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Antimicrobial exposure and clinical outcomes associated with vancomycin -resistant enterococci.

Leon Scott Chavers
University of Alabama at Birmingham

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ANTIMICROBIAL EXPOSURE AND CLINICAL OUTCOMES ASSOCIATED WITH VANCOMYCIN-RESISTANT ENTEROCOCCI

by

LEON SCOTT CHAVERS

A DISSERTATION

Submitted to the graduate faculty of The University of Birmingham,
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ABSTRACT OF DISSERTATION
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Name of Candidate Leon Scott Chavers

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Title Antimicrobial Exposure and Clinical Outcomes Associated with Vancomycin-Resistant Enterococci

Since the 1980s, vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens. We conducted a review of VRE history, a matched case-control study, and a retrospective follow-up study to investigate the epidemiology and progression of VRE in hospitalized patients. We propose a model illustrating the progression of VRE from potential reservoirs to active disease in hospitalized patients. We document clonal transmission of VRE, and we illustrate the limited therapeutic options for VRE treatment by reporting VRE susceptibility to a wide array of antimicrobials.

In the case-control study, antimicrobial exposure and VRE occurrence were evaluated using two control groups: a vancomycin-susceptible enterococci (VSE) group, to assess factors associated with development of VRE, and a non-enterococcus (Non-E) control group, to assess factors associated with positive cultures for enterococci without regard to vancomycin resistance. Controls were matched to 135 VRE cases by hospital location, body site of culture, and date of culture within 30 days. After adjusting for the effect of other antimicrobials, time at risk, and patient morbidity, we found exposures to imipenem (OR = 4.9, 95% CI, 1.6-14.1) and ceftazidime (OR = 2.6, 95% CI, 1.1-6.1) occurred more often in the VRE group than in the VSE group. Exposures to ampicillin (OR = 20.1, 95% CI, 1.5-263.1) and imipenem (OR = 5.1, 95% CI, 1.5-17.1) occurred more

often in the VRE group than in the Non-E group. Neither piperacillin nor vancomycin exposure occurred more often in the VRE group than in either of the two control groups.

In the retrospective follow-up study, a higher hospital mortality rate was observed in VRE patients than in VSE patients ($P = 0.02$). After adjustment for characteristics also shown to be associated with mortality, vancomycin resistance (OR = 2.1; 95% CI, 1.1-4.8) remained a significant predictor of hospital mortality.

In conclusion, our findings are salient to the control and elimination of vancomycin resistance in enterococcus. Vancomycin resistance is an independent predictor of hospital morbidity and mortality. Identifying patients at increased risk for VRE isolation and utilizing antimicrobials with activity against enterococcus could substantially reduce the colonization and spread of VRE.

DEDICATION

I dedicate this dissertation to my parents, Erdist and Shirley Chavers, who taught me the importance of placing family first, and who gave me every chance to succeed; to my wife, Paige Chavers, for her love and support, and for always believing in me, even when I do not; and to my son, Christopher Lance Chavers, who reminds me of the important things in life.

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LIST OF ABBREVIATIONS

APACHE: Acute Physiology and Chronic Health Evaluation score

BSI: blood stream infection

BVAMC: Birmingham Veterans Affairs Medical Center

CDC: Centers for Disease Control and Prevention

CI: confidence interval

GISA: glycopeptide intermediate-resistant *Staphylococcus aureus*

HICPAC: Hospital Infection Control Practices Advisory Committee

ICARE: Intensive Care Antimicrobial Resistance Epidemiology

ICU: intensive care unit

MIC: minimum inhibitory concentrations

MRSA: methicillin-resistant *Staphylococcus aureus*

NNIS: National Nosocomial Infections Surveillance

Non-E: non-enterococci isolates

OR: odds ratio

PFGE: pulse-field gel electrophoresis

U.S.: United States of America

UAB: University of Alabama at Birmingham

VISA: vancomycin intermediate-resistant *Staphylococcus aureus*

VRE: vancomycin-resistant enterococci

VRSA: vancomycin-resistant *Staphylococcus aureus*

VSE: vancomycin-susceptible enterococci

INTRODUCTION

Vancomycin-resistant enterococci (VRE) were first identified in 1986. As the proportion of enterococci that are resistant to vancomycin has increased steadily over the years, VRE have emerged as important nosocomial pathogens. Additionally, VRE were recently implicated in transfer of high-level vancomycin resistance to *Staphylococcus aureus*. An understanding of the epidemiology and progression of VRE is paramount in slowing VRE dissemination and preventing selection of vancomycin-resistant *S. aureus* on a wide scale.

The existing literature indicates that the epidemiology of VRE within health-care institutions is dependent on multiple factors. From this literature, we developed a model for VRE emergence in the hospital setting that is expected to serve as a template for identifying mutable factors that may serve as focal points for intervention. The review also reports the clonal spread of VRE within a large, urban, teaching hospital, and describes the susceptibility of VRE to a large number of antimicrobials.

Questions remain as to the role of specific antimicrobial exposure in the epidemiology of VRE. A matched case-control study was conducted to assess the association between exposure to specific intravenous antimicrobials and the isolation of VRE.

The increasing proportion of enterococcal isolates that are vancomycin resistant may have a major impact on the mortality of hospitalized patients; however, the degree to which VRE contributes to mortality remains controversial and largely unexplored. A retrospective follow-up study was conducted to determine the contribution of

vancomycin resistance to patient mortality and to determine what factors affect the risk of mortality for patients with VRE, while adjusting for disease severity and other factors known to be associated with high hospital mortality.

Our findings and proposed model are salient to the control and elimination of vancomycin resistance in enterococcus. The identification of patients at increased risk for VRE isolation and the utilization of antimicrobials with activity against enterococcus could substantially reduce the colonization and spread of VRE.

VANCOMYCIN-RESISTANT ENTEROCOCCI: FIFTEEN YEARS AND COUNTING

by

L. SCOTT CHAVERS, STEPHEN A. MOSER, WILLIAM H. BENJAMIN. JR., SHAWN E. BANKS, JON R. STEINHAUER, ANITA M. SMITH, CRYSTAL N. JOHNSON, ELLEN FUNKHOUSER, L. PAIGE CHAVERS, ALAN M. STAMM, AND KEN B. WAITES

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ABSTRACT

Vancomycin-resistant enterococci (VRE) have emerged as important nosocomial pathogens since the 1980s due to a wide array of complex factors. Since VRE were first identified, the proportion of enterococci with resistance to vancomycin has increased steadily, and VRE have been implicated in transferring high-level vancomycin resistance to *Staphylococcus aureus*. We review the history of VRE and propose a causal model illustrating the roles of exposure to VRE reservoirs, patient characteristics, antimicrobial exposure, and prevalence of VRE in the progression from potential VRE reservoirs to active disease in hospitalized patients. Differences in VRE colonization and VRE infection are discussed with respect to hospital surveillance methodology and implications for interventions. We further document clonal transmission of VRE in a large, urban, teaching hospital and demonstrate VRE susceptibility to a wide array of antimicrobial agents. This model can guide the identification of mutable factors that are focal points for intervention.

INTRODUCTION

The year 2003 marks the fifteenth anniversary of the first published report of clinical strains of vancomycin-resistant enterococci (VRE) [1]. Since that time, VRE have emerged as important nosocomial pathogens, stimulating a large body of research to elucidate the epidemiology and causal factors responsible for this progression in an organism not generally thought of as an especially virulent pathogen. This previous research indicates that the appearance and spread of VRE within health care institutions are dependent on multiple factors, some of which are amenable to targeted interventions. In

this review, we present a model for VRE emergence based on prior investigations of VRE epidemiology.

BACKGROUND

Vancomycin

Vancomycin is a tricyclic glycopeptide antibiotic derived from the actinomycete *Amycolatopsis orientalis*. Vancomycin's primary mode of bactericidal action in gram-positive organisms involves disruption of peptidoglycan polymerization through binding to peptides containing D-alanyl-D-alanine, the substrate of peptidoglycan synthetase. Vancomycin was first produced in the late 1950s to treat staphylococcal infections, but it was not heavily used until the late 1970s when methicillin-resistant *Staphylococcus aureus* (MRSA) became prevalent. In 1995, the Centers for Disease Control and Prevention (CDC) published guidelines for appropriate use of vancomycin in hospitals as a direct response to concerns regarding the development of vancomycin resistance in enterococci and in other organisms, including the staphylococci [2]. Vancomycin is an appropriate therapeutic choice for treating MRSA, coagulase-negative staphylococci, and meningitis due to *Streptococcus pneumoniae* with reduced susceptibility to beta-lactams, as well as suspected gram-positive bacterial infections in persons who are allergic to beta-lactams. Vancomycin may also be used for treating other conditions, including infective endocarditis, and as prophylaxis against infection in patients undergoing cardiac or vascular surgical procedures. Although once used for treating staphylococcal enterocolitis and antibiotic-associated colitis, oral vancomycin use is strongly discouraged because of concerns over the increase in VRE. Increased use of vancomycin for empiric

and directed treatment of MRSA infections has directly paralleled the emergence and spread of VRE, and may be responsible for recent reports of vancomycin-resistant *S. aureus* (VRSA) [3].

The Age of VRE

In 1986, the first clinical strains of VRE were isolated in England and France [1], and one year later, the first VRE were documented in the United States (U.S.) [4]. Over the years, the proportion of enterococcal isolates exhibiting resistance to vancomycin has increased steadily. The National Nosocomial Infections Surveillance System (NNIS) reported that the percentage of enterococcal isolates exhibiting vancomycin resistance increased from 0.3% to 7.9% overall, and from 0.4% to 13.6% in intensive care units (ICUs) in the U.S. between 1989 and 1993 [5]. By 1999, the percentage of ICU isolates exhibiting vancomycin resistance reached 25.2% of 1579 isolates tested, a 43% increase in the mean rate of resistance compared to the years 1994-1998 [6]. By the year 2000, the percentage of vancomycin-resistant isolates reached 26.3% of 2575 isolates tested from ICUs, representing a 31% increase in the mean rate of resistance compared to the years 1995-1999 [7].

Mortality. The increasing proportion of enterococcal isolates that are vancomycin resistant may have a major impact on the mortality of hospitalized patients [8]. However, the degree to which VRE contributes to mortality has proven difficult to estimate because of comorbidities often present with VRE. The question remains as to whether VRE acquisition increases a patient's severity of illness or whether a patient's severity of illness

increases the likelihood of VRE acquisition. One study estimated the mortality associated with VRE to be as high as 71% in ICUs, whereas mortality associated with vancomycin-susceptible enterococci (VSE) has been estimated at 41% [9], despite similar severity of illness scores. However, others have been unable to demonstrate an increased mortality among persons with bacteremia due to VRE as opposed to VSE when adjustments are made for severity of illness [10, 11], suggesting there is no increase in virulence attributable to VRE.

The emergence and dissemination of VRE during the 1990s has also had a significant impact on health care systems. Patients with VRE have been shown to have an average length of stay of 34.8 days vs. 16.7 days for VSE, despite similar severity of illness scores, and patients with VRE had mean hospital costs of $27,000 per episode in excess of those patients with VSE ($83,000 vs. $56,000) [9].

The VISA-VRSA-VRE connection. In 1996, the first reports of intermediate resistance to vancomycin in *S. aureus* (VISA) with minimum inhibitory concentrations (MICs) of 8 μg/ml were described from patients who received long-term vancomycin treatments for MRSA [12]. In vitro studies had previously demonstrated the transfer of *vanA* resistance genes from enterococci to *S. aureus*, *S. epidermidis*, and other gram-positive organisms via plasmid-mediated conjugation [13, 14], so it was assumed initially that this mechanism might have been operative. However, none of these isolates, or subsequent isolates from the U.S. and other countries demonstrated the presence of *vanA* [15, 16]. Eventually it was determined that these initial VISA isolates possessed altered cell walls that were apparently responsible for their relative insensitivity to vancomycin [17].

In June 2002, the first documented case of infection caused by VRSA (MIC $\geq$ 32 μg/ml) was reported in a patient from the U.S. [18]. A surveillance culture also identified concomitant infection as a result of VRE. This VRSA isolate contained the *vanA* gene, which suggests that the resistance determinant might have been acquired from VRE. Because the initial VISA isolates had reduced susceptibility to both vancomycin and teicoplanin, they have also been referred to as glycopeptide intermediate-resistant *S. aureus* (GISA). The identification of VRSA, GISA, and VISA has substantial implications for patient morbidity and mortality because of the limited therapeutic options for treating these infections. The potential for VRE to pass genes conferring vancomycin resistance to *S. aureus* underscores the importance of understanding VRE epidemiology [18].

The Organism and Its Habitat

The preferred ecologic niche of enterococci is the intestine. These organisms have been identified in humans and many animals, as well as on outdoor vegetation and in surface water. The presence of VRE on outdoor vegetation and in surface water is may be attributed to contamination of these areas by animal excrement or untreated sewage [19]. Enterococci are extremely hearty organisms that are able to survive under environmental conditions often deleterious to other organisms. They tolerate a wide range of environmental conditions: temperature ranges of 10°C to 45°C; hypotonic and hypertonic; acidic and alkaline; and anaerobic and aerobic conditions [20, 21]. Enterococci also have the ability to tolerate sodium azide and concentrated bile salts, which kill or inhibit the growth of most microorganisms. Their ability to colonize persons for long periods [22-25], often without ill effects, to survive on inanimate objects [22, 24-37], and

their intrinsic resistance to many antimicrobial agents [38] guarantee their success as nosocomial pathogens. The resilience of enterococci under hostile environmental conditions enables colonization of areas uninhabitable by other organisms and increases the number of potential enterococcal reservoirs [19].

Clinical Epidemiology

The species distribution of clinical enterococcal isolates varies both internationally [39] and between health care facilities, depending on the characteristics of the health care facility, infection control practices, and antimicrobial use [6, 7, 40-46]. Historically, *E. faecalis* accounted for approximately 90% of all enterococcal isolates; however, the decreasing susceptibility of *E. faecium* to vancomycin and ampicillin has resulted in an increasing proportion of *E. faecium* and a decreasing proportion of *E. faecalis* [47]. According to 1997-1999 data from the SENTRY Antimicrobial Surveillance Program, *E. faecalis* accounted for between 57.2% and 76.8% of clinical isolates in the U.S., Canada, Latin America, Europe, and Asia-Pacific [39]. *E. faecium* accounted for between 4.6% and 19.3% of clinical isolates, while other species accounted for less than 5% of enterococcal isolates.

According to NNIS data from January 1992 to July 1998 [42], *Enterococcus* species was the fourth most common pathogen associated with nosocomial infection in intensive care unit (ICU) patients. Among ICU patients, enterococci ranked first in surgical site infections, and third in urinary tract infections and blood stream infections (BSIs). According to the Surveillance and Control of Pathogens of Epidemiologic Importance program, *Enterococcus* species accounted for 11.7% of all blood stream isolates in 1996

[48]. In 1997, the SENTRY Antimicrobial Surveillance Program showed that enterococci were the forth most common cause of nosocomial BSIs in the U.S. and Canada, accounting for 9.1% to 9.6% of BSIs; however, enterococci were identified in only 2.9% of BSIs in Latin America [39].

Antimicrobial Resistance and Therapeutic Alternatives

Enterococci present a therapeutic challenge because no single agent is bactericidal and because enterococci are to some degree intrinsically resistant to all cephalosporins, clindamycin, trimethoprim, sulfonamides, and to low levels of aminoglycosides [49]. Many enterococci have adapted to antimicrobial exposure in the gastrointestinal tract by acquiring resistance to penicillins, erythromycin, tetracycline, and high levels of aminoglycosides, as well as vancomycin [50, 51]. The propensity of enterococci to acquire resistance may relate to their ability to participate in various forms of conjugation, which can result in the spread of genes as part of conjugative transposons, pheromone-responsive plasmids, or broad host-range plasmids [51]. Although the great majority of *E. faecalis* isolates still exhibit some susceptibility to ampicillin and penicillin, over 70% of *E. faecium* may be resistant to these agents, even if vancomycin susceptibility is maintained [38, 52, 53]. Beta lactamase-mediated penicillin resistance has been reported in enterococci, but most resistance to beta-lactams is by reduced affinity of penicillin binding proteins [54]. Ureidopenicillins, such as piperacillin, are more active against enterococci than are carboxypenicillins, such as ticarcillin, but are generally less active than ampicillin or penicillin G. High-level resistance to gentamicin and/or streptomycin

also occurs in some isolates, further limiting treatment alternatives for serious infections [38, 55, 56].

Before the availability of the streptogramins quinupristin-dalfopristin and the oxazolidinone linezolid, there were often no drugs to which VRE were susceptible in vitro. Recent susceptibility data from the University of Alabama at Birmingham (UAB) Hospital and the Birmingham Veterans Affairs Medical Center (BVAMC) underscore the scarcity of available drugs to treat VRE infections (table 1). Although still extremely rare, VRE with resistance to linezolid [57] or quinupristin/dalfopristin [58] have been described. As these drugs achieve wider usage, further development of resistance can be anticipated.

Table 1. Comparative in vitro susceptibilities of 17 antimicrobials against 185 vancomycin-resistant *E. faecium*

Antimicrobial	MIC* 90%	% Susceptible	% Intermediate	% Resistant
Vancomycin	> 128	0	0	100
Imipenem	> 32	0	0	100
Ticarcillin/Clavulanate	> 128/2	0	0	100
Piperacillin/Tazobactam	> 128/4	0	0	100
Penicillin	> 32	0	0	100
Erythromycin	> 16	0	0	100
Tetracycline	= 32	17.7	3.5	78.8
Nitrofurantoin	> 128	12.4	24.3	63.3
Chloramphenicol	= 16	74.6	20.5	4.9
Rifampin	= 16	66.5	7.5	26
Ciprofloxacin	> 16	1.6	0	98.4
Levofloxacin	> 16	2	0.5	97.5
Gatifloxacin	> 16	1	1.6	97.4
Quinupristin/Dalfopristin	= 0.5	100.0	0	0
Linezolid	= 2	100.0	0	0
Gentamicin 500 μg	> 500	15	NA	85
Streptomycin 1000 μg	> 1000	2.7	NA	97.3

NOTE. Susceptibility interpretations were based on 2002 NCCLS breakpoints [59]; MIC, minimum inhibitory concentration.

The hardiness of enterococci likely adds to their ability to develop resistance by facilitating survival in the gastrointestinal environment and thus enhancing the potential spread from person to person as a multidrug-resistant clone. The combination of these attributes suggests that enterococci and their resistance to antimicrobial drugs will continue to pose a serious challenge for the health care field [38].

