Vancomycin-Resistant Enterococci in King Chulalongkorn Memorial Hospital : A 5-Year Study

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Abstract

The emergence of hospital acquired infections with bacteria resistant to antimicrobials such as vancomycin resistant enterococci (VRE) has become a worldwide concern. The authors studied the prevalence and surveillance of 5 years study of VRE in King Chulalongkon Memorial Hospital and phenotype of these resistance strains. A total of enterococci 1854 isolates were collected from clinical specimens from 1995 to 1999. Screening vancomycin resistance was identified by the agar plated method and minimal inhibitory concentration (MIC) of vancomycin was determined for vancomycin-resistance strains by E-test.

The results demonstated that 15 (0.81%) VRE were isolated from 1,854 specimens. Fourteen VRE were identified as *Enterococcus faecium* and one strain was *Enterococcus faecalis*. All of these strains, carrying the VanB phenotype, were susceptible to teicoplanin.

Similar to other studies, most VRE strains are *E. faecium*. To the authors' knowledge, this is the first VRE study carried out in King Chulalongkorn Memorial Hospital. The results showed a low prevalence of VRE and surveillance of 5 years study demonstated a gradual increase of VRE. Therefore, it is important to continue periodic surveys of VRE to prevent the spread of VRE in hospitals.

Key word : VRE, Phenotype

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Enterococci are normal inhabitants of the gastrointestinal tract of humans and animals and may also be recovered from the oral cavity, skin, and vagina. *Enterococcus faecalis* and *Enterococcus faecium* are the species most often responsible for human disease. Enterococci are often resistant to most antibiotics in clinical practice, except for glycopeptide antibiotic vancomycin that has been regarded as the ultimate treatment of enterococci infections. Recently, strains of enterococci resistant to vancomycin (VRE) have been isolated and become increasingly more common in Europe and America[1,2]. VRE pose a unique public health threat because it makes treatment difficult, and because of the potential for this plasmid-mediated vancomycin resistance trait to be transferred to other microorganisms[3-5]. The mechanisms by which enterococci resist vancomycin are complex including the VanA phenotype with high-level vancomycin and teicoplanin resistance, and the VanB phenotype with moderate to high-level resistance to vancomycin but continued susceptibility to teicoplanin.

VRE have emerged in the past few years as epidemiologically important pathogens. VRE were first reported in France in 1988[6]. Since then, these organisms have been reported as common causes of human infection in the USA, UK, Germany, The Netherlands, Spain and Saudi Arabia[1,2,7]. From 1989 through 1993, the percentage of nosocomial enterococcal infections due to VRE reported by the Centers for Disease Control and Prevention National Nosocomial Infections Surveillance system was increased from 0.3 to 7.9 per cent[8]. Recently, Aswapokee N, et al[9] reported that there was no vancomycin resistance strain in Siriraj Hospital in two periods, one decade apart (1985 and 1995). The question is, are VRE infections a major problem in our community? Therefore, this study investigated the prevalence and surveillance of 5 years study of VRE isolated in King Chulalongkorn Memorial Hospital and phenotype of these resistance strains.

MATERIAL AND METHOD

Strains

A total of 1,854 enterococci isolated from patient specimens at King Chulalongkorn Memorial Hospital from 1995-1999 were studied. Of the 1,854 isolates, 17 were isolated from blood, 33 from cerebrospinal fluid (CSF), 124 from tissue, 165 from body fluid, 311 from pus, 893 from urine, and 216 from the cervix, 12 from ENT, 51 from catheters and 8 from dialysate. All strains were presumptive identified genus by pyrrolidonyl arylamidase activity[10]. Tolerance to bile esculin and growth in 6.5 per cent NaCl were determined as described[11]. The vancomycin resistance strains were identified the species by analytical profile index (API) (Biomerieux, 69280 Marcy-l'Etoile France)

Antimicrobial susceptibility tests

Screening vancomycin resistance strains were initially identified by agar plate (MIC > 6 μg/ml) on Muller-Hinton agar with 6 μg/ml vancomycin. An inoculum 10 μl of 0.5 McFarland of each strain was applied to the surface of the antibiotic-containing agar. The plates were incubated for 18 hours in air at 35°C. MICs of vancomycin was determined for vancomycin-resistant strains by E-test (AB-biodisk, Sweden).

