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Full Length Research Paper

Prevalence, antimicrobial resistance profiles of *Listeria monocytognes* from various foods in Gaborone, Botswana

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Listeria monocytogenes **is known to cause epidemic and sporadic cases of listeriosis. The present study investigated the occurrence, antibiograms and molecular serotypes of the organism in various retail outlets in Gaborone, Botswana. Food samples were obtained randomly from selected supermarkets and street vendors in 5 geographical areas of Gaborone from May, 2007 to September 2007. *L. monocytogenes* was isolated and positively identified by using morphological and biochemical tests. From a total of 1324 food samples tested 57(4.3 %) were positive for *L. monocytogenes*. Out of the 57 isolates of *L. monocytogenes* 7 (12.3%), 3 (5.3%), 0 (0%), 27 (47.4%) and 20 (35.1%) were isolated from cheese, raw milk, meat (biltong), frozen cabbage and salad (coleslaw), respectively. From the 5 geographical areas selected for sampling in this study, Gaborone South recorded the highest number 19 (33.3%) of *L. monocytogenes* isolates while Gaborone West recorded the least, 7 (12.3%). The findings in this study reveal the presence of *L. monocytogenes* serotypes 1/2a and 1/2b in ready to eat food and highlight the need for education and training programmes in food safety in Gaborone, Botswana.**

Key words: *Listeria monocytogenes*, antibiotic resistance, molecular serotyping, food, Botswana.

INTRODUCTION

Listeria monocytogenes causes a very serious illness known as listeriosis. Individuals who are particularly susceptible to this condition are those who are immunocompromised (as in HIV/AIDS infection), pregnant women, newborn babies, and the elderly (Farber and Peterkin, 1991; McLauchlin et al., 2004). Although the incidence of listerosis is low, what is significant is that very high fatalities ranging from 20 to 30% have been reported (Mead et al., 1999).

L. monocytogenes is widely distributed in nature and has been isolated from a wide array of food products. The organism is considered hazardous in the food industry due to its ability to grow in gas or vacuum-packaged products at refrigeration temperatures (Duffy et al., 1994), low water activity (Nolan et al., 1992) as well as low pH (Buchanan et al., 1993) and all these measures are important in the control of food pathogens.

L. monocytogenes is also problematic due to its resistance to antibiotics. The first multiresistant strain of *L. monocytogenes* was isolated in France in 1988 (Poyart - Salmeron et al., 1990), thereafter *L. monocytogenes* strains resistant to one or more antibiotics have since been isolated (Franco Albuin et al., 1994; Charpentier et al., 1995).

It has been shown by various studies that listeriosis is a food-mediated illness (Embarek, 1994; Slutsker and Schuchat, 1999). A wide range of foods such as salads, seafoods, meat, and dairy have been implicated in liste-

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riosis (Bell and Kyriakides, 1998; Schlech, 2000), which follows the oral ingestion of the contaminated food (Finlay, 2001). Studies have shown that the number of *L. monocytogenes* cells can rise following refrigeration from fewer than 100 cells per gram, and this is the dose that is generally accepted for healthy people (Huss et al., 2000; Buchanan et al., 2000).

Serotyping has been used extensively to characterize *L. monocytogenes* (Wieldmann, 2002; Wagner and Allerberger, 2003). Thirteen *L. monocytogenes* serotypes (serovars) have been characterized in this species by using specific and standardized sera (Seeliger and Langer, 1979). Although most clinical isolates belong to serovars 1/2a, 1/2b, and 4b are the majority of strains which have caused large outbreaks were serovar 4b (Kathariou**,** 2000), and serovar 1/2a (Jacquet et al., 2002; Zhang and Knabel, 2005). Serovar identification by serological tests has remained popular. However, numerous molecular biology methods such as multiplex PCR (Doumith et al., 2004) have come to the fore in the characterization of *L. monocytogenes* serotypes. Even though a recent study (Manani et al., 2006) reported the occurrence of *L. monocytogenes* in frozen vegetables in this country, there is little data on the occurrence of this pathogen in foods in Botswana. This investigation was carried out to unravel the prevalence and scope of antimicrobial resistance profiles of *L. monocytogenes* serovars isolated from various retail foods in Gaborone, Botswana.

