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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, Attach 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 484stool 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 49salmonella 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* bu*t 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. 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 percentage of 20.5 %. Table 1and 2. The result as follow

Table 1: The prevalence of total samples *vs.* Positive samples

Month	total samples	Positive samples
January	**22**	**4**
February	**10**	**9**
March	**11**	**6**
April	**6**	**3**
May	**14**	**3**
June	**18**	**2**
July	**28**	**4**
August	**56**	**19**
September	**31**	**12**
October	**100**	**18**
November	**70**	**13**
December	**118**	**6**
Total	**484**	**99**

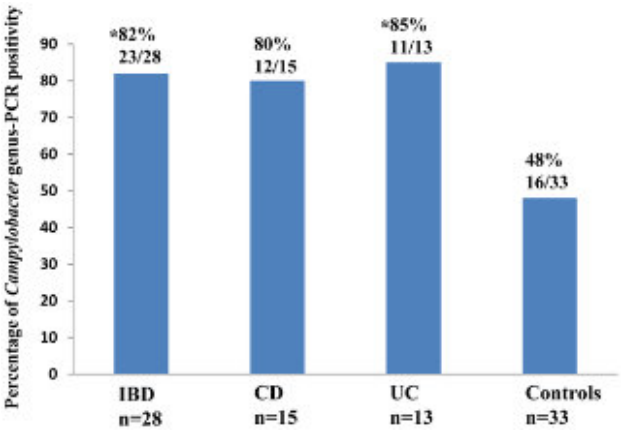
Table 2: Micro-organism isolated in stool samples

Agents	Total (484)	%
Salmonella group B	12	12.12
Salmonella group C2	13	13.13
Salmonella group D	4	4.04
Salmonella group E	13	13.13
Salmonella tyhpimurium	2	2.02
Salmonella enteritidis	5	5.05
Shigella soni	34	34.4
Shigella flexneri	14	14.1
Shigella boydi	2	2.02
Total	99	20.5%

The high number of negative results due to mis identification 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.

For example the prevalance 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 in 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 *Eschericha coli* comprise a single species. However, to facilitate disease surveillance the shigellae 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 Atotal of 484 stool samples were tested for 49salmonella 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 awide 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 n this study, serogroup D (*Sh. sonnei*) was the dominant *Shigella* serogroup,followed by group B (*Sh. flexeneri*), 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 tensemus are mainly caused by *Sh. Dysenteriae* type 1 and *Sh. flexenri*, 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.

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