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ASSESSMENT OF MILK PROCESSING FACILITIES FOR THE PRESENCE OF *LISTERIA* SPECIES AND *LISTERIA MONOCYTOGENES*

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Abstract
This study was designed to determine the occurrence of *Listeria* species and *Listeria monocytogenes* in three yoghurt production facilities in Kaduna metropolis, Nigeria. A total of 156 samples were collected from processing environment (floor drains biofilms, floors, walls and tables), processing equipment (fermentation tanks) and wastewaters. Fifty-four (54) samples were collected from each factory before and during production. *Listeria* species were assessed using *Listeria* selective agar while speciation of the isolates was done using Microgen *Listeria* ID kit. The *Listeria* count, before and during production, were done and expressed as $\log_{10}$mean ± SEM cfu/ml. Before production, the highest count was observed in factory A (5.11 ± 0.32) and lowest in factory C (3.60 ± 0.30). During production, *Listeria* count was highest in factory B (5.68 ± 0.53) and least in factory C (3.49 ± 0.12). No significant difference ($p > 0.05$) in the counts obtained before and during production in each of the three factories. *Listeria* species were detected in 6 (11.54%) of samples from factory A and 12 (23.08) were found in factory B. No *Listeria* was detected in samples from factory C. *Listeria monocytogenes* 4 (33.3%) was detected in samples from factory B and 8 (66.7%) of the *Listeria* species were *Listeria grayi*. In factory A, 4 (66.7%) of the *Listeria* species were *Listeria grayi* while 2 (33.3%) were *Listeria ivanovii*. The results of this study demonstrate possible risk of contamination of yoghurt produced by these factories owing to the occurrence of *Listeria* species and *Listeria monocytogenes* in wastewater from the production line.
Keywords: Biofilm, Contamination, Factory, *Listeria monocytogenes*, yoghurt.production

Introduction
Contamination of foods by *Listeria monocytogenes* has become an issue of global concern because of its public health implication (Eyles, 1995; WHO, 2002). *Listeria monocytogenes* is a bacterium that causes a disease called listeriosis. Mild symptoms in humans include diarrhoea, fever, headache and myalgia but in cases of invasive listeriosis, meningitis and septicaemia are commonest form of manifestation of the disease. Listeriosis in pregnant women can lead to abortion or stillbirth (Aygun and Pehlivanlar, 2006). Pregnant women, infants, immunocompromised individuals and the elderly are at greatest risk of listeriosis (Gillespie *et al.*, 2010). Evidence is accumulating that the organism is a food-borne pathogen, and dairy products have been implicated (El-Marnissi *et al.*, 2013). In dairy industry, *Listeria* can contaminate either directly or indirectly the products and the environment through contaminated raw milk and wastewater resulting in huge losses both in terms of public health and economic consequences.

Milk-borne disease outbreaks associated with *Listeria monocytogenes* have been reported in developed countries but limited information is available in most African countries (WHO, 2002). In Nigeria, like other African countries, HIV/AIDS has high prevalence; hence listeriosis may be a silent killer among the immunocompromised individuals who may depend on yoghurt for protein intake. Several studies have shown that the organism may survive in fermented milk for several weeks, especially if the milk was initially heavily contaminated and, and, if the product was stored under refrigeration (Lovett *et al.*, 1990; Zuniga *et al.*, 1995; Mugampoza *et al.*, 2011). This study

was therefore carried out to assess the occurrence of *Listeria* species and *Listeria monocytogenes* in yoghurt production lines in Kaduna metropolis, Nigeria.

Materials and Methods

***Sample Collection*:** A total of 156 samples were collected from processing environments (floor drains biofilms, floors, walls and tables), processing equipment (fermentation tanks) and wastewaters. Fifty-four (54) samples were collected from each factory before and during production. Floor drains biofilms, working surfaces, tanks, walls and tables in the three factories (A, B and C) were sampled by swabbing with 7.5cm x 7.5cm sterile gauze-pads pre-moistened in sterile 0.1% peptone water. This method is referred to as the sponge sampling technique. Wastewaters from floor drains were examined by collecting 25 ml of drain water.

The swabs were stored in 10 ml sterile tryptone soy broth and transported to the laboratory in the Department of Microbiology, Faculty of Science, Ahmadu Bello University, Zaria, Nigeria for processing. All samples were analysed within 24 – 48 hours after collection and stored at 4°C to improve recovery of *Listeria monocytogenes*.

Sample Processing

***Physico-chemical analyses*:** pH of all the wastewater samples collected was determined using hand held pH meter (Extech, Japan). Temperature of samples was also taken with a digital thermometer (HI 9154C, Singapore) during each sampling session and recorded accordingly.

