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Original Article

Investigation of vancomycin resistant *Enterococcus faecium* outbreak in neonatal intensive care unit

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Abstract: Enterococci are one of the major agents of community-acquired and nosocomial infections. In this study we aimed to analyze the clonal relation of the vancomycin-resistant *Enterococci* outbreak seen at the Neonate Intensive Care Unit (NICU) of Uludag University Hospital. Vancomycin resistance gene was investigated in the *Enterococcus faecium* strains and pulsed field gel electrophoresis (PFGE) was used to investigate the genetic relation between outbreak strains. Enterococci grown in all patient samples were identified as *Enterococcus faecium* by BD Phoenix 100 (Becton Dickinson, USA). We found *vanA* resistance gene in all of the swab samples by Xpert VanA/B test on Cepheid (Cepheid, USA). PFGE band patterns revealed two different strains, of which the majority of them (22/24) had the same clonal origin. The common clonal origin was also isolated from rectal probes. Perianal swab culture positivity was evaluated as colonization but culture growth in two blood cultures, two urine cultures and one wound culture was evaluated as infection and treated with linezolid. All of the patients survived the outbreak. Besides the infection control precautions determining the genetic relation between outbreak strains which can be done in the microbiology laboratory is necessary to control an outbreak. PFGE is a reliable method in the microbiologic analysis of outbreaks. Molecular microbiologic analysis of outbreak strains will contribute to prove the epidemiologic and evolution of outbreaks.

Keywords: *Enterococcus,* neonatal, outbreak, PFGE

Introduction

Enterococci are one of the major agents of community-acquired and nosocomial infections. Antimicrobial resistance in *Enterococci* has posed challenges for treatment in recent years [1, 2]. Vancomycin-resistant *Enterococci* (VRE) emerged in the 1970s and are a great problem in hospitals because of their ability to colonize and infect high risk patients [3]. Vancomycin-resistance is usually concomitant with penicilin and aminoglycoside resistance which restricts treatment options [2]. The capacity of enterococci to survive outside the human body for prolonged periods of time contributes to cross-contamination, through either the hands of healthcare workers, equipment, or surfaces, leading to nosocomial infections and outbreaks [4, 5]. VRE is an epidemiologically important pathogen and contact precautions should be implemented in VRE colonized and infected patients [6]. Nosocomial infections are considered to be caused by direct contact with the hands of medical personnel [7].

In the management of outbreaks, rapid and reliable determination of genetic relation between outbreak strains is a very important tool to organize the infection control precautions [8]. Pulsed field gel electrophoresis is the gold standard method to investigate the genetic relation [5, 8]. In this study we aimed to analyze the clonal relation of the VRE outbreak seen at the Neonate Intensive Care Unit (NICU) of Uludag University Hospital.

Materials and methods

Patients

Patients were newborn premature infants, who were being treated at the Neonate Intensive

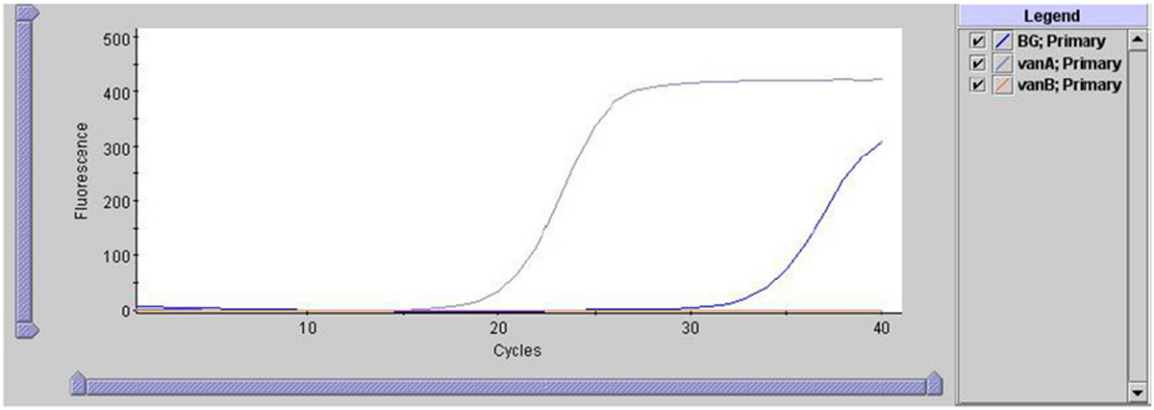

Figure 1. Determination of *vanA* resistance gene in all of the swab samples by Xpert VanA/B test on Cepheid (Cepheid, USA).

