

## Mini Review

### An overview of *Listeria* species in Nigeria

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#### Abstract

Despite the world-wide reports of outbreaks of food-borne listeriosis, the occurrence of *Listeria* is still not widely reported in Nigeria. This is possibly due to lack of a large cold storage food chain and the absence of a comprehensive surveillance system for food-borne pathogens. Searches carried out on major databases revealed that *Listeria* has been reported in humans, animals, environment and food in Nigeria. In Nigeria, the organism has been reported in pregnant women and neonates while ruminants dominate reports of occurrence in animals. In food especially fish, *L. monocytogenes* is reported more than any other *Listeria* species. The organism has been isolated from water bodies and soils from different environments in Nigeria. However, all reports on the occurrence of *Listeria* spp. were based on classical serotyping, biochemical tests and dark colouration of media due to hydrolysis of aesculin with no emerging pattern of infection or dominant molecular serotype. There is an opportunity to utilize the current polymerase chain reaction based molecular techniques to characterize *Listeria* spp. so that accurate information on existing *Listeria* strains and sources of infection can be established in all regions in Nigeria.

#### Keywords

Molecular typing  
Prevalence  
Food and environment  
Humans  
Animals

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#### Introduction

The genus *Listeria* is a Gram-positive, non-spore forming, rod-shaped bacteria of 0.4-0.5x0.5-2 µm in size with rounded ends and can also be coccoid at times, occurring singly or in short chains and not encapsulated (Holt *et al.*, 2000). *Listeria monocytogenes* is reported to be pathogenic in humans and animals and is the causative agent of listeriosis. It is also an agent of several food-borne disease outbreaks (Briandet *et al.*, 1999) and causes serious infection in the elderly, neonates, pregnant women and immune-compromised persons (Farber and Peterkin, 1991). The disease has a high fatality (20 to 30%) rate and infected persons may show signs of meningitis and septicaemia (Nakamura *et al.*, 2006). The organism is resistant to stress (Djordjevic *et al.*, 2002; O'Bryan *et al.*, 2009) and is widely distributed in the environment by virtue of its occurrence in water, soil and plant material (Welshimer, 1968; Rocourt *et al.*, 2003). *L. monocytogenes* is regarded largely as a bacterial pathogen of animals (Low and Donachie, 1997) and causes the affected animals to show uncoordinated posture and circular movements, abortion and/or septicaemia. In humans, the affected persons may show fatigue, headache, muscular and joint pain and gastro-enteritis at the onset of listeriosis (Liu and Busse, 2009).

The species *L. monocytogenes* is widely studied

in the developed world and is known as an entero-invasive gastrointestinal pathogen (Barbuddhe and Chakraborty, 2009). There are no strains of *L. monocytogenes* with unique properties that lead to persistence (Carpentier and Cerf, 2011) and there are mechanisms that can protect the organism when present in acidic juices, yogurt, salad dressings, mayonnaise, and modified CO<sub>2</sub> atmospheres (Smith *et al.*, 2013). Humans can become infected when contaminated food is ingested because the acidic stomach environment and its surface proteins (Bierne and Cossart, 2007) can help the organism to attach to the gut and multiply in the host's cell- cytosol (Pizarro-Cerdá and Cossart, 2009). Attachment of *Listeria* on stainless steel at temperatures below 25°C is aided by flagella (Vatanyoopaisarn *et al.*, 2000). The organism has recently been suggested as a model for understanding how an environmental bacterium switches to life within human cells (Xayarath and Freitag, 2012).

Listeriosis may be caused by unsanitary handling of food products in commercial food processing plants or in homes. To detect the organism and study strains associated with outbreaks, serological and genomic techniques are usually employed. *Listeria* can be subdivided into 15 serovars (Liu and Busse, 2009) and various sub-typing studies have shown that the serovars of *Listeria* can be divided into four evolutionary lineages (Wiedman *et al.*, 1997; Ward

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*et al.*, 2008; Orsi *et al.*, 2011). Some strains have had their whole genome sequenced (Glaser *et al.*, 2001; Chen *et al.*, 2011; Briers *et al.*, 2011) with many more in progress.

