Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.5, No.13, 2015

Antibiotic Susceptibility Testing for *Clostridium Difficile* Iraqi Isolation by using Disk Diffusion Method

Luma Yousif Mehdi[1*] Mona 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
Multidrug resistance were investigated in 75 Clostridium difficile clinical isolates collected during the period of first of June 2013 till the end of april 2014. These isolates show (100%) resistance to Colistin, Nalidixic acid, Cefotaxime, gentamycin, and high resistance to Clindamycin(95%), Ciprofloxacin(88%) , and moderate resistance to Erythromycin (65%), Ampicillin (53%), while show good sensitive to chloramphenicol (80%), and among these antibiotics, Vancomycin and metronidazol was the most effective antibiotic against Cdifficile isolates with high sensitivity (100%) ,(95%) respectively.
Keywords: antibiotics, resistance , Clostridium difficile.

Introduction

Clostridium difficile has been traditionally regarded as a nosocomial human pathogen.
In fact, several authors reported that this bacterium is an important cause of infectious diarrhea that usually develops in patients after hospitalization and antibiotic treatment.

The symptoms of infection range from asymptomatic colonization to mild diarrhea and severe life threatening pseudomembranous colitis.(Kra *et al*.,2014).

In the community setting, there is substantial variation in the risk of CDI associated with different antimicrobial classes. Avoidance of high-risk antibiotics (such as clindamycin, fluoroquinolones) in favor of lower-risk antibiotics (such as tetracyclines) may help reduce the incidence of CDI(Kevin *et al*.,2013; Daniel *et a*l.,2015).

C. difficile is an anaerobic, Gram-positive bacterium that has been implicated as the leading cause of antibiotic-associated diarrhea ,the pathogenic effects of *C. difficile* are mucosal damage to the colon that is caused by the production of toxin A (308 kDa) and/or toxin B (270 kDa)(Victor *et al*.,2014) . Metronidazole is currently the first-line treatment for mild to moderate *C. difficile* infections. Recent reports have identified treatment failure and relapse post metronidazole therapy, as well as reduced susceptible or metronidazole-resistant *C. difficile* strains from clinical isolates(Chong *et al.,*2014). Antibiotic exposure was an important risk factor for community-associated infection , but the risk was different amongst different antibiotic classes.

The risk was greatest with clindamycin followed by fluoroquinolones and cephalosporins, whereas tetracyclines were not associated with an increased risk (Abhishek *et al.,*2013; Daniel *et al*.,2015). This emphasizes the need for antimicrobial susceptibility testing of *C. difficile* and for a simple susceptibility testing method for the routine clinical microbiology laboratory. Disk diffusion is inexpensive and simple to perform and a few studies have evaluated disk diffusion for antimicrobial susceptibility testing of *C. difficile*(Huhulescu *et al.,*2011; Erikstrup *et al.,*2012).

Material and Methods.

Bacterial isolates and identification:

Four hundred thirty stool samples were collected from Iraqi patient ,children and adults suffering from antibiotic associated diarrhea ,and apparently healthy children and adults as showing in table (1) .Stool samples were streaked on selective media(CCFA) +7%horse blood as (George *et al*.,1979) ,incubation in anaerobic conditions at $37C^0$ for 48hrs ,and isolates were presumptively identified (Gram stain , and Malachite green for spore) ,definitive identification was performed by Api20A kit (BioMerieux ,USA), and detection of two toxins A& B in stool samples by ELISA Kit (Premier toxin A&B from Meridian Bioscience ,USA), according to the manufacturer's recommendations. Seventy five isolates positive for *Clostridium difficile* were selected for study .

Antibiotic dick: Disk diffusion was performed with Oxoid disks (Oxoid, UK).

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing to 11 antimicrobials(colistin, nalidixic acid, ,cefotaxime, clindamycin , gentamycin, ciprofloxacin, erythromycin, ampicillin, and chloramphenicol, vancomycin and metronidazol) was carried out for 75 isolates for *C. difficile* by the disk diffusion method on Muellere Hinton agar+ 5% blood(HiMedia, India) (Ebrahim et al., ,2014) , according to Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI ,2011).The antimicrobial agents tested were chosen because of emergence of reduced susceptibility.

Results and Discussion.

Seventy five *C. difficile* isolates were tested for antibiotic sensitivity. All isolates shown 100% resistant to each of Colistin, Nalidixic acid, ,Cefotaxime, Gentamycin. Also high resistance to Clindamycin , Ciprofloxacin was 95% and 88% respectively. The moderate resistance show to Erythromycin 65%, Ampicillin 53%. Chloramphenicol has good sensitive 80% , while excellent sensitivity was showed to vancomycin and metronidazole 100% and 95% respectively. (table.1), figure(1). There is significant association as $p< 0.01$ between *C.difficile* isolates and these antibiotics except Ampicillin 47% sensitive has not significant.

