An alternative method to the infection detection for differentiation of 3 and 4 MRGN by use of GC IMS-Spectral analysis

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Introduction

Routine clinical tests on resistant germs are essentially due to the increasing number of multi-resistant germs but needs a couple of time and resources. A fast and cheap screening test for reservation of necessary exact diagnosis will help to save time and money. Especially identification of MRGN becomes more relevant.

It is well-known that bacteria release volatile markers during growth. Several kinds of spectrometry, e.g. GC, GC-IMS are capable to detect these markers. The identification of a multi-resistant subpopulations of a bacterial strain still was not published for these methods.

The aim of the study was to prove the specifity of differentiation of different strains as well as subtypes of the strain.

Methods

Using a GC-IMS (STEP-Pockau) headspaces of agar and bacterial liquid cultures were analysed. The IMS-System is a combination of a heated multi-capillary column and an ion mobility spectrometer The following different bacteria and strains were used to match the realistic clinical questions:

Pseudomonas aerug. (7), E. coli (4), Enterobacter (5), Klebsiella (6), Citrobacter (9), each known as 3 MRGN or 4 MRGN, indicated with the second digit.

The detected peaks of all measurements were calculated by cluster analysis with support vector machine. Sensitivity and specifity of differentiation was evaluated by Leave-One-Out cross validation method.

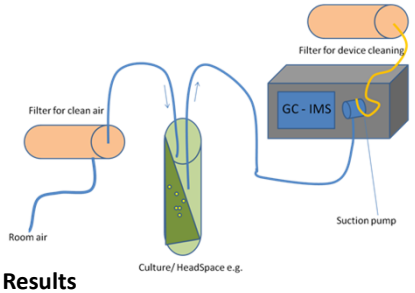


Fig. 1: Schematic diagram of setup for collection of head-space from bacterial cultures.

Results

In comparison of a strain to control-media were identified about 900 different clusters of VOC. i.e. different chemical ions of in headspace of cultures. At least up to 5 were needed to differentiate 3 and 4 MRGN. The sensitivity for discrimination of bacterial growth in itself was nearly 100%.

Fig. 2: The differentiation of Pseudomonas aerug. 3 and 4 MRGN using three different clusters of IMS-spectra (p≤ 0.05)

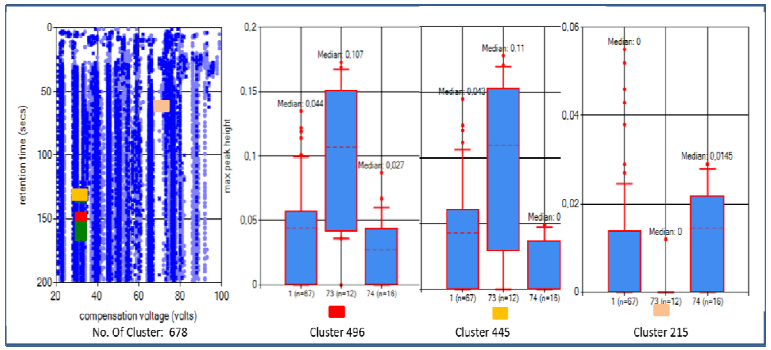
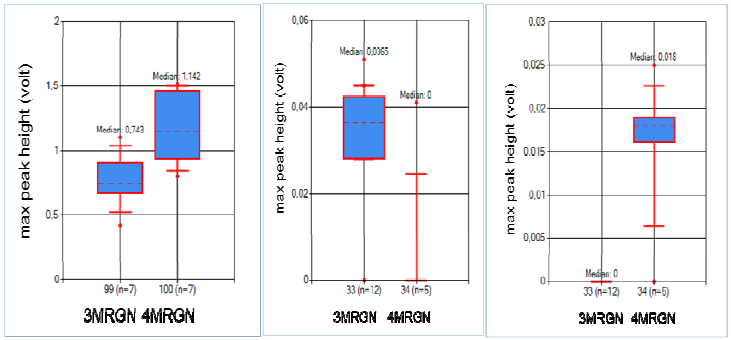


Fig. 3: Differentiation of 3 and 4 MRGN of:

Klebsiella pneumoniae (p≤0.05) E.Coli (p≤ 0.05)



Similar constellations of different clusters between 3 and 4MRGN also were found in headspaces of cultures from Enterobacter and Citrobacter (p≤ 0.05).

Discussion

Different subtypes of bacteria species are possible to differentiate highly specific using VOC-peaks from liquid-culture headspaces. Surprisingly also different pattern between 3 and 4 MRGN were found for several germs.

The methods seem to be effective for rapid screening of bacterial growth, which may help for faster tests and cost savings by reducing needed number of PCR-tests.

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[2] R. Purkhart: Klassifikation von Ausatemluft anhand ihrer differenziellen Ionenbeweglichkeitsspektrogramme. Diplomarbeit, HUB 2010.
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[4] T. Raessler: Validierung des Systems „Multimarkermonitor" auf der Basis eines GC-IMS für die Nutzung in der medizinischen Diagnostik als Screeningmethode. Masterarbeit, Hochschule Mittweida 2015.

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