Journal of Pharmaceutical Research International

33(60B): 1383-1391, 2021; Article no.JPRI.80357
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antibiotic Resistance in Clostridium Difficile: A Rapidly Evolving Threat

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/JPRI/2021/v33i60B34757

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/80357

Review Article

Received 15 October 2021
Accepted 20 December 2021
Published 23 December 2021

ABSTRACT

The bacteria, *Clostridium difficile* the main pathogen associated with nosocomial infections is a gram positive, anaerobic and forms spores. The host and pathogen reaction results in a wide array of manifestation of the disease which ranges at one end as asymptomatic carriage to the other end as severe; toxic megacolon and thus making the treatment with antibiotic a challenge. To add upon the *Clostridium difficile* shows antibiotic resistance making its eradication almost impossible. This antibiotic resistance of *Clostridium difficile* has made it a global challenge over the decades, making it impossible for us to come to a conventional antibiotic to treat infections by *Clostridium difficile*. Several new strains of *Clostridium difficile* have emerged following the unscrupulous use of antimicrobial agents and subsequent transfer of resistance-causing genes between virulent strains the subsequent generations. The resistance to antibiotics is still rampant and not in control, rather it is on rise as the bacteria has greater access to new host due to its mobility and very infectious nature. With the use of extended antimicrobial therapy, an acute danger of creating and spreading new resistant and multi-drug resistant strains always looms. *Clostridium difficile,* during its life cycle, produces spores, which give the organism the capacity to resist extremes of change in the environment. In a country such as India, with a high population density and thus forming a high

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potential platform for the spread of multidrug-resistant strains of *Clostridium difficile* isusually addressed. An understanding of the emergence of antibiotic resistance in *Clostridium difficile,* the mechanisms by which the *Clostridium difficile* acquires it and sensitivity to antibiotics becomes very important. Therefore, we present a review of the antibiotic resistance seen in *Clostridium difficile.* RT027 has emerged as the most virulent strain all the strain of *Clostridium difficile.* Drug resistance is via multiple pathways, including *erm, cfr, tet,* and *rpo B* genes, as well as *Gyr A* and *Gyr B*. Of these, tet is transferable to non-resistant strains. Thus, indiscriminate use of antibiotics has created multiple antibiotic resistant strains of *Clostridium difficile.* Judicious and planned antibiotic treatment is advisable.

Keywords: Clostridium difficile; multidrug resistance; hospital-acquired diarrhea; fluroquinolones; vancomycin; metronidazole; virulent strains.

1. INTRODUCTION

1.1 Background

Clostridium difficile, which has been named as Clostriioides difficile recently, is a gram positive, anaerobic and forms, spores. Even healthy infants harbour it in its gut flora. The organism spreads via the fecal-oral route and is amongst the most common fecal-oral bacteria that are associated with nosocomial infections. The principal cause of diarrhea amongst the hospitalized patients has been attributed to *Clostridium difficile* infection [1]. Clostridium difficile infection has varied manifestations in infected people, in which it can be asymptomatic but it can also be presented as life threatening condition such as toxic megacolon. This varied manifestation has been attributed to host and pathogen interactions [1]. The management of the *Clostridium difficile* has been a difficult task for the clinicians not only because of its various manifestations but also due to the rapid evolve of new strains of *Clostridium difficile* which are resistant to the existing antibiotics regime [1].

