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RESEARCH ARTICLE

Surveillance of Vancomycin-resistant *Enterococci* colonization with GeneXpert *vanA/vanB* test and culture method

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ABSTRACT

Objectives: Early detection of vancomycin resistant *enterococci* (VRE) colonization in hospitalized patients plays an important role in controlling enterococcal infections. For this purpose, the detection of VRE from rectal swab specimens can be performed with various methods.

Methods: In this study, VRE colonization in the rectal swab samples of patients from intensive care units, burn unit, and infectious diseases clinic in our hospital were evaluated. In total, 2,394 rectal swab samples from 1,627 patients were collected between January 2012 and 2013. A commercial product (Enterococcosel agar) was used for cultivating, and the growing bacteria were identified with conventional methods and/or the VITEK 2 Compact system (BioMerieux, France). The samples of the patients transferred from another hospital and who had a history of VRE infection or carriage were examined with an automated real-time PCR system (Gene Xpert™ *vanA*/*vanB*, Cepheid, Sunnyvale, CA).

Results: A total of 59 (3.6%) patients were positive for VRE, 25 were diagnosed with PCR, and 34 were diagnosed with culture methods. Among the samples identified by PCR, 19 were only *vanA* harboring VRE, five were only *vanB* harboring VRE, and one was both *vanA* and *vanB* harboring VRE. All of the VRE isolates were identified as *E. faecium*. The number of patients administered with vancomycin therapy before being diagnosed with VRE was 11(18.6%).

Conclusions: It is important to determine the appropriate surveillance method for the early diagnosis of VRE carriers among patients at risk according to their patient profile, needs, and resources. *J Microbiol Infect Dis 2014; 4(3): 97-101*

Key words: Vancomycin-resistant *enterococci*, *vanA*, *vanB*, Gene Xpert™

GeneXpert *vanA/vanB* testi ve kültür metodu ile Vankomisin dirençli Enterokok kolonizasyonunun sürveyansı

ÖZET

Amaç: Hastanede yatan hastalarda vankomisin dirençli enterokok (VRE) kolonizasyonunun erken tespiti, enterokok enfeksiyonlarının kontrolünde önemli bir yer tutmaktadır. Bu amaçla rektal sürüntü örneklerinde çeşitli yöntemlerle VRE tespiti yapılabilmektedir.

Yöntemler: Bu çalışmada hastanemizde Ocak 2012-2013 tarihleri arasında, yoğun bakım ünitelerinde, yanık ve enfeksiyon hastalıkları kliniklerinde yatmakta olan 1627 hastaya ait 2394 rektal sürüntü örneğinde VRE kolonizasyonu araştırıldı. Bu amaçla kültür için Enterokokosel agar kullanıldı. Üreyen bakteriler konvansiyonel yöntemler ve/veya VITEK 2 Compact (BioMerieux, Fransa) ticari sistemi kullanılarak tür düzeyinde tanımlandı. Başka bir hastaneden nakil gelen ve daha önce VRE enfeksiyonu veya taşıyıcılığı öyküsü olanlarda rektal örnekler otomatize gerçek zamanlı polimeraz zincir reaksiyonu (Realtime PCR) cihazı (Gene Xpert™ *vanA*/*vanB*, Cepheid, Sunnyvale, CA) ile incelendi.

Bulgular: Toplam 59 hastada VRE pozitifliği (%3,6) saptandı. Bunların 25'i PCR, 34'ü ise kültür ile çalışıldı. PCR ile tanı konulanlardan 19'u *vanA* pozitif, 5'i *vanB* pozitif, biri ise *vanA+vanB* pozitif olarak saptandı. Vankomisin dirençli enterokok olarak belirlenen tüm suşlar *E. faecium* olarak tanımlandı. Hastalardan 11(%18.6)'nın VRE tanısı öncesinde tedavisinde vankomisin kullandığı saptandı.

Sonuç: Riskli hastalarda VRE taşıyıcılığını erken dönemde saptayabilmek için her hastanenin kendi hasta profiline, ihtiyaçlarına ve kaynaklarına göre uygun sürveyans yöntemini belirlemesi gerektiğini düşünmekteyiz.

