Journal of Health, Medicine and Nursing
ISSN 2422-8419 An International Peer-reviewed Journal
Vol.37, 2017

Determination the Pathogenicity of C.difficile Isolates among Mice

Luma Yousif Mehdi [1*] Muna Turkey AL-Mossawei [2]
1.College of Health and Medical Technology,Baghdad ,Iraq
2.Biology Department ,College of Science for women,Baghdad University,Iraq

Abstract
Clostridium difficile is the main causative agent of antibiotic-associated diarrhea and colitis in hospitalized patients, the disease is caused mainly by two exotoxins, TcdA and TcdB, produced by the bacteria. Recurrent *C. difficile* infection (CDI) constitutes one of the most significant clinical issues of this disease . **This study aimed to** Determination the pathological effects of *C. difficile* in mice, the results shown that bacterial dose $1x10^6$ CFU\ml led to 50% death within (2-4) days post infection and 100% developed diarrhea ,that would cause infection but not universal lethality . In conclusion, we have established a mouse CDI model that allows for future investigations of the role of the host immuneresponse in the disease's pathogenesis and permits critical testing of new therapeutics targeting recurrent disease.
Keywords: *C.difficile*, dose, pathological effects, mouse.

Introduction

Clostridium difficile (*C. difficile*) infections (CDI) are a clinical concern and are one of the leading causes of antibiotic - associated diarrhea of nosocomial outbreaks, CDI is dramatically increasing in both the prevalence and clinical severity of cases**.** (McFarland, 2015)

C. difficile is a Gram positive, rod-shaped, sporforming, anaerobic and toxin producing bacteria, commonly isolated from soil, humans, and other mammals (Kuijper *et al.,* 2006 ;McFee and Abdelsayed , 2009) . Also is a multidrug-resistant pathogen, flourishes in the colon after the gut microbiota has been altered by antibiotic therapy (Darkoh *et al*.,2015). Acquired either from the environment or the fecal- oral route (Khanna *et al.*,2012; Julia *et al*.,2013). About (15- 25)% of the episodes of antibiotic associated diarrhea (AAD) is linked with the pathogenic

strains , also 86% of *C. difficile* isolates from the suspected cases of *C. difficile* associated diarrhea (CDAD) were characterized as toxigenic .(Barbut *et al.,* 2007; Cohen *et al.,*2010) . The CDI were associated with two virulence factors potential toxins including enterotoxin A and cytotoxin B (linked to the *tcdA* and *tcdB* genes, respectively), producing from pathogenic strains of *C. difficile* (Sunenshine and McDonald , 2006 ; Kuehne *et al*., 2011) .Another toxin known as *C. difficile* binary toxin (cdt) has been isolated from some certain strains that associated with severe infectious form of disease in human (McEllistrem *et al*., 2005 ; Songer , 2010 ; Lessa *et al*., 2012).

Clinically, there is a wide spectrum of *C. difficile* presentations ranging from asymptomatic carriage to severe, life threatening, fulminant colitis, and toxic megacolon (Bartlett and Cerding ,2008; Surawicz,2013).The severity of CDI ranges from mild diarrhea to pseudo membranous colitis (PMC) and can result in death (Alcala ,2013), reaching (95–100)% among patients with documented antibiotic-associated colitis (Christina *et al.*,2013). Recurrent CDI occurs in more than 20% of patients (Sun *et al*.,2011), that become more frequent, more severe, more refractory to standard treatment , and more likely to relapse (Mattila *et al*.,2012; Khanna and pardi , 2014) . The aims of this study is to Determination pathological study of *C. difficile* in mice.

Materials and Methods

C.difficile isolate:

C. difficile were isolation and identification from stool samples were collected from hospital in Baghdad previously by :1.selective media. 2.Gram stain ,Malachite green for spore,Api20A Kit(BioMerieux,USA). 3.detection of two toxins A&B in stool samples by ELISA Kit (primier toxin A&B from Meridian Bioscience ,USA).4. Detection of toxins A&B genes by PCR method .(Mehdi and Al-Mossawei,2015).

