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Antimicrobial resistance in Clostridium difficile ribotype 017

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Structured abstract

Introduction

Antimicrobial resistance (AMR) played an important role in the initial outbreaks of *Clostridium difficile* infection (CDI) in the 1970s. *C. difficile* ribotype (RT) 017 has emerged as the major strain of *C. difficile* in Asia, where antimicrobial use is poorly regulated. This strain has also caused CDI outbreaks around the world for almost 30 years. Many of these outbreaks were associated with clindamycin and fluoroquinolone resistance. AMR and selective pressure is likely to be responsible for the success of this RT and may drive future outbreaks.

Areas covered

This narrative review summarizes the prevalence and mechanisms of AMR in *C. difficile* RT 017 and transmission of these AMR mechanisms. To address these topics, reports of outbreaks due to *C. difficile* RT 017, epidemiologic studies with antimicrobial susceptibility results, studies on resistance mechanisms found in *C. difficile* and related publications available through Pubmed until September 2019 were collated and the findings discussed.

Expert opinion

Primary prevention is the key to control CDI. This should be achieved by developing antimicrobial stewardship in medical, veterinary and agricultural practices. AMR is the key factor that drives CDI outbreaks, and methods for the early detection of AMR can facilitate the control of outbreaks.

Keywords: antimicrobial resistance, *Clostridium difficile*, outbreak,prevention, ribotype 017

Highlights

- Most outbreaks of *C. difficile* infection (CDI) in the past have been associated with antimicrobial resistance (AMR).
- *C. difficile* ribotype (RT) 017 displays a higher prevalence of AMR than other RTs.
- An increase in AMR prevalence in *C. difficile* RT 017 increases the risk of future outbreaks and may complicate treatment options for CDI.

1. Introduction

Clostridium difficile is an important cause of diarrhea associated with antimicrobial use worldwide [1]. This anaerobic Gram-positive bacillus is capable of producing spores that can withstand desiccation and heat, and persist in the environment for long periods of time [2]. The organism has been recently renamed *Clostridioides difficile* [3], however, both *Clostridium difficile* and *Clostridioides difficile* remain valid names, and either can be used when referring to the bacterium [4]. Disease caused by *C. difficile* ranges from self-limiting diarrhea to life-threatening pseudomembranous colitis (PMC), toxic megacolon and death. Since the early 2000s, *C. difficile* has been a major public health threat worldwide, responsible for almost half a million cases of diarrhea and 29,000 deaths annually in the United States alone [5].

C. difficile infection (CDI) is mainly mediated by toxins in the gastrointestinal tract (GIT). Currently, there are three major toxins recognized: toxin A, toxin B and binary toxin [6,7]. Growth of *C. difficile* in the GIT is generally suppressed by the intestinal microbiota, a process known as colonization resistance, and it does not cause disease under normal circumstances. It is only when the patient is exposed to antimicrobials, or some other agent that disturbs the intestinal microbiota, that surviving ingested *C. difficile* spores can germinate, replicate, produce toxins and cause disease [1]. Antimicrobial resistance (AMR) has been an important factor contributing to the pathogenesis of CDI and the spread of *C. difficile*, and intrinsic cephalosporin resistance was critical in the rise of CDI as a hospital-acquired infection in the 1980s [8-10].

Besides intrinsic resistance to cephalosporins, acquired AMR has been a key factor in driving genetic diversity and epidemiological changes in CDI around the world, with many well-publicized outbreaks. Acquired resistance to clindamycin was associated with early CDI outbreaks in the USA [11-13]. Since the early 2000s, strains belonging to the 'hyper-virulent' *C. difficile* ribotype (RT) 027 have caused multiple outbreaks of CDI, initially in North America and subsequently Europe [14,15]. These outbreaks were driven by fluoroquinolone and rifampicin resistance [16,17]. Outbreaks of infection with *C. difficile* RT 078, another epidemic RT commonly associated with zoonotic transmission, have been associated with tetracycline resistance [18,19]. More recently epidemic strains of *C. difficile* RT 018 have been reported in Italy, and in Korea, with both reports noting high-level fluoroquinolone and clindamycin resistance [20,21]. Lastly, a recent outbreak of CDI in Costa Rica was caused by a multidrug-resistant (MDR) lineage of *C. difficile* carrying multiple resistance genes on various mobile genetic elements, including Tn*5397* (containing *tetM*) and Tn*5398* (containing *ermB*) [22].

