INTRODUCTION

Campylobacter jejuni is generally considered commensals of livestock, domestic pet animals and birds. C. jejuni is the main cause of human bacterial intestinal disease identified in many industrial countries [1]. The infection with C. jejuni is associated with acute enteritis and abdominal pain lasting for 7 days or more [2]. Many species of Vibrio that caused diarrhea can grow in thiosulfate citrate, bile salts, sucrose agar as yellow colonies and do not agglutinate with V. cholerae O1 and O139 anti-sera [3]. These species are broadly defined as non-agglutinating (NAG) vibrios [4]. V cholerae has been responsible for several cholera outbreaks in developing countries [3].

Lipopolysaccharide (LPS) is the main outer membrane component of gram negative bacteria which constitutes about 75% of the surface [5] and 5–10% of the total dry weight of gram negative bacteria [6]. Their basic structure consists of three parts: lipid A, core oligosaccharide and repetitive polysaccharide designated as “O” antigen. Lipid A is highly conserved and exerts the endotoxic act-

ABSTRACT

Lipopolysaccharide was extracted from Campylobacter jejuni and Vibrio cholerae (NAG) by EDTA-heating method. Anti-sera against both LPS were raised in rabbit. Passive hemagglutination was used to check the LPS-antigenic relationship between both bacterial species. The results showed that there is no cross reaction relationship between LPS of C. jejuni and LPS of V. cholerae.

Keywords: Campylobacter jejuni, cross reaction, Lipopolysaccharide, Vibrio cholerae.


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itivity, while the "O" antigen carbohydrate chain is a polymer of repeating oligosaccharides, which differs between species and is responsible for the serological specificity of bacteria [7]. LPS causes pathophysiological effects such as fever, leucopenia, leucocytosis and Shwartzman reactivity [8]. It plays an important role with temperature to modulate the inflammatory immune response [9]. The present study aimed to find the LPS-antigenic relationship between two species that mainly responsible for diarrhea in our country (Iraq).

MATERIALS AND METHODS

Bacterial strains

Clinical isolates of \textit{C. jejuni} and \textit{V. cholerae} were procured from department of Biology, college of science university of Baghdad, Baghdad, Iraq. The isolated was stored in lyophilized tubes at -20 and re-cultured in nutrient broth before using in experiment.

LPS purification

The \textit{C. jejuni} and \textit{V. cholerae} LPS was extracted by EDTA method that mentioned clearly in previous study [10]. The LPS was purified by running the extract material through separose-4B gel.

Anti-LPS preparation

Clinical isolates of \textit{C. jejuni} and \textit{V. cholerae} were cultured into nutrient broth for overnight. The bacterial growth was harvested and washed three times with phosphate buffer saline (PBS, 0.1 M, pH 7). The bacterial count was adjusted to $10^{10}$ cell/ml. Rabbits were immunized with bacterial suspension according to standard previous method [11].

Preparation of sensitized sheep red blood cells

The standard method of Zgair [12] was followed to coat sheep red blood cells (SRBCs) with either \textit{C. jejuni} LPS or \textit{V. cholerae} LPS.

Hemagglutination technique

Serial dilutions of complement inactive anti-LPS (1/10, 1/20 up to 1/1280) were prepared in circle bottom microtiter plate (50 µl in each well). Fifty micro-liter of sensitized SRBCs (1%) was added to each well. The microtiter late was incubated for 37 °C at room temperature after mixing gently. The red clear well bottom indicates the positive results, while the dot at the well bottom was indicated for negative result. The titer of each anti-sera was the last positive dilution in each raw. Negative control (PBS and unimmunized rabbit serum) was used. The presence of cross reaction was dependent on the threshold value for agglutination reaction.

Statistical analysis

Data are expressed as mean±SD. The following equation was followed to calculate the threshold value [threshold value = mean + 2(standard deviation)].

RESULTS

The calculation of threshold value for anti-\textit{C. jejuni} showed that the threshold value for anti-\textit{C. jejuni} was 31.1 and 19.4. This finding depict the values of threshold was higher than the titer of hemagglutination of anti-\textit{C. jejuni} and LPS- \textit{V. cholerae}. That proved there was no cross reaction between anti-\textit{C. jejuni} and \textit{V. cholerae} LPS. Similar observation was found in case of hemagglutination between anti-\textit{V. cholerae} and \textit{C. jejuni} LPS (Table 1).

Table 1. The hemagglutination titers of between anti-bacteria (\textit{C. jejuni} and anti \textit{V. cholerae}) and SRBCs that coated with bacterial LPS (\textit{C. jejuni} and \textit{V. cholerae}).

<table>
<thead>
<tr>
<th>Anti- \textit{C. jejuni}</th>
<th>Anti- \textit{V. cholerae}</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS- \textit{C. jejuni}</td>
<td>1024-256</td>
</tr>
<tr>
<td>LPS- \textit{V. cholerae}</td>
<td>16-8</td>
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<td></td>
<td>512-256</td>
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</tbody>
</table>

Discussion

Previous study showed the immunological relationship between the LPS of \textit{Campylobacter} and 11 members of Enterobacteriaceae, and \textit{Vibrio} [13]. In current study, we try to find the relationship between the anti-sera against whole cells of studied bacteria (\textit{V. cholerae} (NAG) and \textit{C. jejuni}) and LPS prepared from \textit{V. cholerae} (NAG) and \textit{C. jejuni}. We found that no cross reaction between prepared LPS that coated on SRBCs and anti-sera.

Previous study on LPS that prepared from \textit{C. jejuni} showed the structure of lipid A composed of GlcN3N that carried two groups of phosphate in location 1 and 4 [14] and some fatty acid such as octadecenoic acid and cyclopropane acid [15]. Moreover, presence a compound in core region called Neu5ac [16]. The lateral chains composed of two kinds of polysaccharide one is low molecular weight and another high molecular weight. \textit{C. jejuni} LPS differs from \textit{V. cholerae} LPS. The last one composes of phosphorylethanolamine in the lipid a position and high amount of fructose [17]. The LPS of \textit{V. cholerae} characterized with low lateral chain and composed of different kinds of sugar molecules. Thus, structurally the LPS of \textit{C. jejuni} differ from LPS of \textit{V. cholerae} that explain there is no immunological relation ship (cross reaction)
between the both LPS and bacteria. Not only LPS can stimulate the immune system in host other antigen like flagellin can also stimulate the immune system [18]. LPS stimulates the immune system strongly, and that happens through T and B cells. The activation of both cells especially T cells may produce autoimmune phenomena [19]. We suggested used other antigens to study the immunological relationship between two genuses. This work is going on in our laboratories. It can be concluded that according to LPS immunological relationship, there is no immunological relation ship between C. jejuni and V. cholerae.

Conflict of interest

The author declares that he has no conflict of interests.

REFERENCES


