

PUBLIC HEALTHWORKER SAFETY

Title: Epidemiology, Toxino- and Geno-typing of *Clostridium difficile* in swine at farm, slaughter and retail - NPB #07-044 revised

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Scientific Abstract

Clostridium difficile is a gram-positive spore-forming anaerobic bacillus pathogenic to humans and animals. The role of pork in *C. difficile* dissemination has not been thoroughly investigated. Specific aims of this project are to investigate whether pigs carry hypervirulent strains of *C. difficile* and to compare phenotypic and genotypic attributes of strains of human and porcine origin. Fecal samples (n=251) were collected from swine farms in Ohio (n=3 farms) and North Carolina [NC] (n= 5 farms) at farrowing, nursery, and finishing stages. Bacteriology was done using conventional approaches. Antimicrobial susceptibility was tested using Epsilometric test for ciprofloxacin, erythromycin, metronidazole, vancomycin, tetracycline, and ampicillin. Prevalence of 74.5% within farrowing piglets, 0.45% within nursery pigs, and 0% within finishing pigs were found. Within farrowing pigs, the prevalence was significantly higher in Ohio (88.5%) than in NC (65.6%). Multi-drug resistance was uncommon although most samples, 81.5% (106/130) were resistant to ciprofloxacin with MIC >32 mg/L. Genotypically, the majority of isolates, 82.6% (428/518), were toxin A+B+. Majority of the isolates (82.5%; 161/195) were found to carry *C. difficile* strains which are Toxinotype V, binary toxin positive and have a 39 bp deletion in the *tcdC*, a down regulator gene for enterotoxin and cytotoxin (*tcdA* and *tcdB* respectively) production. None of the isolates were of the epidemic strain of human health significance that shows 18bp deletion in the downregulator, *tcdC* gene. Twenty-four pigs (12.3%) were also found to carry more than one strain of *C. difficile*. Characterization of human isolates (n=24) showed two strain types similar to those found in pigs: Toxinotype O and nontoxigenic. Pulsed Field Gel Electrophoresis (PFGE) findings show a high level of genotypic diversity among isolates of swine origin, with clustering among different farms. These results also show a group of Toxinotype V strains of swine origin with 100% similarity to CDC NAP7 isolate of human origin, a designated strain but distinctly different from the epidemic one (NAP-1). While the absence of *C. difficile* at finishing stage of production is encouraging, the occurrence of hypervirulent strains in swine indicates the public health significance of *C. difficile* of porcine origin.

These research results were submitted in fulfillment of checkoff-funded research projects. This report is published directly as submitted by the project's principal investigator. This report has not been peer-reviewed.

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