C. difficile RT 017 is another epidemic RT of *C. difficile* that has caused outbreaks globally since the late 1990s, with disease severity no different to other epidemic *C. difficile* RTs [23]. Many of these past outbreaks caused by *C. difficile* RT 017 were also associated with AMR [24-28]. In non-outbreak settings, *C. difficile* RT 017 has also been reported to have higher rates of resistance to many antimicrobial agents [29-31]. This review summarises AMR found in *C. difficile* RT 017, its role in outbreaks and global spread during the preceding decades, the risk it poses for future outbreaks and the problems it may cause in the treatment of CDI in the future. It should be noted that *C. difficile* RT 017 is more prevalent in Asia and the number of reported strains from other regions is limited [23].

2. Literature Search Methodology

A literature search was performed looking for publications that either (1) reported outbreaks of CDI (both in general and specifically caused by *C. difficile* RT 017) with comments on the role of AMR, (2) reported the prevalence of resistance in *C. difficile* RT 017 to at least one of the key antimicrobials (clindamycin, moxifloxacin, meropenem, linezolid, doxycycline and rifaximin), or (3) reported the mechanisms of resistance of *C. difficile* to these agents and cephalosporins. This review included studies published up to September 2019 that were available through Pubmed, as well as relevant articles cited in those studies.

3. Intrinsic Resistance to Cephalosporins

Cephalosporins are beta-lactam antimicrobials which inhibit bacterial cell wall synthesis. These antimicrobials, especially third-generation cephalosporins, are among the most commonly misused antimicrobials in both medical and agricultural practices due to their broad-spectrum activity [32]. Exposure to cephalosporins is also a significant, perhaps the most significant, risk factor for the development of CDI [9,33]. All *C. difficile* are intrinsically resistant to penicillins and cephalosporins, and a recent study suggested that this resistance is conferred by multiple mechanisms, one of which relates to *C. difficile* class D beta-lactamases (CDD-1 and CDD-2) [8]. Genes encoding putative CDD-1 and CDD-2 beta-lactamases are found in the majority of sequenced *C. difficile* genomes, including RT 017 strains, and the purified proteins were shown to have catalytic activity against a broad range of beta-lactam antimicrobials, including penicillins and first- to fourth-generation cephalosporins. They have limited catalytic activity against cephamycins and carbapenems [8]. Even though whether CDD-1 and CDD-2 can be inhibited by beta-lactamase inhibitors, such as clavulanic acid, was not specified in the study, *C. difficile* has been previously reported to be susceptible to the amoxicillin-clavulanate combination [29], suggesting that the enzymes may be inhibited by beta-lactamase inhibitors, a characteristic which is different from other class D beta-lactamases [34].

4. Acquired AMR Genotypes in *C. difficile*

To date, various studies have investigated the mechanisms of AMR in *C. difficile*. A number of acquired resistance genes targeting various classes of antimicrobial agents has been identified. Table 1 summarizes common resistance genotypes found in *C. difficile* with examples that can be found in *C. difficile* strain M68, a whole-genome sequenced reference strain of *C. difficile* RT 017 [35]. Resistance genotypes can be divided into two groups. Resistance can be conferred by accessory genes located on mobile genetic elements such as conjugative transposons (Tns) [36-41]. These elements are capable of changing their position within the genome and can be transferred horizontally both within and between species, e.g. between *C. difficile* and *Enterococcus faecalis* [42]. Resistance can also be conferred by point mutations in existing chromosomally located genes [28,43-47]. Although incapable of horizontal transfer, these mutations can be transferred vertically and cause clonal outbreaks of resistant organisms.

4.1. Clindamycin

Clindamycin belongs to the macrolide-lincosamide-streptogramin B (MLS_B) group of antimicrobial agents. These agents target the bacterial 50S ribosome and inhibit bacterial protein synthesis. The MLS_B resistance phenotype is common in *C. difficile*; 65.9% – 90.9% of *C. difficile* strains in Asia and 49.6% – 56.6% in Europe are resistant to clindamycin [29,30,48-51]. Resistance is principally conferred by a 23S rRNA methyltransferase encoded by the *ermB* (erythromycin ribosomal methylase B) gene [30]. Methylation of the 23S rRNA of the bacterial 50S ribosomal subunit reduces the binding affinity of MLS_B class antimicrobials. This *ermB* gene is carried on Tn*6194*, Tn*6215*, Tn*6218* and Tn*5398* [36], the latter having two copies [37]. In *C. difficile* strain M68, the *ermB* gene is found on Tn*6194* —a 28k bp Tn, which is the most common *ermB*-containing element found in European clinical isolates of *C. difficile* [52]. The *ermB* gene is capable of horizontal transfer and *C. difficile* can acquire *ermB* from different sources, making it possible for *C. difficile* in different regions of the world to independently acquire an MLS_B resistance phenotype. Besides the

