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Diagnostic Comparison of PCR, Chromogenic Agar and Culture for *Vancomycin-Resistant Enterococci* in Intensive Care Units

Yoğun Bakım Ünitelerinde Vankomisine Dirençli Enterokokların Tanısında PCR, Kromojenik Agar ve Kültür Yöntemlerinin Karşılaştırılması

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ABSTRACT

Objective: This study aimed to compare the diagnostic performance and cost-effectiveness of classical culture, chromogenic agar, and the Smart-Cycle I-CORE real-time polymerase chain reaction (PCR) method for detecting *vancomycin-resistant enterococci (VRE)* in intensive care unit (ICU) patient and environmental samples.

Methods: In a prospective surveillance design conducted in adult medical, surgical, and general ICUs, perianal/rectal swab samples from patients hospitalized ≥48 hours and high-touch environmental surface samples were obtained. Each specimen was tested in parallel using classical culture, chromogenic agar, and real-time PCR targeting vanA/vanB. Sensitivity, specificity, positive and negative predictive values (PPV/NPV), turnaround time, and per-test costs were calculated.

Results: In patient samples, PCR, chromogenic agar, and culture achieved sensitivity/specificity of 100%/100%, 100%/80%, and 95.2%/86.4%, respectively. In environmental samples, PCR showed 100%/100%, chromogenic agar 100%/92%, and culture 94%/100%, respectively. PCR provided a markedly shorter time-to-result (~4 h), compared with chromogenic agar (~24 h) and classical culture (~48 h). vanA was the predominant genotype (≈82%), followed by vanB (≈18%). Although PCR was the most costly method, its rapid turnaround time contributed to earlier isolation decisions and to a reduction in environmental positivity rates.

Conclusion: Smart-Cycle I-CORE PCR offers the highest diagnostic accuracy and the fastest reporting among currently available surveillance methods, while chromogenic agar represents a reliable and cost-effective option. A two-step strategy—chromogenic agar for

ÖZ

Amaç: Bu araştırmada, yoğun bakım ünitesi (YBÜ) hasta ve çevresel örneklerinde *vankomisin dirençli enterokok (VRE)* saptanmasında klasik kültür, kromojenik agar ve Smart-Cycle I-CORE gerçek zamanlı polimeraz zincir reaksiyonu (PZR) yöntemlerinin tanısal performans ve maliyet etkinliklerinin karşılaştırılması amaçlanmıştır.

Yöntemler: Prospektif tasarımla dahili, cerrahi ve genel YBÜ'lerde ≥48 saat yatan yetişkin hastalardan perianal/rektal sürüntü örnekleri ile sık temaslı yüzeylerden çevresel örnekler alınmıştır. Tüm örnekler klasik kültür, kromojenik agar ve vanA/vanB genlerini hedefleyen gerçek zamanlı PZR ile eşzamanlı olarak çalışılmıştır. Duyarlılık, özgüllük, pozitif ve negatif öngörü değerleri ile sonuç alma süresi ve test başı maliyet hesaplanmıştır.

Bulgular: Hasta örneklerinde PZR'nin duyarlılığı ve özgüllüğü %100/%100, kromojenik agarın %100/%80, klasik kültürün %95,2/%86,4 olarak bulunmuştur. Çevresel örneklerde ise PZR %100/%100, kromojenik agar %100/%92, klasik kültür %94/%100 doğruluk göstermiştir. Sonuç alma süresi PZR'de ~4 saat, kromojenik agarda ~24 saat ve klasik kültürde ~48 saat olarak saptanmıştır. En sık saptanan genotip vanA (%82) olup bunu vanB (%18) izlemiştir. PZR, maliyet açısından en yüksek olmakla birlikte hızlı sonuç vermesi nedeniyle erken izolasyon kararlarına katkı sağlamıştır.

Sonuç: Smart-Cycle I-CORE PZR yöntemi, *VRE* sürveyansında en yüksek tanısal doğruluk ve en kısa sonuç süresini sağlamaktadır. Kromojenik agar ise tarama için güvenilir ve maliyet açısından uygun bir alternatiftir. "Kromojenik agar ile tarama, PZR ile doğrulama"

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ABSTRACT

routine screening with PCR confirmation—balances accuracy, speed, and cost in ICU *VRE* surveillance.

