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A stool- based detection of Listeria antigen among adult patients with acute gastroenteritis

Saad hasan Mohammad Ali, Khalil Ismaiel A. Mohammed*, Suha A. Auda AlFukar, Huda Q. Mohammed Abu-AL-ess, Wifaq M. Ali Al-Wattar and Jinan M. Mousa

Clinical Communicable Disease Research Unit, College of Medicine, University of Baghdad, Iraq.

* Corresponding author: Khalil Ismaiel A. Mohammed; e-mail: dr.alkarkhi@gmail.com

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ABSTRACT

Diarrheal diseases are one of the social problems in developing countries. The pathogens commonly associated with Patients are *Salmonella, Clostridium difficile*, *Shigella, Yersinia, Escherichia coli and Listeria*. The aim of the study was to evaluate the presence of *Listeria* antigen in acute gastroenteritis in patients admitted to hospital. The study was performed on freshly collected stool samples among 71 acute diarrheal patients admitted to AL-Khadymia and AL-Elweya teaching hospitals from May 2015 to January 2016. A questionnaire was completed for each patient name, age, gender, clinical data like fever, nausea, Vomiting, abdominal pain. The criteria included hemorrhagic fresh stool sample in addition to containing parasites agent. Fresh stool samples were tested by Immunochromatographic assay for antigenic detection of *Listeria.* Listeria antigen identified in one stool samples for females. most patients show fever , vomiting and abdominal pain ,while the stool consistency distributed to 24 % watery and 76% loosely .stool samples show 100 % without blood,100 % with pus , 90.2 % with mucous and 9.8 % without mucous, eight cases (12.6%) with cyst of *E.histolytica*. Antigen present in one stool sample, most patients showed vomiting, fever, abdominal pain, for all the cases without blood. All cases with pus.78 cases with mucous in comparison with 16 without mucous and 8 cases (12.6%) with cyst of *E.histolytica*

Keywords: Acute diarrhea, *Listeria,* Abdominal pain, Immunochromatography.

1. INTRODUCTION

Listeriosis, a serious infection usually caused by eating food contaminated with the bacterium Listeria monocytogenes, is an important public health problem. The disease primarily affects older adults, pregnant women, newborns, and adults with weakened immune systems. However, rarely, persons without these risk factors can also be affected (1).

Listeria is a genus of bacteria that contains ten species. Named after the English pioneer of sterile surgery Joseph Lister, the genus received its current name in 1940. Listeria species are facultatively anaerobic, Gram-positive bacilli (2). The major human pathogen in the Listeria genus is L. monocytogenes. The first documented case of Listeria was in 1924. In the late 1920s, two researchers independently identified Listeria monocytogenes from animal outbreaks. They proposed the genus Listerella in honor of surgeon and early antiseptic advocate Joseph Lister. Eventually, the genus Listeria was proposed and accepted. All species within the Listeria genus are Gram-positive, non-spore forming, catalase-positive rods. The genus Listeria was classified in the family Corynebacteriaceae. The 16S rRNA cataloging studies have demonstrated that L. monocytogenes is a distinct taxon within the Lactobacillus-Bacillus. In 2004, the genus was placed in the newly created Family Listeriaceae. The only other genus in the family is Brochothrix (3).

The genus Listeria contains ten species: *L. fleischmannii*, *L. grayi*, *L. innocua, L. ivanovii, L. marthii, L. monocytogenes, L. rocourtiae, L. seeligeri, L.*

weihenstephanensis and *L. welshimeri*. Listeria dinitrificans, previously thought to be part of the Listeria genus, was reclassified into the new genus Jonesia.(4).

Listeria can be found in soil, which can lead to vegetable contamination. Animals can also be carriers. Listeria has been found in uncooked meats; uncooked vegetables, fruit such as cantaloupes, pasteurized or unpasteurized milk, foods made from milk, and processed foods. Pasteurization and sufficient cooking kill Listeria; however, contamination may occur after cooking and before packaging. Listeria monocytogenes is commonly found in soil, stream water, sewage, plants, and food) (5). Listeria is responsible for Listeriosis, a rare but potentially lethal food-borne infection. The case fatality rate for those with a severe form of infection may approach 25% (6). Although Listeria monocytogenes has low infectivity, it is hardy and can grow in temperatures from 4 °C (39.2 °F) (the temperature of a refrigerator), to 37 °C (98.6 °F), (the body's internal temperature)(5). Listeriosis is a serious illness, and the disease may manifest as meningitis, or affect newborns due to its ability to penetrate the endothelial layer of the placenta (6).

