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Review Article

CLOSTRIDIUM DIFFICILE: A GROWING HEALTH CONCERN

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ABSTRACT

Clostridium difficile is a potent nosocomial pathogen being one of the major causes of Antibiotic Associated Diarrhea. Excessive utilization of broad spectrum antibiotics destabilizes the gut microflora and compromises immunity thereby facilitating the growth and proliferation of this bacterium culminating in the disease. Infection is usually transmitted through transfer of spores via the fecal oral route. Toxin A and B are the predominant toxins responsible for the disease that disseminate intestinal barrier and initiate Rho GTPases activated Interleukin mediated inflammatory pathway. Improper sanitary conditions, contaminated food, animal manure and unclean medical setups are the primary sources of infection. This microorganism is generally diagnosed on taurocholate enrichment agar in combination with advanced techniques including Real Time PCR, enzyme immunoassays and restriction endonuclease analysis. Although Vancomycin and Metronidazole are the first lines of treatment, their efficacy has been found to be compromised mainly because of emergence of novel strains with increased virulence as well as resistance. New drugs including Fidaxomicin and Ribaxamase and advanced techniques like Fecal Microbiota Transplantation are now often used in combination with the former in order to curtail the above. Microbial replacement and utilization of outer membrane of the pathogen are recent breakthroughs towards containment of the disease. Since *Clostridium difficile* has now become one of the major health concerns, knowledge of pathogenesis, minimization of risk factor and development of alternative therapeutics may reduce the negative health consequences inferred by this infectious pathogen.

Keywords: Antibiotics, *Clostridium difficile*, fecal-oral route, resistance, toxins, virulence

1. INTRODUCTION

Clostridium difficile is an opportunistic pathogen often associated with disorders ranging from diarrhea to severe inflammation of the colon. This bacterium is gram positive, anaerobic, rod shaped and motile with peritrichous flagella. The human intestine serves as an ideal environment for inhabitance and growth of this bacterium which generally reproduces by binary fission. Furthermore, this organism is non-hemolytic, non-proteolytic, non-lipolytic and forms endospores under unfavourable conditions which are either sub-terminal or terminal. The genome consists of 4,290,252 base pairs and a circular plasmid of approximately 8000 base pairs. A notable feature is the presence of transposons which equip the bacteria with several characteristics including heightened virulence and antibiotic resistance. *C difficile* satisfies its energy requirement chiefly through fermentative metabolism of amino acids glycine, valine, trytophan, leucine, isoleucine and proline.

Antibiotic Associated Diarrhea (AAD) is a common manifestation of excessive as well as non-judicious use of antibiotics in therapy. Although many cases of AAD are mild and display no microbial associations, few of them may be due to *C difficile* infestation which can thereafter transform into a serious infection [1]. Although *C difficile* may be naturally associated with the gut microbiota of adults as well as children, their number is kept under control by the action of probiotic bacteria present in a healthy and immunocompetent gastrointestinal tract [2]. *Clostridium difficile* disease manifestation generally occurs in individuals who have undergone extensive antibiotic therapy especially using broad spectrum antibiotics, post digestive surgery including cholectomy or ileostomy, elderly, people working in healthcare setups or those possessing a compromised immunity and having a history of Inflammatory Bowel Disease (IBD), kidney disorders or cancer [3]. Indeed, *C difficile* has been associated with 25% of AAD, the severity of which may range from mild diarrhea to intestinal membrane colitis, mesocolonic edema, typhlocolitis, colon toxicity, organ failure and even mortality in extreme and aggravated conditions [4, 5]. The symptoms generally include watery diarrhea,

blood in stool, nausea, abdominal pain, fever, dehydration and weight loss. Additionally, symptoms may also extend to accelerated heart rate, peritonitis and kidney failure accompanied by elevated WBCs and creatinine. Excessive use of antibiotics leads to microbial dysbiosis thereby favoring multiplication and spread of the above. This disease is highly communicable until 48 hours post clearance of the symptoms. The present study aims to address the potent risk factors of *Clostridium Difficile* infection along with the disease pathogenesis as well as current diagnostic and therapeutic approaches employed. An increased awareness and complete understanding of the same would help in preventing the infection along with aiding in research towards generation of novel therapeutic approaches.