ermB gene, a recent study reported a novel *ermG* gene in 11 *C. difficile* RT 017 isolates. This gene also confers an MLS_B resistance phenotype and is located on a mobile genetic element capable of interspecies horizontal gene transfer [53].

The prevalence of clindamycin-resistant *C. difficile* RT 017 is high throughout the world (Figure 1A). Almost all (92.9 - 100.0%) *C. difficile* RT 017 strains from studies in China, Korea and Europe were resistant to clindamycin [30,48,49]. In Thailand, the prevalence of resistance was lower (66.7% - 86.4%), and comparable to other *C. difficile* RTs in the same study, such as *C. difficile* RT 014/020 and non-toxigenic strains [29,54]. In Europe, *C. difficile* RT 017 was reported to have a higher average MIC for clindamycin than other common RTs [51].

Clindamycin was the first antimicrobial agent to be associated with CDI. It was introduced into the clinical environment in the late 1960s as the improved 7-chloro derivative of lincomycin [55]. Only a few years after its release, clindamycin was reported to be associated with PMC and toxic megacolon [56], before it was known that *C. difficile* was the major causative agent of PMC [57]. Indeed, the apparent transmissibility of PMC sparked an international search for the cause that finally resulted in *C. difficile* being implicated [58,59]. In the case of *C. difficile* RT 017, clindamycin resistance was most likely the major factor driving a number of outbreaks of CDI that occurred during the period 1995 to 2000 [24-26]. Evidence of clindamycin resistance is documented in at least three out of five outbreaks during this period. Studies of the outbreaks in Poland and Argentina reported a high prevalence of *ermB* positive strains [24,25]. In addition, despite lacking genetic information, an investigation of the Netherlands outbreak suggested an association between the use of clindamycin for antimicrobial prophylaxis and the development of *C. difficile* RT 017 infection [26].

4.2. Fluoroquinolones

Fluoroquinolones are bactericidal antimicrobials which inhibit bacterial DNA synthesis by targeting DNA gyrase. Although older fluoroquinolones, such as ciprofloxacin, have limited activity against anaerobic bacteria, including *C. difficile*, third- and fourth-generation fluoroquinolones, such

as moxifloxacin, have greater activity [30]. However, exposure of *C. difficile* to these agents creates selective pressure that drives the rapid development of resistance [60]. During 1991 – 1997, before the introduction of moxifloxacin [61], only 6.6 % (13/198) of *C. difficile* strains from Europe were resistant to moxifloxacin [62]. The prevalence of moxifloxacin resistance among *C. difficile* increased to 37.5% (131/349) in 2005 [63], and subsequently to 39.9% (total n = 918) in 2011 – 2012 [50]. In Asia, the prevalence of resistance was higher, 46.4% (26/56) in China in 2007 – 2008 and over 80% in some Korean studies [64,65].

Resistance to fluoroquinolones in *C. difficile* is typically due to a missense mutation in the quinolone resistance determining region (QRDR) of the DNA gyrase subunit genes (*gyr*), either *gyrA* or *gyrB* [28,43,44]. The *gyrA* mutation is responsible for the majority of fluoroquinolone resistance with the most frequent amino acid substitution in GyrA being at T82I [43], which is close to the S83Y and S83I substitutions found in *Escherichia coli* [66]. This substitution is responsible for fluoroquinolone resistance in the greatest proportion of *C. difficile* RT 017 [43]. The same substitution was also found in two epidemic lineages of *C. difficile* RT 027 and thought to be the main factor that drove outbreaks in North America and Europe [16]. In 2003, a novel substitution in GyrB was discovered in *C. difficile* RT 017 (D426V) which was thought to be driving an outbreak in Ireland [27,28]. *C. difficile* strain M68, which was isolated from Ireland in 2006, also has a D426V substitution in GyrB [35].

