

Resistant Enterococci: A Decade Difference[†]

Nalinee Aswapokee, M.D.*

Surapee Tiengrim, M.Sc. (Microbiol)*

Busaba Charoensook, Cert (Microbiol)**

Kantima Sangsiriwut, B.Sc.**

Abstract

Resistant enterococci are growing concern globally, especially those resistant to vancomycin. We studied resistant patterns of enterococci in two periods of a decade apart (1985 and 1995). Fifty isolates of enterococci were randomly selected from each period. Susceptibilities were performed with standard antimicrobial agents (ampicillin, gentamicin and streptomycin) and alternative agents (amoxicillin-clavulanate, ciprofloxacin, fosfomicin, imipenem, nitrofurantoin, rifampin and vancomycin).

Resistance to ampicillin, imipenem and nitrofurantoin was not found in 1985. These were slightly increase in 1995 (10%, 16% and 4%, respectively). There was no vancomycin resistance in both periods, but the intermediately susceptible strains did increase from 10 percent in 1985 to 34 percent in 1995. High level resistance to gentamicin and streptomycin did not change in a decade, being approximately 50 percent for both agents in both periods. Enterococci were not susceptible to ciprofloxacin and rifampin (only 20% and 14% in 1985). Enterococci in 1995 which were resistant to ampicillin had very high MIC (> 256 µg/ml). None of these strains produced beta-lactamases. All such strains showed high level resistance to streptomycin.

Patterns of resistance of enterococci in this university hospital did not change dramatically. Up to 1995, there was no vancomycin resistance, but the intermediate resistant strains did increase quite rapidly. (*J Infect Dis Antimicrob Agents* 2000;17:7-11.)

INTRODUCTION

Resistant enterococci are growing concerns on a global scale. Although these bacteria are the so-called "second-rate" organisms, the important issues are that there were progressively increase in morbidity and mortality of the disease caused by this bacteria.¹ In addition, these organisms notoriously possess intrinsic low-level resistance to virtually all groups of antibiotics available in clinical use. These are β -lactams, lincosamides, aminoglycosides and

quinolones.^{1,2} This situation was made even worse by the rapid acquisition of acquired high-level resistance to various antibiotics, including the agents with low-level resistance and also the glycopeptides.²⁻⁷ The worst possible scenario which is the extreme concern by the people involving in the discipline of infectious diseases and antimicrobial treatment is the capability to transfer the glycopeptide resistance to the "first-rate" organisms, especially *S. aureus*, which is most possible. To our knowledge, entero-

* Division of Infectious Diseases and Tropical Medicine, Department of Medicine,

** Preventive and Social Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

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Reprint request: Nalinee Aswapokee, M.D., Division of Infectious Diseases and Tropical Medicine, Department of Medicine, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

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coccal infections and their resistance problems are not, at the present, a big issue in Southeast Asian countries when compared to the western hemisphere. We studied the resistant patterns of enterococci in two periods of ten-year apart. It was found that resistance to antimicrobial agents was slightly increased and that there was no high level vancomycin resistance among clinical isolates of enterococci.

MATERIALS AND METHODS

Bacterial isolates

Clinical isolates of enterococci obtained from patients hospitalized in Siriraj Hospital, Mahidol University, Bangkok, Thailand, a 2,000-bed tertiary care medical school were used. A total of 100 strains were randomly selected from panels collected in brain heart infusion broth with 20 percent glycerine and kept at -80°C . Fifty strains were isolations in 1985 and another 50 strains were from 1995.

Antimicrobial agents

Ampicillin, amoxicillin-clavulanate, fosfomycin, gentamicin, streptomycin, rifampin, ciprofloxacin, nitrofurantoin and vancomycin were used. Standard powders of gentamicin and streptomycin were purchased from Sigma Chemical Co. The rests of antimicrobial agents were susceptibility discs which were either kindly provided by the respective manufacturers or purchased from BBL Microbiology System.

Susceptibility testings

Standard disc diffusion test for enterococci which describe by NCCLS 1995 was used.⁹ Briefly, inocula were plated on Mueller-Hinton agar and incubated at 35°C in ambient air for 16-18 hours. With vancomycin, incubation period was 24 hours.