2. RISK FACTORS

Antimicrobial and antibiotic treatments are the most influential risk factor for development of *Clostridium difficile* Infection (CDI) since they disrupt the gut microbiome [6]. Antibiotics have been known to increase the risk of the above, with the most potent being Clindamycin, Fluoroquinolones, Cephalosorins and second generation Penicillins (Ampicillin and Amoxicillin). These drugs disrupt the intestinal ecosystem leading to development of AAD especially by *C difficile* [7]. They interfere with bile acid homesotasis and production as well as utilization of Short Chain Fatty Acids (SCFA) thereby leading to osmotic diarrhea accompanied by colonization of the pathogenic strain [8]. Moreover, intake of Proton Pump Inhibitors (PPI) and histamine-2 receptor antagonists has been observed to increase the survival of vegetative cells as well as spores of *C difficile*. These PPIs decrease the gastric acidity and compromise the host immunity, hence facilitating pathogen colonization. All these factors facilitate adherence of the pathogen, toxin production followed by intestinal inflammation leading to the symptoms of the disease. The predominant toxins produced are toxin A and B which are encoded by the pathogenicity locus of this microorganism. Prolonged hospitalization, emergency surgery, kidney injury, diabetes mellitus, cystic fibrosis, liver disease, increased age, malnutrition, regular alcohol consumption and contact with an infected person are also the known risk factors for the same [9, 10]. Furthermore, contaminated food, animal manure and improper hygiene notably predispose towards this infection [11]. Previous reports have indeed documented increased propensity of the disease owing to failure in maintaining adequate hygienic practices [12]. Unclean toilet surfaces are one of the major reservoirs of *C difficile* infection. Although bleaching powder is routinely employed for cleaning these areas, their efficacy has been found to be insufficient to remove the spores of this organism. Moreover, bleaching requires a two step procedure failure of which may lead to safety concerns. However, recent studies have discovered that cleaning of public toilets with hydrogen peroxide lead to better outcome in terms of reducing the microbial load including the spores [13]. Therefore awareness about the various risk factors may improve the preventive strategies adopted against the disease.

3. SOURCES

C difficile has a wide span of occurrence, being predominantly found in soil, water, air, animal and human stools as well as food. Contamination usually occurs by the fecal oral route especially in environments associated with poor hygienic and sanitary conditions [14, 15]. The spore forming capability of this bacterium under adverse environmental conditions facilitates its survival and infectivity since transmission majorly occurs via spores. Once inside a novel host, the bacterium multiplies and generates toxin which thereafter produces the symptoms of the disease. Recent studies have observed that food products serve as one of the chief routes of transmission of the above with the most common ribotypes being 027, 078 and 017, especially in Asia, Europe, North America and Germany [16]. Sea food, especially shell fish has been shown to carry this pathogen. Moreover, this organism also spreads through contaminated water used in food industries and waste water treatment plants. Furthermore, animal wastes, traditionally used as fertilizers have been found to transmit the disease [17]. Therefore, improperly washed or cooked food potentiates a possible risk of the same. Even meats and carcases have shown the presence of *C difficile* spores. These spores have been detected in meats of pigs, cattle as well as chicken with their numbers reducing with an increase in the age of the animal [18]. Kitchen surfaces, refrigerators and food handlers of both the domestic and industrial sectors are possible sources of infection. Factors that influence transmission of this pathogen via food include cross contamination, improper storage, inappropriate hygiene, processing as well as transport. Apart from meat and seafood, raw salads and vegetables have also been documented to harbor the above. Although till date the spore levels found in the above food products are low, yet they are a cause of concern owing to their resistance towards the strong

physical treatments used in food processing [19]. Nonetheless, *C difficile* disease is one of the predominant causes of nosocomial diarrhea which generally has a high rate of transmission in hospitals and health care units particularly devoid of adequate hygiene.

