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S. Bejaoui ¹, S. Persson ², J. Larsen ², D. Frees ¹.

¹University of Copenhagen, Department of Veterinary and Animal Sciences, Section of Food Safety and Zoonoses - Frederiksberg (Denmark), ²Statens Serum Institut, Department of Bacteria, Parasites and Fungi - Copenhagen (Denmark)

Background

Clostridioides difficile is a toxin- and spore-forming bacillus cause of severe and recurrent enteric infections. PCR ribotype 078 (RT078) and its main sequence type 11 (ST11), is associated with a rising number of infections onset in the community, in young and healthy individuals. Farm animals have recently been identified as RT078 reservoirs. In particular, intensive antibiotics use in farming leads to increased resistance and a selective pressure for spreading this pathogen to humans. The aim of this study is to investigate the zoonotic potential of *C. difficile* and its role in horizontal transfer of antimicrobial resistance genes.

Methods

A total of 524 fecal samples were received in two batches from pig farms across Denmark during 2020 and 2021. Batch A (n=330) included samples from sows, piglets and slaughter pigs from thirteen farms. Samples in batch B (n=194) were collected during slaughtering. Samples were screened for *C. difficile* presence and positive isolates were subjected to whole genome sequencing (Illumina) to determine their multilocus sequence type (MLST), toxins and resistance genes. Core genome MLST (cgMLST) was used to compare the veterinary isolates to 921 human clinical isolates from the same period.

Results

Currently, *C. difficile* was isolated from 22 samples - twenty from batch A and two from batch B. All isolates were toxigenic (TcdA+, TcdB+), ten were also binary toxin (cdtA/B) positive. Nine different sequence types (ST) were found (numbers in brackets): ST11(11), ST6(3), ST7(1), ST8(1), ST13(1), ST14(1), ST36(1), ST44(1), ST49(1), all also common in humans (Figure 1). cgMLST distance between pig and the closest human isolate varied and was minimal for the ten ST11, i.e. zero to three alleles difference (Figure 2). Twenty isolates contained at least one resistance gene and in total six different genes were observed, most common were genes towards vancomycin, macrolides and tetracycline, which were also found in humans.

Conclusions

ST11 was the most common overlapping type in porcine and humans in Denmark and eleven isolates were within possible cgMLST transmission range. The presence of multiple and shared resistance genes indicate that *C. difficile* plays a role as a zoonotic reservoir of antimicrobial resistance and in gene exchange.

National *C. difficile* sentinel-surveillance based

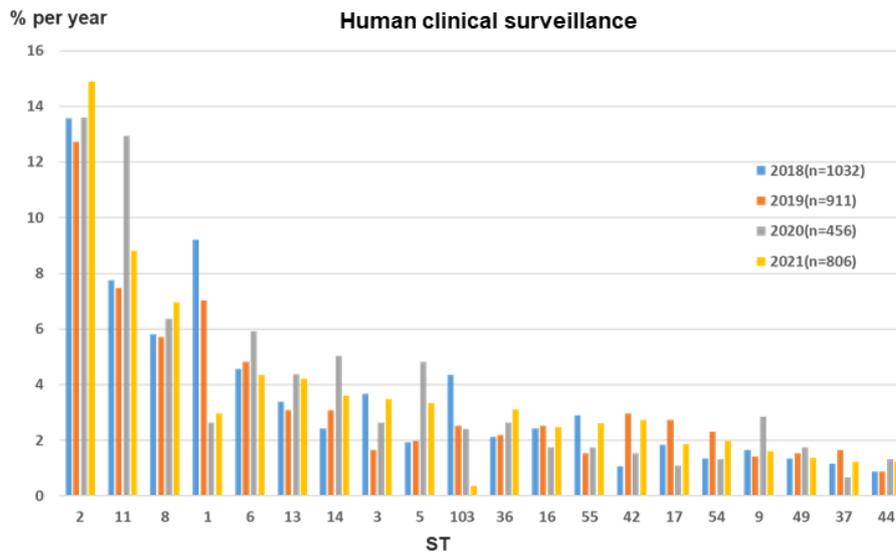


Figure 1. Data on four years of national *C. difficile* sentinel-surveillance based on WGS/MLST

cgMLST comparison

