



Antibiogram for listeria spp. Recovered from raw ruminant milk

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ABSTRACT

In this study, 512 milk samples from different sources were examined. The incidence of *Listeria* (*L.*) among the different ruminant species revealed the isolation of isolates where the percentage of *Listeria* isolation was 6.1%. The isolated *Listeria* spp. were distributed as 9 strains from cows, 8 strains from buffaloes, 6 strains from goats, 8 strains from ewes and 4 strains from she-camels raw milk. The *Listeria* spp. were related to 7 strains of *L. monocytogenes*, 4 strains *L. innocua*, 8 strains *L. welshimeri*, 7 strains *L. seelegeri* and 5 strains *L. grayi* in a percentage of 1.4%, 0.8%, 1.6%, 1.4% and 0.98%; respectively. The antibiogram for *Listeria* isolates revealed that all isolates showed resistance for the following antibiotics: cloxacillin, oxacillin, pefloxacin, flumoquine, cephalosporin, bacitracin, lincomycin, clindamycin. *Listeria* spp. consider as an important environmental and opportunistic pathogens for ruminant raw milk with some evidence for multidrug resistance strains due to miss use of antibiotics.

Key words : (*Listeria* , Incidence , Antibiogram).

INTRODUCTION

L. monocytogenes was widely found in the environment and animals; it has a competitive advantage in salty or cold environments. Although much is known about the growth and survival in various ecosystems, transmission and principle sources are poorly understood, perhaps because it has developed different mechanisms in different systems. It appears that *L. monocytogenes* represents an opportunistic human pathogen and that human infections are likely to contribute little if anything to the ecological success or dispersal of *L. monocytogenes*. The term listeriosis encompasses a wide variety of disease symptoms that are similar in animals and humans. *L. monocytogenes* causes listeriosis in animals and humans; *L. ivanovii* causes the disease in animals only, mainly sheep. Encephalitis is the most common form of the disease in ruminant animals. In young animals, visceral or septicemic infections often occur. Intra-uterine infection of the fetus via the placenta frequently results in abortion in sheep and cattle. Until about 1960, *L. monocytogenes* was thought to be associated almost exclusively with infections in animals, and less frequently in humans (Todar, 2012).

Unprocessed, raw milk from dairy farms is the starting point in the production-consumption

chain; therefore, a reduction of bulk tank milk contamination with *L. monocytogenes* is important. Although pasteurization destroys pathogenic bacteria and makes milk safe for consumption, the presence of *L. monocytogenes* in bulk tank milk may represent a risk of contamination for milk processing plants and the subsequent post pasteurization contamination of milk. One of the routes from which *L. monocytogenes* may initially enter food processing plants (and subsequently colonize the plant environment) is through raw materials (Vogel et al., 2001). Hence, the control of *L. monocytogenes* at the farm level to prevent the entrance of the pathogen into the food chain through raw animal products, including milk, is warranted. *L. monocytogenes* is nearly ubiquitous on dairy farms and its presence in milk and milk filters has been frequently reported (Nightingale et al., 2004). *L. monocytogenes* has been isolated from many locations within dairy farms, including feces (Lyautey et al., 2007), animal drinking water (Latorre et al., 2009), feeds or feed components (Mohammed et al., 2009), and milking equipment (Latorre et al., 2010). A potential link between animal production systems and human listeriosis cases exists, as was suggested by previous studies that described the presence of

matched those observed for isolates from human listeriosis cases (Fugett et al., 2007).

L. monocytogenes is usually susceptible to a wide range of antibiotics (Hof et al., 1997), but since the first isolation of a multiresistant strain in France in 1988 (Poyart-Salmeron et al., 1990), other strains resistant to one or more antibiotics have been recovered from food, environment and from sporadic cases of human listeriosis (Charpentier et al., 1995). Antibiotics to which some *L. monocytogenes* are resistant include tetracycline, gentamicin, penicillin, ampicillin, streptomycin, erythromycin, kanamycin, sulphonamide, trimethoprim, and rifampicin (Charpentier and Courvalin, 1999). Little information is available on the antimicrobial susceptibility of *L. monocytogenes*, particularly on strains isolated from buffalo, cow, goat, ewe and she-camel raw milk indicating the necessity of monitoring the antimicrobial susceptibility of this pathogen. Pasteurization is a highly effective method to eliminate pathogens from milk, it might not always be 100% effective concerning *Listeria* spp (Fleming et al., 1985).

