POTENTIAL VULNERABILITY FACTORS IDENTIFIED IN LARGE CLOSTRIDIAL TOXIN-NEGATIVE, BINARY TOXIN-PRODUCING C. difficile STRAINS

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BACKGROUND AND AIMS

The relevance of large clostridial toxin-negative, binary toxin-producing A/B-CDT+ C. difficile strains in human infection is controversial. A/B-CDT+ C. difficile strains [Fig 1] are considered clinically irrelevant despite their detection in symptomatic individuals and diarrheic animals.1, Recently, we reported the presence of multiple AMR genes in these strains1. Here, we investigate other putative virulence traits that may contribute to their role in idiopathic diarrhea.

METHODS

• Phenotypic assays [motility, hydrolytic enzymes, in vitro and in vivo studies] were conducted on 148 A/B-CDT+ C. difficile strains comprising 10 ribotypes [RTs 033, 238, 239, 288, 585, 586, QX143, QX444, QX521, QX629] including reference strain RT 078 [Fig 2]. A/B-CDT+ RTs 033 and 288 had deletions in the F2 [gycosylation genes] and F3 [early-stage flagellar genes] regions of their flagellar operon while RTs 238, 585, 586, QX143, QX444, QX521, QX629 lacked the F2 region, retaining F1/F3 regions.
• The flagellar and the motility characteristic was conserved only in RT 239 and C. difficile references RTs 012 and 027 [Fig 2].
• The flagellin and flagella cap genes, flfC and flfD, involved in adenohoe and host colonisation, were conserved in all strains.

EXTRACELLULAR ENZYMES

• All A/B-CDT+ C. difficile isolates produced at least 3 extracellular enzymes [deoxyribonuclease, esterase, mucinase], indicating that these are important extracellular proteins for these strains.
• Hyaluronidase and gelatinase were produced by 93/118 [RTs 033, 238, 288, 585, QX444, QX521] and 25/118 [RTs 033, 238, 288] A/B-CDT+ C. difficile isolates respectively.
• No C. difficile isolates produced lecinthinase, elastase or heparinase.

RESULTS

MOBILITY

• Most [8/10] A/B-CDT+ RTs tested were non-motile [RTs 033, 238, 288, 585, 586, QX143, QX444, QX521, QX629] including reference strain RT 078 [Fig 2]. A/B-CDT+ RTs 033 and 288 had deletions in the F2 [gycosylation genes] and F3 [early-stage flagellar genes] regions of their flagellar operon while RTs 238, 585, 586, QX143, QX444, QX521, QX629 lacked the F2 region, retaining F1/F3 regions.

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In vitro and in vivo assays

• Toxicity of the A/B-CDT+ C. difficile strains was confirmed in Vero cells [Fig 3] but not reproduced in vivo.
• Mice infected with A/B-CDT+ C. difficile strains all survived infection despite detection of high numbers of spores [107 CFU/g] in the faeces at either 24h or 96h post-infection [Fig 4].
• None had diarrhoea with the exception of mice infected with RT UK585 [ES 0618]. These mice had soft faeces/diarrhoea 24h post-infection and showed weight loss, however, they recovered.
• Despite successful colonisation by most of the strains [Fig 4], there was no evident disease phenotype. It is possible that the mouse model does not adequately demonstrate disease caused by A/B-CDT+ C. difficile strains.


FIG 3. Cytolytic effect observed after exposing Vero cells to C. difficile Frlates.

REFERENCES


CONCLUSION

• We have confirmed toxin production and the presence of genes and/or proteins associated with survival, colonisation and disease pathogenesis in toxigenic C. difficile strains 2-6,7,9,10,11
• This study provides the first in-depth analysis of A/B-CDT+ C. difficile strains and highlights the need to further investigate their role in disease.