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Exploring the resistance mechanism of heterogeneous vancomycin-intermediate *Staphylococcus aureus* by comparative proteomics

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Abstract (Click on the text to edit)

Our study found seven differentially expressed proteins in hVISA by comparative proteomics analysis, including GpmA, MenB, FabZ, AphC, Asp23, MsrA2, and IsaA. Except for asp23, all of above differentially expressed genes were significantly up-regulated in most of the six clinical hVISA isolates, as indicated by qRT-PCR. Therefore, the six genes may be related to hVISA resistance.

Objectives

Heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) is associated with clinical treatment failure. To date, the resistance mechanism of hVISA remains incompletely clear.

Table 1. The study isolates, susceptibilities, and typing results.

Isolate[a]	Specimen Type	Phenotype[b]	MIC (μg/ml) for[c]: VAN	TEC	OXA	PAP-AUC	Sccmec Type	Spa Type	MLST
Pair 1									
CN1	sputum	VSSA	0.5	4	>256	0.6	III	t030	ST239
CN2	sputum	hVISA	1	4	256	0.9	III	t030	ST239
Pair 2									
CN3	blood	VSSA	0.5	1	256	0.5	III	t030	ST239
CN4	sputum	hVISA	0.5	2	256	0.9	III	t030	ST239
Pair 3									
CN5	sputum	VSSA	1	2	256	0.5	III	t030	ST239
CN6	sputum	hVISA	1	2	256	0.9	III	t030	ST239
Pair 4									
CN7	blood	VSSA	1	1	256	0.6	III	t030	ST239
CN8	sputum	hVISA	1	2	256	0.9	III	t030	ST239
Pair 5									
CN9	sputum	VSSA	0.5	2	256	0.4	III	t030	ST239
CN10	sputum	hVISA	2	8	256	1.0	III	t030	ST239
Pair 6									
CN11	blood	VSSA	1	2	>256	0.7	III	t030	ST239
CN12	abdominal fluid	hVISA	1	2	>256	0.9	III	t030	ST239

[a]Each pair of isolates belonged to the same pulsed-field gel electrophoresis (PFGE) type.

[b]Defined by population analysis profile (PAP)–area under the curve (AUC) method (PAP-AUC) (see Materials and Methods).

[c]VAN, vancomycin; TEC, teicoplanin; OXA, oxacillin.

Methods

In this study, comparative proteomics were performed on a pair of isogenic hVISA (CN10) and vancomycin-susceptible *S. aureus* (VSSA) (CN9) strains isolated from the same patient. The differentially expressed proteins were evaluated in six pairs of clinical hVISA and VSSA strains isolated from the six patients by real-time quantitative RT–PCR (qRT–PCR).

Table 2. Proteins differentially expressed between hVISA and VSSA identified in this study

Accession Number[a]	Protein Name	Gene	Protein PI[b]	Protein MW[c] (Da)	Protein Score C.I.%[d]	ratio[e]	Protein Function/Pathway
[illegible]	Alkyl hydroperoxide reductase subunit C	ahpC	4.88	20963.3	98.842	1.34	Directly reducing organic hydroperoxides in its reduced dithiol form
[illegible]	Alkaline shock protein 23	asp23	5.13	19178.7	100	10.8	Playing a key role in alkaline pH tolerance
[illegible]	2,3-Bisphosphoglycerate-dependent phosphoglycerate mutase	gpmA	5.23	26663.3	100	3.47	Catalyzing the interconversion of 2-phosphoglycerate and 3-phosphoglycerate Carbohydrate degradation; glycolysis; pyruvate from D-glyceraldehyde 3-phosphate: step 3/5
[illegible]	1,4-Dihydroxy-2-naphthoyl-CoA synthase	menB	5.41	30408.4	100	1.31	Converting o-succinylbenzoyl-CoA (OSB-CoA) to 1,4-dihydroxy-2-naphthoyl-CoA (DHNA-CoA) Cofactor biosynthesis
[illegible]	(3R)-Hydroxymyristoyl-[acyl-carrier-protein] dehydratase	fabZ	5.71	16071.4	99.798	3.00	Involved in saturated fatty acid biosynthesis
[illegible]	Probable transglycosylase isaA precursor	isaA	6.11	24198.5	100	1.56	Cleave peptidoglycan
[illegible]	Peptide methionine sulfoxide reductase 2	msrA2	6.04	20343.9	100	1.28	A repair enzyme for proteins that have been inactivated by oxidation catalyzes the reversible oxidation-reduction of methionine sulfoxide in proteins to methionine

Table 3 Relative *gpmA*, *menB*, *fabZ*, *ahpC*, *asp23*, *msrA2*, and *isaA* gene expression of hVISA strains compared with that of the parent strains, as determined by quantitative real-time PCR and normalized to 16S rRNA expression.

Differentially expressed genes	Relative gene expression (arbitrary unit): CN2/CN1	CN4/CN3	CN6/CN5	CN8/CN7	CN10/CN9	CN12/CN11	*p*-value *
gpmA	0.8	215.7	114.2	13.5	1.8	11.0	0.046
menB	1.9	20.3	25.7	1.7	3.7	11.9	0.028
fabZ	4.7	52.5	142.9	1.7	5.8	5.3	0.028
ahpC	1.7	12.5	29.0	1.1	1.4	2.7	0.046
asp23	0.6	231.1	44.3	1.7	18.6	11.4	0.075
msrA2	1.4	77.2	2.3	1.6	1.2	52.5	0.028
isaA	10.2	72.8	674.2	6.7	12.8	5.8	0.028

**p*-value as determined by Wilcoxon rank sum test.

Results

The comparative proteomics showed seven significant protein abundance changes between VSSA and hVISA, including GpmA, MenB, FabZ, AphC, Asp23, MsrA2, and IsaA. All the proteins were up-regulated in hVISA strain by comparative proteomics, and all the seven differentially expressed genes were enhanced in hVISA (CN10) compared with the VSSA strain (CN9) by qRT-PCR. The qRT–PCR results showed that *menB*, *fabZ*, *msrA2*, and *isaA* were up-regulated in all six hVISA strains, and *gpmA*, *ahpC*, and *asp23* were up-regulated in five hVISA strains compared with the VSSA parental strains. Except for *asp23*, all of the above genes were significantly up-regulated in hVISA strains by statistical analysis.

Conclusions

In conclusion, the enhanced expression of *gpmA*, *menB*, *fabZ*, *ahpC*, *msrA2* and *isaA* are closely related to hVISA resistance.

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