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A cross-sectional multi-centre study on *Clostridium difficile* infections in representative regions of Germany, Ghana, Tanzania and Indonesia

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Background

Clostridium difficile infections (CDI) have become an emerging health threat usually ranging from diarrhea to pseudomembranous colitis which can end up in toxic megacolon with high mortality. Since antibiotics have been identified as a major risk factor, we postulate that prevalence rates of CDI and the distribution of *C. difficile* strains differ between geographical regions depending on the regional use of antibiotics.

Tab. 1: Isolation of *C. difficile*

Location	Patients	*C. difficile* positive	Isolates
Germany	122 with diarrhea	33 (27%)	39
	121 w/o diarrhea	0 (0%)	0
Ghana	176 with diarrhea	8 (5%)	11
	131 w/o diarrhea	7 (5%)	7
Tanzania	140 with diarrhea	7 (5%)	7
	110 w/o diarrhea	0 (0%)	0
Indonesia	170 with diarrhea	26 (15%)	30
	232 w/o diarrhea	3 (1%)	3

Material and methods

A cross-sectional, multi-centre study was performed in representative communities in Germany, Ghana, Tanzania and Indonesia. Patients with diarrhea and asymptomatic control individuals of different age were screened for presence of *C. difficile* in stool samples. Cultured *C. difficile* strains were characterized using PCR ribotyping, MALDI-TOF MS (Fig. 2), genome analysis, toxin genes and toxin production, as well as antibiotic susceptibility testing. Potential risk factors were determined using a standardized questionnaire.

Results

A total of 1,202 stool samples from 608 patients and 594 healthy individual were included (Tab. 1). Prevalence rates of CDI ranged from 5% in Africa to 15% and 27% in diarrhoeal patients from Indonesia and Germany, respectively. Nontoxigenic strains of ribotype 084 were most abundant in Africa (Ghana, Fig. 1). In contrast, toxin A+/B+ ribotypes 001/072 and 078 predominated in Germany. In Indonesia, most strains belonged to toxin A-/B+ ribotype 017 and ribotype SLO160 (Fig. 3). Depending on geographical origin, major differences for mobile genetic elements were seen (Fig. 4). All isolates were susceptible to vancomycin and metronidazole, respectively (Tab. 2). Mirroring the antibiotic use, however, moxifloxacin resistance was absent in African *C. difficile* isolates but present in Indonesian (24%) and German ones (67%).

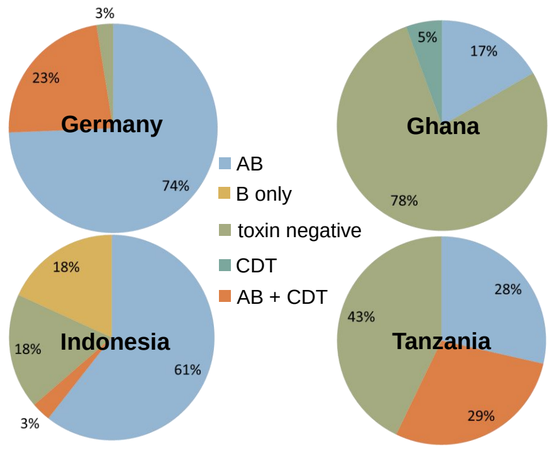

Fig. 1: Toxin distribution. In Indonesia, a high percentage of TcdA-/ TcdB+ strains belonged to ribotype 017. In Ghana as well as in Tanzania nontoxigenic *C. difficile* strains were isolated even from patients suffering from diarrhea.

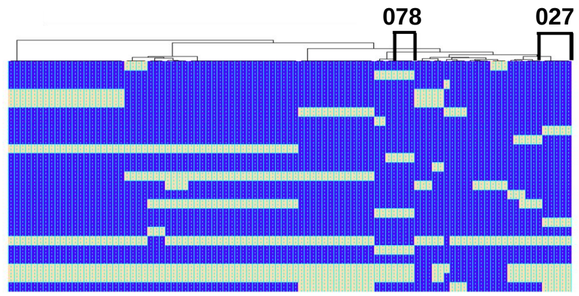

Fig. 2: Typing by MALDI-TOF MS. We reproducibly identified the hypervirulent ribotypes 078 and 027.

Tab. 2: Antibiotic resistance pattern

	VC	MZ	MXF	E
Germany	0%	0%	67%	74%
Indonesia	0%	0%	24%	18%
Ghana	0%	0%	0%	47%
Tanzania	0%	0%	0%	38%

In Germany, a high rate of *C. difficile* isolates are resistant against moxifloxacin (MXF) and erythromycin (E). All isolates were susceptible to vancomycin (VC) and metronidazole (MZ) used in CDI therapy.

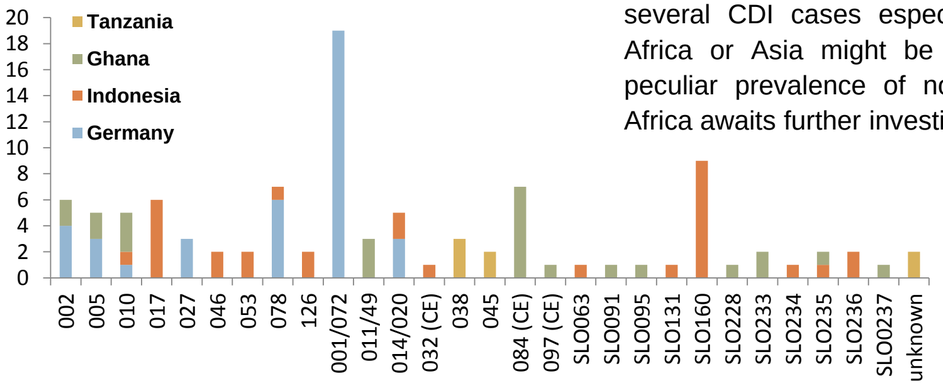

Fig. 3: PCR-Ribotyping. 32 different ribotypes were identified within our 97 isolates.

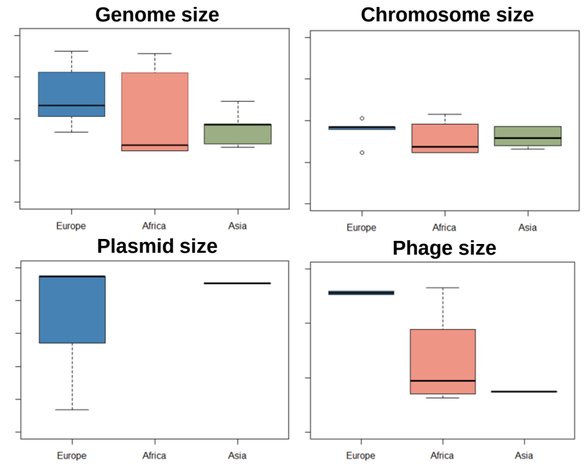

Fig. 4: Genome analysis. Comparison of 15 genomes from three continents revealed differences in genome size and especially in abundance of plasmids and phages.

Conclusions

CDI is a global health threat with geographically different prevalence rates that might reflect distinct use of antibiotics. Significant differences for distributions of mobile genetic elements, ribotypes, toxin production, and antibiotic susceptibilities were observed. If diagnosis relies only on detection of toxin A from stool samples, several CDI cases especially originating from Africa or Asia might be left undetected. The peculiar prevalence of nontoxigenic strains in Africa awaits further investigation.

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