Clostridum difficile infections are amongst the most common cause of diarrhea in nosocomial diarrhea in India, with more than one million cases recorded every year [1]. The causes of the global resistance in *Clostridium difficile* are overcrowded population, increased movement across countries, over-prescription and self-prescription of antibiotics in many countries among human and animal populations , wildlife spread, indiscriminate use of antibiotics without indications as well as poor system for proper sewage disposal and sanitation [2]. Beside being asymptomatic *Clostridium difficile* infection in an individual can manifest as foul-smelling diarrhea, with nausea and fever, three or more times a day for more than two days or toxic megacolon, especially in hospitalized patients [3]. The treatment with antibiotic, which may irradiate the *Clostridium difficile* is a herculean job as the molecular mechanism, which makes the Clostridium difficile more susceptible to a particular antibiotic, is still unraveled. The new strains that prop up in subsequent generations, which show resistant to the antibiotics forms a very crucial key to the basis of the steps in the diagnostic and management of the *Clostridium difficile* [4]. The molecular basis to identify *Clostridium difficile* genotype includes a variety of molecular methods like ; PCR ribotyping, multilocus sequence typing (MSLT), pulsed-field gel electrophoresis (PFGE), multilocus variable-number tandem-repeat analysis (MLVA), and sequencing of functional genes such as slpA and tcdC [5]. Each of these typing methods has its own advantages and disadvantages, and may be applied in different occasions according to the study Table 1 [3]. A definitive diagnosis may be made through a polymerase chain reaction test for the presence of specific bacterial genes [6].

Clostridium difficile form spores, which facilitate the spread of infection among the population. The spread of this aerobic bacteria is further facilitated by its production of toxins; enterotoxin, Toxin A and cytotoxin, Toxin B . *Clostridium difficile* is well known for its resistance to antibiotics including broad spectrum. It is stated that, previous history of indiscriminate antibiotic use is a predisposing risk factor for antibiotic resistance [14]. Fluoroquinolones and cephalosporins, in particular, which has been used in the treatment of *Clostridium difficile* infection has been associated with the evolution of antibiotic resistance in *Clostridium difficile.* Medicines like proton pump inhibitors like esomaprazole, and H2 receptor antagonist, famotidie or ranitidine increase the risk of resistance to multiple times [15]. Infection with

Table 1. Diagnostic method for *Clostridum difficile* and target

Testing Method	Target(s)	Notes	Advantages	Disadvantages	Sensitivity and s66pecificity percentage
Rapid Diagnostic Tests					
Real Time-PCR [7]	tcdB or tcdC genes	• for tcdA- / tcdB+ strains cause disease	Rapid and accurate	No major disadvantages of the test as such.	Sensitivity of 73.3% and a specificity of 99.2%.
EIA [8]	GDH Glutamate Dehydrogenase EIA	Cannot be used alone and has to be paired with another test for confirmation	Rapid highly sensitive	Not for use as a standard test since toxin must be confirmed	sensitivity and specificity of 93% and 89%, respectively.
EIA [9]	Toxins A or B	• Fast with Variable specificity and sensitivity	moderate specificity	Technically sensitive and requires trained manpower	50% to 90% and 70% to 95%, respectively
NAAT [10]		Highly sensitive and specific for Toxicogenic *Clostridium difficile* but the test is costly	High sensitivity fast test	Besides infections may also detect colonization of *Clostridium difficile*	Over 90% or even close to 100%; these values depend on the test that was used as a reference test
LAMP [11]	*tcdA* or *tcdB* genes	Rapid	High Sensitive		
Routine Gold Standard Tests					
Toxigenic Culture [12 and 13]	Toxigenic *Clostridium difficile*	•Reference standard •Difficult to perform	High sensitivity; the organism recovered can bee used for genotyping and thus facilitate in the antimicrobial susceptibility studies mechanism	• long time for the final result	sensitivity and specificity of 93% and 89%, respectively

Abbreviations: CDI, Clostridium difficile infection; EIA, enzyme immunoassay; GDH, glutamate dehydrogenase; LAMP, loop-mediated isothermal amplification; NAAT, nucleic acid amplification testing; RT-PCR, real-time polymerase chain reaction

Clostridium difficile strains resistant to multiple antibiotics is associated with an increased burden on the health system, expenses on part of the patient, and poor prognosis leading to even death [16].