4. PATHOGENESIS

C difficile is normally present in the gastrointestinal tract of many individuals. However, the microbial homeostasis in the gut maintains normal physiology and prevents the initiation of this disease thereby controlling the activity of this pathogen. Prolonged exposure to antibiotics weakens this microbial equilibrium favoring the growth and multiplication of the above. The now virulent pathogen not only causes a disease in the affected individuals but may also affect healthy people via transmission through stools of the former. Once outside the body, the bacteria transform into spores which can survive for long periods on hands, surfaces, objects and clothing unless disinfected. Spores of *C difficile* that are transmitted and ingested via the fecal-oral route colonize the human small intestine owing to their resistance towards the low pH of stomach [20]. These spores thereafter germinate into vegetative cells under the influence of the bile acids in the intestine. Disruption of the gut microbiota is accompanied by attachment of the pathogen to the enterocyte receptors followed by proliferation and production of toxins. The two major exotoxins produced by *C. difficile* in the colon *viz.*; toxin A and B are highly potent and predominant factors responsible for the disease [21]. Certain strains also express an additional toxin called the binary toxin that has been correlated with heightened pathogenesis [22]. The pathogenic strains of *C difficile* have been found to possess a pathogenicity locus containing tcdABCDE. TcdA encodes for an enterotoxin whereas tcdB codes for a cytotoxin; both being responsible for the symptoms of *C difficile* associated diarrhea. tcdC and tcdD produce negative and positive modulators respectively that function in determining the toxin levels during infection. Furthermore, tcdE aids in the release of toxin from the cell wall during pathogenesis [23]. Toxin A and B genes are the main culprits of the infection which ultimately damage the intestinal membrane leading to loss of mechanical barrier. These toxins cause glucosylation and subsequent inactivation of Rho-GTPases (Rho, rac and Cdc42) hence dismantling actin polymerization, tight junctions and integrity of the epithelial cells [24]. Additionally, Toxin A and Toxin B upregulate the production of Tumor Necrosis Factor α (TNF α) and Interleukins (ILs) facilitating macrophage and neutrophil proliferation to the effected sites, further aiding in damage to intestinal membrane through acute inflammation and increase in permeability culminating in apoptotic death of the same [25]. Toxin A has been shown to activate NFκB dependent Interleukin 8 (IL-8) mediated inflammatory pathways leading to destruction of the mucus membrane [26]. This potent toxin activates Activator Protein-1 (AP-1) and Cyclic-AMP Response Binding Protein (CREB). While AP-1 helps in the IL-8 mediated inflammatory pathway, CREB activates the expression of prostaglandin E2 through induction of Cyclooxygenase 2 (Cox 2). The activated prostaglandin E2 accelerates fluid secretion and initiates diarrhea in the affected individuals [27]. These toxins have been reported to inhibit the network of F-actin thereby affecting the membrane structure by covalently modifying Rho GTPases and inhibiting the downstream Phophatidylinositol Kinase-3 (PI3K) pathway. It is the damaged intestinal mucosa and excess mucus production that potentiate the predominant symptoms of CDI including watery diarrhea, colitis, mesocolonic edema, inflammatory bowel disease, toxic megacolon, abdominal pain, malaise, fever and anorexia [20]. These signs generally manifest between the second and third day of the infection. Moreover, excessive usage of antibiotics has been shown to reduce intestinal cell motility and peristalsis helping in the accumulation of the pathogens in the gut. Since *C difficile* incidences are often secondary manifestations of diarrhea, they therefore utilize the reduced contractibility and immunity brought about by extensive antibiotic usage to treat the former to their advantage resulting in adverse outcomes. [28]. In these cases, the symptoms generally appear between the fifth and tenth day of antibiotic usage [29]. The severity of the symptoms depends not only on the virulence of the pathogen but also on the health of the individual.

