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Intrinsic and acquired antimicrobial resistance repertoire of Australian isolates of *Clostridium difficile* identified on whole genome sequencing

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OBJECTIVES: A 2013 report from the CDC ranks *Clostridium difficile* as the most important antimicrobial resistant threat to public health in the USA (1). In this study, whole genome sequencing (WGS) was used to investigate intrinsic and acquired antimicrobial resistance in *C. difficile* in Australia.

METHODS: A total of 171 *C. difficile* strains isolated from humans (*n*=104) and livestock (*n*=67) in the last decade was studied. WGS was performed using standard illumina protocols (2). Acquired antimicrobial resistance genes were identified using SRST2 (3). To identify intrinsic resistance, and to characterise the genetic elements underlying any acquired resistance, annotated *de novo* assemblies were interrogated against a custom Tn/recombinase sequence library with manual curation (4-5). Minimum inhibitory concentrations were determined using the CLSI agar dilution methodology and CLSI/EUCAST breakpoints (6-8).

RESULTS: Fluoroquinolone resistance was observed in 8.2% of isolates (*n*=14, all human sequence type (ST) 11) and conferred by mutations in *gyrA and gyrB.* Forty-six isolates (26.9%) showed a MLS_B phenotype, 29 of which (human ST11 and porcine STs 2,13 and 49) harboured *ermB* genes carried on *Tn*6194-like elements. The remaining 17 isolates with a MLS_B phenotype were negative for *ermB*/*Tn*6194. Tetracycline resistance (tetR) was observed in 29.2% of isolates (*n*=50). *tetM* was found in 47 of 50 tetR isolates, carried on *Tn*916-like (human ST11) and *Tn*5397-like (porcine STs 2,13 and 49) elements. Of the *tetM* positive strains, 48.0% (*n*=24) harboured the efflux gene *tet40* from *Strep. suis*, whilst 14.0% (*n*=7) carried *tetW* from *Tn*B1230 of the ruminent anaerobe *Butyrivibrio flavescens*. Despite *C. difficile* being inherently resistant to aminoglycosides, 23.0% (*n*=40) of isolates carried an aminoglycoside-streptothricin resistance cassette (*ant6-Ia,aph3-III,Sat4A*). All isolates harboured gene clusters for bacitracin and tellurium resistance and carried the *blaR* gene conferring intrinsic resistance to penicillins and nearly all cephalosporins. Notably, all pig isolates carried the cryptic *cme* gene which confers multidrug resistance in *E. faecalis.* All isolates were susceptible to vancomycin, metronidazole, rifaximin, fidaxomicin, meropenem, Augmentin and piperacillin-tazobactam *in vitro*.

CONCLUSIONS: WGS demonstrated that *C. difficile* posseses a diverse repetoire of intrinsic and acquired mechanisms of resistance to clinically relevant antimicrobials, some of which are expressed phenotypically.

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