Isolate and Molecular Study of Pathogenic Bacteria and Study the Activity of Silver Nanoparticle and Lactobacillus acidophilus on it.

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Abstract

Diarrhea is the passage of remarkably loose or watery stools, generally more than three times within 24 hour. Hence this training marked to identify the causative agents in children by using phenotypic and molecular methods. A total of 200 stool samples were collected from patients suffering from diarrhea during a period from August 2017 to December 2017, in a Woman and children BABIL Hospital in Hila province, found 127 samples (63.5%) were positive for bacterial culture, while, 73 samples (36.5%) showed negative results. The result of the biochemical test revealed that 40/127 (31%) of bacterial isolates were E.coli, 10/127 (7.8%) of isolates were Proteus spp, and 20/127 (15.7%) isolate were Klebsilla spp, 26/127 (20%) isolate were and Enterobacter spp, Shigella identification in 10/127 (7.8%), Salmonella in 12/127 (8.6%), P. aeruginosa in 10/127 (7.8) isolate. The Molecular technique used to detect the presence of genes that responsible for E.coli resistant to antibiotics by using specific primers are (aac, tet, ctxm, parc). The results showed that 40 of E. coli isolates carried these genes; the amplification of aac gene was observed in (1-4, 6, 8, 9, 10) sample number, ctxm was observed in (1-13), parc was in (1-11), parc were in (1-4, 7, 8, 9, 10) sample number. In this study, it has been detected antimicrobial effect of silver nanoparticles and lactobacillus acidophilus probiotic on the bacterial isolate, the result showed that Proteus spp isolate were the highly affected while, the antibiotic activity result showed that P. aeruginosa and Shigella spp were the highly affected.

Keywords: Diarrhea, E. coli, aac, tet, ctxm, parc, Silver nanoparticles, lactobacillus acidophilus

INTRODUCTION

The Diarrhea is a sudden increase in incidence and looseness of bowel energy. It is hard to describe diarrhea exactly, since there is great individual difference in normal stool frequency. Pointers of diarrhea comprise a unexpected increase in number of stool occurrence, a reduction in stool constancy, more watery stool and a tendency for stool to be green(1). There is no agreement on when diarrhea becomes chronic, but the shortest duration after which diarrhea may be termed chronic is probably 4 weeks(2). Enteric viruses are the main etiologic managers of acute gastroenteritis amongst infants and young children worldwide, the main detached off the study was to determine the role of enteric viruses in acute diarrhea in the country (3).

Virulence factor denote to the properties that allow a microorganism to institute itself and duplicate on or within exact host species, and that improve the microbe’s potential to cause disease (4). The resistant of bacteria to the antibiotic increasing amongst the bacterial isolates from patients in both outpatient and in patients, which are considered as a focus infection, this resistance can be developed by modification or by plasmid of resistance genes from other microorganisms (5).

The study was amid to detect the most important pathogenesis of diarrhea and determine the role of common gens responsible for antibiotics resistance by the following objectives.

1. Isolation and certification of bacteria that cause diarrhea in children under 6 year by traditional methods and approve by ViteK-2.
2. Detection the genes that resist to antibiotic by PCR technique.

METHODS

Patients and specimens collection

A total of 200 stool specimens were collected from infants and children present at WOMAN AND CHILDREN BABIL Hospital, who were suffering severe diarrhea. The specimens were collected from patients below six years of age and from both sexes, and put in sterile plastic containers of sufficient size and within a tight fitting leak-proof lid were used. The specimens were delivered to the laboratory, and treated directly or as soon as possible, but often not more than two hours after collection.

Isolation and Identification of Bacterial Isolates

Bacteria diagnosed depending on the morphological characteristics on the culture media and confirm the diagnosis by using biochemical test.

Polymerase chain reaction (PCR) assay

The DNA extracted from samples were tested with PCR for DNA with the general primer-aac, ctxm, tet, parc, and Parc, all samples were analyzed for the presence of this genes and negative controls (water) were used in each batch of PCR and the negative controls were added after all the samples were added to the PCR tubes. None of the negative controls were positive. Amplified PCR products were analyzed by electrophoresis in a 1.5% agarose gel containing TAE buffer and ethidium bromide (Sigma, Castle Hill, Australia). PCR amplifications were size determined under UV light using the GelDoc software (Bio-Rad, Sydney, Australia).

Effect of silver nanoparticle and lactobacillus acidophilus on bacterial isolate

The microorganism was inoculated on brain heart infusion broth at 37c for 24 hours.(first activation). And used the same method for (second activation) also used antimicrobial activity of silver nanoparticle and lactobacillus acidophilus on isolates under study. (6)
Statistical Analysis
Data were analyzed using online statistical software (VassarStats, Vassar College, Poughkeepsie, NY) and tested using the Chi-square test.

RESULT AND DISCUSSION
The present study included 200 clinical stool specimen, 127/200 (63.5%) specimens were positive culture (bacterial causes), and the other 73/200 (36.5%) isolates were considered negative results. The biochemical tests which appeared that of isolates were 40/127 (31%) E. coli, 10/127 (7.87%) isolates were Proteus spp., 20/127 (15.7%) isolates were Klebsella spp., 26/127 (20%) were Enterobacteria spp., Shigella were in 10/127 (7.8%), 11/127 (8.6%) of spp. Salmonella and Pentoea appeared in 10/127 (7.8%) isolates as shown in table (1).