Mechanisms of Vancomycin Resistance

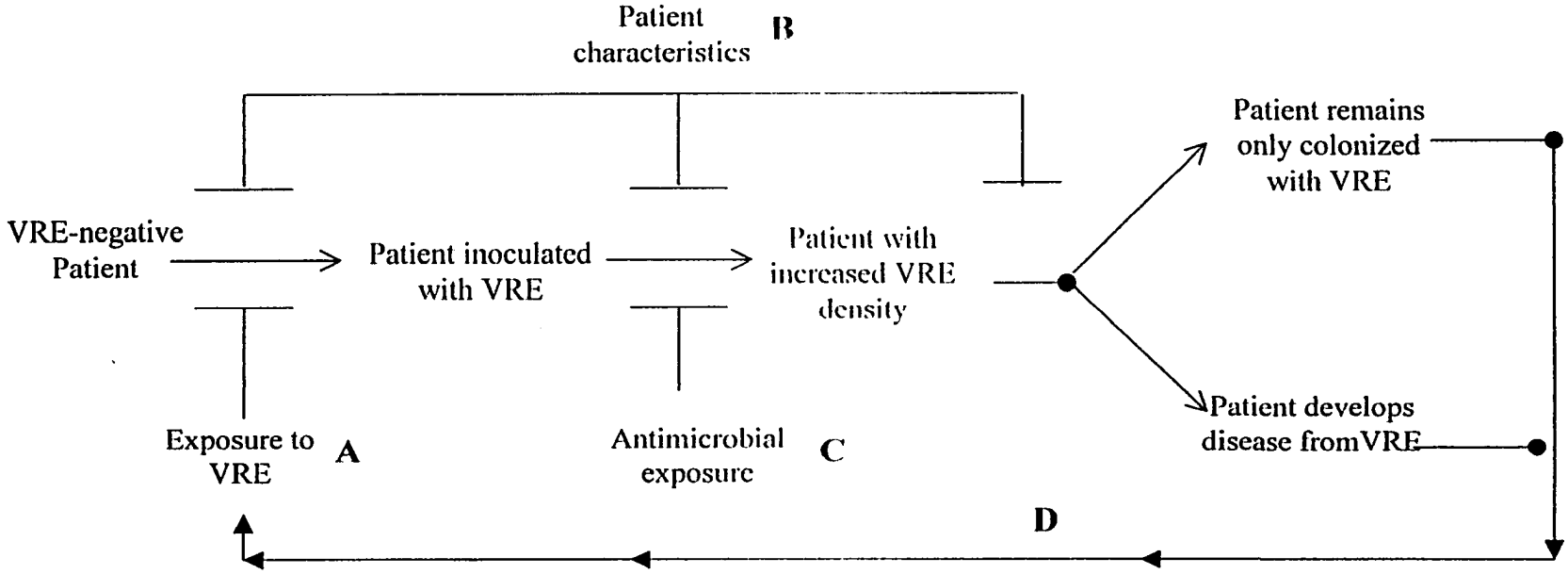
Five phenotypes of vancomycin resistance have been described in enterococci: VanA, VanB, VanC, VanD, and VanE [55]. Of the five phenotypes, the VanA and VanB are the more clinically relevant. Both VanA and VanB phenotypes result from the acquisition of new genetic determinants of resistance carried on transposon Tn *1546.* The origin of these genes remains unknown, but one potential source could be the organisms that produce glycopeptides [60]. Both phenotypes are most frequently found in *E. faecium* and *E. faecalis*. Enterococci with VanA resistance possess high-level resistance to vancomycin (MIC >128 μg/ml) and teicoplanin (MIC >16 μg/ml). Exposure to glycopeptide and non-glycopeptide agents induces the synthesis of several proteins encoded in these genetic determinants that together confer resistance by preventing the binding of vancomycin to its substrate [53].

Enterococci with VanB resistance possess a broad range of resistance to vancomycin (MIC = 4-1,024 μg/ml), and are susceptible to teicoplanin (MIC ≤ 0.5 μg/ml). A constitutive, non-transmissible, chromosomal-based VanC resistance predominantly occurs in commensal species such as *E. casseliflavus* and *E. gallinarum,* although VanA has also been described in the latter species [55]. This type of resistance is typically of lower

magnitude than that mediated by VanA or VanB and results in MIC of 8-16 μg/ml. Because this type of vancomycin resistance has epidemiologic and disease implications different from those of VanA and VanB mediated resistance, it is particularly important for diagnostic laboratories to have adequate methods in place to accurately speciate enterococci and to quantitate their MIC to vancomycin. Recent data from UAB and BVAMC showed that among 198 VRE, 196 were *E. faecium* containing *vanA,* and only two were *E. faecalis* containing *vanB*.

PROPOSED CAUSAL MODEL FOR VRE

In the proposed model (figure 1), a VRE-negative patient is exposed to VRE through one of several reservoirs (A). The patient may or may not become inoculated with VRE, depending, in part, on the characteristics of the patient (B), the route of exposure, and the level of exposure. The VRE inoculum may then increase in density depending on patient characteristics (B) and antimicrobial exposure (C). The increasing VRE density may lead to the patient remaining at the colonization level, or may lead to the development of disease attributable to VRE, again influenced by intrinsic patient characteristics. Patients colonized or infected may then serve as reservoirs for exposing VRE to VRE-negative patients. Transmission is mediated by factors such as patient characteristics (B), antimicrobial use (C), and the prevalence of VRE within the hospital (D). Each of these components in the model is discussed in the following sections.



A. Exposure to VRE

1. Infected/colonized patients
2. Contaminated environmental surfaces
3. Contaminated medical equipment
4. Hands of health care workers
5. Invasive procedures
6. Enteral tube feedings
7. Community exposures

B. Patient characteristics

1. Chronic renal failure
2. Renal insufficiency
3. Organ transplant recipients
4. Neutropenia
5. Liver disease
6. Prolonged hospitalization
7. ICU admission
8. Prolonged ICU stay
9. Intra-hospital transfer
10. Patient overall health

C. Antimicrobial exposure

1. Vancomycin
2. Extended spectrum cephalosporins
3. Antianaerobic antimicrobials
4. Fluoroquinolones

D. Prevalence of VRE

Figure 1. Proposed model for the progression of a VRE-negative patient to VRE colonization or disease; VRE, vancomycin-resistant enterococci; ICU, intensive care unit.

Exposure to VRE Reservoirs (A)

The emergence of VRE through bacterial mutation caused by antibiotic exposure is unlikely because of the complexity of the genetic sequences that are necessary to confer vancomycin resistance. The increase in VRE prevalence in U.S. hospitals during recent years is largely due to clonal spread of VRE to hospitalized patients from potential reservoirs (A). The number of clones within a hospital appears to be related to the length of time VRE is present. Investigations that detected VRE in the early stages of an outbreak generally identified cases caused by a single strain. As VRE become endemic over time, multiple clones are encountered. These multiple clones may include a primary clone coexisting with unrelated strains.

In the U.S., hospitalized patients colonized or infected with VRE appear to be the primary reservoirs for the transmission of VRE within the institutional setting. In an investigation of a monoclonal outbreak of *E. faecium* in an ICU, Boyce et al. [61] reported the primary risk factors for colonization were proximity to another VRE colonized patient and exposure to a nurse who cared for a case on the same shift. A prospective longitudinal cohort study by Bonten et al. [62] of mechanically ventilated patients in an ICU reported that cross-transmission accounted for 85% of colonization among patients initially free of VRE.

Among 185 non-duplicate VRE from UAB and the adjoining BVAMC isolated between 1997-2000, we identified 65 unique pulsed-field gel electrophoretic (PFGE) patterns, each representing a single strain. The remaining 120 isolates (65%) were composed of at least 34 distinct clones containing 2 to 14 isolates each. These findings indicate the apparent spread of individual VRE strains within and between the two adjacent

institutions. Analysis of medical records of patients from whom clonal VRE isolates were obtained revealed significant geographic and temporal associations among these patients. When compared to non-clonal VRE isolates, patients infected by organisms from the same clone were 8 times as likely to have been in the trauma/burn or rehabilitation units, or have had contact with another person with the same clone who had a history of transfer from the trauma/burn or rehabilitation units ($P = 0.04$). Figure 2 shows a dendrogram and PFGE patterns for the VRE isolates from 14 patients representing the largest single clone that persisted at UAB from 1997 through 1999.

Potential reservoirs for VRE exposure may also include contaminated environmental surfaces and medical equipment. Environmental surveillance in hospitals has documented VRE contamination of hospital gowns, doors, cabinets, tables, telephone headsets, floors, bedrails, urinals, bedpans, toilet seats, beds, bed linen, glucose meters, intravenous pumps, electrocardiogram-monitor leads, stethoscope diaphragms, ear-probe thermometers, and blood pressure cuffs [22, 24-37]. Survival of VRE on inanimate objects and medical devices for up to 4 days has been reported [22-25]. However, the contribution of environmental contamination within the hospital as a reservoir of VRE transmission has not been quantified. Two studies have directly implicated medical devices as the source for VRE transmission [29, 32]. Bonten et al. [62] designed a study to evaluate the role environmental contamination plays in VRE transmission. They found that contamination of the environment was transient and that low numbers of colony-forming units were present on each surface. Contact of susceptible patients with the hands of health care workers has been shown as an important determinant in VRE transmission [22, 28, 62]. VRE have been isolated from the hands of health care workers in 0

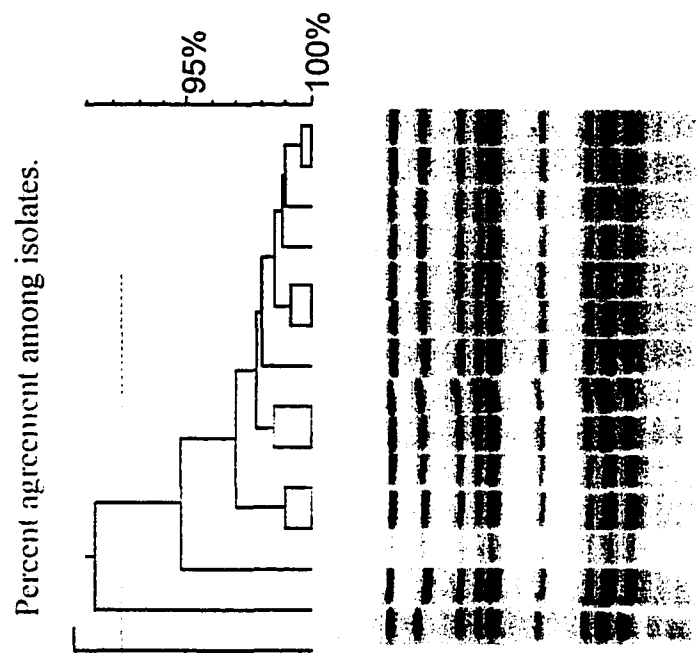


Figure 2. Pulse field gel electrophoresis (PFGE) patterns and dendrogram of a single clone of VRE isolated from 14 patients at University of Alabama at Birmingham Hospital. Five isolates were from 1997; 8 isolates were from 1998; and 1 isolate was from 1999. Patterns were generated using PFGE on SmaI digested genomic DNA and the dendrogram produced using Pearson correlation in GelCompar II (Applied Maths, Kortrijk, Belgium).

to 41% of the hands sampled [27, 28, 63, 64]. Experimental evaluation indicates VRE may contaminate fingers for up to 60 minutes after inoculation [22]. Further studies indicate that health care personnel practiced appropriate hand hygiene only 48% of the time when hand-washing facilities were available [65].

VRE reservoirs outside of the hospital environment have also been identified. These reservoirs were first suspected when with VRE were detected in hospitalized patients from the United Kingdom who had not been previously hospitalized [66]. The common link for several of these patients was residence on farms with livestock colonized with VRE. Subsequent surveillance has identified VRE from both poultry and pork in several European countries. The presence of VRE in farm animals may be due to the incorporation of additives to animal feed [67-70]. Avoparcin, a glycopeptide structurally similar to vancomycin and teicoplanin. was used to promote growth and feed utilization for many years until its use was banned by the European Union in 1997 [71, 72]. Strains of *E. faecium* with decreased susceptibility to avoparcin have been shown to be resistant to vancomycin as well, suggesting that resistance may be mediated by the same gene. In the U.S., avoparcin was not licensed as a feed additive for animals, and limited surveillance has not yet detected the presence of VRE in animals.

Patient Characteristics (B)

Patients with chronic renal failure or other renal insufficiency, neutropenia, cancer, receipt of organ transplant, and lower overall health appear to have an elevated risk for VRE [5, 25, 26, 29, 31, 63, 73]. Other patient characteristics identified to be associated with VRE are prolonged hospitalization [24, 35, 63], prolonged ICU stay [74, 75],

intra-hospital transfer [63], and the use of enteral tube feedings [35]. Risk factors specifically associated with VRE infection as opposed to colonization include the following: malignancy; increased morbidity as measured by an increased Acute Physiology and Chronic Health Evaluation (APACHE) II score; neutropenia; longer hospital stay, renal insufficiency, and hospitalization on a hematologic malignancy/bone marrow transplantation service (reviewed in [55]).

Antimicrobial Exposure (C)

The density of VRE is a function of the antimicrobial pressure exerted by antimicrobials within a given anatomic compartment. Antimicrobials may result in increased VRE density when a particular antimicrobial concentration results in a differential growth rate of VRE by inhibiting the growth of organisms competing for nutrients within the same niche. Factors that determine the ability of an antimicrobial to inhibit growth of VRE in the intestinal tract include the concentration of the antimicrobial in its active form and the susceptibility of the enterococci to it. The bioavailability of the active form of an antimicrobial in the intestinal tract is influenced by biliary or colonic excretion and by the degree to which the antimicrobial is degraded in the intestine [76]. Theoretically, antimicrobials that are excreted in high concentrations in bile and have modest activity against VRE may inhibit small inocula of VRE in the small intestine and prevent colonization. A good example of such an agent is piperacillin. Piperacillin achieves bile concentrations of more than 1000 μg/ml after a 4 g intravenous dose in adults; minimum inhibitory concentrations of many VRE strains are below this level [56]. An antimicrobial may inhibit one enterococcal strain but not another, depending on the susceptibility patterns of the

different strains. For instance, oral vancomycin therapy will inhibit VSE strains in the bowel, but not VRE, resulting in the replacement of susceptible enterococcal strains by VRE [38].

Although the role of antimicrobials in the epidemiology of VRE has been studied extensively, many controversies remain (reviewed in [77]). Antimicrobials that have been implicated in VRE emergence are vancomycin, extended spectrum cephalosporins, antianaerobic agents such as metronidazole, and fluoroquinolones. The use of oral vancomycin has been hypothesized to contribute to the increase in VRE prevalence [28, 78]. In fact, the first documented case of VRE was identified in a patient who received oral vancomycin [1]; however, epidemiologic data on the association between oral vancomycin use and VRE are limited because the use of oral vancomycin therapy has decreased in recent years.

Numerous studies have documented the association between intravenous vancomycin use and VRE [11, 24, 64, 75, 79-87], whereas others have found no association [26, 62, 76, 88-97]. The discrepancies in these findings appear to be due to differences in study design, confounding by length of hospital stay, and, presumably, publication bias, (i.e., positive associations may be more likely to be accepted for publication in peer-reviewed journals than null results [77]). Studies that had control groups representative of the population that gave rise to the VRE colonized or infected patients and that adjusted for length of hospital stay typically revealed that intravenous vancomycin use was not associated with the isolation of VRE. Length of hospital stay is an important confounder in the association between vancomycin use and VRE. As the length of hospital stay increases, the probability of exposure to VRE increases. Likewise, as the length of

hospital stay increases, the probability of exposure to vancomycin increases. Studies not adjusting for length of hospital stay tend to give a biased estimate of the association between vancomycin use and VRE. A recent study was conducted at UAB that was sufficiently powered to evaluate the contribution of intravenous vancomycin exposure and VRE occurrence in 135 patients with VRE and 135 matched controls with VSE. After carefully adjusting for patient comorbidities and length of stay, we found no association between intravenous vancomycin exposure and VRE [98].

Extended-spectrum cephalosporin use has been identified in many studies as an important risk factor for VRE [24, 62, 79, 87, 99-105]. Ostrowsky et al. [91], after adjusting for length of hospital stay, showed that extended-spectrum cephalosporin use was the only antimicrobial exposure associated with VRE in multivariable analysis. A meta-analysis of 19 studies evaluating the association between antibiotic exposure and VRE colonization or infection demonstrated a significant association between receiving extended-spectrum cephalosporins and VRE colonization (OR = 3.4; 95% CI, 2.3-5.0) (reviewed in [77]). The association between extended-spectrum cephalosporin use and VRE has also been demonstrated in studies that evaluated formulary restrictions of cephalosporins on VRE colonization or infection. Bradley et al. [106] reported a decrease in VRE infection and colonization rates in an oncology unit when ceftazidime was replaced with piperacillin-tazobactam. Smith et al. [107] also reported a decline in VRE prevalence when piperacillin-tazobactam was substituted for cephalosporins. May et al. [108] reported an eradication of all VRE infections when piperacillin-tazobactam was substituted for cephalosporins in the trauma/burn unit of the UAB hospital. Finally, Nourse et al. [104] reported a complete eradication of VRE infection and transmission with the restric-

tion of cephalosporins and glycopeptides. Although the decrease in VRE with formulary interventions has been repeatedly demonstrated, the causal association is difficult to estimate because these interventions are often implemented simultaneously with interventions targeting VRE transmission. Additional studies at UAB Hospital have shown that the use of ceftazidime, but not cefotaxime or cefazolin, was associated with occurrence of VRE [98].

The use of antianaerobic agents, such as metronidazole, imipenem, and piperacillin-tazobactam, has also been implicated as a risk factor for VRE [24, 79, 86, 109-112], but the methods of classifying antianaerobic agents have differed among these studies. In the meta-analysis of 14 studies evaluating the association between antianaerobic agents reviewed by Harbarth [77], a significant association was found between receiving antianaerobic agents and VRE colonization or infection (OR = 2.6; 95% CI, 2.0-3.4). Donskey et al. [109] reported an increase in the density of VRE stool colonization with treatment with antianaerobic antimicrobials. They also found that imipenem and piperacillin-tazobactam were associated with increased VRE stool density. These agents have rarely been identified as risk factors for VRE colonization because of their antienterococcal activity. This finding may be due to the inclusion of study patients already colonized with VRE and may not be generalizeable to other patient populations. In a population at UAB that was not limited to patients already colonized with VRE, we identified imipenem as the antimicrobial agent most strongly associated with VRE [98]. In contrast, piperacillin-tazobactam showed no association in this study, which was sufficiently powered to detect a 2-fold association had one been present.