Studies have attributed the elevated instances of *C. difficile* infection to the emergence of a hyper-virulent strain, known as North American pulsed-field type 1 (NAP1), of ribotype 027 and group BI. This strain displays resistance to vancomycin which is the major antibiotic used in *C difficile* therapy. Moreover NAP1 possesses the capability to produce several bacterial toxins apart from the toxins A and B. Additionally, NAP1 also produces heightened levels of toxin A and B as well as the binary toxin compared to the other strains. Furthermore, NAP1 is also associated with a high

probability of gene polymorphism, particularly in the tcdC gene [30]. This bacterial strain has been associated with increased disease virulence, severity as well as recurrence. Pathogen infection is accompanied by a concomitant rise in serum IG A as an attempt to eliminate the toxin and prevent subsequent infection. However, the efficacy of the body's natural defense depends on a number of factors including the immunological and physiological state of the individual [31]. Absence of normal gut microbiota, residual spores of *C difficile* and resistance of the pathogen towards antibiotics used for therapy are the main causes of recurrence which is commonly encountered in approximately 20% of CDI patients treated for initial infection and 60% in recurrent infections [32]. Recently, *C difficile* infection has also been found in patients displaying only nausea and vomiting without any complaints of diarrhea. Therefore, through examination of patient is required to determine the actual cause of the disease [33]. Interestingly, administration of probiotic bacteria *Bacillus coagulans* BC 30 has been potrayed to reduce the percentage of disease recurrence even after withdrawal of antibiotic therapy apart from boosting immunity and reducing inflammation in the infected patients [34].

C difficile infection is generally known to occur in the elderly. In a normal healthy individual, gut microbes protect against the infection. In the upper ileum, microbial hydrolases degrade the bile salts which would otherwise have stimulated growth of this pathogen. Furthermore, secondary bile acids in the lowed ileum further prevent the growth and thereby infestation with *C difficile*. Although infants also possess a compromised immunity, infection with *C difficile* is rare since they lack the receptors for the toxins [35]. However, previous reports have shown that children undergoing cancer therapy are at a risk of *C difficile* infection accompanied by diarrhea due to overgrowth of this microorganism in their intestines [36]. Furthermore, pediatric patients with a history of acute appendicitis have also been reported to be at a risk of the above [37]. Additionally, the bacterial infection has also been reported to spread via fecal discharges and soiled diapers in neonatal Intensive Care Units (ICU) [38]. Hence, maintenance of proper sanitation and minimization of risk factors may undermine disease pathogenesis.

5. DETECTION

Early and accurate diagnosis of *C difficile* is essential to limit the course and severity of the disease. Diagnosis of this pathogen in patient samples is associated with certain challenges mainly because of the limitation of specific and sensitive methods for analysis. Moreover, this pathogen may merely colonize the gut without initiating the development of obvious symptoms thereby making the detection difficult. Radiological analysis is generally not helpful in these circumstances. Enrichment cultures are the preferred means of enumeration of *C difficile* especially from samples where their abundance is low. Sample volume and the incubation time have a major influence on detection efficiency. It has been observed that supplementation of the culture media with cholate salts further improves the yield of the above. Generally, cycloserine-cefoxitin fructose agar (CCFA) and BHI enrichment broth with taurocholate is a method of choice. However, CCFA suffers from the disadvantage of its inability to differentiate between toxigenic and non-toxigenic strains. Previous studies have indeed documented taurocholate agar to perform better in terms of sensitivity and speed in comparison to the former since taurocholate acts as a selective agent aiding in the germination of spores [39]. It has been observed that the recovery of spores in the culture media can be increased by supplementing the same with lysozyme and sodium thioglycolate. The lysozyme has been observed to damage the spore wall thereby aiding in the recovery [40]. Moreover, the thioglycolate in the media has been shown to degrade the disulphide bonds in the spore wall further increasing the efficiency of culture [14]. Enzyme immunnoassays for Glutamate Dehydrogenase (GDH) is also an extremely popular method for identification of this microorganism, since this gene is expressed at a significant level by them. GDH is sensitive, rapid and economical but lacks the ability to differentiate between the toxigenic and non toxigenic forms. Moreover, the sensitivity of this method is limited to 30% [41]. Medical units analyzing stool samples often use stool titration for the purpose of detection of *C difficile* toxin and thereby characterization of the disease. Determination of toxin A and B are also popularly used to characterize the disease with toxin positive isolates correlating with watery stools in patients. Although toxin detection is inexpensive and sensitive, yet there may be problems of denaturation of the same if subjected to improper sample collection and storage [42]. Moreover, advanced techniques such as pulse field gel electrophoresis, capillary gel electrophoresis, enzyme immunoassays for Glutamate Dehydrogenase (GDH), PCR ribotyping, cell culture cytotoxicity, Real Time PCR against Toxin-A and Toxin-