Anahtar kelimeler: Vankomisine dirençli enterokok, *vanA*, *vanB*, Gene Xpert™.

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INTRODUCTION

Vancomycin-resistant *enterococci* (VRE) are important pathogens causing nosocomial epidemics and an increase in mortality and morbidity, especially in intensive care units. It is important to monitor infection in patients colonized with VRE and to prevent the spread of the infection to other patients.[1] One of the most important components of a successful VRE control program is the detection of gastrointestinal colonization. This is due to the fact that the most important VRE reservoirs are the patients that carry VRE in their gastrointestinal systems.[2] Thus, obtaining perirectal swab samples at regular intervals is the gold standard in the determination of VRE.[3] The current study aimed the retrospective evaluation of VRE colonization in rectal swab samples that were obtained from the patients who were hospitalized in intensive care units (ICUs), the Burn Unit and Infectious Disease Clinics of our hospital within the scope of surveillance.

METHODS

Patients, units, schedule of taking samples

Surveillance was conducted in all ICUs in the hospital in order to diagnose patients colonized with VRE by taking rectal swab samples not only from regular patients, but also from newly hospitalized patients once a month during their hospitalization period. In ICUs in which VRE colonization was detected, weekly surveys of VRE were conducted. When all collected perirectal cultures were found to be negative successively for four weeks, this procedure was terminated and surveillance was conducted once a month instead of per week. If a VRE positive patient was transferred from an ICU to a clinic, the weekly surveillance continued in his/her clinic room and the abovementioned procedure was performed in the same way. Although routine VRE surveillance is generally not conducted in Burn Units and Infectious Disease clinics within our hospital, this study also covers these units, as the patients were transferred to these clinics after having been diagnosed with VRE upon their hospitalization in ICUs.

A total of 2,394 rectal swab samples taken from 1,627 patients who were hospitalized in the ICUs, Burn Unit, and Infectious Disease clinic between January 2012 and January 2013 were included in the study. The samples were transported to the laboratory in Amies transport medium. The study was carried out in the Infectious Disease and Clinical Microbiology laboratory. The rectal swabs that were taken for monthly surveys were studied using culture methods.

Laboratory methods

The rectal swab samples taken from patients who were transported from another hospital or patients with VRE infection in their medical history or carriers of VRE were studied using a multiplex real-time PCR (Gene Xpert™ *vanA*/*vanB*, Cepheid, Sunnyvale, CA) device. After mixing the sample with the reagent according to the recommendations of the manufacturer, the rectal swab samples for PCR study were vortexed for 1 minute. This mixture was added to a single-use only cartridge. The cartridge was placed in the device. The Gene Xpert™ Dx module was selected and executed. The results were reported as *vanA* and *vanB* positive or negative. The results that were *vanB* positive were studied again according to the recommendations of the manufacturer. The samples that were evaluated as *vanA* and *vanB* positive were confirmed with cultures.

For culture, the samples were cultivated in BBL enterococcosel agar (Becton Dickinson, USA) in which 6μg/ml vancomycin and 1 μg/ml meropenem were added. They were incubated in aerobic conditions at 37°C for 72 hours. The bacterial growth was controlled daily. Esculin-positive black colored colonies were tested with gram staining, catalase, and growth tests in the medium containing 6.5% NaCl.

The pure cultures of the colonies that were gram-positive, catalase negative, hydrolyzed esculin, and grew in a medium containing 6.5% NaCl were sub-cultured on 5% sheep blood agar. The growing bacteria were defined in species level by using conventional methods and VITEK 2 Compact (BioMerieux, France) system. The resistance of strains that were detected as vancomycin-resistant by VITEK-2 was confirmed by using vancomycin and teicoplanin E-test in accordance with the recommendations of CLSI.[4] The repeated rectal swab samples, as part of the weekly survey, of the patients that were found to be VRE positive with PCR were studied with culture again.