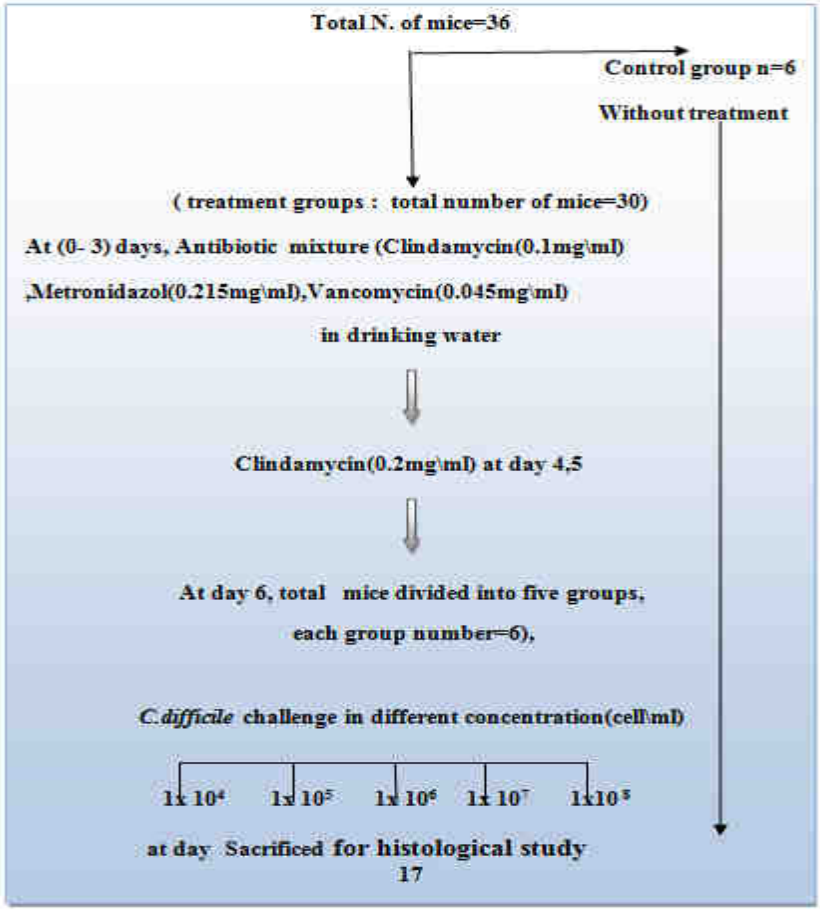
Preparation of *C. difficile* spores :

Five different dose of spores ($1x10^4$,$1x10^5$,$1x10^6$,$1x10^7$,$1x10^8$)spore \ml were used for challenge orally to determined the suitable concentration that will be use to experimental study in mice ,as following: Concentration of spore\ml=(10^6 x4xB)\N
were : B= spores number , N=chamber square counted .
Sporulation of the *C. difficile* was induced on brain heart infusion (BHIS) agar, culture were diluted in fresh media to turbidty equal McFarland (0.5), 0.1ml of this suspension was spread on BHIS agar and anaerobic incubation for seven days, the spores were washed off the plate with phosphate buffered saline, and stored at 4 ^{0}

C ,and concentration determined by serial dilution and using cytometer chamber and McFarland(0.5) (Sorg *et al*.,2010). Each mouse was inoculated intragastrically by sterile 1 ml gavage syringe with 200 µl of the spore suspension containing ($1x10^4$,$1x10^5$,$1x10^6$,$1x10^7$,$1x10^8$) CFU/ml (Sun *et al*.,2011) for challenge .

Experimental infection in mice :
Thirty six(n=36) albino male mice, Mus musculus BALB /C strain aged (5-6)weeks and weighing (20-25) g. were obtained from AL-Nahrain research center ,AL-Nahrain university.

Determination the pathogenicity of *C.difficile* isolates among mice:
Thirty six(n=36) mice were randomly divided into six groups designated as 1, 2, 3,4,5 and 6. Each group consisted of 6 mice, and subjected to the following treatments according to(Sun *et al.*,2011). Scheme(1).
To establish *C.difficile* ,mice were treated at day (0-3) with an antibiotic mixture in drinking water: Clindamycin(0.1mg\ml\Pharma ,USA) ,
Metronidazol(0.215mg\ml\India),Vancomycin(0.045mg\ml\Julphar,U.A.E), followed, at day 4 ,administration 200µl of Clindamycin concentration (0.2mg\ml) orally, and at day 5, administration 500µl of Clindamycin concentration (0.2mg\ml) intraperitoneal injection (i.p.) .(Chen *et al.*,2008) . These drugs that have been used to disrupt intestinal microbial to establish of *C.difficile* infection.



Scheme(1) Experimental study of *C.difficile* Pathogenicity among mice.

Results and Discussion
Pathogenicity of C.difficile among mice : results shown in table (1)bacteria dose of $1x10^6$ CFU\ml led to 50% death within (2-4) days post infection and 100% developed diarrhea ,that would cause infection but not universal lethality .figure (1) that was selected as suitable dose .