Standard agar dilution (NCCLS) was used to determine high level resistance against aminoglycosides.¹⁰ Briefly, multi-inocula were plated on Mueller-Hinton agar and incubated at 35°C in ambient air for 16-18 hours. Vancomycin plates were incubated for 24 hours.

E test was used to determine resistant levels of ampicillin in enterococci against ampicillin and amoxicillin-clavulanate. Quality control strain was *Enterococcus faecalis* ATCC 29212. Beta-lactamase were detected by using chromogenic cephalosporin, Nitrocephin[®].

RESULTS

All 50 isolates randomly selected from the panels of enterococcal strains in 1985 were *E. faecalis*, while 50 strains from 1995 panels included 42 (84%) *E. faecalis* and 8 (16%) *E. faecium*.

Susceptibilities to standard antimicrobial agents of enterococci were summarised in Table 1.

Ampicillin resistance was not found in 1985 and only 10 percent was found in a decade later. Resistance was also not found in imipenem and nitrofurantoin. These were, however, 20 and 10 percent, respectively, in 1995. Startlingly, although there was no vancomycin resistance in both periods, there was only 90

Table 1. Susceptibility patterns of enterococci to standard and alternative antimicrobial agents in 1985 and 1995.

| Antimicrobial Agents | Susceptible | | Intermediate susceptible | | Resistant | |
|-------------------------|-------------|------|--------------------------|------|-----------|------|
| | 1985 | 1995 | 1985 | 1995 | 1985 | 1995 |
| Ampicillin | 100 | 90 | - | - | - | 10 |
| Amoxicillin-clavulanate | 100 | 92 | - | 4 | - | 4 |
| Ciprofloxacin | 26 | 18 | 50 | 54 | 24 | 8 |
| Fosfomycin | 96 | 98 | 4 | 2 | - | - |
| Imipenem | 100 | 80 | - | 4 | - | 16 |
| Nitrofurantoin | 100 | 90 | - | 6 | - | 4 |
| Rifampin | 14 | 32 | 36 | 32 | 50 | 36 |
| Vancomycin | 90 | 66 | 10 | 34 | - | - |

percent full susceptibility in 1985 and only 66 percent in ten years later. The intermediate resistance to vancomycin rose from 10 percent in 1985 to 34 percent in 1995. Susceptibility to fosfomycin was not change in a decade, being 96 and 98 percent in 1985 and 1995, respectively. Enterococci were not susceptible to both ciprofloxacin and rifampin (only 26 and 14 percent in 1985, respectively). Majority of strains showed intermediate susceptibility to ciprofloxacin in both periods, while against rifampin, most strains expressed resistance.

Table 2 shows percentage of high level resistance to aminoglycosides. The prevalences of high level resistance to gentamicin and streptomycin did not significantly change in ten-year period. Approximately half of strains showed resistance to both agents in both 1985 and 1995.

Table 2. Percentage of high level aminoglycoside resistance in enterococci in 1985 and 1995.

| Antimicrobial agents | 1985 | 1995 |
|----------------------|------|------|
| Gentamicin | 52.2 | 52 |
| Streptomycin | 56.8 | 44 |

There were only 5 strains (10%) of ampicillin resistance in the 1995 panels. One strain was *E. faecalis* and the other 4 strains were *E. faecium*. These strains were highly resistant to ampicillin (MIC of > 256 µg/ml) (Table 3). None of these strains produced beta-lactamases as determined by chromogenic cephalosporin. All strains were highly resistant to streptomycin (MIC 100 to > 2,000 µg/ml). *E. faecalis* was resistant to gentamicin only at low level, while 2 of the 4 *E. faecium* strains were highly resistant to gentamicin. Only *E. faecalis* was susceptible to amoxicillin-clavulanate. All 4 strains of *E. faecium* were resistant to this agent.