1.2 Rationale

In a densely populated country such as India, multidrug resistant pathogens can quickly cause an epidemic, since there is a significant potential for rapid spread among vulnerable populations. Therefore, it is empirical that a thorough knowledge of the biochemical mechanism by which the bacteria escapes the antibiotic treatment and develops resistance to an antibiotic must be understood. This mystery of antibiotic resistance unraveled in *Clostridium difficile* would be very useful in arresting the rise of resistant species through monitoring and modification of antibiotic usage policies. Therefore, we decided to put forth few literature put in by scientists and throw a light on the phenomenon of antibiotic resistance in *Clostridium difficile.*

1.3 Objective

The objective was to review state-of-the-art as well as classical literature available regarding the emergence and mechanisms by which the *Clostridium difficile attains* antibiotic resistance*.*

2. METHODOLOGY

2.1 Search Strategy; Study Selection

The electronic online reference databases including PubMed, Scopus, Medline, Embase, web of sciences, cochrane library and Google Scholar were used to explore for literature in relation to antibiotic resistance in *Clostridium difficile*. Research articles using the keywords; *Clostridium difficile*, antibiotic, resistance, strain, ribotype, fluoroquinolone, and genes; along with combinations of the Boolean operators, “AND” and “OR”. Various combinations yielded 10000+ research papers, and further selection was based on content and completeness of data. Since this was a scoping review, literature that reported resistance to a few or specific antibiotics were also included. Preference was given to research papers that covered resistance study for at least nine antibiotics. The search was further refined for recency and relevance using the year of publication choice in Google Scholar, “From 2017- “. Research papers published in reputed journals and with greater number of citations were studied carefully. Finally, the data obtained were summarized to yield an understanding of the situation and arrive at a recommendation.

2.2 Epidemiology of Antibiotic Resistance of *Clostridium difficile*

Initially, in the late 1970’s, Clindamycin was recognized as the single antibiotic that predisposed patients to persistent *Clostridium difficile* infections [17] Clindamycin – resistant *Clostridium difficile* outbreaks were repeatedly reported in the continents of United States of America, Canada and thereafter in Europe [18]. As a result, the use of clindamycin was curtailed in the United States and Europe, leading to a reduced risk of contracting infection by *Clostridium* in the next decade [19].

Cephalosporins were the next class of antibiotics that *Clostridium difficile* became resistant to, in the late 1990’s and early 2000’s [20,21]. 79% of recent virulent strains are resistant to first-generation cephalosporins, while about 35% are resistant to second-generation cephalosporins [22].

There was an epidemic of *Clostridium difficile* infection between 2000 and 2005. This led to the closer examination of the bacterium, since the financial burden and burden upon the health system was considerably high [23]. *Clostridium difficile* – associated diarrhea (CDAD) was attributed during this epidemic to long duration use of antibiotics and the subsequent emergence of highly virulent forms of *Clostridium difficile.*

Over a short period of years, strains of *Clostridium difficile* with increased virulence have evolved across continents. Classification of these strains is based either on the PCR ribotype or on pulsed field gel electrophoresis results combined with specific restriction endonuclease subtypes [24]. In particular, RT 027 evolved because of fluoroquinolone overuse. Other virulent strains include RT 001/072, RT 078, RT 126, and RT 014/020. The evolution of *Clostridium difficile* resistant has been extensively studied in Europe [25, 26]. Several ribotypes have emerged in Europe, with RT027 appearing most frequently. A greater variety of ribotypes were seen in the UK, and in Portugal, Poland, Austria, Switzerland, France, Netherlands, Greece, and Germany, while Slovakia, Cyprus, Denmark, and Hungary had comparatively fewer variants.

Table 2. *Clostridium difficile,* mechanisms of resistance of few antibiotics

Sr. No.	Antibiotics used in the treatment	Resistance developed bio-mechanism	Genes affected	Remarks
1	**Metronidazole (MTZ) [28]**	Biofilm formation		
2	**Ciprofloxacin [29]**	Quinolone resistance-determining regions (QRDRs) showing alterations in the target enzymes through stepwise mutations)	gyrA and gyrB,	Ciprofloxacin
3	**Vancomycin [24]**	biofilm formation		mur G
4	**Chloramphenicol [30]**	Acetyl transferase	Tn4453a and Tn4453b	cat D
5	**Rifamycins [31]**	Altered target		rpo B
6	**Cephalosporins [30]**	Antibiotic enzymatic destruction		Putative β-lactamases and PBPs
7	**Fluoroquinolones [30]**	Altered target	gyr A/gyr B	
8	**Ciprofloxacin [6]**	Mutational alterations in target enzymes through stepwise mutations in the quinolone resistance-determining regions (QRDRs)	gyrA and gyrB,	topoisomerase IV genes

RT027 was the prevailing variant in Hungary and Poland. All European varieties, are however sensitive to fidaxomicin, metronidazole and vancomycin [2].