Table(1):The suitable bacteria dose concentration CFU\ml that would cause severe infection in experimental mice (each group No =6)

Bacterial Dose CFU\ml	Total No .of mice in each group	Death of mice No. within (2-4) day post infection(%)	No. of Developed diarrhea(%)	Chi- square(χ^2)
$1x10^4$	6	0 (0.00%)	2 (33.33%)	9.715 **
$1x10^5$	6	2 (33.33%)	4 (66.67%)	9.715 **
$1x10^6$	**6**	**3 (50.00%)**	**6 (100%)**	11.569 **
$1x10^7$	6	5 (83.33%)	6 (100%)	6.723 **
$1x10^8$	6	6 (100%)	6 (100%)	0.00 NS
Chi- square(χ^2)		15.248 **	11.804 **	---
	** (P<0.01), NS: Non-significant.			

There were highly significant differences between each concentration groups .

C. difficile is responsible of AAD in humans and animals, and is one of the most common nosocomial pathogens. Pathogenic *C. difficile* produces two exotoxins, toxin A and toxin B which induce intestinal inflammation, fluid secretion and mucosal injury (Pothoulakis, 1996).

In conclusion, we have established a mouse CDI model that allows for future investigations of the role of the host immuneresponse in the disease's pathogenesis and permits critical testing of new therapeutics targeting recurrent disease.

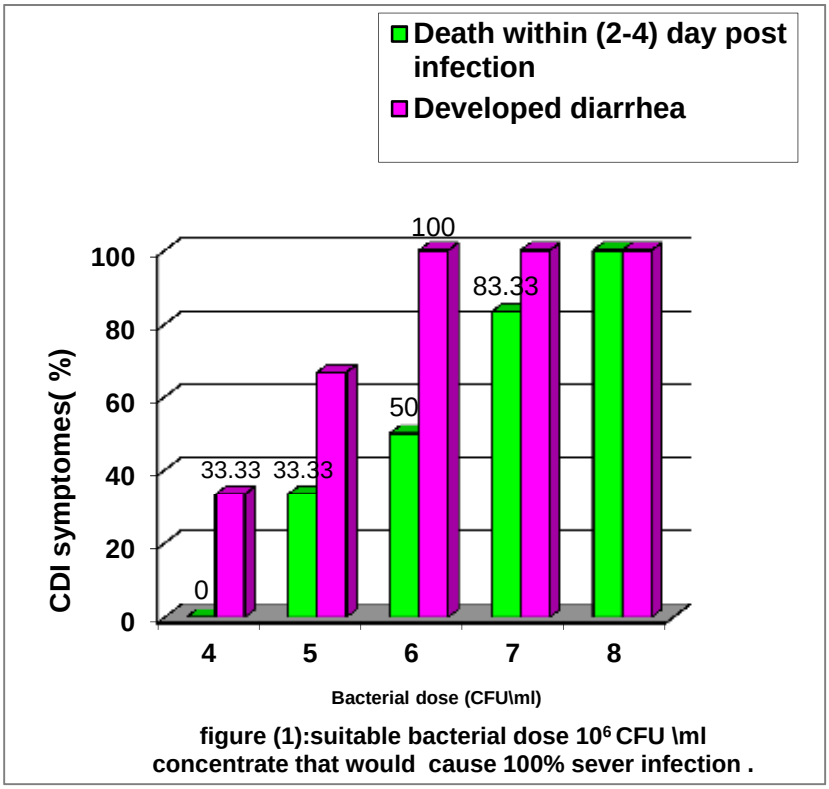


figure (1):suitable bacterial dose 10^6 CFU \ml concentrate that would cause 100% sever infection .

References

McFarland, L. V. (2015).Probiotics for the Primary and Secondary Prevention of *C. difficile* Infections: A Meta-analysis and Systematic Review Antibiotics , *4*:160-178.

Kuijper, E.; Coignard, B. and Tüll, P. (2006). Emergence of *Clostridium difficile* associated disease in North America and Europe. *Clin Microbiol Infect.*,12(6):2‑18.

McFee RB, and Abdelsayed GG. (2009). *Clostridium difficile. Dis. Mon.* ,55: 439‑70.

Darkoh, C.; DuPont H.L.; Norris, S. J. and Kaplan ,H.B. (2015).Toxin Synthesis by *Clostridium difficile* Is Regulated through Quorum Signaling. *M Bio.* , 6(2):e02569-14.

Khanna, S. and Pardi, D . (2012). *Clostridium difficile* infection: new insights into management. *Mayo Clin Proc.*, 87: 1106–1117.

