

HIGH OUTPUT PCR-RIBOTYPING OF Clostridium difficile DURING AN OUTBREAK OF PCR-RIBOTYPE 027 IN VIENNA, AUSTRIA IN 2009

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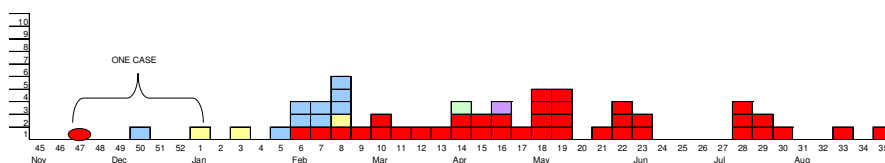
Austrian Agency for Food and Health Safety

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Österreichische Agentur für Gesundheit und Ernährungssicherheit GmbH

Outbreak cases by date of onset and affected hospital



■ Hospital X n=38

■ Hospital 1 n=9

■ Hospital 2 n=3

■ Hospital 3 n=1

■ Hospital 4 n=1

Hospital	Outbreak case	
	New case	Recurrent case
HX	38	-
H1	8	1
H2	2	1
H3	-	1
H4	-	1
Total	48	4

Outbreak



- March-May 2009: A cluster of 95 patients with CDI occurred in a Viennese hospital (HX)
 - HX includes 10 pavilions with a total of 1146 beds
 - Out of these 95 CDI cases, 25 patients with ribotype 027 were identified by the National Reference Laboratory for CD
- Initially, another 2 hospitals were affected with the occurrence of new 027CDI patients
- May 2009: AGES was mandated to investigate the cluster

Data Ascertainment and Laboratory investigation

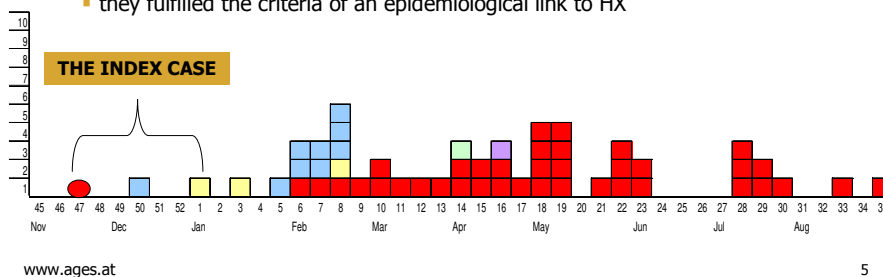


- Medical charts of outbreak cases from HX were reviewed on 30-day outcome and previous antibiotic exposure
- PCR ribotyping of all CD isolates was performed by the NRLCD, AGES until end of August
- The effect of the control measures were assessed by comparing number of CDI cases before and after their implementation (CW 10-30 vs. CW 31-53)

Description of the outbreak until end of August 2009



- **52** outbreak cases found
- Out of these, **38** PCR-ribotype 027-CDI cases occurred in HX
 - In HX, out of the **9** affected pavilions
 - the most affected pavilion included **25** PCR-ribotype 027-CDI cases
- The other **14** outbreak cases occurred in **4** other Viennese hospitals
 - they fulfilled the criteria of an epidemiological link to HX



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Description of the outbreak until end of August 2009

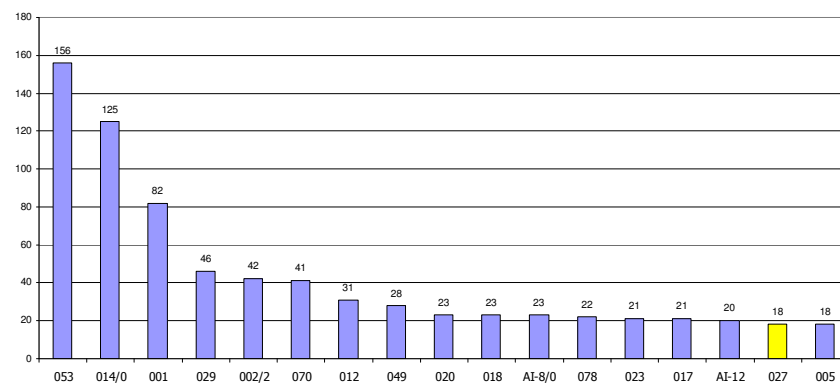


- **Outbreak cases of HX**
 - 24/38 (60%) cases male
 - Median age: 80 years (range: 31-98 years)
 - **30-day mortality: 13/38 (34.2%)**
 - Medical records available for 27/38 (66%) outbreak cases
 - Of these, all 27 cases received antibiotics prior to diarrhoea onset
 - Beta-lactam antibiotics: 55.6% (15/27)
 - Fluoroquinolone: 59.3% (16/27)

