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INCIDENCE AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *LISTERIA MONOCYTOGENES* IN FOUR STREET-FOOD VENDING SITES IN GABORONE, BOTSWANA

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ABSTRACT: *Listeria monocytogenes* is widely distributed in nature and a significant foodborne pathogen. Where outbreaks of listeriosis have occurred, high fatalities ensue especially among the immune suppressed, pregnant women and their fetuses. The current study investigated the occurrence of the *L. monocytogenes* in various foods sold by street vendors in four geographical areas of Gaborone from October 2011 to March 2012. From a total of 396 ready-to-eat street foods cultured, 60 (15.2%) tested positive for the organism. Out of the 60 confirmed isolates of *Listeria monocytogenes*, 48 (12.1%), 6 (1.5%) and 6 (1.5%) were isolated from vegetable, protein and starch food portions, respectively. From the four geographical areas selected for sampling in this study, the UB area recorded the highest number 24 (6.1%) of positive isolates while the bus station area recorded the least, 6 (1.5%). Thirty per cent of the positive isolates were resistant to tetracycline, 10% were resistant streptomycin, chloramphenicol and erythromycin each. The outcomes of the present investigation revealed the presence of *L. monocytogenes* in foods sold by street food vendors in Gaborone. This suggests a need for vigilance in implementing food hygiene training programs in the country.

Key words: *Listeria monocytogenes*, antibiotic resistance, listeriosis, street foods

INTRODUCTION

The gram positive and facultative intracellular microorganism, *Listeria monocytogenes* although less common in cases of sporadic food-borne infections, remains important in the overall burden of food-borne disease.For example, in the United States of America it is said to account for 4% of all hospitalizations and 28% of all deaths from food-borne disease (Mead *et al.*, 2006). The elderly, newborns, pregnant women and the immune suppressed are thought to be the most susceptible to listeriosis. Symptoms of the disease include meningoencephalitis, septicemia, gastroenteritis and abortion as a result of prenatal infection (WHO, 2002). A wide assortment of foods such as meat and meat products, milk and milk products, seafood and salads has been implicated in food-borne listeriosis (Jebelli *et al.*, 2012; Kawasaki *et al.*, 2009). Also, ready-to-eat foods (RTF) may serve as important vehicles in the transmission of the pathogen (Osaili *et al.*, 2011). *L. monocytogenes* is known to thrive under refrigeration conditions and it can survive well in low pH foods such as yoghurt (Gahan *et al.*, 1996) and is also capable of growth on dry surfaces (Wong, 1998). These characteristics particularly make the organism to be well adapted to colonize the food environment. Ever since the first report of antibiotic resistant *L. monocytogenes* in 1988 (Poyart-Salmeron *et al.*, 1998) resistance of the pathogen to antibiotics has become a significant worldwide public health concern (Kovacevic *et al.*, 2013). Infections by *Listeria* are routinely treated by β-lactam antibiotics such as penicillin and ampicillin but in immunocompromised individuals, the treatment regime is augmented with aminoglycosides like trimethoprim (Hof, 2003). Although *L. monocytogenes* has previously been detected in various food products retailing in Botswana (Manani *et al.*, 2006; Letsholo *et al.*, 2008), little data exists on the occurrence of the pathogen in street vended foods. This therefore, necessitates further investigation of this organism

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as well as the disease caused by this bacterium. The aim of the present study was to follow up Morobe *et al.*, (2009) study and assess the susceptibility of 68 isolates of *L. monocytogenes* isolated in street vended foods in order to monitor of its prevalence and antibiotic resistance dynamics.

MATERIALS AND METHODS

Sampling was done from October 2011 to March 2012. A plate of food was bought randomly from street food vendors in four different localities of Gaborone (BBS mall, University of Botswana (UB), Bus Station, and Bus Rank). The plate consisted of starch, protein and vegetable/salad portions. To prevent cross contamination, each plate was placed in a separate properly labeled sampling bag (Whirlpak, Nasco, Fort Atkinson, Wiscosin, USA). The plates were then immediately transported to the lab in a cooler box containing ice packs. Each locality was sampled at least twice during the course of the study. Upon arrival at the lab, a 10g of each portion of the three portions on one plate (that is starch, protein and salad/vegetable) was weighed and transferred to a sterile stomacher bag containing 90ml *Listeria* enrichment broth (Oxoid, Basingstoke, UK) and then homogenized using the stomacher (Seward 400, Tekmar, Cincinnati, Ohio, USA) set at medium speed. The homogenate was then transferred to a sterile conical flask, covered and then allowed to shake at 90rpm on an incubator shaker (Innova 4000, New Brunswick Scientific, Edison, New Jersey, USA) set at 37°C for 24h. A 0.1ml of the overnight culture was aseptically spread plated on modified listeria selective agar (Oxoid) supplemented with listeria selective supplement (Oxoid) and subsequently incubated at 37°C for 24h. Typical *Listeria* colonies appearing dark brown with black zones were sub-cultured on tryptone soy agar (Merck, Darmstadt, Germany) and incubated at 37°C.

