

行政院國家科學委員會專題研究計畫成果報告

台灣新出現之萬古黴素抗藥性腸球菌感染：臨床特徵，藥物感受性和分子流行病學研究

Emergence of Infections Caused by Vancomycin-resistant Susceptibility, and Molecular Epidemiology

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中文摘要

為了了解本院萬古黴素抗藥性之腸球菌(VRE)流行情形。當我們分析了12位病患分離之25株VRE，其臨床特徵菌株之藥物感受性，van A, B和C基因和分子生物學分型。所有之菌株均只含van A基因，有一病患有肛門口膿瘍，且由此傷口分離出12株VRE(分別是4株*E. faecalis*, 7株*E. faecium*, 和1株*E. casseliflavus*)。分子生物學分型之結果顯示，多種species之VRE和多種clones之VRE可同時存在於住院病人之身上，且同一clone之VRE方可長期存活於病人之下腸胃道內。

關鍵詞：萬古黴素抗藥性腸球菌、
菌株散播

ABSTRACT

To understand the epidemiology of vancomycin-resistant enterococci (VRE) in a university hospital in Taipei, Taiwan. A retrospective review over a 27-month period, from March 1996 to May 1998 was performed. Patients with VRE isolated from any body site were identified through hospital microbiology and infection control records. Patient charts were reviewed for clinical and epidemiology data, including age, gender, previous hospital admissions, underlying diseases, types of infection, and recent antibiotic use. Twenty-five isolates of VRE recovered from 12 patients were identified. One patient with a perianal

abscess had 12 isolates of VRE (four isolates of *Enterococcus faecalis*, seven of *E. faecium*, and one of *E. casseliflavus*) recovered from perianal lesions. Among three patients who were hospitalized in the same room, one had a community-acquired cellulitis over the left leg caused by *E. faecalis* and the other two patients both had anal colonization with two isolates of *E. faecalis*. The other eight patients had one *E. faecalis* isolate each from various clinical specimens. All isolates possessed *vanA* resistance phenotype and *vanA* genes. Multiple species of VRE (*E. faecalis*, *E. faecium*, and *E. casseliflavus*) and multiple clones of *E. faecium* could colonize in and/or infect hospitalized patients. In addition, same clones of VRE can persist long-term in patients' lower gastrointestinal tract. Different antibiotypes and RAPD patterns of the isolates from different patients excluded the possibility of nosocomial spread at the hospital. These results extend our knowledge of the coexistence and the persistence of multiple species and multiple clones of VRE in hospitalized patients.

Keywords: Vancomycin-resistant enterococci, clonal dissemination

INTRODUCTION

Enterococci continue to be an important cause of nosocomial infections worldwide. Over the past 20 years there has been a remarkable rise in antimicrobial resistance among enterococci, especially *Enterococcus*

faecium. Outbreaks of VRE infection and long-term stool carriage associated with a clonal strain of *E faecalis* or *E faecium* in hospitalized patients have been reported. However, the simultaneous existence of multiple clones of *E faecium* as well as the presence and persistence of multiple species of VRE (*E faecalis*, *E faecium*, and *E casseliflavus*) in hospitalized patients have not been previously reported.

MATERIALS AND METHODS

Case Definition and Bacterial Isolates

Beginning in March 1996, any patient who was treated at NTUH from whom VRE was isolated from a clinical specimen was subjected to anal swab culture surveys and placed on contact precautions. This included gloves and gowns for all direct contact with the patients and strict adherence to hand washing. Rectal swabs were collected from any patient who resided in a room with known VRE infected patients or evidence of VRE colonization and from health care workers. Environmental samples were taken on premoistened swabs from surfaces (bed rails, bed linen, sink faucets and handles, bedside chart, blood pressure cuffs, electronic thermometers, and stethoscopes) in rooms with patients harboring VRE. The samples were cultured onto vancomycin screen agar (brain heart infusion agar with 6 µg/mL of vancomycin). VRE isolates were characterized by standard microbiological methods. Identification of the organisms to the species level was done using the following three commercial identification systems: API 20 Strep system, API 32 Strep system, and the Vitek GPI system.

Clinical information was obtained by review of data from the medical record, including demographic data, underlying disease and associated condition, length of hospital stay before VRE isolation, the

presence of indwelling devices, surgical procedures done, exposure to antimicrobials in the 30 days before VRE isolation, and outcome. Clinical isolates of VRE were defined as being associated with infection or colonization in accordance with the criteria of the Centers for Disease Control and Prevention.

Antimicrobial Susceptibility Testings

Minimum inhibitory concentrations (MICs) of the 25 isolates of VRE were determined by the E test (PDM Epsilometer, AB Biodisk, Solna, Sweden) according to manufacturer's instructions. Three vancomycin resistant phenotypes were categorized.

Determination of Vancomycin-Resistant Genes by PCR

The oligonucleotide primers used for amplification of the *vanA*, *vanB*, *vanC1*, and *vanC2* were selected from the previously published primer sequences. Restriction fragment length polymorphisms (RFLP) of the amplicons of *vanA*, *vanB*, *vanC1*, and *vanC2* were further digested with *Hinf*I and *Rsa*I.

