
Antimicrobial Susceptibility of *Listeria* Species from Abattoir Effluent in Port Harcourt, Nigeria.

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Abstract

Untreated abattoir effluent constitutes a reservoir for the spread of important pathogens including *Listeria monocytogenes*, the etiologic agent of listeriosis. This study investigated the presence and antimicrobial resistance profile of *Listeria* species from abattoir effluent in some Port Harcourt communities using standard conventional and molecular methods. *Listeria* were detected in 26% of the samples examined. The resulting isolates were identified as *L. grayi* (53.85%), *L. welshimeri* (38.46%) and *L. innocua* (7.69%). The *Listeria* isolates showed varying resistance to cotrimoxazole (44.2%), chloramphenicol (34.6%), tetracycline (46.2%), streptomycin (75%), augmentin (94.2%), gentamycin (19.2%), erythromycin (34.6%) and cloxacillin (100%). All three disinfectants were not effective against *Listeria* species at concentrations of 12% and 25%. At 50% and 100% disinfectant concentration, dettol was the most effective followed by JIK and lastly, izal. The presence of *Listeria* species in abattoir effluent and levels of resistance to commonly used antibiotic and disinfectants portends danger to animals and human population hence the need for adequate treatment of effluent before discharged into the environment.

Keywords: Abattoir effluent; antibiotics; disinfectant; *Listeria* species.

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1. Introduction

Facilities for animal slaughtering activities referred to as Abattoirs, are most commonly located very close to natural water bodies like rivers, streams and lakes for the ease of discharge of the effluent which consist majorly of intestinal content, urine and blood of animals into them [1, 2]. The waste effluent discharged from abattoirs is most times led into water bodies without prior treatment; making the possible source of water for humans and other animals down the stream or lake to become polluted. Water for domestic and recreational activities has been incriminated in the transmission of pathogens, and it was opined that the source of contamination could be either sewage or infected animals [3, 4].

The genus *Listeria*, is a group of closely related Gram-positive, facultative intracellular anaerobic, non-spore forming, rod shaped, motile bacteria. Currently, it is widely accepted that the core phylogeny of *Listeria* consists of six (6) different species which are: *Listeria monocytogenes*, *L. innocua*, *L. seeligeri*, *L. welshimeri*, *L. grayii* and *L. ivanovii*. [5]. *Listeria monocytogenes* is the principal pathogen in humans and animals while *L. ivanovii* is a pathogen of animals only but is occasionally however implicated in human disease. The other four *Listeria* species are generally considered non-pathogenic [6, 7]. *Listeria monocytogenes*, the causative agent of listeriosis, is ubiquitous in the environment and has been recognized as animal pathogen since the 1920s but in the past two decades, it has been implicated in several outbreaks of food-borne illness in humans [8, 9]. Proliferation of *Listeria* is promoted by high humidity and nutrient waste in certain food production plants [10].

With bacteria having a remarkable ability to develop resistance to every antibiotic, it can be anticipated that even bacterial species such as *Listeria*, which were considered to be susceptible to ampicillin, aminoglycosides, tetracycline, macrolides, vancomycin, carbenicillin, cephaloridine, chloramphenicol, erythromycin, furazolidone, methicillin, neomycin, novobiocin, oleandomycin, ticarcillin, azlocillin and less susceptible to chlortetracycline, oxytetracycline, tetracycline, gentamicin, kanamycin, nitrofurantoin, penicillin G, streptomycin, will evolve towards multi- resistance [11, 12, 13, 14]. Since the isolation of the first multiresistant strain of *L. monocytogenes* in France in 1988 [15], *L. monocytogenes* strains resistant to one or more antibiotics have been recovered from food, the environment and sporadic cases of human listeriosis.

Disinfection is one of the basic components of any infection control program especially against nosocomial infections but the widespread use of disinfectant products has prompted some speculation on the development of microbial resistance since their activity are concentration-dependant [16, 17]. There is paucity of information in literature on the activity of disinfectants on *Listeria* species.

This study is therefore aimed at determining the presence and response of *Listeria* species from abattoir effluent in Port Harcourt to antibacterial agents.

2. Materials and Methods

2.1 Collection of samples

The effluent samples were collected from the drainage, just after the slaughter slab into sterile sample collection

bottles. One hundred abattoir effluent samples were examined for the presence of *Listeria* species. The samples were collected from five (5) cattle abattoirs located in Aluu, Choba, Alakahia, Emuoha and Rumuosi communities in the environs of the University of Port Harcourt, Nigeria. The samples were transported to the University of Port Harcourt Environmental Microbiology Laboratory immediately for analysis. The *Listeria monocytogenes* PCM 2191 serovar 01/2 which served as positive control was obtained from the Polish Collection of Microorganisms, Poland.