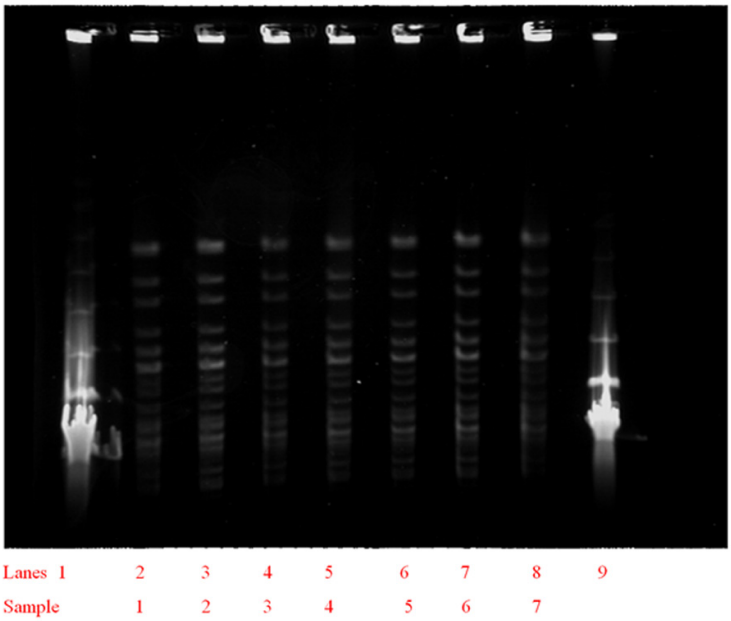

Figure 2. Strain relation according to PFGE (Lane 1and 9: Molecular marker) (PCR 20 bp Low Ladder, Sigma-Aldrich, USA).

Care Unit (NICU) of Uludag University Hospital. Patient 4 and 6 had additonal diseases; patient 4 had a congenital heart disease and, patient 6 had bronchopulmonary dysplasia. Patient 1 was born in a different medical center and referred to our hospital on the 1st day of his life.

Culture samples and Enterococci strains

Routine VRE screening as an infection control policy has been established in our instutition for many years. During our routine screening, we investigated 15 perianal swab samples from the patients at the NICU; we also investigated two blood culture samples, two urine samples and a wound sample from 2 other patients and totally 17 patients were investigated for an outbreak at the NICU. We also investigated four swab samples from three rectal temperature probes. Bacterial identification and antibiotic susceptibility tests were done by BD Phoenix 100 (Becton Dickinson, USA) system. Vancomycin resistance tests were repeated according to Clinical and Laboratory Standards Institute (CLSI) recommendations by Vancomycin E-test [9].

Vancomycin resistance gene detection by real-time PCR

Perianal swabs were analyzed for vancomycin resistance gene by Xpert VanA/B test on Cepheid (Cepheid, USA) according to the manufacturer's instructions. Xpert VanA/B test detects Vancomycin resistance genes transmitted by transposons. These plasmids are usually found in *E. faecium* strains. Cepheid Xpert VanA/B is a qualitative in vitro diagnostic test for rapid detection of Vancomycin resistance genes (The primers and probes used was not given in test manual as it is a commercial secret).

Pulsed field gel electrophoresis (PFGE)

Colonies grown on solid media were transferred to cell suspension buffer including 10 mM Tris-HCl, 50 mM EDTA and 20 mM NaCl and then

