Clostridioides difficile in GB pigs and risks to the food chain

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1. Introduction

Clostridioides difficile infection (CDI) in humans is usually associated with hospital settings. A world-wide increase in community acquired CDI indicates a source of *C. difficile* exposure outside of hospital settings. The bacterium's emergence in animals, farms, and food classifies as a One Health pathogen. Global prevalence in pigs has been well documented in Australia, USA, Canada and European countries, which aid in developing interventions to limit contamination and transmission. However, no prevalence information is available for pig and pork production in the Great Britain. This poster presents preliminary findings.

3. Objectives

3.1 Sampling of farms and abattoirs for *C. difficile.* Recruitment of 22 farms and 9 abattoirs to the study for sample collection and *C. difficile* isolation.

3.2 Analysis of *C. difficile* isolates To characterise *C. difficile* isolates by toxigenicity, antimicrobial profiling and ribotyping.

3.3 Whole Genome Sequencing. To compare human, pig and environmental isolates and ,where relevant, determine directionality of transmission.

2. Aims

This project aims to establish a baseline prevalence of *C. difficile* in GB pig farms and abattoirs, to better understand bacterial transmission in different biomes, and risk to the food chain.

4. Methodology

4.1 On-farm sampling

Floor faeces from indoor farrowing crates, and straw bedding from outdoor farrowing arcs with piglets ≤ 1 week old were collected. Soil and puddle water from around pig sheds and surrounding area were collected. *C. difficile* was isolated by enrichment and selective culture anaerobically using a Whitley workstation (Don Whitley) and identified by MALDI-TOF mass spectrometry (MS) (**Fig. 1**).

4.2 Abattoir sampling

Scald tank water (STW), caecal and carcass swabs of slaughtered pigs were sampled. *C. difficile* was isolated and identified as above (**Fig. 1**)

4.3 Analysis of *C. difficile*

Isolates were characterised by multiplex polymerase chain reaction (PCR) for toxin profiling, and ribotyped. Selected ribotypes will be sequenced by whole genome sequencing (**Fig. 1**)

4.4 Antimicrobial resistance

Isolates were characterised for antimicrobial susceptibility by agar dilution against 5 antimicrobials (CLSI and Eucast) (**Fig. 1**)