Julia, S. S.; Philip, T .and Theoklis, E. Z,(2013). *Clostridium difficile* Infection in Children. *JAMA Pediatr.*,167(6):567-573.

Barbut, F; Mastrantonio, P.; Delmee, M.; Braziar, J.; Kuijper, E. and Poxton, I. (2007). Prospective study of *Clostridium difficile* infections in Europe with phenotypic and genotypic characterisation of the isolates.*Clin Microbiol Infec*.,13 :1048‑1057.

Cohen, S.H. ; Gerding, D.N; Johnson, S.; Kelly, C. P. ; Loo V. G. ; McDonald, L. C. ; Pepin, J. and Wilcox, M. H. (2010).Clinical practice guidelines for *Clostridium difficile* infection in adults: 2010 update by the society for healthcare epidemiology of America SHEA) and the infectious diseases society of America(IDSA). *Infect Control Hosp Epidemiol* ., 31: 431-55.

Sunenshine, R. H. and McDonald, L. C. (2006). "*Clostridium difficile*-associated disease: new challenges from an established pathogen*." Cleve Clinic J Med*. ,73(2): 187-197.

Kuehne, S.A; Cartman, S.T. and Minton, N.P. (2011).Both, toxin A and toxin B, are important in *Clostridium difficile* infection. *Gut Microbes*, 2(4): 252–255.

McEllistrem, M.C.; Carman, R.J.; Gerding, D.N.; Genheimer ,C.W.; and Zheng, L.(2005). A hospital outbreak of *Clostridium difficile* disease associated with isolates carrying binary toxin genes. *Clin Infect Dis.*, 40:265‑272.

Songer, J.G.(2010) .Clostridia as agents of zoonotic disease. *Vet Microbiol*.,140: 399‑404.

Lessa, F.C. ; Gould, C.V. and McDonald, L.C. (2012). Current status of *Clostridium difficile* infection epidemiology. *Clin Infect Dis.*, 55(2):65-70.

Bartlett, J. G and Cerding, D. N. (2008). Clinical Recognition and Diagnosis of *Clostridium difficile* Infection . *Clin. Infect. Dis*.,46: 12-18.

Surawicz, C. M. ; Brandt, L.J. ; Binion, D. G. and Ananthakrishnan A. N. (2013). Guidelines for Diagnosis, Treatment, and Prevention of *Clostridium difficile* Infections. *Am J Gastroenterol*., (108):478–498.

Alcala, L.(2013). Laboratory tests for diagnosis of *Clostridium difficile* infection: Past, present, and future. *Enferm Infect Microbiol Clin.*,31(2):65–67).

Christina, M. S.; Lawrence, J. B .; David, G. B. (2013). Guidelines for Diagnosis, Treatment, and Prevention of *Clostridium difficile* Infections. *Am J Gastroenterol* .,9(1): 108;478–498.

Sun, X.; Wang, H.; Zhang, Y.; Chen, K.; Davis, B. and Feng, H.(2011). Mouse relapse model of *Clostridium difficile* infection. *Infect Immun*., 79:2856–2864.

Mattila, E.; Seppala, R. and Lehtola ,L. (2012). Fecal Transplantation, Through Colonoscopy, Is Effective Therapy for Recurrent Clostridium difficile Infection. Gasteroenterol.,142 :490–496.

Khanna, S. and Pardi, D.(2014). *Clostridium difficile* infection: management strategies for a difficult disease. *Ther Adv Gastroenterol*., 7(2): 72–86.

Mehdi L.Y. and AL-Mossawei M.T.(2015)[a].PCR for Detection of *Clostridium difficile* toxin A(*tcdA*)and toxin B (*tcdB*)genes in Iraq. *Journal of Health ,Medicine and Nursing.* 15,ISSN 2422-8419.

Sorg, J. A. and Sonenshein, A. L.(2010). Inhibiting the initiation of *Clostridium difficile* spore germination using analogs of chenodeoxycholic acid, a bile acid. *J. Bacteriol*.,192: 4983–4990.

Chen, X.; Katchar, K.; Goldsmith, J.D.; Nanthakumar, N.; Cheknis, A.; Gerding ,D.N.and Kelly, C.P. (2008) A mouse model of *Clostridium difficile*-associated disease. *Gastroenterol.*, 135:1984–1992.

Pothoulakis, C. (1996). Pathogenesis of *Clostridium difficile* associated diarrhoea. *Eur J Gastroenterol Hepatol* .,8: 1041-1047.