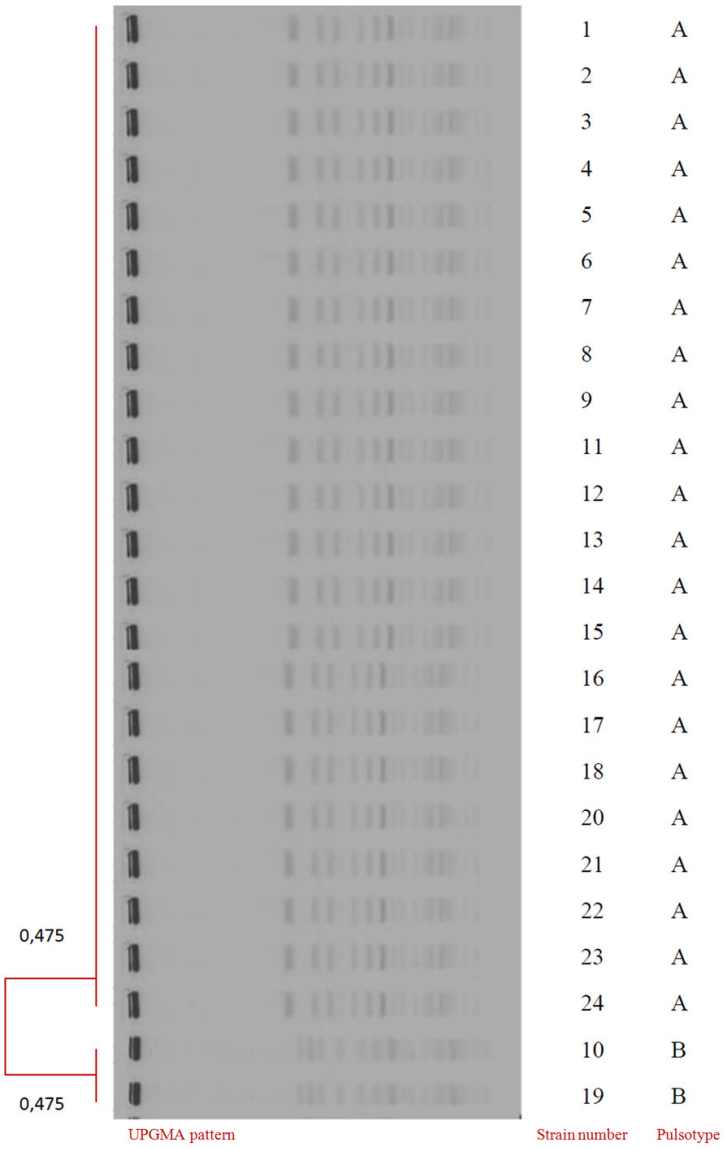

Figure 3. UPGMA patterns of strains; strain numbers and pulsotypes.

centrifuged at 13.000 g at 4°C for two minutes. Pellet was added into 2% low melt agarose and after adding 2 µl lysostaphine (10 mg/ml), it was incubated at 4°C until solidification. Cells in the agarose were lysed by cell lysis solution 1 including 10 mM Tris- HCl, 50 mM NaCl, 50 mM EDTA-0.2% sodyum deoxicholate-0.5% sarkosyl. After lysis agarose was incubated on ice for solidification. Cells in the agarose were lysed by cell lysis solution 2 including 30 µl proteinase K (50 mg/ml) and 5970 µl ES solution (250 mM EDTA, 1% sarkosyl). Agarose molds were washed three times at 50°C for 30 minutes by Tris-HCl- EDTA buffer (10 mM Tris-HCl, 0.1 mM EDTA). Plug molds were divided into two by lancet of which, one was used for PFGE and the other was kept as a spare. Plug molds were incubated at 30°C by SmaI in water bath. Plug molds were loaded on electrophoresis gel. All gels were electrophoresed in 0.5x TBE buffer at 6 V/cm for 20 hours 14°C with a pulse duration of 5.3 to 34.9 seconds ramped linearly in CHEF-DR II system (Bio-Rad Laboratories, Belgium). Gels were stained with etidium bromide, destained in distilled water and photographed with Biometra Gel Documentation Module (Biometra GmBH, Germany). Band profiles were inspected by Bio Doc Analyze Software (Biometra GmBH, Germany). These data were used to produce dendograms showing unwighted pair group method with mathematical averaging (UPGMA) cluster analysis of Dice similarity coefficients produced from pair-wise comparisons of the coded profiles which was taken as 1%. Results were interpreted accoding to Tenover criteria [10].