B and restriction endonuclease analysis accompanied with genome sequencing have been shown to serve as improved methods of identification and determination of disease prognosis. Recent technologies have identified the effectiveness of Real Time Multiplex PCR as an improved method for diagnosis with respect to reproducibility, sensitivity and specificity. It has been shown to be capable of detecting toxin levels as low as 0.5 CFU/ml from stool samples. Moreover, this method has the advantage of being able to detect the toxins directly from stool samples thereby omitting the step of extraction of DNA. Recently, a DNAzyme has been developed which possesses the ability to selectively recognize and cleave the RNA of pathogenic strains of *C difficile* [43]. A patient is diagnosed as *C difficile* positive when either the stool sample shows presence of the toxin genes or endoscopic and histology determination confirms manifestation of pseudomembraneous colitis.

6. PREVENTION AND TREATMENT

The incidences of *C difficile* have alarmingly increased over the recent decades, thereby necessitating the requirement of effective prevention and treatment procedures. Preventative strategies should mainly focus on maintenance of hygiene, cleanliness and contact precautions to reduce the risk factors of infection since *C difficile* is highly communicable. Moreover, care should also be taken to practice the proper cooking and storage guidelines of a food substance to prevent the spread of the infection via food [44]. Furthermore, excessive and non-judicial use of antibiotics should also be avoided to reduce the risk of developing antibiotic associated *C difficile* disease. Therapy may either include administration of antibiotics, withdrawal of antibiotics thought to potentiate the disease or even minor surgery in cases of serious infections. Broad spectrum antibiotics including Vancomycin and Metronidazole are the common means of treatment of CDI. Vancomycin is administered either orally or through rectum whereas the route of dispension of Metronidazole is oral or intravenous. Since Vancomycin is poorely absorbed by the gut epithelium, intravenous suspension is avoided in this case. However, these drugs promote gut dysbiosis thereby further worsening the situation in several instances and demanding the requirement of specific anti microbial agents. Moreover, traditional antibiotic thereby are also ineffective because of gene mutation leading to generation of new strains with increased virulence which are responsible for heightened cases of recurrent infections and drug resistance. Vancomycin interferes with the terminal step of cell wall synthesis by the bacteria. However, *C difficile* has been shown to exhibit resistance against the same owing to the presence of vanGcd cluster in their genomes which is able to synthesize D-Ala-D-ser precursors instead of D-Ala-D-ala in response to sublethal concentrations of drug [45]. Furthermore, *C difficile* mutants that lack the vanGcd cluster have been attributed to higher sensitivity towards Vancomycin [46]. However, owing to higher percentage of resistance towards Metronidazole, Vancomycin still has remained the first line of treatment in several medical units. Recently a new macrocyclic antibiotic Fidaxomicin has been shown to be effective, although the efficacy is limited to the NAP1/027 strain. Generally, a repeated course of vancomycin is also recommended for the first cycle of relapse which is followed by pulsed oral vancomycin administration with or without probiotics if a second relapse occurs. All future relapses are normally treated with Fidaxomicin [47]. Another novel β lactamase drug Ribaxamase is often co-administered with traditional broad spectrum antibiotics in certain cases. Intravenous delivery of immunoglobulins especially monoclonal IgG is also used in order to boost the host immunity [48]. Administration of probiotics as an attempt to replenish the gut microbiota is also a method of choice [49]. It has been reported that a combination of antibiotic therapy with probiotics, especially *Lactobacillus plantarum 299v* results in improved prognosis [50]. Cellular overexpression of Rho GTPases (Rho A, Rhob and Rho C) in cultured cells has been found to prevent the cytotoxic effects of this microorganism [50]. Additionally, non pathogenic strains of *E coli* and *Bacillus coagulans* have also been reported to improve the symptoms of CDI. *B coagulans* GBI-30 has been found to improve stool consistency and colonic inflammation as well as reduce the incidences of CDI mediated mortality in mouse models. This probiotic bacterium has also been found to reduce the pathogen numbers as well as toxin levels in the stool samples of the treated patients. Inhibition of IL-8 activated NFκB inflammatory pathway was shown to be inhibited by administration of *B coagulans* thereby helping in the amelioration of the disease symptoms including weight loss, watery diarrhea and colonic histopathology [51]. Currently, stool transplantation has shown promising results in control of the above especially in Intensive Care Unit patients [52]. This includes collection of stool from healthy individual, isolation of beneficial microbes followed by their introduction into the gut of the patient [53]. Fecal