Keywords: *Vancomycin-resistant enterococci*, real-time PCR, chromogenic agar, surveillance, intensive care units

ÖZ

yaklaşımı, yoğun bakım koşullarında hız, doğruluk ve maliyet dengesi açısından önerilebilir.

Anahtar Sözcükler: *Vankomisin dirençli enterokok*, gerçek zamanlı PZR, kromojenik agar, sürveyans, yoğun bakım üniteleri

INTRODUCTION

Vancomycin-resistant enterococci (VRE) have emerged as important healthcare-associated pathogens since their first description in the late 1980s. Their ability to acquire and transfer resistance determinants, survive for prolonged periods on inanimate surfaces, and rapidly disseminate under the selective pressure of broad-spectrum antibiotic use has led to endemic circulation particularly in intensive care units (ICUs) (1-3).

Enterococci are Gram-positive cocci commonly found in the gastrointestinal microbiota. *Enterococcus faecalis (E. faecalis)* and *Enterococcus faecium (E. faecium)* represent the most clinically relevant species and may persist on medical equipment and environmental surfaces, thereby contributing to nosocomial transmission. Of particular concern is the increasing vancomycin resistance observed predominantly among *E. faecium* isolates in recent years, posing a significant public health challenge worldwide (4,5).

Rapid and accurate detection of *VRE* colonization is essential for timely implementation of infection control measures, including patient isolation and environmental decontamination, to prevent hospital outbreaks. Conventional culture-based methods remain widely available and relatively inexpensive; however, they require prolonged incubation periods that may delay infection control interventions. Chromogenic agar has been introduced as a more rapid screening method allowing presumptive identification of *VRE* colonies based on color differentiation, although its diagnostic performance may vary depending on laboratory conditions. In contrast, molecular assays such as real-time polymerase chain reaction (PCR) targeting resistance genes including vanA and vanB provide high sensitivity and specificity and can substantially reduce diagnostic turnaround time (6,7).

Although the molecular epidemiology of *VRE* has evolved over the past decade, the fundamental diagnostic strategies used for *VRE* detection—culture-based screening, chromogenic agar, and PCR—remain central components of infection control programs in many healthcare settings. The data used in the present study were obtained from a prospective surveillance program conducted in the ICUs of a tertiary-care university hospital. Therefore, the primary aim of this study was to compare the diagnostic performance, turnaround time, and relative cost of classical culture, chromogenic agar, and Smart-Cycle I-CORE real-time PCR for detecting *VRE* in patient and environmental samples, to provide evidence supporting the optimization of surveillance strategies in critical care environments.

MATERIALS AND METHODS

Study Design and Ethical Approval

This prospective diagnostic validation study was conducted in the ICUs of a tertiary-care university hospital to compare classical culture, chromogenic agar, and Smart-Cycle I-CORE real-time PCR for *VRE* surveillance. The study protocol was approved by the Mersin University Clinical Research Ethics Committee (approval number: 2008/91, date: 17/10/2008) and was carried out in accordance with the Declaration of Helsinki. Written informed consent was waived because the study did not include identifiable patient data.

Setting and Sample Collection

The study was performed in the medical, surgical, and general ICUs of Mersin University Medical Faculty Hospital. Adult patients hospitalized for ≥48 hours were eligible. During the study period, 210 patient samples and 185 environmental samples were collected. Environmental swabs were obtained from high-touch surfaces in rooms of *VRE*-positive or suspected patients (e.g., bed rails, monitor keypads, nightstand surfaces).

Specimen Sampling and Transport

Patient samples consisted of perianal or rectal specimens collected with sterile flocked swabs and transported in appropriate media according to manufacturer's recommendations. Environmental sampling was performed with sterile swabs applied to frequently touched surfaces under similar transport conditions. Initial screening was conducted 48 hours after ICU admission. Weekly follow-up samples were obtained from *VRE*-positive patients. All specimens were processed within two hours of receipt.

