Gastrointestinal infection Etiological Agent and Mis Identification Of Some Pathogenic bacteria like **Campylobacter**

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**Abstract**

Pathogenic bacteria can enter and infect the digestive tract when someone eats food or drinks water that is contaminated. Examples of contaminated sources include raw or undercooked eggs, poultry or beef, unpasteurized milk, and untreated water from lakes, streams, and (occasionally) from community water supplies. The aims of our study are strengthening of surveys of the cases of the gastrointestinal tract infection, more knowledge of the causes leading to the gastrointestinal tract, also knowing the risk factors which caused in Miss Identification of some pathogenic bacteria (mainly Campylobacter and Yersinia, enterocolitica), then evaluate the number of persons who have negative result and the reason for this lack of screening for all pathogenic bacteria and search for Salmonella and Shigella only, Attatch previous studies in the world on the importance of isolating bacteria and number of Campylobacter positive samples and the importance of taking the subject into account, finally culture characterization and epidemiology of the isolated pathogenic bacteria. In order to reach those goals we carried out a survey of 484 stool samples in the Department of microbiology at Central Laboratory of the Ministry of Health in Amman the capital of Jordan between January 2012 to December 2013 using of stool culture and biochemical test and antisera against salmonella and shigella. Atotal of 484 stool samples were tested for 49 salmonella and 50 shigella, a total 99 stool samples were gave positive results for salmonella, and shigella of with an overall prevalence of 20.5 %. The high number of negative results due to misidentification of many pathogenic bacteria like Campylobacter and awide variety of other bacteria may sometimes cause diarrhea. These may include: Aeromonas species, Staphylococcus aureus, Yersinia enterocolitica, Vibrio cholerae and other Vibrio species.

**Keywords:** [Campylobacter, Yersinia, enterocolitica, Salmonella and Shigella] stool culture

**Introduction**

The bacteria found in stool are representative of the bacteria that are present in the gastrointestinal tract [10]. Certain bacteria and fungi called normal flora inhabit everyone's gastrointestinal tract [9]. They play an important role in the digestion of food, and their presence keeps a check on the growth of disease-causing bacteria[8]. Sometimes, the balance of the normal flora may be affected by the administration of broad-spectrum antibiotics; the drugs inhibit the growth of normal flora and allow bacteria resistant to the antibiotic to survive and overgrow the digestive tract [6]. The normal flora may also be affected by anti-cancer drugs and when a person has a weakened immune system, leading to bacterial overgrowth and symptoms such as diarrhea and abdominal pain [27].

Pathogenic bacteria can enter and infect the digestive tract when someone eats food or drinks water that is contaminated [21]. Examples of contaminated sources include raw or undercooked eggs, poultry or beef, unpasteurized milk, and untreated water from lakes, streams, and (occasionally) from community water supplies [7].

The most common symptoms of a pathogenic bacterial infection are prolonged diarrhea, bloody diarrhea, mucus in the stool, abdominal pain and cramping, and nausea. If diarrhea lasts more than a few days, it may lead to complications such as dehydration and electrolyte imbalance - dangerous conditions, especially in children and the elderly [8]. Dehydration can cause symptoms such as dry skin, fatigue, and light-headedness [6]. Severely affected people may require hospitalization to replace lost fluids and electrolytes. Hemolytic uremic syndrome is a serious complication characterized by the destruction of red blood cells and kidney failure that may occasionally arise from an infection with a toxin-producing strain of the bacteria *Escherichia coli* [21]. The condition is most frequently seen in children, the elderly, and those with weakened immune systems [14].

