Antimicrobial Susceptibility testing of *Enterococcus* spp. in a VanB-VRE outbreak setting.

P. Christoffer Lindemann[1], Torunn S. Haukeland[1], Helge Kolstad[1], Bjørg C. Haldorsen[2], Kristin Hegstad[2, 3]

1: Department of Microbiology, Haukeland University Hospital, Bergen, Norway
2: Norwegian National Advisory Unit on Detection of Antimicrobial Resistance (K-res), Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway
3: Research Group of Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, University of Tromsø -The Artic University of Norway, Tromsø, Norway.

Background

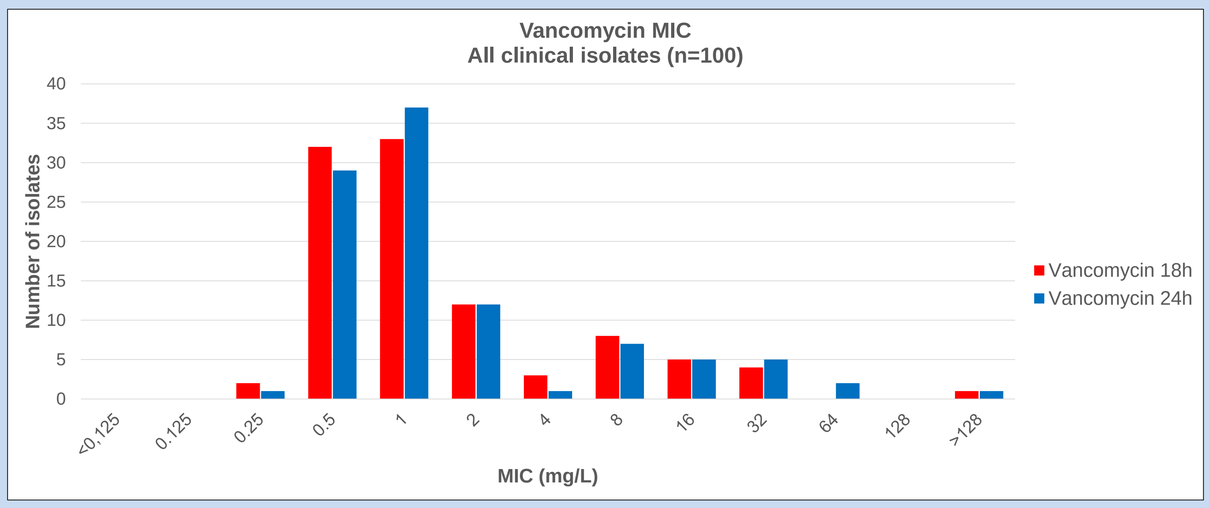
Vancomycin resistant enterococci (VRE) continues to challenge laboratories and health care institutions worldwide. Low-MIC *vanB* isolates are especially difficult to detect using phenotypic methods. EUCAST recently issued a warning against gradient tests underestimating vancomycin MIC in low-level resistant strains, and routine laboratories are recommended to use more laborious methods, like broth micro dilution (BMD). At the Department of Microbiology at Haukeland University Hospital (Bergen, Norway), we wanted to validate the new ComASP™ test from Liofilchem® for use in routine diagnostics. An ongoing outbreak of *vanB* VRE at our hospital makes a challenging setting for phenotypic antimicrobial susceptibility testing.

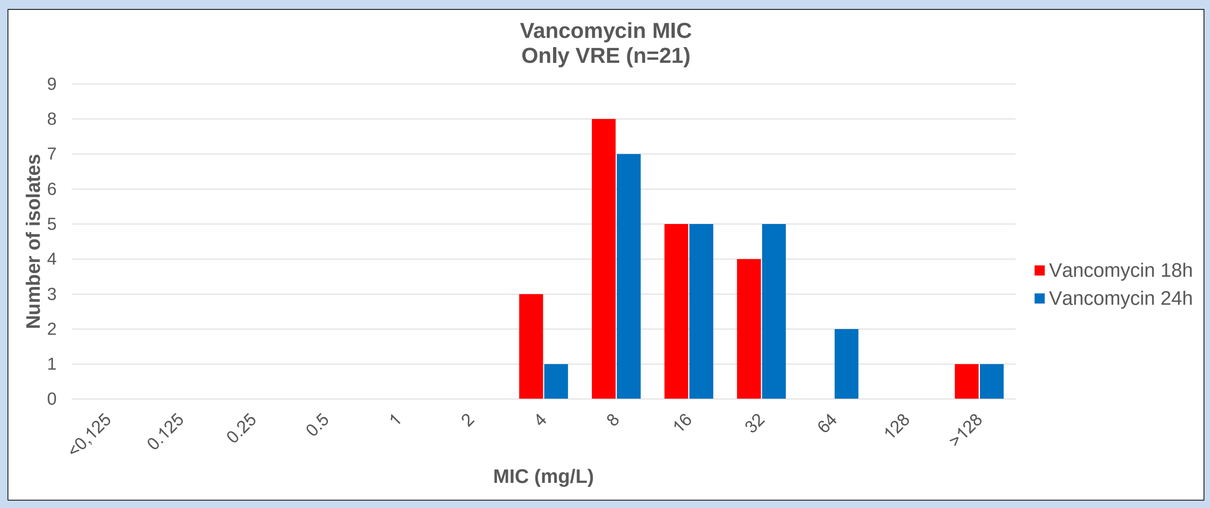
Materials & Methods

Two collections were included:

1) A challenging panel of thirteen clinical isolates with *vanA* (n=1) or *vanB* (n=12), four clinical isolates without acquired glycopeptide resistance and two quality control strains (ATCC 29212 and ATCC 51299-*vanB)* was used to compare ComASP™ to standard BMD.
2) One hundred isolates of *Enterococcus spp*. from routine diagnostics and VRE-screening were included to compare ComASP™ to established phenotypic and genotypic methods. 21 of these isolates were VRE (20 *vanB*, 1 *vanA*).

