ORIGINAL ARTICLE

Clostridium difficile in Antibiotic-Associated Diarrhoea and Detection of Toxin Producing Strains in a Tertiary Care Hospital in Western Maharashtra

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Abstract:

Background: Rampant and injudicious use of broad spectrum antibiotic in hospitalized patients has increased the incidence of *Clostridium difficile* Associated Diarrhea (CDAD). In recent years, *Clostridium difficile* Infection (CDI) has become more frequent, severe, and difficult to treat. *Aim and Objective:* A prospective, study was conducted to isolate *C. difficile* in Antibiotic-associated Diarrhoea (AAD) and to detect toxin producing strains of *C. difficile* from faecal samples of patients suspected to have CDI. *Material and Methods:* A total of 111 hospitalized patients who developed diarrhoea after >72 hours of admission and suspected of CDI were enrolled for investigation. The samples were subjected to anaerobic culture and toxin assay. *Results:* The total sample size of the study was 111 patients who were having antibiotic associated diarrhoea. Majority of the patients were from the age group 21-30 years and 41-50 years i.e., 23 (20.7%). Males 64 (57.7%) were affected more as compared to females 47 (42.3%). Third generation cephalosporins were the most common group of antibiotics associated with both AAD 36 (32.4%) and CDAD 9 (42.85%) cases, followed by carbapenem fluroquinolones in combination 3 (12.5%). Culture positivity was seen in 12 (10.81%) of the 111 stool samples and 39 (35.13%) were toxin producers. *Conclusion:* The use of several medications was found to be associated with an increased risk of CDAD. The only way to reduce *Cl. difficile* infection is to judiciously use antibiotics, strictly adhere to antibiotic policy and to give prime importance to strict infection control measures.

Keywords: *Clostridium difficile, Clostridium difficile* Associated Diarrhea, Antibiotic-associated Diarrhoea

Introduction:

Clostridium difficile (Cl. difficile) is an important cause of antibiotic-associated diarrhea and one of the most common healthcare associated infections all around the world. It accounts for 15–25% of cases of nosocomial antibiotic-associated diarrhoea, 50-75% of antibiotic associated colitis and 90-100% of antibiotic associated pseudomembranous colitis [1-3]. Rampant and injudicious use of broad spectrum antibiotic in hospitalized patients has increased the incidence of *Cl. difficile* Associated Diarrhea (CDAD) [1].

Cl. difficile is a Gram-positive, spore-forming, anaerobic rod shaped bacteria. It can be a part of the normal intestinal microbial flora and some individuals, particularly at the extremes of age, may be asymptomatically colonized. *Cl. difficle* causes a wide spectrum of illness ranging from asymptomatic colonization or mild diarrhea to fulminant disease characterized by toxic megacolon, sepsis, pseudomembranous colitis and death. Major risk factors for *Cl. difficile* infection include advanced age, hospitalization, and exposure to antibiotics [3].

Antibiotic-associated Diarrhoea (AAD) is defined as otherwise unexplained diarrhea that occurs in association with the administration of antibiotics [4-5]. Antibiotic-associated diarrhea is a common

side effect of treatment with the antibiotic agents. It is estimated that 10-15% of all hospitalized patients treated with antibiotics will develop diarrhoea [4]. Almost all antibiotics have been associated with it.

The antibiotics commonly associated with diarrhoea are ampicillin, amoxycillin, clindamycin and cephalosporin, occasionally macrolides, penicillins, cotrimoxazole, lincomycin, tetracyclines, aminogylcosides and fluroquinolones. The frequency of diarrhoea varies with the antibacterial agents. Diarrhoea occurs in approximately 5-10% of patients who are treated with ampicillin, 10-25% of those who are treated with amoxicillin–clavulanate, 15-20% of those who receive cefixime, and 2-5% of those who are treated with cephalosporins, fluoroquinolones, azithromycin, clarithromycin, erythromycin, and tetracycline [4-5]. The rates of diarrhoea associated with parenterally administered antibiotics, especially those with enterohepatic circulation, are similar to the rates associated with orally administered agents [6].

CDAD is a life threatening disease with an attributeable mortality of 6-15% and up to 25% in frail elderly people [7]. In recent years, *Cl. difficile* Infection (CDI) has become more frequent, severe, and difficult to treat [8-10]. Emergence of hypervirulent strains like NAP1/B1/027 of *Cl. difficile* has made infections even more difficult to treat and a major public health concern.

