Resistance profiling and ribotyping of *Clostridium difficile* isolates in Germany: Results of the PEG study 2013/2014

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Introduction

Clostridium difficile is the most prevalent pathogen causing diarrhoea in hospitals; however, also in the community *C. difficile* may cause symptomatic infections, especially following antibiotic therapies. In recent years epidemiology of *C. difficile* was characterized by the emergence of new hypervirulent strains with multiresistance to standard antibiotic while still sensitive to antibiotics used for specific *C. difficile* therapy (metronidazole and vancomycin). Selection of outbreak strains and also the response to antibiotic treatment may depend on antibiotic resistance profiles of circulating strains. In 2013 fidaxomicin received approval for treatment of severe *C. difficile* infection but until now there are no antibiotic resistance data available for isolates in Germany.

Methods

In the present multicentre study conducted by the Paul Ehrlich Society (PEG), Germany, a representative selection of *C. difficile* strains were included. Isolates were collected in 45 laboratories from in- and -outpatients with diarrhoea. *C. difficile* was confirmed by Maldi-ToF and PCR ribotyping was performed using capillary gel electrophoresis and automated strain assignment (bionumerics). For the current 027 outbreak strains subtyping was included using Multi-locus variable tandem number of repeats analysis (MLVA). Antibiotic susceptibility testing was performed using agar dilution method according to standard protocols (CLSI). MICs were interpreted by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints or epidemiological cut-off (ECOFF) values if available.

Results

Tab 1: Preliminary results of susceptibility testing was shown for 210 isolates derived from hospitalized patients. The percentage of sensitive (S), intermediate (I) and resistant (R) isolates was shown in combination with minimal inhibitory concentrations (MIC) of the 50% and 90% percentile. Sensitivity to the standard antibiotics (metronidazol and vacomycin) was 100%, and also for fidaxomicin a low MIC was found without exception (clinical breakpoints are not available).

Drug	MIC_{50} (mg/L)	MIC_{90} (mg/L)	S (%)	I (%)	R (%)	n
Clindamycin*	8	≥1024	68.6	-	31.4	210
Fidaxomicin	0.25	0,5	-	-	-	210
Metronidazol	0.5	1	100	-	0	210
Levofloxacin	8	256	-	-	-	210
Moxifloxacin*	2	32	58.6	-	41.4	210
Rifamixin	0.004	0.015	-	-	-	210
Vancomycin	1	1	100	-	0	210

***EUCAST ECOFF-value was used**

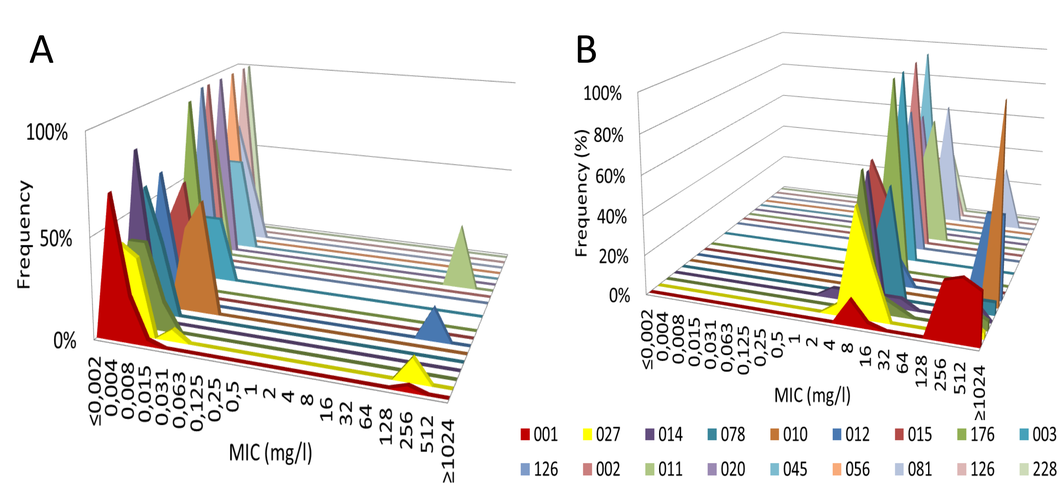

Fig. 1: Minimal inhibitory concentrations (MICs) of rifaximin (A) and clindamycin (B) for the initial 210 *C. difficile* isolates of hospitalized patients . Sporadic antibiotic resistance was detected for some ribotypes while other ribotypes were always sensitive in the actual strain collection. Ribotype related characteristic resistance profiles were found for other antibiotics reflected e.g. by moxifloxacin resistance of the predominant nosocomial strains (ribotype 001 and 027)

