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Isolation of *Aeromonas caviae* from yoghurt ("*nono*") sold in Jos, Nigeria

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Abstract

A total of 30 *nono* samples were collected from Farin Gada, Bauchi Road and Gangare markets in Jos and analysed for the presence of *Aeromonas* species. 3 (10%) of the 30 *nono* samples were positive for *Aeromonas* species; which was found to be *Aeromonas caviae*. All the isolates were obtained from non-heat treated *nono* samples; all isolates were found to be sensitive to streptomycin and gentamicin but resistant to amoxycillin, augmentin, cloxacillin, cotrimoxazole, erythromycin, and tetracycline

Keywords: Aeromonas; Isolation; Yoghurt (*Nono*).

Introduction

The genus *Aeromonas* consists of aquatic bacteria and sometimes pathogens of fish and cold-blooded vertebrates that inhabit wet environments (Hazen *et al.*, 1978). However, the aeromonads have been isolated in large numbers from various foods such as green vegetables, raw milk, ice cream, meat (beef, pork, lamb, and poultry) and sea foods (Merino *et al.*, 1995). Like *Listeria monocytogenes, Plesiomonas shigelloides* and *Yersinia enterocolitica*, *Aeromonas* species have attracted attention primarily because of their ability to grow at chill temperatures (4^0C) prompting the concern that any threat they might pose will increase with increasing use of chilled foods (Ingham, 1990). The role of *Aeromonas* spp as human pathogens and their transmission have been revised during the past few years. According to some authors, the isolation of highly virulent strains is increasing, thus these bacteria can no longer be classified among the opportunistic agents as they were in the past (Janda and Abbott 1998). Currently, *Aeromonas hydrophilia, Aeromonas caviae*, and *Aeromonas sobria* have been classified as food borne pathogens of emerging importance (Holmberg *et al;* 1986). Clinical manifestations of infections caused by *Aeromonas* spp range from skin and soft tissue infections, bacteremia to gastroenteritis (Jones and Wilcox, 1995). However acute watery diarrhea with a short duration is the most common clinical feature (Albert *et al.*, 2000).

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"*Nono*" is a type of yoghurt produced locally from fermented cow milk mainly by the nomadic Fulani herdsmen. In Nigeria, especially in the northern region, *nono* is regarded as one of the staple foods consumed by a high percentage of the population. Fermented products like *nono* are generally safe; risk however increases due to faulty procedures, contamination during processing, handling and storage at ambient temperatures and use of raw materials as starter cultures (Olusupo *et al.*, 2002).

In Jos Nigeria *Aeromonas* spp have been isolated from stools of infants with diarrhea who had recently been introduced to solid food. However, none were isolated from exclusively breast fed infants (Ochai, 2003). This result might be suggestive of transmission through contaminated foods/water. This study was therefore undertaken to isolate *Aeromonas* spp from *nono* sold in Jos and to determine the antibiogram of recovered isolates.

Experimental

Samples. The samples analysed in this study included a total of 30 (24 heat-treated and 6 non-heat treated) *nono* samples, collected from three markets (Gangare, Farin Gada and Bauchi Park) in Jos. The samples were collected in sterile leak proof universal bottles labeled appropriately and transported to the laboratory within 1-2 hours after collection in coolers containing ice.

Processing of specimens. The samples were processed according to guidelines provided by Senior (1989) for examination of foods for food-poisoning bacteria. These include enrichment, Gram-stain, motility, biochemical testing and antimicrobial sensitivity testing.

Enrichment. 5ml of *nono* samples were inoculated into 45ml peptone water containing 10ug/ml ampicillin, and incubated at 28^{0}C for 24 hours. After the incubation period, 1ml of the culture was inoculated directed onto the surface of starch-ampicillin agar plates (containing 10μg, ampicillin per ml medium). Another 1ml of a 1 to ten dilution of the culture in peptone water was also inoculated onto the surface of starch-ampicillin agar plates. The plates were incubated at 28^{0}C for 24 hours.

Biochemical testing. Isolates were identified as *Aeromonas* spp using the following standard tests: - oxidase test, motility test, indole test, carbohydrate fermentation test, catalase test, urease test and citrate utilization test. All tests were as described by Collee and Miles (1989) and Porter and Duguid (1989).

Characterization of species. Isolates were characterized to the species level based on seven biochemical tests as described by Carnahan *et al* (1991). These included aesculin hydrolysis, gas from glucose, acid from arabinose, indole production, acid from sucrose, Voges-Proskauer reaction and resistance to cephalothin (30μg).