L. monocytogenes rarely develops acquired resistance to antibiotics. However, some studies have recently reported an increased rate of resistance to one or several clinically relevant antibiotics in environmental isolates (Conteret et al., 2009) and less frequently in clinical strains (Morvan et al., 2010). Yet, this probably remains a marginal phenomenon for clinical strains, although only a limited number of studies have focused on the evaluation of antimicrobial resistance in *Listeria* (Hansen et al., 2005).

So the aims of the present study are recording of *Listeria* spp incidence in ruminant raw milk with special attention to its antibiogram.

MATERIALS AND METHODS

• Sample, isolation and identification

A total of 512 samples of raw milk (103 cows, 100 buffaloes, 107 goats, 102 ewes and 100 she-camels) collected from different Governorates (El Giza, El Kaluobia, EL Fayoum, El Sharkia, El Behaira).

• Isolation and identification of *Listeria* spp.

For the isolation and identification of *Listeria* species in the milk samples, the techniques recommended by the International Organization for Standards (ISO 11290-1, 1996) were implemented. Isolation and identification of *Listeria* was performed using the double enrichment procedure, the first (Half Fraser) and

the second (Fraser) enrichment broths. As stated by (Ahmed Orabi et al., 2016)

• Antibiogram for *Listeria* isolates

All purified isolates were tested by the standard disc diffusion method (CLSI, 2014) and were subjected to a susceptibility panel of 28 antibiotics (Oxoid) belonging to 11 drug classes (Table 6). Isolates were cultured in trypticase soy broth (TSB) supplemented with 0.6% yeast extract, and transferred to Mueller–Hinton agar (Oxoid). The plates were incubated at 37°C for 48 hours

RESULTS

A total number of 512 examined samples that were distributed as 103 samples from cow, 100 samples from buffaloes, 107 samples from goats, 102 samples from ewes and 100 samples from she-camels, the incidence of *Listeria* among the different ruminant species revealed that the percentage of *Listeria* isolation reached 6.1%. The isolated *Listeria* spp. were distributed as 9 strains from cows, 8 strains from buffaloes, 6 strains from goats, 8 strains from ewes and 4 strains from she-camels raw milk. The *Listeria* spp. were distributed as 7 strains of *L. monocytogenes*, 4 strains *L. innocua*, 8 strains *L. welshimeri*, 7 strains *L. seelegeri* and 5 strains *L. grayi* in a percentage of 1.4%, 0.8%, 1.6%, 1.4% and 0.98%, respectively. *L. monocytogenes* isolates antimicrobial patterns showed resistance for the following antibiotics: cloxacillin, oxacillin, pefloxacin, flumequine, cephalosporin, bacitracin, lincomycin, clindamycin, while other *Listeria* isolates were resistant to penicillins G, cloxacillins, oxacillins, pefloxacin, flumequine, lincomycins, clindamycin and cephalosporins, sulphatrimethoprim and rifampicin.

DISCUSSION

L. monocytogenes is a Gram +ve, non spore forming, rod-shaped flagellate. As Gram-positive non-spore forming, catalase-positive rods, the genus *Listeria* was classified in the family Corynebacteriaceae through the seventh edition of Bergey's Manual. 16S rRNA cataloging studies of Stackebrandt et al. (1983) demonstrated that *Listeria monocytogenes* was a distinct taxon within the Lactobacillus-Bacillus branch of the bacterial phylogeny constructed by Woese (1981). In 2000 the Family Listeriaceae was created within the expanding Order Bacillales, which also include

Staphylococcaceae, Bacillaceae and others (Todar, 2012).

Listeriosis outbreaks have mostly been linked to consumption of raw milk or cheese made of unpasteurized milk. Previous outbreaks of listeriosis have been linked to a variety of foods especially processed meats (such as hot dogs, deli meats, and pate). Today, most are linked to consumption of raw milk or cheese made from unpasteurized milk. The public health importance of listeriosis is not always recognized, particularly since *Listeria* is a relatively rare disease compared with other common foodborne illnesses such as salmonellosis or botulism. However, because of its high case fatality rate, listeriosis ranks among the most frequent causes of death due to foodborne illness: second after salmonellosis. Changes in the manner food is produced, distributed and stored have created the potential for widespread outbreaks involving many countries. Pasteurization of raw milk, which destroys the *L. monocytogenes*, does not eliminate later risk of *L. monocytogenes* contamination in dairy produce. In their recent review of data concerning human listeriosis, the Group of work of the (WHO, 1988), it is considered food infection is transmitted predominantly in an ape-zootica. Because, although the soil is the source, transmission to man is mainly produced from the environment through animals and food surfaces. In this way, *L. monocytogenes* should be considered as environmental bacteria whose transmission to humans occurs mainly through consumption of foods that have been contaminated during its manufacture and production (WHO, 1988).