Detection of aac gene in E. coli
Polymerase chain reaction technique of E. coli clinical isolates revealed that aac gene were gave positive result for this gene with product size 490 bp. This study is similar to the study by (8) who detected E. coli isolates from different sources.

Detection of ctxm gene in E. coli
Polymerase chain reaction technique of E. coli isolates revealed that ctxm gene were gave positive result for this gene with product size (543bp) as shown in figure (2).

Table 1 - The positive result of the bacterial isolate from diarrheal children

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>No</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>40</td>
<td>31%</td>
</tr>
<tr>
<td>Proteus spp</td>
<td>10</td>
<td>7.87%</td>
</tr>
<tr>
<td>Klebsilla spp</td>
<td>20</td>
<td>15.7%</td>
</tr>
<tr>
<td>Enterobacter spp</td>
<td>26</td>
<td>20%</td>
</tr>
<tr>
<td>Shigella spp</td>
<td>10</td>
<td>7.8%</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>11</td>
<td>8.6%</td>
</tr>
<tr>
<td>Pentoea spp</td>
<td>10</td>
<td>7.8%</td>
</tr>
</tbody>
</table>

The result of our study is like to the study of (9) who found that blaCTXM β-lactamase was the most prevalent among the ESBL producing G-ve isolates; followed by blaTEM β-lactamases were the less. In India (10), reported that blaCTXM β-lactamases were more common enzymes than blaTEM in clinical negative isolates (11). PCR is considered an efficient method for ESBL detection, because it is faster than phenotypic detection method (12), in addition to detect the presence of poorly or non-expressed (silent) genes, that is difficult to determine by phenotype methods; PCR may also be used to directly test samples as an early predictor of infection (13).
Detection of \textit{tet} gene in \textit{E.coli}

The gene \textit{tet} of \textit{E.coli} appear as positive result by PCR technique with product size (337bp) as shown in figure (3). Tetracycline is clinically known as broad spectrum antibiotic and is effective against many Gram-positive and -negative bacteria. Tetracycline mostly act by stopping the binding of tRNA to the A site of the 30S rRNA, causing misreading of the mRNA code or inhibition of the initiation step of protein synthesis \cite{14}.

Detection of \textit{Parc} gene in \textit{E.coli}

\textit{Parc} gene of \textit{E.coli} give a positive result by PCR Technique with size(264bp). The first is the targeting mutations in the quinolone resistance-determining region (QRDR) of two \textit{gyrA} and \textit{gyrB} genes, which encode for the subunits of DNA gyrase, and/or in \textit{parC} and/or \textit{parE} subunit of topoisomerase IV \cite{15}.
Pathogenic bacteria can gain resistance to antibiotics through horizontal gene transfer of naturally occurring antibiotic resistance genes (ARGs) native to other microbes or de novo mutations under environmental pressure. The Plasmid-mediated quinolone resistance mechanism captures a variant of aminoglycoside acetyl-transferase aac(6')-Ib-cr gene, which can diminish a fluoroquinolone activity by adding an acetyl group to this antimicrobial agent. The success of the CTX-Ms over the classical ESBL-enzymes is linked to the way by which CTX-M enzymes were spread. Resistance to quinolone has been classified into two mechanisms. The first is the targeting mutations in the quinolone resistance-determining region (QRDR) of two gyrA and gyrB genes, which encode for the subunits of DNA gyrase, and/or in parC and/or parE subunit of topoisomerase IV.

**Antimicrobial activity of silver nanoparticles and Lactobacillus acidophilus on bacterial isolate**

The effect of silver nanoparticles and L. acidophilus revealed that 25% of E. coli isolates are effected by silver nanoparticles and 15 isolates are effected by L. acidophilus, 10 of klebsilla isolates are effected by silver nanoparticles and 10 are effected by L. acidophilus, 9 of salmonella isolates are effected by silver nanoparticles and 2 are effected by L. acidophilus, 6 of shigella isolates are effected by silver nanoparticles and 4 are effected by L. acidophilus, 8 isolates of Proteus isolate are effected by silver nanoparticles and 2 are effected by L. acidophilus, and 8 isolates of Proteus isolate are effected by silver nanoparticles and 2 are effected by L. acidophilus as shown in table (3).

**Table (3) Antimicrobial activity of silver nanoparticles and L. acidophilus on bacterial isolate**

<table>
<thead>
<tr>
<th>Type of bacteria</th>
<th>Silver</th>
<th>L. acidophilus</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>Klebsilla</td>
<td>8</td>
<td>10</td>
</tr>
<tr>
<td>Salmonella</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Shigella</td>
<td>8</td>
<td>7</td>
</tr>
<tr>
<td>Proteus</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Enterobacter</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Pentoea</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

**CONCLUSION**

In end, our outcomes tested the attendance of the pathogenic bacteria showed the most common pathogens are E. coli then proteus and the less Pentoea. The Molecular technique appeared four genes (aac, tet, ctXM, parc) observed in more isolates of E. coli and the (aac gene ) Is the most common in isolates than others. Proteus spp were the highly effected by silver nanoparticles and lactobacillus acidophilus.

**REFERENCES**