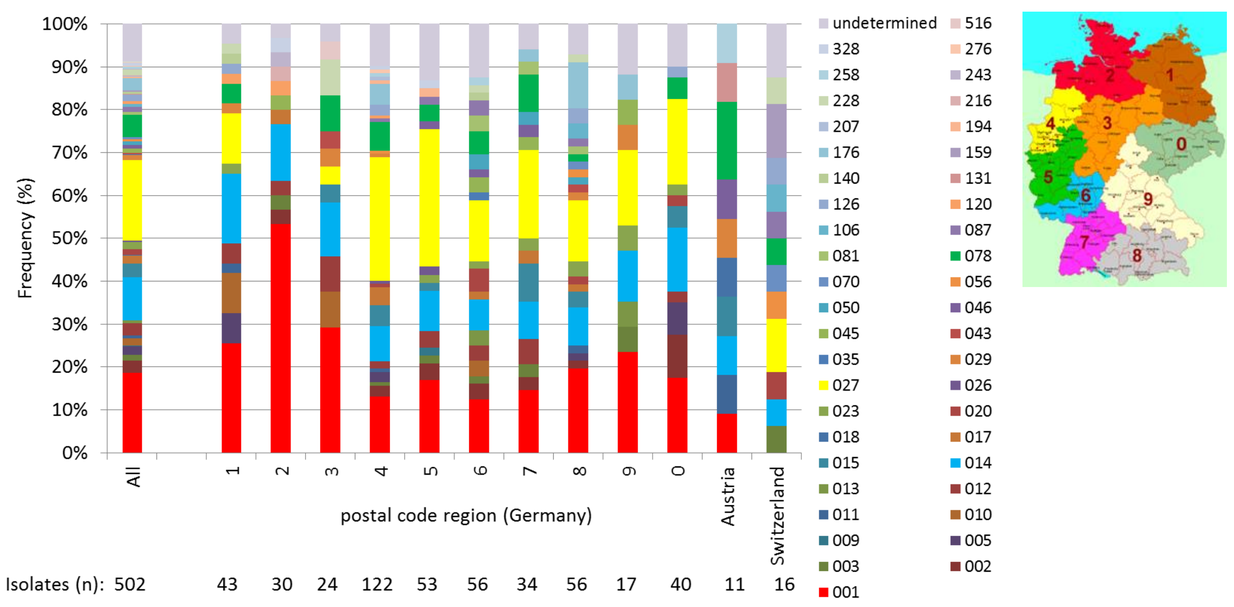

Fig. 2: Geographic distribution of *C. difficiole* ribotypes in Germany (with postal code region) and in few centers in Austria and Switzerland.

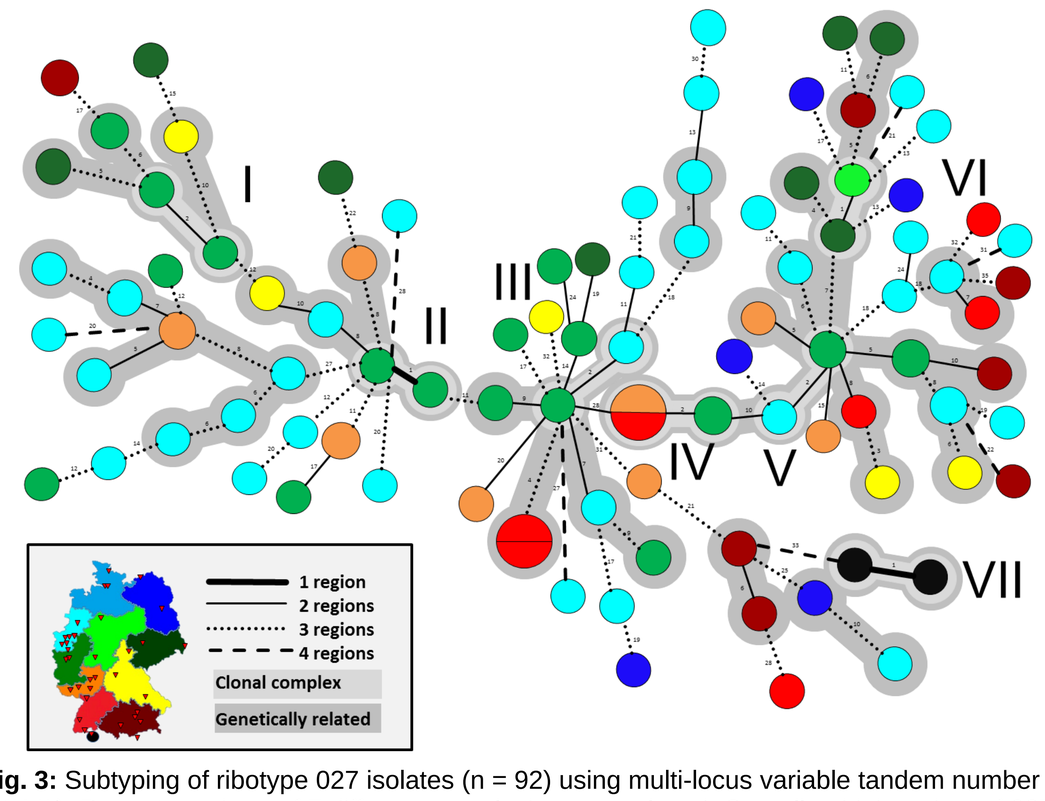

Fig. 3: Subtyping of ribotype 027 isolates (n = 92) using multi-locus variable tandem number of repeats (MLVA): Repeat numbers of 7 different gene loci were analyzed. Overall, a high variety of 027 strains can be found independent from geographic regions. Strains of the same clonal complex can be found in different geographic region (e.g. cluster IV) as a sign of interregional spreading but also in the same hospital (e.g. cluster VII).

Conclusions

- Low MICs for standard antibiotics (vancomycin, metronidazol) and also for fidaxomicin is the base for successful calculated treatment without regular antibiotic resistance testing.
- Antibiotic resistance to other antibiotics appears preferably for isolates of certain ribotypes. Antibiotic resistance to indicator antibiotic substances are characteristic for ribotypes predominantly found in hospitals (001 and 027).
- The new epidemic and hypervirulent outbreak strain ribotype 027 is highly prevalent in Germany, except for the North (postal code 2), however, also other new ribotypes with a similar toxin gene repertoire (tcdA, tcdB, cdtA and cdtB) and the potential capacity for global spreading can be regularly found in Germany (e.g. ribotype 176).
- Regular national and international surveillance of *C. difficile* is required to evaluate the dynamics of epidemic spreading and also the emergence of antibiotic resistance.