Our findings are presented in this paper.

Material and Methods

Stool specimens from 4276 patients at King Faisal Specialist Hospital and Research Centre were collected

during the period from March 1, 1995, to February 29, 1996. The specimens were received in the laboratory in wide-mouthed, water-tight plastic containers with tight-fitting lids. All stool specimens received during the study period of one year for bacterial cultures from both outpatients as well as inpatients from all services (e.g., ICU, oncology, surgery, ob/gyn, medicine, etc.) were analyzed for the presence of VRE. The hospital is a 500-bed tertiary care facility which also serves as a referral center for Saudi Arabia and the Middle East. Specimens were inoculated on 5% sheep blood agar (BAP), McConkey agar and *Campy* BAP (containing 10 mg vancomycin/L). All plates were incubated at 35 °C for 24-48 hours, BAP and McConkey under aerobic and *Campy* BAP under microaerophilic conditions. Suspected colonies of enterococci were selected from either BAP and/or *Campy* BAP, and identified by morphology, cultural characteristics and biochemical tests as per standard procedure¹⁶ and confirmed by the GPI (Gram positive identification) system (Vitek Systems, Inc., Hazelwood, MO, USA). Initial susceptibility to antimicrobial agents was tested by disk-diffusion test according to the recommendations of the National Committee for Clinical Laboratory Standards.¹⁷ Minimum inhibitory concentration (MIC) of *Enterococcus* against antimicrobials was determined by Vitek Systems and confirmed by standard broth dilution method.¹⁸ For MIC determination, Mueller-Hinton broth, pH 7.2-7.4, was used as the test medium and inoculated with the turbidity-adjusted suspension of the isolate to achieve a final inoculum of 10⁵ CFU/mL.¹⁸ *S. faecalis* ATCC 29212 was used as a quality control organism during MIC determination. Results were found to be within the expected ranges.¹⁸

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Results and Discussion

During the period from March 1, 1995, to February 29, 1996, we examined stool specimens from 4276 patients for the presence of VRE. Ninety-one percent of the specimens processed were from inpatients, with an age range of one month to 76 years old. VRE were found in six patients and all were identified as *E. faecium*. Medical records of these six patients were reviewed and their clinical features are summarized in Table 1. The patients ranged from a baby boy of three months with acute lymphocytic leukemia to a 49-year-old female with irregular menstrual bleeding. All the patients except patient #5 were inpatients when VRE was detected in their stool specimen. Patient #5 was grossly obese (height 120 cm, weight 45 kg), with a long history of colic and recurrent diarrhea, who was seen at the hospital employee health clinic as an outpatient. None of the patients grew enterococci or VRE from any other clinical specimens. High resistance to vancomycin (MIC >256 mg/L) was found in all the six isolates, and to gentamicin in five isolates (Table 2). Four and six isolates had therapeutically achievable levels of MICs for ciprofloxacin and augmentin, respectively.

Although the epidemiology of VRE has not been completely elucidated, we know that certain patients are at increased risk for VRE colonization or infection. These include critically sick patients, those with severe underlying disease or immunosuppression, patients in the oncology or transplant wards, those with intra-abdominal or cardiothoracic surgical procedures, patients with indwelling urinary or central venous catheters, and those with prolonged hospitalization or receiving broad-spectrum antimicrobial and/or vancomycin therapy.¹⁹⁻²²

According to the Centers for Disease Control and Prevention (CDC) of Nosocomial Infections Surveillance System, the percentage of nosocomial enterococci resistant to vancomycin increased from 0.3% in 1989 to 7.9% in 1993. Among patients in ICUs with nosocomial infections, the percentage of VRE increased from 0.4% in 1989 to 13.6% in 1993.²³ The rate of colonization has varied at different geographic locations. Initially, Uttley et al.⁹ reported the largest clusters of nosocomial infections or colonization involving 45 strains of three species of VRE in the UK. Jorden et al.²⁴ examined 354 stool specimens at the John Radcliffe Hospital (UK) and found that 19 (5.4%) patients were colonized by VRE. The colonization rate of 3.5% in Belgium and up to 86% in liver transplant and oncology units in the US have been reported.^{15,25,26} Since our first report describing isolation of VRE from a wound infection three years ago,¹⁰ we have isolated VRE from the blood of two patients, and from the mitral valve and the urine of one patient each. We know of only one other report of VRE from Saudi Arabia which described VRE colonization in a patient with acute myeloid leukemia, who

TABLE 1. Clinical features of patients with VRE colonization.