Despite an increasing rate of listeriosis reported in several European countries in recent years (Allerberger and Wagner, 2010), and other outbreaks in the United States (Cartwright *et al.*, 2013), Canada (Taillefer *et al.*, 2010; Clark *et al.*, 2010) and China (Wang *et al.*, 2013), the occurrence and prevalence of the organism in Nigeria is hardly reported. The aim of this review was to evaluate what has been reported in literature to date, describe emerging trends and prevalence of *L. monocytogenes* in Nigeria and identify opportunities for the use of current and better research methods for studies on the organism. To this end, literature search and evaluation of articles on databases like Pubmed, Scopus and Web of Science was carried out using phrases such as *Listeria* and Nigeria, etc. Since not too many articles have been indexed by the databases searched, the search was extended to other open access journals that contain articles on *Listeria* studies in Nigeria.

#### *Occurrence of Listeria species in humans in Nigeria*

Sequence typing confirmed that a predominant food borne *L. monocytogenes* clone caused human listeriosis cases and outbreaks in Canada from 1988 to 2010 (Knabel *et al.*, 2012). Even though there have been cases of severe outbreaks of food infections with diarrhoea in Nigeria, there has been no outbreak attributed to a dominant strain or clone of *Listeria* which led to many people being admitted to the hospital for treatment. The organism has been reported in groups normally associated with *Listeria* e.g. neonates and pregnant women (Table 1). The first evidence of prevalence of *Listeria* in Nigeria was provided by Njoku-Obi and Njoku-Obi (1965) through serological evidence. They found it puzzling that no case of listeriosis was recorded in Nigeria as of 1965 and attributed this to non-recognition of the organism or confusion with other organisms that can decolourize the *Listeria* selective media. These authors studied the population served by Lagos University Teaching Hospital to ascertain if *Listeria* was common enough to warrant intensive efforts to isolate the organism. In their study, they determined the levels of specific agglutinating antibodies of *Listeria* in presumed healthy blood donors, staff and students. Although they did not isolate the organism in 580 Nigerians studied, the authors found that the positive results provided by levels of somatic agglutinating and complement fixing antibodies were strong enough to provide evidence that there was

wide spread occurrence of the organism in Nigeria and emphasized that complete proof would be the isolation of the organism from future clinical samples.

The organism was finally isolated in Nigeria by Eyo *et al.* (1969) when they reported *Listeria leptomeningitis* in an adult female. The lady was successfully treated in the hospital with chloramphenicol and prednisone and to the best knowledge of the authors this was the first proven case from Nigeria and the West African sub-continent as a whole. The first case of neonatal listeriosis was reported by Onyemelukwe and Lawande (1982) when they isolated *L. monocytogenes* of the same serotype from a 2-day old neonate who developed *Listeria meningitis* after contracting the organism from the mother. The mother and child were both effectively treated with ampicillin. Ako-Nai *et al.* (1999) suggested that *L. monocytogenes* was emerging as an agent in the aetiology of neonatal septicaemia in Nigeria when they isolated the organism among other dominant organisms in incidences of septicaemia in Ile-Ife. Furthermore, Adejuyigbe (2001) reported 5 positive cases of *L. monocytogenes* out of 66 septicemic neonates. However, studies carried out by Ojukwu *et al.* (2006) showed no isolation of the organism in 33 septicemic neonates out of 138 neonates screened and recently Nwadioha *et al.* (2013) found no *Listeria* in a 3 year retrospective study of 1500 paediatric patients.

In another study with adults, Onyemelukwe *et al.* (1983) recorded a 27% mortality rate from 19 patients that tested positive for *L. monocytogenes* in a 1 year prospective study. The clinical conditions in which the organism was isolated included meningitis and meningoencephalitis. Many years later, Emele (2000) screened 1097 cerebrospinal fluid samples submitted for analysis in Sokoto State and found that 0.4% of the samples were infected with *L. monocytogenes*. For other clinical samples, Esumeh and Odugbemi (1992) screened 420 faecal specimens from patients with acute gastroenteritis for *L. monocytogenes* and found no positive samples. However, they found the mannitol fermenting sub-species *L. grayii* in 4 samples.

In their clinical study, Bolarinwa *et al.* (2011) carried out a random microbiological screening of 162 blood samples donated for transfusion at a teaching hospital in Ile-Ife by performing colony morphology, Gram and spore stains and standard biochemical tests and found that *Listeria* spp. was among the Gram-positive organisms isolated. They pointed out that even though the organism was widely distributed in nature, it is rarely a body commensal in humans and suggested that environmental contamination, false-

positive laboratory results, and skin contamination could not be completely ruled out as reasons for *Listeria* detection. The disease has been noted to be largely undiagnosed and under reported in India (Barbuddhe *et al.*, 2012) and this can be said to be the same situation in Nigeria.

