Int J Clin Exp Pathol 2014;7(9):6274-6277
www.ijcep.com /ISSN:1936-2625/IJCEP0001457

Brief Communication

Liofilchem® Chromatic VRE and vancomycin MIC Test Strip detected glycopeptide resistance in a *van*B neonatal *Enterococcus faecium* isolate showing alternate vancomycin susceptibility and resistance with bioMérieux Vitek2

Vincenzo Savini[1], Roberta Marrollo[1], Eleonora Coclite[2], Paola Fusilli[2], Carmine D'Incecco[2], Paolo Fazii[1], Giovanni Gherardi[3]

[1]Clinical Microbiology and Virology, Spirito Santo Hospital, Pescara (Pe), Italy; [2]Neonatal Intensive Care Unit and Neonatal Pathology, Spirito Santo Hospital, Pescara (PE), Italy; [3]Integrated Research Centre, University Campus Biomedico, Rome (RM), Italy

Received July 18, 2014; Accepted August 23, 2014; Epub August 15, 2014; Published September 1, 2014

Abstract: A 1-month old neonate urine sample yielded *van*B *Enterococcus faecium*; nevertheless, the isolate alternatively showed susceptibility and resistance to vancomycin with bioMérieux Vitek2 (cards AST592, AST632, AST586), while glycopeptide resistance was detected by Liofilchem® vancomycin MIC Test Strip and disc along with the Chromatic VRE chromogenic medium. This communication emphasizes that, as *van*B gene may be heterogeneously expressed within a given *Enterococcus* population, glycopeptide resistance may be missed when using automated systems for antibiotic susceptibility testing. We suggest therefore that vancomycin *in vitro* activity be studied on all clinical isolates through agar methods, including use of chromogenic media.

Keywords: *Enterococcus faecium*, *van*B, vancomycin, glycopeptide, neonate, newborn

A 1-month old male child with fever (37.5°C) underwent urine culture that yielded rare *Enterococcus*-like α-hemolytic colonies. Although considered to be of no clinical relevance, the strain was identified to the species level and screened for antimicrobial resistance with the purpose to detect *van* elements, if any [1-3].

Particularly, the strain showed group D antigen agglutination that was detected by both the Oxoid streptococcal grouping kit (Thermo Fisher, USA) and the Strep-Check kit (Liofilchem®, Italy) and identification as *Enterococcus faecium* was achieved by Vitek2 GP card (bioMérieux, France) and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) using Bruker Biotyper software 2.0 (Bruker Daltonics, Germany) as well as by sequencing of a 16S rRNA gene 900-bp amplicon (100% identity to the *E. faecium* strain KF358453.1 was obtained). Antibiotic susceptibility testing performed with Vitek2 AST592 card (along with cards AST632 and AST586) showed the strain to be alternatively vancomycin susceptible or resistant, with MIC (Minimum Inhibitory Concentration) values ranging between ≤ 0.5 and 8 μg/mL. Disc test (Liofilchem®) for vancomycin produced a 19 mm inhibition zone diameter, that was in the susceptibility range according to the European Committee for Antibiotic Susceptibility Testing [EUCAST] 2014 guidelines. Nevertheless, a fuzzy zone edge otherwise microcolonies within the inhibition area were observed (**Figures 1**, **2**), meaning resistance to the glycopeptide (based on EUCAST criteria). Accordingly, vancomycin MIC Test Strip (MTS-Liofilchem®) provided a 2 μg/mL MIC value at 24 h incubation (in the susceptibility range based on EUCAST), but microcolonies grew inside the elliptic inhibition zone, clearly confirming resistance (**Figure 3**). Accordingly, the isolate formed blue colonies on Liofilchem® Chromatic VRE (**Figure 4**), a chro-

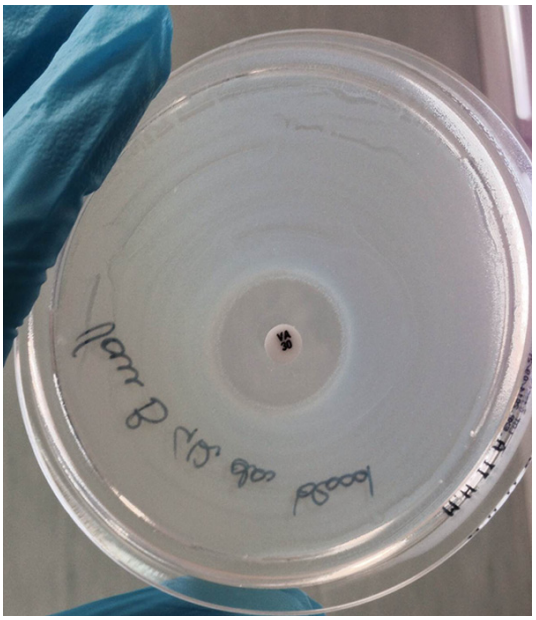

Figure 1. Fuzzy zone edge obtained with vancomycin (Liofilchem®) on Mueller Hinton II agar (Liofilchem®).