Random Amplified Polymorphic DNA (RAPD) Patterns

Two oligonucleotide primers: M13 (5'-GAGGGTGGCGGTTCT-3') and ERIC1 (5'- GTGAATCCCCAGGAGCTTACAT-3') were used.

RESULTS

Characterization of Patients

A total of 25 isolates of VRE from 12 patients were identified: 17 of *E faecalis*, 7 of *E faecium*, and one of *E casseliflavus*. Among these 12 patients, VRE isolates were associated with clinical diseases in seven patients (two bacteremia, two wound infections, two perianal infections, and one

catheter-related sepsis). One patient (patient 12) had a refractory perianal abscess and 12 isolates were recovered from the abscess fluid, debrided tissue, and swab specimens from the lesion. Three of these seven patients had other microorganisms (coisolates) recovered from the same clinical specimens (other than anal swab specimens) which were positive for VRE (*Aeromonas hydrophila* in patient 4, *Escherichia coli* in patient 9, and *Prototheca* spp. in patient 8). Patients 10 and 11 were found to have VRE colonization during culture surveillance after patient 9 was discovered to have a positive culture from a cellulitis lesion. These three patients resided in the same medical ward for several days.

The duration of hospitalization before the first isolation of VRE from these patients' specimens (except for the wound culture of patient 9) ranged from 4 days to 6 months. The VRE strain, isolated on the first hospital day from the cellulitis lesion of patient 9, who was not hospitalized in the two years prior to this admission, was considered to be community-acquired. The rectal swab cultures of patients 1 to 6, 8, and 9 were negative for VRE.

The majority of patients had underlying immunocompromised conditions and/or had undergone surgical intervention with indwelling device implantation. Prior to acquisition of VRE, all of these patients had received one or more antimicrobial agents, including β -lactams, aminoglycosides, or quinolones, for days or weeks within the previous 30 days for treatment of various infections.

RAPD patterns

Among the 25 VRE isolates, 17 RAPD patterns produced by the two primers were identified: 12 patterns for *E faecalis*, four for *E faecium*, and one for *E casseliflavus*. Isolates recovered from different patients, including the three patients (patients 9, 10,

and 11) who were hospitalized in the same room, displayed different RAPD patterns. Two *E faecalis* isolates recovered from patient 10 had identical antibiotypes and RAPD patterns. Two isolates of *E faecalis* recovered from patient 11 were identical phenotypically and genotypically. This is also true for the four isolates of *E faecalis* in patient 12. The time intervals of the recovery of identical *E faecalis* clones in these three patients ranged from 7 days (patients 10 and 11) to 30 days (patient 12). In patient 12, four RAPD patterns (patterns m to p) were identified in the seven isolates of *E faecium*: two (isolates L5 and L10) of the pattern m isolates were recovered within an interval of seven weeks.

Characterization of Vancomycin-Resistant Genes

All isolates possessed only the *vanA* gene and had identical DNA fingerprinting of the gene by *HinfI* and *RsaI*.

Antimicrobial Susceptibilities

All isolates possessed the *vanA* resistance phenotype. The VRE isolates belonging to different clones had different antibiotypes.

DISCUSSION

Based on the microbiological and molecular epidemiological data on the 25 VRE isolates, four important points were elucidated. First, though these isolates were found within a 27-month period at the hospital, nosocomial transmission of these *vanA* strains of VRE did not occur. Second, one of our patients had community-acquired VRE infection, suggesting that the VRE strain did exist outside of the hospital environment in Taiwan. Third, a single clone of vancomycin-resistant *E faecalis* or *E faecium* could persist for weeks in the lower gastrointestinal tract or in the perianal lesions

of hospitalized patients. Fourth, multiple species (*E faecalis*, *E faecium*, and *E casseliflavus*) and multiple clones of the same species of VRE (*E faecium*) could simultaneously infect or colonize the perianal area of in-patients.

Previous studies have suggested that antimicrobial pressure, particularly receipt of agents with activity against anaerobes (clindamycin and metronidazole) and glycopeptides (vancomycin and teicoplanin), might increase the rate of stool carriage of VRE and promote the development of VRE infection. However, only one-third of our patients with infection or colonization with VRE had been previously exposed to glycopeptides. In addition, previous studies have shown that when weekly surveillance rectal cultures were done, 48% of patients had gut colonization of VRE before a clinical VRE isolate was recovered. However, among the six patients in our study in whom VRE was isolated from body sites rather than the bowel, all rectal swab cultures were negative for VRE. Insufficient sensitivity of a single rectal swab to detect VRE gut colonization among patients with clinical VRE isolations might partly contribute to this finding.

In summary, this study extends our knowledge of the persistence and the coexistence of multiple species and multiple clones of VRE in hospitalized patients, and emphasizes the importance of strain typing in characterizing the evolving VRE epidemic. Elucidating the factors contributing to the organism's survival in the hospital environment as well as its persistence in the hospitalized patients may be essential for understanding the acquisition and transmission of nosocomial VRE infections.

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