



Prevalence and antibiotic susceptibility of *Listeria monocytogenes* in raw milk from cattle herds within Sokoto Metropolis, Nigeria

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Abstract

One hundred and ninety two raw milk samples were collected from lactating cows identified in Fulani herds and small scale dairy farms within Sokoto metropolis in order to investigate the presence and determine the antibiotic susceptibility of *Listeria monocytogenes* in the milk. Selective culture and identification method was employed for the bacterial isolation and Kirby-Bauer technique was used for the antibiotic susceptibility test. Seventy six samples (39.58%) were positive for *Listeria* species, which upon biochemical characterization 39(51.3%) were *Listeria innocua*, 14(18.4%) *Listeria ivanovii*, 17(22.4%) *Listeria monocytogenes*, 4(5.3%) *Listeria welshimeri* and 2(2.6%) *Listeria seeligeri*. Antibiotic susceptibility test of the isolates revealed high resistance to ampicillin (100%), and streptomycin (80%), followed by ampiclox (70%), tetracycline (30%), then gentamycin (20%) while, there was no resistance to ciprofloxacin and chloranphenicol. The findings of this study necessitate the need for extension personnel to educate the Fulani herdsmen, milk handlers and other livestock producers on the significance of hygiene especially during milking and the effect of indiscriminate use of drugs particularly antibiotics. There is also need for the agencies concerned such as the National Agency for Food and Drugs Administration and Control (NAFDAC) to regulate the sales and use of both human and veterinary drugs by drug hawkers and other non-professional veterinary practitioners.

Keywords: antibiotic susceptibility, *Listeria monocytogenes*, prevalence, raw milk.

Introduction

Listeria monocytogenes is a Gram-positive, facultative anaerobic, non-spore-forming, rod-shaped bacterium that belongs to the genus *Listeria* and family *Listeriaceae* (OIE, 2004). Other members of the genus include *Listeria ivanovii*, *Listeria innocua*, *Listeria welshimeri*, *Listeria seeligeri* and *Listeria grayi*. The organism is ubiquitous in nature often found in animal products such as raw milk and raw meat (Johansson *et al.*, 1999) due to unsanitary practices during milking and slaughtering (Schchat *et al.*, 1991). The organism causes infection (listeriosis) in man and in different animal species such as cattle, sheep and goats (Mathakiya *et al.*, 2011). Human infection is more devastating in immunosuppressed individuals, pregnant women, infants, geriatrics, HIV-AIDS patients, etc (Acha and Szyfres, 2003).

Pregnant women may experience abortion, stillbirth, premature birth or septicemia in the newborn (Acha and Szyfres, 2003). The elderly and infants suffer from meningitis, meningoencephalitis or, less frequently, septicaemia (Acha and Szyfres, 2003). While the immunocompetent individuals develop rashes as a result of direct contact with contaminated tissues or food items; or suffers from a syndrome called febrile gastroenteritis (Salamina, 1996). Listeriosis in animals present with clinical syndromes of abortion or neonatal septicemia, encephalitis localized to the brain stem or spinal cord in adult animals (Blood *et al.*, 2000). This study was designed to investigate the presence and determine the antibiotic susceptibility pattern of *Listeria monocytogenes*.

Materials and methods

Study area

Sokoto is the capital of Sokoto State, located in the North Western part of Nigeria. With a land area of approximately 56,000 square kilometers, Sokoto is located between longitude 5° 15" East and latitude 13° 05" North (Anon, 2012). The state is bordered to the North by Niger Republic, to the East by Zamfara State and to the South and West by and Kebbi State (Anon, 2012). Sokoto metropolis is mainly made up of Sokoto north and Sokoto south local government areas, however, some parts of Dange-shuni and Wammako local government areas are found within the metropolis. Sokoto is located in the sudan savannah vegetation belt with sandy soil and a humidity of below 40% year round except during the rainy season when it rises to 60% (Anon, 2012). The two dominant seasons are the wet and dry seasons. Harmattan, which is usually dry, cold and dusty, is experienced between November and February. The mean annual rainfall ranges between 500mm and 1,300mm (Anon, 2012).

According to the provisional figures of the 2006 National Population Census, Sokoto has population of 427,760 people principally made up of two major tribes, namely; Hausa and Fulani. The state is blessed with livestock resources and ranked second in livestock population in Nigeria (NPC, 2006).

Study design and sample collection

A cross-sectional study was conducted within Sokoto metropolis from January 2010 to October 2010 and

a total of 192 milk samples were collected from lactating cows in farms and Fulani herds within the metropolis. While milking, a minimum of 30mls was collected directly into a sterile bottle from each lactating animal identified and the samples were conveyed in an ice-packed container to the Public Health Laboratory of the Usmanu Danfodiyo University, Sokoto.

Culture and identification

Using culture and identification method as recommended by the International Organization for Standardization (ISO 11290-1, 1996), 25mls of each sample was enriched using 225mls of ONE Broth-Listeria (Oxoid, Hampshire United Kingdom) and incubated aerobically at 30°C for 48 hours. The broth-enriched sample was inoculated onto Oxoid Brilliance *Listeria* selective differential media (Oxoid, Hampshire United Kingdom) and incubated aerobically at 37°C for 24 – 48 hours. Blue-green colonies on the media that expressed gram positive coccobacilli on gram staining were presumed to be *Listeria* species.