Pt.	Sex	Age (yrs)	Underlying disease	Previous antimicrobial therapy
1	M	38	Aortic valve replacement with mitral valve repair	Augmentin, rifampin
2	F	3	Down syndrome, bronchial pneumonia	Ceftriaxone, augmentin
3	M	36	Hepatoblastoma/neuroblastoma	Vancomycin, ceftazidime, amikacin, metronidazole
4	F	49	Menometrorrhagia, D/C	None
5	M	10	Grossly obese, recurrent diarrhea	Augmentin, amoxicillin
6	M	3 months	Acute lymphocytic leukemia	Vancomycin, imipenem

TABLE 2. Antimicrobial susceptibility of VRE isolates.

Patients #	Minimum inhibitory concentration (mg/L)				
	Van.	Gen.	Amp.	Aug.	Cipro.
1	>256	>256	>256	2.0	2.0
2	>256	16	>256	2.0	4.0
3	>256	>256	>256	2.0	8.0
4	>256	>256	>256	2.0	2.0
5	>256	>256	128	2.0	2.0
6	>256	>256	>256	2.0	2.0

Van.=vancomycin; gen.=gentamicin; amp.=ampicillin; aug.=augmentin; cipro.=ciprofloxacin.

subsequently developed VRE septicemia.²⁷ With the exception of one of our patients (#5 in this paper), all other infected or colonized patients in Saudi Arabia had underlying diseases. Only one of them (patient #4 in this report) did not have a history of broad-spectrum antimicrobial administration.

Although we have found VRE infection in five patients and fecal colonization in six of the 4276 patients, prevalence of VRE appears to be very low in Saudi Arabia. Informal discussions with the laboratory staff and infectious diseases practitioners of most large hospitals in the Kingdom indicated that none have seen VRE except a reported case in Riyadh Military Hospital.²⁷ However, one of the primary problems in the West in tracing the history of VRE has been failure to look for VRE in many centers until the 1990s. During a 1993 survey of all the accredited New York hospitals and laboratories, Frieden et al.³³ found that 76 of the 81 hospitals tested all isolates for VRE, three only blood isolates, and two did not test enterococci for vancomycin susceptibility. Fifteen percent of the 48 nonhospital-based laboratories in the survey did not look for VRE. Similarly, commonly used techniques vary in their ability to detect VRE. In a recent study,³⁴ it was found that NCCLS-recommended methods^{17,18} showed a specificity and sensitivity of 100%, compared with 98%

and 73%, respectively, for Vitek (BioMerieux, Hazelwood, MO, USA) and 98% and 93% respectively, for Microscan (Baxter, West Sacramento, CA, USA). Therefore, disc diffusion or dilution methods^{17,18} appear to be the preferred procedures for screening VRE.

Since most of the enterococcal infections are endogenous, it appears that VRE may be present in the community and become part of the intestinal flora of patients entering hospitals. VRE have been isolated from sewage treatment plants,^{28,29} from the fecal samples of broiler chicken, thawing liquids of commercially produced frozen chicken and turkey, chicken and pig farms and minced meat of pigs in Europe.³⁰⁻³² In almost all the cases, VRE were associated with the use of glycopeptide avoparcin (Avotan, Cyanamid International) as a growth promoter in the animal feed, and were not found in farms or meat samples of chicken that were fed without avoparcin.³⁰⁻³² These findings suggest that hospitals may not be the only source of VRE and other factors may exert a selective pressure. We believe that investigation of environmental sources, poultry farms and cattle farms in Saudi Arabia for the presence of VRE would indicate the extent of the problem in this part of the world.

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