This results coincides with pervious study shown 100% resistant to Colistin, Gentamycin,(Norakhoda *et al.,*2010) and 100% resistant to Cefotaxime, Clindamycin89% (Mehdi *et al.*,2013) and (Alexander *et al.,* 2007) shown 100% resistance to Clindamycin . Pervious study shown high resistance of *C. difficile* to Clindamycin, Gentamycin, Nalidixic acid, Ciprofloxacin, but in agreement with them that *C.difficile* high resistance to Erythromycin and Ampicillin (Ebrahim *et al.,*2014).

Our result agreement with (Mehdi *et al.*,2013) that shown moderate resistance to Erythromycin 57%, and with(**P**atrizia *et al.,*2011) that shown a good sensitive to Chloramphenicol, but in agreement about resistance to Clindamycin 48%, Erythromycin 48%. Previous studies (Norakhoda *et al.,*2010;Kang *et al*.,2012) shown moderate resistance to Clindamycin.

The current results coincides with previous studies (Alexander *et al.,* 2007;; Patrizia *et al.,*2011 ; Kang *et al*.,2012 ;; Ebrahim *et al.,*2014) they found all isolates100% were sensitive to Metronidazole and Vancomycin. Our resealts similar to previous studies in Iran(Norakhoda *et al.,*2010)with high sensitivity to vancomycin (100%) and metronidazol(91%), and the same as (Mehdi *et al.*, 2013) that show high sensitive to Metronidazol (94%), and Vancomycin(92%). and with(Norakhoda *et al.,*2010 ;**P**atrizia *et al.,*2011) that shown a good sensitive to Chloramphenicol,

Metronidazole is highly active against most strains of pathogenic *C difficile* with only rare reports of antibiotic resistance.(Johnson *et al*., 2000) . Increasing evidence suggests, that prolonged exposure to metronidazole can lead to resistance(Pelaez *et al*.,2008) and that susceptibility decreases over time(.Baines *et al.,*2008) For this reason, surveillance of antibiotic resistance in *C difficile* is ongoing and resistance could limit the use of this antibiotic in the future. Metronidazole is generally recommended as the first-line treatment of *C difficile*. It induces microbial cell death by DNA disruption and subsequent inhibition of nucleic acid synthesis. Metronidazole is most effective in anaerobic sites such as the human colonic lumen. In addition to its antimicrobial properties, metronidazole also appears to have anti-inflammatory, antioxidant, and immunomodulatory effects (Baines *et al*,2008) .Vancomycin inhibits bacterial cell wall synthesis has broad activity against gram-positive bacteria, but it essentially has no effect on gram-negative bacteria or fungi. Vancomycin is highly active against all strains of pathogenic *C difficile*, and resistance has been reported in only a single study. Vancomycin is recommended as first-line therapy in pregnant women, in children younger than 10 years of age, and for severe infections. Metronidazole and vancomycin still seem to be most effective drugs for treatment CDI.(Bourgault *et al.,*2006 ; Moellering *et al.,*2006; Zar FA *et al,*2007).

Table 1. Distribution of sample study according to antibiotic sensitive

Antibiotic µg\disc	Sensitive		Intermediate		Resistance		Chi-square- χ^2
	No.	(%)	No.	(%)	No.	(%)	
Vancomycin, 30	75	100	0	0	0	0.00	14.39 **
Metronidazole,5	71	95.0	0	0	4	5.00	13.70 **
Clindamycin,10	4	5.00	0	0	71	95.0	13.74 **
Gentamycin,10	0	0.00	0	0	75	100	14.50 **
Ampicillin,25	35	47.0	0	0	40	53.0	1.09 NS
Chloramphenicol,10	60	80.0	0	0	15	20.00	10.66 **
Erythromycin,15	26	35.0	0	0	49	65.00	9.27 **
Colistin ,10	0	0.00	0	0	75	100	14.50 **
Nalidixicacid,30	0	0.00	0	0	75	100	14.50 **
Ciprofloxacin,10	9	12.0	0	0	66	88.00	12.94 **
Cefotaxime,10	0	0.00	0	0	75	100	14.50 **
Chi-square- χ^2	----	16.38 **	---	0.00 NS	----	16.38 **	----
** (P<0.01), NS: Non-significant.							

