Incidence and antibiotic susceptibility pattern of *Listeria monocytogenes* isolates from milk of West African Dwarf and Red Sokoto breeds of goat from Southwestern Nigeria

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Abstract: Listeriosis is an emerging zoonosis requiring continuous surveillance in order to prevent outbreaks in humans. The incidence of *Listeria monocytogenes* was evaluated from milk samples obtained from mastitic West African dwarf and Red sokoto breed of goats. A total of sixty samples was evaluated. An incidence rate of 12 (20%) was observed for *Listeria monocytogenes.* Antibiotic sensitivity of the 12 isolates against eight different antibiotics using the Disc Diffusion Method showed that the isolates were most susceptible to Gentamycin with the exception of strains 14B and 53A which were resistant to all the antibiotics used, the human strains used as control were only susceptible to Gentamycin and share a strong positive correlation at $P<0.01$ with strains 41A and 43A, however complete resistance to all the isolates was observed with Augmentin and Cloxacillin. The effectiveness of the antibiotics was in this order; Gentamycin > Chloramphenicol > Erythromycin > Streptomycin > Tetracycline > Cotrimoxazole > Augmentin >Cloxacillin. Hence, a continued surveillance of emerging antimicrobial resistance of this pathogen is very important, considering the fact that the samples evaluated were obtained from goat milk which presents several public health implications on humans.
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Introduction

Listeria monocytogenes, is a Gram-positive, rod shaped bacterium, which has been reported to be pathogenic for humans and animals (Fleming et al., 1985; Linnan et al., 1988). It is widely disseminated in the environment: soil, sewage, raw and decaying vegetables (Seeliger and Jones, 1986) and has recently been implicated in several food-borne epidemic listeriosis (Aureli et al., 2000). *L. monocytogenes* principally causes meningitides, encephalitis, abortion; in adult animals, septicaemia; in neonates (Nieman and Lorber, 1980; Schuchat et al., 1991), ophthalmitis which is associated with bacterial contamination of the cornea from the feed source. Some lactating ruminants may also have clinical mastitis associated with listeriosis, only a few cases of listeria mastitis have been reported in the literature (Gitter et al., 1980; Sharp, 1987). These observations indicate that either the organism does not readily invade the udder or cases of listerial mastitis are not being detected in routine laboratory examination of mastitic milk samples. However, when it occurs, the animal may have prolonged shedding of the bacteria in the milk.

Application of faeces or dung slurries of infected (or carrier) animals onto agricultural land as manure can serve as source of the organism (Chukwu et al., 2006; Nicholas et al., 2000). It could be transmitted to healthy animals while browsing and humans through contaminated vegetables or fruits (Schlech et al., 1983; Chukwu et al., 2004). Its virulence potential has been long established in diverse studies (Gellin and Broome, 1989).

The West African Dwarf (WAD) goat is widely distributed across the rainforest belt of Southern Nigeria. They are short-legged and small-bodied animals, weighing between 22 and 26kg. They also present variable coat colours, ranging from black, brown, gray, red and white, and sometimes combinations of these in a variety of patterns (Mourad *et al.,* 2000). While the Red sokoto (Maradi) breed is found in the Northern parts of the country; among others like the long-legged Sahel goats (Borno White or Kyalla), a relatively small sized goat (60 cm height and weight of 27 kg), prominent forehead; mucous membrane is black; both sexes have short to medium horns; known for the good quality skin (Wilson, 1991), reputed for high quality leather; used in the leather industry both locally and internationally (Akpa *et al.,* 1998).

Antibiotic resistance is a global public health problem. Although all countries are affected, the extent of the problem in the developing nations is certainly much more widespread and most of the data about the resistant bacterial strains are almost unknown (R*ichet* et al., 2001).

Although antibiotic resistance is a natural expression of evolution and bacterial genetics, certain

factors are thought to contribute immensely to enhance the expression and spreading of this bacterial inherent potentiality.