There are very few Indian studies that report the prevalence, epidemiology, diagnosis, molecular characterisation and risk factors of CDI in hospitalized patients with diarrhoea. CDI among hospitalized patients is a growing concern in India. Frequent outbreaks of CDI can occur due to the presence of *Cl. difficile* along with the number of people receiving antibiotics and other drugs in the hospitals. In this prospective study, considering the importance of CDIs in hospital settings, a prospective, laboratory based observational study was conducted to culture for *Cl. difficile* and Toxin A and Toxin B detection was done from faecal samples of patients suspected to have CDI.

Material and Methods:

The study was conducted from 1st May 2017-30th June 2018 in the Department of Microbiology, Krishna Institute of Medical Sciences, Karad, Maharashtra, India. The study was approved by the Institutional Ethics Committee. Written informed consent was obtained from all patients or their parents/guardians in case of minors.

A total of 111 hospitalized patients who developed diarrhoea after >72 h of admission and suspected of CDI were enrolled for the study. Diarrhoea was defined as the occurrence of three or more loose stools per day lasting for at least two days. Patients with incomplete data, pregnant women and children less than two years of age were excluded from the study.

Stool samples were collected from the patients admitted in the hospital for reason other than diarrhoea and developed diarrhea after 72 h of antibiotic administration.

Demographic and clinical data of the patients including clinical diagnosis, age, sex, frequency and duration of diarrhoea, stool consistency and presence of blood and mucous in the stool were recorded. These patients admitted to various wards (Surgery, ICU, medical ward, etc) of the hospital were undergoing treatment for underlying disease conditions. Information on antibiotics and other drugs received by them during the past two weeks was noted at the time of

sample collection. The patients were evaluated for other signs and symptoms of CDI inclusive of fever and pain abdomen. The patients were categorized according to their age into groups.

Single faecal samples from patients suspected of CDI were received in the department of Microbiology. Stool sample were inoculated on the routine culture media; BHIA and selective media Cycloserine Cefoxitin Fructose Agar (CCFA) (Fig.1). Colonies were identified by Gram staining (Fig. 2). Identification of isolates was done by culture appearance, Gram staining, and biochemical tests. Detection of enterotoxin and cytotoxin (Toxin A and Toxin B) of *Cl. difficile* was performed on the stool specimen according to manufacturer's instructions by a double sandwich enzyme-linked immunosorbent assay technique using a commercial kit. (Premier® Toxins A and B-Meridian Bioscience Europe).

A cut off OD value of 0.150 at a wavelength of 450 nm was taken for result interpretation. A diagnosis of CDAD was made in all patients with stool samples positive for toxins A and B. The data were entered into database programme and analyzed by SPSS version 20.0 (SPSS Inc., Chicago, IL, USA).

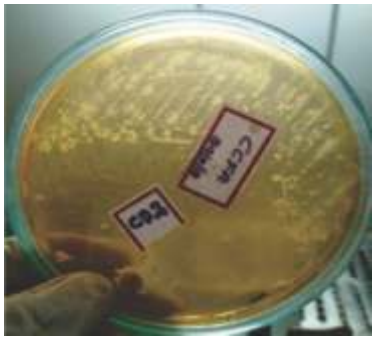

Fig. 1: Cycloserine Cefoxitin Fructose Agar

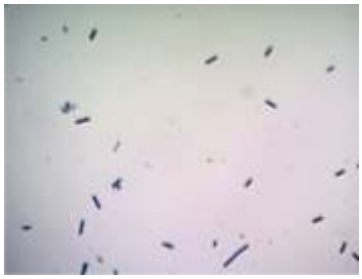

Fig. 2: Colonies were identified by Gram Staining

Results:

The total sample size of the study was 111 patients who were having AAD. Of total 111 patients admitted for the reason other than diarrhea and were given antibiotics and developed diarrhea after 72 h of antibiotics administration, were included in the study group i.e. AAD cases.

Table 1 shows age and sex wise distribution of AAD cases. Of the 111 patients analyzed in the study, 64 (57.7%) were males and 47 (42.3%) females with age ranging from 10 to 87 year (mean age ± SD: 44.44 ± 17.54 year). Majority of the patients were from the age group 21-30 years and 41-50 yrs i.e. 23 (20.7%). Males 64 (57.7%) were affected more as compared to females 47 (42.3%).