All isolates were screened for vancomycin resistance using Brain Heart Infusion (BHI) agar screen with 6 mg/L vancomycin. The ComASP™ tests were set up according to the manufacturer and additionally read after 24 hours incubation. Standard BMD was done at the Norwegian reference laboratory (K-res). All isolates were subjected to *vanA/B*-PCR.



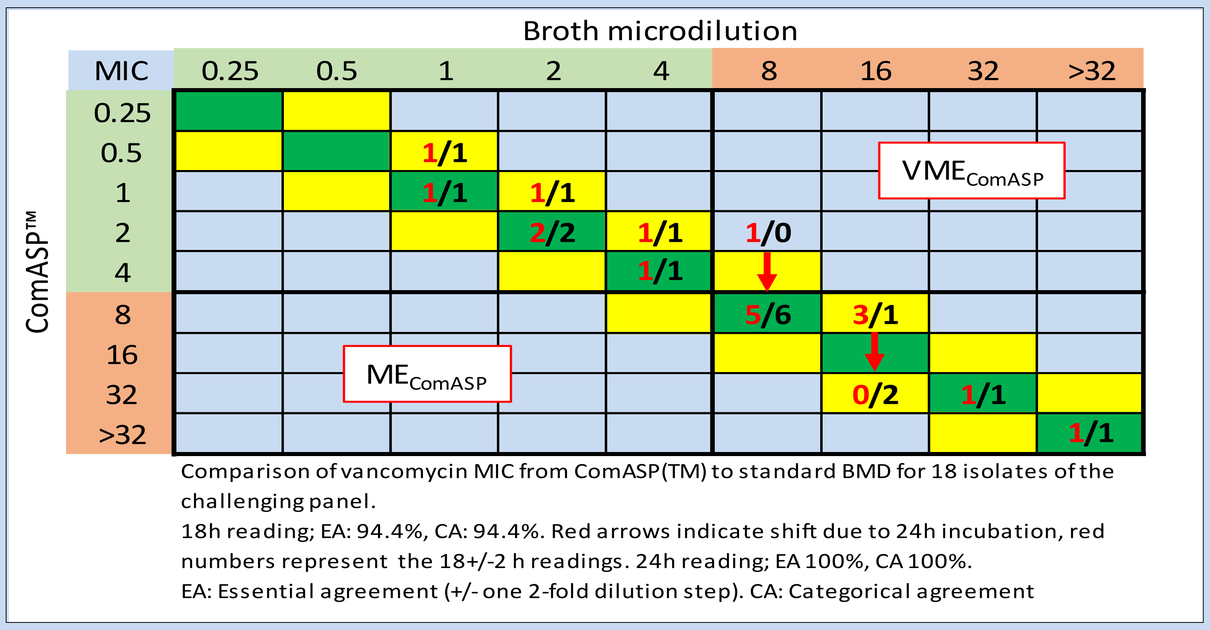


Results

For the challenging collection, 94.7% were correctly categorised according to the expected phenotype using agar screen. The essential and categorical agreements (EA/CA) of the ComASP™ test compared to standard BMD were 94.4% (24h reading). CA was only 84.2% for 18+/- 2h reading.

For the 100 clinical isolates, concordance was perfect across methods when ComASP™ was read after 24 hours. However, one *vanB*-positive isolate with very scarse growth on the BHI-screening agar, had repeated MICs of 4 mg/L.

When comparing 18h and 24h reading of the ComASP™ vancomycin MIC, EA was 99.0% for all clinical isolates, and 95.2% (20/21) for the VRE isolates. CA was 90.5% for the VRE isolates, and 100% for the non-VRE isolates.



Comparison of vancomycin MIC from ComASP(TM) to standard BMD for 18 isolates of the challenging panel.
18h reading; EA: 94.4%, CA: 94.4%. Red arrows indicate shift due to 24h incubation, red numbers represent the 18+/-2 h readings. 24h reading; EA 100%, CA 100%.
EA: Essential agreement (+/- one 2-fold dilution step). CA: Categorical agreement

Conclusions

Agar screen and ComASP™ are reliable methods for detecting vancomycin resistance in enterococci. Both are suitable for use in a routine laboratory.

Vancomycin MIC from ComASP™ should be read after 24 hours.

This study also supports the need for genetic tests for *vanA/B* to ensure all VRE isolates are detected.

Acknowledgements

Liofilchem is acknowledged for their kind donation of ComASP™ for this project.

HELSE BERGEN
Haukeland University Hospital

paul.christoffer.lindemann@helse-bergen.no

THE ARCTIC UNIVERSITY OF NORWAY • UiT •

NASJONAL KOMPETANSETJENESTE
for påvisning av antibiotikaresistens