Enterococcus faecalis American type culture collection (ATCC) 29212 and *Staphylococcus aureus* ATCC 25923 were used as controls.

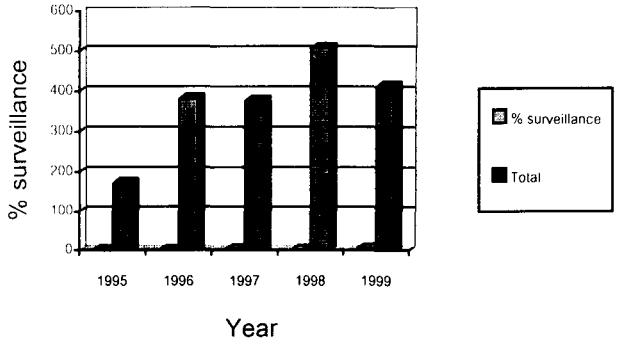
In order to distinguish VanA and VanB phenotypes of VRE, MIC of teicoplanin was determined by E-test.

RESULTS

Vancomycin-resistant enterococci was isolated from 15 (0.81%) of 1,854 clinical samples. Of the 15 VRE, one was isolated from blood, two from body fluid, three from urine, two from tissue, five from pus and two from the cervix. Surveillance of 5 years study is shown in Fig. 1. Fourteen of VRE were identified as *Enterococcus faecium* and a single strain was *Enterococcus faecalis*. All of these strains were shown by MICs of teicoplanin to posses the VanB phenotype (Table 1).

DISCUSSION

In the present study, the prevalence of vancomycin resistance in enterococci isolated from King Chulalongkorn Memorial Hospital was lower, at 0.81 per cent compared with rates of resistance < 3 per cent in Taiwan[12,13] and those in North Amercica and Europe[14] but higher than the previous report from Siriraj Hospital[9]. Several genes, including *van*A, *van*B, *van*C, *van*D and *van*E, contribute to vancomycin resistance in enterococci[15]. *E. faecium* is the most frequently isolated species of VRE and typically produces high vancomycin (MICs ≥ 64 μg/ml) and teicoplanin (MICs ≥ 16 μg/ml). These iso-



Year	%Surveillance
1995	0.58
1996	0.26
1997	1.06
1998	0.59
1999	1.45

Fig. 1. Percentage of VRE surveillance in King Chulalongkorn Memmorial Hospital in a 5-year study.

Table. 1. *In vitro* vancomycin and teicoplanin susceptibility and phenotype of 15 Enterococci strains.

Strain No.	Species	MIC (μg/ml)		Phenotype characteristic
		Vancomycin	Teicoplanin	
106	*E. faecalis*	≥256	1.0	VanB
305	*E. faecium*	8.0	1.0	VanB
653	*E. faecium*	8.0	1.0	VanB
659	*E. faecium*	8.0	1.0	VanB
687	*E. faecium*	12.0	2.0	VanB
693	*E. faecium*	12.0	2.0	VanB
1345	*E. faecium*	8.0	1.0	VanB
1378	*E. faecium*	8.0	0.5	VanB
1468	*E. faecium*	8.0	1.0	VanB
1568	*E. faecium*	8.0	1.0	VanB
1598	*E. faecium*	8.0	1.0	VanB
1604	*E. faecium*	8.0	1.0	VanB
1678	*E. faecium*	8.0	1.0	VanB
1736	*E. faecium*	8.0	2.0	VanB
1847	*E. faecium*	8.0	2.0	VanB
Reference ATCC29212	*E. faecalis*	4.0	0.25	
Reference ATCC25923	*S. aureus*	4.0	0.25	