Results

Enterococci grown in all patient samples were identified as *Enterococcus faecium* by BD Phoenix 100 (Becton Dickinson, USA). All of the strains were resistant to vancomycin, teicoplanin, penicilin, ampicillin and susceptible to daptomycin, linezolid and quinopristin-dalfopristin. We found *vanA* resistance gene in all of the swab samples by Xpert VanA/B test on Cepheid (Cepheid, USA) (**Figure 1**). PFGE band patterns are shown in **Figure 2** and dendograms are shown in **Figure 3**. Dendogram profiles revealed two different strains, of which the majority of them (22/24) had the same clonal origin. The other two strains had the same clonal origin which were isolated from perianal swabs of two different patients. The common clonal origin was also isolated from rectal probes. The results are summarized in **Table 1**. Perianal swab culture positivity was evaluated as colonization but culture growth in two blood

Table 1. Specimen type, number and pulsotype according to patients

Patient/Probe No	Sample No	Specimen	Pulsotype
1	1	PAS	A
2	2	PAS	A
3	3	PAS	A
4	4	PAS	A
4	5	Blood culture	A
5	6	PAS	A
6	7	PAS	A
7	8	PAS	A
7	9	Blood culture	A
8	10	PAS	B
9	11	PAS	A
10	12	PAS	A
10	13	Urine	A
11	14	PAS	A
12	15	Urine	A
13	16	PAS	A
14	17	Wound	A
15	18	PAS	A
16	19	PAS	B
17	20	PAS	A
Probe 1	21	Swab	A
Probe 2	22	Swab	A
Probe 2	23	Swab	A
Probe 3	24	Swab	A

PAS, Perianal swab; A: Pulsotype of common isolate, B: Pulsotype of other isolates.

cultures, two urine cultures and one wound culture was evaluated as infection. These findings revealed the presence of an outbreak which began on Semptember 2013 and ended on December 2013. Infected patients were treated with linezolid. All of the patients survived the outbreak. The first outbreak patient was referred to our hospital from a different healthcare center.

Discussion

This study revealed a close relationship between VRE colonization and VRE symptomatic infections; similar pulsotypes were identified in patients both asymptomatic and clinically manifested VRE. Five patients were infected after colonization. These findings reveal that infection control policies should include active surveillence which should monitor both VRE colonization and VRE infection [11, 12].

Typing of bacterial strains is important in the suspicion of an outbreak and proving the nosocomial spread of infections. Phenotypic and genotypic methods can be used for strain typing, but now molecular methods are being used generally [10, 13, 14]. PFGE, rep-PCR and RFLP methods are the common methods for molecular typing. PFGE is more discriminative than rep-PCR in defining clonal relation. Though PFGE turnaround time is longer than rep-PCR, PFGE costs less than rep-PCR [15]. In this study we used PFGE to fingerprint DNA of VRE. DNA was digested by *Sma*I restriction enzyme and divided into 10 to 20 pieces differing from 10 to 800 kilobase pairs which was electrophoresed by iso-electric focusing [13, 14, 16].

Infection control precautions could not be implemented until the time passed during isolation, identification and reporting of the first strain, which resulted in spread of VRE to other neonates and led to an outbreak. As the first strain was imported from a diffent center, strict infection control precautins could be implemented for a time, as there was not a VRE colonization in our NICU. The probes which were shown to be colonized with the same VRE strain played an important role in the outbreak besides the hands of the health care staff. Though all the strains were phenotypically showed concordant, PFGE revealed genetical discrepancy between the strains. It was thought that, phenotypical methods are not able to determine genetical discrepancies. That's why many laboratories are increasingly using molecular techniques, as they offer higher sensitivity and faster turnaround time than traditional culture methods [17, 18].

Multiple approaches were used to curtail the outbreak. As per usual all VRE infected or colonized patients were subjected to control precautions, enhanced infection control and prevention measures were undertaken including the use of personel protective equipment, strategies to increase adherence to hand hygiene recommendations, no more new patient was imported to the neonatal unit and none of the patients were exported from neonatal unit to other clinics during outbreak, education about infection control precautions was given to all workers and rectal probes were treated by disinfectants for a longer duration and higher concentration [17, 19, 20]. After the

treatment of the last outbreak patient and no more VRE growth in culture plates, it was thought that the outbreak was terminated.

Implementation of molecular methods have an important role in the management and analysis of outbreaks. PFGE, which may be used in outbreaks has a high correlation with epidemiological data and can be used as the gold standard molecular method. PFGE usage is restricted because of the facts that; PFGE is labor intensive, needs authorized personnel and it needs an expensive device.

During an outbreak, determining the genetic relation between outbreak strains which can be done in the microbiology laboratory as an infection control precaution is necessary. Molecular microbiologic analysis of outbreak strains may prove the epidemiologic and evolution of outbreak strains.

Disclosure of conflict of interest

None.

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