#### Occurrence of *Listeria* species in animals in Nigeria

The early researchers on *Listeria* in Nigeria concentrated on the occurrence of the organism in humans and this may be the possible reason why studies on the organism as it affects animals actually kicked off in the late eighties. At the moment, the most implicated animal is cattle but there are reports of *Listeria* in many other animals (Table 1). Most reported cases are from the north of the country where the majority of livestock production for food consumption is carried out. Oni *et al.* (1989) concluded that *L. monocytogenes* infection is widespread in domestic animals in Nigeria when they carried out a survey to determine the antibody prevalence to serotypes 1/2a, 1/2b, 1/2c, 3a and 4b in 1,190 serum samples from various animal sources in Kano and Kaduna States.

An outbreak of listeriosis was reported in a herd of cattle by Akpavie and Ikheloa (1992) from the south-western city of Ibadan. The organism was isolated in pure culture and the infected animals were associated with still birth, abortion and nervous signs before death. No micro abscesses in the brain were observed when histopathology was carried out but purulent meningitis was seen. In another unusual clinical case (Chukwu *et al.*, 2006), the organism was isolated from specimens of blood and vaginal discharge of an African buffalo (*Syncerus caffer*) presented with septicaemia and abortion. The animal recovered after treatment and the authors pointed out the need for further investigation of listeriosis in wildlife since it was the first recorded case of *Listeria* infection found in wild life in Nigeria.

In Sokoto State, Yakubu *et al.* (2012) found *Listeria* in 39 out of 192 raw milk samples collected from lactating cows identified in nomadic herds and small scale dairy farms. After biochemical characterization, 5 species of *Listeria* were found to be present with *Listeria innocua* being the most abundant, followed by *L. ivanovii*, *L. monocytogenes*, *L. welshimeri* and *L. selegeri*, respectively. The authors suggested that *Listeria* infection may have occurred as a result of unhygienic milking. Another report identified that poor methods of pasteurization contribute to high microbial counts in milk (Lawan *et al.*, 2012). Occurrence of the organism has also been reported in other animals that produce milk.

Table 1. Various sources of *Listeria* in Nigeria

Source /Origin	Reference
<b>Human</b>	
Patients with meningitis, septicaemia.	Onyemelukwe <i>et al.</i> (1983); Emele (2000).
Neonate and mother	Onyemelukwe and Lawande (1982).
Neonates	Ako-Nai <i>et al.</i> (1999).
Blood samples	Bolarinwa <i>et al.</i> (2011).
Faecal specimen	Esumeh and Odugbemi (1992).
<b>Animals</b>	
Cow faeces	David and Odeyemi (2007).
African buffalo,	Chukwu <i>et al.</i> (2006a).
Guinea pig	Chukwu <i>et al.</i> (2006c).
Pig, goat, cattle, horse, dog, camel, chicken	Oni <i>et al.</i> (1989); Akpavie and Ikheloa (1992).
Animal droppings	Umeh and Okpokwasili (2009).
Raw cattle milk	Oni <i>et al.</i> (1989); Yakubu <i>et al.</i> (2012).
Raw goat milk	A detunji and Olaoye (2012).
Domestic cats	Magaji <i>et al.</i> (2008).
Cockroach	Adeleke <i>et al.</i> (2012).
<b>Environment</b>	
Butcher's table	Ikeh <i>et al.</i> (2010); Adetunji and Ishola (2011).
Lake	Nwachukwu <i>et al.</i> (2010).
Irrigation water	Mawak <i>et al.</i> (2009).
Naira currency notes	Kawo <i>et al.</i> (2009).
Veterinary surgical material	Tambuwal <i>et al.</i> (2009).
Soil	David and Odeyemi (2007).
<b>Food</b>	
Fish	Chukwu <i>et al.</i> (2006b); Mawak <i>et al.</i> (2007); Ehizibolo <i>et al.</i> (2007); Salihu <i>et al.</i> (2008); and Olayinka (2012); Nwachukwu and Ma (2012); Shinkafi and Ukwaja (2010).
Vegetables	Pondei and Ogbonna (2004); David and Odeyemi (2007); Nwachukwu <i>et al.</i> (2010b); Ikeh <i>et al.</i>
Dried beef <i>Kilishi</i>	Yusuf and TengkuHaziayamin (2013).
<i>Kunu</i> Drink	Nwachukwu <i>et al.</i> (2009).
<i>Wara</i> soft cheese	A detunji and Adegoke (2008)
Fermented food ( <i>gari</i> etc.)	Ijabadeniya (2007); Osho <i>et al.</i> (2010).
Frozen poultry	A detunji and Odetokun (2012).