Clostridium difficile has also been isolated from domestic animals in Europe, particularly from pigs (62 strains) and dogs (39 strains). The most common *Clostridium difficile* ribotypes found these cases were 078 and 010.Type 010, found in dogs, is non-toxicogenic. RT078, 150, 127, 014/020, 126, and 031/1 were antibiotic-resistant. Similarly, *Clostridium difficile* exhibited multiple ribotypes in United Sates of America, with increased resistance to metronidazole, but not vancomycin. Global studies have found an increasing resistance to metronidazole and vancomycin [27].

The drugs of choice thus remain Metronidazole and Vancomycin. For example, cephalosporins are destroyed enzymatically via altered beta-lactamase action, while 23 S RNA methylases and RNA methyltransferases render Erythromycin ineffectual. Efflux mechanisms may or may not be involved in this resistance. *Clostridium difficile* presents an altered target to fluoroquinolones, with the gyr A/ gyr B locus involved [28]. Rifamycins and Vancomycin also find their targets altered in resistant strains. Fidaxomycin and vancomycin target bacterial RNA polymerases, and thus in resistant varieties, rpoB or rpoC may be mutated [29]. Additionally, altered peptidoglycan synthesis may also be involved in vancomycin resistance through mutations in the MurG gene (Figs. 1, Table 2).

Multiple amino acid changes in bacterial proteins have been associated with antibiotic resistance. Particularly, Threonin at position 82 is a hotspot, with amino acids changing commonly to Isoleucine, alanine or valine. Multiple mechanisms thus exist in the antimicrobial resistance exhibited by *Clostridium difficile.* Usually, antibiotic resistance can be through reduced uptake, increased efflux, or enzymatic [30]. The mechanisms employed by multiple-antibiotic-resistant *Clostridium difficile* are heterogenous. An important aspect is the mobility of resistance-conferring portions of the genome between bacteria [30]. The ribotype variation seen to occur in *Clostridium difficile* is maintained via mobile genetic elements, particularly, transposons, which participate in transduction, transformation and conjugation. Mobile genetic elements almost constitute as much as 11% of the *Clostridium difficile* genome and this is the reason for the constantly changing face that the bacterium presents to antibiotics [31]. Tn5398, Tn5215, and Tn6194 ribotypes together confer resistance to erythromycin and other MLSB (macrolide-lincosamide-streptogramin B) family antibiotics. Transposons Tn5397, Tn916, and Tn6164 transfer tet genes to the recipient bacterium, conferring tertracycline resistance. It is possible that this element was acquired in *Clostridium difficile* from another bacterium, such as Bifidobacterium. Transposons Tn4453a and Tn4453b, make the catP gene mobile, conferring chloramphenicol resistance upon the recipient via enhancing acetyltransferase activity [27].

The resistance exhibited to metronidazole is of special interest, since metronidazole is popularly prescribed and is an economical alternative, compared to vancomycin. The response is of a heterogeneous and inducible nature. One method employed by the bacterium is to produce a thick impenetrable biofilm matrix made of proteins, polysachharides and DNA, and regulated by the Cwp84 cysteine protease component (32). Metabolic pathways involved in conferring metronidazole resistance are poorly understood, but are thought to involve nitroreductases and DNA repair mechanisms, and, additionally, iron uptake (24). GyrA and GyrB genes may also be involved in fluoroquinolone resistance. GyrA/GyrB represent two subunits of a DNA gyrase that prevents fluoroquinolone-induced damage in *Helicobacter pylori* as well as *Clostridium difficile* [33].

