MOLECULAR TYPING OF THE BACTERIA CLOSTRIDIUM DIFFICILE STRAINS ISOLATED FROM PATIENTS WITH ACUTE INTESTINAL INFECTION RIBOTYPING-PCR TECHNIQUE

Elnaz Nafisi*

Young Researchers and Elite Club, Ahar Branch, Islamic Azad University, Ahar, Iran.

ABSTRACT

Clostridium difficile infection is common in hospitals growing problem in recent years-have been reported. Detection of Clostridium difficile infection source control and prevent the spread of nosocomial infection caused by it is very important. Since PCR ribotyping method recently as an effective method to study the epidemiology of Clostridium difficile strains has been suggested, this study was conducted. Methods: A descriptive study was conducted over a 12 month period. 17 samples of patients suspected of infection with Clostridium difficile Clostridium difficile were isolated. All stool samples were treated with shock alcohol and yeast extract medium. The suspension of the broth treated Cephalexin cycloserine fructose agar (CCFA) enriched with 5% sheep blood culture method is linear up to 5 days were incubated in anaerobic conditions. The prevalence of isolates to determine the actual rate (Confidence Interval) of the population was estimated. Definitive identification, cdd-3 gene and determine the toxin profile of genes tcdA, tcdB were identified using PCR method. Clostridium difficile to identify Ribotype on samples obtained from hospitalized patients were PCR using primers specific ribosomal genes. Results: Of 89 samples, 17 (7/15%) samples were positive. The frequency of isolates with profiles toxin A + B + 12 (59/70%), A + B- 1 (9/5%) and A-B + 4 (9/23%) were reported. Ribotyping analyzed isolates showed that all of them have a different banding pattern. Conclusion: It seems that Ribotypes circulating varies in different parts of hospital infection may be due to endogenous or business strains pathogenic strains of the environment.

KEYWORDS: Clostridium difficile bacteria, toxins, techniques Ribotyping-PCR.
INTRODUCTION
Diarrhea is one of the most common complications are treated with antibiotics. Since the early seventies AD, especially after the introduction of antibiotics clindamycin, antibiotic-associated colitis and certain forms of severe clinical signs increased Pseudomembranous colitis. Clostridium difficile is the most common bacterial pathogens causing diarrhea after antibiotic use toxin A and toxin B, respectively, and by strains causing toxin (Toxigenic) makes this form of the disease. Toxin A and toxin B is a cytopreparatory an enterotoxin toxin in the body, both of which reinforce each other and act as synergistic. In recent years the incidence of diseases associated with Clostridium difficile (C. difficile associated disease) has expanded considerably in the world and this increase with the emergence of highly aggressive strains (virulent) bacteria in North America and Europe is followed.\(^1\)

Since all strains of Clostridium difficile toxins produce about 2% of healthy adults and 50% of children younger than 2 years may infect the, Identification toxins A and B, better detection and isolation of the bacterium in clinical diagnosis is.\(^2\)

APPLICATION
1. in hospitalized patients with frequent passage of stool loose, abdominal pain, fever or nausea during or after a course of antibiotics or surgery of the digestive system that has been done, there.\(^3\)

2. In the case of outpatient above symptoms within 6 or 8 weeks after receiving the antibiotic, a few days after chemotherapy or in individuals who have a chronic disease of the digestive system and doctors suspected the worsening of the disease Clostridium difficile is.\(^4\)

3. This test may detect persistent diarrhea with no pathological causes such as parasites or pathogenic bacteria have been found to be helpful.\(^5\)

4. If the disease or colitis treated for diarrhea associated with antibiotic use, Again relapse and symptoms appear, the measurement of Clostridium difficile toxins is possible to confirm the disease, is requested again.\(^6\)

