J Infect Chemother (1999) 5:46–48

NOTE

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Analysis of cross infection using genomic fingerprinting in nosocomial urinary tract infection caused by *Enterococcus faecalis*

Received: April 8, 1998 / Accepted: October 14, 1998

Abstract
To evaluate cross infection and a possible outbreak of *Enterococcus faecalis* urinary tract infection (UTI) in our urology ward, we studied the DNA fingerprinting of *E. faecalis* strains isolated from nosocomial UTI patients, in the period 1982–1996, using arbitrarily primed polymerase chain reaction (AP-PCR) analysis. The serovar and amplified products of DNA extracted from clinically isolated urinary *E. faecalis* strains by the AP-PCR method were analyzed, and the respective isolation periods of *E. faecalis*-positive UTI patients were investigated. There were nine patients with *E. faecalis* UTI between March and May 1994 and all strains isolated from their urine specimens were serovar type 7. AP-PCR revealed that five of the nine isolates had the same pattern. It appeared that these strains had caused the outbreak of *E. faecalis* UTI. Cross-infection between patients with *E. faecalis* UTI was demonstrated by genomic fingerprinting, suggesting that cross infection had occurred via urinary catheters or by hand contact in our ward. We may, therefore, reasonably conclude that we should beware of the transmission of urinary *E. faecalis* and take countermeasures against its dissemination.

Key words Cross infection · Nosocomial infection · Urinary tract infection · *E. faecalis* · AP-PCR

Introduction

Resistance to many antibiotics is already common among enteroccoci. In recent years, vancomycin (VCM)-resistant enterococci (VRE) have increased alarmingly in many countries.[1] Since 1988, a rapid increase in the incidence of infection and colonization by VRE has been reported in United States hospitals, although this is still rare in Japan. Enterococci commonly cause nosocomial infections. Outbreaks and endemic infections caused by enterococci, including VRE, indicate that patient-to-patient transmission of the microorganisms can occur, either through direct contact via the hands of personnel, contaminated patient-care equipment, or environmental surfaces.[2]

In the 1980s, we found an increased frequency of *Enterococcus faecalis* urinary tract infection (UTI) in our ward. While this increase may be linked with the selection of resistant strains of enterococci from intestinal flora as a result of cephalosporins overuse, there have been reports of cross infection between patients[3,4] caused by urinary *E. faecalis*.

Genomic polymorphism of microorganisms at the strain level has been described, demonstrated by the use of a single arbitrary primer in the arbitrarily primed polymerase chain reaction (AP-PCR)[5] or in the random amplification of polymorphic DNA (RAPD).[6] These studies showed that strains could be distinguished by comparing polymorphisms in genomic fingerprints. In epidemiological investigations, this technique has been widely used to genotype a variety of microorganisms,[7,8] and is well suited to the study of genetic analysis and monitoring of the spread of nosocomial pathogens. In comparison with ribotyping, AP-PCR has the additional advantages of speed and simplicity.[9]

We report here an investigation of urinary *E. faecalis* cross infection using genomic fingerprinting with AP-PCR analysis.

Patients and methods

We examined the distribution pattern of patients with UTI caused by *E. faecalis* and strains isolated from urine in the urology ward of Sapporo Medical University Hospital from 1982 to 1996. Positive urine culture was defined as more

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than 10000 cfu/ml. Urine samples were incubated for 24 h at 35°C with a sheep blood agar plate (Seiken, CD; Denka Seiken, Tokyo, Japan), and identified by Walk Away type 96 (Dade Microscan; Sacramento, CA, USA). We reviewed the patients' isolation periods, room numbers, and whether or not they had an indwelling urinary catheter, and examined the serovar and DNA polymorphisms by AP-PCR analysis of the strain.

Serovar analysis

Serological typing was done as reported by Maekawa et al.[10]

AP-PCR analysis

E. faecalis strains were grown for 6 h in 5 ml of Todd-Hewitt broth at 37°C. Genomic DNA was extracted with Instagene Purification Matrix (Bio-Rad Laboratories, Hercules, CA, USA). After preliminary trials using various primers in our laboratory, we selected a single arbitrary primer; 5′-TGGAATCCAGGGGAAACACTG-3′, which was designed as mouse interleukin 1 alpha antisense primer.[11] We obtained similar results with other arbitrary primers, such as OPA-02 and OPA-17 (Operon Technologies, Alameda, CA, USA). The AP-PCR was performed in a volume of 10 μl that contained 1 μl of the sample DNA, 2.0 μM of the primer, 3.5 mM $MgCl_2$, dNTP mix, PCR buffer, and 0.25 U of Ampli Taq Gold (Perkin-Elmer Cetus, Norwalk, CN, USA). The amplification was performed in a Gene Amp PCR System 9600-R (Perkin-Elmer Cetus). The cycles were performed by the method of Fang and colleagues,[12] with a pre-heat cycle added. This method was well suited to discriminate genetic fingerprinting in our analysis. The pre-heat cycle included activation of Ampli Taq Gold at 95°C for 9 min. Two low-stringency cycles initially included a denaturation step at 92°C for 5 min, an annealing step at 40°C for 5 min, and an extension step at 72°C for 5 min. Then 40 high-stringency cycles included a denaturation step at 92°C for 1 min, an annealing step at 60°C for 1 min, and an elongation step at 72°C for 2 min.[12] After amplification, a total of 10 μl of each amplified sample was electrophoresed on 3% agarose gel at 100 V and stained with ethidium bromide. A Low DNA Mass Ladder (GIBCO BRL, Life Technologies, Rockville, MD, USA) was electrophoresed with the PCR products as a molecular weight standard. The mobility of each amplification product was determined under ultraviolet light.