This was established when an evaluation of 60 milk samples from West African dwarf and Red Sokoto breed of goats showed an incidence rate of 12% for *Listeria* (Adetunji and Olaoye, 2012).

Animal droppings have been widely implicated in the occurrence of the organism. David and Odeyemi (2007) found *L. monocytogenes* in cattle faeces used for manure in Ado-Ekiti in Ekiti State while in a wider study, Umeh and Okpokwasili (2009) screened a total of 1000 fresh faecal samples of livestock from different animals namely cattle, sheep, goat, chicken and pigs for prevalence of *L. monocytogenes* and found that the organism occurred highest in faeces from cattle (30%) and lowest in pig faeces. Nwachukwu and Orji (2012) called for veterinary surveillance of poultry droppings when they found high occurrence of *L. monocytogenes* in 3 farms in Okigwe, Imo State region of Nigeria. Particularly worrisome was the resistance of the isolated strains to chloramphenicol and ampicillin.

The organism has been found in animals not normally associated with *Listeria*. Magaji *et al.* (2008) studied the microflora from the buccal cavity of 26 stray cats and found 3 *Listeria* spp. out of the 51 bacteria isolates identified in the study while Adeleke

*et al.* (2012) collected cockroaches from residential areas and hospital vicinities to determine the microbial flora they harbour and found *L. monocytogenes* among other micro-organisms isolated.

#### *Prevalence of Listeria species in the environment in Nigeria*

The diverse environment in Nigeria provides favourable conditions for *Listeria* to thrive and contaminate food sold in the open especially ready-to-eat (RTE) foods. The tropical weather is warm and humid all year round and many rural places are not very hygienic and have poor water sanitation. Environmental studies of *Listeria* in Nigeria have shown that the organism occurs in known *Listeria* sources such as soil, lakes and other sources that are not commonly mentioned in literature like veterinary surgical material (Tambuwal *et al.*, 2009) and Naira currency notes (Kawo *et al.*, 2009). *Listeria* from soil has also been identified by Ikeh *et al.* (2010) who examined soil samples from a field where cows and pigs were kept before slaughter and found *Listeria* in all samples examined. The authors attributed the presence of *Listeria* to faecal droppings from the animals while David and Odeyemi (2007) found the organism in soil used for farming and also named faecal dropping as the source.

A study of two anthropogenic lakes in Abia State in south east Nigeria by Nwachukwu *et al.* (2010) showed a prevalence rate of 91.67 and 79.17% for 24 samples analysed for each lake. The authors pointed out the ubiquity of the organism in nature and the organism's reputation as a water and food-borne bacterial pathogen as reasons for the high prevalence. Another study by Mawak *et al.* (2009) used the 2-step enrichment method to analyse natural water bodies including rivers, streams and ponds used for irrigation in Jos, Plateau State in the middle belt region of Nigeria. They found that four species of *Listeria* namely *L. monocytogenes*, *L. innocua*, *L. ivanovii* and *L. grayii* was present in 10 out of 30 samples analysed. Their report suggested that dry season farmers should be educated on measures that would reduce the hazards associated with *Listeria*.

The butcher's table from where fresh beef is sold has also been implicated as a vehicle for *Listeria*. Five surface swabs from butcher's tables taken by Ikeh *et al.* (2010) in Nsukka, south eastern Nigeria showed occurrence of *Listeria* in all the samples while Adetunji and Ishola (2011) enumerated *Listeria* on meat tables before and after sales of meat in Ibadan municipal abattoir in Nigeria. The latter found that there was an increase in *Listeria* count after meat sales. They attributed this to the wooden table used

which may entrap bacteria and encourage cross contamination and highlighted that the results could reflect poor hygienic conditions of the meat tables.