Table 1: Age and Sex wise Distribution of AAD Cases

Age Group (Yrs)	Male (%)	Female (%)	Total (%)
1-10	0	0	0
11-20	1 (0.9)	6 (6.4)	7 (5.3)
21-30	8 (7.2)	15 (13.5)	23 (20.7)
31-40	8 (7.2)	9 (8.1)	17 (15.3)
41-50	14 (12.6)	9 (8.1)	23 (20.7)
51-60	14 (12.6)	6 (5.4)	20 (18.0)
>61	19 (17.1)	2 (1.8)	21 (18.9)
Total	64 (57.7)	47 (42.3)	111 (100)

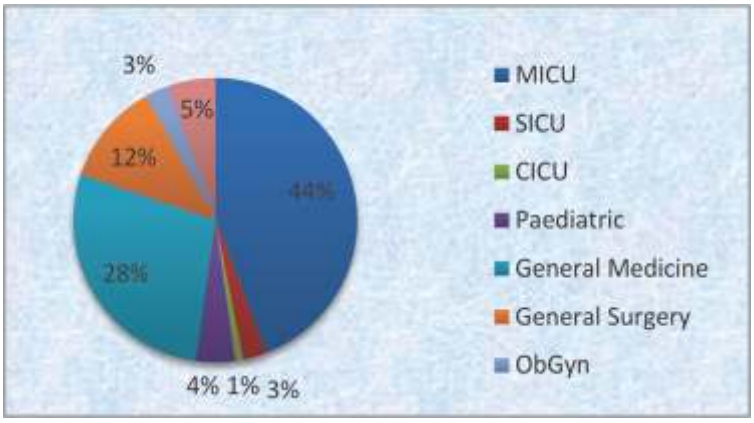

Fig. 3: Distribution of AAD Cases in Various Departments

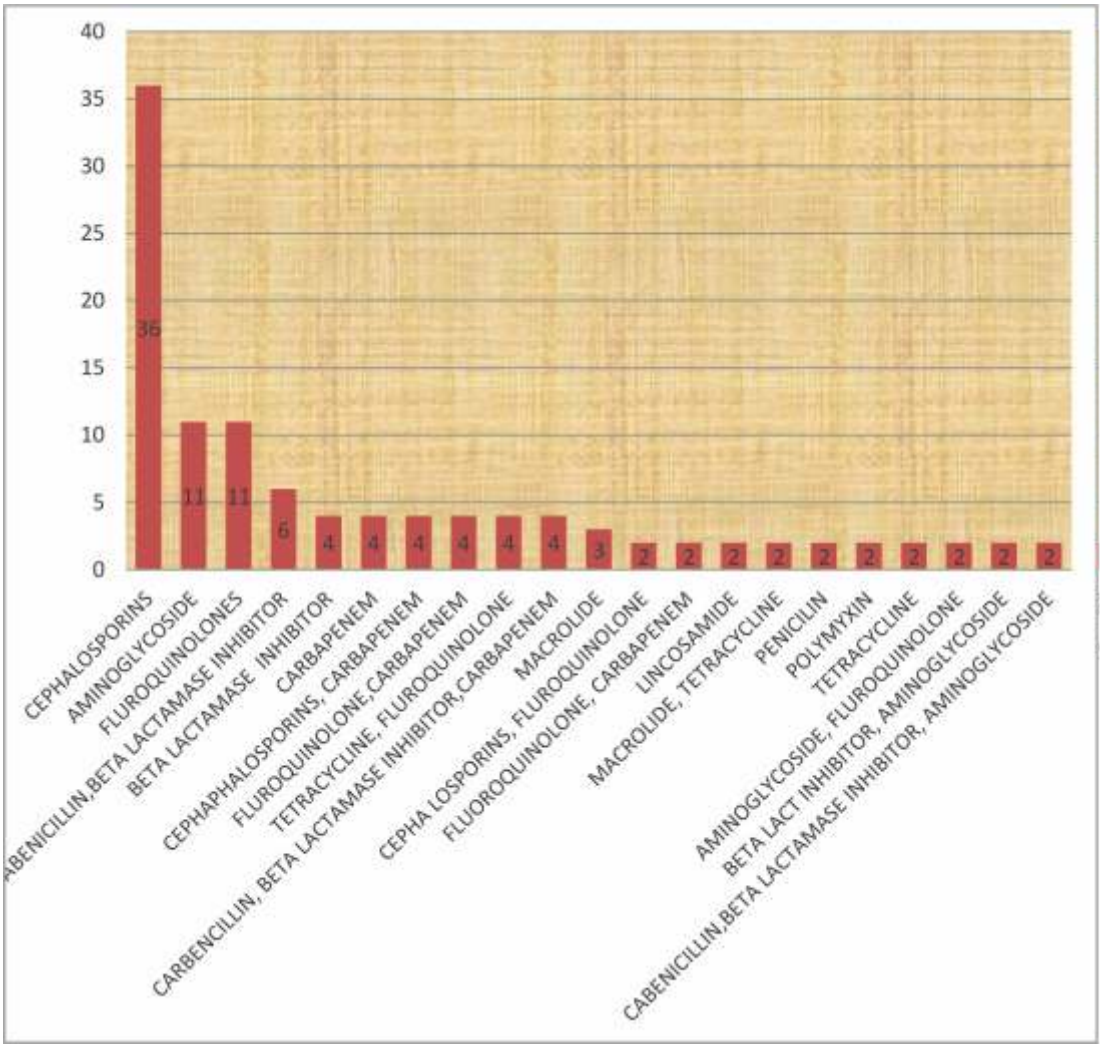

Fig. 4: Categorization of Antibiotics in AAD Cases

Fig. 3 shows distribution of AAD cases in various departments. Majority of the patients were from medical ICU 49 (44.14%) followed by general medicine 31 (27.2%). Least number of cases was from CICU 1 (0.9%). Predominant patients presenting clinical symptoms as watery diarrhoea in 66 (59.46%), pain abdomen in 35 (31.53%) and fever in 42 (37.84%). The average duration of diarrhoea was 1.86±0.84 days, and was not different in various age groups.

Categorization of antibiotics received by patients is shown in Fig 4. Of the 111 patients, the major antibiotic groups in use were cephalosporines (32.4%, n=36), aminoglycosides (9.9%, n=11), fluroquinolones (9.9%, n=11), were associated with antibiotic associated diarrhoea cases.

Table 2 shows antibiotics in CDAD. Third generation cephalosporins were the most common group of antibiotics associated with both AAD 36 (32.4%) and CDAD 9 (42.85%) cases, followed by carbapenem fluroquinolones in combination 3 (12.5%) and fluroquinolones, tetracycline and fluroquinolone in combination 2 (9.52%) and macrolides, carbapenem, sulfonamides, cephalosporins, polymyxin 1 each (4.76%). *Cl. difficile* was isolated from 12 (10.81%) of