Application of hyphenated techniques for the determination and identification of antibiotics and microorganisms from clinical samples

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INTRODUCTION

Microorganisms occupy all known ecosystems – from natural environments those changes by humans to living organisms. The presence of a microbiome is necessary for the proper functioning and development of the body. Understanding the physiology and genetics of pathogenic bacterial species is important for disease prevention and faster microbiological diagnosis. Biochemical, serologic or chemotaxomic methods and also, in recent years, spectroscopic, spectrometric an genomic tools are routinely used to identify microorganisms in the context of clinical microbiology. One of the most frequently use method for the identification of microorganisms is the MALDI TOF-MS technique.

PROJECT GOALS

Application of spectrometric method for identification of bacterial cell isolated from patient under antibiotics therapy.

Development of chromatographic method for isolation, determination and identification of selected antibiotics and their metabolites from biological samples.

MATERIALS & METHODS

1. Biological material was postoperative wound swabs from patient treated with antibiotics.
2. For bacteria culture non-selective (BHI, MHA) and selective (BCP, VRE) culture media were used and for identification of microorganisms MALDI TOF-MS (with α-HCCA as a matrix) was used.
3. Isolated bacteria strains: *E. coli*, *S. aureus* and *E. faecalis* were incubated for 4, 6 and 24h and two culture media TBS and BD BALCTEL were used (standard and Sepsityper methods).
4. Antibiotics and their metabolites were identified in Full Scan, SIM, Product Ion and MRM modes. Analyzes in conjunction with HPLC were performed using an ACE5 C18 300 column, mobile phases: (A) water + 0.1% formic acid + 2 mM ammonium formate; (B) acetonitrile.
5. The isolation of the tested compounds was carried out with the use of MEPS sorbents and an eVol® automatic electronic syringe.

CONCLUSIONS

Microorgnisms identification

- *Staphylococcus* and *Enterococcus* genus were the largest contributors to postoperative wound infections
- The highest identification rates were obtained for *S. aureus* incubated 6h on liquid TSB medium, for which the extracts were analyzed by the standard method
- The use of universal microbiological media has a positive effect on the quality of protein profiles. Additional components found in selective media deteriorate the quality of MS spectra.

Antibiotics and their metabolites

- Conditions for the chromatographic determination of selected compounds as well as HPLC-MS/MS operating conditions were developed and selected, CCD was used to develop and select the MS/MS operating conditions for the tested drugs and their metabolites.
- The parameters of isolation and enrichment of selected compounds from standard solutions and real samples were developed, the C18 sorbent and the acetonitrile:methanol:water mixture (5:3:2; *v/v/v*) were selected (average recovery 98.16% ± 1.75%) as an elution medium.
- It has been proven that the use of mass spectrometry to determine and identify potential metabolites of selected antibiotics is possible.

RESULTS

Microorgnisms identification:

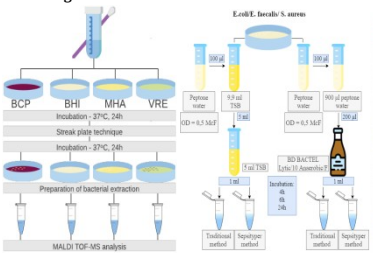


Fig. 1. Scheme of bacterial culture (left side) and scheme of selecting conditions in the method of sample preparation for MALDI TOF-MS analysis (right side).

Tab. 1. Comparison of the obtained identification indices of the tested bacterial species in terms of the applied liquid culture media and methods of sample preparation.

Bacterial strain	Times	Standard method		Sepsityper method	
		Vials	TBS	Vials	TBS
S. aureus	4h			1,73	2,07
	6h	2,16	2,16	2,14	2,21
	24h	2,19	1,89	2	2,16
E. coli	4h	2,31	2,15		1,79
	6h	1,32	2,12		
	24h	1,99	2,05	1,57	1,9
E.faecalis	4h		2,32		2,35
	6h	1,55	1,7	2,25	1,48
	24h	2,25	2,22	2	

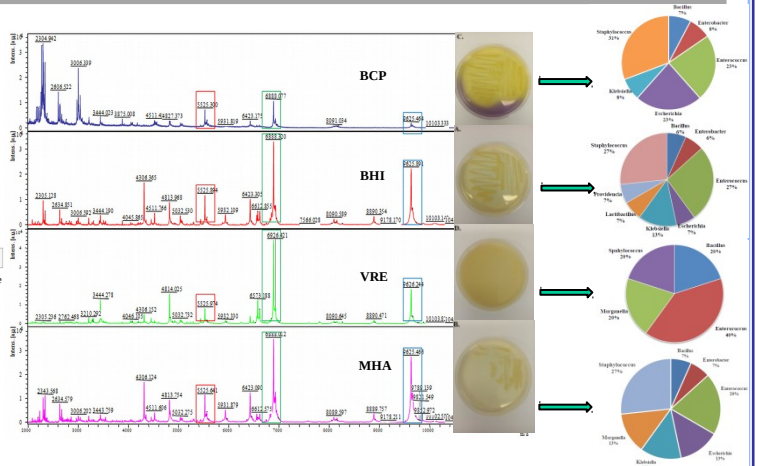
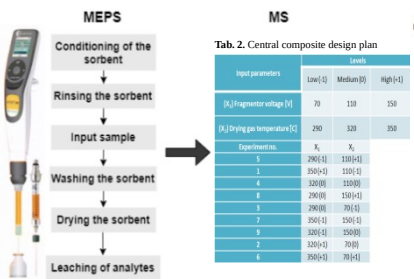


Fig. 2. Comparison of MS spectra for *S. aureus* bacteria isolated on four different culture media (BHI, BCP, MHA, VRE). Comparison of the morphology of *S. aureus* obtained on the microbiological media used: BHI (A), MHA (B), BCP (C) and VRE (D) and the percentage of bacterial strains isolated on each of the media

Antibiotics and their metabolites determination:



Tab. 2. Central composite design plan

Input parameters	Levels		
	Low (-1)	Medium (0)	High (+1)
[X_1] Fragmentor voltage [V]	70	110	150
[X_2] Drying gas temperature [C]	290	320	350
Experiment no.	X_1	X_2	
5	290 [-1]	110 [+1]	
1	350 [+1]	110 [-1]	
4	320 [0]	110 [0]	
8	290 [0]	150 [+1]	
3	290 [0]	70 [-1]	
7	350 [-1]	150 [-1]	
9	320 [-1]	150 [0]	
2	320 [+1]	70 [0]	
6	350 [+1]	70 [+1]	

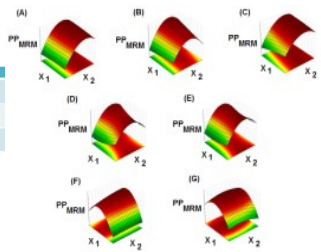


Fig. 3. Response surface area for the baseline value (PP_{MRM}) depending on the voltage at the fragmentor (X_1) and the drying gas temperature (X_2) for (A) AMOX and its metabolites (B, C), (D) CIP and its metabolite (E), MET and its metabolite (G).

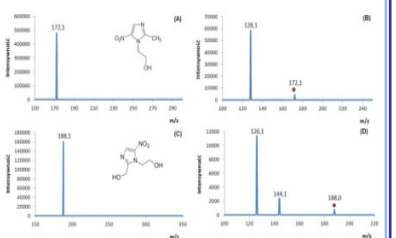


Fig. 4. MS spectra and fragmentation spectra for MET (A, B) and the identified metabolite - MET-OH (C, D) in a patient's plasma sample (dose 250 mg).