RESULTS

A total of 2,394 rectal swab samples taken from 1,627 patients between January 2012 and January 2013 were included in the study. Of all of the samples, 437 (18.2%) were samples taken from patients hospitalized in the neonatal ICU, 349 (14.6%) were in the pediatric ICU, 453 (18.9%) were in the

anesthesiology ICU, 464 (19.4%) were in the neurosurgical and reanimation ICU, 324 (13.5%) were in the neurology ICU, 338 (14.1%) were in internal medicine ICU, 22 (0.9%) were in the burn unit, and 7 (0.2%) were those in the infectious diseases departments.

Vancomycin resistant *enterococci* were detected in 124 of a total of 2394 rectal samples. The repeated positive results detected in the same patient were eliminated and then a total of 59 patients samples that were VRE positive (3.6%) were evaluated. When the epidemiological data of the VRE-positive patients were evaluated, it was determined that six were children and 53 were adults. Thirty-six (61%) of the patients were female and 23 (39%) were male. The mean age of adult patients was 64.6 years (range: 30-94 years) and the mean age of the pediatric patients was 1.5 years (range: 1-2 years). It was found that 11 (18.6%) patients used vancomycin treatment prior to the diagnosis of VRE.

Table 1. The distribution of culture and PCR positivities of rectal swab samples according to the departments.

Unity	n (%)	Culture(+)*	PCR (+)*	Total
NSICU	464 (19.4)	1	8	9
ARICU	453 (18.9)	15	6	22
NBICU	437 (18.2)	2	1	3
PICU	349 (14.6)	-	1	1
NICU	324 (13.5)	3	5	8
IMICU	338 (14.1)	13	-	13
Burn Unit	22 (0.9)	-	1	1
Infectious diseases	7 (0.3)	-	3	3
TOTAL	2394	34	25	59

n: Number of rectal swab samples; *Only one of the repeated culture/PCR positivities for each patient was evaluated; NSICU: Neurosurgical ICU; ARICU: Anesthesiology and Reanimation ICU; NBICU: Neonatal ICU; PICU: Pediatric ICU; NICU: Neurology ICU; IMICU: Internal Medicine ICU

It was determined that out of total 59 patients, 18 (30.5%) had neurological disorders (central nervous system disorders, such as cerebrovascular disease, epilepsy, or hydrocephalus), 13 (22%) had nephrologic diseases (chronic renal failure, acute renal failure), seven (11.8%) had congestive heart failure, eight (13.5%) had respiratory system diseases (pneumonia, chronic obstructive pulmonary disease), and four (6.7%) had orthopedic problems (traffic accident, femur fracture, or cervical disc pathologies), two (3.3%) had acute abdomen, and nine (15.2%) had other diseases (ovarian carcinoma, suicide, fever of unknown origin, or gastrointestinal hemorrhage).

Vancomycin resistant *enterococci* positivity was detected with PCR in 25 (42.3%) patients and with cultures in 34 (57.6%) patients out of 59 patients. Among the samples that the diagnosis was done with PCR, one was *vanA+vanB* positive, five were *vanB* positive, and 19 were *vanA* positive. All VRE positive strains were defined as *E. faecium*.

The VRE positivity of rectal swab samples that was detected by culture and PCR is presented in Table 1.

DISCUSSION

Surveillance studies are very important in the detection of VRE colonization. In our hospital, routine surveillance is performed in ICUs for the early detection of patients that are colonized with VRE and for immediate implementation of the necessary contact isolation precautions.

When VRE colonization was detected, weekly surveys of VRE began. When all of the perirectal cultures that were taken consecutively for four weeks, become negative, surveillance studies were conducted once a month.[5]

In previous studies, it was mentioned that the risk factors for VRE colonization were linked with long hospitalization process, medical history of hospitalization in intensive care, dialysis and transplantation units, renal failure, enteral feeding, hematological malignancies, neutropenia, antibiotic use (especially cephalosporins, antibiotics effective on anaerobes, quinolone, and vancomycin), contact with VRE positive patients, or the health care personnel who attend to these patients, and medical instruments contaminated with VRE.[6-9] In the current study, there were risk factors consistent with this data. Most of the patients were hospitalized in the ICU, acute or chronic renal failures were present in 22% of the patients. There was a history of vancomycin use before the diagnosis of VRE in 18.6% of cases. Different from the previous studies, 30.5% of the patients in the present study had neurological diseases.