MATERIALS AND METHODS

Sampling

Samples were obtained randomly from selected supermarkets and street vendors in 5 geographical areas of Gaborone (east, west, north, south, and central). Samples collected were; raw vegetables (cabbage), salads, raw milk, cheese and meat (biltong). In this study, 250 - 300 samples per product were obtained. The samples were put in separate properly labeled sterile specimen bags and placed into a cooler box containing ice packs. Gloves were worn to avoid cross-contamination between samples from different supermarkets and street vendors. Aseptic technique was followed to avoid contamination during transport of the samples from the supermarkets to the laboratory.

Enrichment, culturing, morphological and biochemical identification

On arrival at the laboratory, the samples were transferred to properly labeled stomacher bags and then homogenised with the Stomacher (Seward 400, Tekmar, and Cincinnati Ohio, USA) set at medium speed. The homogenised samples were enriched by placing 25 g of the sample into 225 ml enrichment broth (Mast Diagnostcs, Merseyside, UK) and incubated at 30˚C for 48 h on Innova 4000 Newbrunswick Scientific incubator shaker. A loop full of culture was sub-cultured on Modified Listeria Selective Agar (Oxoid, Basingstoke, UK) supplemented with Listeria Selective Enrichment Supplement (Oxoid) and then incubated at 37ºC for 24 h. Dark brown colonies with black zones characteristic of *Listeria* were sub-cultured on nutrient agar. A Gram stain was performed on suspected colonies and Gram positive short rods colonies were sub-cultured onto tryptose soy agar (Merck, Darmstadt, Germany) slants and these were maintained at 4℃. Following this, the catalase test was performed on all the isolates and catalase positive isolates were identified to the species level by API Listeria (Oxoid). Isolates that were confirmed as *L. monocytogenes* were preserved in a solution containing 80% tryptose soy broth (Oxoid) and 20% glycerol at -80℃ for use in the steps that followed.

Table 1. Incidence of *Listeria monocytogenes* in various food products.

Food product	No. of isolates	% of positive isolates
Cheese	7	2.75
Raw milk	3	1.08
Biltong	0	0.00
Frozen cabbage	27	10.11
Salads (coleslaw)	20	7.41

Antibiotic susceptibility testing

Isolates that were confirmed as *L. monocytogenes* were inoculated on Mueller-Hinton broth (Oxoid, Basingstoke, Hampshire, England). The flasks were incubated at 37˚C on a Gallenkamp shaker (200 rpm) for 24 h. The turbidity of the actively growing broth culture was adjusted with sterile saline to obtain turbidity optically comparable to that of the 0.5 McFarland standard. One milliliter of the cell suspension was then transferred onto the surface of Mueller-Hinton agar (Oxoid, Basingstoke,Hampshire, England) and then spread evenly. The susceptibilities of all isolates to different antimicrobial agents were tested by the disk-agar method as standardized by the National Committee for Clinical Laboratory Standards (NCCLS, 1998). The following panel of antimicrobial disks and concentrations were used; chloramphenicol (25 µg), erythromycin (5 µg), fusidic acid (10 µg), methicillin (10 µg), novobicin (5 µg), penicillin G (1 U), streptomycin (10 µg), tetracycline (25 µg) (Mast Diagnostics, Merseyside, UK) as well as ampcillin (25 µg), cephalothin (30 µg), sulphamethaxozole/trimethoprim (25 µg), gentamicin (30 µg), and nitrofurantoin (10 µg) (Oxoid). They were obtained from the South African Bureau of Standards (Pretoria, South Africa) *L. monocytogenes* ATCC 19115 was used as the reference strain.

RESULTS

Prevalence of *L. monocytogenes*

L. monocytogenes was found in all food products except biltong. Table 1 shows that among the food products, the organism was frequently isolated from frozen cabbage (10.11%), while raw milk recorded the least number of isolates (1.08%). *L. monocytogenes* was not isolated at all from biltong.