Antimicrobial resistance is a zoonotic health resulted threat. As in humans, the use of antimicrobial agents in animals resulted in the emergence and spread of resistant bacteria. Resistant bacteria of animals may be passed to humans via the food chain or direct animal contact, and may result in resistant infections. Increasing prevalence of resistance to antimicrobial agents such as fluoroquinolones and third-generation cephalosporins, which are important for the treatment of infections caused by enteric pathogens, has significant public health implications. Antibiotic use whether for treatment or prophylaxis, or as performance enhancers will result in antibiotic resistant micro-organisms, not only among pathogens, but also among bacteria of the endogenous microflora of animals (Van Den Bogaard and Stobberingh, 1999).

Antibiotic resistance and inefficient empirical treatment of *Listeria* infections could be responsible for this increased mortality (Charpentier and Courvalin, 1999). Since the first multi-resistant *Listeria monocytogenes* strain was observed in France (Poyart-Salmeron, et al., 1990), different antibiotic resistance patterns in environmental, food, and clinical sources have been reported (Walsh, et al., 2001; Paciorek, 2004; Hansen, et al.,2005).

The purpose of this study was to determine the presence of *Listeria* spp. in milk drawn from goats and to determine the sensitivities of the *L. monocytogenes* isolates to commonly used antimicrobial agents.

Materials and methods

Collection of Samples:

A total of sixty goat milk samples were collected at random (from West African dwarf and Red sokoto breeds of goats). The samples were collected using sterile universal bottles, and transported on ice to the laboratory for bacteriological analysis.

Isolation and Identification Procedures:

The culture method was on the basis of international standard (ISO 11290-1-1997) as previously described by Narang, 2004. One millilitre (1.0ml) of each sample, measured using sterile pipettes were serially diluted in 0.1% peptone water to 10-3 dilution level, 0.1ml of the this dilution was inoculated onto Listeria Selective Agar supplemented with modified oxford antibiotic supplement (acriflavine, nalidixic acid and cycloheximide) (Becton, Dickinson and company) plates and were incubated for 48hrs at $37^{o}C\pm 2^{o}C$ under microaerophilic conditions. Suspected listeria colonies were counted using a digital colony counter, *L. monocytogenes* isolates were identified, using specific antisera. Isolates showing coagulation reactions with a drop of *L. monocytogenes* polyserotypic antiserum (difco bd, USA) was reported as positive.

Antibiotic sensitivity test

The antibiotic sensitivity of the isolates was determined by the disk diffusion method of CLSI(2005) on Mueller- Hilton agar using the following antibiotics (Difco,USA), Augmentin(Aug) (30 µg), Chloramphenicol(Chl) (10 µg), Cloxacillin(Cxc) (5 µg), Cotrimoxazole(Cot) (25 µg), Erythromycin(Ery) (5 µg), Gentamycin(Gen) (10 µg), Streptomycin(Strep) (10 µg) and Tetracycline(Tet) (10 µg). 0.1 ml each of over- night broth culture of the isolates was inoculated on Mueller- Hilton agar plates and allowed to dry before the disk was inserted and then incubated at 30-37^{o}C for 18-24 hrs, two *Listeria* strains from human was also tested, for comparison in sensitivity to the antibiotics. The diameter of the zone of clearance (including the diameter of the disk) was measured to the nearest whole millimetre and interpreted on the basis of CLSI guideline (CLSI, 2005).