A limited number of studies have examined the association between fluoroquinolones exposure and VRE colonization. Unlike vancomycin, extended-spectrum cephalosporins, and antianaerobic agents, most fluoroquinolones used over the past several years have relatively poor antianaerobic activity and therefore should not promote increases in VRE density [113], an observation we have confirmed independently [98]. There is evidence, however, that the effect of fluoroquinolones on the fecal flora may be more pronounced in special patient populations, such as bone marrow transplant recipients and patients undergoing gastrointestinal surgery, than in other populations [114].

Colonization vs. Disease

A distinction must be made between colonization with VRE and infection with VRE. Infection occurs when a patient carries VRE and presents with clinical signs or symptoms of disease, whereas colonized patients lack the signs or symptoms evident in VRE infection. Colonization is most frequently observed in the gastrointestinal tract and to a lesser extent the urinary tract. The distinction between colonization and infection is important for VRE surveillance and for estimation of disease burden and overall VRE prevalence within an institution as illustrated in the proposed casual model (D). Colonized individuals are potential reservoirs for transmission of VRE and should be identified and included in infection control measures because they constitute a major route of exposure. These patients may remain colonized for weeks, months, or even years because of the asymptomatic nature of the colonization. Patients with intestinal colonization are at increased risk for developing infection with VRE, and are a potential source for spread of VRE to the hands of health care workers, to the environment, and to other

patients, especially if they are fecal-incontinent and/or have diarrhea [61]. The prevalence of VRE colonization or infection in a hospital was identified as the most important factor in the spread of VRE [115]. Once the prevalence of VRE exceeds 50%, the other hypothesized predictors of median time to colonization have only a slight effect. When the prevalence of VRE reaches this magnitude, lapses in infection control practices are more likely to result in cross-transmission of VRE.

Patients with higher concentrations of VRE in their feces present a potentially greater risk for spread of VRE. Green et al. [116] reported that approximately 60% of liver-transplant patients remained colonized for 12 weeks or more, and Livornese et al. [29] found a majority of patients remained colonized for more than 3 months. Lai et al. [117] reported that two patients were colonized for at least 281 days. Hospital workers themselves do not appear to be a significant reservoir for VRE, but have been implicated in the spread of VRE from one patient to another via hand carriage [23, 24, 61, 118].

PREVENTION AND CONTROL OF VRE

As exhibited by our VRE causal model, prevention and control measures have several points for potential intervention. Effective VRE prevention and control must incorporate many elements in order to halt the progression of VRE reservoirs to non-colonized patients. Central to VRE prevention are: (1) timely screening of all enterococcal isolates for vancomycin resistance and prompt reporting of vancomycin resistance by the clinical laboratory using methods shown to be accurate and reliable; (2) instituting and mandating compliance with infection control measures known to reduce spread of VRE among patients in the hospital and ambulatory care settings, including identification

of colonized patients by appropriate screening procedures and by flagging charts of re-admitted persons who had VRE in prior hospitalizations; (3) education of hospital personnel including physicians nurses, pharmacists, laboratory personnel, other healthcare workers, ancillary personnel, students, patients, and families; and (4) judicious antimicrobial use, not limited to vancomycin, but also including other agents known increase VRE density, specifically cephalosporins, and antianaerobic agents.

CONCLUSION

History has demonstrated that VRE have been and will remain a problem in the hospital setting. Once a drug-resistant nosocomial pathogen, such as VRE, that is easily transmitted from person to person becomes endemic in an institution, it is never completely eradicated and is likely to continue to increase in prevalence over time. With the recent emergence of high-level vancomycin resistance in *S. aureus*, the control of VRE has taken on even greater importance. An understanding of the epidemiology and progression of VRE from colonized/infected patients to uncolonized patients is paramount in slowing VRE dissemination. Continued reliance on antimicrobials alone in controlling VRE has become an outdated infection-control measure, as evidenced by the fact the resistance in both VRE and MRSA has already been documented in the oxazolidinone linezolid. The age of antimicrobial resistance officially began the day the first antimicrobial agent was administered, and will remain with us through the indefinite future. Only through prompt attention to detection of new cases of VRE colonization and disease, and through rigorous institutional infection control, pharmacy, and educational policies can

we hope to maintain control of this pathogen and prevent the emergence of VRSA on a wide scale.

REFERENCES

1. Leclercq R, Derlot E, Duval J, et al. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N Engl J Med **1988**; 319:157-61.

2. Recommendations for preventing the spread of vancomycin resistance: recommendations of the Hospital Infection Control Practices Advisory Committee (HICPAC). Am J Infect Control **1995**; 23:87-94.

3. Fridkin SK, Gaynes RP. Antimicrobial resistance in intensive care units. Clin Chest Med **1999**; 20:303-16.

4. Uttley AH, Collins CH, Naidoo J, et al. Vancomycin-resistant enterococci. Lancet **1988**; 1:57-8.

5. Nosocomial enterococci resistant to vancomycin--United States, 1989-1993. MMWR **1993**; 42:597-9.

6. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1990-May 1999, issued June 1999. Am J Infect Control **1999**; 27:520-32.

7. National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January 1992-June 2001, issued August 2001. Am J Infect Control **2001**; 29:404-21.

8. Vergis EN, Hayden MK, Chow JW, et al. Determinants of vancomycin resistance and mortality rates in enterococcal bacteremia. a prospective multicenter study. Ann Intern Med **2001**; 135:484-92.

9. Stosor V, Peterson LR, Postelnick M, et al. *Enterococcus faecium* bacteremia: does vancomycin resistance make a difference? Arch Intern Med **1998**; 158:522-7.

10. Garbutt JM, Ventrapragada M, Littenberg B, et al. Association between resistance to vancomycin and death in cases of *Enterococcus faecium* bacteremia. Clin Infect Dis **2000**; 30:466-72.

11. Lautenbach E, Bilker WB, Brennan PJ. Enterococcal bacteremia: risk factors for vancomycin resistance and predictors of mortality. Infect Control Hosp Epidemiol **1999**; 20:318-23.

12. Hiramatsu K. The emergence of *Staphylococcus aureus* with reduced susceptibility to vancomycin in Japan. Am J Med **1998**; 104:7S-10S.

13. Arthur M, Courvalin P. Genetics and mechanisms of glycopeptide resistance in enterococci. Antimicrob Agents Chemother **1993**; 37:1563-1571.

14. Heaton MP, Handwerger S. Conjugative mobilization of a vancomycin resistance plasmid by a putative *Enterococcus faecium* sex pheromone response plasmid. Microb Drug Resist **1995**; 1:177-83.

15. Diekema DJ, Pfaller MA, Schmitz FJ, et al. Survey of infections due to *Staphylococcus* species: frequency of occurrence and antimicrobial susceptibility of isolates collected in the United States, Canada, Latin America, Europe, and the Western Pacific region for the SENTRY Antimicrobial Surveillance Program, 1997- 1999. Clin Infect Dis **2001**; 32 Suppl 2:S114-32.

16. Tenover FC, Biddle JW, Lancaster MV. Increasing resistance to vancomycin and other glycopeptides in *Staphylococcus aureus*. Emerg Infect Dis **2001**; 7:327-32.

17. Perl TM. The threat of vancomycin resistance. Am J Med **1999**; 106:26S-37S; discussion 48S-52S.

18. *Staphylococcus aureus* resistant to vancomycin--United States, 2002. MMWR **2002**; 51:565-7.

19. Huycke M, Sahm D, Gilmore S. Multiple-drug resistant enterococci: The nature of the problem and an agenda for the future. Emerg Infect Dis **1998**; 4:1218-1231.

20. Freeman R, Kearns AM, Lightfoot NF. Heat resistance of nosocomial enterococci. Lancet **1994**; 344:64-5.

21. Kearns AM, Freeman R, Lightfoot NF. Nosocomial enterococci: resistance to heat and sodium hypochlorite. J Hosp Infect **1995**; 30:193-9.

22. Noskin GA, Stosor V, Cooper I, et al. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. Infect Control Hosp Epidemiol **1995**; 16:577-81.

23. Wade JJ. The emergence of *Enterococcus faecium* resistant to glycopeptides and other standard agents--a preliminary report. J Hosp Infect **1995**; 30 Suppl:483-93.

24. Morris JG, Jr., Shay DK, Hebden JN, et al. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establishment of endemicity in a university medical center. Ann Intern Med **1995**; 123:250-9.

25. Boyce JM, Opal SM, Chow JW. Outbreak of multi-drug resistant *Enterococcus faecium* with transferable vanB class vancomycin resistance. J Clin Microbiol **1994**; 32:1148-53.

26. Edmond M, Ober J, Weinbaum D, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. Clin Infect Dis **1995**; 20:1134-1136.

27. Bonilla HF, Zervos MA, Lyons MJ, et al. Colonization with vancomycin-resistant *Enterococcus faecium*: comparison of a long-term-care unit with an acute-care hospital. Infect Control Hosp Epidemiol **1997**; 18:333-9.

28. Boyce JM, Mermel LA, Zervos MJ, et al. Controlling vancomycin-resistant enterococci. Infect Control Hosp Epidemiol **1995**; 16:634-7.

29. Livornese LLJ, Dias S, Samel C. Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic thermometers. Ann Intern Med **1992**; 117:112-116.

30. Porwancher R, Sheth A, Remphrey S, et al. Epidemiologic study of hospital-acquired infection with vancomycin-resistant *Enterococcus faecium*: possible transmission by an electronic ear-probe thermometer. .

31. Karanfil LV, Murphy M, Josephson A. A cluster of vancomycin-resistant *Enterococcus faecium* in an intensive care unit. Infect Control Hosp Epiemiol **1993**; 13:195-200.

32. Falk PS, Winnike J, Woodmansee C, et al. Outbreak of vancomycin-resistant enterococci in a burn unit. Infect Control Hosp Epidemiol **2000**; 21:575-82.

33. Gould FK, Freeman R. Nosocomial infection with microsphere beds. Lancet **1993**; 342:241-2.

34. Wilcox MH, Jones BL. Enterococci and hospital laundry. Lancet **1995**; 345:594.

35. Slaughter S, Hayden MK, Nathan C, et al. A comparison of the effect of universal use of gloves and gowns with that of glove use alone on acquisition of vancomycin-resistant enterococci in a medical intensive care unit. Ann Intern Med **1996**; 125:448-56.

36. Mato R, de Lencastre H, Roberts RB, et al. Multiplicity of genetic backgrounds among vancomycin-resistant *Enterococcus faecium* isolates recovered from an outbreak in a New York City hospital. Microb Drug Resist **1996**; 2:309-17.

37. Freeman R, Gould FK, Ryan DW, et al. Nosocomial infection due to enterococci attributed to a fluidized microsphere bed. The value of pyrolysis mass spectrometry. J Hosp Infect **1994**; 27:187-93.

38. Murray B. Diversity among multidrug-resistant enterococci. Emerging Infectious Diseases **1998**; 4:34-47.

39. Low DE, Keller N, Barth A, et al. Clinical prevalence, antimicrobial susceptibility, and geographic resistance patterns of enterococci: results from the SENTRY Antimicrobial Surveillance Program, 1997-1999. Clin Infect Dis **2001**; 32 Suppl 2:S133-45.

40. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992-April 2000, issued June 2000. Am J Infect Control **2000**; 28:429-48.

41. Intensive Care Antimicrobial Resistance Epidemiology (ICARE) Surveillance Report, data summary from January 1996 through December 1997: A report from the National Nosocomial Infections Surveillance (NNIS) System. Am J Infect Control **1999**; 27:279-84.

42. National Nosocomial Infections Surveillance (NNIS) System report, data summary from October 1986-April 1998, issued June 1998. Am J Infect Control **1998**; 26:522-33.

43. National Nosocomial Infections Surveillance (NNIS) report, data summary from October 1986-April 1997, issued May 1997. A report from the NNIS System. Am J Infect Control **1997**; 25:477-87.

44. National Nosocomial Infections Surveillance (NNIS) report, data summary from October 1986-April 1996, issued May 1996. A report from the National Nosocomial Infections Surveillance (NNIS) System. Am J Infect Control **1996**; 24:380-8.

45. Martone WJ, Gaynes RP, Horan TC, et al. National Nosocomial Infections Surveillance (NNIS) semiannual report, May 1995. A report from the National Nosocomial Infections Surveillance (NNIS) System. Am J Infect Control **1995**; 23:377-85.

46. Nosocomial infection rates for interhospital comparison: limitations and possible solutions. A Report from the National Nosocomial Infections Surveillance (NNIS) System. Infect Control Hosp Epidemiol **1991**; 12:609-21.

47. Mundy LM. Enterococci: susceptibility, resistance, and now dependence on vancomycin. Am J Clin Pathol **1998**; 109:367-70.

48. Jones RN, Marshall SA, Pfaller MA, et al. Nosocomial enterococcal blood stream infections in the SCOPE Program: antimicrobial resistance, species occurrence, molecular testing results, and laboratory testing accuracy. SCOPE Hospital Study Group. Diagn Microbiol Infect Dis **1997**; 29:95-102.

49. Edmond MB. Multidrug-resistant enterococci and the threat of vancomycin resistant *Staphylococcus aureus*. In: Prevention and Control of Nosocomial Infections; **1997**. p. 339-355.

50. Edmond MB, Ober JF, Dawson JD, et al. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. Clin Infect Dis **1996**; 23:1234-9.

51. Murray B. Enterococci: How to make a second-rate pathogen into a first-rate problem [abstract 473]. In: 34th Annual meeting of the Infectious Diseases Society of America (New Orleans). Alexandria VA: Infectious Disease Society of America, 1996.

52. Murray BE. The life and times of the *Enterococcus*. Clin Microbiol Rev **1990**; 3:46-65.

53. Murray BE. Vancomycin-resistant Enterococci. Am J Med **1997**; 101:284-293.

54. Rice LB. Emergence of vancomycin-resistant enterococci. Emerg Infect Dis **2001**; 7:183-7.

55. Cetinkaya Y, Falk P, Mayhall CG. Vancomycin-resistant enterococci. Clin Microbiol Rev **2000**; 13:686-707.

56. Donskey CJ, Rice LB. The influence of antibiotics on spread of vancomycin-resistant enterococci: the potential role of selective use of antibiotics as a control measure. Clinical Microbiology Newsletter **1999**; 23:1020-1025.

57. Prystowsky J, Siddiqui F, Chosay J, et al. Resistance to linezolid: characterization of mutations in rRNA and comparison of their occurrences in vancomycin-resistant enterococci. Antimicrob Agents Chemother **2001**; 45:2154-6.

58. Karlowsky JA, Zhanel GG, Hoban DJ. Vancomycin-resistant enterococci (VRE) colonization of high-risk patients in tertiary care Canadian hospitals. Canadian VRE Surveillance Group. Diagn Microbiol Infect Dis **1999**; 35:1-7.

59. Performance Standards for Antimicrobial Susceptibility Testing: 12th Informational Supplement. Wayne, PA: NCCLS; **2002**.

60. Leclercq R. Heterospecific transfer and expression of enterococcal resistance to glycopeptides. In: Bacterial resistance toy glycopeptides. Brun-Buisson C, Eliopoulos G, Leclercq R, editors. Paris, France: Medecine-Sciences Flammarion; **1998**. p. 39-46.

61. Boyce JM, Opal SM, Chow JW, et al. Outbreak of multidrug-resistant *Enterococcus faecium* with transferable vanB class vancomycin resistance. J Clin Microbiol **1994**; 32:1148-53.

62. Bonten MJ, Hayden MK, Nathan C, et al. Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. Lancet **1996**; 348:1615-9.

63. Tornieporth NG, Roberts RB, John J, et al. Risk factors associated with vancomycin-resistant *Enterococcus faecium* infection or colonization in 145 matched case patients and control patients. Clin Infect Dis **1996**; 23:767-72.

64. Rubin LG, Tucci V, Cercenado E, et al. Vancomycin-resistant *Enterococcus faecium* in hospitalized children. Infect Control Hosp Epidemiol **1992**; 13:700-705.

65. Pittet D, Mourouga P, Perneger TV. Compliance with handwashing in a teaching hospital. Infection Control Program. Ann Intern Med **1999**; 130:126-30.

66. Bates J. Jordens Z. Selkon JB. Evidence for an animal origin of vancomycin-resistant enterococci. Lancet **1993**; 342:490-1.

67. Kampf G, Hofer M, Wendt C. Efficacy of hand disinfectants against vancomycin-resistant enterococci in vitro. J Hosp Infect **1999**; 42:143-50.

68. Klare I, Badstubner D, Konstabel C, et al. Decreased incidence of VanA-type vancomycin-resistant enterococci isolated from poultry meat and from fecal samples of humans in the community after discontinuation of avoparcin usage in animal husbandry. Microb Drug Resist **1999**; 5:45-52.

69. Klare I, Heier H, Claus H, et al. *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol Lett **1995**; 125:165-71.

70. Klare I, Heier H, Claus H, et al. *Enterococcus faecium* strains with *vanA*-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. Microb Drug Resist **1995**; 1:265-72.

71. Witte W, Klare I. Glycopeptide-resistant *Enterococcus faecium* outside Hospitals: a commentary. Microb Drug Resist **1995**; 1:259-63.

72. Aarestrup FM. Occurrence of glycopeptide resistance among *Enterococcus faecium* isolates from conventional and ecological poultry farms. Microb Drug Resist **1995**; 1:255-7.

73. Jordens JZ, Bates J, Griffiths DT. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J Antimicrob Chemother **1994**; 34:515-28.

74. Montecalvo MA. Natural history of colonization with vancomycin resistant *Enterococcus*. Infect Control Hosp Epidemiol **1995**; 16:680-685.

75. Papanicolaou GA, Meyers BR, Meyers J, et al. Nosocomial infections with vancomycin-resistant *Enterococcus faecium* in liver transplant recipients: risk factors for acquisition and mortality. Clin Infect Dis **1996**; 23:760-6.

76. Donskey CJ, Schreiber JR, Jacobs MR, et al. A polyclonal outbreak of predominantly VanB vancomycin-resistant enterococci in northeast Ohio. Northeast Ohio Vancomycin-Resistant Enterococcus Surveillance Program. Clin Infect Dis **1999**; 29:573-9.