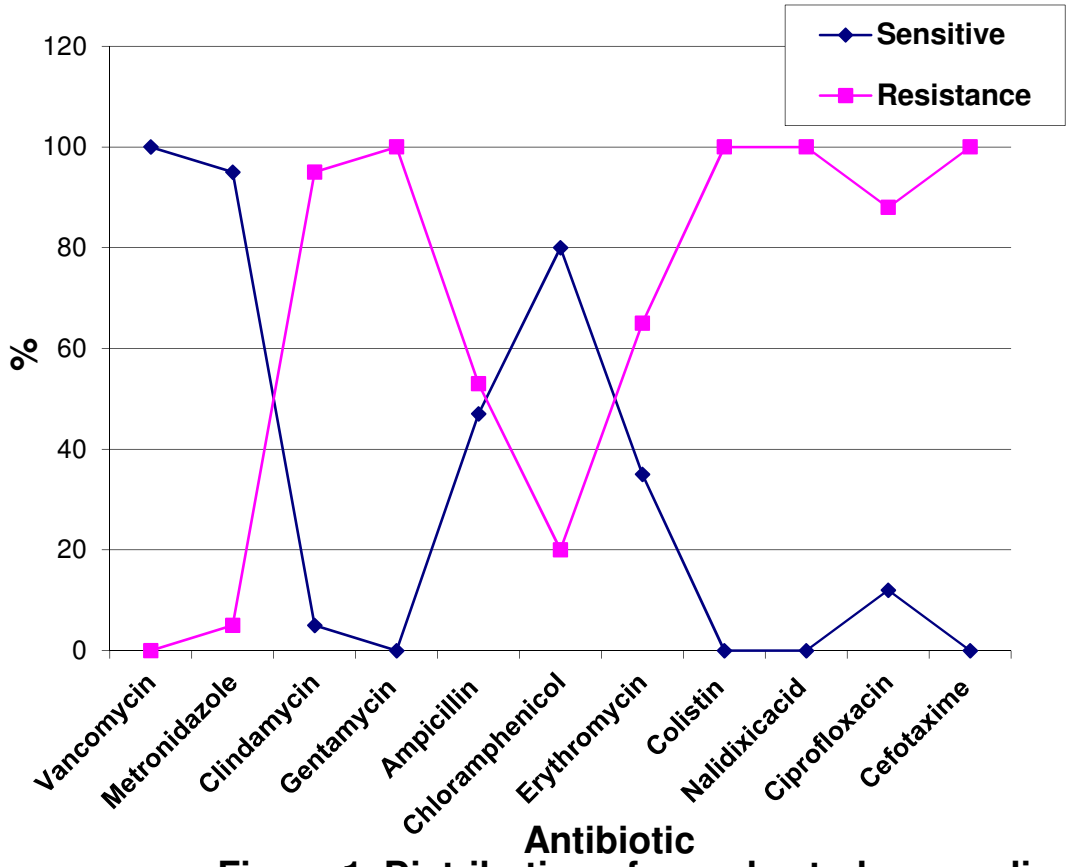

Figure 1. Distribution of sample study according to antibiotic sensitive (%)

References

1 - Kra A. K. , Adj_ ehi T. D. , Kouadio F. N. , Koffi M. D. , Yao G. L. ,(2014). Clostridium perfringens and Clostridium difficile in cooked beef sold in C^ote d'Ivoire and their antimicrobial susceptibility. Anaerobe 28 , 90e94.

2- Kevin A. Brown, N. K., Nick D., and David N. F. .(2013). Meta- Analysis of Antibiotics and the Risk of Community-Associated *Clostridium difficile* Infection. Antimicrob Agents Chemother. May 2013; 57(5): 2326–2332.

3- Daniel A. L.; Thomas J. L.,(2015). *Clostridium difficile* Infection. The New England Journal of Medicine. ;372:1539-48.

4- Victor R. C. M., Viviane N., Sydney M. F., and Mario J. A..(2014). Genes Encoding Toxin of *Clostridium difficile* in Children with and without Diarrhea. Hindawi Publishing Corporation Scientifica Volume 2014, Article ID 594014, 4 pages.

5- Chong PM, Lynch T, McCorrister S, Kibsey P, Miller M, et al. (2014). Proteomic Analysis of a NAP1 Clostridium difficile Clinical Isolate Resistant to Metronidazole. PLoS ONE 9(1): e82622. doi:10.1371/journal.pone.0082622.

6-. Abhishek D., Vinay P., Priyaleela T., Chaitanya P., David D. K. R., Thomas J. S, Adrian V. H. and Curtis J. D.(2013). Community-associated Clostridium difficile infection and antibiotics: a meta- analysis. Antimicrob Chemother; 68: 1951–1961.

7- Huhulescu S., Sagel U., Fiedler A. et al.,(2011). Rifaximin disc diffusion test for in vitro susceptibility testing of Clostridium difficile. J Med Microbiol; 60: 1206–1212.

