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Screening for *Listeria monocytogenes* and other related species from faecal specimens at the Lagos University Teaching Hospital, Lagos

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Summary

Four hundred and twenty faecal specimens from patients with acute gastroenteritis and apparently healthy persons who reported at the Lagos University Teaching Hospital (LUTH) between October, 1988 and May, 1989 were investigated for faecal carriage of *Listeria monocytogenes* and other related species. Of these specimens, none was positive for *Listeria* species. However, the mannitol – fermenting *Listeria* species now *Murraya grayi* sub species *grayi* and *Murraya grayi* sub spp. *murrayi* representing 0.95% (4 out of 420) were isolated. Other well known enteric pathogens isolated in the course of this study were *Escherichia coli* (11.4%), *Salmonella typhi* (7.1%) and *Yersinia* species (1.4%).

Résumé

Quatre cent vingt échantillons faecal obtenu des malades avec un gastroenterite intense et apparement des personnes en santé qui se sont presentés au centre Universitaire de Lagos de Science et de la Santé (LUTH) entre le mois d'octobre 1988 et Mai, 1989 ont été examiné pour le port faeal de *Listéria monocytogènes* et d'autres espèces relatif. De tous ces échantillons, aucun a été positive pour les espèces de *Listéria*. Cependant, les espèces de *Listéria* qui fermente le mannitol maintenant *Murraya grayi* sousespèce *murrayi* representant 0.95% (4 sur 420) ont été isolés. D'autre pathogènes bien connu isolé en cours de cette étude sont *Escherichia coli* (11.4%), *Salmonella typhi* (7.1%) et les espèces de *Yersinia* (1.4%).

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Introduction

The ubiquitous nature of *Listeria* species will favour easy spread and colonisation [1]. Pathogenic and non- pathogenic species have been cultured from a wide variety of sources including fishes, birds, animals and water [1, 2, 3]. In general, organisms in the genus are small, gram-positive, non spore-forming, motile rods. The systematics of *Listeria* species had been undergoing some changes. The mannitol – fermenting species have been assigned to a new genus *Murraya* [4], and *Listeria monocytogenes sensu lacto* have been classified into five species [1].

Listeriosis caused by *Listeria monocytogenes* is primarily a disease of animals [5] and the occurrence in a human subject or zoonosis [2, 5, 6, 7] have contributed to the continuous upsurge on reports of *L. monocytogenes* in human infections. In recent times, significant increase in cases of listeriosis as a foodborne pathogen has been recorded, and on the list of these incriminated foods and food products are icecream and cheese [7, 8], unpasteurized milk [9], leafy vegetables [10], and chickens both raw [7] and ready cooked [11].

In humans, the clinical syndrome may present as abortion, meningitis or meningoencephalitis, diarrhoea, septicaemia, or endocarditis especially in neonates, the elderly and immuno- compromised patients [12, 13, 14]. However, most human infections according to Bortolussi *et al.* [15] are manifested as sepsis or meningitis in neonates and immuno-compromised patients, sepsis or non-specific flu-like illness in healthy women during pregnancy, which can lead to infection of the foetus.

Listeria meningitis with or without focal neurological signs is the commonest mode of the central nervous system (CNS) infection. In the United States, *L. monocytogenes* is the fifth most common cause of bacterial meningitis and also ranks second as the commonest cause of neonatal meningitis [15]. This severe infection can affect all age groups, but

there is a very high preponderance for neonates, the elderly, and immuno-compromised patients [12, 14].

With listeria infection of the foetus *in utero* there could be an early abortion during the second to fourth months of pregnancy [3] or late abortion between the fifth to ninth months of pregnancy often resulting in still birth, or if alive prematurity or meningitis may ensue [13]. According to Bojsen- Moller [6] and Robertson [13] source of infection in pregnant women are due to colonisation of the vagina or cervix through the oral route with subsequent transperineal spread from anus to vulva. Evidence of venereal route transmission by the demonstration of *Listeria* in semen of husband of habitual aborters have been reported [6].

There have been also reports of the occurrence of *Listeria* spp. in the faeces of healthy persons [2, 6, 16] and in association with diarrhoeagenic cases [6, 11]. Bojsen-Moller [6] on the occurrence of *Listeria* species in the human intestinal tract is of the view that at least one per cent of the population at any given time is colonised; nonetheless, in two separate studies, he [6] reported the incidence of 23% and 8.3% with 15% and 0.5% respectively in association with diarrhoea. A significantly increased carrier rate in persons with close contact or association with animals have been documented [6, 16]. Man has been known to contract listeriosis after contact with an infected dog [17].

To our knowledge however, there is no documented case indicating human listeric infections or isolation of *L. monocytogenes* and other related species in the faeces of both healthy and diarrhoeagenic persons in our environment.

Materials and methods

Specimens

A total of 420 faecal specimens submitted to our hospital routine enteric/parasitology laboratory between October, 1988 and May, 1989 were examined irrespective of the patients' sex and age. Two hundred and seventy two (65%) of these specimens were obtained from patients presenting with gastroenteritis, and the remaining 148 (35%) came from apparently healthy persons or control cases. The first 350 faecal specimens were specifically processed for *Listeria monocytogenes* and other related species, while other well known enteric pathogens were inclusive in the remaining 70 specimens.