The prevalence of fluoroquinolone-resistant strains of *C. difficile* RT 017 is higher than in other RTs (Figure 1B). More than half the *C. difficile* RT 017 isolates from China in 2012 – 2013 (58.8%; 20/34), Thailand in 2010 – 2015 (83.3%; 10/12 and 77.3%; 17/22), South Korea in 2000 – 2009 (85.3%; 29/34) and South Africa in 2014 – 2015 (97.6%; 124/127) were resistant to moxifloxacin [29,48,49,54,67]. The study in South Korea also reported an association between the introduction of moxifloxacin in 2003 and a shift in the molecular epidemiology of CDI in that country where the prevalence of *C. difficile* RT 001 (9.6%; 5/52 moxifloxacin resistance) decreased and

C. difficile RT 017 (85.3%; 29/34 moxifloxacin resistance) increased [49]. Although the number of isolates was low (19 isolates), all European *C. difficile* RT 017 isolates in 2005 were resistant to moxifloxacin [63]. In a later study focussing on the period 2011 – 2014, *C. difficile* RT 017 had the highest average MIC for moxifloxacin compared to other common RTs in Europe [51].

Fluoroquinolones are among the most commonly abused antimicrobials due to their broad-spectrum and bactericidal activity [68,69]. Third- and fourth-generation fluoroquinolones are also considered antimicrobials at high risk of causing CDI, partly because of the outbreaks of *C. difficile* RT 027 infection in North America and Europe [33]. The risks associated with third- and fourth-generation fluoroquinolones are more related to the development of resistance in *C. difficile* than other factors. Given the evidence of outbreaks associated with the use of moxifloxacin in the past and the increase in moxifloxacin resistance in both *C. difficile* RT 017 and other RTs, the use of third- and fourth- generation fluoroquinolones should be carefully monitored.

4.3. Carbapenems

Carbapenems are bactericidal antimicrobials which inhibit bacterial cell wall synthesis by targeting penicillin-binding proteins. They are broad-spectrum and are generally used for the treatment of nosocomial infections caused by MDR pathogens. Historically, *C. difficile* has been susceptible to imipenem and other carbapenems, however, recent studies suggest that many strains, including *C. difficile* RT 017, are developing resistance (Figure 1C). *C. difficile* RT 017 in China and South Korea was reported to have a higher rate of resistance to imipenem compared to *C. difficile* RTs 001, 012 and 014, although the resistance rates remained low (8.0% – 12.0%) [48,49]. As carbapenems are commonly used for treating nosocomial infections, carbapenem resistance may promote the spread of *C. difficile* in hospitals in the future.

A recent study identified two missense mutations (A555T and Y721S) near the active site of the penicillin-binding protein genes (*pbp1* and *pbp3*) that are associated with imipenem resistance in *C. difficile* RT 017. This study also reported a new class of penicillin-binding protein gene (*pbp5*) that

193 was unique to *C. difficile* RT 017. However, *pbp5* was found in both resistant and susceptible
194 *C. difficile* RT 017 strains and a role for this gene was not identified [45].

195 **4.4. Linezolid and Cadazolid**

196 Linezolid is the first oxazolidinone antimicrobial agent that inhibits bacterial protein
197 synthesis at an early stage [70]. It is commonly used to treat infections caused by MDR Gram-
198 positive bacteria [71]. In general, *C. difficile* is susceptible to linezolid and only a few *C. difficile*
199 strains have been reported to be resistant. A study in Germany suggested an overall rate of
200 resistance to linezolid in *C. difficile* of 5.7% (11/192) [72], while in Spain only one of 44 *C. difficile*
201 strains (2.3%) was linezolid-resistant [73]. In a follow-up study that included 891 *C. difficile* strains,
202 there were only nine linezolid-resistant strains (1.0%), however, six of these (66.7%) were *C. difficile*
203 RT 017 [41]. Further study revealed that all six linezolid-resistant *C. difficile* RT 017 strains harbored a
204 *cfr* (chloramphenicol-florfenicol resistance) methyltransferase gene found on mobile genetic
205 elements [41]. The *cfr* methyltransferase gene is the only linezolid resistance determinant known to
206 be transferred horizontally. Besides linezolid, methylation of 23S rRNA by Cfr also confers resistance
207 to phenicols, lincosamides, pleuromutilins and streptogramin A [74]. Given the increased use of
208 linezolid for the treatment of MDR gram-positive bacterial infections, and the transferable property
209 of the *cfr* gene, it is possible that linezolid resistance will drive an outbreak of *C. difficile* RT 017 in
210 the future [75]. Besides the acquisition of the *cfr* gene, a point mutation in the *rplC* (ribosomal
211 protein L3) gene was also reported to be associated with linezolid resistance in *C. difficile* [46].