Gram staining was performed on the colonies and Gram positive short rods were further grown on sheep blood agar. Isolates that displayed β-hemolytic activity on sheep blood agar were sub-cultured on tryptone soy agar (Merck) slants and incubated at 37°C for 24h and thereafter maintained at 4°C. The strains on slants were then identified using the API *Listeria* identification kit (bioMeriéux, Marcy l'Etoile, France) according to manufacturer's instructions. Positive isolates were further confirmed by the VITEK 2 (bioMerieux, Hazelwood, Missouri, USA) automated identification system according to the instructions from the manufacturer. This identification system has higher discriminatory power because it identifies bacteria based on 64 biochemical substrates compared to API Listeria which has 20 substrates. Positive *L. monocytogenes* isolates were preserved at -80°C in tryptose soy broth (Merck, Darmstadt, Germany) containing 20% glycerol and the preserved isolates were used in antimicrobial susceptibility testing.

Antimicrobial susceptibility testing was performed on all the confirmed *L. monocytogenes* isolates. The isolates were first standardized by growing them on Mueller-Hinton broth (Oxoid) for 24h and then the turbidity of the physiologically active culture was adjusted with sterile saline solution to obtain optical density comparable to 0.5 McFarland standards. One millimeter of the standardized suspension was then spread evenly on the surface of Mueller-Hinton agar (Oxoid) using a sterile bent glass rod. The susceptibilities of all isolates to different antibiotics were tested by the disk-agar diffusion method using criteria set by the CLINICAL LABORATORY STANDARDS INSTITUTE (CLSI) (2006). The following antimicrobial disks and concentrations were used for susceptibility testing; 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). *Listeria monocytogenes* ATCC 19115 was used as the reference strain. The obtained data was analyzed using the Statistical Package for Social Sciences (SPSS 12.0, SPSS, Chicago, Illinois). Pearson's correlation coefficient was used to determine differences in means among street vended foods obtained in four geographical areas of Gaborone, Botswana (P=0.01).

RESULTS AND DISCUSSION

In this study, from a total of 396 street-vended food samples examined, 60 (15.2%) tested positive for *Listeria monocytogenes*. The organism was detected in all the three food products analyzed, albeit with varying rates. Table 1 shows that out of the 60 positive isolates, salad/vegetable portion recorded the highest incidence (80%) of *Listeria monocytogenes*, whilst both protein and starch parts recorded 10% each. The prevalence of the organism among the foods tested was significantly different ($p<0.01$; $p=0.000$). Of the 60 positively identified *L. monocytogenes* isolates in the four sampling localities, the area around the Bus Rank registered the least number of positive isolates (10%) while street vending sites around the University of Botswana had the highest rates of incidence (24%). That notwithstanding, the differences in the prevalence of the organism among the zones sampled were not statistically significant ($p<0.01$; $p=0.000$). Interestingly, statistical analysis revealed that the locality of sampling did not also have any bearing on the isolation of *L. monocytogenes* from a particular type of food ($p<0.01$).

Antimicrobial susceptibility testing performed for the 60 isolates revealed that resistance was not encountered for methicillin, novobiocin, penicillin G, and fusidic acid (Table-3). Table-3 also shows that the highest incidence of resistance was recorded for tetracycline (30%) whilst streptomycin, chloramphenicol and erythromycin recorded 10% each. Among the 60 *Listeria monocytogenes* isolates, 20 (33.3%) displayed multi-drug resistance. Six resistance antibiograms were documented, with the vegetable or salad portion showing the highest diversity of isolates (five), one of which (S, T) also was common to the starch part. The vegetable or salad portion also had 8 multi-drug resistant isolates that were resistant to more than four antibiotics. The resistance pattern which was noted for the protein part of the food was peculiar only to that food portion (Table- 4).

The prevalence rate of *L. monocytogenes* in ready-to-eat street-vended foods sold in Gaborone, Botswana was found to be 15.2%. This finding is consistent with findings by Cabedo *et al.*, (2008) who established an incidence range of 6.2 to 20.0% of *L. monocytogenes* in ready-to-eat foods in Spain, but much lower than the 23.4% reported in a Belgian market (Van Coillie *et al.*, 2009). A previous investigation (Morobe *et al.*, 2009) found a much lower rate of 4.3% of *L. monocytogenes* in ready-to-eat foods in retail outlets in Botswana, while another study in the country did not detect the microorganism in the food products investigated (Matthews *et al.*, 2013). Other studies also performed in Botswana found a similar prevalence rate of up to 10% [(Manani *et al.*, 2006) of the organism, while Letsholo *et al.*, (2008) established contamination rates of 4 to 12% in street-vended foods. It is important to note that in the latter study, only *Listeria* species other than *L. monocytogenes* were detected. Several studies have proved that the incidence rates of *L. monocytogenes* depends largely on the food product or item under investigation (Cabedo *et al.*, 2008; Cordano and Rocourt, 2001; Mena *et al.*, 2004).

