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MICROBIAL CONTAMINATION OF WEANING FOODS:A RISK FACTOR FOR DIARRHEA AND SUBSEQUENT MALNUTRTION IN CHILDREN IN OSOGBO, NIGERIA

ADELANI TIJANI[1], MICHAEL OOLADEJIFWACN[2] & OGUNGBE OLUWABUNMI[3]

[1]Senior Lecturer, Department of Nursing, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

[2,3]Department of Nursing, Ladoke Akintola University of Technology, Ogbomoso, Nigeria

ABSTRACT

Children in the world die in thousand annualy from diarrheal diseases; hundreds of millions suffer from frequent episodes of diarrhea and consequent impairment of nutritional status. Thus, the aim of the study was to assess the microbial contamination of foods used in weaning as well as the mothers' hygienic practices in food preparation for the children in Osogbo, Nigeria. A weaning foods samples were obtained five areas within Osogbo Local Government Area. An experimental research design was employed. The samples were analysed for microbial load at the microbiology research laboratory, Federal University of Technology, Akure. The experiment involved the use of standard microbiological methods for isolation and identification of existing microorganisms in food samples collected. The results shows the mean bacteria count was $35x\ 10^3 \pm 5 \times 10^2$cfu/ml and the mean fungal count $1.3 \times 10^3 \pm 1.2 \times 102$cfu/ml. These values exceed the standards for food safety limits as set by the Food and Agricultural Organisation(Bacterial load ≤ 10,000cfu/ml; Fungal load≤700 cfu/ml). A total of 30 microorganisms comprising 16 bacterial species, and 14 fungal species (4 yeasts species and 10 mould species) were isolated. The organisms includes bacteria, moulds as well as yeasts. The bacterial species are *Acinetobacter calcaecelicus, Bacillus coagulans, Bacillus substilis, Micrococcus luteus, Aerococcus viridans, Branhamella catarrhalis, Staphylococcus aureus, Corynebacterium xerosis, Bacillus cereus, Lactobacillus lactis, Streptococcus lactis, Lactobacillus ferrentium, Lactobacills plantarum, Zymomonas mobilis, Thiocapsa rosea and Thiocapsa roscopersicina*, the yeasts species isolated are *Candia vini, Saccharomyces cerevisiae and kleokella apiculata*, the moulds species found are *Candida albiclum, Penicillium italicum, Apergillus falvus, Apergillus repens, Articulospora inflata, Pleurothecium recurvatum, Pseudotorula hoterospora, varicosporium inflata and Gonatobotryum sapiculatum.* The results further showed only that 20% of the subjects got their water supply from borehole in which water ran through pipe to supply household. The rest 80% got their water from hygienically doubtful sources: 40% got water from community (open) well where only one water drawer was share by all; 20% from personal household (covered) well; rest 20% got water from municipal water which they stored in big containers at home (to last up to 7 to 10 days). The assessment of the microbial load of the water used to prepare food were found to contain pathogenic microorganisms. The assessment of the microbial load of the water used to prepare food were found to contain pathogenic microorganisms. The environment where food were being prepared was also found to have contributed to food contamination. From the study, 60% of the houses used pit laterines; only 20% hosehold had functional water closet system. In 40% households, there were physical presence of flies in the latrine area and visible stool around the latrine. Evidence of food contamination was revealed by the percentage of the children that had gastrointestinal ailments: 80% of the subjects said they usually visit health facilities within Osogbo four to six times per year on account of diarrheal diseases affecting their children. Similarly, 60% of them have lost babies in the past as a results of unresolved gastrointestinal ailments; and 40% still had family friends and relations having their

children under hospital admission due to various childhood diseases. It was recommended that government should provide portable water on daily basis to the populace through the provision of pipe borne municipal water. It was also recommended that the government should enforce the digging of at least a ventilated improved pit latrine in every household to curtail the spread of faeco-oral infection facilitated by flies.