Results

The result of isolation and identification of isolates based on cultural and morphological appearances reveal that, from the sixty milk samples collected, 29(48.33%) samples were identified to contain *L. monocytogenes* while 12 (41.38%) out of the 29 reacted positively to the specific Listeria antiserum used. Hence bringing about a prevalence rate of 20%. Table(1) . The antibiotics sensitivity pattern of the 12 isolates to 8 antibiotics are presented in Table (2). A hundred percent resistant was observed for Augmentin and cloxacillin, two of the isolates (14B and 53A) showed multi- resistance to all the antibiotics tested. The human strains (Ctrl 2and3) that served as control were only sensitive to Gentamycin. Some of the isolates exhibited bacteriostatic activity to the antibiotics, showing clear zones of inhibition and spotted growth. While the other isolates showed resistance at various levels to the different antibiotics as follows, Chl (29.17%), Cot(91.67%), Ery (29.17%), Gen (16.67%), Strep (33.33%) and Tet (33.33%). Hence, Gen is the most sensitive and effective against this strains of *Listeria monocytogenes*, while Aug and Cxc should not be used. Also, a strong positive correlation existed between the antibiotics with the exception of Aug and Cxc at $P<0.01$ level. The correlation between the strains;1A, 29Ae, 32Cp, 36Cp, 50Ae and 58B is strong and positive, no correlation existed between 14B and 53A strains with the other strains, while a weak positive correlation occurred between stains 13Ae, 38A, 41Ae and 43A at $P<0.01$ level. The relationship between the isolates and human strains used as control strains revealed that; a strong positive relationship existed between the

control strains and strains 41Ae and 43A (0.85 and 1.0 respectively), no correlation exist between the human strains and strains 14B and 53A, while a weak correlation occurred between the other strains (1A, 13Ae, 29Ae, 32Cp, 36Cp,38A, 50Ae and 58B) and the human strains.

Table 1. Occurrence of *L. monocytogenes* in the milk samples studied

Strains	Number(n=60)	percentage%
L. spp	60.00	100.00
L. monocytogenes(doubtful)	29.00	48.33
*L. monocytogenes (*positive to antiserum)	12.00	20.00

Table 2. Percentage antibiotics resistance of isolates of *L. monocytogenes*

Antibiotics	Number (*n=24*)	Percentage resistance(%)
Augmentin	24	100
Chloramphenicol	7	29.17
Cloxacillin	24	100
Cotrimoxazole	22	91.67
Erythromycin	7	29.17
Gentamycin	4	16.67
Streptomycin	8	33.33
Tetracycline	8	33.33

Table 3: ANTIBIOTICS SENSITIVITY PROFILE

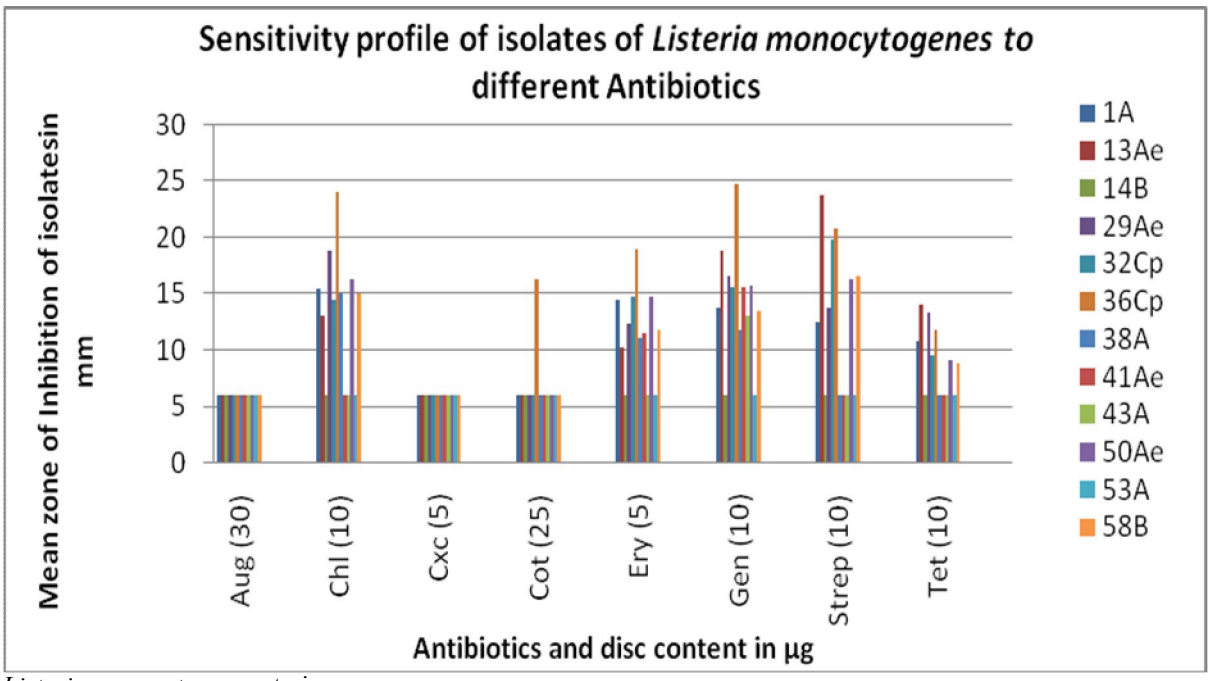
S/N	Strains	Cot	Chl	Cxc	Ery	Gen	Aug	Strep	Tet
1	1A	R	*	R	*	*	R	*	*
2	13Ae	R	✔	R	✔	*	R	*	*
3	14B	R	R	R	R	R	R	R	R
4	29Ae	R	✔	R	*	✔	R	✔	✔
5	32Cp	R	✔	R	✔	✔	R	✔	✔
6	36Cp	✔	✔	R	✔	✔	R	✔	✔
7	38A	R	✔	R	✔	✔	R	R	R
8	41Ae	R	*	R	✔	*	R	*	*
9	43A	R	R	R	R	✔	R	R	R
10	50Ae	R	✔	R	✔	✔	R	✔	✔
11	53A	R	R	R	R	R	R	R	R
12	58B	R	✔	R	✔	✔	R	✔	✔
13	Ctrl 2	R	R	R	R	✔	R	R	R
14	Ctrl3	R	R	R	R	✔	R	R	R

