

# REDUCED $\delta$ -HEMOLYSIN ACTIVITY AND AGR-EXPRESSION CORRELATE WITH AN hVISA/VISA PHENOTYPE

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## INTRODUCTION

hVISA and VISA are a serious public health problem due to the difficulties in the treatment of their infections. Nowadays different, but no easy and too long, screening methods are in use to correctly identify these strains.

## AIM OF THE STUDY

Our study was directed to evaluate a **simple and rapid hVISA and VISA screening method**, based on the  $\delta$ -haemolysin production, to correctly discriminate VSSA, hVISA and VISA.

## METHODS

19 clinical hVISA, 5 clinical NARSA VISA, 8 VSSA (previously assigned by PAP-AUC analysis) of different agr-genotypes, and three prototype microorganisms, i.e. NRS149 (VSSA-agrII), Mu3 (h-VISA-agrII), Mu50 (VISA-agrII) were screened for their  $\delta$ -hemolysin production.

This assay was evaluated, after 24h of incubation at 37°C, on different media i.e. 5% sheep-blood-agar with Columbia (COL), Muller-Hinton (MH), Trypticase soy agar (TSA) medium base manufactured by Oxoid (OX), bioMerieux (BM), Becton Dickinson (BD) and 5% sheep-blood-agar COL added with 6 mg/L vancomycin manufactured by Liofilchem® (LC).

To co-validate our phenotypic data, we also analyzed the *hld* mRNA amount (delta-hemolysin-encoding gene) in the prototype microorganisms by quantitative relative real time RT-PCR.

The expression results were shown as the increment/decrement (folds) of GISA towards VSSA as calibrator and *gyrB*, housekeeping gene, was used as normalizer.

Three to five distinct biological replicates were done and statistical expression analyses were performed using the relative expression software tool REST2009.

Results was presented as means  $\pm$  standard deviations (SDs) and statistical significance was assumed at *P* values of  $\leq 0.05$ .

## TABLES AND FIGURES

Tab.1

SAMPLE	OX COL	BM COL	BD COL	OX MH	BM MH	OX TSA	BM TSA	BD TSA	LC COL+ 6mg/L VAN
VSSA control strain NRS149	++	++	++	+++	+++	+	+	++	++
Clinical VSSA (8)	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8	8/8
GISA (24)									
hVISA control strain MU3	-/+	-/+	-/+	++	++	-/+	-/+	-	-
Clinical hVISA	17/19	15/19	15/19	13/19	12/19	16/19	14/19	14/19	17/19
VISA control strain MU50	-	-	-	-	-	-	-	-	-
NARSA Clinical VISA	4/5	4/5	4/5	4/5	4/5	4/5	4/5	4/5	5/5
TOT. GISA correctly detected	21/24	19/24	19/24	17/24	16/24	20/24	18/24	18/24	22/24
SENSITIVITY (%)	87.5	79.16	79.16	70.8	66.6	83.3	75	75	91.6
SPECIFICITY (%)	100	100	100	100	100	100	100	100	100

### LEGEND

OX COL: 5% sheep blood agar with Columbia by Oxoid  
BM COL: 5% sheep blood agar with Columbia by bioMerieux  
BD COL: 5% sheep blood agar with Columbia by Becton Dickinson  
OX MH: 5% sheep blood agar with Muller Hinton by Oxoid  
BM TSA: 5% sheep blood agar with Trypticase soy by bioMerieux  
BD TSA: 5% sheep blood agar with Trypticase soy by Becton Dickinson  
LC COL+ VAN: 5% sheep blood agar with Columbia added with 6 mg/L VAN by Liofilchem®

Tab.2

Method	Sensitivity (%)	Specificity (%)
LC COL+6mg/L VAN	91.6	100
OX COL	87.5	100
Macro E test (2 McF) *	75.0	100

\* Campanile F et al. Int J Antimicrob Agents. 2010 Nov;36(5):415-9.

Tab. 3

Strains	Fold Change	SE	P(H1)	Result
MU50vsNRS149	0.004	0.003-0.005	0.048	DOWN
MU3vsNRS149	0.025	0.022-0.029	0.000	DOWN
MU50vsMU3	0.163	0.130-0.203	0.000	DOWN

Fig.1

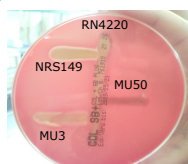
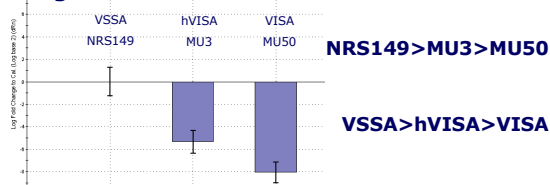


Fig.2



## CONCLUSIONS

Our phenotypic and molecular data suggest the use of the  $\delta$ -hemolysis assay on **Liofilchem® (LC) COL+ 6 mg/L VAN sheep blood agar as easy and rapid screening method to accurately detect hVISA and VISA**

## RESULTS

### Phenotypic assays:

i) In all used media, Mu50 (VISA) showed no  $\delta$ -hemolysis zone (-) whereas NRS149 (VSSA) presented a large one (one or more +). Regardless of the medium-manufacturing company (OX, BM, BD), Mu3 (hVISA) exhibited a low  $\delta$ -hemolysis evidenced by a small zone in COL and TSA blood agar (-/+), but not in MH (++) (Tab.1 - Fig.1).

ii) The addition of 6 mg/L vancomycin in the COL medium resolves discrepancies giving, in most cases, a definitive result in GISA isolates.

**COL base and the presence of 6 mg/L vancomycin rendered clear and easily readable  $\delta$ -hemolysis zones leading to an accurate detection of hVISA and VISA**

iii) In tested media, comparing the  $\delta$ -hemolysin zone of 32 clinical MRSA with those of control strains, and consequently establishing the number of strains that correctly categorized in VSSA, hVISA and VISA as in PAP-analysis, we calculated the media sensitivity (%) and specificity (%) (Tab.2).

**Liofilchem® (LC) COL+ 6 mg/L VAN sheep blood agar showed the highest sensitivity (91.6%) followed by OX COL sheep blood agar (87.5%)**

### Molecular analysis:

The phenotypic data correlated with molecular ones where a gradual and substantial *hld* down-regulation was found in the prototypes as follows: **VSSA < hVISA < VISA** (Tab.3 - Fig.2).