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Original Research Article

Prevalence of *Listeria species* in meat processing environments

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A B S T R A C T

A total of 100 surface swabs (table tops and knives) were collected from meat processing facilities and retail markets in Kerala. These samples were screened for the presence of *Listeria* spp.to provides information on the occurrence of organisms in such environments. Following a two step enrichment procedure and plating in selective agar, confirmation of the isolates were based on biochemical tests. *Listeria innocua* could be detected in three percent of the samples taken which were also isolated from human infections earlier. Reports suggest that *Listeria monocytogenes* and *L. innocua* share the same ecological niche and *L. innocua* could be used as an indicator for the presence of *L. monocytogenes.* The antibiotic sensitivity pattern of isolates showed resistance to more than one antimicrobial agent. The results of this study demonstrated the possibility of equipments and working environment as a source of contamination to the meat and meat products by *Listeria* spp. in food processing environments. Special attention is required with respect to cleaning and sanitation procedures. Further investigations are essential for the determination of main contamination points in food processing facilities so that effective control measures can be implemented based on HACCP principles.

Keywords

Surface swabs; *Listeria* spp ; Selective agar; antimicrobial agent.

Introduction

Listeriosis is an emerging bacterial zoonosis which is capable of causing severe foodborne infections in both humans and animals. Although the disease is rare and accounts for only about 0.02 percent of all foodborne illnesses, listeriosis has got a high mortality rate of 20-30 per cent that increases upto 70 per cent in high risk groups such as pregnant women, neonates and immuno compromised individuals (Tompkin, 2002). The genus Listeria include 6 different species- *Listeria monocytogenes, L. ivanovii, L. seeligeri, L. innocua, L. welshimeri and L. grayi* out of which *L. monocytogenes, L. ivanovii and L. innocua* have been isolated from human infections. Its ubiquitous nature coupled with high

mortality rate make this pathogen a serious public health issue.

The widespread nature of Listeria allow easy access to a variety of raw foods including vegetables (Sunil *et al.*,2012*)*, fruits, meat, milk, seafoods (Sunil *et al*., 2013) and food products during various phases of production, processing, manufacturing and distribution. Due to the hardy growth characteristics of Listeria, it is able to contaminate and thrive in food processing facilities by forming biofilms (Donlan and Costerton, 2002). Once biofilms are formed, it persists there for many years and can act as a continuous source of contamination. By controlling the establishment and multiplication of Listeria in these environments, it is possible to minimize or prevent the risk of food contamination with proper sanitation procedures. The purpose of the present study was to generate information on the incidence of *Listeria* spp. in meat processing plant and retail markets in Kerala.

Materials and Methods

Surface swabs were taken from meat processing plant at Eranakulam and retail shops at Mannuthy and Thrissur. A total of 100 swabs were taken and brief description of all samples are given in table 1. Premoistened swabs were firmly rubbed over the surface to be examined by using parallel strokes and rotation of the swab. These swabs were transferred to 25 ml of peptone water immediately after collection. The methodology selected for the detection of *Listeria* spp. was that recommended by the United States Department of Agriculture (Mcclain and Lee, 1988) with modifications. In the primary enrichment phase, 25 ml of the initial dilution was transferred to 225 ml of University of Vermont Medium I (UVM I, Himedia, India) and incubated at 30 ^{0}C for 24 h. 0.1 ml of UVM I was transferred to UVM II (Himedia, India) and incubated at 30 ^{0}C for 24 h. Then it was streaked to Polymyxin acriflavin lithium chloride ceftazidime aesculin mannitol(PALCAM, Himedia,India) agar plates and incubated at 37 ^{0}C for 48 h.

Identification of *Listeria* spp.

The colonies with a grayish green glistening appearance surrounded by a black zone of aesculin hydrolysis on PALCAM agar were selected for further confirmation. Atleast five suspected colonies were selected and subcultured on Brain heart infusion (BHI) agar. Morphologically typical colonies were verified by Gram's staining, catalase reaction, tumbling motility at 20-25^{0}C, methyl red-Voges Proskauer (MR-VP) reactions, nitrate reduction, fermentation of sugars (rhamnose, xylose, mannitol and dextrose) and hemolysis on blood agar.