2.2 Isolation of *Listeria* species

The isolation of *Listeria* species from the effluent was carried out in accordance with the International Organization for Standardization (ISO) recommended procedure. [18]. Ten milliliters of each sample was aseptically transferred to 90ml of sterile half-strength Fraser *Listeria* enrichment broth and incubated at 35°C for 24h. One milliliter was transferred from the primary enrichment broth to 10ml of full-strength Fraser *Listeria* enrichment broth with *Listeria* selective enrichment supplement in duplicate test tubes and incubated at 37°C for 24 to 48h. From the culture obtained in Fraser broth showing evidence of darkening due to aesculin hydrolysis by *Listeria* spp., 0.1ml was transferred onto duplicate PALCAM plates. After spreading, plates were incubated at 37°C for 24- 48h. The plates were examined for the presence of characteristic colonies presumed to be *Listeria* spp- 2mm grayish-green colonies with a black sunken centre and a black halo on a cherry-red background, following aesculin hydrolysis and mannitol fermentation. Five typical colonies were selected randomly from a pair of PALCAM plates for confirmation and subsequent identification.

2.3. Confirmation and identification of *Listeria* species

Colonies suspected to be *Listeria* were transferred onto trypticase soy agar (Becton, Dickinson & Company, France) with 0.6% yeast extract (LAB M, UK) and incubated at 37°C for 18 to 24h, before being subjected to standard physiological and biochemical tests (Gram staining, catalase reaction, oxidase reaction, beta haemolysis on sheep blood agar and acid production from mannitol, rhamnose and xylose) and molecular identification involving the use of oligonucleotide primer described by Border *and his colleagues* [19] synthesized by Biomers.net GmbH, Germany (Table 1). DNA was extracted by the boiling method without Triton X-100 [20]. The reactions involving U1, LI1, LM1 and LM2 were carried out in a final volume of 25µl, containing 2.5 µl 10×PCR buffer, 1.5 µl MgCl₂, 0.5µl dNTP (deoxynucleoside triphosphate), 0.25 µl each of appropriate primer, 0.1 µl AmphiTaq DNA polymerase (All products of Solis BioDyne, UK), 1.5µl of appropriate DNA preparation and 18.4µl sterile distilled water. Amplification following an initial denaturation at 95°C for 3min was performed in 30 cycles, at 95°C for 30s, 50°C for 60s and 72°C for 60s in a thermo cycler (Mastercycler-Eppendorf, Vapo-product, Germany). A final extension was performed for 10min at 72°C. A 8µl aliquot of PCR product mixed with a loading dye (10mM, EDTA, 10% glycerol, 0.015% bromo phenol blue and 0.017% sodium dodecyl sulphate (SDS), made up to 100ml) were checked in an ethidium bromide stained 1.5% agarose (Fermentas, Life Science, Germany) and the gel visualised in a UV transilluminator (GenoSens 1500, Clinx Science Instruments Co.Ltd, China). Reaction mixture with the DNA of *L. monocytogenes* PCM 2191 serovar 01/2 template served as positive control while a reaction mixture without DNA template was incorporated as a negative control in each reaction.

Table 1: Sequences of oligonucleotide primer.

Primer	Derived from + or – strand	Sequence(5'-3')	Location on 16SrRNA
U1	+	CAGCMGCCGCGGTAATWC	519-536
LI1	-	CTCCATAAAGGTGACCCT	1457-1440
LM1	+	CCTAAGACGCCAATCGAA	
LM2	-	AAGCGCTTGCAACTGCTC	

M denotes A or C; W denotes A or T; R denotes A or G

2.4Antibiotics sensitivity testing

The identified isolates were subjected to antibiotics susceptibility testing, using the disk diffusion technique as described by Bauer-Kirby [21]. Antibiotics disk containing the following antibiotics: tetracycline (10µg), streptomycin (10µg), augmentin (30µg), gentamycin (10µg), erythromycin (5µg), cloxacillin (5µg), chloramphenicol (30 µg) and cotrimoxazole (10 µg) were employed (Abtek biological,). From an overnight culture in brain heart infusion broth, a 10⁸ cell/ml (0.5 MacFarland turbidity standards) bacterial culture was prepared in sterile saline, from which 0.1ml was inoculated onto Mueller Hinton agar. Thereafter, antibiotic discs were aseptically placed on the surface of the agar and plates incubated at 37⁰C for 24h. Zone of inhibition was measured in millimeter.

