

A new reliable screening method for the evaluation of VISA and hVISA strains by "Vancomycin-Teicoplanin MIC Test Strip" (VTMTS)

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Background

Staphylococcus aureus with reduced susceptibility to vancomycin have been isolated with increasing frequency since 1997 (Hiramatsu et al. 1997) and have generally arisen in methicillin-resistant strains. These strains, known as vancomycin-intermediate *S.aureus* – VISA, have been reported from Japan, the United States and Europe. Although these isolates, with homogeneous resistance to vancomycin (MICs ≥ 4 mg/L), continue to be rare, there are increasing reports of strains showing heteroresistance (hVISA), often with vancomycin MICs in the 1-2 mg/L range (Campanile et al. 2010; Appelbaum P.C. 2007; Wootton M. et al., 2007), and comparing different methods of detection (van Hal S.J. April 2011; Satola S. Jan. 2011). Heteroresistance to glycopeptides, in which subpopulations with reduced susceptibility (approximately 10^{-6}) coexists in a seemingly susceptible phenotype, have been associated with clinical failure (Charles PG. et al, 2004; Sakoulas G. et al 2004).

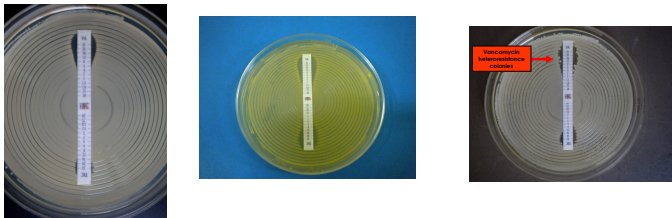
Methods

The sample consisted of 23 MRSA strains already characterised as hVISA and VISA by population analysis (PAP/AUC), belonging to the main HA-MRSA and CA-MRSA clones diffused in Italy. Mu50 and NRS403 (VISA), Mu3 and NRS22 (hVISA), ATCC 29213 (VSSA) and 9 clinical VSSA, were also included as control strains.

Vancomycin-Teicoplanin MIC Test Strip (VTMTS) consists of a double-sided gradient strip of VA 32-0.5 mg/L and TP 32-0.5 mg/L (Liofilchem srl, Italy). The screening was performed in BHI plates (BD, Diagnostic Systems), and tested in parallel with the GRD Etest (AB bioMérieux, France), and compared with macrodilution method Etest (MET). Sensitivity and specificity were calculated using PAP analysis as golden-standard (Table 1).

The interpretive MIC cutoffs used at 24 and 48h were used according to the international guidelines (CLSI 2010; EAS 003).

Figure 1: VTMTS test and detection of heteroresistant sub-populations



Objectives

Heteroresistance to glycopeptides, in which subpopulations with reduced susceptibility coexist in a seemingly susceptible phenotype, have been recently associated with clinical failure.

Our objective was the validation of an alternative phenotypic test to identify VISA and hVISA strains in parallel with other standardized methods already described and confirmed by population analysis (PAP/AUC) considered the "gold-standard".

Table 1: Sensitivity and specificity values for the different methods in determining VISA/hVISA strains

| METHOD | PAP VISA/hVISA N°/27 | % SENS | PAP-VSSA N°/10 | % SPEC |
|-------------------|----------------------|--------|----------------|--------|
| Macro Etest (MET) | 20 | 74.0% | 10 | 100% |
| GRD | 14 | 51.85% | 10 | 100% |
| VTMTS | 22 | 81.48% | 10 | 100% |

| Strain | Code | Antibiogram | Quality Control | Ref. (n°) |
|------------|------------|-------------|-----------------|-----------|
| ATCC 29213 | ATCC 29213 | ATCC 29213 | ATCC 29213 | 10 |
| ATCC 10541 | ATCC 10541 | ATCC 10541 | ATCC 10541 | 10 |
| ATCC 10688 | ATCC 10688 | ATCC 10688 | ATCC 10688 | 10 |

* Refer to MIC Test Strip Technical Sheet (CSE - hVISA)

Table 2: hVISA stratification of strains compared with MIC values distribution for vancomycin and teicoplanin.

| Vancomycin Range 0.5 - 8 mg/L | N° strains | MIC BMC | | | | |
|-------------------------------|------------|---------|----|---|---|---|
| | | 0.50 | 1 | 2 | 4 | 8 |
| GRD Etest | 37 | 7 | 19 | 7 | 2 | 2 |
| VTMTS | 14 | 0 | 8 | 3 | 1 | 2 |
| Macro Etest | 22 | 2 | 11 | 3 | 2 | 2 |
| PAP | 27 | 4 | 13 | 6 | 2 | 2 |

| Teicoplanin Range 0.12 - 8 mg/L | N° strains | MIC BMC | | | | | | | | |
|---------------------------------|------------|---------|------|------|---|---|----|---|----|----|
| | | 0.12 | 0.25 | 0.50 | 1 | 2 | 4 | 8 | 16 | 32 |
| GRD Etest | 37 | 2 | 2 | 14 | 6 | 2 | 10 | 3 | 0 | 0 |
| VTMTS | 14 | 0 | 0 | 3 | 0 | 0 | 5 | 3 | 0 | 0 |
| Macro Etest | 20 | 0 | 0 | 8 | 0 | 2 | 9 | 3 | 0 | 0 |
| PAP | 27 | 0 | 0 | 10 | 3 | 2 | 9 | 3 | 0 | 0 |

MICs for vancomycin and teicoplanin were determined according to CLSI guidelines (CLSI, 2010).

Results and conclusions

In this study we validated a new phenotypic test for the screening of VISA and hVISA strains. It was analyzed in parallel with other standardized methods already described, and confirmed by population analysis (PAP/AUC) considered the "gold-standard".

VTMTS was able to define 81.48% (n° 22/27) hVISA and VISA strains (table 1), confirming to be a reliable test, correlating well with the PAP analysis. All VSSA were also confirmed negative. The test was replicated three times and by two different operators and was found to be highly reproducible.

Our results demonstrated that the new VTMTS method is a good, reproducible and easy-to-use method for detection of heteroresistance in *Staphylococcus aureus*, and an efficient alternative for clinical and epidemiological purposes.

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