Microbiota Translantation (FMT) has been approved by United States Food and Drug Administration in 2013. Stool transplantation has been found to generate a cure rate in the range of 80-90 % [54]. Appropriate donor selection, blood tests and stool examination are required in order to achieve the expected outcome as well as reduce the associated risks. Furthermore, the feces should also be processed and the recipient prepared according to the guidelines [55]. FMT has shown to produce promising results even in the elderly [56]. Although this method is relatively safe and cost effective having mild side effects, yet it may pose a possible risk of associated complications. Indeed certain negative impacts of FMT have been reported which include infections, perforations and even death [57]. Moreover, introduction of the same requires invasive procedures. Interestingly, attempt is being made to develop capsulated versions of fecal microbes in order to ease the route of administration. Passive and active immunizations with anti-toxins are amongst the methods of treatment [47]. Recent researches are being carried out using the outer envelope of the pathogen for development of a new drug. The outer wall of this pathogen contains three components viz; branched penta-glycosylphosphate repeating unit, hexa-glycosylphosphate unit and lipid associated glycosylphosphate unit. These three polymers are now being utilized as targets for drug delivery because of their easy accessibility. Moreover the scope of the protein rich surface layer (S layer) of this bacterium is also being investigated [45]. Apart from these, scrotase-anchored proteins, cysteine proteases and other components of the outer layer of *C difficile* are amongst the few novel therapeutic targets that are being studies in order to address the existing disadvantages of the current treatment strategies. Passive as well as active immunization with anti toxin B antibody in addition to conventional antibiotic delivery has also been attempted for improved prognosis. Moreover, previous studies have developed a synthetic peptide (poly β peptide) that was shown to be capable of preventing the outgrowth of spores and multiplication of vegetative cells at a concentration of 3.13−12.5 μg/mL [58]. However, the effectiveness of the same has only been demonstrated *in vitro*. Recent strategies further include the microbial replacement therapy where non-toxigenic strains of C *difficile* are administered to the affected individuals with the purpose of out-competing the pathogenic ones from the gut microenvironment. This approach has indeed been shown to significantly reduce the recurrence rate of CDI compared to placebo controls [59]. Nonetheless, the effectiveness of the treatment protocols can be further strengthened either by preventing the risk factors or by reinforcing adequate sanitation and hygiene.

7. CONCLUSION

Prolonged antibiotic administration to combat primary infection has often been associated with negative health side effects including reduced immunity, diarrhea and risk of secondary infection. *Clostridium difficile* is one of the major pathogens that opportunistically invade patients associated with the above and multiply within the host initiating severe symptoms of the disease such as watery stools, organ toxicity and even death in extreme conditions. Since the spores of this bacterium are transmitted through the fecal oral route, maintenance of proper hygienic practices and complete knowledge of the risk factors as well as disease pathway are useful in limiting the spread of the infection. Moreover, owing to the high rate of mutation and genetic recombination, this bacterium has recently been found to occur in altered forms, especially the NAP1/027 strain that display heightened virulence and resistance towards the traditional therapeutic methods employed for its removal. Researchers are now exploring alternative targets of treatment which include the outer envelope of *C Difficile*, S layer, anchored protein and proteases. Current methods of treatment also employ stool transplantation, which on one hand has been implicated to improve prognosis but on the other hand has also been associated with some negative side effects. Therefore, thorough investigation of the pathogenic course and the associated risk factors may generate novel avenues for the control and treatment of the above thereby improving the present scenario of disease management.

