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# Comparison of four different molecular methods for Clostridium difficile outbreak investigations

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## Background

*Clostridium difficile* infection is an urgent public threat and outbreaks increased markedly in hospitals in recent years. Ribotyping of strains has been the most widely used molecular tool to distinguish an outbreak from a coincidental accumulation. As ribotyping is time-consuming we investigated alternative methods for outbreak analysis.

## Methods

14 *Clostridium difficile* bacterial isolates from different patients were collected in a cardiac surgery clinic within one month. The number of CDI in the hospital was much higher than the average of the previous month suggesting a *C. difficile* outbreak. Capillary gel electrophoresis-based PCR ribotyping was performed and resulting peak patterns were assigned to PCR ribotypes using the Webribo database. The results were compared to a subtyping dendrogram generated by MALDI-TOF mass spectrometry using Biotyper software. Random amplified polymorphic DNA (RAPD)-PCR was also performed. As RAPD-PCR is often used for subtyping Gram-negative bacteria, while the more recent method of Fourier transformation-Infrared Spectroscopy (FTIR) was published for different Gram-positive isolates (e.g. *Corynebacterium ulcerans*), we added this to the investigative panel.

Tab.1

| Strain | Age | Gender | Species | tcdA | tcdB | Ribotype | RAPD-PCR | FT-IR | MALDI |
|---|---|---|---|---|---|---|---|---|---|
| EL402860 | 62 | m | Clostridium difficile | pos | pos | 046 | A | | |
| EL402903 | 84 | m | Clostridium difficile | pos | pos | 400 | B | | |
| EL402945 | 72 | m | Clostridium difficile | pos | pos | 014 | C | | |
| EL402952 | 33 | w | Clostridium difficile | pos | pos | 005 | D | | |
| EL402975 | 71 | w | Clostridium difficile | pos | pos | n RT | E | | |
| EL402979 | 59 | m | Clostridium difficile | pos | pos | 106 | E | | |
| EL403022 | 80 | w | Clostridium difficile | pos | pos | 400 | F | | |
| EL403180 | 74 | w | Clostridium difficile | pos | pos | 400 | D | | |
| EL403181 | 84 | m | Clostridium difficile | pos | pos | 078 | G | | |
| EL403210 | 73 | w | Clostridium difficile | pos | pos | 400 | D | | |
| EL403313 | 43 | w | Clostridium difficile | neg | neg | 010 | H | | |
| EL403461 | 48 | w | Clostridium hathaway | nd | nd | nd | I | | |
| EL403467 | 29 | m | Clostridium difficile | pos | pos | 078 | J | | |
| EL403488 | 74 | w | Clostridium difficile | pos | pos | 400* | K | | |
| EL403625 | 81 | m | Clostridium difficile | pos | pos | 555 | J | | |
| EL305725 | co | - | Clostridium difficile | pos | pos | 400 | K | | |
| EL305692 | co | - | Clostridium difficile | neg | neg | n RT | M | | |

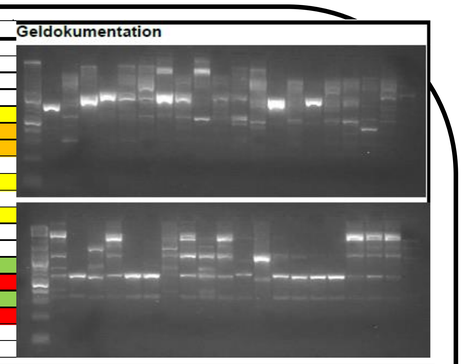
[co: control; nd: not done; n RT: new Ribotype]

## Results

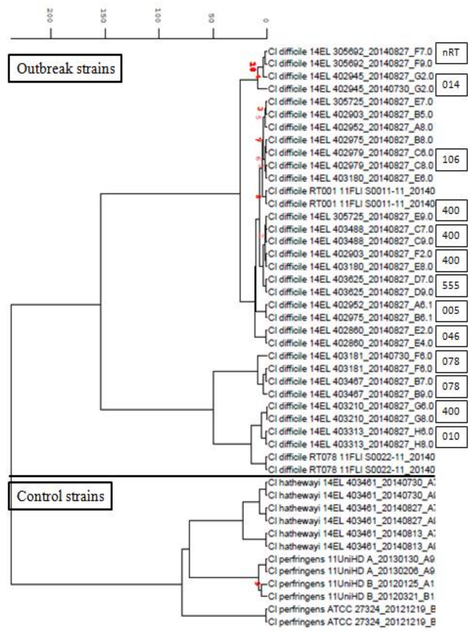
14 C. difficile strains (and 3 controls) were typed by Ribotyping and toxin analysis (Tab. 1), RAPD-PCR (Fig. A), Fourier transformation-Infraed Spectroscopy (Fig. B) and by MALDI-ToF MS (Bruker™ microflex; Fig. C) and the results clustered (as far as rules for strain typing already exists).

A

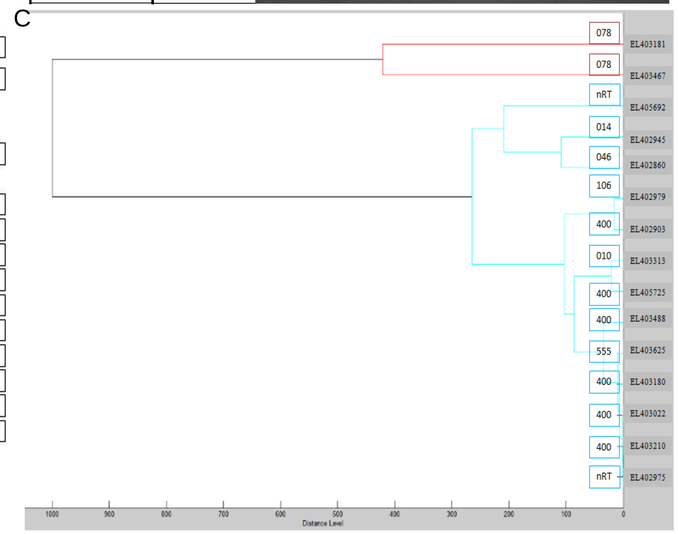
| Labornummer | Ergebnis |
|---|---|
| Ladder | |
| EL402860 | A |
| EL402903 | B |
| EL402945 | C |
| EL402952 | D |
| EL402975 | E |
| EL402979 | E |
| EL403022 | F |
| EL403180 | D |
| EL403181 | G |
| EL403210 | D |
| EL403313 | H |
| EL403461 | I |
| EL403467 | J |
| EL403488 | K |
| EL403635 | J |
| EL305725 | K |
| EL305692 | L |
| EL302326 | M |




B




C



## Conclusions

The subsumed evaluation of the four methods clearly showed that CDI threat on the ward was more likely a coincidental accumulation than a confirmed outbreak, although single transmission (e.g. 078 strains) might have occurred. Even if the data for the alternative methods RAPD-PCR, mass spectrometry and FT-IR might be preliminary, it showed promising strength in differentiating the strains on a molecular level. Ribotyping seems to be the standard methods as comparison, for the other methods agreed interpretation rules are necessary.

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