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Occurrence of enteropathogens in traditional weaning cereal pastes in Jos, Nigeria

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A study was carried out to determine the occurrence of enteropathogens with emphasis on *Escherichia coli, Salmonella* spp, *and Shigella* spp in traditional weaning cereal pastes in Jos, Nigeria. A total of 50 uncooked weaning cereal pastes including (maize (n = 18), Guinea corn (n = 15), millet (n = 11), and millet and corn (n = 6)) were obtained from different locations within Jos metropolis and examined using standard microscopy and culture. Of the total number examined, 17(34%) were positive with an average microbial load of $1.31x10^{10}$cfu/g. A total of 30 isolates were recovered and identified which included: *E coli 11(36.67%),* Shigella *dysenteriae* 7(23.33%), *Shigella* spp 5(16.67%), *E. coli* O157:H7 4(13.33%), and *Salmonella typhi* 3(10.00%). Contamination was found to depend on handlers rather than on location. All isolates were susceptible to ciprofloxacin and ofloxacin and all showed multiple drug resistance. Proper processing of weaning cereals is indicated to prevent cases of infantile diarrhea

Keywords: Cereals, enteropathogens, isolates, occurrence, weaning foods.

INTRODUCTION

In developing countries, infantile diarrhea is one of the major precipitating factors of child morbidity and mortality and is associated with bacterial contamination of weaning foods (UNICEF, 1998). This is because at weaning, children are gradually withdrawn from breast milk and are introduced to solid food. Amongst the bacterial causative agents of infantile diarrhea are *Escherichia coli, Salmonella* spp, *Shigella* spp *and Campylobacter* spp (Suan *et al.,* 1985).

The locally available infant weaning cereals in Jos and environs include fresh cow milk and pap (ogi, akamu, or koko), which is thin cereal gruel made from different kinds of cereals such as *Zea mays* (maize), *Sorghum bicolor* (Guinea corn) and *Pennisetum americanum* (millet) (Ikeh *et al.,* 2001). The age of introduction of weaning cereals in Nigeria is 3-6 months (Onofiok and Nnanyelugo, 1998). Because weaning cereals have a low nutritional value due to the processing methods (Onofiok and Nnanyelugo, 1998), most mothers add other food items such as sugar, groundnut, cray fish, half cooked egg, Soybeans, honey, and ground fish (Ikeh *et al.,* 2001).

Several factors which could lead to contamination of weaning foods include: their source, mode of preparation and storage, the use of contaminated water and utensils during the preparation of the food and exposure of the prepared food to the atmosphere. Several studies have reported the occurrence of food borne pathogens in samples of food fed to weaning infants (Black *et al.,* 1992; Erku and Ashenafi,

1995; Baylis and Petit, 1997; Fawole and Oso, 1998).

The aim of this study was to determine the occurrence of enteropathogens in local weaning cereal paste in Jos, Nigeria as well as determine the antibiotic susceptibility profile of the recovered isolates.

MATERIALS AND METHODS

Study area

The study area for the research was Jos metropolis, Nigeria and was divided into two groups (rural and urban settlements) based on availability of potable water. The rural areas included: Fudawa, Yan Shanu, Eto Baba, and Congo Russia while the urban areas included: Anguwan-Rukuba, Nassarawa, Apata, and Bauchi Road.

Sample collection

A total of 50 samples of the various types of raw, locally prepared infant weaning cereal pastes were collected aseptically into sterile dry universal bottles and analysed. Twenty-eight (56%) of the samples were collected from mothers while 22(44%) samples were collected from stalls and were grouped into four based on the cereal used for their production. The groups included: A- maize (n = 18) B- Guinea corn (n = 15) C- millet (n = 11) and D- millet and maize (n = 6). The samples were collected from Yan Shanu (n = 7), Eto-Baba (n=6), Congo Russia (n = 6), Apata (n = 6), Bauchi road (n = 6), Anguwan-Rukuba (n = 6) and Nassarawa (n = 7). The mode of storage and water used for processing were noted.

Determination of pH

One gramme of sample was dissolved in 10ml of distilled water (pH 7.0) and the pH determined using a pH meter.

Pre-Enrichment Food homogenate was prepared by dissolving 1g of the sample in 10ml of nutrient broth as described by FAO (1979). This was incubated at 37°C for 24h.

Total plate count

One millilitre of the food homogenate was transferred into a tube containing 9ml of nutrient broth. This procedure was repeated by making serial dilutions with nutrient broth up to 10^{-10}. Twenty-five microlitres of each dilution was inoculated unto the surfaces of nutrient agar and blood agar. The plates were incubated at 37°C for 24h.