8. REFERENCES

1. Kaltsas A, Simon M, Unruh LH, Son C, et al. *Journal of Clinical Microbiology*, 2012; **50**:1303-1307.
2. Leffler DA, Lamont JT. *N Engl J Med.*, 2015; **372**:1539-1548.
3. Lavallée C, Laufer B, Pepin J, Mitchell A, et al. *Clin Microbiol Infect.*, 2009; **15**:1093-1099.
4. Kelly CP, LaMont JT. *N Engl J Med.*, 2008; **359**:1932-1940.
5. McDonald LC, Coignard B, Dubberke E, Song X, et al. *Infect. Control Hosp. Epidemiol.*, 2007; **28**:140-145.
6. Yablon SA, Krotenberg R, Fruhmann K. *Am J Phys Med Rehabil.*, 1992; **71**:102-107.

7. Barbut F, Petit JC. *Clin Microbiol Infect*., 2001; **7**: 405-410.
8. Mullish BH, Williams HRT. *Clin Med (Lond.)*, 2018; **18**:237-241.
9. McFarland LV. *Dig Dis*., 1998; **16**:292-307.
10. Kochhar G, Edge P, Blomme C, Wu XR, et al. *Inflamm Bowel Dis*., 2018, **24**:402-409.
11. Killeen S, Martin ST, Hyland J, O' Connell PR, et al. *Surgeon*., 2014; **12**:256-262.
12. Al-Tawfiq JA, Treble M, Abdrabalnabi R, Okeahialam C, et al. *Epidemiol. Infect.*, 2018; **146**:276-282.
13. Alfa MJ, Lo E, Wald A, Dueck C, et al. *BMC Infectious Diseases*, 2010; **10**:268-276.
14. Lund BM, Peck MW. *Foodborne Pathog Dis*., 2015; **12**:177-182.
15. Rodriguez DC, Seyboldt C, Rupnik *M. Adv Exp Med Biol*., 2018; **1050**:227-243.
16. Collins DA, Riley TV. *Trop. Med Infect. Dis.*, 2019; **4**:1-12.
17. Gauvry E, Mathot AG, Leguérinel I, Couvert, et al. *Res Microbiol.*, 2016; **168**:369-378.
18. Dubberke ER, Haslam DB, Lanzas C, Bobo LD, et al. *Zoonoses Public Health*, 2011; **58**:4-20.
19. Candel-Pérez C, Ros-Berruezo G, Martínez-Graciá C. *Food Microbiol.*, 2019; **77**:118-129.
20. Poutanen SM, Simor AE. *CMAJ*, 2004; **171**:51-58.
21. Martin-Verstraete I, Peltier J, Dupuy B. *Toxins (Basel.)*, 2016; **8**:1-24.
22. Eckert C, Emirian A, Le Monnier A, Cathala L, et al. *New Microbes New Infect.*, 2015; **3**:12-17.
23. Voth DE, Ballard JD. *Clin. Microbiol. Rev*., 2005; **18**:247-263.
24. Smits WK, Lyras D, Lacy DB, Wilcox MH, et al. *Nat Rev Dis Primers*., 2016; **2**:1-47.
25. Poxton IR, McCoubrey JM, Blair G. *Clinical Microbiology and Infection,* 2001; **7**:421-427.
26. Kim JM, Lee JY, Yoon YM, Oh YK, et al. *Scandinavian Journal of Immunology*, 2006; **63**:453-460.
27. Kim H, Rhee SH, Kokkotou E, Na X, et al. *J Biol Chem*., 2005; **280**:21237-21245.
28. Percy WH, Christensen J. *Gastroenterology*, 1985; **88**:964-70.
29. Tedesco J. *Medical Clinics of North America*, 1982; **66:**655-664.