2.5.Disinfectant sensitivity testing

The sensitivity of the isolates to phenolic compounds (izal and dettol) and a hypochlorite (JIK) was determined, using the agar well diffusion method as described by Awodeleand his colleagues [22]. Different concentrations of the disinfectants (100%, 50%, 25% and 12%) were employed [22]. The zones of inhibitions were compared to that previously reported by Okesola and Olola [17].

3. Results and Discussion

The public health concern over the sources and nature of water for consumption, food preparation, irrigation and recreation activities in any locality is worldwide. This is due to the fact that water is known to be most potent vehicle of transmission of infectious diseases [23]. Abattoir effluent when discharged finds its way into natural water ways [2] and could therefore be a source of contamination of water for consumption and recreational activities [24]. *Listeria* species were detected in 26% of the samples examined. The highest occurrence was from Aluu (45%) followed by Alakahia (25%) while the lowest recorded was from Emuoha (5%) (Table 2). This finding is comparable to reports of 21.1% occurrence in slaughter houses in Parana State, Brazil, 22% occurrence in the feaces of cattle and 13% occurrence in ruminants in Maiduguri [24, 25, 26].

The resulting *Listeria* isolates were identified as *L. grayi*70 (53.85%), *L. welshimeri*50 (38.46%)and *L.*

*innocua*10 (7.69%). *Listeria monocytogenes*, the pathogenic species was not detected as no bands were obtained from the Primer LM1 and LM2 specific for *L. monocytogenes*. The isolates were however, confirmed to be *Listeria* by the U1 and LI1 primer combination for all *Listeria* species producing the expected 938base paired band (Plate 1). This may not be unconnected with its absence in raw beef and goat meat as previously reported [27, 28, 29, 30 31]. According to Carvalheira and his colleagues [32], the inhibition of *L. monocytogenes* by *L. innocua* is frequently related to the decrease in the growth rate of the species inhibited or to the inhibitory activity caused by bacteriocins produced by *L. innocua*. Hence, the presence of any *Listeria* species in a specific environment can indicate the presence of others, including *L.monocytogenes*, suggesting that *L. innocua* could be an excellent indicator for this pathogen.

Table 2: The occurrence of *Listeria* in the different abattoir

Abattoir sampled	Number of samples examined	Number of positive samples	Percentage occurrence
Aluu	20	9	45
Choba	20	5	25
Alakahia	20	7	35
Rumuosi	20	4	20
Emuoha	20	1	5
Total	100	26	