the 111 stool samples. *Cl. difficile* isolate positivity the minimum age 16 years and 62 was maximum age of patient. The male to female ratio for *Cl. difficile* isolate positive patient was 2:1. There was no significant difference between the mean age of patients with *C. difficile* isolates and those negative for *Cl. difficile. Cl. difficile* toxin A and B ELISA was performed on 111 fresh stool samples, 39 (35.13%) were toxin producers. all culture positive samples were ELISA positive.

Discussion:

Cl. difficile is an important cause of AAD and one of the most common healthcare associated infections all around the world. The studies on CDI throughout Asia [11] and particularly India are limited. Most previous studies about CDAD in India have shown prevalence rates ranging from

Table 2: Categorization of Antibiotics Causing CDAD

Antibiotic Group	CDAD Cases	Percentage
Cephalosporins	9	42.85
Fluroquinolones	2	9.52
Macrolides	1	4.76
Sulfonamides, Cephalosporins	1	4.76
Carbapenem	1	4.76
Polymyxin	1	4.76
Fluroquinolone, Carbapenem	3	12.5
Cephalosporins, Carbapenem	1	4.76
Tetracycline, Fluroquinolone	2	9.52

7.1-26.6% [11-20]. Niyogi *et al*. (1991) have isolated *Cl. difficile* in 11% hospitalized patients with diarrhoea [18]. Gupta *et al*. (1985) have reported 25.3% isolation of *Cl. difficile* from diarrheal patients of all age groups [19]. Bhattacharya *et al*. (1991) have investigated 233 patients with acute diarrhoea and isolated *Cl. difficile* as a sole pathogen from 7.3%, of which, 82.4% produced cytotoxin [20]. In these studies the different methods of detecting *Cl. difficile* were used. In our study we found a prevalence rate of 10.81% by culture and 35.13% by ELISA.