Description of the outbreak until end of August 2009



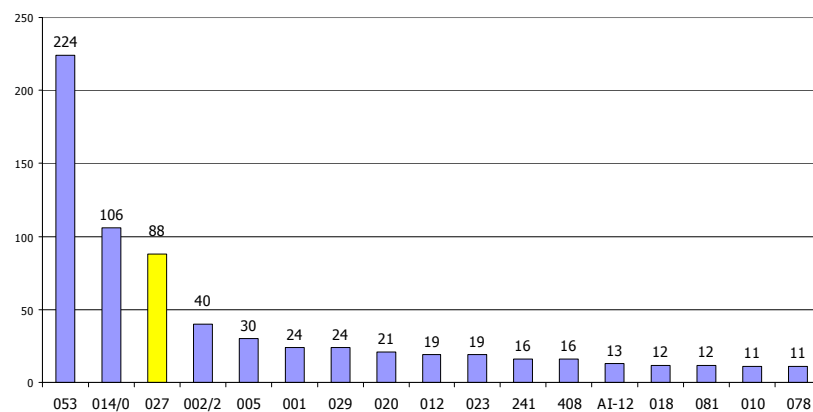
PCR-ribotypes found in Austria 2008



Description of the outbreak until end of August 2009



PCR-ribotypes found in Vienna between March and August 2009



Description of the outbreak until end of August 2009



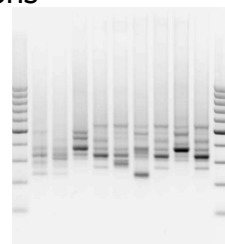
- between March and August **720** samples were send by various Viennese Hospitals to our Lab
- in total **1320** C. difficile samples from Austria were analyzed during this period
 - of these, **1042** C. difficile samples could be cultivated
- capillary gel electrophoresis PCR-ribotyping was used in combination with AGES-WEBRIBO (webribo.ages.at)
 - **152** different PCR-ribotypes found
 - all **52** PCR-ribotype 027 positive samples were also MLVA-typed
- this work was done by two part time technicians
- average time to final lab-results was **6 days**

Capillary gel electrophoresis based PCR ribotyping



Conventional Clostridium difficile PCR-Ribotyping

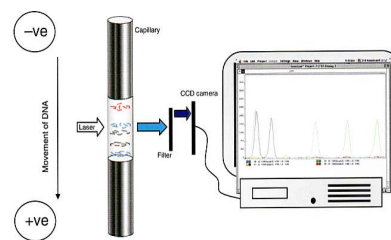
- problematic inter-laboratory exchange
 - variable use of laboratory specific nomenclature
- no international database
- every sample has to be compared with controls processed under the same conditions
- inconsistent data interpretation of similar band patterns
 - e.g. PCR-ribotype 066
 - known with Toxinotype V and VI
 - untypable 001 subtypes



Capillary gel electrophoresis based PCR ribotyping



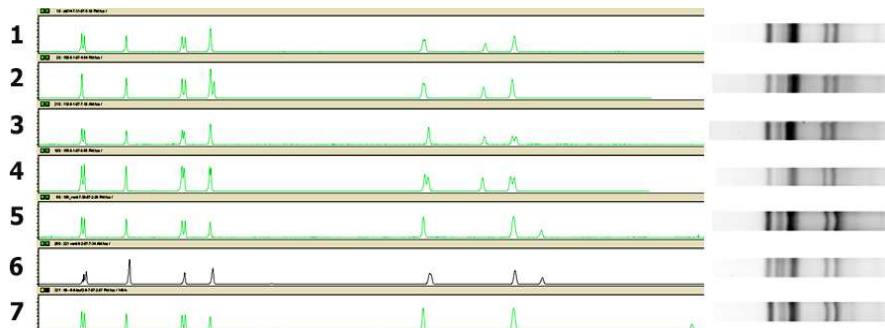
- high reproducibility by using
 - a fluorescence labeled primer
 - a uniform size-standards within every sample
 - reproducible running conditions for every sample
- high throughput
- comparability of data results
- easy-to-use data analysis
- higher discriminatory power
 - fragments with 1.5 bases difference or more can be differentiated



Capillary gel electrophoresis based PCR ribotyping



- 6 different 014 subtypes including 020
- Toxinotype V and VI of PCR-ribotype 066 can be differentiated



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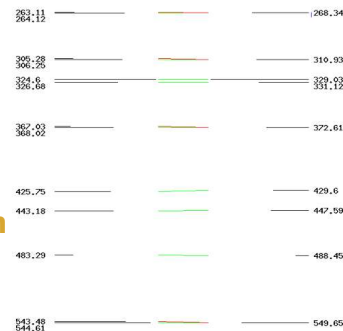
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AGES-WEBRIBO webribo.ages.at



- **freely accessible** database for *Clostridium difficile* PCR-ribotyping
- **no commercial** software is needed (Bionumerics, Genemapper, Ridom-Staphtype ..)
- **all ABI** gained .fsa files can be analyzed automatically
- **reliable results** regardless of any size-standard or sequencing machine used
- **99% correct** identification of samples analysed with
 - **different** size-standards
 - **different** sequencing machines
 - **different** primers (Stubbs/Bidet)
 - a **supervised learning algorithm** copes with double-peaks due to PCR-artefacts

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- is capable of **differentiating** ribotypes 014 and 020
 - even five more 014 subtypes can be separated
- is capable of **differentiating** 4 of the suspected 8 PCR-ribotype 001 subtypes first described by Northey et al.



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Conclusions



The findings

- underline how rapidly a highly virulent CD strain can spread within and between hospitals
- prove the reliability of **capillary gel electrophoresis PCR-ribotyping** in combination with **AGES-WEBRIBO** as high output tool for outbreak investigation
- show the importance of PCR-ribotyping in elucidating links between separate hospital clusters and following the implementation of evidence-based control measures

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