KEYWORDS: Microbial Contamination of Weaning Foods

INTRODUCTION

According to WHO report in the African region, diarrheal diseases are the leading causse of morbidity and mortality in children under five years of age (WHO African Report, 1996). It was indicated that each child in the African region has five episodes of diarrhea per year and that 800,000 children die each year from diarrhea and dehydration. Of the estimated total 10.6 million deaths among children younger than five years of age world wide, 42% occur in the African region. Global estimates of the number of deaths due to diarrhea have showm a steady decline, from 4.6 million in the 1980s to 3.3 million in the1990s to 2.5 million in the year 2000(Policy Project/Nigeria, 2002 and UNICEF 2004).Contaminated weaning foods account for a substantial proportion of diarrhoeal diseases among infants and young children, especially in developing countries. Worldwide (excluding China), it is estimated that 1400 million episodes of diarrhoea occur annually in children under the age of 5 year. In 1990 alone, over 3 million children died from contaminated weaning foods (Esrey, 1990). Several studies have linked the the pathogen causing diarrhoea to contaminated weaning foods in developing countries. According to Black, Brown, Becker, Abdul-Alim and Merson (1989), Gomes (1991) and Oluwafemi Ibeh (2011), up to 70% of diarrhoeal episodes in children under weaning could be due to pathogens transmitted through food and water. Unfortunately, the importance of food safety in the prevention of diarrhoeal diseases is often overlooked or neglected among Africans. It is often observed that the strategies for prevention of diarrhoeal disease and associated malnutrition are limited to promotion of breastfeeding and improving water supply and sanitation, thus neglecting the need to educate foodhandlers, particularly mothers, in food safety (Henry, 1989). Not infrequently, studies of why children suffer from diarrhoea overlook the relevant factors related to food safety (WHO, 1992). Exclusive breastfeeding for the first four months, and if possible for six months, is the recommended method of feeding full-term infants by healthy, well nourished mothers. The advantages that breast milk accord to the baby are well-documented. However, after six months, breast milk alone is not sufficient both in quantity and quality to meet the nutritional requirements of the child, especially in energy and micronutrients such as iron, zinc and vitamin A. As the child grows older, therefore, it is necessary to supplement the breast milk with other foodswhich start as liquid foods and slowly progress to solids. During the weaning process, the introduction of foods other than breast milk into an infant's diet begins andgradually, breastfeeding sessions is reduced until finally stop (Kikanfunda, Walker andTumwine, 2003). The weaning period isvery critical in the life of a child and if not well managed, might lead to malnutrition and other health implications (Okafor, Ozumba, Oribanjo, Onu, Dauda and Olatunji, 2008). Breast-feeding as a major source of infant nutrition has been shown to protect children against the development of diarrhea in Africa and other parts of developing world (Uwaegbute, 1990). In contrast, foods given as complementary feeding probably contribute to occurrence of diarrhea in infants. There are ampoule studies that demonstrated the relationship of hygiene practices to increase risk of diarrhea. Moterjemi, Kaferstein, Moy and Quevedo (1993) showed that early introduction of milk-formula or solid food increases exposure to enteropathogens and has been associated with increased rates of acute diarrhea. Studies in The Gambia in the 1970s demonstrated heavy contamination of gruels used as complementary foods in breast-fed infants (Barell and Rowland,

1992). Millet flour, cooking water, empty serving bowls, and even simmering gruel were all found to be contaminated with *E. coli*; colony counts of this and other organisms increased steadily with storage at room temperature. Although the investigators speculated that contamination of complementary foods increased the risk of diarrhea, a subsequent study by the same group failed to document an association between water or weaning food contamination and higher rates of diarrheal morbidity.Two more recent studies have found an increased risk of diarrhea associated with the consumption of maize-based weaning foods. However, in one of these studies, the association was only significant in children living in rural communities. The failure to demonstrate an association between the contamination of complementary foods and diarrhea in these studies may be due to a less comprehensive search for specific enteropathogens. The use of fermented cereal-based weaning foods, which have been demonstrated to inhibit the growth of potentially pathogenic bacteria, especially enteropathogenic *E. coli*, represents a simple, locally acceptable measure that may prove to be useful in curtailing the proliferation of diarrheal disease pathogens in food. Storage of food in proximity to household defecation sites was evaluated in one study in Nigeria and found to be significantly associated with acute diarrhea among residents (Kikanfunda et al., 2003). In a study carried ou by Mannam and Rahman (2001), it was discovered that hygienic practices of mothers during preparation of weaning foods have significant correlation with the occurrence of diarrhoea in children. In Africa, the first solid food and the most popular weaning food is a thin cereal gruel that has different names depending on the type of cerealsand locations within the West African sub region. In various parts of Nigeria, children weaned between the ages of 3-24 months, the usual first weaning food is called pap, *akamu*, *ogi*, or *koko* and is made from maize (*Zea mays*), millet (*Pennisetum americanum*), or guinea corn (*Sorghum* spp.). Available reports show thatpap from corn or sorghum does not usually meet the nutritional requirements of the infant (Okafor et. Al., 2008; and Ayo, Oluwalana, Idowu, Ikuomola, Umor and Yusuf, 2011). Traditional weaning foods in West Africa are known to be of low nutritive value and are generally characterized by low protein, low energy density, and high bulk. Maize pap or *koko* has been implicated in the aetiology of protein-energy malnutrition in children during the weaning period. Cereal-based diets have lower nutritional value than animal-based ones. The problems inherent in the traditional West African weaning foods and feeding practices predispose the infant to malnutrition, growth retardation, infection, diarrhea and high mortality (Ayoet al., 2011). It is however sickening that globally, out of 10 million children under the age of fivethat die each year, more than half of the deaths are attributable to diarrhea and malnutrition. If adequate health systems were put in place, two-third of the deaths could be prevented (WISHH, 2006). Amongst the primary functions of the health care workers is to promote appropriate feeding practices for infants and young children. According to Murray and Loppez (1996), diarrhea is one of the top three causes of childhood mortality in Sub-Saharan Africa. In 2000 alone, approximately 40% of childhood deaths in Sub-Sahara Africa was attributed to diarrheal disease (WHO, 2005). Vulnerability of children to the disease, which posed a strong threat to the lives of African children, is strongly associated with parental care and household environmental factors among others. For instance, household environmental factors, particularly water and toilet facilities have been found to be significantly related to the incidence of childhood diarrhea (Murray and Lopez (1998); Fayehun and Omololu, 2008).The association between diarrhoeal diseases and malnutrition has been the subject of extensive studies, and these have been reviewed by Sheth and Dwivedi (2006). Despite the complex nature of the interaction between infectious diseases and malnutrition, it is generally accepted that infectious diseases can affect children's growth once weaning is initiated (WHO, 2003). Children can develop multiple nutrient deficiencies and it will be important to improve all nutritional parameters at the same time in order to have the best impact on health outcomes. For instance, protein-energy malnutrition can impair iron absorption. Providing iron without addressing protein-energy malnutrition is