212 A close relative of linezolid, cadazolid, has been developed for the treatment of CDI [76].
213 Resistance mechanisms for linezolid and cadazolid are different and linezolid-resistant *C. difficile*
214 may remain susceptible to cadazolid [46], however, in a recent phase III trial the clinical cure rate for
215 cadazolid was inferior to that of vancomycin, the current standard treatment [77], and the future of
216 cadazolid use in the treatment of CDI is now uncertain [78].

4.5. Tetracyclines

Tetracyclines inhibit bacterial growth by targeting the 30S ribosome. Resistance to these agents is common in *C. difficile* and mediated by efflux and ribosomal protective proteins encoded by an array of *tet* (tetracycline) genes such as *tetM*, *tetW*, *tetA(P)* and *tetB(P)* [79]. These genes encode proteins that mimic ribosomal elongation factors thus protecting against the anti-translational activity of tetracyclines. The *tetM* gene is the most common element associated with tetracycline resistance and can be found on Tn*5397*, Tn*5398* and Tn*916*-like elements [38-40,80]. These are among the most widespread Tns that can be transferred between different bacterial species in the colon [81]. Tetracycline-resistance in *C. difficile* RT 017 is most commonly associated with a Tn*916*-like element carrying *tetM* [40]. In *C. difficile* strain M68, the *tetM* gene is found on an 18kb element, Tn*6190,* one of the Tn*916*-like elements.

Tetracycline resistance is more prevalent in Asian compared to European *C. difficile* RT 017 (Figure 1D). More than half the *C. difficile* RT 017 isolates from two studies in China (85.7%; 12/14 and 82.4%; 28/34) and 45.5% (10/22) of strains in Thailand were resistant to tetracycline [30,48,54], while only 27.8% of European isolates were tetracycline-resistant. Nevertheless, the latter was still higher than the prevalence among other RTs from the same region (0.0% – 3.4%) [63].

The overuse of tetracycline has been reported to be a major driver of a clonal expansion of tetracycline-resistant *C. difficile*. A recent study reported that the rapid spread of tetracycline-resistant *C. difficile* RT 078 was associated with the presence of the *tetM* gene and increased agricultural use of tetracycline [19]. The high prevalence of tetracycline resistance in *C. difficile* RT 017 in Asia is also likely associated with the high usage of these agents in the region. Tetracyclines are agents of choice for the treatment of many tropical infections endemic in South-East Asia especially, as well as sexually-transmitted infections [82-84]. These infections are usually caused by non-culturable pathogens and treatment is commonly prescribed without laboratory confirmation of

causative pathogens, leading to an overuse of tetracyclines. In addition, doxycycline is recommended as a chemoprophylactic agent for malaria in various guidelines [85].

4.6. Rifaximin

Rifaximin is a derivative of rifampicin, an antimicrobial that inhibits RNA synthesis. Initially, it was proposed as adjunctive therapy for CDI due to its potent *in vitro* activity against *C. difficile* and low systemic absorption [86]. However, the prevalence of rifampicin and rifaximin resistance has gradually increased and resistance was associated with CDI outbreaks in the United States [17,87]. *C. difficile* RT 017 has been reported to have a higher prevalence of resistance to rifaximin than other common RTs in both Europe and Asia [29,51]. Missense mutations in the *rpoB* gene, which encodes a beta subunit of the RNA polymerase enzyme, reduce the affinity of the enzyme to both rifaximin and rifampicin and confer cross-resistance to the agents. Several amino acid substitutions in *rpoB* have been associated with rifaximin resistance in *C. difficile* [47]. A genomic study of *C. difficile* RT 017 documented R505K and H502N mutations in a third of *C. difficile* RT 017 strains (32.5%; 90/277 and 33.2%; 92/277, respectively). Interestingly, one isolate in this study remained susceptible to rifampicin despite having both the R505K and H502N mutations [88].