In the present study tested *Listeria* among the different ruminant species revealed that the percentage of *Listeria* isolation reached 6.1%. The isolated *Listeria* spp. were distributed as 9 strains from cows, 8 strains from buffaloes, 6 strains from goats, 8 strains from ewes and 4 strains from she –camels raw milk. The *Listeria* spp. were distributed as 7 strains of *L. monocytogenes*, 4 strains *L. innocua*, 8 strains *L. welshimeri*, 7 strains *L. seelegeri* and 5 strains *L. grayi* in a percentage of 1.4%, 0.8%, 1.6%, 1.4% and 0.98%, respectively (Ahmed Orabi et al.,2016).

In Iran, Rahimiet al. (2012) were found that 21 isolate to be resistant to one or more antimicrobial agents. Six strains (28.6%) were

resistant to a single and 5 strains (23.8%) showed resistance to two antimicrobial agents. Multi-drug resistance was established in 23.8% of *Listeria* strains. Resistance to nalidixic acid was the commonest finding (85.7%), followed by resistance to penicillin (47.6%), and tetracycline (33.3%). The antimicrobial susceptibilities of the 64 isolate of *Listeria* spp. were examined by the standard disk diffusion method in Thailand by Stonsaovapak and Boonyaratanakornkit (2010). *Listeria* spp. was resistant to penicillin (6.3%), chloramphenicol (3.1%) and tetracycline (1.6%), but sensitive to amoxicillin, vancomycin, ampicillin, rifampicin and sulfamethoxazole.

The study of Davis and Jackson (2009) compared antimicrobial susceptibility of *Listeria innocua*, *L. welshimeri*, and *L. monocytogenes* isolated from various sources. Antimicrobial susceptibility testing was performed using a micro broth procedure with Sensititre® minimum inhibitory concentration plates containing 18 antimicrobials. Resistant isolates were analyzed for the presence of antimicrobial resistance genes using PCR. The majority of *L. monocytogenes* isolates were resistant to oxacillin (99%, 89/90) and ceftriaxone (72%, 65/90), while few isolates were resistant to clindamycin (21%, 19/90) and ciprofloxacin (2%, 2/90). When selected sources of *L. monocytogenes* are compared, resistance to ceftriaxone, clindamycin, and oxacillin ranged from 27% to 86%, 7% to 43%, and 96% to 100%, respectively. Resistance to ciprofloxacin (6%, 2/34), quinupristin/dalfopristin (7%, 1/14), and tetracycline (7%, 1/15) was observed with *L. monocytogenes* isolated from food, animal, and environmental sources, respectively. All *L. welshimeri* isolates (6/6) were resistant to streptomycin, quinupristin/dalfopristin, ciprofloxacin, rifampin, oxacillin, penicillin, and clindamycin, while most isolates (67%, 4/6) were resistant to trimethoprim/sulfamethoxazole. All *L. innocua* isolates (4/4) were resistant to oxacillin and penicillin, whereas 75% (3/4) of isolates were resistant to tetracycline, ceftriaxone, and clindamycin.

CONCLUSION

Listeria spp. consider as an important environmental and opportunistic pathogens for

ruminant raw milk with some evidence for multidrug resistance strains due to miss use of

antibiotics.

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الملخص العربي

البيوجرام الحيوي لأنواع الليستريا المعزولة من ألبان المجترات الخام

في هذه الدراسة تم فحص 512 عينة لبن من المجترات المختلفة لتحديد نسبة تواجد ميكروب الليستريا فيه و كانت 1.6 % مقسمة الي (9 معزولات من لبن الأبقار و 8 معزولات من لبن الجاموس و 6 معزولات من لبن الماعز و 4 معزولات من لبن الجمال) و تم تصنيف هذه المعزولات الي (7 عترات لىستريا مونوسيتوجينز و 4 عترات لىستريا انوكا و 8 عترات لىستريا ولىشيميري و 7 عترات لىستريا سيليجيري و 5 عترات لىستريا جراي) بنسبة 98%، 1.4 %، 1.6 %، 0.8%، 1.4 % بالترتيب و بدراسة حساسية هذه العترات للمضادات الحيوية أظهرت مقاومة للكلوكساسيلين والاووكساسيلين والبي فلوكساسين و الفلوموكوين و السيفالواسبورين والباكتريوسين و اللينكومايسين و الكليندامايسين الكلمات الدالة : (الليستريا - البيوجرام - نسبة الحدوث)