Listeria uses the cellular machinery to move around inside the host cell: It induces directed polymerization of actin by the ActAtransmembrane protein, thus pushing the bacterial cell around (7).

Listeria monocytogenes, for example, encodes virulence genes that are thermo regulated. The expression of virulence factor is optimal at 39 °C, and is controlled by a transcriptional activator, PrfA, whose expression is thermo regulated by the PrfA thermo regulator UTR element. At low temperatures, the PrfA transcript is not translated due to structural elements near the ribosome binding site. As the bacteria infect the host, the temperature of the host melts the structure and allows translation initiation for the virulent genes.

The majority of Listeria bacteria are targeted by the immune system before they are able to cause infection. Those that escape the immune system's initial response, however, spread through intracellular mechanisms and are, therefore, guarded against circulating immune factors (6).

To invade, Listeria induces macrophage phagocytic uptake by displaying D-galactose in their teichoic acids that are then bound by the macrophage's polysaccharide receptors. Once phagocytosed, the bacterium is encapsulated by the host cell's acidic phagolyso some organelle.Listeria, however, escapes the phagolyso some by lysing the vacuole's entire membrane with secreted hemolysin (8), now characterized as the exotoxin listeriolysin O (5). The bacteria then replicate inside the host cell's cytoplasm (6).

Listeriosis is a serious disease for humans; the overt form of the disease has a mortality rate of about 20 percent (9). Manifestations of Listeriosis are host-dependent. In addition, Listeriosis can present in different ways depending on the type of infection. In older adults and persons with immunocompromising conditions, septicemia and meningitis are the most common clinical presentations. Pregnant women may experience a fever and other non-specific symptoms, such as fatigue and aches, followed by fetal loss or bacteremia and meningitis in their newborns. Meningitis is often complicated by encephalitis, a pathology that is unusual for bacterial infections. Immunocompetent persons may experience acute febrile gastroenteritis or no symptoms. The symptoms of Listeriosis are usually fever and muscle aches that sometimes proceeded by diarrhea or other gastrointestinal symptoms. Almost those who are diagnosed with Listeriosis has "invasive" infection, in which the bacteria spread beyond the gastrointestinal tract (1) Listeria is an opportunistic pathogen: It is most prevalent in the elderly, pregnant mothers, and AIDS patients. With improved healthcare leading to a growing elderly population and extended life expectancies for AIDS patients, physicians are more likely to encounter this otherwise-rare infection (only 7 per 1,000,000 healthy people are infected with virulent Listeria each year)(5).

2. MATERIALS AND METHODS

2.1 Study population

During a period of eight months from May 2015 to January 2016, a study was conducted at two teaching hospitals in Baghdad. AL-Elweya and Al-Khadymia teaching hospitals on freshly collected stool samples from a total number of 71 cases of acute diarrhea among adult patients.

A questionnaire was completed for each patients containing the following information: name, age, gender, clinical data (fever, nausea, vomiting, abdominal pain, and diarrhea), macroscopic and microscopic laboratory examinations of stool samples. The inclusion criteria was to include in this study watery stool samples (at macroscopic examination) and a parasite –free stool samples at microscopic examination (using saline and iodine preparations) from the diarrheal cases that were not lasting more than seven days after the onset of illness. The criteria were also, to include reported hemorrhagic fresh stool samples containing parasitic agents (*Giardia lamblia or Entamoeba histolytica*) in their stools. Stool samples were collected in a labeled screw cap clean container. Stool samples were tested by Immunochromatographic assay (purchased from CerTest Biotech, Spain) for antigenic detection of *Listeria* and were done according to instructions of the manufacturers. Allowing the card –device, test reagents and stool samples to reach to room temperature prior to testing. A separate stool collection tube and device were used for each sample and the assay was done right after collection. To detect *Listeria*, approximately 100mg or 100 microtiter of

stool sample was put and shaken in collection tube containing the diluents. Four drops or 100µl was dispensed in the circular window of the card. The results (appearance of the colored bands) were read after 10 minutes.

This CerTest-*Listeria* KIT is qualitative Immunochromatographic assay for determination of rotavirus in fecal samples. The membrane on the test band region is pre coated with mouse monoclonal antibodies against *Listeria* antigens. During testing, the sample is allowed to react with the colored conjugates (anti-Listeria mouse monoclonal antibodies-red microspheres) which were pre-dried on the test. The mixture then moves upward on the membrane by capillary action. As the sample flows through the test membrane, the colored particles migrate. In the case of positive result, the specific antibodies present on the membrane will capture the colored particles and a red colored line becomes visible. The mixture captures the colored particles and a red colored line becomes visible. The mixture continues to move across the membrane to the immobilized antibody placed in the control band region, a green-colored band always appear. The presence of this green band serves as 1-verification that sufficient volume is added, 2-that proper flow is obtained and 3-as an internal control for the reagents. Insufficient specimen volume, incorrect procedural or deterioration of the reagents is the most likely reasons for control line failure. Negative results were indicated by only one green band (control line).