#### *Isolation of Listeria species from foods in Nigeria*

According to McLauchlin (1996), foods associated with transmission of *Listeria* have generally been highly processed, have extended shelf life at refrigeration temperatures, are capable of supporting the growth of *L. monocytogenes* and consumed without further cooking. Adzitey and Huda (2010) pointed out that studies on *L. monocytogenes* and its association with foods is important to create more awareness in order to reduce its colonisation, transmission, cross contaminations and infections. Even though the reasons for the increasing number of pathogens causing food and water diseases in North America are found in Nigeria, occurrence of food-borne *Listerial* infection is not well reported. The reasons for the increasing number of pathogens include improved ability to isolate and identify organisms, import of a variety of products from abroad, large animal feeding stations and an increase in the number of immune compromised persons (Wadhwa *et al.*, 2002).

Hoelzer *et al.* (2012) have reported that one major determinant of the listeriosis risk is the ability of a food to support the growth of *L. monocytogenes* during storage but data regarding the ability to support growth of the organism are scarce or non-existent for many produce commodities. This may be another reason why the occurrence of *Listeria* is hardly reported in Nigeria because there is the absence of an extensive cold food storage system with little information on the ability of the numerous Nigerian processed food products to support *Listeria* growth. Amongst the different kinds of foods consumed in Nigeria, fish is mostly associated with the occurrence of *Listeria* (Table 1).

The higher number of literature citations for fish compared with other food commodities could indicate the type of food researchers have been mostly working on and may not mean that the organism occurs in fish more than any other foods found in Nigeria. Work reported has been mainly on dry fish, which is obtained by open air roasting of fresh fish for preservation. The organism has been isolated from different fish varieties and across different sites in Nigeria and several *Listeria* species which includes *L. monocytogenes*, *L. innocua*, *L. ivanovii* and *L. grayii* has been reported. However, in a recent study on the storage stability of smoke-dried African catfish (*Clarias gariepinus*) stored for two months by Adeyemi *et al.* (2013), no *Listeria* was found despite

the fact that 7 bacteria and 6 fungal species were isolated. The general consensus amongst authors on *Listeria* occurrence in fish is that poor hygiene may increase the chances of *Listeria* colonisation.

Several locally processed foods have been named as a source of *Listeria*. Ijabadeniyi (2007) carried out a microbiological survey on some fermented food products including gari and lafun made from cassava (*Manihot esculenta*) and ogiri (*Ricinus communis*). While no *Listeria* was recorded for gari and ogiri, the count recorded for lafun was considered low and not enough to pose any health risk. A similar study by Osho *et al.* (2010) using gari, elubo-isu from yam (*Dioscorea rotundata*) and iru from locust bean seed (*Parkia* spp) recorded *Listeria* in low numbers for only elubo-isu.

The absence of the organism in gari is good news for Nigerians as gari is a staple food consumed by millions of Nigerians. An attempt by this author to isolate the organism from gari using methods of Doumith *et al.* (2004) after primary and secondary enrichments yielded no *Listeria* (data not shown). Oguntoyinbo and Dodd (2010) carried out a comprehensive total bacterial community fingerprinting at different fermentation times during gari production using denaturing gradient gel electrophoresis (DGGE) and found no *Listeria* indicating that the organism may not survive gari processing.

The organism has been isolated from kilishi, a snack food made by sun drying beef (Moshood and TengkuHaziyaamin 2013), kunu, a popular traditional drink (Nwachukwu *et al.*, 2009) and Wara, a type of soft cheese made from coagulating fresh cows' milk (Adetunji and Adegoke, 2008). Vegetables (Pondei and Ogbonna, 2004) and frozen poultry (Adetunji and Odetokun, 2012) have also been implicated as major sources of *Listeria* in Nigeria.

#### *Future perspectives*

The existing food safety status of many developing countries has been adjudged to be distressing due to inadequate assessment of foodborne illness diseases such as botulism, shigellosis, campylobacteriosis, *Escherichia coli* infection, *Staphylococcus aureus* infection, salmonellosis, listeriosis and cholera which pose a major threat to human health (Akhtar *et al.*, 2012).