Culture

The cold enrichment method as described by Gray *et al.* [18] was employed using two sets of tryptose phosphate broth, one with and the other without the antibiotics Polymixin (25 units). Both sets of faecal broth culture were subcultured on the 7, 28, and 56 days of holding at 4°C respectively onto two sets of Polymyxin Blood Agar, PBA [6], Acriflavine-Ceftazidime Agar, AC medium [19], and 5% blood enriched AC medium, ACBA. These plates were incubated at 37°C under increased carbon dioxide (CO_2) tension in candle extinction jar for 18 – 36 hours.

MacConkey agar was included to the above listed media in the processing of the last 70 specimens, and cultures on this medium were made on days 1, 7 and 28 and then incubated in air at 37°C for 18 – 48 hours.

Identification

All colonies resembling *Listeria* species on the aforementioned PBA, AC, and ACBA plates were subcultured onto fresh antibiotic – free blood agar, incubated at 37°C in an enhanced CO_2 environment for 18 – 24 hours, and screened for *Listeria monocytogenes* and other related species by their characteristic gram-positive coccobacilli morphology, catalase production, 'tumbling' motility at room temperature and various other biochemical tests as described by Hollis and Weaver [20]. Isolates were also subjected to the CAMP (Christie, Atkins, and Munch-Peterson) test using *S. aureus* (ATCC 25923) strain on sheep blood agar plates.

Six reference strains of *L. monocytogenes* (F9499, F9475, F9035, F8825, KC1709 and KC668) kindly provided by Dr. Robert Weaver of the Special Pathogens Laboratory, Center for Disease Control, (CDC), Atlanta, USA were included as control for the screening process.

Identification of other enteric pathogens was carried out using standard procedures [21].

Results

Four (0.95%) of the 420 faecal specimens grew culture of *Murraya species*. Three of these isolates belong to the species *Murraya grayi* sub species *murrayi* (formerly *Listeria murrayi*), and the other species is *Murraya grayi* sub spp. *grayi* (formerly *L. grayi*). The biochemical characteristics of these isolates are shown in Table 1. None of the isolates was positive for the CAMP test. None of the specimens yielded cultures of the other species now in the genus *Listeria*.

The clinical findings associated with two of the patients positive for *Murraya* spp. were typhoid-like

Table 1: Biochemical characteristics of *Murraya* isolates

Tests	Positive Number ($n = 4$)
1. β-haemolysis on sheep blood agar	0/4
2. Gram-positive coccobacilli	4/4
3. Catalase	4/4
4. 'Tumbling' motility at room temperature	4/4
5. Motility on semi-solid medium at room temp.	4/4
6. Indole	0/4
7. Urease	0/4
8. Hydrogen sulphide (H_2S)	0/4
9. Action on Kligler Iron Agar (KIA): Butt	4/4
Slant	4/4
Gas from glucose	0/4
10. Methyl Red (MR)	4/4
11. Voges Proskauer (VP)	4/4
12. Oxidase	0/4
13. Citrate utilisation	0/4
14. Nitrate reduction	3/4
15. Acid from: Salicin	2/4
Trehalose	4/4
Xylose	0/4
Rhamnose	0/4
Mannitol	4/4

fever and gastroenteritis, while the other two were control cases.

As regards the three media used for screening of *Listeria* and other related organisms, there was no significant difference in selectivity between the PBA and AC media. However, the enriched AC medium, ACBA had a significant decrease in selectivity.

Fourteen of the 70 specimens processed for *Listeria* organisms and other enteric pathogens yielded cultures of *Escherichia coli* (11.4%), *Salmonella typhi* (7.1%) and *Yersinia* spp (1.4%). The 14 isolates were all from cases of gastroenteritis.

Discussion

Listeria monocytogenes and other related species have been isolated from routine stool cultures of both asymptomatic and symptomatic cases of gastroenteritis. Bojsen- Moller [6] reported that at least one per cent of normal people may excrete *Listeria* with faeces at any given time. Our study reveals an isolation rate of 0.95% for *Murraya* species with 0.24% in association with diarrhoea. However, the role of this organism in this disease state could not be ascertained since other known enteric pathogens such as viruses was not sought from the specimen that yielded this culture.

The results of this study demonstrates the rare occurrence of *Listeria* and *Murraya* species in the faecal specimens of our studies population, when contrasted to results of Bojsen- Moller [6] who reported isolation rates of 23% and 8.3% with 15% and 0.5% respectively in association with diarrhoea. This opinion is further buttressed, when one considers the relative common occurrence in the faeces of other known enteric pathogens amply shown by an isolation rate of 20% (14 out of 70 specimens) for *Escherichia coli, Salmonella typhi* and *Yersinia* species in this study.

The rare occurrence of *L. monocytogenes* and other related species here, contributes to the growing knowledge that his group of organisms are not resident normal bacterial flora of the human gut. This view is particularly true considering the numerous cases of listeriosis arising from exogenous sources, particularly from consumption of contaminated food and food products [7, 8, 11]. This low rate of occurrence in Nigerians and the apparent lack of reports to our knowledge incriminating this opportunistic pa-

thogen, *L. moncytogenes* in infections can be attributed to one or more factors such as good living conditions, good management of immuno-compromised patients and indiscriminate chemoprophylaxis. Arguably, likened to the urban life of persons examined, these factors may perhaps account for this observation.

It is therefore our view that screening of food products and persons in some rural settings, as well as those with close contact with animals might help to provide more information on prevalence of *L. moncytogenes* and other related species in Nigeria. Thus, this study would serve to create a baseline information and also provide necessary awareness of the possible role of *Listeria* and other related species in diarrhoeal diseases in Nigeria.

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