77. Harbarth S, Cosgrove S, Carmeli Y. Effects of antibiotics on nosocomial epidemiology of vancomycin- resistant enterococci. Antimicrob Agents Chemother **2002**; 46:1619-28.

78. Lam S. Singer C, Tucci V. et al. The challenge of vancomycin-resistant enterococci: a clinical and epidemiologic study. Am J Infect Control **1995**; 23:170-180.

79. Beezhold DW, Slaughter S, Hayden MK, et al. Skin colonization with vancomycin-resistant enterococci among hospitalized patients with bacteremia. Clin Infect Dis **1997**; 24:704-6.

80. Boyle JF, Soumakis SA, Rendo A, et al. Epidemiologic analysis and genotypic characterization of a nosocomial outbreak of vancomycin-resistant enterococci. J Clin Microbiol **1993**; 31:1280-5.

81. D'Agata EM, Green WK, Schulman G, et al. Vancomycin-resistant enterococci among chronic hemodialysis patients: a prospective study of acquisition. Clin Infect Dis **2001**; 32:23-9.

82. Newell KA, Millis JM, Arnow PM, et al. Incidence and outcome of infection by vancomycin-resistant *Enterococcus* following orthotopic liver transplantation. Transplantation **1998**; 65:439-42.

83. Orloff SL, Busch AM, Olyaei AJ, et al. Vancomycin-resistant *Enterococcus* in liver transplant patients. Am J Surg **1999**; 177:418-22.

84. Peset V, Tallon P, Sola C, et al. Epidemiological, microbiological, clinical, and prognostic factors of bacteremia caused by high-level vancomycin-resistant *Enterococcus* species. Eur J Clin Microbiol Infect Dis **2000**; 19:742-9.

85. Rao GG, Ojo F, Kolokithas D. Vancomycin-resistant gram-positive cocci: risk factors for faecal carriage. J Hosp Infect **1997**; 35:63-9.

86. Shay DK, Maloney SA, Montecalvo M, et al. Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. J Infect Dis **1995**; 172:993-1000.

87. Singh-Naz N, Sleemi A, Pikis A, et al. Vancomycin-resistant *Enterococcus faecium* colonization in children. J Clin Microbiol **1999**; 37:413-6.

88. Dembry LM, Uzokwe K, Zervos MJ. Control of endemic glycopeptide-resistant enterococci. Infect Control Hosp Epidemiol **1996**; 17:286-92.

89. McManus AT, Goodwin CW, Pruitt BA, Jr. Observations on the risk of resistance with the extended use of vancomycin. Arch Surg **1998**; 133:1207-11.

90. Ostrowsky BE, Trick WE, Sohn AH, et al. Control of vancomycin-resistant enterococcus in health care facilities in a region. N Engl J Med **2001**; 344:1427-33.

91. Ostrowsky BE, Venkataraman L, D'Agata EM, et al. Vancomycin-resistant enterococci in intensive care units: high frequency of stool carriage during a non-outbreak period. Arch Intern Med **1999**; 159:1467-72.

92. Pfundstein J, Roghmann MC, Schwalbe RS, et al. A randomized trial of surgical antimicrobial prophylaxis with and without vancomycin in organ transplant patients. Clin Transplant **1999**; 13:245-52.

93. Donskey CJ, Hoyen CK, Das SM, et al. Effect of oral Bacillus coagulans administration on the density of vancomycin-resistant enterococci in the stool of colonized mice. Lett Appl Microbiol **2001**; 33:84-8.

94. Toye B, Shymanski J, Bobrowska M, et al. Clinical and epidemiological significance of enterococci intrinsically resistant to vancomycin (possessing the *vanC* genotype). J Clin Microbiol **1997**; 35:3166-70.

95. Trabulsi A, Glover AM, Reising SF, et al. Absence of rectal colonization with vancomycin-resistant enterococci among high-risk pediatric patients. Infect Control Hosp Epidemiol **1998**; 19:109-12.

96. Wendt C, Krause C, Xander LU, et al. Prevalence of colonization with vancomycin-resistant enterococci in various population groups in Berlin, Germany. J Hosp Infect **1999**; 42:193-200.

97. Carmeli Y, Samore MH, Huskins C. The association between antecedent vancomycin treatment and hospital-acquired vancomycin-resistant enterococci: a meta-analysis. Arch Intern Med **1999**; 159:2461-8.

98. Chavers S, Moser S, Benjamin W, et al. Antibiotic Exposure as a risk factor for development of vancomycin-resistant enterococcus, December 16-19. In: 41st Interscience Conference on Antimicrobial Agents and Chemotherapy; Chicago, IL; **2001**.

99. Dahms RA, Johnson EM, Statz CL, et al. Third-generation cephalosporins and vancomycin as risk factors for postoperative vancomycin-resistant enterococcus infection. Arch Surg **1998**; 133:1343-6.

100. Loeb M, Salama S, Armstrong-Evans M, et al. A case-control study to detect modifiable risk factors for colonization with vancomycin-resistant enterococci. Infect Control Hosp Epidemiol **1999**; 20:760-3.

101. Montecalvo MA, Horowitz H, Gedris C. Outbreak of vancomycin-, ampicillin-, and aminoglycoside-resistant *Enterococcus faecium* bacteremia in an adult oncology unit. Antimicrob Agents Chemother **1994**; 38:1363-1367.

102. Moreno F, Grota P, Crisp C, et al. Clinical and molecular epidemiology of vancomycin-resistant *Enterococcus faecium* during its emergence in a city in southern Texas. Clin Infect Dis **1995**; 21:1234-7.

103. Moulin F, Dumontier S, Saulnier P, et al. Surveillance of intestinal colonization and of infection by vancomycin- resistant enterococci in hospitalized cancer patients. Clin Microbiol Infect **1996**; 2:192-201.

104. Nourse C, Murphy H, Byrne C, et al. Control of a nosocomial outbreak of vancomycin resistant *Enterococcus faecium* in a paediatric oncology unit: risk factors for colonisation. Eur J Pediatr **1998**; 157:20-7.

105. Quale J, Landman D, Saurina G, et al. Manipulation of a hospital antimicrobial formulary to control an outbreak of vancomycin-resistant enterococci. Clin Infect Dis **1996**; 23:1020-5.

106. Bradley SJ. Control of glycopeptide-resistant enterococci in an oncology unit. Pharmacotherapy **2000**; 20:203S-212S.

107. Smith DW. Decreased antimicrobial resistance after changes in antibiotic use. Pharmacotherapy **1999**; 19:129S-132S.

108. May AK, Melton SM, McGwin G, et al. Reduction of vancomycin-resistant enterococcal infections by limitation of broad-spectrum cephalosporin use in a trauma and burn intensive care unit. Shock **2000**; 14:259-64.

109. Donskey CJ, Chowdhry TK, Hecker MT, et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. N Engl J Med **2000**; 343:1925-32.

110. Lucas GM, Lechtzin N, Puryear DW, et al. Vancomycin-resistant and vancomycin-susceptible enterococcal bacteremia: comparison of clinical features and outcomes. Clin Infect Dis **1998**; 26:1127-33.

111. Pegues DA, Pegues CF, Hibberd PL, et al. Emergence and dissemination of a highly vancomycin-resistant vanA strain of *Enterococcus faecium* at a large teaching hospital. J Clin Microbiol **1997**; 35:1565-70.

112. Tokars JI, Satake S, Rimland D, et al. The prevalence of colonization with vancomycin-resistant *Enterococcus* at a Veterans' Affairs institution. Infect Control Hosp Epidemiol **1999**; 20:171-5.

113. Donskey CJ, Hanrahan JA, Hutton RA, et al. Effect of parenteral antibiotic administration on persistence of vancomycin-resistant *Enterococcus faecium* in the mouse gastrointestinal tract. J Infect Dis **1999**; 180:384-90.

114. Korten V, Murray BE. Impact of the fluoroquinolones on gastrointestinal flora. Drugs **1993**: 45:125-33.

115. Bonten MJ, Slaughter S, Ambergen AW, et al. The role of "colonization pressure" in the spread of vancomycin- resistant enterococci: an important infection control variable. Arch Intern Med **1998**; 158:1127-32.

116. Green M, Barbadora K, Michaels M. Recovery of vancomycin-resistant gram-positive cocci from pediatric liver transplant recipients. J Clin Microbiol **1991**; 29:2503-6.

117. Lai KK, Kelley AL, Melvin ZS, et al. Failure to eradicate vancomycin-resistant enterococci in a university hospital and the cost of barrier precautions. Infect Control Hosp Epidemiol **1998**; 19:647-52.

118. Wade J, Baillie L, Rolando N, et al. Pristinamycin for *Enterococcus faecium* resistant to vancomycin and gentamicin. Lancet **1992**; 339:312-3.

THE ASSOCIATION BETWEEN ANTECEDENT INTRAVENOUS ANTIMICROBIAL EXPOSURE AND VANCOMYCIN-RESISTANT ENTEROCOCCI ISOLATION FROM PATIENTS

by

L. SCOTT CHAVERS, STEPHEN A. MOSER, ELLEN FUNKHOUSER, WILLIAM H. BENJAMIN, JR., L. PAIGE CHAVERS, ALAN M. STAMM, AND KEN B. WAITES

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ABSTRACT

Since the 1980s, vancomycin-resistant enterococci (VRE) have emerged as important hospital-acquired pathogens in the United States. This study evaluated the association between a variety of intravenous antimicrobial exposures and the isolation of VRE.

Antimicrobial exposure and VRE occurrence were evaluated using two control groups: a vancomycin-susceptible enterococci (VSE) group, to assess factors associated with development of VRE, and a non-enterococcus (Non-E) control group, to assess factors associated with positive cultures for enterococci without regard to vancomycin resistance. Both sets of controls were matched to 135 VRE cases by hospital location, body site of culture, and date of culture within 30 days. Odds ratios (OR) and 95% confidence intervals (CI) were computed.

After adjusting for the effect of other antimicrobials, time at risk, and patient morbidity, exposure to imipenem (OR= 4.9, 95% CI: 1.6-14.1) and ceftazidime (OR = 2.6, 95% CI: 1.1-6.1) occurred more often in VRE cases than VSE controls. Exposure to ampicillin (OR = 20.1, 95% CI: 1.5-263.1) and imipenem (OR = 5.1, 95% CI: 1.5-17.1) occurred more often in VRE cases than in Non-E controls. Neither piperacillin, nor vancomycin occurred more often in VRE cases than in either control group.

Our study offers further evidence that replacing broad-spectrum cephalosporins with extended-spectrum penicillins, specifically piperacillin, may be effective in reducing VRE.

INTRODUCTION

Since first identified in 1986, VRE have emerged as important hospital-acquired pathogens in the United States. Patients with VRE have experienced higher mortality, longer hospital stays, and increased hospital charges than have patients with vancomycin-susceptible enterococci (VSE) [1]. Increased VRE prevalence has been linked to cross-transmission from the hands of health care workers, contaminated equipment, and contaminated environmental surfaces [2-5]. Excessive use of antimicrobials has been identified as one of the most important modifiable risk factors for VRE occurrence within the hospital [6, 7]. Antimicrobial agents with differential activities against competing organisms in the gastrointestinal tract may select for increased density of VRE. The identification of individual antimicrobial agents or individual classes of drugs as risk factors may lead to interventions or to restriction of antimicrobial use that could decrease the emergence of VRE [8].

While it is clear that use of antimicrobials plays an important role in the occurrence of VRE, many controversies remain [9]. In particular, the impact of the use of vancomycin, piperacillin with or without tazobactam, and imipenem on the growth of VRE remains uncertain. Conflicting results may be due to small study sizes [10], the use of control groups that are healthier than cases [11], and lack of adjustment for potential confounders [9]. The purpose of our study was to evaluate the association between exposure to specific intravenous antimicrobial agents and combination of agents, and the isolation of VRE using a more rigorous case-control design.

METHODS

Study Design

We conducted a hospital-based case-control study in a large (greater than 800 beds), urban, tertiary-care teaching hospital using risk-set sampling of controls. Between January 1998 and August 2000, 1486 *Enterococcus faecalis* or *E. faecium* isolates representing 858 patients were identified. We identified both cases and controls from this population using the clinical microbiology laboratory computerized database.

Bacterial Identification and Susceptibility Testing

Enterococcus identification and susceptibility testing was performed using standard biochemical procedures and broth microdilution in the MicroScan WalkAway 96 (Dade MicroScan, West Sacramento. CA). Minimum inhibitory concentrations (MIC) were interpreted according to breakpoints established by the National Center for Clinical Laboratory Standards that were in effect during the time the study was performed [12]. Enterococci with vancomycin MIC $\geq$ 32 μg/ml were designated as VRE, whereas those with MICs $\leq$ 4 μg/ml were designated as VSE. Occasionally, *E. casseliflavus* and *E. gallinarum* are detected in clinical specimens and demonstrate intermediate resistance to vancomycin (MIC 8-16 μg/ml). Because these organisms are usually commensals and are rarely associated with true infection, and because this type of chromosomally-mediated resistance is not considered transmissible within the hospital setting, these species were excluded from the study. No *E. faecalis* or *E. faecium* with intermediate MIC for vancomycin were included in the analyses. Laboratory policy mandates that all sites, including urine, are identified only to genus level. However, all isolates, regardless en-

terococci from sterile sites be identified to species level, whereas isolates from non-sterile of origin, are screened for vancomycin resistance using brain heart infusion agar containing 6 μg/ml vancomycin (Remel, Inc., Lenexa, KS). Organisms that grow on this medium are then identified to species level using the MicroScan WalkAway 96 as previously described. The magnitude of vancomycin resistance is confirmed for all isolates using the agar gradient diffusion (Etest) technique (AB BIODISK, Solna, Sweden), which measures MIC up to 256 μg/ml.

Cases

Individuals from which VRE were isolated were eligible cases. A master list of all potential cases was compiled from the laboratory database and checked for duplicates. Patients with isolates initially susceptible to vancomycin, but from whom VRE were subsequently isolated were included as eligible cases. Among the 858 patients with enterococcal isolates reported by the microbiology laboratory during the study period, 174 had isolates with vancomycin MICs $\geq$ 32 μg/ml. Of these 174 patients, 39 had cultures collected within 72 h of hospital admission. These 39 patients were considered to have community-acquired VRE, possibly related to prior hospitalization, and were excluded from the sample, resulting in 135 VRE cases.

Controls

Among the 858 patients with culture-confirmed *E. faecium* or *E. faecalis*, 684 had VSE. There were 656 patients identified as having VSE from cultures collected after 72 h of admission who were suitable for inclusion as potential controls in the VSE control

group. A second control group was selected from 2,561 potential controls who were found to be negative for enterococcus by culture. The 2,561 potential enterococcus-negative controls (Non-E) were identified after removing individuals cultured within 72 h of hospital admission. Each potential control was matched to the case according to location within the hospital, by body site of specimen collection, and by specimen collection date within 30 days. Incidence density sampling was used to select controls from each group; that is, one patient was randomly selected from each pool of matched potential controls to serve as the control for the case [13, 14]. The VSE control group was used to assess factors associated with development of VRE. The Non-E control group was used to assess factors associated with positive cultures for enterococci without regard to vancomycin resistance.

Variables

Medical record numbers for cases and controls were used to link microbiology laboratory data to hospital records to obtain information on hypothesized risk factors. Factors hypothesized to be associated with development of VRE were antimicrobial exposure, patient demographic characteristics, underlying illnesses and severity of illness, invasive procedures, and length of time at risk. All intravenous antimicrobial agents used by $\geq$5% of the study population within the designated time period were evaluated as potential risk factors for development of VRE. Length of time at risk was defined as the time between date of hospital admission and date of culture. Severity of illness on the date of specimen collection was estimated using the Acute Physiology, Age, Chronic Health Evaluation III (APACHE III) morbidity score [15]. The calculation of the

APACHE III score was based on the most severe values within 24 h of the day of interest.

Statistical Analyses

Data analyses were performed using SAS software, release 8.0 (SAS Institute Inc., Cary, North Carolina). The distribution of all hypothesized risk factors were obtained for VRE cases and for each set of controls. The primary statistical analysis was conditional logistic regression. Two sets of analyses were conducted. In one set of analyses, the dependent variable indicated the isolation of VRE from patients compared with VSE isolation. In the second set of analyses, the dependent variable indicated the isolation of VRE from patients compared with no enterococcus isolation. Each hypothesized risk factor for VRE isolation was evaluated individually using a univariable conditional logistic regression model. The effect of each antimicrobial exposure on VRE isolation was evaluated using a single multivariable model that simultaneously adjusted for exposure to other antimicrobials, time at risk of greater than 7 days, and an APACHE III score greater than 50. Odds Ratios (OR) and 95% confidence intervals (CI) were calculated. Statistical significance was indicated by 95% CI excluding the null value of 1. Human subject use and research design were reviewed and approved by the University of Alabama at Birmingham Institutional Review Board.

RESULTS

Study Population

VRE were isolated from the urinary tract (40%), blood or other sterile body fluids (37%), wounds (14%) and stool (9%). VRE were identified from both intensive care

(ICU) (38%) and non-intensive care units. Both control groups had the same distribution for body site of isolates and ICU status because of the matching of the controls to cases based on these characteristics. There was no significant difference in the distribution of cases and controls with respect to age, sex, payer status, or hospital service (table 1). The distribution of antibiotic exposure among VRE cases and each control group is shown in figure 1.

Underlying illness

Hematologic malignancy was the only underlying illness to be significantly more common in VRE cases than in VSE controls. VRE cases were more likely than Non-E controls to have a time at risk longer than 7 days, an APACHE III greater than 50, congestive heart failure, cellulitis, and urinary tract infections (table 2).

Procedures

Both lumbar puncture and thoracenteses were performed significantly more often in VRE cases than in VSE controls (table 3). There were 10 thoracenteses procedures among VRE cases and none among VSE controls ($P < 0.01$). VRE cases were more likely than were Non-E controls to have had a lumbar puncture, abdominal surgery, and skin or wound debridement prior to specimen collection date.