Dutta *et al.* [16] have reported an isolation rate of 3.6%. Ingle *et al.* [17] have reported a prevalence rate of 17% using ELISA for *Cl. difficile* toxins A and B in a retrospective study of all in-patients and out-patients of their hospital.

In our study we had maximum cases from MICU 49(44.14%) followed by general medicine and general surgery. Chaudhary *et al.* (2008) [1] have reported 524 samples were analyzed for *Cl. difficile* toxin, total of 37 (7.1%) specimens were toxin producers. Highest number of toxin producers were from hematology/oncology ward (67.5%) followed by surgery and neurology wards. The most common antibiotics implicated in hospital-acquired CDI include cephalosporins, ampicillin/amoxicillin and clindamycin even though all antibiotics have been implicated at one time or the other [21]. Pakyz *et al.* [22] have reported aminoglycosides 95%, β lactamase inhibitors 42.0%, first generation cephalosporins 26.5%, second generation cephalosporins 4.7%, third or fourth generation cephalosporins 50.7%, and fluoroquinolones 46.8%. Lv *et al.* [23] have reported β-lactam/β-lactamase inhibitor compounds 12 (26.67%), cephalosporins 29 (64.44%), carbapenem 5 (11.11%), glycopeptides 1 (2.22%), quinolone 10 (22.22%), amino-glycosides 5 (11.11%), lincosamides 6 (13.33%), macrolides 2 (4.44%).

In our study, cephalosporins 36 (32.4%), aminoglycosides 11 (9.9%), fluroquinolones 11 (3.6%), β-lactamse inhibitors 4 (3.6%) were the major group of antibiotics causing AAD. Third generation cephalosporins 9 (42.85%) were the major group of antibiotics causing CDAD followed by fluroquinolones 2 (9.52%) and macrolides 1 (4.76%). The reasons for lesser frequency of CDI in India could be due to frequent use of freely available metronidazole, incomplete antibiotic treatment, a good immune response towards *Cl. difficile* and high-fibre diet consumption. Apart from these, absence of virulent NAP1 could also contribute to lesser prevalence of CDI [24]. Limited documentation of culture or toxin proven CDI in India could also be because of inadequate facilities for culturing anaerobic pathogens in many of the hospitals [25].

Conclusion:

C. difficile infection is an important nosocomial infection with associated morbidity, mortality and cost. Hospitalized patients receiving antibiotics for their ailments are at a great risk of acquiring CDAD. Local surveillance is important. The use of several medications was found to be associated with an increased risk of CDAD. The only way to reduce *Cl. difficile* infection is to judiciously use antibiotics, strictly adhere to antibiotic policy and to give prime importance to strict infection control measures. Continuous surveillance for *Cl. difficile* infections needs to be done to monitor progress in prevention. The most successful control measure to reduce the asymptomatic diseases is to restrict antimicrobial use.