Keys: R- resistant, *-intermediate, ✔ -very susceptible

Table 4. Interpretation standards for disc diffusion susceptibility testing/results

Antibiotics and disc content (µg)	Interpretation (*N=24*)			Diameter zones of inhibition (in mm) of *L. monocytogenes* isolate (given by mean± standard error of mean)											
	Resistant (n)	Intermediate (n)	Sensitive (n)	1A	13Ae	14B	29Ae	32Cp	36Cp	38A	41Ae	43A	50Ae	53A	
Aug (30)	24	-	-	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000
Chl (10)	7	3	14	15.45 ±5.514	13.00 ±1.472	5.90 ±0.000	18.75 ±0.947	14.50 ±1.041	24.00 ±0.408	15.00 ±0.408	5.90 ±0.000	5.90 ±0.000	16.25 ±1.031	5.90 ±0.000	15.00 ±0.408
Cxc (5)	24	-	-	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000
Cot (25)	22	-	2	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	16.25 ±0.629	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000
Ery (5)	11	3	14	14.45 ±4.953	10.25 ±0.853	5.90 ±0.000	12.25 ±0.629	14.75 ±1.031	19.00 ±1.000	11.00 ±1.000	11.50 ±0.866	5.90 ±0.000	14.75 ±2.057	5.90 ±0.000	11.75 ±0.250
Gen (10)	4	6	14	13.75 ±2.496	18.75 ±0.479	5.90 ±0.000	16.50 ±0.957	15.50 ±0.646	24.75 ±0.629	11.75 ±1.182	15.50 ±0.289	13.00 ±0.408	15.75 ±0.750	5.90 ±0.000	13.50 ±0.500
Strep (10)	8	6	10	12.50 ±0.500	23.75 ±0.479	5.90 ±0.000	13.75 ±0.479	19.75 ±1.548	20.75 ±0.750	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	16.25 ±1.250	5.90 ±0.000	16.50 ±1.709
Tet (10)	8	5	11	10.75 ±0.750	14.00 ±0.408	5.90 ±0.000	13.25 ±0.250	9.50 ±0.500	11.75 ±0.854	5.90 ±0.000	5.90 ±0.000	5.90 ±0.000	9.00 ±0.577	5.90 ±0.000	8.75 ±0.479