The number of colonies on the plates was taken and the total plate count was determined by multiplying the number of colonies with the dilution factor (Gaffa and Azoro, 2005).

Isolation and identification

From the pre-enriched bottles, loopfuls of food homogenates were sub-cultured onto MacConkey agar (MCA) and Rappaport-Vassiliadis enrichment broth (RVEB) and then incubated at 37°C for 24h. After this, non-lactose fermenting colonies on the MCA and loopfuls from the RVEB were sub-cultured unto Salmonella-Shigella agar (SSA). These were incubated at 37°C for 24h after which the isolates were identified using the descriptions of Chessbrough (1998). For each biochemical tests, a control experiment was set up.

Escherichia coli colonies were screened for *E. coli* 0157:H7 by subculturing a colony on sorbitol MacConkey agar (SMA) (Oxoid, CM813) for sorbitol fermentation and latex agglutination using latex test reagent (DR620). Isolates that did not ferment sorbitol within 24h and latex agglutination test positive were identified as *E. coli* 0157: H7 (March and Ratnam, 1989)

Antimicrobial sensitivity test

All isolates were tested for sensitivity to

the following antibiotics: ampicillin (10μg), ciprofloxacin (5μg), cotrimoxazole (25μg), gentamicin (10μg), nalidixic acid (30μg), nitrofurantoin (300μg), ofloxacin (30μg), and tetracycline (30μg) while only *Salmonella typhi* was tested for sensitivity to chloramphenicol (30μg).

Sensitivity of isolates to antimicrobial agents was determined on nutrient agar plate using the disc diffusion method as described by WHO (2005). The isolates were inoculated into nutrient broth and were incubated at 37°C for 24h. These were then seeded unto the surface of nutrient agar using a sterile swab. The plates were allowed to dry with the lids in place. Using sterile forceps, the antimicrobial discs were placed on the agar plates. These were then incubated at 37°C for 24h.

Zones of inhibition >21mm (ciprofloxacin), >19mm (tetracycline, nalidixic acid), >18mm (chloramphenicol), >17mm (ampicillin, ofloxacin, nitrofurantoin), ≥ 16mm (cotrimoxazole), >15mm (gentamicin) were considered sensitive while zones of inhibition <10mm (cotrimoxazole), <12mm(gentamicin), <13mm (ampicillin, ofloxacin, nalidixic acid), <14mm (nitrofurantoin, tetracycline), <15mm (ciprofloxacin) were considered resistant.

Statistical analysis

Analysis of variance (ANOVA) and the Chi-square test were used to analyse the results obtained.

RESULTS

A total of 50 samples were analysed out of which 17 (34%) samples were positive for one or more of the enteropathogens sought for. Of the seventeen samples, 6(35.29%) had pure isolates of one organism, 9(52.94%) samples had two isolates while 2(11.77%) of the sample had three isolates (Table 1).

Figure 1 shows the percentage of positive samples with respect to the cereal type. Millet had the highest positive cases 6(54.54%), followed by Guinea corn and maize 5(33.33%) and 2(33.33%), respectively and then millet and corn 4(22.22%).

Table 2 shows the mean plate count in relation to the location. From the results, the samples collected from Apata (an urban area) had the lowest mean plate count (6.85×10^9) while the samples collected from Yan Shanu (a rural area) had the highest mean plate count (2.31×10^{10}). Other areas had the following mean plate counts: Bauchi road- urban (1.92×10^{10}), Congo Russia- rural (1.73×10^{10}), Fudawa-rural (9.85×10^9), Eto Baba- rural (9.76×10^9), Angwan Rukuba-urban (9.47×10^9), and Nasarawa- urban (9.28×10^9). However, the difference in the mean plate counts in relation to location was not statistically significant ($P>0.05$)

Table 3 shows the pH range and distribution of enteropathogens in relation to the type of cereal. The millet based weaning food had the widest pH range (2.3 - 3.0), while maize and corn had the lowest range (2.9- 3.1). Maize had a pH range of 2.5-2.9 and guinea corn had a pH range of 2.4- 2.8. The difference in the pH ranges was not statistically significant ($P>0.05$)

A total of 30 isolates comprising of 5 species / strains of bacteria were recovered. *E. coli* had the highest frequency of 11(36.67%), followed by *Shigella dysenteriae* 7(23.33%), *Shigella* spp 5(16.67%), *E. coli* O157:H7 4(13.33%), and *Salmonella typhi* had the least frequency 3(10.00%). Millet had the highest number of isolates 13(43.33%), while Guinea corn had the least with 4(13.33%). Others had the following

rates: millet and corn 8(26.67%), and maize 5(16.67%) (Table 3). The analysis of variance showed that, there was a significant difference in the level of contamination ($p<0.05$).

