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Monitoring *Clostridium difficile* types in clinical routine using MALDI-TOF

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Introduction

Clostridium difficile (CD) is a common cause of diarrhea in patients with antibiotic treatment and long hospital stay. The severity of the symptoms varies and depends on the patient's condition but also on which type of CD causes the infection. To suspect and confirm the dissemination of a certain clone within a ward or a hospital, CD must be cultured and isolates has to be typed.

In 2015 Rizzardi et al published a novel method where CD isolates were typed with MALDI-TOF and classified into High molecular weight (HMW)-profiles according to their surface layer proteins (Kristina Rizzardi, Thomas Åkerlund. High Molecular Weight Typing with MALDI-TOF MS - A Novel Method for Rapid Typing of Clostridium difficile. Published: April 29, 2015. DOI:10.1371/journal.pone.0122457). The HMW-profiles correlates partly with ribotypes. Using this method, we retrospectively analyzed all CD isolated between Sep 2013 and March 2016

Methods

The study encompassed 489 isolates cultured from CD toxin-positive feces samples collected between Sep 2013 and March 2016 from four different hospitals in two different counties in Sweden. The material did not contain repeat isolates. All isolates were analyzed according to the published method, with the addition of an internal calibrant to guarantee the correct masses. Generated spectra were automatically interpreted to a HMW-profile by software provided by the Swedish Health Agency.

Objective

The aim of this project was to: 1) evaluate the capability of the method to differentiate between CD strains, 2) map the diversity of CD types between and within four hospitals with only little exchange of patients 3) see if the method could detect two previously identified CD outbreaks (ribotypes 027 and 002), and 4) point to other, hitherto unknown outbreaks.

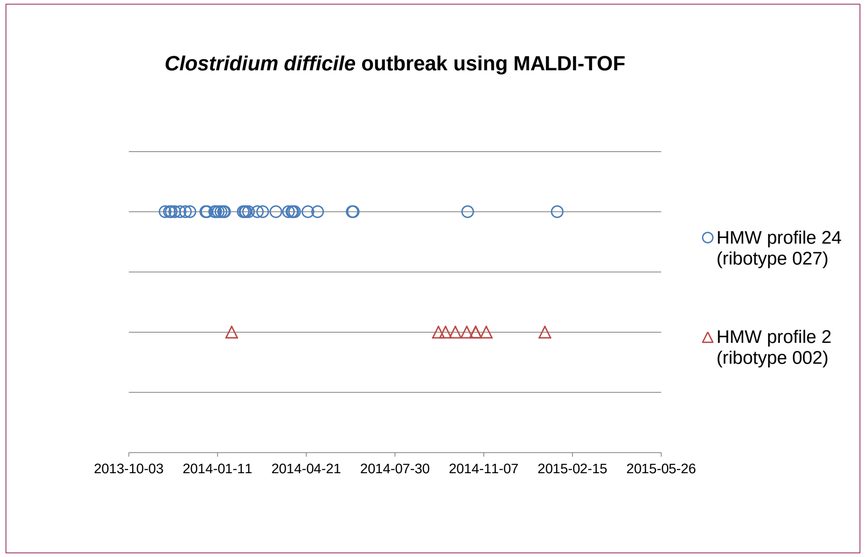

Figure 1. Visualisation of the two confirmed outbreaks of ribotypes 027 and 002 respectively. Each plot represent an infected patient.

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Results

Among the 489 isolates, 23 of 32 possible HMW-profiles were represented. The predominant HMW-profile was 14 (which correlates to ribotypes 011, 014/077, 020, 095, 103, 106, 220, x144), followed by 13 (ribotypes 011, 029, 039, 043, 078, 097,x48, x4, x119, x78, x90, x25) and 5 (ribotypes 005, 015, 023, 050, 054, 075, 080, 116, 234, 375 ,x25, x50, x6). To increase the granularity of HMW-typing these profiles can be subtyped further. Plotting the HMW-profiles over a time line revealed the two known CD outbreaks as clusters (Figure 1 and 2).More clusters were identified and further investigations using Next Generation Sequencing is being performed.

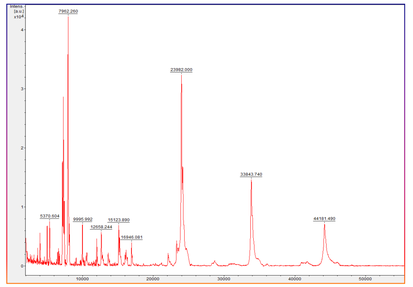

Figure 2. Spectrum of HMW profil 24 (ribotype 027)

Conclusions

HMW-typing is a simple, rapid and cheap method to monitor the spread of CD types in healthcare settings. If performed as a real time service it can provide early detection, and thus prevention, of larger CD outbreaks. HMW-types can be correlated to more virulent ribotypes, such as 027, in support of infection control measures.