The geographical areas from which *L. monocytogenes* was sampled seemed to affect the incidence of the organism (Table 2). Of the geographical zones sampled in this study, Gaborone South recorded the most number (33.33%) whilst Gaborone West recorded the least

Table 2. Prevalence of *L. monocytogenes* in different localities in Gaborone.

Geographical area	No. of positive isolates	% of positive isolates
Gaborone East	9	15.79
Gaborone West	7	12.28
Gaborone North	9	15.79
Gaborone South	19	33.33
Gaborone Central	13	22.81

(12.28%) with Gaborone East and North recording slightly higher than Gaborone West.

Antimicrobial susceptibility testing of *L. monocytogenes*

Antimicrobial susceptibility testing was performed on all the 57 confirmed *L. monocytogenes* isolates. Of these isolates, 31 (54.39%) were found to be resistant to one or more antibiotic. Resistant rates to penicillin G, sulphamethaxozole/trimethoprim, chloramphenicol, and tetracycline were encountered in 42.11, 29.82, 28.30, and 22.81%, respectively (Table 3). Antibiotic resistance was not encountered for fusidic acid, erythromycin, methicillin, ampicillin and cephalothin. In all, 15 different resistance patterns were observed. Of the food products tested, frozen cabbage and salads recorded the highest diversity of resistance patterns. From the resistance patterns, only one pattern (penicillin G and tetracycline) was common among all the food products that tested positive for *L. monocytogenes* (Table 4). Otherwise, the rest of the resistant patterns were unique or peculiar to the different food products that were tested.

DISCUSSION

In the present study contamination rates of food products with *L. monocytogenes* were 2.75, 1.08, 0.00, 10.11, and 7.41% for cheese, raw milk, biltong, frozen cabbage, and salads (coleslaw), respectively. *L. monocytogenes* is known to contaminate milk and milk products such as cheese because of the complex nature of these products (Cimmons, 2001). The non-occurrence of *L. monocytogenes* in biltong is significant in public health because the product is a ready-to-eat (RTE) and dry meat. Most reports on the occurrence of this food borne pathogen in meats have tended to concentrate on raw meat, meat mixed with salads and poultry. Low water activity had been shown to profoundly limit the growth and multiplication of the pathogen in some conditions (Vermeulen et al., 2007). Biltong from this country is exported to the EU markets and the absence of the organism may be due to the zero tolerance limits in the processing of the meat.

The incidence rate (2.75%) of *L. monocytogenes* in cheese was less than the 8.2% reported in the United Kingdom (Greenwood et al., 1991) and the 6.4% reported in Germany (Rudolph and Scherer, 2001). In a previous study in Iran (Moshtaghi and Mohamadpour, 2007), the incidence of *L. monocytogenes* in raw milk was found to be 1.6%, and is similar to the 1.08% in the present study. The prevalence of the organism in cheese is hazardous to consumers because cheese is a ready to eat (RTE) food. Although raw milk is heat treated (pasteurization) before consumption, where the raw milk is processed to dairy products before pasteurization it poses a health risk to the consuming population.

Of public health concern is the occurrence of the microorganism in frozen cabbage (10.11%) and salads (7.41%). A recent study (Little et al., 2007) found a lower proportion (6.0%) of salads to be contaminated with *L. monocytogenes* while another study conducted in the United States of America (Prazak et al. 2002) found an even lower number (2.34%) of cabbage samples to be contaminated. The high incidence of the microorganism in salads and cabbage are alarming because the salads (coleslaw) are ready to eat and the cabbage is sometimes eaten raw when it is mixed with other vegetables to make salads. According to Little et al. (2007), the occurrence of *L. monocytogenes* in pre-packaged mixed salads could result from the original contamination of raw material, cross contamination during processing, packaging or at retail. In chilled foods such as coleslaw and frozen cabbage, temperature is the principal controlling factor for their safety. However, *L. monocytogenes* has been shown to thrive under refrigeration temperatures (Duffy et al., 1994).