Microbiological analyses: *Listeria* species were detected and enumerated according to the procedure described by Loncarevic *et al.* (1996). One millilitre (1 ml) of the samples preserved in 10 ml of tryptone soy broth was inoculated in 9 ml of sterile primary *Listeria* enrichment broth, shaken and incubated at 30°C for 24 hours in duplicate tunes. One millilitre (1 ml) of sample from the primary enrichment tube was transferred to 9 ml Fraser secondary enrichment broth. Blackening of the medium was presumptively indicative of the presence of *Listeria* species. In order to identify *Listeria monocytogenes*, presumptive colonies were sub-cultured on plates containing Oxford formulation medium. The resulting colonies were counted and recorded. Five colonies were then taken from each plate and transferred to tryptone soya agar with yeast extract and incubated at 35°C for 24 – 48 hours. Trypticase soya agar with 0.6% yeast extract slant was inoculated and incubated at 35°C for 24 hours and stored at 4°C for further use. Suspected colonies (black halos and sunken centres) from selective agar plates were confirmed by Gram staining, catalase, oxidase and motility tests. Microgen *Listeria* ID kit (Microgen Bioproducts, Surrey, UK) was used to identify different species of *Listeria* present.

Statistical Analyses: Data were analysed using Statistical Package for Social Sciences (SPSS) version 20 (SPSS Inc., U.S.A.). Bacterial counts were normalised by expressing as $\log_{10}$cfu/ml and summary statistics calculated for all continuous variables. Mean values from the three factories were compared using Duncan's Multiple Range Test (DMRT).

Results

There was no *Listeria* species detected in the samples collected from the floor and equipment in the three factories. On the other hand, floor drain biofilms and wastewaters analysed revealed the presence of *Listeria* species. Table 1 presents the average temperature and pH of wastewater sampled from the three factories. Comparison of the temperature and pH showed that factory A had the highest temperature of 31.18°C while factory B had the least (29.50°C); the perceived difference in temperature was not significant ($F_{df=2}$ = 0.507; p = 0.617). There was significant variation ($F_{df=2}$ = 4.414; p = 0.042) in the pH of wastewater obtained from the three factories with

factory A having the highest pH of 10.06 followed by factory C (8.18) and factory B with a near neutral pH (7.69) as the least. Bacterial counts of *Listeria* species in wastewater obtained from floor drains were taken before commencement of production and during production and the results were as presented in Table 2. In all the factories, there was no significant difference in bacterial counts of wastewater before and during production ($t = 1.412$, $p = 0.294$; $t = -2.097$, $p = 0.069$ and $t = 0.262$, $p = 0.818$ for factories A, B and C respectively).Bacterial counts obtained from wastewaters before production in the three factories were compared using analysis of variance and it was found that there was significant difference ($F_{df=2.0} = 5.497$, $p = 0.017$) in the counts observed with factory A having the highest count of *Listeria* species and factory C had the least. Similarly, counts obtained from wastewater during production showed that there was no significant difference ($F_{df=2.0} = 2.864$, $p = 0.093$) (Figure 1).

Distribution profile of *Listeria* species in the three factories presented in Table 3 showed that factory B had the highest occurrence of *Listeria* species 12 (23.1%) followed by factory A, 6 (11.6%) while no *Listeria* was detected in samples obtained from factory C. Biochemical characterisation and identification profile using Microgen identification kit showed that 4 (33.3%) of the 12 *Listeria* species detected from wastewater in factory B were *Listeria monocytogenes* and the 8 (66.7%) were *Listeria grayi*. Factory A on the other hand had no *Listeria monocytogenes* detected in its wastewater but 4 (66.7%) of the 6 *Listeria* species isolated were *Listeria grayi* while the remaining 2 (33.3%) were *Listeria ivanovii* (Table 3).

Table 1: Physico-chemical properties of wastewater collected before and during production

Factory	***Mean ± SEM***	
	Temperature (°C)	**pH**
A	31.18 ± 1.58	10.06 ± 0.77^{a}
B	29.50 ± 1.24	7.69 ± 0.40^{b}
C	29.73 ± 0.78	8.18 ± 0.46^{ab}
Statistics:	F = 0.507; p = 0.617	F = 4.414; p = 0.042*

Mean values with different superscript in the same column are significantly different. Mean values were separated using Duncan multiple range test.
* = Significant difference exists at $p \leq 0.05$. A = factory A; B = factory B and C = factory C.

Table 2: Comparison of bacterial counts obtained from samples collected before and during production

Factory	**No. sampled**	**Counts on LSA (log_{10}cfu/ml) Mean ± SEM**		**t**	**P**
		Before production	**During production**		
A	52	5.11 ± 0.32	4.41 ± 0.73	1.412	0.294
B	52	4.32 ± 0.21	5.68 ± 0.53	-2;097	0.069
C	52	3.60 ± 0.30	3.49 ± 0.12	0.262	0.818

LSA = *Listeria* selective agar; cfu/ml = coliform forming unit per ml; SEM standard error of mean; t = value of calculated t-test; p = level of significance; A = factory A; B = factory B and C = factory C.

Table 3: Occurrence of *Listeria* species in samples collected from processing lined of three factories

Factory	No. sampled	Number positive (%)			
		***Listeria* spp**	***L. monocytogenes***	***L. grayi***	***L. ivanovii***
A	52	6 (11.5)	0 (0.0)	4 (66.7)	2 (33.3)
B	52	12 (23.1)	4 (33.3)	8 (66.7)	0 (0.0)
C	52	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	156	18(11.5)	4(22.2)	12(66.7)	2(11.1)

A = factory A; B = factory B and C = factory C.