Antimicrobial use

VRE was significantly associated with receiving imipenem or ceftazidime in the univariable and multivariable models (table 4). No other associations were observed with

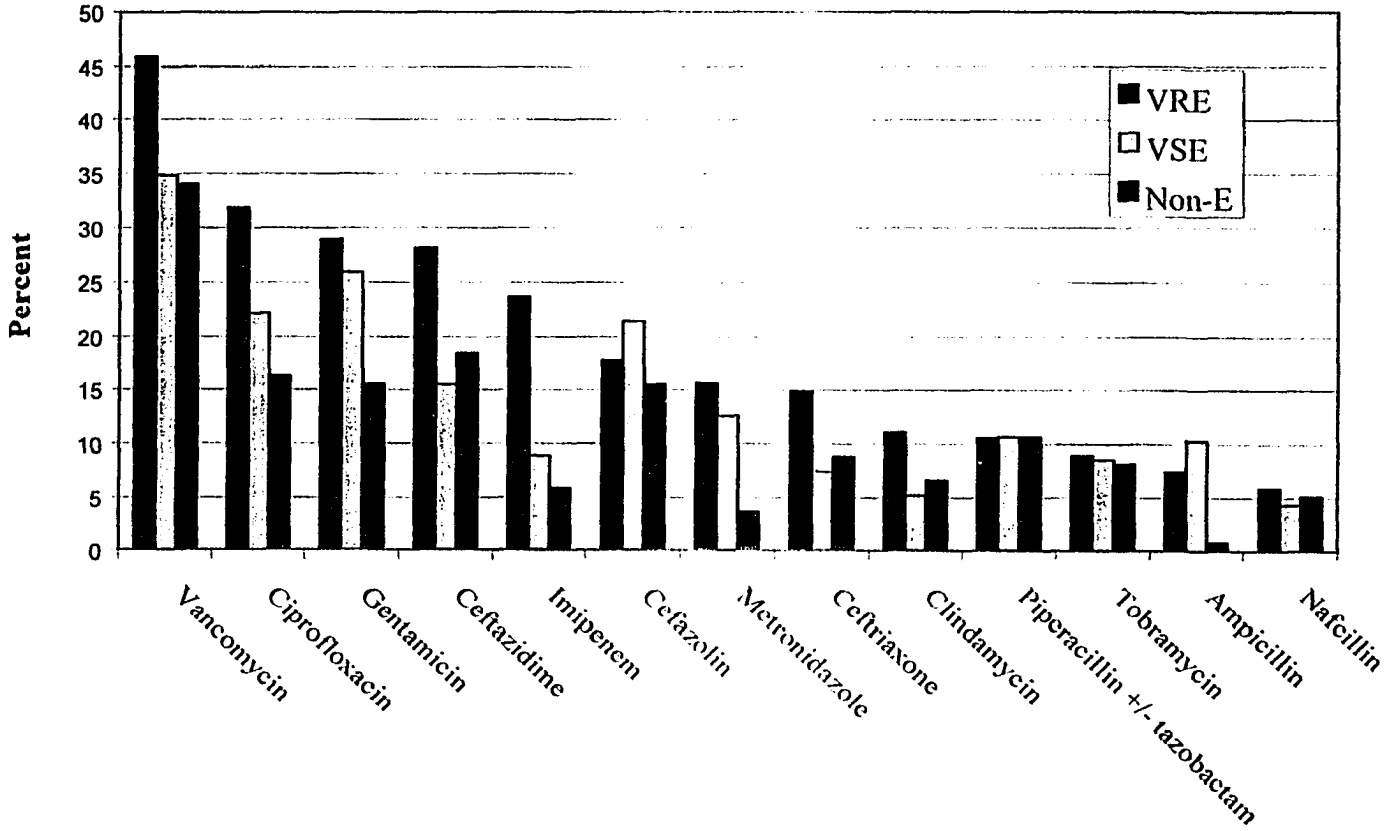

Figure 1. Percentage of 135 VRE cases, 135 VSE controls, and 135 Non-E controls exposed to each antimicrobial; VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci; Non-E, no enterococci identified.

Table 1. Comparison of demographic characteristics of 135 cases (VRE) and 135 matched controls (VSE and Non-E).

	VRE		VSE			Non-E		
Characteristic	N	%	N	%	OR (95% CI)[a]	N	%	OR (95% CI)[b]
Age distribution								
≤ 45 years of age	37	(27)	33	(24)	Reference	37	(27)	Reference
45-54 years of age	26	(19)	33	(24)	0.9 (0.7-1.3)	28	(21)	1.1 (0.8-1.5)
55-64 years of age	34	(25)	26	(19)	1.0 (0.7-1.5)	36	(27)	1.0 (0.8-1.4)
≥ 65 years of age	38	(28)	43	(32)	0.9 (0.6-1.3)	34	(25)	1.0 (0.7-1.5)
Sex								
Male	56	(8)	80	(59)	Reference	79	(59)	Reference
Female	79	(41)	55	(41)	1.2 (0.9-1.5)	56	(41)	1.2 (0.9-1.5)
Payer status								
HMO	71	(53)	70	(52)	Reference	72	(53)	Reference
Medicare	53	(39)	58	(43)	1.0 (0.8-1.2)	50	(37)	1.0 (0.8-1.3)
Medicaid	11	(8)	7	(5)	1.1 (0.7-1.8)	13	(10)	1.0 (0.6-1.5)
Service								
Surgery	48	(36)	51	(38)	Reference	47	(35)	Reference
Medicine	87	(64)	84	(62)	1.0 (0.8-1.3)	88	(65)	1.0 (0.8-1.3)

NOTE. VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci, Non-E, cultures with no enterococci identified.

[a] OR comparing vancomycin-resistant enterococci cases to vancomycin susceptible enterococci controls.

[b] OR comparing vancomycin-resistant enterococci cases to controls with no Enterococci identified.

Table 2. Comparison of underlying illnesses and morbidity between 135 cases (VRE) and 135 matched controls (VSE and Non-E).

	VRE		VSE			Non-E		
Underlying illness	N	(%)	N	(%)	OR (95% CI)[a]	N	%	OR (95% CI)[b]
Time at risk[f] >7 days	115	(85)	109	(81)	1.3 (0.7-2.7)	82	(61)	4.3 (2.1-8.5)*
APACHE III score on date of culture >	82	(60)	74	(55)	1.4 (0.8-2.3)	45	(33)	3.6 (2.0-6.6)*
Neoplasms	10	(7)	14	(10)	0.7 (0.3-1.6)	11	(8)	0.9 (0.4-2.1)
Hematologic malignancy	12	(9)	3	(2)	10.0 (1.3-	12	(9)	1.0 (0.4-2.7)
Diabetes	33	(24)	32	(24)	1.0 (0.6-1.9)	31	(23)	1.1 (0.6-2.0)
White blood cell disorder	2	(1)	3	(2)	0.7 (0.1-4.0)	5	(4)	0.4 (0.1-2.1)
Congestive heart failure	27	(20)	27	(20)	1.0 (0.5-1.8)	14	(10)	2.3 (1.1-4.8)*
Cardiovascular disease	3	(2)	8	(6)	0.3 (0.1-1.4)	8	(6)	0.3 (0.1-1.4)
Pneumonia	40	(30)	32	(24)	1.5 (0.8-2.7)	38	(28)	1.2 (0.6-2.4)
Peritonitis	6	(4)	3	(2)	2.0 (0.5-8.0)	2	(1)	3.0 (0.6-14.9)
Liver disease	15	(11)	8	(6)	2.0 (0.8-4.9)	9	(7)	2.0 (0.7-5.3)
Pancreatitis	12	(9)	10	(7)	1.2 (0.5-2.9)	7	(5)	2.0 (0.7-5.8)
Acute renal failure	27	(20)	30	(22)	0.8 (0.4-1.7)	27	(20)	1.0 (0.5-2.1)
Cellulitis	30	(22)	23	(17)	1.4 (0.7-2.6)	15	(11)	2.4 (1.2-4.8)*
Urinary tract infection	44	(33)	37	(27)	1.4 (0.7-2.5)	22	(16)	2.7 (1.4-5.1)*
History of alcohol abuse	5	(4)	1	(1)	5.0 (0.6-42.8)	3	(2)	1.7 (0.4-7.0)

NOTE. VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci, Non-E, cultures with no enterococci identified; indicates significant association (*).

[a] OR comparing vancomycin-resistant enterococci cases to vancomycin susceptible enterococci controls.

[b] OR comparing vancomycin-resistant enterococci cases to controls with no Enterococci identified.

Table 3. Comparison of invasive procedures performed on 135 cases (VRE) and 135 matched controls (VSE and Non-E).

	VRE		VSE			Non-E		
Procedures	N	(%)	N	(%)	OR (95% CI)[a]	N	(%)	OR (95% CI)[b]
Lumbar puncture	16	(12)	2	(1)	8.0 (1.8-34.8)*	6	(4)	2.7 (1.1-6.8)*
Cardiothoracic surgery	10	(7)	8	(6)	1.3 (0.5-3.4)	9	(7)	1.1 (0.4-3.2)
Vascular access device	36	(27)	33	(24)	1.0 (0.6-1.8)	26	(19)	1.5 (0.8-2.5)
Tracheostomy	22	(16)	23	(17)	0.9 (0.5-1.7)	17	(13)	1.4 (0.7-3.0)
Lung biopsy	14	(10)	9	(7)	1.8 (0.7-4.9)	24	(18)	0.4 (0.2-1.0)
Thoracentesis	10	(7)	0	(0)	†	3	(2)	3.3 (0.9-12.1)
Ventilator >95 hours	26	(19)	28	(21)	0.9 (0.5-1.7)	26	(19)	1.0 (0.5-2.0)
Abdominal surgery	53	(39)	38	(28)	1.7 (0.8-3.6)	23	(17)	3.0 (1.4-8.0)*
Renal surgery	6	(4)	2	(1)	3.0 (0.6-14.9)	3	(2)	2.0 (0.5-8.0)
Hemodialysis	23	(17)	26	(19)	1.2 (0.6-2.2)	17	(13)	1.8 (0.9-3.5)
Solid organ transplant	6	(4)	8	(6)	0.7 (0.3-2.2)	6	(4)	1.0 (0.2-4.0)
Bone/joint surgery	13	(10)	9	(7)	1.6 (0.6-4.0)	9	(7)	1.6 (0.6-4.0)
Skin/wound debridement	25	(19)	18	(13)	1.8 (0.8-4.0)	9	(7)	3.3 (1.4-7.6)*

NOTE. VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci, Non-E, cultures with no enterococci identified; indicates significant association (*); p-value from $X^2 < 0.05$ (†).

[a] OR comparing vancomycin-resistant enterococci cases to vancomycin susceptible enterococci controls.

[b] OR comparing vancomycin-resistant enterococci cases to controls with no Enterococci identified.

Table 4. Comparison of exposure to specific antimicrobials between 135 cases (VRE) and 135 matched controls (VSE and Non-E).

	VSE		Non-E	
Antimicrobials	Crude OR (95% CI)[a]	Adjusted OR (95% CI)[b]	Crude OR (95% CI)[c]	Adjusted OR (95% CI)[d]
Ampicillin	0.7 (0.3-1.6)	0.5 (0.2-1.5)	10 (1.3-78.0)*	20.1 (1.5-263.1)*
Nafcillin	1.4 (0.4-4.4)	1.1 (0.3-4.1)	1.2 (0.4-3.9)	0.7 (0.1-3.7)
Piperacillin +/- tazobactam	0.9 (0.4-1.9)	1.0 (0.4-2.5)	1.0 (0.5-2.1)	1.0 (0.3-3.4)
Cefazolin	0.8 (0.4-1.4)	0.7 (0.3-1.4)	1.2 (0.6-2.5)	2.0 (0.7-5.5)
Ceftazidime	2.2 (1.2-4.2)*	2.6 (1.1-6.1)*	1.7 (1.0-3.0)	0.9 (0.4-2.1)
Ceftriaxone	2.0 (0.9-4.3)	2.0 (0.8-5.0)	2.0 (0.8-4.7)	2.2 (0.7-7.1)
Ciprofloxacin	1.8 (1.0-3.3)	1.6 (0.7-3.6)	2.4 (1.3-4.4)*	1.8 (0.8-4.4)
Clindamycin	2.6 (0.9-7.3)	1.3 (0.4-5.0)	1.7 (0.7-4.2)	0.8 (0.2-2.9)
Gentamicin	1.1 (0.7-2.0)	1.2 (0.6-2.5)	2.5 (1.3-4.7)*	1.5 (0.6-4.3)
Metronidazole	1.2 (0.7-2.4)	1.0 (0.4-2.5)	4.2 (1.6-11.1)*	2.8 (0.7-10.9)
Imipenem	3.9 (1.7-8.8)*	4.5 (1.8-11.9)*	5.0 (2.1-12.0)*	5.1 (1.5-17.1)*
Tobramycin	1.1 (0.4-2.9)	0.4 (0.1-1.3)	1.1 (0.4-2.7)	0.2 (0.1-0.8)*
Vancomycin	1.8 (1.0-3.1)	1.1 (0.5-2.3)	1.7 (1.0-2.8)	1.0 (0.4-2.5)

NOTE: Only antimicrobials with a minimum use of 5% within the population were evaluated. VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci. Non-E, cultures with no enterococci identified; indicates significant association (*); p-value from X^2 <0.05 (†).

[a]OR comparing vancomycin-resistant enterococci cases to vancomycin susceptible enterococci controls using univariable model

[b] OR comparing vancomycin-resistant enterococci cases to vancomycin susceptible enterococci controls using multivariable model adjusting for other antimicrobials in the table, APACHE III >50, and time at risk >7.

[c]OR comparing vancomycin-resistant enterococci cases to controls with no *Enterococci* identified using univariable model

[d]OR comparing vancomycin-resistant enterococci cases to controls with no *Enterococci* identified using univariable model using multivariable model adjusting for other antimicrobials in the table, APACHE III >50, and time at risk >7.

exposure to other antimicrobials. The univariable model indicated that VRE cases were more likely than were Non-E controls to have exposure to ampicillin, ciprofloxacin, gentamicin, imipenem, and metronidazole. The multivariable model indicated that VRE cases were more likely than were Non-E cases to be exposed to ampicillin and imipenem. Non-E controls were more likely than were VRE cases to be exposed to tobramycin.

DISCUSSION

The objectives of this study were (1) to identify patient characteristics associated with the isolation of VRE and the isolation of enterococci in general; and (2) to quantify the association between patient exposure to specific antimicrobials and the isolation of VRE, as well as the isolation of enterococci in general. Our findings support four conclusions: (1) underlying conditions and severity of illness were differentially associated with VRE isolation and enterococcal isolation in general; (2) patients identified with enterococcal isolation were more likely to have undergone invasive procedures; (3) specific antimicrobial agents were differentially associated with vancomycin resistance and enterococcal isolation in general; and (4) no association was observed between VRE isolation or enterococcal isolation and vancomycin or piperacillin +/- tazobactam.

The identification of VRE was associated with an underlying condition of hematologic malignancy. Among these patients, VRE colonization may be persistent. Patients with hematologic malignancies are often repeatedly admitted to the hospital and are frequently treated with antibiotics to prevent possible infections. These patients are often admitted to the ICU on entrance to the hospital. Each of these factors increases the probability of VRE exposure. The observed differences may be explained by differences in

severity of illness not adjusted for in the design and past exposure to antibiotics not observed in the study.

Severity of illness, as estimated by the APACHE III score, was associated with vancomycin resistance. The findings are consistent with results from previous studies that identified an association between comorbidity and VRE [16, 17]. The findings that severity of illness is associated with enterococcal growth in general is consistent with the findings that more severe conditions require more invasive procedures, longer hospital stay, and greater exposure to antimicrobial therapy [9].

Vancomycin resistance was associated with the lumbar puncture procedure. This association is most likely due to the widespread empiric use of vancomycin for the treatment of suspected beta-lactam-resistant *Streptococcus pneumoniae* meningitis among patients undergoing the lumbar puncture procedure. VRE was also associated with a thoracentesis; however, when evaluated within the context of no increased risk with cardiothoracic surgery or pneumonia across the three groups, the increase is probably due to severity of illness not accounted for in the study design or analysis. Enterococcal growth in general was associated with lumbar punctures, abdominal surgery, and skin or wound debridement.

In multivariable analysis, use of ceftazidime or imipenem was found to be associated with VRE isolation. The association between ceftazidime and VRE in our study is consistent with one study in which limiting hospital-wide use of cefotaxime, ceftazidime, vancomycin, and clindamycin was associated with a significant decrease in the prevalence of fecal colonization with VRE from 47 to 15% [18]. Another study showed that restricting third-generation cephalosporins in febrile, neutropenic patients was associated

with a reduction in VRE prevalence. This same study showed piperacillin-tazobactam use decreased the prevalence of VRE colonization; however, this decrease was reversed upon return to ceftazidime use [19]. In our trauma and burn ICU, we have also observed a decrease in VRE colonization and disease after limiting broad-spectrum cephalosporin use [20].

The association between imipenem use and high-density stool colonization with VRE was first identified by Donskey, et al. in 2000 [21]. Donskey's study was based primarily on patients with already detectible levels of VRE colonization. Our study was not restricted to these patients. We included both colonized and diseased patients. Imipenem has both anti-enterococcal and anti-anaerobic activity, which in combination have rarely been identified as risk factors for VRE colonization [9]. A possible explanation for the association between imipenem and VRE in our study may be the different susceptibility levels of enterococcal species to imipenem. The majority of vancomycin resistance is found in *E. faecium*, whereas imipenem has greater activity against enterococci, which is more likely to be susceptible to vancomycin [22].

In our analysis, vancomycin use was not associated with VRE. Previous studies have identified intravenous vancomycin use as a risk factor for VRE [16, 23-34]; however, other studies found no effect [35-46]. The different results produced by these studies are due to the selection of different control groups and not adjusting for length of time at risk. In our study and others with appropriate selection of controls and adjustment for length of time at risk, the association between vancomycin treatment and VRE was small and nonsignificant. The explanation of this negative finding may be that a large number

of patients with VRE colonization serve as reservoirs for transmission to other patients who have not necessarily received vancomycin.

Several antimicrobial agents were found to be associated with enterococcal isolation. Ampicillin, ceftriaxone, gentamicin, and metronidazole have broad activity against many bacterial species, but limited activity against *E. faecium*, facilitating the increase of *E. faecium* within a given ecologic niche by suppressing competing organisms.

There was no association between piperacillin, with or without tazobactam, and VRE. In one other study by Donskey, et al. [21], piperacillin-tazobactam has been associated with increased stool colonization density with VRE. In that study, identification of piperacillin-tazobactam as a risk factor was based on patients already colonized with VRE such that the results may not be generalizeable. Like imipenem, piperacillin-tazobactam has both anti-enterococcal activity and anti-anaerobic activity. Previous studies using antimicrobial formulary interventions in which piperacillin-tazobactam was used in place of ceftazidime [19] or in place of all cephalosporins [20, 47] showed a decrease in VRE acquisition, VRE prevalence, or eradication of all VRE infections.