MATERIALS AND METHODS
Cross-sectional study on 89 cases of acute bacterial infection took place. Information about each patient were collected using a questionnaire. Data collected included age, sex, clinical symptoms and the location.\(^7\)
To kill bacteria, stool specimens were grown quickly in specific environments. Fecal samples into two parts, for culture and PCR tests were divided. All the stool samples of the alcohol and yeast extract with shock, were treated. In shock, alcohol, first, 1 g of feces with a small volume of 95% ethanol, mixed gently and then was incubated at room temperature for 2 minutes. Then this environment, the specific environment Cephalexin cycloserine fructose agar (CCFA), enriched with 5% sheep blood and the antibiotic cycloserine (250μg/ml) and Cephalexin (8μg/ml) and Clostridium difficile medium environment, enriched with 5% sheep blood were cultured in a linear way. The pellets were examined after 48 hours. If Colonies the round, dull gray and white and smell like horse stables and feature fluorescent under ultraviolet light, for the diagnosis of Gram stain and specific PCR was used.

The growth of colonies on the plate, for final identification and also to determine the type of toxin, DNA of bacteria using a kit QIAamp, was extracted and 280 nm in a
spectrophotometer, the optical density was evaluated. If confirmed by the quality, DNA in TE buffer was maintained at a temperature of -20 degrees.[12] To detect and identify bacteria Clostridium difficile-specific primers and Struppi6 Tim6 product takara japan, cdd-3 was used for gene amplification. Using these primers a fragment of 622 bp in length was amplified.[13] PCR products on a 1.2% agarose gel with a concentration in terms of the potential difference of 80 volts, for 2 hours to separate and then stained with ethidium bromide solution Banding was observed.[14] All isolated by PCR, CDD-3 gene was positive in terms of encoding toxins types A and B were studied.[15]

Table 1: The sequence of the primers used for amplification of genes Cdd-3, TcdA, TcdB.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Nucleotide sequence</th>
<th>During parts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cdd-3</td>
<td>Tim6</td>
<td>5''TCCAATATAATAAATTAGCATTCCA3''</td>
<td>622bp</td>
</tr>
<tr>
<td></td>
<td>Struppi6</td>
<td>5''GGCTATTACACGTAATCCAGATA3''</td>
<td></td>
</tr>
<tr>
<td>TcdA</td>
<td>TA1</td>
<td>5''ATGATAAGGCAACTTCAGTGG3''</td>
<td>624bp</td>
</tr>
<tr>
<td></td>
<td>TA2</td>
<td>5''TAAGTTCCTCCTGCTCCATCAA3''</td>
<td></td>
</tr>
<tr>
<td>TcdB</td>
<td>TB1</td>
<td>5''GAGCTGCTTCAATTGGAGAGA3''</td>
<td>412bp</td>
</tr>
<tr>
<td></td>
<td>TB2</td>
<td>5''GTAACCTACTTTTACAAACCCG3''</td>
<td></td>
</tr>
</tbody>
</table>

For PCR, in response to the volume of 100μl, the amount of template 5μl, 10μl of buffer 10x, 2μl of dNTP, 3μl of MgCl2, 5μl of primers P3 and P5 10μM concentration and 0.3μl of DNA Taq and add others to Dyonayzer volume of 100μl with distilled water, was completed. PCR for the amplification of ribosomal genes was repeated for 35 cycles.[16]

![Figure 3: sequencing primers ribosome in bacteria Clostridium difficile.](image-url)
Figure 4: protein-coding regions of the bacterium Clostridium difficile.
RESULTS
89 patients with acute bacterial infection of the digestive system, and given the prevalence of infection with Clostridium difficile, the actual rate of diarrheal disease by 90% probability of at least 10 to 21.4% respectively.\textsuperscript{[17]} All patients had diarrhea. 85% of patients had a history of antibiotic use and 15% of patients were not taking any antibiotics, but the incidence of Clostridium difficile toxins A and B, and both were positive. Most of the patients in ICU and oncology wards had the lowest prevalence of patients.\textsuperscript{[18]}

DISCUSSION
The project showed that Ribotyping PCR technique can more accurately than other molecular techniques, the bacteria Clostridium difficile to multiply and nucleotide sequences in
ribosomes 5S, 16S, 23S to evaluate protein synthesis and the formation of colonies. So this technique is a useful method for achieving fast and high quality diagnosis of acute diarrhea in patients with gastrointestinal disorders, and will be.[19]

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