Session: OS202 MALDI-TOF - diagnostics for the micro lab in the 21st century

Category: 4b. Diagnostic bacteriology – non-culture based, including molecular and MALDI-TOF

25 April 2017, 15:06 - 15:16
OS1034

Subtyping of vancomycin-resistant enterococci with MALDI-TOF mass spectrometry

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Background: Vancomycin-resistant enterococci (VRE) are important nosocomial pathogens causing outbreaks in health care institutions. In Denmark, the incidence of vancomycin-resistant *Enterococcus faecium* (VREfm) has rapidly increased since 2012. Typing methods are essential for understanding outbreak epidemiology and rapid and widely available methods are especially valuable for outbreak management to prevent further spread. We therefore aimed to investigate the usefulness of Matrix Assisted Laser Desorption/Ionization – Time Of Flight mass spectrometry (MALDI-TOF MS) subtyping of VRE isolates in a nosocomial outbreak setting.

Material/methods: Representative VREfm isolates which had been characterized by whole genome sequencing (WGS) in an earlier nationwide study were analysed by MALDI-TOF MS subtyping. Reproducibility was analysed by repeated analysis and lead to the following protocol: Ethanol extraction was performed as a triplet and analysis was undertaken on three different target spots with three consecutive runs, thus resulting in 27 spectra per isolate to compensate for peak variation. Spectra were evaluated manually, indicator peaks were defined and a peak scheme was established to distinguish between three major WGS clusters defined by single nucleotide polymorphism (SNP)-analysis. Furthermore, representative isolates were examined using a direct method based on adding up a total of 400, 800 and 1600 shots respectively.

Results: Thirty Danish VRE isolates from January 2012 to April 2013 were included in the study. Twenty-one isolates belonged to three major WGS clusters, while the remaining nine were unrelated. Using seven reproducible indicator peaks, the three WGS groups could be distinguished from each other with only one isolate being misclassified. This isolate belonged to the cluster, where WGS had shown greatest genetic diversity. However, five of the nine isolates not belonging to the three WGS clusters could not reliably be classified as non-outbreak isolates. Using the direct method did not add information as no new indicator peaks were found.

Conclusions: MALDI-TOF MS spectra of the three main WGS clusters were distinguishable from each other. However, MALDI-TOF MS subtyping was not able to reliably distinguish non-outbreak isolates from the three major WGS clusters, thus limiting the practical value of this typing method for outbreak management.