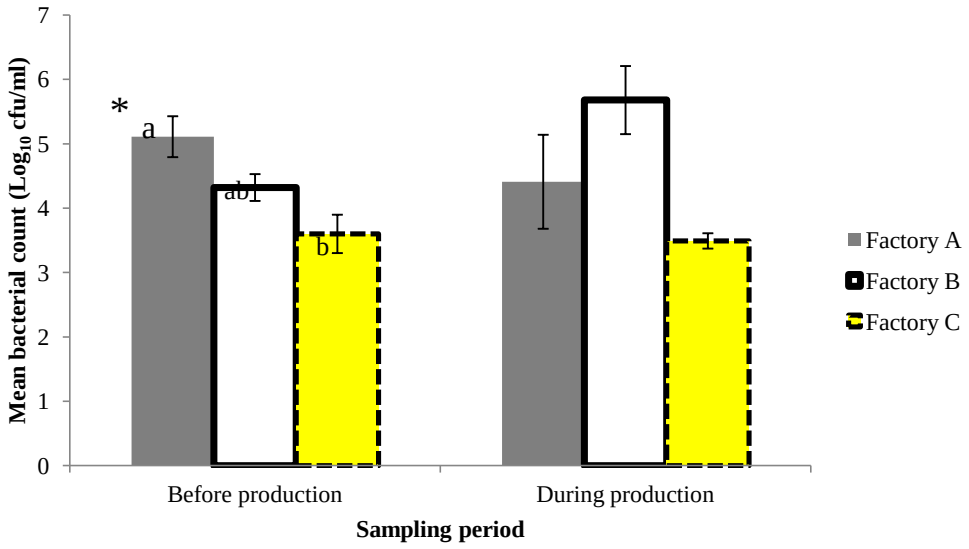

Figure 1: Comparison of counts of *Listeria* species obtained from the three factories before and after production. * = significant difference exists at $p \leq 0.05$ (Before production: $F = 5.497$, $p = 0.017$* and During production: $F = 2.864$, $p = 0.093$). Bars with different superscripts are significantly different.

Discussion

In the present study, a total of 156 samples of main drain biofilm and swabs were collected. All the swab samples showed no presence of *Listeria* species. The *Listeria* counts expressed as $\log_{10}$ cfu/ml obtained from factory A (5.11 ± 0.32), B (4.32 ± 0.21) and C (3.60 ± 0.30) before production were not significantly different ($p > 0.05$) from that obtained during production in factories A (4.41 ± 0.73), B (5.68 ± 0.53) and C (3.49 ± 0.12), this indicates that the presence of *Listeria* in the main drain does not change significantly production. The comparison of *Listeria* counts obtained from samples from the three factories before production showed that factory A had a significantly higher ($p < 0.05$) *Listeria* counts, followed by factory B while factory C had the least count. The perceived differences could be largely due to the difference in the hygienic and sanitary practices employed by the operators of the factories. This implies that factory C had the best

hygienic practice. Similar comparison of *Listeria* count obtained from samples collected during production revealed no significant difference ($p > 0.05$) between the three factories.

The mean temperature of samples obtained from the three yoghurt producing factories range from 29.50 – 31.18°C and pH of 7.69 – 10.06. These ranges of physicochemical parameters provide conducive growth condition for the growth of *Listeria* species given the fact that the active or base ingredient of yoghurt is milk which is a proteinous substance that serves as nutrient for utilisation by the bacteria that form biofilm in the drains and stainless steel pipe network of the production lines. Mosteller and Bishop (1993) reported that *Listeria monocytogenes* adhere to polymeric materials such as plastic and stainless steel pipe, especially at temperatures greater than 30°C and low pH (4 to 7). Quaternary ammonium compounds, hypochlorite and iodophores are common sanitizers used for cleaning many dairy utensils, working environment and equipment. A number of these compounds provide inadequate reduction in numbers of *Listeria* cells that adhere to milk biofilms (Mosteller and Bishop, 1993). Therefore, inadequate cleaning of the containers, production environment and equipment may lead to contamination of the subsequent batch of the product.

Of the total samples collected, 11.5% of the main drain samples were positive for *Listeria* species. This prevalence is comparable with the 11.9% (42/353) reported by Hosein *et al.* (2008) but in disagreement with the results reported by El-Sharef *et al.* (2006) and Arsalan and Ozdemir (2008). However, Beak *et al.* (2000) and El-Sharef *et al.* (2006) reported lower incidence rates of *Listeria monocytogenes* (4 and 6.2% respectively). The incidence of *Listeria grayi* in the present study is higher than 5.8% reported for *L. grayii* by Hosein *et al.* (2008).

Conclusion

The research showed that *Listeria* species were shown to be present in the main drains of the milk processing facilities. The results of this study revealed the potential risk of contamination of yoghurt produced by factory B where *Listeria monocytogenes* was detected. Extensive prevalence studies on the occurrence of *Listeria* species and *Listeria monocytogenes* among raw milk handlers and yoghurt processing factory workers is recommended in order to assess and elucidate the epidemiology of listeriosis in Kaduna metropolis.

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