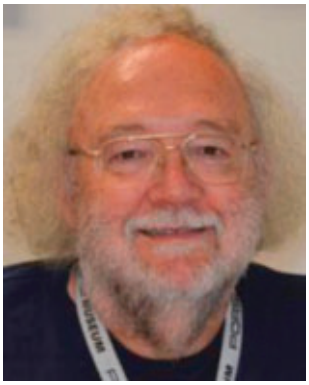
The impact of antimicrobial resistance on induction, transmission and treatment of *Clostridium difficile* infection





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***Clostridium difficile* infection (CDI) of the gastrointestinal (GI) tract is a potentially life-threatening disease that has surpassed multi-drug-resistant *Staphylococcus aureus* as the commonest antimicrobial-resistant organism associated with healthcare[1]. This obligate anaerobic spore-forming Gram-positive bacillus colonises the GI tract and its numbers increase after disruption of the commensal GI microbiota often induced by exposure to antimicrobial agents[2]. Paradoxically, the disease that may follow its outgrowth necessitates further antimicrobial treatment. Already a major challenge to infection prevention and control strategies, there are indications that *C. difficile* is developing further resistance to currently used antimicrobial agents.**

C. difficile (Figure 1) produces toxins that cause a wide spectrum of disease, ranging from mild to self-limiting diarrhoea through to severe complications including pseudomembranous colitis (PMC) (Figure 2), toxic megacolon and death[2]. Historically, clindamycin and third-generation cephalosporins have been recognised for their propensity to promote CDI[3]; however, almost all antimicrobial agents have been implicated. In Australia, treatment with metronidazole and vancomycin remains the preferred options as first-line therapy drugs for mild-to-moderate and severe cases of CDI, respectively[4]. Resistance to multiple antimicrobials represents a selective advantage for the emergence of new *C. difficile* strains.

The prevalence of CDI outbreaks has risen since the early 2000s associated with the appearance in multiple countries of an epidemic strain of *C. difficile*, PCR ribotype (RT) 027[2]. This strain displays high levels of fluoroquinolone (FQ) resistance not seen previously and infection has been associated with increased

Figure 1. Sporulating *Clostridium difficile* (courtesy of Professor SP Borriello).

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Figure 2. Pseudomembranous colitis, a serious outcome of *C. difficile* infection.

morbidity and mortality leading to description of the strain as 'hyper-virulent'[2]. The increased virulence may be due to enhanced production of toxins A and B *in vitro*, in addition to the presence of binary toxin. Despite improved clinical management strategies for CDI, healthcare costs for treating CDI remain high. *C. difficile* is now recognised by the Centers for Disease Control and Prevention as the most urgent public health threat in the USA, causing more than 453 000 infections per year with approximately 29 300 deaths and $4.8 billion in excess medical costs per year[5].

The emergence and rapid transmission of *C. difficile* strains resistant to multiple antimicrobials is now a significant problem worldwide. *C. difficile* has evolved multiple mechanisms for antimicrobial resistance (AMR) including chromosomal mutations and acquired mobile genetic elements (MGE), causing alterations in the targets of antimicrobials and/or in metabolic pathways. Rates of AMR in *C. difficile* vary considerably between studies, related to geography and antimicrobial policy. In this short review, important classes of antimicrobial agents will be discussed in relation to their role in inducing and treating CDI.

Antimicrobials that incite CDI

β-Lactams

Broad spectrum β-lactam antimicrobials such as aminopenicillins and cephalosporins are known for their propensity to cause CDI[3]. Cephalosporin resistance is a hallmark of *C. difficile;* data extrapolated from previous studies revealed that the majority of *C. difficile* isolates tested were resistant (95% second generation; 38% third generation) and there is usually significant clustering around the breakpoint[6]. Thus, *C. difficile* is often described as 'intrinsically resistant' to cephalosporins, mediated by class D β-lactamases and a β-lactam inducing penicillin binding protein encoded on *cdd* and *blaR* genes, respectively[7,8].