Results

We examined the relation between the room numbers of patients with *E. faecalis* UTI and the *E. faecalis* isolation period by the serovar. Between March and May 1994, nine patients had serovar type 7 *E. faecalis* UTI, which strains were presumed to have caused an outbreak of *E. faecalis* UTI.

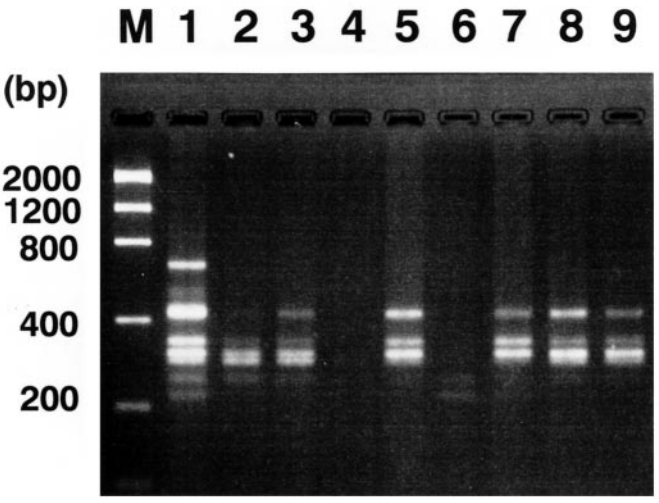

Fig. 1. Amplified products by arbitrarily primed polymerase chain reaction analysis of nine strains of *Enterococcus faecalis* serovar type 7 isolated from nine patients with *E. faecalis* urinary tract infection. *Lane M*, molecular weight marker; *lanes 1–9*, the nine patients

Figure 1 shows the genomic DNA products of the nine strains isolated from the nine patients, amplified by AP-PCR. Strains from patients 3, 5, 7, 8, and 9 had a common pattern. Patients 5, 7, and 8 had indwelling urinary catheters at the time of isolation. The other two patients did not have urinary catheters, but had malignant urinary tract diseases. The pattern in patient 2 was similar to that in patient 3, whereas the DNA pattern in patient 1 was clearly different from the others. Patients 4 and 6 showed very faint bands and the pattern differed from the others.

Discussion

Enterococci, in particular *E. faecalis* and *E. faecium*, are indigenous flora in the human bowel. Although these pathogens are rarely associated with primary infections in the noncompromised host, they commonly cause nosocomial infections in hospitalized or immunocompromised patients.

The frequency of *E. faecalis* UTI was shown to have risen from 6% in 1975 to 16% in 1984 in one study,[13] and from 3.2% in 1982 to 12.6% in 1989 in our study. During that period, we overused cephalosporins in our ward. Because *E. faecalis* had lower susceptibility to the cephalosporins, we speculated that they had propagated, becoming selected. In the 1990s, we regulated the use of antimicrobial agents, which resulted in a decrease in doses, compared with doses used in the 1980s. As drug use decreased, the number of patients with *E. faecalis* UTI patients also decreased.

However, in 1994, there was a higher frequency of *E. faecalis* UTI patients than in the other 1990s. We suspected an outbreak of *E. faecalis* UTI and examined the serovars of strains isolated from urine specimens. Nine strains of

serovar type 7 were isolated from nine patients from March to May and we used AP-PCR analysis to discriminate them. The amplified products showed that five of the nine strains had the same pattern, one of the strains had a pattern similar to that of the first five strains, and the other three strains were different. The results indicated that, in the five UTI patients with *E. faecalis* strains of the same pattern, cross infection was implicated.

Hall and colleagues[14] reported that direct cross-infection of *E. faecalis* UTI occurred rarely, if at all, based on their examination of DNA fragment patterns with the restriction enzyme *Sst* I. In our study, the amplified products of AP-PCR analysis suggested that there was cross infection between *E. faecalis* UTI patients; that is to say, it seemed reasonable to suppose that there was an outbreak of urinary *E. faecalis*. Clinically, three of the five patients had an indwelling urinary catheter. Two others had undergone urinary tract treatment just before the isolation of urinary *E. faecalis*. Therefore, cross infection may have occurred via the urinary catheter or by hand contact. This suggested that possible future infection by VRE might be extremely troublesome, because of the possibility of cross infection by urinary *E. faecalis*, which is pathogenic in the urinary tract.[15] In this series, no strains were resistant to vancomycin. We found faint bands in two of the patients with *E. faecalis* UTI, and speculated that the low volume of extracted DNA led to these unclear faint bands.

Simple and reproducible fingerprints of complex genomes can be generated using single arbitrarily chosen primers and PCR.[5,6] The different strains of various microorganisms can be discriminated by AP-PCR or RAPD.[7–9] Such DNA analyses using a single arbitrarily chosen primer can effectively provide an epidemiological marker. AP-PCR analysis is easy to perform, the only difficulty being selection of an effective and clearly discriminative primer. Thus, we believe that examination of polymorphism in genomic fingerprinting by single arbitrarily primed PCR can provide clear and useful epidemiological analysis of urinary *E. faecalis*.

Conclusion

To evaluate cross infection by urinary *E. faecalis*, we studied DNA polymorphism in clinically isolated urinary *E. faecalis*, using the AP-PCR method. The DNA pattern indicated that there was cross-infection between patients with *E. faecalis* UTI. The cross-infection seemed to have occurred via urinary catheters or hand contact. Thus, we should beware of the transmission of urinary *E. faecalis* and establish effective countermeasures.

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