For positive result, in addition to the green control band, a red band also appeared on the site of result line. A total absence of the control colored band (green) regardless the appearance or not of the result line (red) was evaluated as an invalid result.

3. RESULTS AND DISCUSSION

3.1 Gender

Diarrheal according to their gender Patients with acute diarrhea, were studied, among them 43 were males and 28were females. Males to females ratio was 0.87. Listeria antigen was revealed in 71 of fecal samples. Among that studied who has Listeria antigen positive diarrhea only one (Table 1). The results show statistical difference between Listeria positive antigen in both males and females group using chi square test.

Table 1: Diarrheal patients according to their gender and Listeria infection.

Listeria Antigen	Males No.%	Females No.%	Total No.%
Listeria + ve Antigen	1(100%)	--------	1 (100%)
Listeria - ve Antigen	42(60%)	28(40%)	70 (100%)
Total	43(60.5%)	28(39.5%)	71 (100%)

3.2 Fever

Patients with acute diarrhea whom fecal specimens were positive to *Listeria* antigen or negative develops fever more than those without fever (67.6% versus 32.4%). The result revealed statistically significant difference ($p<0.01$).

Table 2: Patients with acute diarrhea according to fever in their bodies.

Listeria Antigen	Fever positive No.%	Fever negative No.%	Total No.%
Listeria + ve Antigen	1(100%)	--------	1 (100%)
Listeria - ve Antigen	47 (67%)	23 (33%)	70 (100%)
Total	48 (67.6%)	23 (32.4%)	71 (100%)

3.3 Abdominal pain

Patients with acute diarrhea whom fecal specimens were *Listeria* positive antigen and negative antigen develops. Abdominal pain more than those without abdominal pain (70.4% versus 29.6%).The result revealed significant difference ($p<0.01$).

Table 3: Diarrheal patients according to abdominal pain.

Listeria Antigen	Abdominal pain positive No.%	Abdominal pain negative No.%	Total No.%
Listeria + ve Antigen	1(100%)	--------	1 (100%)
Listeria - ve Antigen	49 (70%)	21 (30%)	70 (100%)
Total	50 (70.4%)	21 (29.6%)	71 (100%)

Table 4: Diarrheal patients according to vomiting.

Listeria Antigen	Vomiting No.%	Total No.%
Listeria + ve Antigen	1(100%)	1 (100%)
Listeria - ve Antigen	70 (100%)	70 (100%)
Total	71 (100%)	71 (100%)

3.4 Vomiting

All patients with acute diarrheal whom fecal specimens were positive to *Listeria* antigen or negative develops vomiting (100%).

3.5 Stool color

Patients with acute diarrhea whom fecal specimens were *Listeria* psitive antigen or *Listeria* antigen negative varies in the stool color 92.9 % were brown, and 7.1% were yellowish. Result shows significant difference ($p<0.01$) between the groups.

Table 5: Diarrheal patients according to stool color.

Listeria Antigen	Color		Total
	Brown	Yellow	
Listeria + ve Antigen	1(100%)	--------	1 (100%)
Listeria - ve Antigen	65 (92.8%)	5 (7.2%)	70 (100%)
Total	66 (92.9%)	5(7.1%)	71 (100%)

3.6 Stool consistency

Patients with acute diarrhea whom fecal specimens were *Listeria* positive antigen and Listeria negative antigen develop watery stool more than loose stool (76% versus 24%). The results indicated significant difference ($p<0.01$) between groups.

Table 6: Diarrheal patients according to the consistency.

Listeria Antigen	Consistency		Total
	Loose	Watery	
Listeria + ve Antigen	1(100%)	--------	1 (100%)
Listeria - ve Antigen	53 (75.7%)	17(24.3%)	70 (100%)
Total	54 (76%)	17(24%)	71 (100%)

3.7 Blood

A patients with acute diarrhea whom fecal specimens were *Listeria* positive antigen and *Listeria* negative antigen show (100%)negative bloody stool. The results indicated significant difference ($p<0.01$) between groups.

Table 7: Diarrheal patients according to blood in their stool.