Our findings must be interpreted within the strengths and limitations of the study design. The study was retrospective in nature and could not evaluate the moment of acquisition of VRE and VSE, and, hence, the most critical periods of antimicrobial exposure. Our quantification of exposure to each antimicrobial was ever/never quantification, and did not take into consideration antimicrobial dosage, duration, or appropriateness of use. Therefore, we have not evaluated actual bioavailability of each antimicrobial. Each of these exposures is important in its own right and should be evaluated. Our study did not differentiate colonization with VRE from infection with VRE. However, we do not

believe that this distinction is relevant to the hypothesized mechanism being tested. The proposed mechanism of antimicrobial pressure increasing VRE density should be independent of the whether the patient presents with symptoms of disease.

Studies focusing on risk factors for antimicrobial resistance have been criticized when controls were selected from a group of individuals with the susceptible form of the organism in question [8]. Since individuals with the susceptible form of the organism constitute a small portion of the population giving rise to the cases, their exclusive use as control subjects may lead to a biased estimate of risk because of a distorted estimate of exposure frequency in the source population. Harris et al. [8] suggested the most appropriate comparison group would come from a random sample of the hospitalized cohort.

We initially considered the use of a random sample of the hospital population as a comparison group in our study; however, it was our belief that the population from which the cases arise is a subset of the entire hospital population. This subset consists of individuals with an increased probability of acquisition of enterococci through transmission, more severe underlying conditions, and a greater number of invasive procedures. Our matching criteria and restriction of one control group to VSE patients was an attempt to equalize the distribution of these factors within the VRE and VSE groups. A comparison between the VRE cases and VSE controls indicated no significant differences in the adjusted factor, apart from hematologic malignancy. In contrast, the Non-E group was significantly healthier, had fewer underlying illnesses, and had fewer invasive procedures than did the VRE cases. A hospitalized cohort would over-sample this group, producing controls significantly healthier than the cases, and producing antimicrobial associations for enterococcus isolation, as opposed to VRE isolation.

A second methodological criticism is that previous studies using susceptible forms of an organism as controls have introduced selection bias that distorts the estimate of effect of antimicrobials active against susceptible organisms, but not against resistant organisms. Treatment with active antimicrobials is likely to inhibit the growth of susceptible organisms, making this exposure less frequent among susceptible organisms identified by culture than among patients in the source population. The implication to our study is that vancomycin, and any other antimicrobial differentially active against VSE, would reduce the probability of VSE being detected, and therefore should not be included as a potential control. In our study, these controls would enter the Non-E control group. Our findings that the association between VRE and both control groups with respect to vancomycin exposure remained consistent indicates a robust estimate of the risk. For imipenem, the true estimate of association probably lies between the estimates indicated by the two control groups because of imipenem's differential activity against enterococcus species and because of the differential nature of vancomycin resistance across enterococcus species.

Our findings are salient to the control and elimination of vancomycin resistance in enterococcus. The distribution of the VRE cases in our study by source of specimen is comparable to the distributions reported nationally in the NNIS system [48], with the majority of cases having VRE isolates from the bloodstream and urinary tract, and wound and stool isolates accounting for the minority of cases. This is due to the use of clinical isolates as opposed to surveillance isolates. In general, clinical specimens are more likely to be taken from the bloodstream and urinary system than from stool and wounds. Surveillance samples have identified wounds and stool as important reservoirs for VRE. The

implication to our study is the underestimation of the number of VRE cases and potential controls by excluding asymptomatic patients [49], and by including only those cases that represent patients in which medical conditions warranted the ordering of cultures.

Our large number of cases provided an opportunity to adjust for exposure to multiple antimicrobials, severity of underlying illness, and length of hospital stay, thereby allowing us to focus on the independent association between specific antimicrobials and the occurrence of VRE. Our study offers further evidence that the replacement of broad-spectrum cephalosporins by extended-spectrum penicillins may be effective in reducing VRE.

The increasing proportion of enterococcal isolates resistant to vancomycin poses a substantial problem to the hospitalized patient as well as to healthcare delivery systems because there are limited treatment alternatives. Identifying patients at increased risk for VRE isolation and utilizing antimicrobials with activity against enterococcus could substantially reduce the colonization and spread of VRE.

REFERENCES

1. Edmond MB, Ober JF, Dawson JD, et al. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. Clin Infect Dis **1996**; 23:1234-9.

2. Falk PS, Winnike J, Woodmansee C, et al. Outbreak of vancomycin-resistant enterococci in a burn unit. Infect Control Hosp Epidemiol **2000**; 21:575-82.

3. Montecalvo MA, Jarvis WR, Uman J, et al. Infection-control measures reduce transmission of vancomycin-resistant enterococci in an endemic setting. Ann Intern Med **1999**; 131:269-72.

4. Livornese LLJ, Dias S, Samel C. Hospital-acquired infection with vancomycin-resistant Enterococcus faecium transmitted by electronic thermometers. Ann Intern Med **1992**; 117:112-116.

5. Noskin GA, Stosor V, Cooper I, et al. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. Infect Control Hosp Epidemiol **1995**; 16:577-81.

6. Weinstein RA. Controlling antimicrobial resistance in hospitals: infection control and use of antibiotics. Emerg Infect Dis **2001**; 7:188-92.

7. Murray BE. Vancomycin-resistant enterococcal infections. N Engl J Med **2000**; 342:710-21.

8. Harris AD, Karchmer TB, Carmeli Y, et al. Methodological principles of case-control studies that analyzed risk factors for antibiotic resistance: a systematic review. Clin Infect Dis **2001**; 32:1055-61.

9. Harbarth S, Cosgrove S, Carmeli Y. Effects of antibiotics on nosocomial epidemiology of vancomycin- resistant enterococci. Antimicrob Agents Chemother **2002**; 46:1619-28.

10. Carmeli Y, Samore MH, Huskins C. The association between antecedent vancomycin treatment and hospital- acquired vancomycin-resistant enterococci: a meta-analysis. Arch Intern Med **1999**; 159:2461-8.

11. Tornieporth NG, Roberts RB, John J, et al. Risk factors associated with vancomycin-resistant *Enterococcus faecium* infection or colonization in 145 matched case patients and control patients. Clin Infect Dis **1996**; 23:767-72.

12. Performance Standards for Antimicrobial Susceptibility Testing: 12th Informational Supplement. Wayne, PA: NCCLS, **2002**.

13. Sheehe PR. Dynamic risk analysis in retrospective matched-pair studies of disease. Biometrics **1962**; 18:323-341.

14. Miettinen OS. Estimability and estimation in case-referent studies. Am J Epidemiol **1976**; 103:226-235.

15. Knaus WA, Wagner DP, Draper EA, et al. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. Chest **1991**; 100:1619-36.

16. Morris JG, Jr., Shay DK, Hebden JN, et al. Enterococci resistant to multiple antimicrobial agents, including vancomycin. Establishment of endemicity in a university medical center. Ann Intern Med **1995**; 123:250-9.

17. Shay DK, Goldmann DA, Jarvis WR. Reducing the spread of antimicrobial-resistant microorganisms. Control of vancomycin-resistant enterococci. Pediatr Clin North Am **1995**; 42:703-16.

18. Quale J, Landman D, Saurina G, et al. Manipulation of a hospital antimicrobial formulary to control an outbreak of vancomycin-resistant enterococci. Clin Infect Dis **1996**; 23:1020-5.

19. Bradley SJ, Wilson AL, Allen MC, et al. The control of hyperendemic glycopeptide-resistant *Enterococcus spp.* on a haematology unit by changing antibiotic usage. J Antimicrob Chemother **1999**; 43:261-6.

20. May AK, Melton SM, McGwin G, et al. Reduction of vancomycin-resistant enterococcal infections by limitation of broad-spectrum cephalosporin use in a trauma and burn intensive care unit. Shock **2000**; 14:259-64.

21. Donskey CJ, Chowdhry TK, Hecker MT, et al. Effect of antibiotic therapy on the density of vancomycin-resistant enterococci in the stool of colonized patients. N Engl J Med **2000**; 343:1925-32.

22. Weinstein MP. Comparative evaluation of penicillin, ampicillin, and imipenem MICs and susceptibility breakpoints for vancomycin-susceptible and vancomycin-resistant *Enterococcus faecalis* and *Enterococcus faecium*. J Clin Microbiol **2001**; 39:2729-31.

23. Beezhold DW, Slaughter S, Hayden MK, et al. Skin colonization with vancomycin-resistant enterococci among hospitalized patients with bacteremia. Clin Infect Dis **1997**; 24:704-6.

24. Boyle JF, Soumakis SA, Rendo A, et al. Epidemiologic analysis and genotypic characterization of a nosocomial outbreak of vancomycin-resistant enterococci. J Clin Microbiol **1993**; 31:1280-5.

25. D'Agata EM, Green WK, Schulman G, et al. Vancomycin-resistant enterococci among chronic hemodialysis patients: a prospective study of acquisition. Clin Infect Dis **2001**; 32:23-9.

26. Lautenbach E, Bilker WB, Brennan PJ. Enterococcal bacteremia: risk factors for vancomycin resistance and predictors of mortality. Infect Control Hosp Epidemiol **1999**; 20:318-23.

27. Newell KA, Millis JM, Arnow PM, et al. Incidence and outcome of infection by vancomycin-resistant Enterococcus following orthotopic liver transplantation. Transplantation **1998**; 65:439-42.

28. Orloff SL, Busch AM, Olyaei AJ, et al. Vancomycin-resistant Enterococcus in liver transplant patients. Am J Surg **1999**; 177:418-22.

29. Papanicolaou GA, Meyers BR, Meyers J, et al. Nosocomial infections with vancomycin-resistant *Enterococcus faecium* in liver transplant recipients: risk factors for acquisition and mortality. Clin Infect Dis **1996**; 23:760-6.

30. Peset V, Tallon P, Sola C, et al. Epidemiological, microbiological, clinical, and prognostic factors of bacteremia caused by high-level vancomycin-resistant Enterococcus species. Eur J Clin Microbiol Infect Dis **2000**; 19:742-9.

31. Rao GG, Ojo F, Kolokithas D. Vancomycin-resistant gram-positive cocci: risk factors for faecal carriage. J Hosp Infect **1997**; 35:63-9.

32. Rubin LG, Tucci V, Cercenado E, et al. Vancomycin-resistant *Enterococcus faecium* in hospitalized children. Infect Control Hosp Epidemiol **1992**; 13:700-705.

33. Shay DK, Maloney SA, Montecalvo M, et al. Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. J Infect Dis **1995**; 172:993-1000.

34. Singh-Naz N, Sleemi A, Pikis A, et al. Vancomycin-resistant *Enterococcus faecium* colonization in children. J Clin Microbiol **1999**; 37:413-6.

35. Bonten MJ, Hayden MK, Nathan C, et al. Epidemiology of colonization of patients and environment with vancomycin-resistant enterococci. Lancet **1996**; 348:1615-9.

36. Dembry LM, Uzokwe K, Zervos MJ. Control of endemic glycopeptide-resistant enterococci. Infect Control Hosp Epidemiol **1996**; 17:286-92.

37. Donskey CJ, Schreiber JR, Jacobs MR, et al. A polyclonal outbreak of predominantly VanB vancomycin-resistant enterococci in northeast Ohio. Northeast Ohio Vancomycin-Resistant Enterococcus Surveillance Program. Clin Infect Dis **1999**; 29:573-9.

38. Edmond M, Ober J, Weinbaum D, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. Clin Infect Dis **1995**; 20:1134-1136.

39. McManus AT, Goodwin CW, Pruitt BA, Jr. Observations on the risk of resistance with the extended use of vancomycin. Arch Surg **1998**; 133:1207-11.

40. Ostrowsky BE, Trick WE, Sohn AH, et al. Control of vancomycin-resistant enterococcus in health care facilities in a region. N Engl J Med **2001**; 344:1427-33.

41. Ostrowsky BE, Venkataraman L, D'Agata EM, et al. Vancomycin-resistant enterococci in intensive care units: high frequency of stool carriage during a non-outbreak period. Arch Intern Med **1999**; 159:1467-72.

42. Pfundstein J, Roghmann MC, Schwalbe RS, et al. A randomized trial of surgical antimicrobial prophylaxis with and without vancomycin in organ transplant patients. Clin Transplant **1999**; 13:245-52.

43. Donskey CJ, Hoyen CK, Das SM, et al. Effect of oral Bacillus coagulans administration on the density of vancomycin-resistant enterococci in the stool of colonized mice. Lett Appl Microbiol **2001**; 33:84-8.

44. Toye B, Shymanski J, Bobrowska M, et al. Clinical and epidemiological significance of enterococci intrinsically resistant to vancomycin (possessing the *vanC* genotype). J Clin Microbiol **1997**; 35:3166-70.

45. Trabulsi A, Glover AM, Reising SF, et al. Absence of rectal colonization with vancomycin-resistant enterococci among high-risk pediatric patients. Infect Control Hosp Epidemiol **1998**; 19:109-12.

46. Wendt C, Krause C, Xander LU, et al. Prevalence of colonization with vancomycin-resistant enterococci in various population groups in Berlin, Germany. J Hosp Infect **1999**; 42:193-200.

47. Smith DW. Decreased antimicrobial resistance after changes in antibiotic use. Pharmacotherapy **1999**; 19:129S-132S.

48. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992-April 2000, issued June 2000. Am J Infect Control **2000**; 28:429-48.

49. Montecalvo MA. Natural history of colonization with vancomycin resistant Enterococcus. Infect Control Hosp Epidemiol **1995**; 16:680-685.

MORBIDITY AND MORTALITY ASSOCIATED WITH VANCOMYCIN-RESISTANT ENTEROCOCCI

by

L. SCOTT CHAVERS, MICHAEL H. HARDIN, ELLEN FUNKHOUSER, STEPHEN A. MOSER, WILLIAM H. BENJAMIN, JR., PAIGE CHAVERS, ALAN M. STAMM, AND KEN B. WAITES

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ABSTRACT

Since the 1980s, vancomycin-resistant enterococci (VRE) have emerged as important hospital-acquired pathogens in the United States; however, the independent association between vancomycin resistance and hospitalized patient morbidity and mortality remains controversial. We investigated the contribution of vancomycin resistance to patient morbidity and mortality, while adjusting for disease severity and other factors known to be associated with high hospital mortality.

During 1998 to 2000, 135 patients with VRE were identified. Patients with vancomycin-susceptible enterococci (VSE) were matched to VRE patients by hospital location, body site of culture, and date of culture within 30 days. Differences between VRE and VSE cohorts were analyzed using the Wilcoxon rank sum statistic, Kaplan-Meyer survival analysis, and unconditional logistic regression. Odds Ratios (OR) and 95% confidence intervals (CI) were calculated.

A significantly higher hospital mortality rate was observed in VRE patients than in VSE patients ($P = 0.02$). Vancomycin resistance (OR = 2.1; 95% CI, 1.1-4.8) remained a significant predictor of hospital mortality in multivariable analysis. The present study provides evidence that vancomycin resistance is associated with increased mortality, independent of other factors known to be associated with hospital mortality.

INTRODUCTION

The first clinical strains of vancomycin-resistant enterococci (VRE) were identified in 1986. Since then, the proportion of enterococci that are resistant to vancomycin has risen steadily [1-7]. In 1998, the SCOPE study reported the proportion of enterococ-

cal vancomycin resistance was approaching 14% overall, and the rate of vancomycin resistance among *Enterococcus faecium* in the northeast United States was 63% [8].

Enterococcus species are particularly well equipped to survive in the clinical setting because of their ability to withstand hostile environmental conditions [9, 10] and because of both intrinsic and acquired forms of resistance to most available antimicrobials. Enterococci are intrinsically resistant to some degree to all cephalosporins, clindamycin, trimethoprim, sulfonamides, and low levels of aminoglycosides, and many enterococci have adapted to antimicrobial exposure by acquiring resistance to penicillins, erythromycin, tetracycline, and high levels of aminoglycosides [11]. Historically, enterococci were not considered important nosocomial pathogens [12]; however, the acquisition of vancomycin resistance, in combination with high level ampicillin and aminoglycoside resistance. has limited therapeutic options to the streptogramin quinupristin-dalfopristin and the oxazolidinone linezolid in many cases.

The lack of therapeutic options. along with the increasing proportion of VRE. has led to concerns about the morbidity and mortality caused by VRE among hospitalized patients [13, 14]. Estimates of mortality among hospitalized patients with VRE bacteremia have ranged from 37% to 76% [15]; however, the morbidity and mortality independently attributable to VRE remains controversial. The objectives of the present study were: (1) to determine the contribution of vancomycin resistance to patient morbidity and mortality; and (2) to determine what factors affect the risk of mortality for patients with VRE, while adjusting for disease severity and other factors known to be associated with high hospital mortality.

METHODS

Study Design

A hospital-based, retrospective, follow-up study was conducted in a large (greater than 800 beds), urban, tertiary-care, teaching hospital among patients previously identified in case-control study evaluating predictors of VRE identification. Between January 1998 and August 2000, 1486 *Enterococcus faecalis* or *E. faecium* isolates representing 858 patients were identified from the clinical laboratory database. Patients with VRE and VSE were identified from this population.

Bacterial Identification and Susceptibility Testing

Enterococcus identification and susceptibility testing were performed using standard biochemical procedures and broth microdilution in the MicroScan WalkAway 96 (Dade MicroScan, West Sacramento, CA). Minimum inhibitory concentrations (MICs) were interpreted according to breakpoints established by the NCCLS that were in effect during the time the study was performed [16]. Enterococci with vancomycin MIC $\geq$ 32 μg/ml were designated as VRE, whereas those with MICs $\leq$ 4 μg/ml were designated as vancomycin-susceptible (VSE). Occasionally *E. casseliflavus* and *E. gallinarum* are detected in clinical specimens that will demonstrate intermediate resistance to vancomycin (MIC 8-16 μg/ml). Because these organisms are usually commensals, are rarely associated with true infection, and because this type of chromosomally mediated resistance is not considered transmissible within the hospital setting, these species were excluded from the study.

Patients

A master list of all potential VRE patients was compiled from the laboratory database and checked for duplicates. Among the 858 patients with enterococcal isolates reported by the microbiology laboratory during the study period, 174 had isolates with vancomycin MIC ≥ 32 μg/ml. Of these 174 patients, 39 had cultures collected within 72 h of hospital admission. These 39 patients were considered to have community-acquired VRE, possibly related to prior hospitalization, and were excluded, resulting in a VRE cohort of 135 patients.