lates typically contain *van*A genes. A *van*B-containing isolate normally produces a lower level of resistance to vancomycin (MICs 16 to 64 μg/ml) and is susceptible to teicoplanin (MIC ≤ 1 μg/ml). It has been reported that levels of vancomycin resistance among *van*B isolates ranged from 4 to ≥ 1,000 μg/ml, whereas, susceptibility to teicoplanin was retained[15]. *E. gallinarum* and *E. casseliflavus* isolates, causing VanC phenotype, are intrinsically resistant to vancomycin with the MICs of 4 to 32 μg/ml and are susceptible to teicoplanin[15]. The use of vancomycin and teicoplaninMICs to distringuish VRE phenotype has some limitation. A recent report from Ostrowsky BE, et al[16] demonstated, a few *van*D-containing isolates of *E. faecium* with a moderate level of resistance to vancomycin (MICs 64 to 128 μg/ml) and teicoplanin (MICs 4-8 μg/ml). In addition, the *van*E-containing *E. faecalis* has recently been reported. This strain is resistant to low levels of vancomycin (MIC 16 μg/ml) and susceptible to teicoplanin (MIC 0.5 μg/ml)[17]. To the authors' knowledge this is the first VRE carried out in King Chulalongkorn Memorial Hos-

pital. Vancomycin resistance in enterococci is a concern not only because of the challenge of treating patients who may be infected with VRE, but also because of the potential for the vancomycin resistance genes to be spread to other organisms such as *Staphylococcus aureus*. Enterococci are part of the normal flora of the gastrointestinal tract and the female genital tract. Therefore, most infections with these organisms have been attributed to the patient's endogenous flora[18]. However, recent reports have demonstrated that enterococci, including VRE, can be spread by direct patient-to-patient contact or indirectly *via* transient carriage on the hands of personnel[19], contaminated environmental surfaces[19,20], or patient care equipment[21].

It is not always easy to assess the clinical significance of VRE in routine cultures or to differentiate colonization from infection. This is especially true for urine or when VRE are part of a polymicrobial infection. In some cases, attempts at treatment are not indicated. The extent to which VRE causes morbidity and mortality is often difficult to determine, because most affected patients have serious underlying diseases that cause substantial morbidity and death and VRE are often recovered in mixed cultures with other potential pathogens[22]. Although VRE infection in our community seems not to be serious, periodic prevalence surveys in King Chulalongkorn Memorial Hospital should be performed to control the spread of VRE in this institute.

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การศึกษาความชุกของเอ็นเตโรค็อคไคสายพันธุ์ดื้อต่อแวนโคมัยซินในโรงพยาบาลจุฬาลงกรณ์ (ศึกษา 5 ปี)

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โรคติดเชื้อในโรงพยาบาลที่เกิดจากเชื้อแบคทีเรียที่ดื้อยา เช่น vancomycin resistant enterococci (VRE) ได้เป็นที่สนใจกันอย่างแพร่หลาย คณะผู้วิจัยจึงทำการศึกษา ความชุกของการดื้อต่อ vancomycin และลักษณะแสดงออกทางพันธุกรรมในเชื้อ enterococci จำนวน 1,854 สายพันธุ์ ซึ่งแยกได้จากผู้ป่วยโรงพยาบาลจุฬาลงกรณ์ตั้งแต่ พ.ศ. 2538 – พ.ศ. 2542 พบเชื้อจำนวน 15 สายพันธุ์ (ร้อยละ 0.81) ดื้อต่อ vancomycin ซึ่งยืนยันโดยการทดสอบ E-test โดย 14 สายพันธุ์ เป็น *Enterococcus faecium* มี MICs อยู่ระหว่าง 8–12 μg/ml และ 1 สายพันธุ์ เป็น *Enteococcus faecalis* มี MIC > 256 μg/ml และทุกสายพันธุ์มีฟีโนไทป์เป็น *van*B โดยไวต่อยา teicoplanin

E. faecium เป็น VRE ที่พบได้มากที่สุดใน enteroocci เช่นเดียวกับการศึกษาจากรายงานอื่น ๆ VRE ที่พบจากการศึกษานี้ถือเป็นรายงานแรกในโรงพยาบาลจุฬาลงกรณ์ โดยมีความชุกอยู่ในระดับต่ำเมื่อเปรียบเทียบกับบางประเทศ แต่ surveillance ใน 5 ปีมีแนวโน้มค่อย ๆ เพิ่มขึ้น ดังนั้น การติดตามการดื้อยาของเชื้อกลุ่มนี้เป็นระยะ ๆ จะช่วยป้องกันการแพร่กระจายของ VRE ในโรงพยาบาลได้

คำสำคัญ : เอ็นเตโรค็อคไคสายพันธุ์ดื้อต่อแวนโคมัยซิน, ฟีโนไทป์

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