Antimicrobial susceptibility testing. Sensitivity of isolates to antimicrobial agents was determined on Mueller-Hinton agar plates using the disc diffusion method of Scott (1989). From a pure culture of the isolate to be tested, a uniform streak was made on the agar plate. The antibiotic discs (Antec Diagnostics UK) were placed on the plates and incubated at 37^{0}C overnight. The diameter of the zone of inhibition for each test antibiotic was measured and sensitivity or resistance estimated by comparing with zone-diameter interpretive standard (Sommers, 1980). All isolates were tested for sensitivity to the following antibiotics, Augmentin (30 mcg), Gentamicin (10 mcg), Cotrimoxazole (25 mcg), Cloxacillin (5 mcg), Tetracycline (10mcg), Erythromycin (5 mcg), Amoxycillin (25 mcg), Streptomycin (10 mcg).

TABLE 1: Number of *Aeromonas* species isolated from nono samples analysed.

Type of sample	Number tested	Number (%) Aeromonas isolated
Raw	6	3 (50.0)
Boiled	24	0 (0.0)
Total	30	3 (10.0)

Table 2: *In vitro* antibiotics susceptibility pattern of *Aeromonas* species isolated from *nono*

Antibiotics	Concentration(mcg/ml)	Number of isolates tested	Number (%) sensitive
Amoxycillin	25	3	0 (0.0)
Augmentin	30	3	0 (0.0)
Cloxacillin	5	3	0 (0.0)
Cotrimoxazole	25	3	0 (0.0)
Erythromycin	5	3	0 (0.0)
Gentamicin	10	3	3 (100)
Streptomycin	10	3	3 (100)
Tetracycline	10	3	0 (0.0)

TABLE 3: Biochemical reactions used in the identification of *Aeromonas caviae*

Biochemical tests	Isolate 1	Isolate 2	Isolate 3
Oxidase	+	+	+
Indole Production	+	+	+
Voges-Proskauer reaction	-	-	-
Aesculin hydrolysis	+	+	+
Gas from glucose	-	-	-
Acid from arabinose	+	+	+
Acid from sucrose	+	+	+

Key: + = Positive; - = Negative

Results

A total of 30 *nono* samples consisting of 6 raw (non-heat treated) and 24 boiled (heat treated) were collected from three markets in Jos and analysed. 3 (50%) of the raw samples yielded *Aeromonas* species while none was isolated from the boiled samples. Thus the total number from 30 *nono* samples was 3 (10%) (Table 1). An *in vitro* antibiotic susceptibility pattern of the 3 isolates was determined (Table 2). All isolates were sensitive to gentamicin and streptomycin. None was sensitive to amoxicillin, augmentin, cloxacillin, cotrimoxazole, erythromycin and tetracycline. All isolates were found to be *Aeromonas caviae* based upon the biochemical tests used to speciate them (Table 3).

Discussion

The isolation of *Aeromonas* from *nono* is in agreement with reported results from several researchers who isolated the bacterium although in low numbers from milk and dairy products (Ibrahim and MacRae, 1991; Khali, 1997). The isolation rate of 10% found in this study is higher than the 5% reported by Ibrahim and Mac Rae (1991) for milk sample in Brisbane, Australia. Variations in isolation rates might be due to the degree of contamination of the milk product. Sources of contamination of the product might be from the handlers involved in the preparation, utensils used or hawkers (sellers), etc. The animal (cow) from which the milk was obtained might also be a probable source of contamination since *Aeromonas* have been isolated from a variety of animals as well as animal products (Gray, 1984; Majeed, *et al*; 1989). Milk is normally sterile when it is secreted, but when it leaves the cow's udder, it is rapidly inoculated with a variety of organisms usually from the environment (Hayes, 1981). *Aeromonas* was isolated only

from non-heat treated *nono* samples. This result probably suggests that boiling may have killed the organisms if present. However only 50% of the raw samples contained *Aeromonas* spp, therefore the degree of contamination of the product may also be a contributory factor in this instance.

The antimicrobial sensitivity pattern showed that the isolates were resistant to all antibiotics tested with the exception of streptomycin and gentamicin (Table 2). *Aeromonas* isolated from the stool of patients in Jos also showed similar antibiogram with isolates being resistant to augmentin, cotrimoxazole, and penicillin (Ochai, 2003). Other agents of gastroenteritis e.g. *Yersinia enterocolitica* isolated in Jos were also found to be resistant to the three antibiotics (Kandakai-Olukemi *et al.*, 2004). Multiple resistances of isolates to some of these drugs in Nigeria could be attributed to the fact that the antibiotics have been abused due to constant and indiscriminate usage (Omonigho *et al.*, 1999).

The findings that all isolates exhibited multiple drug resistance might also indicate that the source of contamination of the *nono* samples may probably have been from humans. *Aeromonas* spp isolated from natural environment that have not been polluted by humans should normally not show multiple drug resistance. All isolates were found to be *Aeromonas caviae*. Several reports have indicated that this is the most prevalent species found in heavily polluted environments (Araujo *et al.*, 1991).

In conclusion, this study has clearly shown that *Aeromonas* species can be isolated from *nono* sold in Jos metropolis. This presents cause for concern since *nono* is widely consumed by the populace and the *Aeromonas* species isolated (*Aeromonas caviae*) has been reported to cause gastroenteritis in humans.

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