References

1. Chaudhry R, Joshy L, Kumar L, Dhawan B. Changing pattern of Clostridium difficile associated diarrhoea in a tertiary care hospital: a 5 year retrospective study. *Ind J Med Res* 2008; 127(4): 377.
2. Hedge DD, Strain JD, Heins JR, Farver DK. New advances in the treatment of Clostridium difficile infection (CDI). *Ther Clin Risk Manage* 2008; 4(5):949.
3. Vaishnavi C. Established and potential risk factors for Clostridum difficile infection. *Ind J Med Microbiol* 2009; 27(4):289.
4. Bartlett JG. Antibiotic-associated diarrhea. *Clin Infect Dis* 1992; 15(4):573-81.
5. Gilbert DN. Aspects of the safety profile of oral antibiotic agents. *Infect Dis Clin Pract* 1995; 4(2):S103.
6. Wiström J, Norrby SR, Myhre EB, Eriksson S, Granström G, Lagergren L, *et al*. Frequency of antibiotic-associated diarrhoea in 2462 antibiotic-treated hospitalized patients: a prospective study. *J Antimicrob Chemother* 2001; 47(1):43-50.
7. Vaishnavi C. Clostridium difficile infection: clinical spectrum and approach to management. *Ind J Gastroenterol* 2011; 30(6):245-54.
8. Dallal RM, Harbrecht BG, Boujoukas AJ, Sirio CA, Farkas LM, Lee KK, *et al*. Fulminant Clostridium difficile: an underappreciated and increasing cause of death and complications. *Ann Surg* 2002; 235(3):363-72.
9. McDonald LC, Killgore GE, Thompson A, Owens Jr RC, Kazakova SV, Sambol SP, *et al*. An epidemic, toxin gene–variant strain of Clostridium difficile. *New Engl J Med* 2005; 353(23):2433-41.
10. Pépin J, Valiquette L, Cossette B. Mortality attributable to nosocomial Clostridium difficile–associated disease during an epidemic caused by a hypervirulent strain in Quebec. *CMAJ* 2005; 173(9):1037-42.
11. Collins DA, Hawkey PM, Riley TV. Epidemiology of Clostridium difficile infection in Asia. *Antimicrob Resist Infect Control* 2013; 2(1):21.
12. Ayyagari A, Agarwal J, Garg A. Antibiotic associated diarrhoea: Infectious causes. *Ind J Med Microbiol* 2003; 21(1):6-11
13. Rupnik M. How to detect Clostridium difficile variant strains in a routine laboratory. *Clin Microbiol Infect* 2001; 7(8):417-20.
14. Burke KE, Lamont JT. Clostridium difficile infection: a worldwide disease. *Gut Liver* 2014; 8(1):1-6.
15. Vaishnavi C, Kaur S, Singh K. Clostridium perfringens type A & antibiotic associated diarrhoea. *Ind J Med Res* 2005; 122(1):52-6.
16. Dutta P, Niyogi SK, Mitra U, Rasaily R, Bhattacharya MK, Chakraborty S, *et al*. Clostridium difficile in antibiotic associated pediatric diarrhea. *Ind Ped* 1994; 31(2):121-6.
17. Ingle M, Deshmukh A, Desai D, Abraham P, Joshi A, Rodrigues C. Prevalence and clinical course of Clostridium difficile infection in a tertiary-care hospital: a retrospective analysis. *Ind J Gastroenterol* 2011; 30(2):89-93.
18. Niyogi SK, Bhattacharya SK, Dutta P, Naik TN, De SP, Sen D, *et al*. Prevalence of Clostridium difficile in hospitalised patients with acute diarrhoea in Calcutta. *J Diarrhoeal Dis Res* 1991; 9(1):16-9.
19. Gupta U, Yadav RN. Clostridium difficile in hospital patients. *Ind J Med Res* 1985; 82:398.
20. Bhattacharya MK, Niyogi SK, Rasaily R, Bhattacharya SK, Dutta P, Nag A, *et al*. Clinical manifestation of Clostridium difficile enteritis in Calcutta. *JAPI* 1991; 39(9):683-4.
21. Vaishnavi C, Bhasin D, Kochhar R, Singh K. Clostridium difficile toxin and faecal lactoferrin assays in adult patients. *Microbes Infect* 2000; 2(15): 1827-30.
22. Pakyz AL, Jawahar R, Wang Q, Harpe SE. Medication risk factors associated with healthcare-associated Clostridium difficile infection: a multilevel model case–control study among 64 US academic medical centres. *J Antimicrob Chemother* 2013; 69(4):1127-31.
23. Lv Z, Peng GL, Su JR. Factors associated with Clostridium difficile diarrhea in a hospital in Beijing, China. *Brazilian J Med Biol Res* 2014; 47(12):1085-90.
24. Vaishnavi C, Singh M, Mahmood S, Kochhar R. Prevalence and molecular types of Clostridium difficile isolates from faecal specimens of patients in a tertiary care centre. *J Med Microbiol* 2015; 64(11): 1297-304.
25. Lovely J, Rama C, Benu D. Detection and characterization of Clostridium difficile from patients with antibiotic-associated diarrhoea in a tertiary care hospital in North India. *J Med Microbiol* 2009; 58(12):1657-9.

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