Diagnostic Procedures

Classical culture, chromogenic agar culture, and real-time PCR were performed simultaneously on each specimen.

Classical Culture: Enterococcus-selective media (e.g., Enterococcosel/BEA) and 6.5% NaCl media were inoculated and incubated at 37 °C for 24–48 h. Identification was performed using Gram staining, catalase testing, and PYR testing. Vancomycin susceptibility was assessed phenotypically according to CLSI criteria.

ChromID *VRE* chromogenic agar (bioMérieux, France) was inoculated and incubated at 37 °C for 24 h. Color differentiation provided presumptive identification (pink–red colonies suggestive of *E. faecium*; blue–green colonies suggestive of *E. faecalis*). Presumptive *VRE* isolates were confirmed using molecular testing.

Real-time PCR was performed using the Smart-Cycle I-CORE Real-Time PCR System (Cepheid, USA) to detect the *vanA* and *vanB* genes. DNA extraction and amplification followed the manufacturer's instructions. Cycle threshold (Ct) ≤35 was interpreted as positive. Positive and negative controls were included in each run.

Quality Control and Internal Validation

Quality control included *E. faecium* ATCC 51559 (vanA positive) and *E. faecalis* ATCC 29212 (vancomycin susceptible). At least one positive and one negative control were included per assay run. Runs

with invalid controls were repeated. Daily calibration and weekly maintenance of instruments were documented.

Cost Analysis

Direct diagnostic costs were calculated per test based on institutional cost components, including reagents, consumables, personnel time (processing, analysis, reporting), and instrument utilization (energy and service allocation). Costs were expressed in Turkish Lira (”). All diagnostic methods were compared using identical unit prices.

Statistical Analysis

Statistical analysis was performed using SPSS version 25 (IBM Corp., USA). χ^2 or Fisher’s exact tests were applied to compare categorical variables. Agreement between methods was assessed using Pearson correlation analysis. Diagnostic performance metrics (sensitivity, specificity, PPV, NPV) were calculated using standard definitions. A p-value <0.05 was considered statistically significant.

RESULTS

Between May and October 2010, a total of 420 ICU patients were screened for *VRE* colonization using perianal swab specimens. Of these, 268 (63.8%) were male and 152 (36.2%) were female. *VRE* colonization was detected in 21 patients (5.0%), including 11 males (52.4%) and 10 females (47.6%). The median age of colonized patients was 56.1 ± 5.07 years (range, 30–80). Mean age was 61.8 ± 12.3 years in males and 49.0 ± 30.8 years in females.

Intensive Care Unit Distribution and Clinical Characteristics

Among all screened patients, 146 (34.7%) were admitted to medical ICUs, 137 (32.6%) to surgical ICUs, and 137 (32.6%) to general ICUs. Of the 21 *VRE*-positive cases, 3 (14.3%) were identified in medical ICUs, 9 (42.9%) in surgical ICUs, and 9 (42.9%) in general ICUs.

Chronic comorbidities were present in 18 patients (85.7%); 3 patients (14.3%) had no comorbidities. Of these, at least two comorbidities were identified in 7 patients (33.3%). Diabetes mellitus was the most frequent underlying disease (33.3%), followed by solid organ malignancies (23.8%) and cardiovascular diseases (19.0%).

Total parenteral nutrition was administered to 18 patients (85.7%), and enteral nutrition was used concomitantly in 14 patients (66.7%). Previous surgery was recorded in 16 patients (76.2%), most commonly gastrointestinal procedures (42.8%), followed by genitourinary procedures (19.0%), cranial procedures (9.5%), and thoracic procedures (4.0%).

Central venous catheterization was documented in 20 patients (95.2%) at the time of *VRE* detection. Diarrhea was present in 6 patients (28.6%). Immunosuppression was observed in 9 patients (42.9%).

Within the preceding three months, 10 patients (47.6%) had been hospitalized and 4 (19.0%) had stayed in an ICU. The mean ICU length of stay among *VRE*-positive patients was 17.4 ± 11.0 days (range, 6–50) (Table 1).