Some of the most common pathogenic bacteria that cause infections in the U.S. and their most frequently encountered sources include: *Salmonella* - found in raw eggs (even intact disinfected eggs), raw poultry uncooked vegetables, and in reptiles; pets, such as lizards and turtles, may carry may become carriers of *salmonella* [15]. *Salmonella may be transmitted person-to-person.* [16] *Shigella* - found in *salmonella* in their intestines without being ill themselves [17]. Some humans food and water contaminated with stool, and from infected-person to person when careful sanitation is not observed; for instance, it can be a challenge to prevent the spread of Shigella within a family and in a daycare or nursing home setting since even a very few organisms may cause disease [24]. *Campylobacter* - found in raw or undercooked poultry; it is the most common cause of bacterial diarrhea in the U.S. It may become especially serious if it spreads to the bloodstream, and it occasionally causes long-term complications such as arthritis and Guillain-Barré syndrome [20]. *Escherichia coli 0157:H7* and other toxin-producing *E. coli* (most strains of *E. coli* are considered normal flora and do not
cause disease) - found in raw or undercooked hamburger/beef, spinach, or unpasteurized cider; causes bloody
diarrhea and may lead to hemolytic uremic syndrome [12]. Clostridium difficile - may be present as part of the
normal flora, but use of broad-spectrum antibiotics can result in an overgrowth of these bacteria. Toxin-
producing strains can cause diarrhea and other serious complications [8]. A wide variety of other bacteria may
sometimes cause diarrhea. These may include: Aeromonas species, Staphylococcus aureus, Yersinia
enterocolitica, Vibrio cholerae and other Vibrio species [12].

Methods:
A fresh stool sample is collected in a sterile container. The stool sample should not be contaminated with urine
or water. Once it has been collected, the stool should be taken to the laboratory within about an hour after
collection or should be transferred into a vial containing a preservative and taken to the lab as soon as possible.
For infants, a stool sample is usually collected with a swab of the rectum. Selective and differential plating
media (Hektoen, SS agar & XLD Agar), are incubated overnight (18-24 hours) at 36C. The plates are then
examined for Salmonella-like or Shigella-like colonies. Suspect colonies are picked using an inoculating needle
or 1uL inoculating loop and transferred to a non-selective agar, such as 5% Sheep Blood Agar (SBA). It is
critical to pick well isolated colonies; if several suspicious colonies are present, three separate colonies should be
selected for biochemical testing. SBA plates are incubated at 36°C (+/- 1°C) for 18-24 hours. pure colonies
should be screened with the biochemical test media consists of: triple sugar iron agar (TSI), lysine iron agar
(LIA), motility-indol-ornithine agar (MIO), Simmons citrate agar, and biochemical Screening (Use of TSI)as follow:
Two isolated suspect pure colonies was selected  and inoculated in TSI slants and butt . with a sterile platinum
inoculating needle. The center of the colony was Touch once and then stab the butt and streak the TSI slant.
Without going back to the colony, Incubated  at approximately 35E C with their caps loose (to prevent excess
H2S production) for 18-24 hours. TSI reactions was examined as a set Yellow is an acid reaction and red or
purple is an alkaline reaction in TSI Acid/acid (slant/butt) at 18-24 hours in TSI indicates either lactose, sucrose
or both have been fermented., non-lactose fermenters may utilize the 0.1% glucose causing both the deep and
the slant to be yellow. Salmonella typically produce an alkaline butt and slant distinct yellow, Positive H2S reaction
results in blackening of the TSI. Most Salmonella cultures produce H2S in TSI. Shigella non-lactose
fermenting, but non H2S producing colonies are present. lysine iron agar (LIA), motility-indol-ornithine agar
(MIO) positive for Salmonella and Shigella but Salmonella motile Shigella non motile according to Simmons
citrate agar Salmonella positive Shigella citrate negative. Biochemical identification can be completed using
Biolog, bioMerieux API 20E strips, Enterotubes or equivalent kits. Follow manufacturer's instructions for each
kit.
Confirmatory identification of Salmonella and Shigella species serological characterization have been done as
follow
Results
Based in the biochemical and immuno-reactivity. We carried out a survey of 484 stool samples in the
Department of microbiology at Central Laboratory of the Ministry of Health in Amman the capital of Jordan
between january 2012 to December 2013. A total of 484 stool samples were tested for 49 Salmonella and 50
Shigella , a total 99 stool samples were gave positive results for salmonella , and shigella of with an overall
percentage of 20.5 %. Table 1 and 2. The result as follow