Macrolide-lincosamide-streptogramin B (MLS_B)

The macrolide-lincosamide-streptogramin (MLS) B class of antimicrobials contains structurally different but functionally similar drugs, that all bind to the 50S ribosomal subunit[9]. Clindamycin was the first antimicrobial agent linked to PMC in the mid-1970s[2]. Although the use of clindamycin has declined since its close association with CDI was first described, clindamycin resistance remains common[10]. In a recent review by Spigaglia *et al.*, 51.1% of *C. difficile* clinical isolates in 46 papers published between 2012 and 2017 were resistant to clindamycin[6]. Resistant strains exhibit varying minimal inhibitory concentrations (MICs) ranging from 16 to ≥265 mg/L between RTs and often according to the geographical location[6].

In *C. difficile*, resistance to the MLS_B family is usually conferred by erythromycin methylase B, encoded by *ermB*, which prevents drug binding by methylation of bacterial 23S rRNA. The *ermB* gene is often located on MGEs such as Tn*5398* and Tn*6194*, with multiple genetic organisations with no clear RT association[11]. However, clindamycin-resistant *ermB* negative and clindamycin-susceptible *ermB* positive strains of *C. difficile* have been reported[12]. Thus, alternative mechanisms of resistance may be present in *C. difficile*.

Erythromycin is a macrolide that inhibits protein synthesis. Macrolides alone do not have a strong association with CDI; however, the true risks are likely underestimated given that macrolides are often co-administered with other antimicrobial agents[13]. Similar to clindamycin, erythromycin resistance may be mediated through the expression of *ermB*, as well as *ermFS*, mutation in 23S rDNA and a possible *cme* efflux pump[11,14,15].

FQs

FQ resistance is well documented in *C. difficile* owing to the FQ-resistant epidemic strain of *C. difficile* RT027. FQs such as ciprofloxacin and moxifloxacin target DNA gyrase (*gryA* and *gryB*) and prevent the synthesis of enzymes responsible for supercoiling bacterial DNA. Resistance to FQs in *C. difficile* is invariably due to alterations in drug target structure via nucleotide substitutions of *gyrA* and/or *gyrB* within the quinolone-resistance determining region of DNA gyrase subunits. The selective pressure for resistance was likely due to heavy reliance on FQs in a clinical environment, particularly with levofloxacin being one of the most commonly prescribed antimicrobials in North America during the late 1990s and early 2000s[16]. More recently, Freeman *et al.* reported 35.8% of 2694 of *C. difficile* isolates collected over 3 years were resistant to

moxifloxacin, in multiple RTs but particularly in RTs 001, 018, 356, 017 and 198[10]. The detection of FQ resistance is now an important epidemiological target for the identification of epidemic *C. difficile* strains.

Antimicrobials for CDI treatment

Metronidazole

Metronidazole remains the primary drug of choice in the treatment of mild-to-moderate CDI in Australia although in the USA metronidazole is no longer recommended for first-line treatment[17]. The key mode of action is direct DNA damage following reduction of its nitro group once inside the bacterium[18]. Since metronidazole has been a mainstay of therapy for CDI for over 30 years, reduced efficacy has been reported. In a prospective observational study of 207 CDI patients, only 50% were successfully treated, and 22% continued to experience symptomatic CDI despite ≥10 days of treatment. Furthermore, 28% of patients experienced symptomatic recurrence within 90 days[19]. Publications from France[20], Spain[21] and elsewhere have reported clinical isolates with reduced susceptibility to metronidazole, however, observations of metronidazole resistance *in vitro* are scarce. A recent pan-European survey of antimicrobial susceptibility in 2694 *C. difficile* isolates over 3 years reported 0.2% were resistant to metronidazole, mostly RT027 and its close relative RT198[10]. In addition, resistance to metronidazole appears to be heterogeneous within a population and MICs were largely dependent on the antimicrobial susceptibility method[21]. More important, *C. difficile* strains isolated from patients who failed metronidazole therapy appeared to have similar MICs to those isolated from successfully treated cases. Thus, reduced efficacy of metronidazole in the clinical setting was unlikely to be attributed to decreased susceptibility and more likely linked to other bacterial and host factors that remain to be elucidated[22]. Currently, oral metronidazole remains the preferred antimicrobial agent for mild CDI in Europe due to low costs[4].