Table 1. Demographic and clinical characteristics of *VRE*-positive patients (n = 21).

Parameter	n (%)	Details / mean ± SD
Age (years)	–	56.1 ± 5.07 (range, 30–80)
Sex (male/female)	11 (52.4) / 10 (47.6)	–
ICU type	–	Medical: 3 (14.3%), surgical: 9 (42.9%), general: 9 (42.9%)
Total patients screened	420	–
VRE positivity	21 (5.0)	–
Comorbidities	18 (85.7)	DM: 7 (33.3%), malignancy: 5 (23.8%), cardiovascular: 4 (19.0)
Total parenteral nutrition	18 (85.7)	–
Enteral feeding	14 (66.7)	–
Previous surgery	16 (76.2)	GI: 9 (42.8%), GU: 4 (19%), cranial: 2 (9.5%), Thoracic: 1 (4%)
Central venous catheter	20 (95.2)	–
Diarrhea	6 (28.6)	–
Immunosuppression	9 (42.9)	–
Previous hospitalization (last 3 months)	10 (47.6)	Prior ICU stay: 4 (19.0)
ICU length of stay (days)	–	17.4 ± 11.0 (range, 6–50)
Common infection types*	–	Pneumonia (n = 11), surgical site (n = 9), bacteremia (n = 8), UTI (n = 3)
VRE species	–	*E. faecium* 81%, *E. faecalis* 19%
Environmental positivity	21/113 (18.3)	Bed: 9, nightstand: 5, monitor/pump: 5, Cart: 2

*Infection types listed in this table (e.g., pneumonia, surgical site infection, bacteremia, and urinary tract infection) represent the primary clinical conditions leading to ICU admission and are not necessarily infections caused by *VRE*.
GI: Gastrointestinal surgery, GU: Genitourinary surgery, ICU: Intensive care unit, DM: Diabetes mellitus, *VRE: Vancomycin-resistant enterococci, SD: Standard deviation.*

Antibiotic Use

All *VRE*-positive patients were receiving parenteral antimicrobial therapy at the time of detection. The most frequently administered agents were carbapenems (19%), carbapenem plus glycopeptide combinations (23.8%), ampicillin–sulbactam (9.5%), carbapenem plus aminoglycoside combinations (9.5%),and ampicillin–sulbactam plus metronidazole (9.5%), whereas glycopeptide monotherapy was used in 9.5% of cases. Among patients receiving glycopeptides, six (85.7%) were treated with teicoplanin and one (14.3%) was treated with vancomycin. Antimicrobial treatment indications included bacteremia (n = 8), urinary tract infection, and other suspected or confirmed infections.

E-test results showed that all isolates exhibited high-level vancomycin resistance (MIC >256 µg/mL), while teicoplanin resistance was observed in 75% of isolates (MIC >16 µg/mL).

Environmental Samples

Among 113 environmental samples obtained from rooms of *VRE*-positive patients, 21 (18.3%) yielded *VRE*. Environmental colonization persisted during follow-up in 14 patients and lasted for a mean of 7 ± 5.4 days (range, 7–21). No environmental contamination was detected at the end of the study. The most frequently contaminated surfaces were bed rails (n = 9), nightstands (n = 5), and monitor/pump surfaces (n = 5), followed by clinical carts (n = 2).

Only one patient with perianal *VRE* colonization developed clinical infection; therefore, separate risk factor analyses for colonization versus infection could not be performed. Environmental positivity was not significantly associated with the duration of colonization (p = 0.6). The infection types listed in Table 1 (e.g., pneumonia, surgical site infection, bacteremia, and urinary tract infection) represent the primary clinical diagnoses leading to ICU admission, rather than infections caused by *VRE*.

Table 2. Distribution of *VRE* species.

Species	n	%
E. faecalis	4	19.0
E. faecium	17	81.0
Total	21	100.0

E. faecium: Enterococcus faecium, *E. faecalis: Enterococcus faecalis, VRE: Vancomycin-resistant enterococci.*

Species Distribution and Antimicrobial Susceptibility

Of the 21 *VRE* isolates, 81% were *E. faecium* and 19% were *E. faecalis* (Table 2). All isolates demonstrated high-level gentamicin resistance.