Table-1: Incidence of *Listeria monocytogenes* in ready-to-eat food products

Food Product	**% of Positive Isolates**
Vegetable	12.1
Protein	1.5
Starch	1.5

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

Geographical zone	**% of positive isolates**
BBS mall	20
Bus station	10
Bus rank	30
University of Botswana	40

Table-3: Antibiotic resistance of *Listeria monocytogenes* isolates

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Methicillin	100	-	-
Novobicin	100	-	-
Penicillin G	100	-	-
Streptomycin	50	40	10
Tetracycline	50	20	30
Chloramphenicol	70	20	10
Erythromycin	80	10	10
Fusidic acid	70	5	5

Table-4: Resistance antibiograms of *L. monocytogenes* isolates from three food types.

Food portion	No. of isolates	Resistance pattern[a]	No. of resistant strains	% Resistant
Salad/Vegetable	16	S, T ,C , E, FC	8	50
		S, T, C, E	2	12.5
		S, T	2	12.5
		T, E	2	12.5
		S, E	2	12.5
Protein	2	S, T, E	2	100
Starch	2	S, T	2	100

[a] Abbreviations; Methicillin (MT), Novobicin (NO), Penicillin G (PG), Streptomycin (S), Tetracycline (T), Chloramphenicol (C), Erythromycin (E), Fusidic acid (FC).

This trend was indeed observed in the present study where the salad/vegetable, protein and starch components of the street-vended foods were found to account for 12.1% and 1.5% each, respectively. Morobe *et al.*, (2009) found salads (7.4%) to be contaminated by *L. monocytogenes* while Velani and Roberts (1991) reported the incidence of the pathogen in 6.7% of ready to eat salads and all these results do not correlate to those in the current study. The differences in the occurrence and distribution of the organism in salads and vegetables may be due to sampling in different time points and geographical specificity. The presence of *L. monocytogenes* in salads is of imperative public health implication because they are consumed raw, and therefore pose a risk to consumers. The occurrence of the pathogen in salads could result from contamination of the original salad raw material, cross contamination during processing and transport, packaging or by street vendors at the point of sale. The organism is also known to proliferate under refrigeration temperatures, so refrigerators may serve as avenues that may seed the organism to food products (Cox *et al.*, 1989; Sergelidis *et al.*, 1997).

On the other hand the low incidence in the protein and starch components may be due to inactivation during cooking. Normal pasteurization processes have proved effective in the elimination of this pathogen, so conventional cooking would be expected to be particularly destructive (Norrung, 2000). Five geographical areas were sampled in this study, with street vendors based at the University of Botswana recording the highest incidence of *L. monocytogenes* (40%). Interestingly, this sampling area consisted entirely of ambulatory street vendors whose structures were also not permanent. The other three zones were housed in permanent structures built by the city council connected with water and electricity. The street vendors at the University of Botswana could have, as a result, had their food more exposed to dust in the streets and also had no access to facilities that could keep the food at a controlled temperature. Due to the ubiquitous presence of *L. monocytogenes,* the presence of the strains in the samples collected can be

represented by a large variety of animal and vegetable food products (Mead *et al.*, 1999). These factors could have led to enhanced contamination of their food by *L. monocytogenes*.

Antimicrobial susceptibility testing revealed complete sensitivity to penicillins (penicillin G and methicillin) and novobiocin. Notably, the highest resistance recorded was for tetracycline with chloramphenicol, streptomycin and erythromycin recording 10% each (Table 3). The results presented in the present report are not in concordance with a previous study (Morobe *et al.*, 2009) and may represent a periodic shift in antibiotic resistance dynamics of the pathogen. Various reports exist on the isolation of tetracycline and streptomycin resistant *L. monocytogenes* from food and other sources (Zhang *et al.*, 2007; Lyon *et al.*, 2008; Yang *et al.*, 2008). Resistance to tetracycline especially remains the most worrisome since the first report of antibiotic resistant strains of *L. monocytogenes* (Kovacevic *et al.*, 2013). Fluoroquinolones such as tetracycline are members of the quinolone group of antibiotics licensed to treat diseases in humans and animals in Botswana, and an increase in their resistance in these bacteria is therefore of significant concern (Kilonzo-Nthenge *et al.*, 2008). Importantly, 33.3% of *L. monocytogenes* were multi-drug resistant, including 8 isolates that were resistant to more than four antimicrobials. Our results are consistent with previous reports (Zhang *et al.*, 2007; Safdar and Armstrong, 2003) which show a continuing pattern of *L. monocytogenes* isolates that are multi-drug resistant. Thus present study reports on the occurrence of antibiotic resistant *L. monocytogenes* in ready-to-eat foods sold by street vendors in Botswana, which may be of potential public health risk to consumers. To reduce the risk of food-borne listeriosis, appropriate sanitation practices such as hazard analysis critical control point programs in street food vending are required. These programs can minimize the contamination of food products from original sources, during processing and at the point of sale.

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