Living in deprived conditions has been cited by Mook *et al.* (2010) as a possible cause for the increasing prevalence of *Listeria* among ethnic minorities in England and Wales. These authors pointed out that increased immigration and/or economic migration in recent years appear to have

altered the population at risk of pregnancy-related listeriosis in England and Wales and that these changes need to be taken into account in order to target risk communication strategies appropriately. These minorities include families from Asia and Africa and since no data is available on the actual prevalence of human listeriosis in Nigeria, it is possible that the trend of increasing prevalence of listeriosis among immigrants residing in the developed world is also replicated in developing countries such as Nigeria where many people in the rural areas live in deprived conditions.

The occurrence of *Listeria* is taken very seriously in the developed world and many countries have adopted the risk-based policy in setting *Listeria* limits except the US that has adopted a zero-tolerance approach (Warriner and Namvar, 2009). The reports from Nigeria cited in this paper were limited by methods that were used for identification of the organism. This is because most reports from Nigeria on the occurrence and identification of the organism were mainly based on primary detection methods (HPA, 2007), classical biochemical testing and the occurrence of black coloration of *Listeria* selective media due to aesculin hydrolysis. Although these methods are standard and all *Listeria* species hydrolyse aesculin, it has been shown that selection of *Listeria* colonies based on black colouration due to aesculin hydrolysis of media can give false positives (Fraser and Sperber, 1988). Also it has been found by Devriese *et al.* (1999) that other organisms such as *Streptococcus pluranimalium* from subclinical intramammary infections in dairy cows can cause brown discoloration due to aesculin hydrolysis.

However, Dwivedi and Jaykus (2011) acknowledged that culture-based techniques to detect food-borne pathogens are still considered to be the "gold-standard," but these remain cumbersome and time consuming. These authors advised the need to eliminate or reduce cultural enrichment in the process of detecting food-borne pathogens. The methods used can be improved by applying molecular techniques. Gasanov *et al.* (2005) stated that standard methods are being replaced by molecular tests which are more accurate and reflect genetic relationships between isolates. Also, Nightingale *et al.* (2007) used multiplex PCR to show improved discriminatory power and reliability of *L. monocytogenes* molecular serotyping methods and concluded that these are now accepted as reliable replacements of the classical serotyping procedures. This was confirmed by Zhang and Knabel (2005) who suggested that multiplex PCR simplifies serotyping and sequencing.

The classification of *L. monocytogenes* has

continued to evolve as shown by Ward *et al.* (2010) who developed a 30-probe assay for simultaneous classification of *L. monocytogenes* by lineage, major serotypes and epidemic clone types to facilitate rapid strain characterization and the integration of subtype data into risk-based inspection programs. These new methods can be applied in Nigeria because there is the need to develop more robust epidemiological monitoring for the organism in order to ascertain prevalence of the major serotypes and lineages in Nigeria. It is known that initiating new lines of investigations ensures lower occurrence of listeriosis and increased efforts by food processors and food regulatory agencies in the developed world to control *L. monocytogenes* in high risk foods have resulted in significant decrease in the incidence of sporadic listeriosis (Swaminathan and Gerner-Smidt, 2007). Therefore, more efforts in studying the organism would ensure that the adverse health effects of human *L. monocytogenes* infections in Nigeria would be established and documented in databases similar to many countries around the world (e.g. United Kingdom) where there are well documented databases spanning almost 40 years of surveillance with incidence data available for various age and risk groups (McLauchlin *et al.*, 2004). It is generally agreed that the more you look, the more you see.

Since PCR-based molecular typing methods have not been used in literature on the occurrence of *Listeria* in Nigeria as cited in this work, it was very difficult to establish any emerging pattern or trend using current *Listeria* molecular serotypes classification. Although there has been an improvement in methods used in the study of the organism in the country in the last decade, there is a whole new opportunity to characterize the *Listeria* strains in Nigeria with current molecular and nano biotechnology methods proposed by Theron *et al.* (2010) in order to identify the strains that dominate the environment. This may lead to other novel applications like phage typing (Loessner, 1990; Rees and Dodd, 2006) that would enable a greater understanding of pathogenic *Listeria*, control of their virulence determinants and establishment of procedures to control this bacterium in food as well as in infected animals (McLauchlin, 1997).

## Conclusions

If researchers in Nigeria increase their collaborative studies with established laboratories in the developed world, it will only be a matter of time before many existing novel molecular methods are applied to *Listeria* research in the country.

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