Table 4 shows the sensitivity of the various isolates to different antibiotics. All isolates showed 100% sensitivity to ciprofloxacin, and ofloxacin and a 100% resistance to nalidixic acid an older generation quinolone. *E. coli, E. coli O157:H7* and *Shigella dysenteriae* showed moderate sensitivity to gentamycin and ampicillin and total resistance to tetracycline, nitrofurantoin, and cotrimoxazole. *Shigella* spp showed moderate sensitivity to ampicillin, cotrimoxazole, gentamycin, nitrofurantoin, and tetracycline while *Salmonella typhi* showed moderate sensitivity to ampicillin, cotrimoxazole, and nitrofurantoin.

Table 1: Distribution of samples positive for enteropathogens in relation to location and average microbial load.

Location	No. examined		Total	No. positive (%)
	Mother	Stalls		
Rural				
Congo Russia	2	4	6	1(16.67)
Eto Baba	3	3	6	2(33.33)
Fudawa	5	1	6	3(50.00)
Yan Shanu	3	4	7	4(57.14)
Sub-total	13	12	25	10(40.00)
Urban Angwan Rukuba	4	2	6	1(16.67)
Apata	4	2	6	1(16.67)
Bauchi road	2	4	6	2(33.33)
Nassarawa	5	2	7	3(42.86)
Sub total	15	10	25	7(28.00)
Total	28	22	50	17(34.00)
Average microbial load /g	8.6×10^9	1.76×10^{10}	1.31×10^{10}	

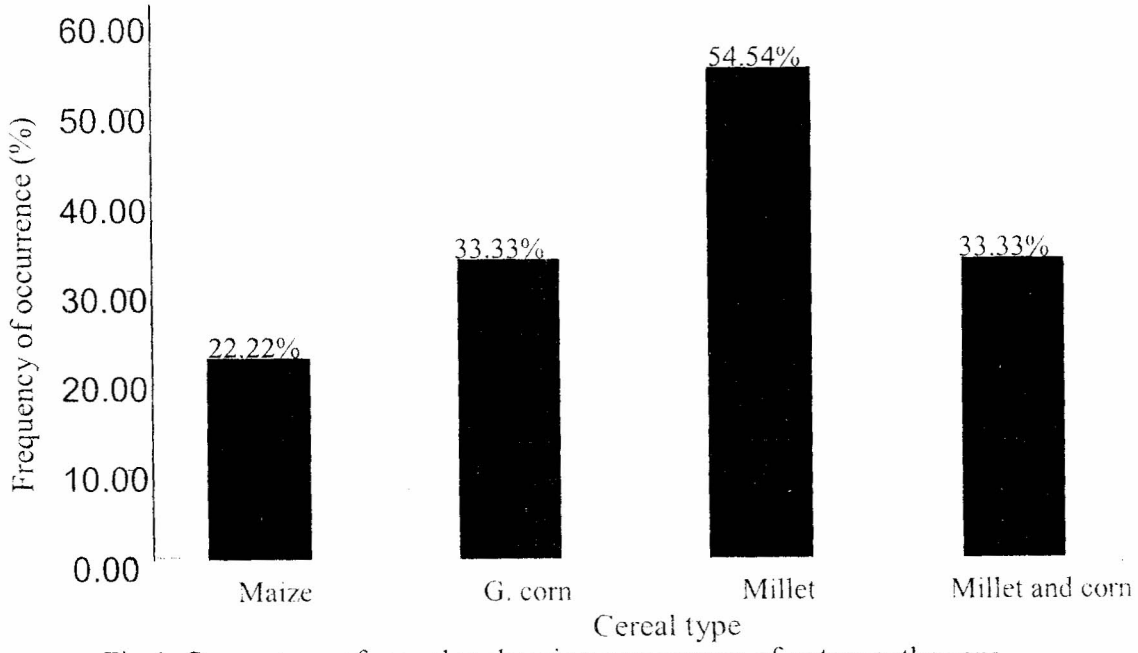

Fig 1: Percentage of samples showing occurrence of enteropathogens

Table 2: Mean plate count in relation to location

Location	Mean plate count (cfu/g)
Nassarawa	9.28×10^9
Congo Russia	1.73×10^{10}
Eto Baba	9.76×10^9
Fudawa	9.85×10^9
Yan Shanu	2.31×10^{10}
Angwan Rukuba	9.47×10^9
Apata	6.85×10^9
Bauchi Road	1.92×10^{10}