3. DISCUSSION

The variants seen across continents are of significance in understanding the epidemiology, behavior, and spread of *Clostridium difficile* among various populations. Agar dilution tests, broth dilution tests, MIC gradient diffusion, and other antibiotic susceptibility tests help determine whether an emerging variant is one of concern. Additionally, susceptibility studies help understand the nature of variations and the possible mechanisms of transfer of antibiotic resistance in *Clostridium difficile* [1].

This rapid rate and variety of mutations conferring both increased virulence and antibiotic resistance is of concern, since serious infections with *Clostridium difficile* may lead to debilitation and death [34]. A high economic cost is also involved with the administration of third generation antibiotics and specialized

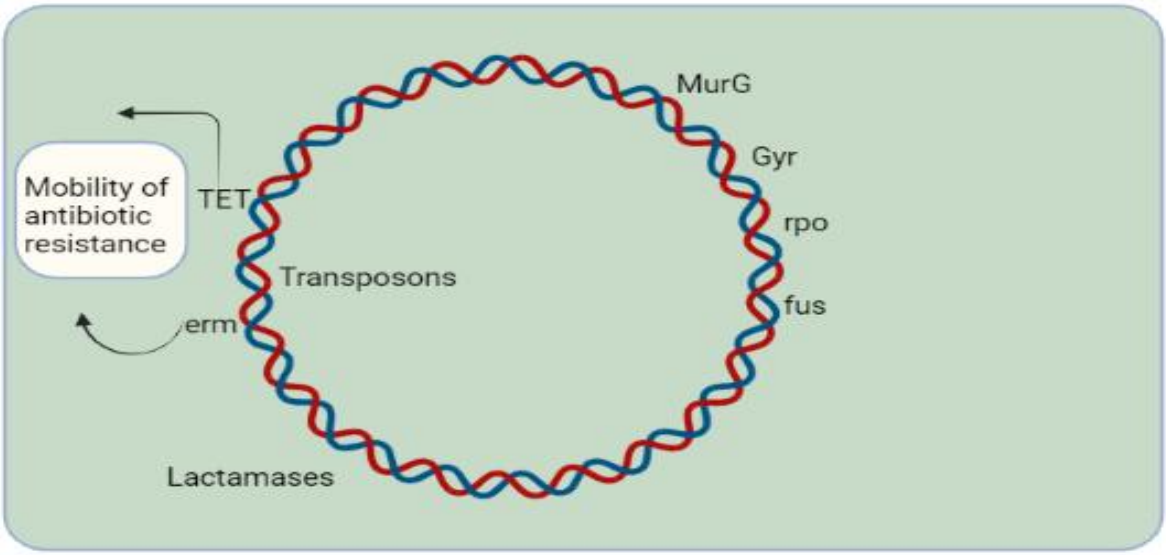

Fig. 1. The resistance developed after the mutation of the RNA polymerase enzyme of the *Clostridium difficile*

formulations. Mobile elements within the bacterial genome ensure the rapid emergence of resistant variants, while already acquired resistances are retained by selection against a background of indiscriminate antibiotic use [35-39]. The increased morbidity and mortality associated with the newer, more virulent strains, coupled with the multi-drug resistance seen to evolve, underscore the need for understanding the molecular bases behind the mechanisms of harm avoidance employed by the numerous strains, as well as the development of newer antibiotics and alternate treatment strategies [14].

4. CONCLUSION

The greatest burden of *Clostridium difficile* infections globally is its resistance to antibiotics, which has become a matter of utmost importance since its major cause of nosocomial diarrhea. To add, further, to the burden, new strains of the *Clostridium difficile* have evolved following unscrupulous use of antibiotics that over the years have increased in incidence and newer strains keep on emerging. The underlying biochemical mechanism inherent in *Clostridium difficile* is an interesting mystery to unravel which will certainly bring a drop in the incidence of this burden and help scientists in the development of new drugs against it. Finally, attention has to be drawn towards the clinicians and self-administrated populations towards the blind use of antibiotics, which will in return facilitate to decrease the occurrence as well as spread of the drug resistant *Clostridium difficile* infection.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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