In a study that was conducted on 2,115 patients in the USA, the rate of VRE colonization was found to be 4.7%, and in another study[11] on 1,362 patients

this value was found to be 10%. VRE colonization was detected at a rate of 2%[12] in 624 patients in Holland, and at a rate of 3.5%[13] in 636 patients in Belgium. In the studies from Turkey, Ergani Özcan et al.[14] reported the VRE colonization rate as 1.5% in 2,488 rectal swab samples, whereas Aygün et al.[15] reported this rate as 1.9% in 467 patients. In the current study, the rate of VRE colonization in our hospital was found to be 3.6%. Although this rate is lower than the data in the United States, it is found to be consistent with Europe and Turkey.

The resistance of *enterococci* to glycopeptide antibiotics was first reported by Uttley et al.[16] in 1988. There are six types of resistance genes among *enterococci*: A, B, C, D, E, and G. While the strains with the *vanA* phenotype had high levels of resistance to vancomycin and teicoplanin, those with the *vanB* phenotype were moderately or highly resistant to vancomycin and were sensitive to teicoplanin.[17] The resistance in both phenotypes is *transferrable* to other species and inducible. In studies that were conducted with human-induced *enterococci* in Turkey, the most frequently detected resistance phenotype was *vanA*.[14,18-20] In the current study, the researchers also detected the *vanA* phenotype most frequently (76%). Although *vanB* resistance is rarely seen in Turkey, it was first detected by Coşkun et al.[21] in an *E. faecium* strain. In the current study, the positivity of *vanA* and *vanB* together was seen in one (1.69%) strain and *vanB* positivity was detected in five (8.4%) strains. Some studies have shown that some anaerobic bacteria other than *enterococci* (such as *Clostridium bolteae, C. hathewayi, C. innocuum-like, C. lavalense, C. symbiosum, Eggerthella lenta,* and *Ruminococcus lactaris-like, C. clostridioforme* and *Atopobium minutum*) may also cause *vanB* positivity in rectal samples.[22-26] Thus, it is important to confirm the results with the cultures in strains that were detected as only *vanB* positive. In the current study, the samples that were detected as *vanB* positive were examined with PCR again and the positive samples were confirmed with cultures.

VRE positivity was detected with PCR at a rate of 42.3% and 57.6% was detected with cultures. In the current study, as PCR and culture methods were not used together in the same sample, the superiority of the two methods in the detection of VRE could not be compared.

The use of the PCR method in the detection of glycopeptide resistance was first defined by Dutka-Malen et al.[27] in 1995. Today the use of PCR in clinical microbiology laboratories for the detection of VRE has gradually increased. The detection of VRE with culture takes 2-4 days. There are advantages of PCR over culture, such as saving time required for the detection of VRE and providing the application of control interventions in a shorter time. Gene Xpert™ is a real-time device in which the lysis, extraction, amplification, and detection procedures are performed by using a single-use only cartridge.[28] The time to obtain results takes 45 minutes and provides the opportunity to take immediate precautions after isolation. In a study by Gazin et al.[29] in which they compared different PCR methods, they found that the Gene Xpert™ device is effective in the detection of VRE in low concentrations such as 10-100 CFU/ml. In another study, the sensitivity, specificity, positive predictive value, and negative predictive value of this device were reported as 96.4%, 93%, 92%, and 96.9%, respectively.[30] The PCR method is a favored method, as it yields the results within a short period of time. However, this method is costly and it was applied to patient groups who were transferred from another hospital or who had a previous medical history of VRE infection or who were carriers.

Recently, acquired antibiotic resistance has been observed in *enterococci*. It is important to detect VRE colonization early in hospitalized patients to control infections. The current study detected 42.3% of VRE colonization with PCR and 57.6% with the culture method. However, the PCR and culture methods were not applied together, as this study did not aim to compare the two methods. In conclusion, each hospital should determine the appropriate surveillance method according to their patient profile, needs, and the sources for the early detection of VRE porters in high risk patients.

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All authors declare that they have no conflict of interest.

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