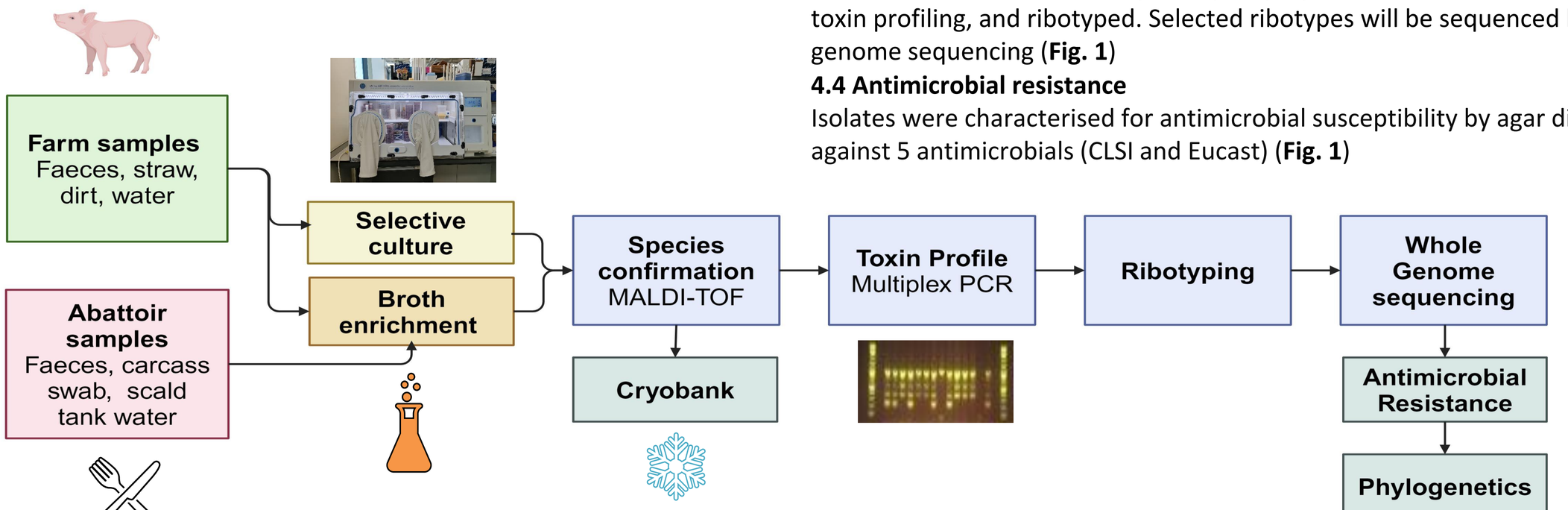

Figure 1. Sampling methods for isolation and identification of *C. difficile*

5. Results

C. difficile was isolated from all farm and abattoir sample types collected so far; 73 of 76 farm isolates were toxigenic and 18 of 19 abattoir isolates were toxigenic **(Table 1)**. Ribotyping of selected isolates revealed unique or overlapping farm and abattoir prevalences **(Table 2)**. Antimicrobial susceptibility profile of isolates showed 10.5% of farm and 33.3% of abattoir were multidrug resistant **(Table 3)**.

Table 1. Farm and abattoir samples positive for *C. difficile*

Positivity	Farm				Abattoir		
	Faecal	Straw	Soil	Water	STW	Ceacal	Carcass
Number	66/92	70/77	54/63	12/66	6/21	9/360	6/362
%	71.7	90.0	85.7	18.2	28	2.5	1.8

Table 2. Ribotype prevalence (%) on farms and in abattoirs

Ribotype	Farm	Abattoir
078	66.1	16.7
193	1.7	5.6
023	1.7	5.6
018	1.7	5.6
045	13.5	0
005, 002	5.1	0
038	3.4	0
026	1.7	0
015	0	44.1
014	0	11.1
087	0	5.6
081	0	5.6

Table 3. Antimicrobial resistance of farm and abattoir isolates

Antibiotic	% farm (n = 86)	% abattoir (n = 18)
Ciprofloxacin	3.5	5.6
Vancomycin	8.2	11.1
Metronidazole	9.3	11.1
Cefotaxime	33.7	77.8
Tetracycline	54.7	55.6

References

1. Knight & Riley (2019). Genomic Delineation of Zoonotic Origins of *Clostridium difficile*. *Frontiers in Public Health* Jun 20:7:164.

2. Lim et al., (2020). *Clostridium difficile* and One Health. *Clinical Microbiology and Infection* Jul;26(7):857-863.

3. Candel-Perez et al., (2019). A review of *Clostridioides* (Clostridium) *difficile* occurrence through the food chain. *Food Microbiology* Feb:77:118-129.

6. Discussion

- Preliminary prevalences of *C. difficile* in breeding farms with piglets ≤ 1 week old, and in environmental samples are similar to other reports ≤100 % and ≤87.5%, respectively) [1].
- Prevalence of *C. difficile* in abattoirs is similar to other reports (≤ 28%) [3].
- RT078 was most prevalent in farms [1], and RT015 in abattoirs.
- 11% of isolates were resistant to CDI treatment antimicrobials, greater than those reported in Europe (< 9%) [2].
- Methodology, sample sites, and age of piglet may contribute to differences in detection.

7. Future work

- Recruit more farms for sampling
- Further toxicity, ribotyping and antimicrobial profiling of isolates
- Whole genome sequencing
- Genomic comparisons between pig and human isolates

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