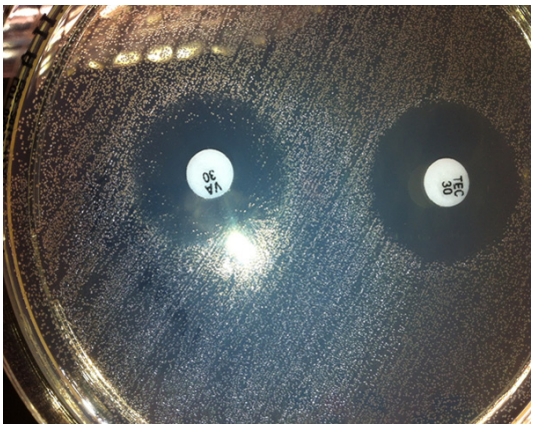

Figure 2. Microcolonies within the vancomycin (VA disc) halo - microcolonies are absent in the teicoplanin (TE disc) inhibition zone (discs and Mueller Hinton II agar are provided by Liofilchem®).

mogenic medium where such a color indicates glycopeptide resistant *E. faecium.* As a confirmation of vancomycin resistance, a real-time polymerase chain reaction (PCR) targeting the *van*A and *van*B genes [2, 3] was performed with the Xpert *van*A/*van*B Assay on a GeneXpert® Instrument system (Cepheid, US) that detected the *van*B element [3].

Enterococci inhabit gut of humans and animals and are among the major agents of hospital-acquired infections [2, 3]. Recently, the wide

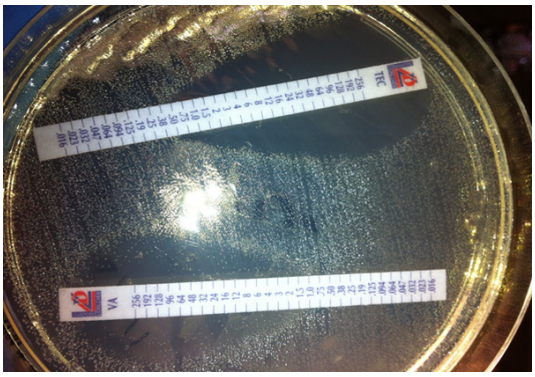

Figure 3. Microcolonies within the vancomycin (VA strip) halo - microcolonies are absent in the teicoplanin (TE strip) inhibition zone (strips and Mueller Hinton II agar by Liofilchem®).

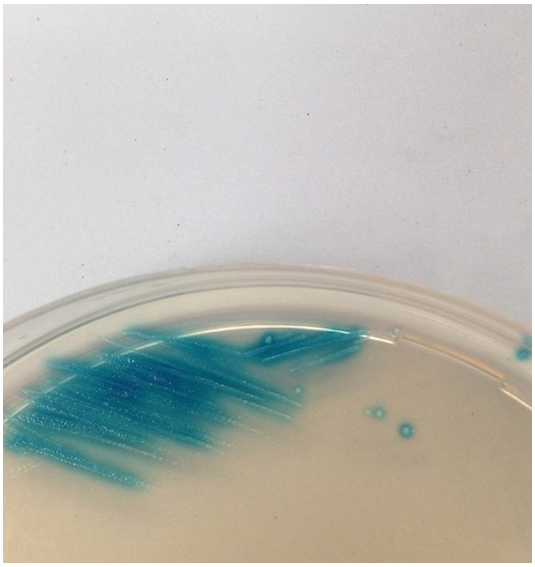

Figure 4. Colonies of the studied isolate on Liofilchem® Chromatic VRE medium.

use of antibiotics and immunosoppressive treatments along with the increasing use of invasive therapeutic procedures, have brought these organisms, notably *E. faecium,* to gain emerging clinical relevance [2]. *Enterococcus* has been known to be intrinsically resistant to cephalosporins, and is considered not to respond to aminoglycosides, under treatment. In fact, even though these classes of drugs may display *in vitro* activity, they are expected to fail *in vivo* [2]. Again, multidrug resistance has been widely reported within the genus and, as antimicrobial resistance varies among *Enterococcus* species, precise identification is necessary for clinical strains, as it supports selec-

tion of proper antibiotics [2]. In particular, a significantly higher prevalence of resistance to penicillin, ampicillin, rifampicin, ciprofloxacin, levofloxacin, fosfomycin, erythromycin and nitrofurantoin is found in *E. faecium* rather than *Enterococcus faecalis*, whereas that to chloramphenicol, quinupristin/dalfopristin, minocycline and tetracycline is mostly observed in the second (quinupristin/dalfopristin, in particular, is intrinsically poorly effective versus *E. faecalis*) [2]. Furthermore, a low prevalence of linezolid, vancomycin and teicoplanin resistance in both species is reported, globally, and the mentioned three compounds are then currently and widely used for treatment [2]. Resistance to glycopeptides is however described and mainly due to alteration of the peptidoglycan precursors, which leads to drug failure in inhibiting the cell wall synthesis [2, 3]. The *van*A gene may be transferred to *Staphylococcus aureus*, too, therefore care is needed when vancomycin is administered to manage enterococci [2].