Chart 1



Listeria monocytogenes strains =
(1A, 13Ae,14B,29Ae,32Cp,36Cp,38A,41Ae,43A,50Ae,53Ae,58B)

Discussion

The incidence rate of 20% of *Listeria monocytogenes* observed in our study is very low compared to levels observed in water samples from anthropogenic lakes by (Nwachukwu, et al., 2010), however similar prevalence rate were recorded by Salihu et al., (2008) in smoked fish and values as low as (18 (4.7%) out of 380 samples)were obtained in five categories of foods: meat and meat products, dairy and dairy products, fresh vegetables, fresh seafood, and ready-to-eat food (Stonsaovapak and Boonyaratanakornkit, 2010). The low incidence could also explain the fact that several organisms aside *Listeria monocytogenes* can cause mastitis in goats. However, it is still of public health importance as these goat milk could be taken raw there by putting the public at risk of infection with listeriosis.

The complete resistance shown by Aug and Cxc (100%) indicates that, these new and widely used drugs are ineffective in the treatment of Listeriosis, this is similar to reports from a study in Ado-Ekiti where environmental isolates were resistant to Aug and Cxc (David and Odeyemi, 2007), also, the resistance to antibiotics can be due to selective antibiotic pressure (Hanchung et al., 2004) or intergons and other insertion elements (Didier et al., 2000).

Multi-drug resistance shown by 14B and 53A strains could be attributed to similarities in the strains. This is also capable of complicating the management of listeriosis as the plasmid regulated resistance will usually be transferred to other organisms co-operating with *Listeria monocytogenes* to establish diseases (Brooks et al., 2001). The emergence of antibiotic resistant isolates from raw milk obtained from goats is of medical and public health importance because of the ability of the organisms to interact with man, either through contact or consumption (Hanchun et al., 2004), thus initiating listeriosis ,which will defy almost all the antibiotic chemotherapy.

The susceptibility of the human strains used in this study to Gentamycin alone when compared to other strains reveals a wide disparity in the genetic characteristics of the strains isolated and the human strains (control strains). However, the strong correlation that occurred between the control strains and 41A and 43A isolates depicts a close genetic similarity in the strains.

All the isolates were susceptible to Gen (% resistance of 16.67) except for strains 14B and 53A, hence Gen could be regarded as the drug of choice in this study followed closely by Chl and Ery (29.17% resistance) then Strep and Tet (33.33% resistance). This strongly supports earlier report by Schald (1983)

that *L. monocytogenes* isolates are susceptible to Chloramphenicol and Gentamycin, similar trends of antibiotic resistance were also observed by Khachatourians (1998). Although Chloramphenicol is no longer in use in Nigeria Erythromycin could therefore be used in place. It is also obvious from the results that majority of the isolates (1A, 29Ae, 32Cp, 36Cp, 50Ae and 58B) share similar characteristics by the strong positive correlation that existed between them. Notwithstanding strains 14B and 53A differs from all the other isolates, thus, further studies is needed to elucidate the characteristics responsible for it multi-resistance, because of its public health importance. Therefore, findings from our study follows trends observed from environmental isolates of *L. monocytogenes* from; Ado-Ekiti (David and Odeyemi, 2007) and anthropogenic lakes in Lopkpa-Ukwu, in Abia state, Nigeria (Nwachukwu, et al., 2010*)*.

It can be concluded that, *Listeria monocytogenes* is slowly becoming antibiotic resistant with the emergence of strains showing multi- resistance. Hence, a continued surveillance of emerging antimicrobial resistance of this pathogen is very important: Considering the fact that the study involved raw goat milk which is readily available to the public for direct consumption without heat treatment, the risk of infection with human listeriosis, it is therefore important to effectively heat treat milk to prevent outbreak of this emerging zoonosis in Nigeria. The data from this study therefore, serves as a baseline for the improvement of studies on the antibiotics resistant strains of *L. monocytogenes* strains isolated from goat milk and for further epidemiological and public health surveillance.

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