30. O'Connor JR, Johnson S, Gerding DN. *Gastroenterology*, 2009; **136**:1913-1924.
31. Johnson S, Sypura WD, Gerding DN, Ewing SL, et al. *Infect Immun*., 1995; **63**:3166-3173.
32. Wilcox M, Fawley W, Settle A, Davidson A. *J Hosp Infect*., 1998; **38**:93-100.
33. Abid H, Bischof E. *Cureus*, 2019; **11**:e4162.
34. Fitzpatrick LR, Small JS, Greene WH, Karpa KD, et al. *Gut Pathogens*, 2012; **4**:13-21.
35. Murabata M, Kato H, Yano H, Ogura M, et al. *Kansenshogaku Zasshi*, 2008; **82**:419-426.
36. Vidrine SR, Cortina C, Black M, Vidrine SB. *J La State Med Soc*., 2012; **164**:265-267.
37. Faden HS, Dryja D et al. *Am J Infect Control,* 2015; **43**:887-888.
38. Li B. *Int J Biomed Sci*., 2016; **12**:83-88.
39. Bliss DZ, Johnson S, Clabots CR, Savik K, et al. *Diagn Microbiol Infect Dis.,* 1997; **29**:1-4.
40. Permpoonpattana P, Phetcharaburanin J, Mikelson A, Dembek, M, et al. *J Bacteriol.,* 2013; **195**:1492-1503.
41. Sloan LM, Duresko BJ, Gustafson DR, Rosenblatt JE. *J Clin Microbiol*., 2008; **46**:1996-2001.
42. Yaeger M, Funk N, Hoffman L. *J Vet Diagn Invest.*, 2002; **14**:281-287.
43. Shen Z, Wu Z, Chang D, Zhang W, et al. Angew Chem Int Ed., 2016; **55**:2431-2434.
44. Cataldo MA, Granata G, Petrosillo N. *Expert Rev Anti Infect Ther.*, 2017; **15**:1027-1040.
45. Kirk JA, Banerji O, Fagan RP. *Microb Biotechnol.*, 2017; **10**:76-90.
46. Ammam F, Marvaud, JC, Lambert T. *Can J Microbiol*., 2012; **58:**547-551.
47. Austin M, Mellow M, Tierney WM. *Am J Med.*, 2014; **127**:479-483.
48. Tariq R, Khanna S. *Indian J Gastroenterol.*, 2017; **36**:3-10.
49. Kujawa-Szewieczek A, Adamczak M, Kwiecień K, Dudzicz S, et al. *Nutrients*; 2015; **7**:10179-10188.
50. Giry M, Popoff MR, Eichel-Streiber CV, Boquet P. *Infect Immun.*, 1995; **63**:4063-4071.
51. Fitzpatrick LR, Small JS, Greene WH, Karpa KD. *Gut Pathogens*, 2011; **3**:1-9.
52. Ebigbo A, Messmann H. *Med Klin Intensivmed Notfmed.*, 2013; **108**:624-627.
53. Arbel LT, Hsu E, McNally K. *Cureus*, 2017; **9**:e1599.
54. Rao K, Safdar N. *J Hosp Med.*, 2016, **11**:56-61.
55. Wang JW, Kuo CH, Kuo FC, Wang YK, et al. *J Formos Med Assoc.*, 2019; **118 Suppl 1:**S23-S31.
56. Friedman-Korn T, Livovsky DM, Maharshak N, Aviv Cohen N, et al. *Dig Dis Sci*., 2018; **63**:198-203.

57. Baxter M, Colville A. *J Hosp Infect*., 2016; **92**:117-127.
58. Liu R, Suárez JM, Weisblum B, Gellman SH, et al. *J Am Chem Soc.*, 2014; **136**:144498-144504.
59. Gerding DN, Meyer T, Lee C, Cohen SH, et al. *JAMA*, 2015; **313**:19-27.