8- Erikstrup L. T. , Danielsen T. K. L., Hall V., Olsen K. E. P. , Kristensen B. , Kahlmeter G. , Fuursted K. and Justesen U. S..**(2012).** Antimicrobial susceptibility testing of Clostridium difficile using EUCAST epidemiological cut-off values and disk diffusion correlates. Clin Microbiol Infect; 18: E266–E272.

9-George WL,SutterVL,CitronD,finegold SM,(1979).Selective and differential medium for isolation of *C.difficile .J Clin Microbiol* 9:214-219.

10- CLSI. Performance standards for antimicrobial susceptibility testing.(2011). CLSI document M100-S20. Wayne, PA: Clinical and Laboratory Standards Institute; 2011.

11-Mehdi G.; Hossein G. ; Masoud A.; Masoumeh A. R. ; Farahnaz S. S. M. ; Mohammad R. Z. ; and Mohammad M. A. .(2013).Antimicrobial Susceptibility of Clostridium Difficile Clinical Isolates in Iran. Iranian Red Crescent Medical JournalAugust; 15(8): 704-711.
12-Alexander R.P., Henry R. S., Todd D., and J. Scott W.(2007). *Clostridium difficile* in Retail Ground Meat, Canada Emerg Infect Dis. Mar; 13(3): 485–487.
13-Patrizia S., Fabrizio B., Paola M. (2011). Multidrug resistance in European *Clostridium difficile* clinical isolates. J. Antimicrob. Chemother. *doi: 10.1093.*
14- J. Kang, H. Pai, J. Kim, J.H. Hwang, T.Y. Choi (Guri, Seoul, KR) (2012). Comparison of the Clostridium difficile agar and Brucella agar for the antimicrobial susceptibility testing of Clostridium difficile. Antimicrobial susceptibility testing - EUCAST and beyond2012, 15:30 - 16:30.p679.
15-. Ebrahim R., Mohammad J. and Scott W. J.(2014). Prevalence of *Clostridium difficile* in raw beef, cow, sheep, goat, camel and buffalo meat in Iran. *BMC Public Health* .14:119.
16- Norakhoda S., Mohammad H. S., Mohammad R. G., Saeed E., and Farzanah A. H.(2010).The Incidence of Nosocomial Toxigenic *Clostridium difficile* Associated Diarrhea in Tehran Tertiary Medical Centers. *Acta Medica Iranica* 2010; 48(5): 320-325.
17- Johnson S, Sanchez JL, Gerding DN. (2000) .Metronidazole resistance in Clostridium difficile. Clin Infect Dis;31:625–626.
18- Pelaez T, Cercenado E, Alcala L, et al.,(2008) .Metronidazole resistance in Clostridium difficile is heterogeneous. J Clin Microbiol; 46:3028–3032.
19-. Zar FA, Bakkanagari SR, Moorthi KM, Davis MB. (2007). A comparison of vancomycin and metronidazole for the treatment of Clostridium difficile-associated diarrhea, stratified by disease severity Clin Infect Dis;45:302–307.
20- Baines SD, O'Connor R, Freeman J, et al.,(2008). Emergence of reduced susceptibility to metronidazole in Clostridium difficile. J Antimicrob Chemother;62:1046–1052.
21- Bourgault AM, Lamothe F, Loo VG, Poirier L.(2006). In vitro susceptibility of Clostridium difficile clinical isolates from a multi-institutional outbreak in Southern Quebec, Canada. Antimicrob Agents Chemother 2006;50:3473–3475.
22-. Moellering RC Jr. Vancomycin: a 50-year reassessment.(2006). Clin Infect Dis 2006;42(Suppl 1):S3–S4.

The IISTE is a pioneer in the Open-Access hosting service and academic event management. The aim of the firm is Accelerating Global Knowledge Sharing.

More information about the firm can be found on the homepage:
http://www.iiste.org

CALL FOR JOURNAL PAPERS

There are more than 30 peer-reviewed academic journals hosted under the hosting platform.

Prospective authors of journals can find the submission instruction on the following page: http://www.iiste.org/journals/ All the journals articles are available online to the readers all over the world without financial, legal, or technical barriers other than those inseparable from gaining access to the internet itself. Paper version of the journals is also available upon request of readers and authors.

MORE RESOURCES

Book publication information: http://www.iiste.org/book/

Academic conference: http://www.iiste.org/conference/upcoming-conferences-call-for-paper/

IISTE Knowledge Sharing Partners

EBSCO, Index Copernicus, Ulrich's Periodicals Directory, JournalTOCS, PKP Open Archives Harvester, Bielefeld Academic Search Engine, Elektronische Zeitschriftenbibliothek EZB, Open J-Gate, OCLC WorldCat, Universe Digtial Library , NewJour, Google Scholar