Rifampicin is one of the main agents used for the treatment of tuberculosis [89]. Patients with tuberculosis are exposed to rifampicin for at least 6 months and this exposure places selective pressure on any *C. difficile* that may be colonizing these patients. There have been reports of a high prevalence of *C. difficile* RT 017 in tuberculosis hospitals in South Africa [67,90,91]. There is also a high prevalence of tuberculosis in South-East Asia [92]. This and rifampicin usage may be impacting rifaximin resistance among *C. difficile* RT 017 and compromising the possible use of rifaximin for the treatment of CDI due to *C. difficile* RT 017. An example has been reported in *Staphylococcus aureus*, where a rifampicin-resistant subpopulation of colonizing bacteria proliferated within only 2 weeks of the initiation of antituberculous therapy [93].

4.7. Multidrug-resistant (MDR) *C. difficile* RT 017

MDR *C. difficile* refers to *C. difficile* isolates that have acquired resistance to at least three antimicrobial agents [29]. MDR *C. difficile* RT 017 has been a growing problem for more than a decade. A study in European countries in 2005 revealed that *C. difficile* RT 017 was the second most common RT associated with MDR (18.3%; 15/82) with seven isolates (8.5%) being resistant to the MLS_B group of antimicrobials (represented by clindamycin and erythromycin) and moxifloxacin, while eight isolates (9.8%) were also resistant to rifampicin [94]. In 2015, two-thirds (8/12) of *C. difficile* RT 017 isolates in Thailand were MDR [29]. This number was higher than other toxigenic strains in the same study. Furthermore, three clinical studies of *C. difficile* RT 017 infection concluded that resistance to many antimicrobial agents, such as clindamycin, rifampin, co-trimoxazole, first-generation cephalosporins and fluoroquinolones, had a stronger association with *C. difficile* RT 017 compared to other *C. difficile* strains [95-97]. This suggests that the rate of resistance to these antimicrobial agents is higher in *C. difficile* RT 017 than in other RTs in the same region.

5. Impact of AMR on CDI Outbreaks

From the first descriptions of *C. difficile* as a cause of PMC and antimicrobial-associated diarrhea, outbreaks of CDI have been associated with AMR [11-13,16-18,20-22]. Antimicrobial overuse provides selective pressure on *C. difficile* often at a time when the gut microbiota has been depleted. AMR can be easily acquired via horizontal transfer of accessory genes from either another strain of *C. difficile* or other bacteria sharing the gut environment. Some examples include the acquisition of the *ermB* gene and clindamycin resistance phenotype that was associated with multiple CDI outbreaks in the late 1990s [11-13,24-26], or the acquisition of the *tet* gene family and tetracycline resistance phenotype that has shaped the evolution of *C. difficile* RT 078 into an important epidemic RT [18,19].

Some AMR is acquired by point mutations that can be transferred vertically and these have importance in the evolution of various *C. difficile* lineages. The most significant example of this has

been the emergence of fluoroquinolone-resistant lineages of *C. difficile* RT 027 that caused outbreaks in the northern hemisphere in the early 2000s following mutations in the *gyrA* gene that occurred in the early to mid-1990s [16]. Point mutations conferring resistance to both fluoroquinolones and rifamycins are often associated with a low fitness cost that facilitates maintenance of the resistance phenotype in the absence of selective pressure [98,99].

An increase in the prevalence of MDR bacteria is inevitable as more broad-spectrum antimicrobials are used to treat infections. Resistance to these antimicrobials will provide a survival advantage to *C. difficile* and shape its evolution. There is already evidence that this is occurring in *C. difficile* RT 017. *C. difficile* RT 017 is resistant to many antimicrobials [29-31], and it is highly prevalent in Asia where the use of antimicrobials is poorly controlled [23,100]. This could easily lead to further expansion of RT 017 in the region and possible outbreaks in the future. Besides clindamycin and fluoroquinolones that have already been associated with RT 017 outbreaks [24-28], resistance to other antimicrobials, notably carbapenems and linezolid, has the potential to drive outbreaks as there has been greater use of these antimicrobials due to the increased prevalence of MDR organisms [101]. The mechanisms of resistance against these antimicrobials have already been described for RT 017 [41,45], and such an outbreak would be a serious public health threat further complicating a disease that already has a high morbidity and mortality rate [102]. Thus, it is likely that *C. difficile* RT 017 will continue to be a successful RT, not only in Asia but around the world.

6. Conclusion

C. difficile RT 017 is associated with a high prevalence of resistance to multiple antimicrobials. Most resistance can be transferred both horizontally (including between species residing in the colon) and vertically. Some resistance has a low fitness cost and can persist even without selective pressure. AMR has been the major factor behind the success of *C. difficile* RT 017 and it is likely that the high rates of AMR in *C. difficile* RT 017 will drive future outbreaks of infection caused by this RT. In addition, rifaximin resistance may also limit the treatment options for CDI due to *C. difficile* RT 017 in the future.

