The Solid-Phase MicroExtraction for detection of antibiotic resistance in *Staphylococcus aureus* strains

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Introduction

Bloodstream infections are commonly caused by *Staphylococcus aureus* strains [1]. Such disease entity correlates with an elevated risk of sepsis, which poses a threat to a patient's health and life. The standard microbiological diagnostics of bloodstream infection takes from 3 days to 7 days, during which the patient receives broad-spectrum antibiotics what contributes to drug resistance [2]. **The application of the Solid-Phase Microextraction method (SPME) in sepsis diagnosis may reduce diagnostics time up to 2 hours.**

The aim of this study was to investigate the suitability of the Solid-Phase Microextraction method in the differentiation of methicillin-susceptible *Staphylococcus aureus* (MSSA) from methicillin-resistant (MRSA) strains based on the volatile compounds (VOCs) secreted by these bacteria.

Materials and Methods

5 MSSA and 5 MRSA strains were cultivated in defibrinated sheep blood in headspace vials containing a magnetic stir bar and a screw cap sealed with a polytetrafluoroethylene/silicone. Volatile compounds were isolated using a 2 cm length fiber, from the headspace above the bacterial suspension. VOCs were distributed and analyzed using gas chromatography combined with mass spectrometry thanks to the GCMS QP-2020 Shimadzu system. The compounds were identified using the National Institute of Standards and Technology (NIST) Mass Spectral Reference Library (NIST Mass Spectral Library, version 17a, 2017). Statistical analysis was performed using the TIBCO Software Inc. (2017) Statistica (data analysis software system), version 13. http://statistica.io. Acquired results were analyzed by ANOVA rank, Kruskal-Wallis ANOVA by ranks, and t-test. Results $p \leq 0,05$ were considered statistically significant.

Results

By means of the Solid-Phase Microextraction method 352 volatile compounds were isolated from the head-space phase of planktonic culture in the blood of *Staphylococcus aureus*. For further analysis, 40 compounds were selected, isolated from minimum 70% of the tested samples. Table 1 presents volatile compounds whose concentration differed the majority of MSSA vs. MRSA samples. The concentration of Undecane, 4-methyl was significantly higher in MRSA than in MSSA samples.

Table 1. Compounds differentiating between methicillin susceptible Staphylococcus aureus MSSA and methicillin resistance Staphylococcus aureus MRSA samples.

Compound Name	Molecular Formula MF	Molecular Weight MW [g/mol]	Retention Time [min]	Average area of the peak/number of cells		Test t Significance level p
				MSSA	MRSA	
Undecane, 4-methyl-	**$C_{12}H_{26}$**	**170,33**	**8,7**	**12584**	**27284**	**0,012**
Hexadecane	$C_{16}H_{34}$	226,44	7,2	45104	69265	0,079
Undecane, 5-methyl-	$C_{12}H_{26}$	170,33	8,9	66354	108531	0,116
Heptane, 2-methyl-	C_8H_{18}	114,23	3,1	641876	931875	0,131
Undecane, 3-methyl-	$C_{12}H_{26}$	170,33	7,27	134134	40688	0,135
Undecane	$C_{11}H_{24}$	156,31	6,4	115164	47654	0,146
Heptadecane	$C_{17}H_{36}$	240,50	8,8	93412	37260	0,1811

Conclusion

The comparison of profiles of secreted volatile metabolites revealed the significant differences between the MRSA and MSSA metabolomes. The results may serve as proof of the concept for further research aiming to create a new analytical method. Shortening the time of diagnosis of sepsis to 2 hours will significantly reduce the patient's risk of death.

References

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The research was financially supported by funding SUBZ.D230.22.026 from the IT Simple system of Wroclaw Medical University.