Diagnostic Performance of the Methods

In environmental samples, the turnaround time of PCR was 1 hour, compared with 38.8 ± 6.6 hours for Enterococcosel agar and 24.5 ± 5.9 hours for chromogenic agar. No significant difference was observed between Enterococcosel agar and chromogenic agar for environmental specimens (p > 0.05).

In patient samples, the turnaround time of Enterococcosel agar was 60 ± 4.0 hours, whereas chromogenic agar required a significantly shorter duration of 26.8 ± 3.2 hours (p < 0.038).

When PCR was considered the reference standard, patient samples yielded sensitivities, specificities, PPVs, and NPVs of 100% for PCR; 95.2%, 84.6%, 92%, and 96%, respectively, for Enterococcosel agar; and 100%, 80%, 94%, and 100%, respectively, for chromogenic agar (Table 3).

In environmental samples, with PCR as the reference standard, Enterococcosel agar showed a sensitivity of 94%, specificity of 100%, PPV of 100%, and NPV of 80%, whereas chromogenic agar showed a sensitivity of 100%, specificity of 92%, PPV of 87%, and NPV of 100% (Table 4).

Cost Analysis

The average direct diagnostic cost per test was calculated to be 181 ” for PCR, 113.15 ” for chromogenic agar, and 182.4 ” for classical culture. Although PCR had a higher direct cost per test than chromogenic agar, it provided substantially faster results. This shorter turnaround time may facilitate earlier infection control interventions such as patient isolation and environmental decontamination. From a practical perspective, chromogenic agar may serve as an effective screening method due to its lower cost, whereas PCR may be used as a confirmatory test when rapid and highly accurate detection is required.

Table 3. Diagnostic performance and cost per patient sample.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cost (”/sample)
PCR	100	100	100	100	181
Enterococcosel agar	95.2	86.4	92	96	182.4
Chromogenic agar	100	80	94	100	113.15

PCR: Polymerase chain reaction, PPV: Positive predictive value, NPV: Negative predictive value.

Table 4. Diagnostic performance and cost per environmental sample.

Method	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Cost (”/sample)
PCR	100	100	100	100	181
Enterococcosel agar	94	100	100	80	182.4
Chromogenic agar	100	92	87	100	113.15

PCR: Polymerase chain reaction, PPV: Positive predictive value, NPV: Negative predictive value.

DISCUSSION

This study compared the diagnostic performance, turnaround time, and cost of three commonly used modalities—classical culture, chromogenic agar, and Smart-Cycle I-CORE real-time PCR—for detecting *VRE* colonization in ICUs. Our findings demonstrated that PCR achieved the highest diagnostic accuracy, whereas chromogenic agar emerged as a practical and cost-effective alternative for routine surveillance due to its ease of interpretation and lower implementation costs.

An important consideration when interpreting the findings of this study is the time period during which the data were collected. Although the surveillance was conducted in 2010, the diagnostic approaches evaluated in this study—classical culture, chromogenic agar, and PCR targeting the *vanA/vanB* genes—remain fundamental tools in current clinical microbiology laboratories. Therefore, the results primarily reflect differences in diagnostic performance and turnaround time rather than temporal changes in *VRE* epidemiology.

E. faecium and *E. faecalis* are well-recognized causes of healthcare-associated infections, particularly among critically ill patients with risk factors such as broad-spectrum antibiotic exposure, invasive procedures, and immunosuppression (5-8). The predominance of *VRE* in the medical and surgical ICUs of our cohort is consistent with existing epidemiological trends, and the higher freq*uency* of *E. faecium* isolates is in line with increasingly reported resistance profiles in the literature.

Classical culture remains widely accessible and inexpensive; however, its prolonged incubation period, often exceeding 48 hours, may delay the implementation of infection control interventions. Chromogenic agar offers more rapid presumptive identification based on color differentiation and can be used as an efficient rule-out tool given its high negative predictive value (9,10). In the present study, chromogenic agar performed adequately as a screening method, representing a feasible option for large-scale surveillance.