Detection of the underlying gene for a resistance trait is important in the laboratory practice, although unfortunately molecular methodologies usually require skilled operators and are time-consuming and expensive, then not suitable for the routine activity. Based on the manufacturer's instructions and indications, the Cepheid Xpert *van*A/*van*B Assay is a qualitative *in vitro* diagnostic test designed for fast detection of vancomycin-*van*A/*van*B genes directly from rectal and perianal swabs; the assay is in fact particularly dedicated to patients at risk for gut colonization by glycopeptide-resistant enterococci (mostly vancomycin resistant entercocci [VRE]) through an automated real-time PCR targeting the mentioned genes. As the spread of VRE is through contact with colonized or infected individuals within healthcare facilities, and given the need of identifying and isolating their carriers, the test is mostly intended to screen patients by perianal or rectal swabs at admission, once a week after admission, after antibiotics receipt and, finally, upon discharge.

GeneXpert Instrument Systems automate and integrate sample purification, nucleic acid amplification, and target detection sequence in simple or complex samples by using real-time PCR, that therefore, and interestingly, needs no particularly skilled operators. Agar methodologies, such as Liofilchem® discs, MTS and Chromatic VRE, are however cost-effctive and reliable and permit an accurate diagnostics of *Enterococcus* glycopeptide resistance. In particular, it is crucial to evaluate not only disc-generated inhibition zone diameters and MICs produced by MTS, but to look for microcolonies and/or a fuzzy zone edge, if present, based on EUCAST indications.

Again, we suggest to screen all clinical isolates through the chromogenic medium and agar-based assays, as it emerges from this communication that, due to both inducibility of *van*B gene and its potentially heterogeneous expression (along with variability of phenotypically observed *van*B-mediated vancomycin resistance), this trait may not be detected by Vitek2; with automated systems, in fact, it is likley that the bacterial population that displays the resistance trait may go underecognized.

Moreover, it should be kept in mind that the Cepheid Assay is designed to detect *van*A/B genes only; nevertheless, *van*A/B is exclusively harboured by *E. faecalis* (*van*A/B), *E. faecium* (*van*A/B) and *Enterococcus durans* (*van*A) [3]; again, *E. faecium* and *E. faecalis* may carry *van*D (*E. faecium* and *E. faecalis*), *van*E (*E. faecalis*), *van*G (*E. faecalis*) and *van*M (*E. faecium*) genes, that may confer glycopeptide resistance, but are missed with GeneXpert® [3]; then, working with enterococcal species other than those mentioned or with *E. faecalis/E. faecium* carrying glycopeptide resistance traits other than *van*A/B would provide negative results with this system, in spite of the harboured genetic element potentially encoding resistance.

Enterococci have been considered in the past to cause endogenous infections; later, it became clear that they could commonly spread through colonized healthcare operators. Therefore, VRE-carrying patients, even healthy, should be carefully recognized as they may behave as reservoirs for vancomycin and teicoplanin resistance [3]. Finally, resistance may be expressed by a restricted number of bacterial cells within a given microbial population (the phenomenon is called heteroresistance), therefore it might not be observed with Vitek2, as was in this case, and it is required that agar methods be routinely carried out to support a reliable detection of glycopeptide resistance among clinical enterococci.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Vincenzo Savini, Clinical Microbiology and Virology, *Spirito Santo* Hospital, via Fonte Romana 8, CAP 65124, Pescara (Pe), Italy. Tel: 0039 340-7379737; E-mail: vincenzo_savini@libero.it

References

[1] Birgand G, Ruimy R, Schwarzinger M, Lolom I, Bendjelloul G, Houhou N, Armand-Lefevre L, Andremont A, Yazdanpanah Y, Lucet JC. Rapid detection of glycopeptide resistant enterococci: impact on decision-making and costs. Antimicrob Resist Infect Control 2013; 2: 30.

[2] Jia W, Li G, Wang W. Prevalence and antimicrobial resistance of *Enterococcus* species: a hospital-based study in China. Int J Environ Res Public Health 2014; 11: 3424-3442.

[3] Savini V, Gherardi G, Astolfi D, Polilli E, Dicuonzo G, D'Amario C, Fazii P, D'Antonio D. Insights into airway infections by enterococci: a review. Recent Pat Antiinfect Drug Discov 2012; 7: 36-44.