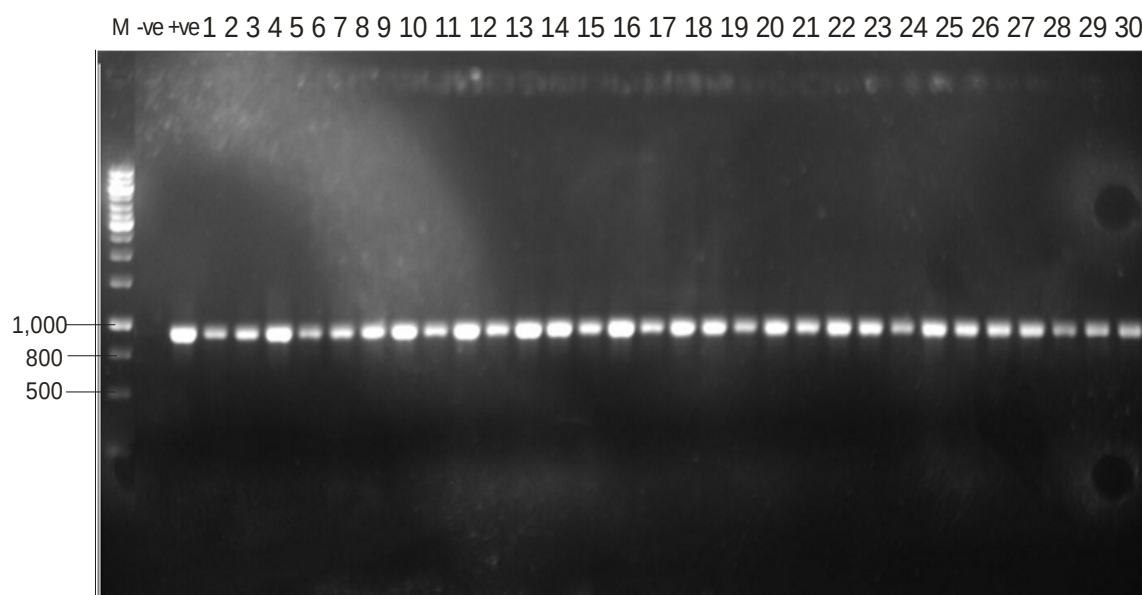


Plate 1: Gel electrophoresis of total genomic DNA from *L. monocytogenes* PCM 2191 serover01/2 strain and isolated *Listeria* species subjected to PCR using LI1 and U1 primer combination for all *Listeria* species. Lanes: M, molecular weight standard; -ve, Negative control; +ve, positive control (*L. monocytogenes* PCM 2191 serover 01/2 strain); 1-30 isolated *Listeria* species.

The results of the antibiotic sensitivity showed varying resistance of the *Listeria* species to cotrimoxazole

(44.2%), chloramphenicol (34.6%), tetracycline (46.2%), streptomycin (75%), augmentin (94.2%), gentamycin (19.2%), erythromycin (34.6%) and cloxacillin (100%). This observation is consistent with previous reports of multiple drug resistance [4, 33]. This result totally agrees with the findings of Adetunji and Isola [34] who reported that *Listeria monocytogenes* from retail meat tables in Ibadan municipal abattoir were highly sensitive to gentamycin and erythromycin. Adetunji and Isola [34] also reported that cloxacillin is the least sensitive drug. The finding of this study is not in agreement with the report that 100% *Listeria* isolates were sensitive to gentamicin [35, 36]. The occurrence of resistance strains is usually attributed to the acquisition of resistance plasmids from other microbial species in the digestive tract [37]. The mere fact that all the antibiotics recorded at least some degree of resistance shows that *Listeria* species are increasingly receiving antibiotic resistance genes which pose as threat to future combat of *Listeria* related infections in both humans and animals.

The disinfectant concentrations employed inhibited the growth of *Listeria* to varying degrees. (Figure 1). All three disinfectants were not effective against *Listeria* species at concentrations of 12% and 25%. Olorode and Okpokwasili [38] have reported that JIK at 10% concentration was not effective against *Salmonella* isolated from abattoirs in Port Harcourt. Okesola and Olola [17] reported the susceptibility of *Pseudomonas aeruginosa* to 10% izal and non-susceptibility to 10% JIK and dettol.

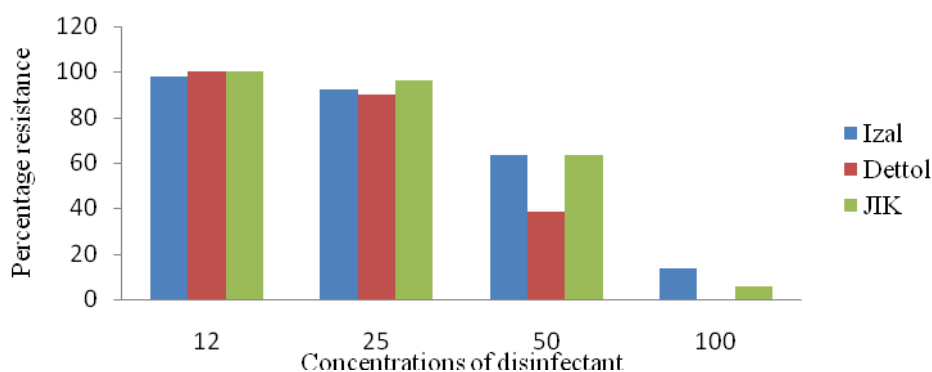


Figure 1: Resistance of *Listeria* to disinfectants

At 50% and 100% disinfectant concentration, dettol was the most effective followed by JIK and lastly, izal. This finding is comparable to report by Olorode and Okpokwasili [38] that 70% dettol was the most effective against *Salmonella* isolates and confirm the notion by Schönberg and Gerigk [10] that *Listeria* removal is almost impossible by disinfection.

4. Conclusion

The study has established the presence of *Listeria* species in abattoir effluent being discharged into bodies of water around the abattoir. The non-isolation of *L. monocytogenes* does not completely eliminate the possibility of contacting listeriosis when water contaminated with non-pathogenic *Listeria* species is consumed. The levels

of resistance to commonly used antibiotic and disinfectants portends danger to animals and human population, hence the need for adequate treatment of effluent before discharged into the environment.

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