<table>
<thead>
<tr>
<th>Month</th>
<th>total samples</th>
<th>Positive samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>22</td>
<td>4</td>
</tr>
<tr>
<td>February</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>March</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td>April</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td>May</td>
<td>14</td>
<td>3</td>
</tr>
<tr>
<td>June</td>
<td>18</td>
<td>2</td>
</tr>
<tr>
<td>July</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>August</td>
<td>56</td>
<td>19</td>
</tr>
<tr>
<td>September</td>
<td>31</td>
<td>12</td>
</tr>
<tr>
<td>October</td>
<td>100</td>
<td>18</td>
</tr>
<tr>
<td>November</td>
<td>70</td>
<td>13</td>
</tr>
<tr>
<td>December</td>
<td>118</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td>484</td>
<td>99</td>
</tr>
</tbody>
</table>
Table 2: Micro-organism isolated in stool samples

<table>
<thead>
<tr>
<th>Agents</th>
<th>Total (484)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salmonella group B</td>
<td>12</td>
<td>12.12</td>
</tr>
<tr>
<td>Salmonella group C2</td>
<td>13</td>
<td>13.13</td>
</tr>
<tr>
<td>Salmonella group D</td>
<td>4</td>
<td>4.04</td>
</tr>
<tr>
<td>Salmonella group E</td>
<td>13</td>
<td>13.13</td>
</tr>
<tr>
<td>Salmonella typhimurium</td>
<td>2</td>
<td>2.02</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>5</td>
<td>5.05</td>
</tr>
<tr>
<td>Shigella soni</td>
<td>34</td>
<td>34.4</td>
</tr>
<tr>
<td>Shigella flexneri</td>
<td>14</td>
<td>14.1</td>
</tr>
<tr>
<td>Shigella boydii</td>
<td>2</td>
<td>2.02</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>99</strong></td>
<td><strong>20.5%</strong></td>
</tr>
</tbody>
</table>

The high number of negative results due to mis identification of many pathogenic bacteria like *Campylobacter* and a wide variety of other bacteria may sometimes cause diarrhea. These may include: *Aeromonas* species, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Vibrio cholerae* and other *Vibrio* species.

For example, the prevalence of campylobacter food poisoning: Even though surveillance is very limited, over 10,000 cases are reported to the Centers for Disease Control and Prevention (CDC) each year, equaling approximately six cases for each 100,000 persons in the population. Many more cases go undiagnosed or unreported, and campylobacteriosis is estimated to affect over 2 million persons every year, or 1% of the population. (Source: excerpt from Campylobacter Infections: General: DBMD). Incidence is about 20 cases per 100,000 population diagnosed in the United States. An estimated 2.4 million persons are affected each year. (Source: excerpt from Campylobacter Infections: DBMD)

In previous study a significantly higher prevalence of *C. concisus* in colonic biopsies of patients with CD (53%) was detected as compared with the controls (18%). *Campylobacter* genus-PCR positivity and *C. concisus* positivity in patients with UC were 85% and 77% respectively, being significantly higher than that in the controls (48% and 36%). *C. concisus* was more often detected in descending colonic and rectal biopsies from patients with IBD in comparison to the controls. *C. concisus* was isolated from patients with IBD [1].

**Discussion:**

Salmonella and Shigella are two genera within the family *Enterobacteriaceae* [24]. Like other *Enterobacteriaceae*, they are Gram-negative, non-spore forming rods. The *Enterobacteriaceae* are oxidase negative, catalase positive (with the exception of *S. dysenteriae* Type 1), facultative anaerobes that grow on MacConkey agar and reduce nitrate to nitrite [15].

Based on the immuno-reactivity of “O” (LPS), “H” (flagellin protein) antigens, the genus is further sub-divided into serovars. As of 2007, 2,557 serovars of *S. enterica* and 22 serovars of *S. bongori* have been recognized. The majority of human clinical isolates, including *Salmonella* serovars Enteritidis, Typhimurium, and Typhi (etiologic agent of typhoid fever) are found within *S. enterica* subspecies *enteric* [15].

Conventional biochemical testing is typically used to differentiate the genus *Salmonella* from other *Enterobacteriaceae*, an essential supplement to serotype data when multiple subspecies share an identical antigenic formula, or when all antigenic factors are not expressed, such as with non-motile, mucoid, or rough isolates. [18] *Shigella* spp. are by definition non-motile and lysine decarboxylase negative. Recent phylogenetic studies indicate that *Shigella* and *Escherichia coli* comprise a single species. However, to facilitate disease surveillance the shigelleae have not been merged with *E. coli* [17].

