MOLECULAR CHARACTERIZATION OF *CLOSTRIDIUM DIFFICILE* STRAINS FROM ELDERLY CARE HOME RESIDENTS

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INTRODUCTION

Clostridium difficile is the primary cause of nosocomial diarrhoea and pseudomembranous colitis after use of antibiotics. Production of toxins A and B are the main virulence factors responsible for its pathogenicity. Increasing age, several co-morbidities, environmental contamination, antibiotic exposure and other intestinal perturbations appear to be the greatest risk factors for *C. difficile* infection (CDI). Therefore, elderly care home residents are considered particularly vulnerable to CDI.

OBJECTIVES

The aim of this study was to evaluate and follow the prevalence of *C. difficile* in a Belgian nursing home and to characterize the *C. difficile* strains isolated from faeces of elderly care home residents

MATERIAL AND METHODS

Samples

During a 4-month period, stool samples from a group of 23 elderly care home residents were collected weekly.

Methods

Culture was carried out using cycloserine cefoxitin fructose taurocholate broth. Isolates were tested using multiplex PCR detection of *tpi, tcdA, tcdB* and *cdtA* genes. Toxic activity was also confirmed by cytotoxic immunoassay. Further characterization was performed by PCR ribotyping and the molecular genetic GenoType CDiff test system. This test made possible the detection of deletions in the regulator gene *tcdC* (18bp and 39bp deletions or single base deletion at position 117) and *gyrA* gen mutation associated with moxifloxacin resistance

RESULTS

A total of 7/13 (30.4%) residents were (at least one week) positives for *C. difficile. C. difficile* was detected in 13/30 (43.3%) episodes of diarrhea and 4/13 (30.7%) residents positives for *C. difficile* had previously received an antibiotic therapy.

Four different PCR ribotypes were identified, including PCR ribotype PCR ribotype 027. Only one PCR-ribotype was negative for the cytotoxicity assay and for all toxin genes while all of the other types were positive for both of toxin genes *tcdA* and *tcdB.* In addition, type 027 contained *cdtA* gene encoding for the binary toxin and 39 bp deletion in the regulator gene *tcdC*, which is associated with an increased production of toxin and hyper-virulent *C. difficile* strains. All of PCR ribotypes 027 isolated and the non-toxigenic PCR ribotypes UCL36 had a mutation in the *Gyr1A* gene. This mutation is related with moxifloxacin resistance.

Table 1. PCR-ribotypes, toxin activity and gene profile of *C. difficile* isolated from stool samples

PCR-ribotype	No. isolates	Detection of toxin genes by PCR			Genotype CDiff test system										
		tcdA	*tcdB*	*cdtA*	*tcdA*	*tcdB*	*cdtA*	*cdtB*	*tcdC*18 bp	*tcdC*39 bp	*tcdC*117 bp	*gyrA* WT	*gyrA* mut 1A	*gyrA* mut 1B	ECP
027	36	POS	POS	POS	POS	POS	POS	POS	POS	neg	POS	neg	POS	neg	POS
UCL16a	9	POS	POS	neg	POS	POS	neg	neg	neg	neg	neg	POS	neg	neg	POS
UCL36	10	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	neg	POS	neg	neg
UCL46	2	POS	POS	neg	POS	POS	neg	neg	neg	neg	neg	POS	neg	neg	POS

CONCLUSIONS

In summary, this study shows that toxigenic and hyper-virulent *C. difficile* strains are present in a Belgian nursing home. Among the types identified, 027 were the most prevalent PCR-ribotypes which are among the four most prevalent ribotypes of *C. difficile* isolated from patients in Europe.

ACKNOWLEDGEMENTS

The authors would like to thank the Nursing Home Sainte-Joséphine (Theux-Belgium) for permission and support to sample.