Among the 858 patients with culture-confirmed *E. faecium* or *E. faecalis*, 684 had VSE. There were 656 patients identified as having VSE from cultures collected after 72 h of admission who were suitable for inclusion in the VSE cohort. VSE patients who matched each VRE patient by location within the hospital, by body site of specimen collection, and by specimen collection date within 30 days were assembled into a potential riskset. One VSE patient was randomly selected from each risk-set to serve as a member of the VSE cohort [17, 18].

Variables

Patient record numbers were used to link microbiology laboratory data to hospital records. The following data were collected for each patient: demographic characteristics; hospital mortality; underlying illnesses; invasive procedures; length of hospitalization before culture date and from culture date to discharge; antibiotic therapy, and severity of illness on date of hospital admission, date of culture, and date of discharge (as estimated by the Acute Physiology, Age, Chronic Health Evaluation III (APACHE III) morbidity

score) [19]. The APACHE III score was based on the most severe values within 24 h of the day of interest.

Statistical Analyses

Demographics were obtained for VRE and VSE patients. Statistical significance was assessed by means of the chi-square tests for categorical variables and Wilcoxon rank-sum test for continuous variables. Differences in morbidity trends between VRE and VSE patients, as estimated by the APACHE III scores, were analyzed by fitting a regression line for each patient, and by averaging the intercepts and slopes over VRE and VSE patients separately. The average intercept represents the average APACHE III score on admission, and the average slope represents the average change in patient morbidity over time. Differences between VRE and VSE cohorts in average morbidity scores on admission and the average change in morbidity scores over time were analyzed using the Wilcoxon rank sum statistic. Kaplan-Meyer survival analysis was used to analyze differences between VRE and VSE patient mortality rates within 30 days of the last positive culture date.

The primary method of analysis for predictors of mortality occurring within 30 days of culture date was unconditional logistic regression. Odds Ratios (OR) and 95% CI were calculated. Significant OR were indicated by 95% CIs excluding the null value of 1. Significant predictors of 30-day mortality from univariable analyses were used to calculate two ordinal variables that separately quantified the number of significant underlying conditions and the number of significant diagnostic or therapeutic procedures for each patient. These composite variables were used in multivariable analysis. Age was ex-

cluded from the multivariable analysis because of its inclusion in the estimation of the APACHE III score. Data analyses were performed by using SAS software, release 8.0 (SAS Institute Inc., Cary, N.C.). Human subject use, analysis, and report of these data were reviewed and approved by the University of Alabama at Birmingham Institutional Review Board.

RESULTS

VRE were isolated from the urinary tract (40%), blood or other sterile body fluids (37%), wounds (14%), and stool (9%). VRE were identified from both intensive care (ICU) (38%), and non-intensive care units (62%). VSE patients had the same distribution for body site origin of isolates and ICU status because of matching on these characteristics. There were no significant differences in the distribution of cases and controls with respect to age, sex, payer status, or hospital service (table 1). The average APACHE III scores for VRE and VSE cohorts on admission were 44.1 and 44.0 respectively ($P = 0.99$), and the average change in APACHE III scores over each time-point was 26.6 for the VRE cohort and 19.4 for the VSE cohort ($P = 0.23$) (figure 1).

The decrement to patient survival related to vancomycin resistance was measured from the last positive VRE or VSE culture (figure 2). The mortality rates for VRE and VSE patients were nearly identical for the first 3 days following the last positive culture; however, the deleterious effect of vancomycin resistance in enterococci was evident longitudinally by the higher mortality rate in the VRE cohort ($P < 0.01$).

Table 1. Demographic characteristics of 135 VRE and 135 matched VSE controls.

Characteristic	VRE N	VRE %	VSE N	VSE %	OR (95% CI)[a]
Age distribution					
≤ 45 years of age	37	(27)	33	(24)	Reference
45-54 years of age	26	(19)	33	(24)	0.9 (0.7-1.3)
55-64 years of age	34	(25)	26	(19)	1.0 (0.7-1.5)
≥ 65 years of age	38	(28)	43	(32)	0.9 (0.6-1.3)
Sex					
Male	56	(8)	80	(59)	Reference
Female	79	(41)	55	(41)	1.2 (0.9-1.5)
Payer status					
HMO	71	(53)	70	(52)	Reference
Medicare	53	(39)	58	(43)	1.0 (0.8-1.2)
Medicaid	11	(8)	7	(5)	1.1 (0.7-1.8)
Service					
Surgery	48	(36)	51	(38)	Reference
Medicine	87	(64)	84	(62)	1.0 (0.8-1.3)
Mortality					
Survived at least 30 days from last	98	(73)	115	(85)	Reference
Died within 30 days of last positive	37	(27)	20	(15)	2.2 (1.2-4.0)

NOTE. VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci.

[a] OR comparing vancomycin-resistant enterococci cases to vancomycin susceptible enterococci controls.

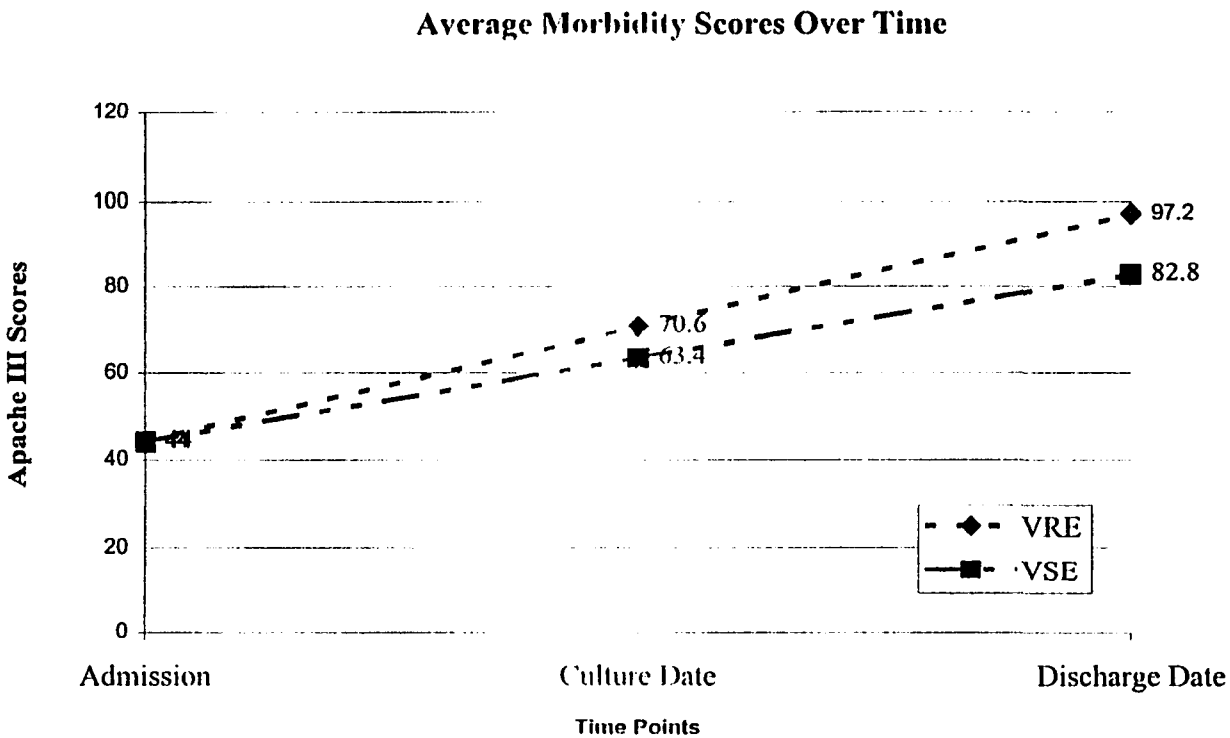

Figure 1. Change in APACHE III score from hospital admission to hospital discharge; VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci.

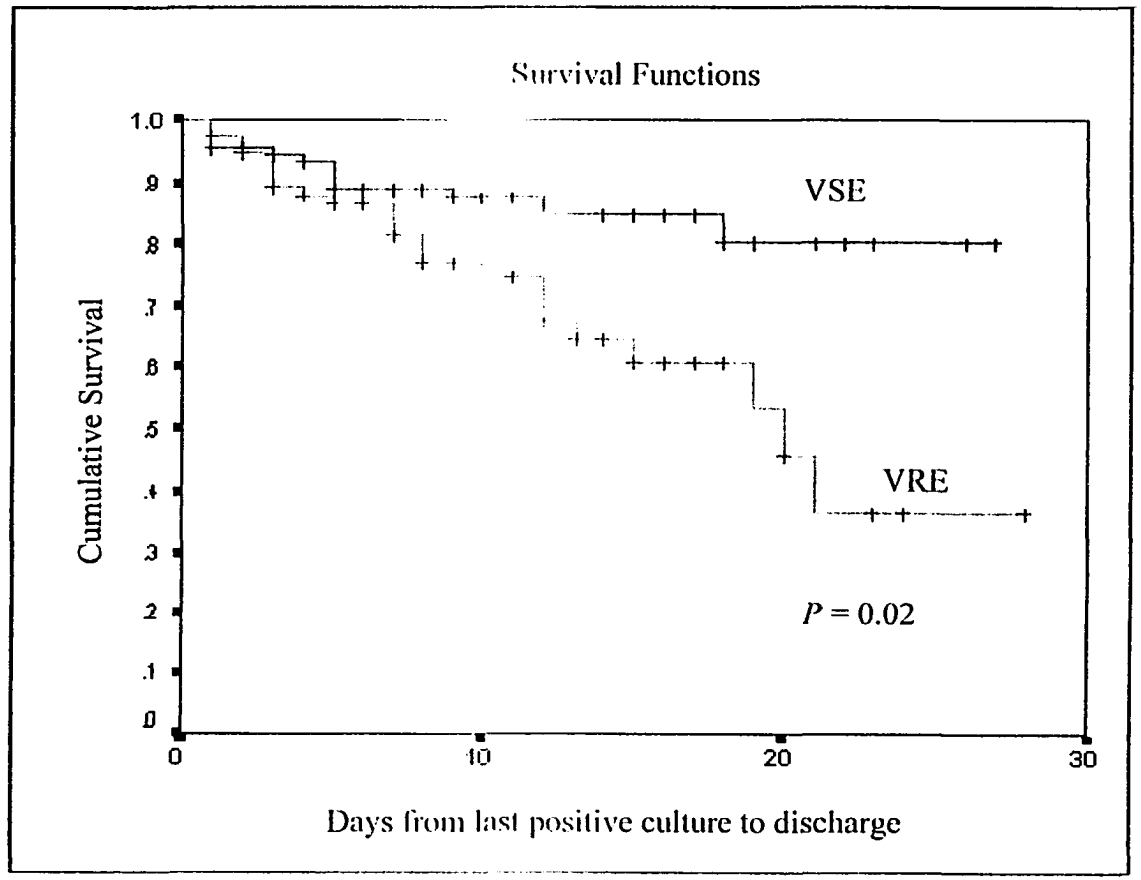

Figure 2. Cumulative survival for VRE and VSE patients. VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci.

Predictors of 30-day hospital mortality from univariable models are shown in tables 2 through 4. The multivariable logistic regression model included significant demographic predictors from the univariable models, excluding age, along with the number of significant underlying conditions and procedures. Independent predictors of 30-day mortality were found to be: vancomycin resistance, surgical service admission, increasing APACHE III scores, and number of significant underlying conditions (table 5.).

Table 2. Univariable analysis of patient demographics associated with 30-day mortality.

	Died		Lived			
Characteristic	N	(%)	N	(%)	OR	(95% CI)
Vancomycin resistance						
VSE	20	(35)	115	(54)	Reference	
VRE	37	(65)	98	(46)	2.2	(1.2-4.0)
Sex						
Male	24	(42)	112	(52)	Reference	
Female	33	(57)	101	(47)	1.5	(0.8-2.7)
Age						
≤ 45 years of age	10	(27)	56	(17)	Reference	
45-54 years of age	20	(35)	39	(18)	2.4	(1.2-4.5)
55-64 years of age	12	(21)	48	(23)	0.9	(0.4-1.8)
≥ 65 years of age	15	(26)	66	(31)	0.8	(0.4-1.5)
Surgical service admission	51	(89)	128	(60)	5.8	(2.4-14.2)
ICU	29	(51)	46	(22)	3.7	(2.0-6.8)
Medicaid	2	(3)	16	(7)	0.4	(0.1-2.0)

NOTE. VRE, vancomycin-resistant enterococci; VSE, vancomycin-susceptible enterococci; ICU, Intensive Care Unit.

Table 3. Univariable analysis of patient underlying illnesses associated with 30-day mortality

	Died		Lived			
Characteristic	N	(%)	N	(%)	OR	(95% CI)
Bacteremia	32	(56)	74	(35)	2.3	(1.3-4.2)
Neoplasms	10	(17)	32	(15)	1.2	(0.5-2.6)
Hematologic malignancy	7	(12)	8	(4)	3.5	(1.2-10.2)
Diabetes	11	(19)	54	(25)	0.7	(0.3-1.4)
White blood cell disorder	2	(3)	3	(1)	2.5	(0.4-15.3)
Congestive heart failure	12	(21)	42	(19)	1.1	(0.5-2.2)
Pneumonia	32	(56)	40	(19)	5.4	(2.9-10.1)
Peritonitis	3	(5)	6	(3)	1.9	(0.5-7.8)
Liver disease	12	(21)	11	(5)	4.8	(2.0-11.6)
Pancreatitis	4	(7)	18	(8)	0.8	(0.3-2.5)
Acute renal failure	26	(45)	31	(14)	4.8	(2.5-9.2)
Urinary tract infection	12	(21)	69	(32)	0.5	(0.3-1.1)
Cardiovascular disease	42	(74)	147	(69)	1.2	(0.6-2.3)
Decubitus ulcer	9	(16)	25	(12)	1.4	(0.6-3.2)
Five point increase in APACHE III at culture date					1.3	(1.2-1.4)

NOTE. APACHE III, Acute Physiology, Age, Chronic Health Evaluation III morbidity score.

Table 4. Univariable analysis of patient diagnostic and therapeutic procedures associated with 30-day mortality.

	Died		Lived			
Characteristic	N	(%)	N	(%)	OR	95% CI
Tracheostomy	14	(24)	31	(14)	1.9	(0.9-3.8)
Cardiothoracic surgery	5	(8)	13	(6)	1.4	(0.5-4.3)
Vascular access devise	4	(7)	5	(3)	3.1	(0.8-11.9)
Hemodialysis	13	(22)	36	(16)	1.4	(0.7-2.9)
Lung biopsy	14	(25)	9	(4)	7.2	(2.9-17.8)
Thoracentesis	7	(12)	3	(1)	9.6	(2.4-38.5)
Abdominal surgery	19	(33)	55	(26)	1.4	(0.7-2.6)
Renal surgery	2	(3)	6	(3)	1.2	(0.2-6.3)
Bone/joint surgery	4	(7)	18	(9)	0.8	(0.3-2.5)
Ventilator	32	(56)	35	(16)	6.4	(3.4-12.0)
Skin/wound debridement	9	(15)	34	(16)	1.0	(0.4-2.2)
Chemotherapy	1	(2)	1	(1)	1.2	(0.1-12.0)
Lumbar puncture	8	(14)	10	(5)	3.2	(1.2-8.7)

Table 5. Multivariable analysis of characteristics associated with 30-day mortality.

Characteristic	OR	95% CI
VRE	2.6	(1.1-6.9)
Surgical service	13.2	(2.4-71.6)
ICU admission	2.1	(0.7-5.5)
APACHE III score on date of culture (for each five point increase)	1.3	(1.1-1.4)
Number of significant underlying conditions	1.7	(1.1-3.0)
Number of significant procedures	1.5	(0.9-2.8)

NOTE. VRE, vancomycin-resistant enterococci; ICU, intensive care unit; APACHE III, Acute Physiology, Age, Chronic Health Evaluation III morbidity score.

DISCUSSION

The present study showed a significantly higher rate of hospital mortality among the VRE cohort than among the VSE cohort. Vancomycin resistance remained a significant predictor of hospital mortality in multivariable analysis after adjustment for characteristics also shown to be associated with mortality. The present study also showed a closer temporal association between the VRE cohort and 30-day hospital mortality after the last positive culture than between the VSE cohort and 30-day mortality. The initial similarity in mortality in the first 3 days following culture and the subsequent divergence of the mortality rates provides strong evidence that vancomycin resistance was a major contributor to mortality.

The present study is consistent with studies that have found higher mortality among VRE patients than among VSE patients [13, 14, 20-22]. Other studies however, have shown no association [15, 23-26]. Previous studies have indicated patients with severe underlying conditions are at greater risk for VRE colonization and subsequent infections [1, 27-32]. These patient characteristics for VRE acquisition make the independent

contribution of vancomycin resistance to morbidity and mortality difficult to estimate because these severe underlying conditions are also associated with increased morbidity and mortality. The different findings among studies evaluating the VRE-associated mortality may be attributable to differences in study designs and to the method of adjusting for the underlying conditions that confound the association between VRE and hospital mortality [13]. Studies that used large multivariable models or stepwise regression models to adjust for severe underlying conditions were less likely to find an independent association between VRE and hospital mortality [13]. Composite scores of severity of illness, such as the APACHE III score, that are designed to predict mortality are the most frequently identified predictors of VRE infection or colonization [15, 20-24, 32-35]. Studies that have used stepwise logistic regression analyses often result in the severity of illness composite score accounting for the greatest degree of variation between VRE and VSE groups, with little variance attributable to vancomycin resistance, particularly when sample sizes are small [15, 23-26]. Other conditions highly associated with mortality, such as bacteremia and pneumonia, often occur concomitantly within a patient. The use of multivariable logistic regression models that include several collinear covariates often produce inflated standard errors that result in imprecise estimates; therefore, the contribution of vancomycin resistance to mortality may not be discernable [15].

The present study used hospital location as a surrogate for patient morbidity and used logistic regression to adjust for differences in morbidity between VRE and VSE patients that remained after matching. The validity of location as a surrogate measure of morbidity was confirmed on analysis of APACHE III. The problem of simultaneous occurrence of significant underlying conditions and procedures was addressed by using a

composite score for the number of significant underlying conditions and procedures. Additionally, the composite scores gave a measure of extent of morbidity that was included in the model along with the severity of morbidity as estimated by the APACHE III score.