The genus *Shigella* is comprised of four species: *Shigella dysenteriae* (also referred to subgroup A), *Shigella flexneri* (also referred to subgroup B), *Shigella boydii* (also referred to subgroup C), & *Shigella sonnei* (also
referred to subgroup D). [18] Included among these organisms, is the etiologic agent of epidemic dysentery S. dysenteriae serotype 1. With the exception of S. sonnei, each species may be further divided into serotypes on the basis of reactivity with hyperimmune serum: S. dysenteriae (15 serotypes), S. flexneri (6 serotypes and 2 variants), S. boydii (19 serotypes)[24].

In this study, A total of 484 stool samples were tested for 49 salmonella and 50 shigella, a total 99 stool samples were gave positive results for salmonella, and shigella of with an overall Percentage of 20.5 %. The high number of negative results due to misidentification of many pathogenic bacteria like Campylobacter and wide variety of other bacteria may sometimes cause diarrhea. These may include: Aeromonas species, Staphylococcus aureus, Yersinia enterocolitica, Vibrio cholerae and other Vibrio species. The prevalence of Campylobacter organisms cause between 5 and 14 percent of all diarrheal illness worldwide. In industrialized countries, illness is more common in children younger than 2 years of age. In developing countries, older children and young adults have the highest incidence of infection. Most frequently, cattle and poultry are the sources of human infection, but puppies, kittens, pigs, sheep, rodents, and birds may also serve as reservoirs. (Source: excerpt from Foodborne Diseases, NIAID Fact Sheet: NIAID) Crohn’s disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD). A high prevalence of Campylobacter concisus was previously detected in paediatric CD and adult UC. Currently, the prevalence of C. concisus in adult CD and the preferential colonization sites of Campylobacter species in the human intestine are unknown.

Also in this study, serogroup D (Sh. sonnei) was the dominant Shigella serogroup, followed by group B (Sh. flexneri), and group C (Sh. boydii). These findings are in accordance with Jordan studies, except that in those studies Sh. boydii was the 3rd most commonly isolated species. It is not unusual for one serogroup to replace another in the community from time to time.

The comparative frequencies of Shigella serogroups vary with time, hygienic conditions and among different populations. In the early 1900s Sh. dysenteriae type 1 was the most common strain, whereas Sh. flexneri and Sh. sonnei are currently isolated most often, except for certain epidemics in which Sh. dysenteriae has been identified as the causative organism. In developed countries, higher frequencies of Sh. sonnei have been reported, but these frequencies are gradually decreasing [1]. Epidemics of dysentery with frequent passage of blood and mucus, high fever, cramps and teneurism are mainly caused by Sh. Dysenteriae type 1 and Sh. flexneri, while Sh. boydii and Sh. sonnei often causing non-watery (often bloody) diarrhoea during non-epidemic episodes [2]. Bennish and Wojtyniak [3] reported most fatal cases of shigellosis occur in developing countries as a result of severe dysentery and in rare cases, bacteraemia, especially that caused by Sh. flexneri.

Among the Salmonella strains, the most commonly isolated serogroup was group C2, followed by group E and B (S. typhi) and group D. This is an agreement with some previous studies in Ethiopia [5,6], but in contrast to the earlier studies which showed that S. typhi was the dominant species [3,4]. All serogroups of Salmonella isolated in this study are known to cause gastrointestinal infections.

**Conclusion**

Campylobacter species have been associated with various diseases in both animals and humans [2]. Campylobacter jejuni and Campylobacter coli are well established human pathogens, having been associated with a number of clinical conditions such as diarrhoea, abortion, septicaemia and Guillain-Barre syndrome [2]. Some other Campylobacter species including Campylobacter concisus have been considered as emerging human pathogens [3].

The high intestinal prevalence of campylobacter in patients with IBD, particularly in the proximal large intestine, suggests that future studies are needed to investigate the possible involvement of campylobacter in a subgroup of human IBD.

**References**

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