Real-time PCR provides the major advantage of directly detecting of *vanA* and *vanB* genes, enabling rapid isolation measures. Previous studies have shown that molecular-based algorithms reduce diagnostic turnaround time from 24–48 hours to 3–5 hours compared with conventional culture, contributing to earlier interruption of the transmission chain (11-13). In the current study, PCR consistently returned results within 4 hours, facilitating patient isolation within approximately 6 hours, thereby supporting timely infection prevention efforts.

Environmental persistence of *VRE* on dry surfaces for prolonged durations highlights the importance of environmental decontamination in prevention strategies (14-16). In our investigation, the environmental positivity rate decreased from 6.7% to 1.2% after the implementation of environmental and isolation precautions, indicating the effectiveness of targeted cleaning interventions.

Although PCR was more expensive than chromogenic agar, it provided substantially faster results (17-19). Consequently, a two-step diagnostic approach—initial screening by chromogenic agar followed by PCR confirmation—may offer an optimal balance between cost and diagnostic reliability, depending on laboratory capacity and workload.

The present findings align with the 2023 guideline by the Turkish Ministry of Health on the prevention of *VRE*, which emphasizes early diagnosis, effective isolation, and meticulous environmental decontamination as key components in controlling endemic *VRE* transmission.

Study Limitations

This study has several limitations, including its single-center design and a relatively small number of isolates. Nevertheless, our results provide important insights into the applicability and cost-effectiveness of molecular diagnostic methods in ICU settings in Türkiye. Larger multicenter studies are warranted to further validate these findings and guide national infection control strategies.

This study has several limitations. First, it was conducted in a single tertiary-care center with a relatively small number of isolates, which may limit the generalizability of the findings. Second, the differentiation between colonization and infection could not be fully evaluated because of the small number of cases of infection. Third, molecular characterization was limited to detection of vanA and vanB, without further genotypic analysis. Therefore, multicenter prospective studies are required to validate our results and better define the epidemiological characteristics of *VRE* circulation in intensive care settings. Another limitation of this study is that the dataset was collected in 2010. Although the fundamental diagnostic methods remain unchanged, the molecular epidemiology of *VRE* may have evolved over time.

CONCLUSION

This study presents a comparative analysis of three diagnostic methods used for the detection of *VRE* in ICUs. Our findings indicate that the Smart-Cycle I-CORE PCR system provides the highest diagnostic accuracy and the shortest turnaround time, whereas chromogenic agar represents an appropriate and practical option for active surveillance due to its lower cost and ease of use.

Early detection, which enabling which enables the rapid implementation of isolation measures, offers a major advantage in preventing nosocomial transmission. Given the high mortality risk predominantly associated with *E. faecium* strains, rapid diagnosis and appropriate isolation remain essential components of infection control strategies.

Although PCR appears more expensive in the short term, it has the potential to reduce additional costs associated with delayed isolation in the long run. Therefore, a two-step diagnostic strategy—screening by chromogenic agar followed by PCR confirmation—may provide an optimal balance among speed, accuracy, and cost, depending on institutional resources.

The selection of diagnostic strategies that ensure an appropriate balance between diagnostic accuracy, rapidity, and cost-effectiveness is crucial for infection prevention, patient safety, and effective resource management in intensive care settings.

Ethics

Ethics Committee Approval: The study protocol was approved by the Mersin University Clinical Research Ethics Committee (approval number: 2008/91, date: 17/10/2008) and was carried out in accordance with the principles of the Declaration of Helsinki.

Informed Consent: Written informed consent was waived because the study was observational and did not involve any identifiable personal data.

Footnotes

Authorship Contributions

Concept: M.U., G.E., Design: M.U., G.E., B.Ç.D., N.M.E., Data Collection or Processing: M.U., G.E., Analysis or Interpretation: M.U., G.E., B.Ç.D., N.M.E., Literature Search: M.U., G.E., B.Ç.D., N.M.E., Writing: M.U., G.E., B.Ç.D., N.M.E.

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