Biochemical Differentiation

Biochemical characterization as recommended in OIE 2008 terrestrial manual was conducted using catalase, xylose, rhamnose and CAMP tests (Christie, Atkins, and Munch-Peterson test) in order to differentiate the various *Listeria* species as shown in table 1.

Table 1: Characterization of *Listeria* species

Species	CAMP-test	Rhamnose	Xylose	Gram stain
<i>L. monocytogenes</i>	+	+	-	+
<i>L. innocua</i>	-	V	-	+
<i>L. ivanovii</i>	-	-	+	+
<i>L. seeligeri</i>	W	-	+	+
<i>L. welshimeri</i>	-	V	+	+
<i>L. grayi</i>	-	V	-	+

Source: OIE Terrestrial Manual, 2008

W: weak haemolysis

V: variable

+: positive reactions

-: no reaction

Antibiotic Susceptibility Tests

Antibiotic susceptibility test using the Kirby-Bauer Disc Diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI, 2006a and 2006b) with *Staphylococcus aureus* ATCC 29213 as control strain was conducted using the commonly used antibiotics in the study area which comprises of ampicillin (25µg), streptomycin (30µg) ampiclox (30µg), gentamycin (10µg), ciprofloxacin

(10µg), chloranphenicol (30µg) and tetracycline (10µg).

Results

Out of the 192 samples collected, 76(39.58%) were positive for *Listeria* species, which upon biochemical characterization 39(51.3%) were *Listeria innocua*, 14(18.4%) *Listeria ivanovii*, 17(22.4%) *Listeria monocytogenes*, 4(5.3%) *Listeria welshimeri* and 2(2.6%) *Listeria seeligeri*. The most common isolates

were *Listeria innocua* while the least isolates were *Listeria seeligeri*. The antibiotic susceptibility test for *Listeria monocytogenes* and other *Listeria* species showed high resistance to ampicillin (100%), and streptomycin (80%), followed by ampiclox (70%), tetracycline (30%), then gentamycin (20%). No

resistance was seen to ciprofloxacin and chloranphenicol. However, multi-drug resistance (MDR) was observed, 20% of the isolates were resistant to two antibiotics and 80% were resistant to more than two antibiotics. In addition, no isolate was seen resistant to only one antibiotic (Table 2).

Table 2: Antibiotic resistance of *Listeria* species isolates from raw milk.

Antibiotics	<i>Listeria species</i> (n=40)	<i>Listeria Monocytogenes</i> (n=8)	<i>Listeria innocua</i> (n=16)	<i>Listeria ivanovii</i> (n=8)	<i>Listeria seeligeri</i> (n=4)	<i>Listeria welshimeri</i> (n=4)
Ampicillin	40(100%)	8(100%)	16(100%)	8(100%)	4(100%)	4(100%)
Gentamycin	8(20.0%)	4(10.0%)	4(10.0%)	0(00.0%)	0(00.0%)	0(00.0%)
Ampiclox	28(70.0%)	8(20.0%)	8(20.0%)	8(20.0%)	0(00.0%)	4(10.0%)
Ciprofloxacin	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)
Streptomycin	32(80.0%)	8(20.0%)	8(20.0%)	8(20.0%)	4(10.0%)	4(10.0%)
Chloranphenicol	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)
Tetracycline	12(30.0%)	0(00.0%)	12(30.0%)	0(00.0%)	0(00.0%)	0(00.0%)
Resistance to one antibiotic	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)	0(00.0%)
Resistance to two antibiotics	8(20.0%)	0(00.0%)	4(10.0%)	0(00.0%)	4(10.0%)	0(00.0%)
Resistance to more than two antibiotics	32(80.0%)	8(20.0%)	12(30.0%)	8(20.0%)	0(00.0%)	4(10.0%)

Discussion

This study has shown the presence of *Listeria monocytogenes* in raw milk on sale within Sokoto metropolis with a prevalence of 22.4%, higher than the findings of Hamdi *et al.*, (2006), who reported a prevalence of 7.7% in Algeria. The high prevalence recorded in this study could be attributed to the unhygienic manner in which milking is being conducted in the study area. Although Parenteral administration of [penicillin](#) or [ampicillin](#) is recommended in the treatment of listeriosis with [trimethoprim-sulfamethoxazole](#) considered the drug of choice in patients allergic to penicillin (Temple and Nahata, 2000), the antibiotic susceptibility test of the isolates showed that *Listeria monocytogenes* is susceptible to ciprofloxacin, chloranphenicol and tetracycline but resistant to ampicillin, streptomycin, ampiclox and gentamycin. This resistance could be attributed to the irrational use of the antibiotics on cattle by the producers or veterinary quacks in the study area, and the finding is similar to that of Nwachukwu *et al.* (2009) in Abia State, who reported the resistance of *Listeria monocytogenes* to ampicillin and its susceptibility to chloranphenicol.

In conclusion, the study has revealed the presence of *Listeria monocytogenes* in raw milk produced within Sokoto metropolis and has indicated the high tendency of listeriosis, which is a disease of public health concern, among the human populace and animals. The study has also showed that ciprofloxacin, chloranphenicol and tetracycline are among the drugs of choice in the treatment of listeriosis. Therefore, there is the need for extension personnel to educate the Fulani herdsman and other milk handlers on the significance of hygiene especially during milking. The agencies concerned such as the National Agency for Food and Drugs Administration and Control (NAFDAC) should regulate the sales and use of both human and veterinary drugs by drug hawkers and other non-professional veterinary practitioners.

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