DISCUSSION

It seems that the problems of resistant enterococci in this location has not yet been advanced as it is in the Western World.^{7,8,11,12} In 1985, we have not found ampicillin-resistant enterococci, while in 1985, *E. faecalis* producing plasmid-mediated β-lactamase was already reported in USA.^{4,13} Ten years later, only 10 percent of enterococci were resistant to ampicillin, and most resistant isolates were *E. faecium*. This may be due to small sample size, or may be due to the change of proportion between *E. faecalis* and *E. faecium*. High level resistance to aminoglycosides was found in half of the isolates in both periods, and the prevalence of resistance was similar for gentamicin and streptomycin. There is no explanation for the stability of prevalence of resistance in 1985 and 1995. It has been shown that high-level gentamicin resistance in enterococci is associated with the production of bifunctional aminoglycoside-modifying enzyme APH(2'') + AAC(6').¹⁴ Recently, Hortiwakul et al, has shown that this enzyme is very common among *S. aureus* in Thailand.¹⁵ It is possible that this enzyme also mediated high-level gentamicin resistance in enterococci in this country.

There was no vancomycin-resistant enterococci both in 1985 and 1995. There was, however, an increase from 10 percent in 1985 to 34 percent in 1995, of vancomycin intermediate susceptible enterococci (VIE). Unfortunately, we have not performed the MIC of VIE. According to the NCCLS guideline for the MIC interpretative standards of enterococci (1998), VIE had the MIC of 8-16 µg/ml.¹⁰ Tenover pointed out that for any strains of *S. aureus* with vancomycin MIC ≥ 4 µg/ml should be regarded as the potential candidates for vancomycin-resistant strains.¹⁶ If this is also true for enterococci, the potential candidates of VRE in our area was rapidly increasing.

Table 3. Level of resistance to other antimicrobial agents of ampicillin-resistant enterococci.

| Organisms | MIC (µg/ml) | | | |
|----------------------------|-------------|-------------------------|------------|--------------|
| | Ampicillin | Amoxicillin-clavulanate | Gentamicin | Streptomycin |
| <i>E. faecalis</i> (E 114) | > 256 | 4 | 7.8 | 2,000 |
| <i>E. faecium</i> (E 104) | > 256 | 12 | 15.6 | 1,000 |
| <i>E. faecium</i> (E 145) | > 256 | 12 | 15.6 | > 2,000 |
| <i>E. faecium</i> (E 112) | > 256 | 32 | > 2,000 | > 2,000 |
| <i>E. faecium</i> (E 123) | > 256 | 64 | > 2,000 | > 2,000 |

Up to 1995, there was no VRE in our institution. There is no clear explanation for this. The fact that vancomycin consumption in this country is low, comparing to the Western World, and that the oral preparation of this agent was not registered for clinical use in this country, may partly explain this.

Before the advent of third and fourth generations quinolones, ciprofloxacin had been claimed to exert some bacteriostatic activity against enterococci and synergistic bactericidal activity has been demonstrated when combined with ampicillin or gentamicin.^{17,18} In this study, we found that very few enterococci were susceptible to ciprofloxacin, and this finding is similar to that reported by Venditti et al.¹⁹ On the contrary, we found that 81 and 90 percent of our recent clinical isolates of enterococci were susceptible to levofloxacin and trovafloxacin, respectively.^{20,21} This finding indicated that new generation quinolones may be more useful clinically for enterococcal infections than ciprofloxacin.

The majority of enterococci were susceptible to nitrofurantoin, making this agent useful in treating enterococcal urinary infection. All enterococci were susceptible to imipenem in 1985, but 20 percent became resistant to this agent. This may reflect the increased prevalence of *E. faecium* in 1995 rather than an acquired resistance in *E. faecalis* to this agent, because all strains resistant to imipenem were *E. faecium*.

Although resistant enterococci in this area have not reached the crisis in 1995, the increase in prevalence of VIE signified that the nightmare is coming. Strict adherence to prudent use of vancomycin, as part of the Hospital Infection Control Practice Committee (HICPAC) recommendation for preventing the spread of vancomycin resistance,²² has been demonstrated to reduce prevalence of VRE.²³ This programme should receive prompt attention in this country. The sooner the implementation of this programme being made, the more likelihood of slowing of emergence and/or spreading of VRE is anticipated.

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