Table 3: The pH and distribution of enteropathogens in relation to the type of weaning cereal

Type of weaning food	No. examined	pH range	*Enteropathogen isolated*					Total
			E. coli	*E. coli* O157:H7	*Shigella* spp	*Shigella dysentriae*	*Salmonella* spp.	
Maize	18	2.5-2.9	2	1	1	0	1	5
Guinea corn	15	2.4-2.8	0	1	1	2	0	4
Millet	11	2.3-3.0	5	2	1	4	1	13
Millet and corn	6	2.9-3.1	4	0	2	1	1	8
Total (%)	50	11(36.67)	4(13.33)	5(16.67)	7(23.33)	3(10.00)	11(36.67)	30

Table 4: *In vitro* susceptibility pattern of isolates from local weaning cereals

Antibiotic (µg)	*Enteropathogens*				
	E. coli n=11	*E coli* O157: H7 n=4	*Shigella dysenteriae* n=7	Shigella spp n=5	*Salmonella typhi n=3*
Ampicillin (10)	8(72.72)	2(50.00)	3(42.86)	1(33.33)	3(60.00)
Chloramphenicol (30)	-	-	-	3(100.00)	-
Ciprofloxacin (5)	11(100.00)	4(100.00)	7(100.00)	3(100.00)	5(100.00)
Cotrimoxazole (25)	0 (0.00)	0(0.00)	0(0.00)	2(66.67)	2(40.00)
Gentamycin (10)	6(54.55)	1(25.00)	4(57.10)	3(100.00)	4(80.00)
Nalidixic acid (30)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Nitrofurantoin (300)	0(0.00)	0(0.00)	0(0.00)	2(66.67)	2(40.00)
Tetracycline (30)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(40.00)
Ofloxacin (30)	11(100.00)	4(100.00)	7(100.00)	3(100.00)	5(100.00)

Key: n, number tested; –, not tested.

DISCUSSION

A total of 50 samples were analysed and 17(34%) were positive for the enteropathogens sought for. This study therefore confirms the occurrence of these enteropathogens in locally prepared weaning cereals available in Jos, Nigeria. All organisms isolated were of intestinal origin indicating the poor sanitary conditions prevailing in both the rural and the urban areas of Jos.

The occurrence of *E. coli* O157:H7 amongst others poses a serious threat to health of weaning children. Reports in the literature estimates that 10% of patients with enterohaemorrhagic *Escherichia coli* (EHEC) O157:H7 may develop haemolytic uraemic syndrome (HUS) with a case fatality rate of 3-5% (WHO, 2005). Although the samples analysed were raw the possibility of cross contamination cannot be over emphasized.

This study showed that millet based weaning paste was more contaminated than other cereal based foods ($P<0.05$). This is probably due to the higher nutritive value of millet as compared with other cereals (Ikeh *et al.*, 2001)

The average microbial load of samples collected from the rural areas was higher than those collected from the urban areas with Yan Shanu having the highest microbial load and Apata having the lowest average microbial load. However, there was no significant difference between the mean microbial loads of the locations ($P>0.05$). This implies that contamination is dependent on handlers rather than on the location or type of settlement. The fact that the sanitary conditions in both the rural and urban areas of Jos are similar supports this assertion.

Despite the relatively low pH of the weaning cereals, it was observed that the organisms thrived in the weaning cereals. The presence of acid tolerant pathogens in weaning cereals heightens level of the public health hazards as this acquired physiological trait could minimize the antimicrobial action of gastric acidity (D'Aoust, 1991). The contamination of these cereals by these pathogens therefore poses a serious public health problem.

From the antimicrobial sensitivity test results, it was observed that ciprofloxacin and ofloxacin could be the first choice antibiotics while ampicillin and gentamicin could be alternative drugs for treatment of infections caused by these isolates. Although *Salmonella typhi* showed susceptibility to inhibition by chloramphenicol, it is not a recommended drug of choice due to its toxicity. Likewise, gentamicin and nitrofurantoin are not recommended for treatment of shigellosis as they are not effective for *in vivo* treatment of shigellosis (WHO, 2004) even though they are susceptible *in vitro*. Tetracycline was effective against none but *Shigella* spp. However, its use is not recommended because of its ineffectiveness against *Shigella dysentriae*. It should only be used when the species of *Shigella* has been identified not to be *S. dysenteriae*; else, it should not be used.

It could be concluded that local weaning cereals in Jos, Nigeria do contain enteropathogens hence the need for significant changes in the processing of weaning cereals to prevent cases of infantile diarrhea.

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