Vancomycin

Vancomycin is the first-line treatment for moderate to severe CDI[4]. Antimicrobial activity is achieved through inhibiting the biosynthesis of the bacterial cell wall peptidoglycan. Unlike metronidazole, vancomycin is poorly absorbed in the gastrointestinal tract after oral administration leading to high concentrations in the gut and rapid suppression of CDI[23]. The mechanism for vancomycin resistance in *C. difficile* is still unclear but is possibly linked to the possession of a *vanG* homolog ($vanG_{Cd}$). The homolog is inducible by vancomycin *in vitro* but does not promote vancomycin resistance in *C. difficile*[24]. Susceptibility to vancomycin remained high with Freeman *et al.* reporting 98.6% of isolates susceptible[10]. However, there have been sporadic reports of elevated MICs ≥4-8 mg/L from a previous pan-European survey[25], although the underlying mechanism of resistance was not determined. There has been a report of a cryptic *vanB2* gene cassette (Tn*1549*-like) in a *C. difficile* strain isolated from an Australian calf[26]. Notably, vancomycin is now the preferred treatment over metronidazole for an initial episode of CDI as recommended by the Infectious Diseases Society of America and Society for Healthcare Epidemiology of America in 2018[17].

Rifamycins

The rifamycins achieve selective toxicity in bacteria by targeting bacterial DNA-dependent RNA polymerase[27]. Both rifampicin and rifaximin have been used to treat CDI due to very low MICs and they are often used as adjunctive post-vancomycin therapy for recurrent CDI[17]. One potential concern about the use of rifaximin is the rapid development of resistance during treatment[27]. Resistance is likely conferred by RpoB amino acid substitutions that appear to be independently derived rather than disseminated from specific resistant clones[27]. Thus, prolonged use of rifamycins is not recommended.

Fidaxomicin

Fidaxomicin is a macrocyclic narrow spectrum bactericidal agent that targets bacterial RNA polymerase[28]. It is the first drug to be licensed and recommended for CDI treatment for adults in over 25 years[28] and it is currently the recommended treatment for an initial episode of CDI in the USA along with vancomycin[17]. Fidaxomicin offers lower recurrence rates in CDI patients compared to vancomycin and metronidazole[29], and reduced susceptibility to fidaxomicin is very rare[17]. It has minimal impact on the native gut microbiota, sparing the *Bacteroides* group, and achieves high concentrations in the gut and faeces. The use of fidaxomicin has been limited due to its high cost.

AMR in *C. difficile* and One Health

C. difficile colonises the gastrointestinal tracts of all animals during the neonatal period, multiplies and is excreted, but cannot/does not compete well when other bacterial species start to colonise. CDI should always have been considered a zoonosis, either direct or indirect. The One Health concept is a worldwide strategy for interdisciplinary collaboration and communication in all aspects of healthcare for humans, animals and the environment[30]. In recent years, 70% of emerging or re-emerging infections have been vector-borne or zoonoses – animal diseases transmissible to humans[31]. Adult humans treated with antimicrobials fool *C. difficile* into thinking it is colonising a neonatal gut. In the