Listeria Antigen	Blood		Total
	Positive	Negative	
Listeria + ve Antigen	----------	1 (100%)	1 (100%)
Listeria - ve Antigen	----------	70 (100%)	70 (100%)
Total	----------	71(100%)	71 (100%)

3.8 Mucous

A Patient with acute diarrhea whom fecal specimens were *Listeria* positive antigen and *Listeria* negative antigen show mucous in their stool ($p<0.01$) in comparison with other groups (90.2 % versus 9.8%).

Table 8: Diarrheal patients according to mucous in their stool.

Listeria Antigen	Mucous		Total
	Positive	Negative	
Listeria + ve Antigen	1 (100%)	---------	1 (100%)
Listeria - ve Antigen	63 (90%)	7 (10 %)	70 (100%)
Total	64 (90.2%)	7 (9.8%)	71 (100%)

Table 9: Diarrheal patients according to presence of pus cells in their stool.

Listeria Antigen	Pus cells		Total
	Positive	Negative	
Listeria + ve Antigen	1 (100%)	---------	1 (100%)
Listeria - ve Antigen	70 (100%)	--------	70 (100%)
Total	71 (100%)	---------	71 (100%)

3.9 Pus cells

A patients with acute diarrhea whom fecal specimens were *Listeria* positive antigen and *Listeria* negative antigen show 100% pus cells in their stool. (Table-9).

3.10 Cysts

A patients with acute diarrhea whom fecal specimens were *Listeria* negative antigen and positive antigen show presence of *Entamoeba histolytica* cyst. The percent was 12.6% versus 87.4% (Table-10).

Table 10: Diarrheal patients according to presence of *Entamoeba histolytica* cyst in their stool.

Listeria Antigen	*E.histolytica* cyst		Total
	Positive	Negative	
Listeria + ve Antigen	1 (100%)	---------	1 (100%)
Listeria - ve Antigen	7 (10 %)	63(90%)	70 (100%)
Total	8 (12.6%)	63(87.4%)	71 (100%)

Listeria was identified in one stool samples of patient out of 71 samples (Table - 1). The infections may be due to lack of sanitary facilities and poor living condition among the major causes of diarrhea. *Listeria* has the third highest mortality rate among all food borne infections. *Listeria* is present in soil, water, some animal sources, raw (unpasteurized) milk, ready-to-eat deli meats, hot dogs, and refrigerated smoked seafood. (10, 11). The result in line with (12) who reported the symptoms most frequently diarrhea (in 33%–88%), of patients with gastroenteritis. In general the most common symptoms of invasive gastroenteritis due to *Listeria monocytogenes* are fever, diarrhea, and headache. In most cases, at least one gastrointestinal symptom such as nausea, diarrhea, and vomiting present (Table-2,3,4). Although *Listeria* does not produce any enterotoxin and the mechanism of pathogenesis is unknown, direct invasion of bacterial protein with E-cadherin on the host cell, leading to diarrhea, fever, and bacteremia, is proposed. (13,14). The possible mechanism may be due to the bacteria act on the vascular and nervous apparatus resulting in increased permeability and decrease tone of the vessel, than upset thermal regulation and vomiting (15,16). Diarrhea is typically non bloody and watery stool (Table - 6,7,8) the result in line with (17) in a general The mechanism by which *L. monocytogenes* causes diarrhea is unknown, but diarrhea is likely the result of direct invasion. The organism is not known to produce any enterotoxins, and invasion is suggested by occasional bloody diarrhea and bacteremia (18) Most patients with diarrhea with positive or negative Listeria antigen show pus, mucous (Table-8,9) in their stools). In a general, patients with Listeriosis and other pathogenic bacteria is often containing mucous, pus specially when the diarrhea is sever (19). Some patients show presence of *Entamoeba histolytica* cyst in their stools (Table - 10). In a general, the Entamoebiasis represented the most common parasites which are transmitted via the ingested unhealthy food (20). However, there is no specific vaccination to prevent neither spread nor infection of the disease (21). Amoebiasis is more risky infectious disease than other while the cyst of Entamoeba can survive for up to a month in a soil or for up to 45 min under fingernails .Invasion of the intestinal lining causes amoebic bloody diarrhea or amoebic colitis. If the parasites reach blood stream it can spread through the body to another sites like liver when it causes amoebic liver abscess (22,23,24).

4. CONCLUSION

Antigen present in one stool sample, most patients show vomiting, fever and abdominal pain in all the cases without blood and all cases with pus, 78 cases with mucous in comparison with 16 without mucous and only eight cases (12.6%) with cyst of *E.histolytica.*

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