7. Expert opinion

This narrative review focuses on the role of AMR in promoting the spread of *C. difficile* and the development of CDI, especially CDI due to *C. difficile* RT 017, rather than antimicrobials for the treatment of CDI. The regulation of these inciting antimicrobials is the key to the primary prevention of CDI. CDI is difficult to treat and patients can suffer multiple recurrences even after receiving appropriate treatment [77]. The development of new antimicrobials is underway, but the efficacy of some of these new treatment options remains questionable and they may not make it to market. Thus, an effective primary prevention strategy, such as antimicrobial stewardship, may be a better approach to resolve the problem of CDI.

AMR plays an important role in the spread of *C. difficile* and outbreaks of CDI. By identifying key inciting antimicrobials, a strategy can be developed to control the use of these antimicrobials and subsequently reduce the spread. A successful example of this can be seen in Australia, where fluoroquinolones are strictly regulated resulting in a relatively low prevalence of fluoroquinolone-resistant organisms [103]. However, the regulation of each key antimicrobial has its own difficulties. For example, regulation of tetracyclines will require a change in guidelines for the diagnosis and treatment of many tropical infections, and rifampicin regulation may impact on the control of tuberculosis.

Besides medical practice, antimicrobial use must also be controlled in veterinary and agricultural practices, as *C. difficile* is a pathogen of One Health importance. Currently, antimicrobial use in production animals and crops is poorly regulated and is believed to be the major source of antimicrobial contamination. Exposure of environmental *C. difficile* to these antimicrobials drives the development of resistance in these strains that then spread to humans and eventually cause CDI.

Currently, detection of AMR determinants is done retrospectively in large research facilities. Now that major determinants are known, as discussed in this review, it is possible to develop

detection methods that can be used in real-time clinical settings. This will identify drug-resistant *C. difficile* with a high risk of spread that can then trigger prevention protocols.

CDI involves the interaction between the pathogen (toxigenic *C. difficile*), host immunity and intestinal microbiota. While this review focuses on the pathogen and AMR, there are other factors that contribute to the successful spread of *C. difficile*. Thus the pathogen *C. difficile* RT 017 is commonly found in Asia, however, the prevalence of CDI is still poorly documented and the disease itself appears to be less severe and with lower mortality. This suggests that there may be unknown host- or microbiota-related factors in this population that are protective against CDI. Currently, very little is known about the *C. difficile* population in Asia and further studies may reveal key factors for the prevention of CDI.

In conclusion, by understanding AMR in *C. difficile*, both in general and in high-risk RTs such as *C. difficile* RT 017, it is possible to develop preventive strategies for CDI, by both regulation of high-risk antimicrobials in humans, animals and environment, and by early detection of AMR *C. difficile* in clinical settings.

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Articles of special interest have been highlighted with an asterisk (*, **).

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628 **Table and Figure**

629 Table 1 – Common acquired antimicrobial resistance genotypes in *C. difficile*

Antimicrobials	**Resistance genotypes**		**Reference strain**
	Accessory genes	**Mutations of existing genes**	***C. difficile* M68 ***
MLS_B group	*ermB*	-	*ermB* in Tn*6194*
Fluoroquinolones	-	*gyr*A and *gyrB* (QRDR)	D426V in GyrB
Carbapenems	-	*pbp1* and *pbp3*	-
Linezolid	*cfr*	*rplC*	-
Tetracyclines	*tet* family	-	*tetM* in Tn*6190*
Rifaximin	-	*rpoB*	-

630 Note: MLS_B group; macrolide-lincosamide-streptogramin B group, *ermB*; erythromycin ribosomal
631 methylase B—acts by methylating 23S rRNA and protecting the protein from the antimicrobials, *gyrA*
632 and *gyrB*; DNA gyrase subunits A and B, QRDR; quinolone resistant determining region, *pbp1* and
633 *pbp3*; penicillin-binding proteins 1 and 3, *cfr*; chloramphenicol-florfenicol resistance, *rplC*; ribosomal
634 protein L3, *tet* family; tetracycline family— encodes a protein that protects the ribosomw against
635 anti-transitional activity of tetracyclines, *rpoB*; beta subunit of RNA polymerase, * *C. difficile* strain
636 M68 is a reference strain for genomic studies of *C. difficile* RT 017 (GenBank accession number:
637 FN668375)