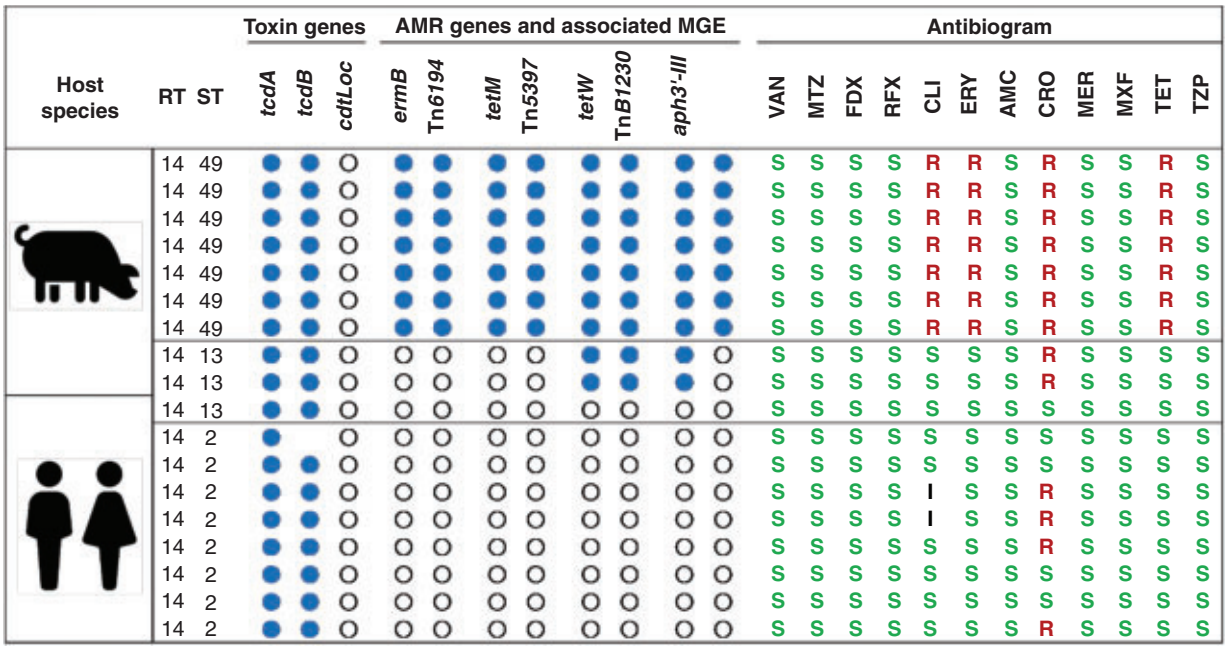

Figure 3. Comparative analysis of AMR genotype and phenotype in *C. difficile* RT014 isolated from pigs and humans with CDI in Victoria, Australia in 2013. All strains also showed MICs of >16 mg/L to trimethoprim, gentamicin, tobramycin and spectinomycin (no breakpoints are currently available, data not shown). The genomic context for aminoglycoside resistance genes (*aph3'-III*, *sat4*, and *ant6*) was a multidrug cassette from *Erysipelothrix rhusiopathiae* isolated from swine. ST13 was found in both humans and pigs. RT, PCR ribotype; ST, multi-locus sequence type; *tcdA*, toxin A gene; *tcdB*, toxin B gene; *cdtLoc*, binary toxin locus; S, susceptible; I, intermediate; R, resistant; VAN, vancomycin; MTZ, metronidazole; FDX, fidaxomicin; RFX, rifaximin; CLI, clindamycin; ERY, erythromycin; AMC, amoxicillin-clavulanate; CRO, ceftriaxone; MEM, meropenem; MXF, moxifloxacin; TET, tetracycline; TZP, piperacillin-tazobactam. (●), Present; (○), absent.

1980s and 90s, there was an expansion of CDI in hospitals driven by cephalosporins to which *C. difficile* is intrinsically resistant (see section on β-lactams). Since 1990 in North America, cephalosporins have been licensed for use in food animals. There has been amplification of *C. difficile* in food animals, with subsequent contamination of meat, and vegetables grown in soil containing animal faeces[32]. Animal strains of *C. difficile* are now infecting humans causing a rise in the incidence of community-acquired CDI[33]. In Australia, tetracyclines are used commonly in food animals[34] and *C. difficile* RT014 human and porcine strains isolated in Australia demonstrated tetracycline resistance[8].

The tetracycline family of antimicrobials has broad activity against Gram-positive and Gram-negative bacteria, including anaerobes, and tetracycline resistance occurs frequently in many organisms[35]. Whilst tetracycline is not usually associated with the induction of CDI, nor used for treatment, tetracycline resistance has been reported in *C. difficile* and is transferable between strains[36]. Resistance in *C. difficile* is mostly mediated by *tet* genes carried on transposable elements related to Tn*916* that encodes the TetM cytoplasmic protein that protect ribosomes from tetracycline binding[35]. Figure 3 is a comparative analysis of AMR genotype and phenotype in *C. difficile* RT014 strains isolated from pigs and humans in Australia in 2013 (Knight *et al*. unpublished), and clearly shows macrolide and tetracycline resistance genes concentrated in porcine isolates.

Conclusion

Antimicrobial agents are an intrinsic part of CDI; in its induction, transmission and treatment. The widespread emergence of hypervirulent strains around the world has highlighted the importance of AMR in facilitating the spread of epidemic *C. difficile* clones. There are worrying trends of increasing resistance to the current therapies used to treat this infection. The contribution of antimicrobial use in production animals and how this contributes to the emergence and spread of new strains of *C. difficile* is underappreciated. A One Health approach could alleviate pressures towards the development of further AMR in this significant healthcare pathogen.

Conflicts of interest

The authors declare no conflicts of interest.

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Biographies

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