Information about the impact of VRE infection on clinical end points other than mortality is lacking [21, 36, 37]. The present study is unique in that APACHE III scores were computed longitudinally to assess the change in morbidity over time. The initial criteria of matching by hospital location resulted in almost identical morbidity scores on admission. There was a greater average estimated morbidity increase longitudinally in the VRE cohort than in the VSE cohort; however, the difference between the two slopes did not reach statistical significance.

The study was limited in that it was retrospective, making the body site of initial colonization of VRE and VSE difficult to determine. Both VRE and VSE cohorts were selected based on cultures of single body sites, and these cultures may not have accurately detected the presence of VRE in other body locations. We also included both colonized and infected patients in each cohort. The inclusion of patients with VRE isolated from urine or stool potentially dilutes the association between VRE and mortality more than if the study were limited to clinical infections.

Another limitation of the study is that we did not match VRE to VSE according to *Enterococcus* species. In our hospital, the vast majority of VRE are *E. faecium*, whereas the majority of VSE are *E. faecalis*. One study has suggested that *E. faecium* itself may be associated with greater morbidity than is *E. faecalis*. Therefore, the species difference may be a confounding variable. Finally, we did not evaluate pharmacological interven-

tions for the treatment of VRE, nor the appropriateness of clinical care provided to each cohort. VRE are inherently more difficult to treat than are VSE because of the dearth of available treatment options. VRE may be a marker of limited therapeutic interventions, and the lack of treatment options, or difficulty in treating VRE may be the driving factor for increased mortality in VRE patients [13].

In summary, in one of the largest single hospital-based studies of VRE morbidity and mortality, we have shown that vancomycin resistance increases mortality risk and the rate of hospital mortality. We were able to analyze the effect of VRE while adjusting for other predictors of mortality using a methodology that maintained model stability. Our findings are supported by the consistency of the three separate statistical methods used.

REFERENCES

1. Nosocomial enterococci resistant to vancomycin--United States, 1989-1993. MMWR **1993**; 42:597-9.

2. National Nosocomial Infections Surveillance (NNIS) report, data summary from October 1986-April 1996, issued May 1996. A report from the National Nosocomial Infections Surveillance (NNIS) System. Am J Infect Control **1996**; 24:380-8.

3. National Nosocomial Infections Surveillance (NNIS) report, data summary from October 1986-April 1997, issued May 1997. A report from the NNIS System. Am J Infect Control **1997**; 25:477-87.

4. National Nosocomial Infections Surveillance (NNIS) System report, data summary from January 1990-May 1999, issued June 1999. Am J Infect Control **1999**; 27:520-32.

5. National Nosocomial Infections Surveillance (NNIS) System report, data summary from October 1986-April 1998, issued June 1998. Am J Infect Control **1998**; 26:522-33.

6. National Nosocomial Infections Surveillance (NNIS) system report, data summary from January 1992-April 2000, issued June 2000. Am J Infect Control **2000**; 28:429-48.

7. National Nosocomial Infections Surveillance (NNIS) System Report, Data Summary from January 1992-June 2001, issued August 2001. Am J Infect Control **2001**; 29:404-21.

8. Jones RN, Marshall SA, Pfaller MA, et al. Nosocomial enterococcal blood stream infections in the SCOPE Program: antimicrobial resistance, species occurrence, molecular testing results, and laboratory testing accuracy. SCOPE Hospital Study Group. Diagn Microbiol Infect Dis **1997**; 29:95-102.

9. Freeman R, Kearns AM, Lightfoot NF. Heat resistance of nosocomial enterococci. Lancet **1994**; 344:64-5.

10. Kearns AM, Freeman R, Lightfoot NF. Nosocomial enterococci: resistance to heat and sodium hypochlorite. J Hosp Infect **1995**; 30:193-9.

11. Edmond MB. Multidrug-resistant enterococci and the threat of vancomycin resistant Staphylococcus aureus. In: Prevention and Control of Nosocomial Infections. Wenzel RH editor; **1997**. p. 339-355.

12. Moellering RC, Jr. Vancomycin-resistant enterococci. Clin Infect Dis **1998**; 26:1196-9.

13. Lodise TP, McKinnon PS, Tam VH, et al. Clinical outcomes for patients with bacteremia caused by vancomycin- resistant enterococcus in a level 1 trauma center. Clin Infect Dis **2002**; 34:922-9.

14. Vergis EN, Hayden MK, Chow JW, et al. Determinants of vancomycin resistance and mortality rates in enterococcal bacteremia. a prospective multicenter study. Ann Intern Med **2001**; 135:484-92.

15. Garbutt JM, Ventrapragada M, Littenberg B, et al. Association between resistance to vancomycin and death in cases of *Enterococcus faecium* bacteremia. Clin Infect Dis **2000**; 30:466-72.

16. Performance Standards for Antimicrobial Susceptibility Testing: 12th Informational Supplement. Wayne, PA: NCCLS; **2002**.

17. Sheehe PR. Dynamic risk analysis in retrospective matched-pair studies of disease. Biometrics **1962**; 18:323-341.

18. Miettinen OS. Estimability and estimation in case-referent studies. Am J Epidemiol **1976**; 103:226-235.

19. Knaus WA, Wagner DP, Draper EA, et al. The APACHE III prognostic system. Risk prediction of hospital mortality for critically ill hospitalized adults. Chest **1991**; 100:1619-36.

20. Linden PK, Pasculle AW, Manez R, et al. Differences in outcomes for patients with bacteremia due to vancomycin-resistant *Enterococcus faecium* or vancomycin-susceptible *E. faecium*. Clinical Infectious Diseases **1996**; 22:663-670.

21. Stosor V, Peterson LR, Postelnick M, et al. *Enterococcus faecium* bacteremia: does vancomycin resistance make a difference? Arch Intern Med **1998**; 158:522-7.

22. Pelz RK, Lipsett PA, Swoboda SM, et al. Vancomycin-sensitive and vancomycin-resistant enterococcal infections in the ICU: attributable costs and outcomes. Intensive Care Med **2002**; 28:692-7.

23. Shay DK, Maloney SA, Montecalvo M, et al. Epidemiology and mortality risk of vancomycin-resistant enterococcal bloodstream infections. J Infect Dis **1995**; 172:993-1000.

24. Lucas GM, Lechtzin N, Puryear DW, et al. Vancomycin-resistant and vancomycin-susceptible enterococcal bacteremia: comparison of clinical features and outcomes. Clin Infect Dis **1998**; 26:1127-33.

25. Stroud L, Edwards J, Danzing L, et al. Risk factors for mortality associated with enterococcal bloodstream infections. Infect Control Hosp Epidemiol **1996**; 17:576-80.

26. Lautenbach E, Bilker WB, Brennan PJ. Enterococcal bacteremia: risk factors for vancomycin resistance and predictors of mortality. Infect Control Hosp Epidemiol **1999**; 20:318-23.

27. Boyce JM, Opal SM, Chow JW. Outbreak of multi-drug resistant *Enterococcus faecium* with transferable vanB class vancomycin resistance. J Clin Microbiol **1994**; 32:1148-53.

28. Edmond M, Ober J, Weinbaum D, et al. Vancomycin-resistant *Enterococcus faecium* bacteremia: risk factors for infection. Clin Infect Dis **1995**; 20:1134-1136.

29. Jordens JZ, Bates J, Griffiths DT. Faecal carriage and nosocomial spread of vancomycin-resistant *Enterococcus faecium*. J Antimicrob Chemother **1994**; 34:515-28.

30. Karanfil LV, Murphy M, Josephson A. A cluster of vancomycin-resistant *Enterococcus faecium* in an intensive care unit. Infect Control Hosp Epiemiol **1993**; 13:195-200.

31. Livornese LLJ, Dias S, Samel C. Hospital-acquired infection with vancomycin-resistant *Enterococcus faecium* transmitted by electronic thermometers. Ann Intern Med **1992**; 117:112-116.

32. Tornieporth NG, Roberts RB, John J, et al. Risk factors associated with vancomycin-resistant *Enterococcus faecium* infection or colonization in 145 matched case patients and control patients. Clin Infect Dis **1996**; 23:767-72.

33. Papanicolaou GA, Meyers BR, Meyers J, et al. Nosocomial infections with vancomycin-resistant *Enterococcus faecium* in liver transplant recipients: risk factors for acquisition and mortality. Clin Infect Dis **1996**; 23:760-6.

34. Noskin GA, Stosor V, Cooper I, et al. Recovery of vancomycin-resistant enterococci on fingertips and environmental surfaces. Infect Control Hosp Epidemiol **1995**; 16:577-81.

35. Bhavnani SM, Drake JA, Forrest A, et al. A nationwide, multicenter, case-control study comparing risk factors, treatment, and outcome for vancomycin-resistant and -susceptible enterococcal bacteremia. Diagn Microbiol Infect Dis **2000**; 36:145-58.

36. Edmond MB, Ober JF, Dawson JD, et al. Vancomycin-resistant enterococcal bacteremia: natural history and attributable mortality. Clin Infect Dis **1996**; 23:1234-9.

37. Caballero-Granado FJ, Becerril B, Cuberos L, et al. Attributable mortality rate and duration of hospital stay associated with enterococcal bacteremia. Clin Infect Dis **2001**; 32:587-94.

SUMMARY DISCUSSION

Overview of Results

These investigations were conducted to evaluate patient demographics, underlying conditions, diagnostic and therapeutic procedures, a variety of antecedent intravenous antimicrobial exposures, and clinical outcomes associated with the isolation of VRE in hospitalized patients. Based on prior investigations of VRE, our study proposed a model illustrating the roles of exposure to VRE reservoirs, patient characteristics, antimicrobial exposure, and prevalence of VRE in the progression from potential VRE reservoirs to active disease within the hospitalized patient. We discussed differences in VRE colonization and VRE infection, as well as how these differences affect hospital surveillance reporting and interventions targeting the spread of VRE. We documented clonal transmission of VRE in a large, urban, teaching hospital, and reported VRE susceptibility to a wide array of antimicrobial agents.

In a hospital-based case-control study of 135 VRE patients compared with 135 vancomycin-susceptible enterococci (VSE) patients and 135 non-enterococcus (Non-E) patients, specific underlying conditions, severity of illness, invasive procedures, and antimicrobial exposures occurred more often in VRE patients than in VSE or Non-E patients. Hematologic malignancy was more common in VRE patients than in VSE patients. When compared to Non-E patients, VRE patients were more likely to have the following: hospitalization longer than 7 days before culture, an APACHE III score

greater than 50, congestive heart failure, cellulites, and urinary tract infections. Lumbar punctures and thoracenteses were performed more often in VRE patients than in VSE patients, and lumbar punctures, abdominal surgery, and skin or wound debridement were performed more often in VRE patients than in Non-E patients. Imipenem or ceftazidime exposure was more frequent in VRE patients than VSE patients, and ampicillin or imipenem exposure was more frequent in VRE patients than in Non-E patients.

In the retrospective follow-up study of patients identified from the case-control study, the VRE cohort had a higher mortality rate than did the VSE cohort. Vancomycin resistance remained a significant predictor of hospital mortality in multivariable analysis after adjusting for characteristics shown to be associated with mortality. The study also identified a significantly higher 30-day hospital mortality rate after the last positive culture in the VRE cohort than in the VSE cohort. Our findings were supported by the consistency of the three separate statistical methods used.

Strengths

Both the case-control study and the retrospective follow-up study used more rigorous methodological design than did previous studies. The VSE patient group was selected from the same population that gave rise to the VRE patient group, a strategy that excluded patients less likely to be exposed to VRE. The case-control study utilized a Non-E group to include patients exposed to antimicrobials that resulted in the elimination of enterococcus and to identify factors that were associated with enterococcal growth. The matching scheme increased the efficiency of evaluating predictors of VRE occurrence and the efficiency of evaluating the effect of vancomycin resistance on hospital

morbidity and mortality by placing similar distributions of the matching factors in the VRE and VSE patient groups.

The large number of VRE patients provided an opportunity to adjust for multiple antimicrobial exposures, severity of underlying illness, and length of hospital stay. Adjusting for these variables allowed us to focus on the independent association between specific antimicrobial monotherapy and the occurrence of VRE. The large number of VRE patients and unique quantification of co-morbidities associated with hospital mortality helped maintain the stability of the model when we evaluated the independent contribution of vancomycin resistance to hospital morbidity and mortality.

Limitations

The study was limited in that it was retrospective, making the body site of initial colonization of VRE and VSE difficult to determine. Both VRE and VSE patients were selected based on cultures of single body sites. This detection method may not accurately detect the presence of VRE in other body locations. The retrospective design also precluded an evaluation of initial VRE and VSE; hence, the most critical periods of antimicrobial exposure could not be ascertained.

The study included both colonized and infected patients. The inclusion of colonized patients potentially dilutes the association between VRE and mortality; however, we do not believe that this distinction is relevant to the analysis of antimicrobial predictors of VRE occurrence. The proposed mechanism of antimicrobial pressure increasing VRE density should be independent of the whether the patient presents with signs or symptoms of disease. The study did not match VRE to VSE according to *Enterococcus*

species. In our hospital, the vast majority of VRE are *E. faecium*, whereas the majority of VSE are *E. faecalis*. Therefore, the species difference may be a confounding variable.

In evaluating morbidity and mortality, the study did not evaluate pharmacological interventions for the treatment of VRE, nor the appropriateness of clinical care provided to each cohort. VRE are inherently more difficult to treat than VSE because of the dearth of available treatment options. In the case-control study, our quantification of exposure to each antimicrobial was ever/never quantification, and did not take into consideration antimicrobial dosage, duration, or appropriateness of use. Therefore, we have not evaluated actual bioavailability of each antimicrobial.

Finally, the study used clinical isolates as opposed to surveillance isolates. The implication to our study is the underestimation of the number of VRE and VSE patients through the exclusion of asymptomatic patients, and through the inclusion of those cases that represent patients in which the medical conditions warranted the ordering of cultures. However, the distribution of the VRE cases in our study by source of specimen is comparable to the distributions reported nationally, implying that our findings are generalizable to most hospital settings.

Implications

The increasing proportion of enterococcal isolates resistant to vancomycin poses a substantial problem to the hospitalized patient as well as to healthcare delivery systems. History has demonstrated that VRE have been and will remain a problem in the hospital setting. Our study provides supporting evidence that vancomycin resistance is an independent contributor to hospital morbidity and mortality. With the recent emergence of

high-level vancomycin resistance in *Staphylococcus aureus*, the control of VRE has taken on even greater importance. An understanding of the epidemiology and progression of VRE from colonized/infected patients to uncolonized patients is paramount in slowing VRE dissemination.

Our findings and proposed model are salient to the control and elimination of vancomycin resistance in enterococcus. Identifying patients at increased risk for VRE and utilizing antimicrobials with activity against enterococcus could substantially reduce the colonization and spread of VRE. Only through prompt detection of VRE, rigorous institutional infection control, and pharmacy and educational policies can we hope to maintain control of this pathogen and prevent selection of VRSA on a wide scale.

APPENDIX

UNIVERSITY OF ALABAMA AT BIRMINGHAM INSTITUTIONAL REVIEW BOARD APPROVAL

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

The Institutional Review Board for Human Use (IRB) has an approved Multiple Project Assurance with the Department of Health and Human Services and is in compliance with 21 CFR Parts 50 and 56 and ICH GCP Guidelines. The Assurance became effective on January 1, 1999 and the approval period is for five years. The Assurance number is M-1149, identification number 01.

Principal Investigator: KEN WAITES/SCOTT CHAVERS

Protocol Number: X000119009

Protocol Title: Molecular Epidemiology of Vancomycin Resistance in Enterococcus in Two Adjac Hospitals and the Effect of Antimicrobial Use on its Development

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The IRB reviewed and approved the above named project on 2/12/00. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project received EXPEDITED review.

IRB Approval Date: 2/12/00

Date IRB Approval Issued: 2/12/00

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

The IRB approved consent form used in the study must contain the IRB approval date and expiration date.

IRB approval is given for one year unless otherwise noted. For projects subject to annual review research activities may not continue past the one year anniversary of the IRB approval date.

Any modifications in the study methodology, protocol and/or consent form must be submitted for review and approval to the IRB prior to implementation.

Adverse Events and/or unanticipated risks to subjects or others at UAB or other participating institutions must be reported promptly to the IRB.

1120 Administration Building
701 20th Street South
934-3789
Fax 934-1301

The University of
Alabama at Birmingham
Mailing Address:
AB 1120
1530 3rd Ave S
BIRMINGHAM AL 35294-0111

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM

Institutional Review Board for Human Use

Form 4: IRB Approval Form
Identification and Certification of Research
Projects Involving Human Subjects

The Institutional Review Board for Human Use (IRB) has an approved Multiple Project Assurance with the Department of Health and Human Services and is in compliance with 21 CFR Parts 50 and 56 and ICH GCP Guidelines. The Assurance became effective on January 1, 1999 and the approval period is for five years. The Assurance number is M-1149.

Principal Investigator:	WAITES, KEN B
Co-Investigator(s):	
Protocol Number:	X000119009
Protocol Title:	*Molecular Epidemiology of Vancomycin Resistance in Entercoccus in Two Adjacent Hospitals and the Effect of Antimicrobial Use on its Development*

The IRB reviewed and approved the above named project on 02/26/01. The review was conducted in accordance with UAB's Assurance of Compliance approved by the Department of Health and Human Services. This Project will be subject to Annual continuing review as provided in that Assurance.

This project recieved EXPEDITED review.

IRB Approval Date: 2/26/01

Date IRB Approval Issued: 02/26/01

Marilyn Doss

Marilyn Doss, M.A.
Vice Chair of the Institutional Review
Board for Human Use (IRB)

Investigators please note:

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GRADUATE SCHOOL
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DISSERTATION APPROVAL FORM
DOCTOR OF PHILOSOPHY

Name of Candidate Leon Scott Chavers

Graduate Program Epidemiology

Title of Dissertation Antimicrobial Exposure and Clinical Outcomes Associated With Vancomycin-resistant Enterococci

I certify that I have read this document and examined the student regarding its content. In my opinion, this dissertation conforms to acceptable standards of scholarly presentation and is adequate in scope and quality, and the attainments of this student are such that he may be recommended for the degree of Doctor of Philosophy.

Dissertation Committee:

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Fabio Barbone	
James Michael Hardin	
Ken B. Waites	
Sten H. Vermund	

Director of Graduate Program

Dean, UAB Graduate School

Date 8-29-07