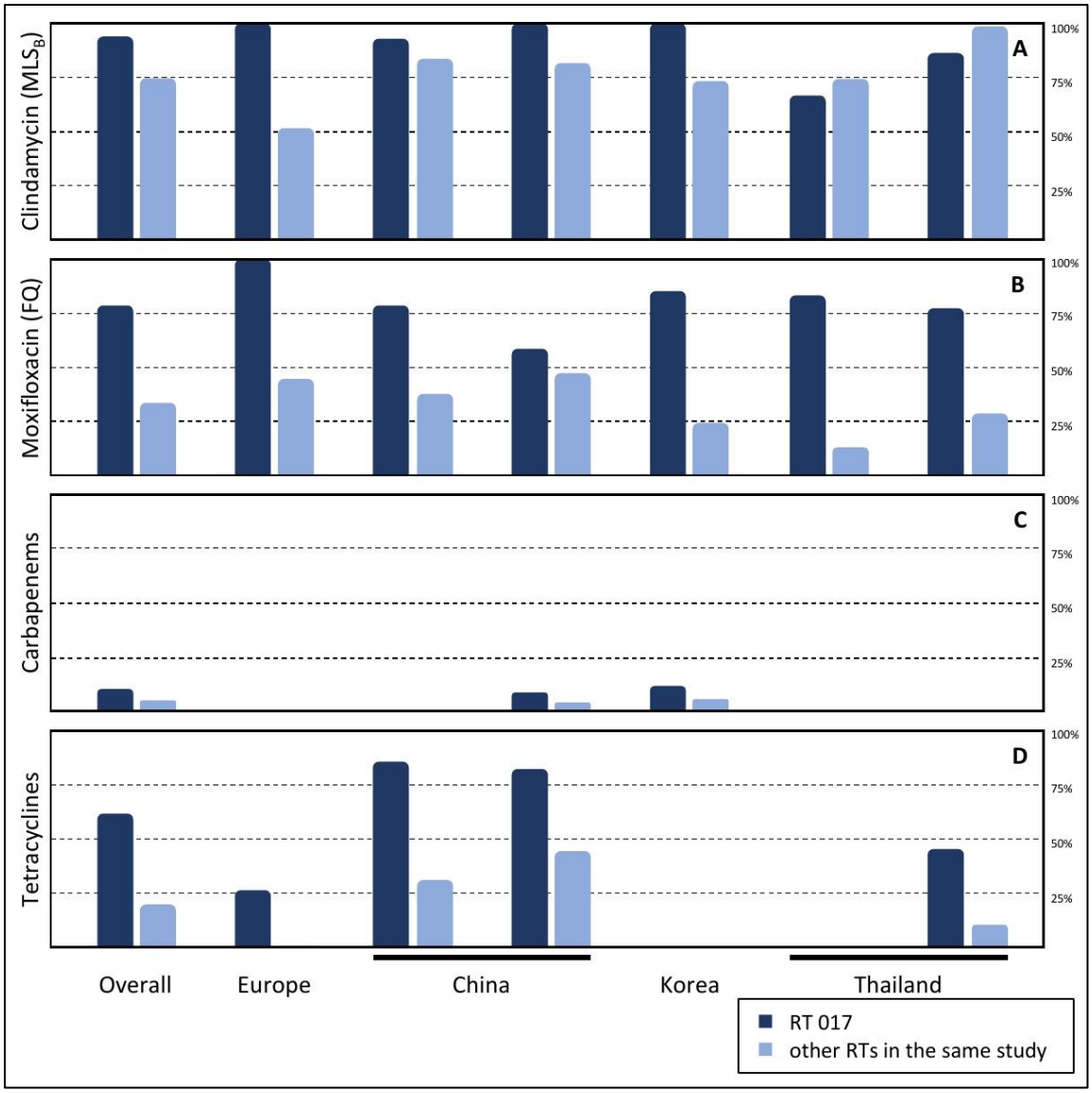

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Figure 1 - Resistance prevalence of *C. difficile* RT 017 (dark blue bars) and other RTs in the studies (light blue bars) from Europe and Asia against four major antimicrobial groups: (A) macrolide-lincosamide-streptogramin B (MLS_B) group (represented by clindamycin), (B) third- and fourth-generation fluoroquinolones (FQ; represented by moxifloxacin), (C) carbapenems and (D) tetracyclines