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

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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 al.,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 37C0 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 Mueller 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 Patrizia 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 reseals 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 ;Patrizia 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 appear s 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

<table>
<thead>
<tr>
<th>Antibiotic µg/disc</th>
<th>Sensitive</th>
<th>Intermediate</th>
<th>Resistance</th>
<th>Chi-square- $\chi^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (% )</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td></td>
</tr>
<tr>
<td>Vancomycin, 30</td>
<td>75 100</td>
<td>0 0</td>
<td>0 0</td>
<td>14.39 **</td>
</tr>
<tr>
<td>Metronidazole,5</td>
<td>71 95.0</td>
<td>0 0</td>
<td>4 5.00</td>
<td>13.70 **</td>
</tr>
<tr>
<td>Clindamycin,10</td>
<td>4 5.00</td>
<td>0 0</td>
<td>71 95.0</td>
<td>13.74 **</td>
</tr>
<tr>
<td>Gentamycin,10</td>
<td>0 0.00</td>
<td>0 0</td>
<td>75 100</td>
<td>14.50 **</td>
</tr>
<tr>
<td>Ampicillin,25</td>
<td>35 47.0</td>
<td>0 0</td>
<td>40 53.0</td>
<td>1.09 NS</td>
</tr>
<tr>
<td>Chloramphenicol,10</td>
<td>60 80.0</td>
<td>0 0</td>
<td>15 20.00</td>
<td>10.66 **</td>
</tr>
<tr>
<td>Erythromycin,15</td>
<td>26 35.0</td>
<td>0 0</td>
<td>49 65.00</td>
<td>9.27 **</td>
</tr>
<tr>
<td>Colistin ,10</td>
<td>0 0.00</td>
<td>0 0</td>
<td>75 100</td>
<td>14.50 **</td>
</tr>
<tr>
<td>Nalidixicacid,30</td>
<td>0 0.00</td>
<td>0 0</td>
<td>75 100</td>
<td>14.50 **</td>
</tr>
<tr>
<td>Ciprofloxacain,10</td>
<td>9 12.0</td>
<td>0 0</td>
<td>66 88.00</td>
<td>12.94 **</td>
</tr>
<tr>
<td>Cefotaxime,10</td>
<td>0 0.00</td>
<td>0 0</td>
<td>75 100</td>
<td>14.50 **</td>
</tr>
<tr>
<td>Chi-square- $\chi^2$</td>
<td>---- 16.38 **</td>
<td>---- 0.00 NS</td>
<td>---- 16.38 **</td>
<td>----</td>
</tr>
</tbody>
</table>

** (P<0.01), NS: Non-significant.
References


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