Antibiotic sensitivity test

All *Listeria* isolates were subjected to antibiotic sensitivity test against 12 different antimicrobial agents by agar diffusion method, as per the procedure described by Bauer *et al* (1966). Listeria isolates were tested against ampicillin (10µg), cefotaxime (30µg), chloramphenicol (30µg), cloxacillin (10µg), cotrimoxazole (25µg), doxycycline (30µg), erythromycin (15µg), gentamicin (10µg), rifampicin (5µg), streptomycin (25µg), enrofloxacin (10µg) and vancomycin (30µg) antibiotic discs (HiMedia). The interpretation of the result was made by comparing diameter of the zone of inhibition with standard zone of inhibition chart provided by the disc

manufacturing company. The clinical breakpoints for *Listeria* susceptibility testing were defined according to the Clinical and Laboratory Standard Institute (CLSI, 2010) and the isolates were grouped as sensitive, intermediary sensitive and resistant, against each antibiotic.

Results and Discussion

Table.1 shows all the collected samples and isolates obtained. In our study, out of 100 swabs taken, three tested positive for *Listeria* spp. thereby indicating a prevalence of three percent. The isolates were obtained from stainless steel table tops of meat plant, Eranakulam. All the three isolates were non-hemolytic and confirmed as *L. innocua* by biochemical analysis. Other species of *Listeria* were not isolated in this study.

All the positive isolates obtained from surface swabs were subjected to antibiotic sensitivity test by standard disc diffusion method. *L. innocua* isolates were sensitive to cefotaxime, chloramphenicol, cotrimoxazole, doxycycline, erythromycin, streptomycin, vancomycin and gentamicin, intermediary sensitive to enrofloxacin and rifampicin and resistant to ampicillin and cloxacillin (Table 2).

A total of 100 surface swabs were collected from meat processing facilities and retail markets in Kerala. These samples were screened for the presence of *Listeria* spp. to provides information on the occurrence of organisms in such environments. Following a two step enrichment procedure and plating in selective agar, confirmation of the isolates were based on biochemical tests. *L.innocua* could be detected in three percent of the samples. Although not regarded as highly pathogenic, *L. innocua* has been isolated from human infections (Perrin *et al*., 2004). Moreover, King *et al*. (1990) suggested that *L. monocytogenes* and *L. innocua* share the same ecological niche and *L. innocua* could be used as an indicator for the presence of *L. monocytogenes.* Curiale and Lewus (1994) reported that *L. innocua* has a shorter generation time than *L. monocytogenes* and the recovery of *L. monocytogenes* using selective broth was lower when *L. innocua* was present.

The use and misuse of antibiotics in human and animal medicine is a major factor that contributes to the development of bacterial resistance. In this study, the isolates were resistant to two antimicrobial agents (ampicillin and cloxacillin). Since *L. innocua* and *L. monocytogenes* share the same ecological niche, resistance genes can be transferred through movable genetic elements such as transposons and plasmids (Poyart-Salmeron et al., 1990) to *L. monocytogenes* and other food borne pathogens and results in failure of antimicrobial therapy. Moreover, the resistant strains from meat tables may find their way into human population through contaminated meat, meat products and occupational exposure.

The cutting equipments and tables are a focal point in the plants for the preparation of food (Kusumaningrum *et al*., 2003). In

Table.1 Surface swabs from food processing environment

Sample	Collection area	Type of surface	Number of samples	Positive samples	Total
Table tops	Eranakulam	Stainless steel	15	3 (*L. innocua*)	57
		Wooden	5	-	
	Thrissur	Wooden	18	-	
	Mannuthy	Wooden	19	-	
Meat cutting knives	Eranakulam		15	-	43
	Thrissur		12	-	
	Mannuthy		16	-	

Table.2 Antibiogram of isolates

Antibiotics	isolate1	isolate 2	isolate 3
Ampicillin	R	R	R
Cotrimoxazole	S	S	S
Chloramphenicol	S	S	S
Cloxacillin	R	R	R
Erythromycin	S	S	S
Enrofloxacin	I	I	I
Rifampicin	I	I	I
Gentamicin	S	S	S
Cefotaxime	S	S	S
Streptomycin	S	S	S
Doxycycline	S	S	S
Vancomycin	S	S	S

this study, three samples which are positive for *Listeria spp*. were taken from stainless steel tabletops of meat processing plant where the raw meat and meat products are continuously coming in contact. These bacteria can directly contaminate the food products by cross contamination or it can persist in the processing environment in the form of biofilms. Therefore it is important to identify the critical control points in meat processing plants and retail markets in order to develop an effective cleaning strategy. An effective cleaning procedure may lead to a significant reduction (upto 99.8 percent) of bacteria occurring on the food processing equipments (Dunsmore et al., 1981). The results of this study demonstrated the possibility of equipments and working environment as a source of contamination by *Listeria spp.* in food processing facilities. Special attention is required with respect to cleaning and sanitation procedures. Further investigations are required for the complete analysis of critical control points in food processing establishments and to implement and maintain hygiene procedures based on HACCP principles.

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