Among the five geographical areas sampled in the present study, the highest prevalence rate was recorded in Gaborone south (33.33%) whereas Gaborone west recorded the least (12.28%). It is important to note that Gaborone South is characterized by overcrowding and the inhabitants are generally of low economic and educational status. An interesting point to note from this study is that there were no significant differences in the prevalence of *L. monocytogenes* in retail supermarkets and street vendors. A previous study in South Africa (Lues et al., 2006) found the microbiological quality of foods served by street vendors to be within acceptable safety limits. In the same study the occurrence of specific microorganisms was thought to be indicative of a degree of ignorance on the part of the food handlers towards proper hygienic practices.

This study found resistance to penicillin G, sulphamethaxozole/trimethoprim, chloramphenicol, and tetracycline to be 42.11, 29.82, 28.30 and 22.81%, respectively. However, no isolate was resistant to fusidic acid, erythromycin, methicillin, ampicillin and cephalothin. In contrast to the present study, Dhanashree et al. (2003) found no strain that was resistant to chloramphenicol in a study in India as did Facinelli et al. (1991) in a survey of Italian meat and dairy products. Tetracycline resistance

Table 3. Susceptibility of *L. monocytogenes* to 13 antimicrobial agents.

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Chloramphenicol	71.70	-	28.30
Fusidic acid	100	-	-
Erythromycin	100	-	-
Methicillin	100	-	-
Novobicin	85.96	-	14.04
Penicillin G	54.79	3.10	42.11
Streptomycin	80.70	-	19.30
Tetracycline	67.84	9.35	22.81
Ampicillin	100	-	-
Cephalothin	100	-	-
Sulphamethaxozole/trimethoprim	70.18	-	29.82
Gentamicin	84.21	-	15.79
Nitrofurantoin	92.98	-	7.02

Table 4. Resistance patterns of *L. monocytogenes* isolates from food sources.

Food source	No. of isolates	Resistant pattern	No. of resistant strains	% Resistant
Cheese	7	PG, T	1	14.29
		C, PG, NO, S	1	14.29
		C, PG, NO, S, T	1	14.29
Raw Milk	3	PG, T	1	33.33
Cabbage	27	PG, T	2	7.41
		S, SXT, T	2	7.41
		C, PG, SXT	3	11.11
		C, PG, NO, T	1	3.70
		C, CN, PG, SXT	5	18.51
		CN, PG, NO, SXT	1	3.70
		C, NI, PG, NO, S, T	1	3.70
Salad	20	PG, T	1	5.00
		NO,PG,T	1	5.00
		PG, S, SXT	3	15.00
		CN,PG,SXT	3	15.00
		C, PG, S, T	1	5.00
		C, NI, PG, T	1	5.00
		C, NI, PG, NO, S	2	10.00

The antibiotics are Chloramphenicol (C), Gentamycin (CN), Novomycin (NO), Ntrofurantoin (NI), Penicillin G (PG), Streptomycin (S) ,Sulphamethoxazole/Trimethoprim (SXT), Tetracyclin (T).

has been the most frequently observed phenotype among *L. monocytogenes* strains (Charpentier et al., 1995). Tetracycline resistance in this study was noted to be much higher than the 8.4% reported USA (Zhang et al., 2007). Tetracycline resistance is thought to originate from the use of antibiotic in animal production (Schroeder et al., 2002). Antibiogram profiles point to the high diversity of the microorganism in the food products tested. Since the first isolation of a multiresistant strain of *L. monocytogenes* in France in 1998 (Poyart -Salmeron et al., 1990) multi-resistant strains have been extensively isolated.

The findings clearly highlight the occurrence of *L. monocytogenes* (serotypes 1/2a and 1/2b) among retailers and street vendors in the 5 geographical areas of Gaborone. The presence of this human pathogen in ready-to-eat foods should be considered as having significant public health implications, particularly among the immunocompromised and HIV/AIDS persons who are at greater risk.

It was evident that *L. monocytogenes* was resistant to chloramphenicol, penicillin G, sulphamethaxazole/ trimethoprim and tetracycline, suggesting that the incorrect use of these antimicrobial agents for therapeutic purposes in animals and humans may lead to the development of antibiotic resistance.

We recommend education of stakeholders such as product suppliers, supermarket management, cleaning staff and hygiene specialists on the biology of food borne infections and the intricate need to maintain the cold chain order to prevent food borne outbreaks.

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