likely to be less effective and have more potential for adverse effects that can occur with iron supplementation. Deficiency in three micronutrients; iodine, iron and vitamin A are widespread affecting more than a third of the world's population (Ogumba, 2012). Other micronutrients of concern are B vitamins, vitamin C and Zinc, and according toKaul, Kaur, Sunita, and Chibber (1996), individuals and families suffer serious consequences including learning disabilities, impaired work capacity, illness and death. To stem the tide therefore,and to improve the public health, there is need for considerable research interest in understanding the etiology of diarrhea and institute preventive measures to safeguard the lives of African children against the disease. Hence, the aim of the study was to assess the weaning foods with a view to identifying possible contributing factors to persistent diarrhoea among under five children in Osogbo, Nigeria.

MATERIAL AND METHODS

The study was carried out from August to October, 2013. To investigate the microbial contamination in weaning foods of children in Osogbo Local Government Area (LGA), an experimental research design was employed. The experiment involved the use of standard microbiological methods in the microbiology research laboratory at the Federal University of Technology, Akure. Microorganisms in food samples collected were isolated, bacteria and yeasts identified by morphological observations of cultures and biochemical tests and microsopic observation of moulds.Osogbo is the capital city of Osun State Nigeria. Osogbo falls within 47km^2 area of South Western Nigeria with, postal code 230. It is within the tropical rain forest region of Nigeria where climate is hot and humid which is influenced by rain-bearing southwest monsoon winds from the ocean and dry northwest winds from the Sahara Desert. Osogbo is situated at latitude $7^0$46' N and longitude 4^0 34' E. It is about 370m above the mean sea level. According to the national populaton census of 2006, the total population of Osogbo LGA was put at 156,694. There are 15 wards within the LGA. (Osogbo LGA Secretary, 2014).

The weaning food samples were sourced from ramdomly selected households in osogbo LGA. A total of 10 visits was made to major wards in the LGA where assessments of households was made from, house to house before deciding on houses to select. The houses selected as sample were chosen based on the following criteria:Houses where there were mothers with children of ages 0 - 2 years;mothers who gave their consent; and mothers who have commenced the weaning foods with the use of complementary foods. The selected houses are situated in Isale osun, Oke-ayepe, Oke-Baale, Kola Balogun and Ogo-Oluwa areas. Mothers from selected households have been informed of the date and time of sample collection. Structured questionnaire were served the respondents. The questionnaire has three sectionson namely: socio-demographic characteristics, household environmental hygiene and health seeking behaviour. The questionnaire was first served and retrived immediately after filling on 25th September, 2013. All the samples were also collected same day in the morning as presented in table 1. The food samples were collected aseptically into tightfitted containers by carefully lifting the lid halfway while the gruel was poured into it as shown in plate 1. The samples were then transported to the microbiology research laboratory, Federal University of Technology, Akure. Simple ramdomization probability method was used for selection of the 5 sampling areas (wards) and for selection of sample source.

Microbial Analysis

The following were carried out on the collected samples:

Media preparation and serial dilution

Isolation of Microorganisms (yeasts, moulds and bacterial)

Total Heterotrophic Count

Colonial Characterization

Gram Staining

Biochemical Tests (Catalase, Oxidase, Citrate, Starch Hydrolysis, Sugar Fermentation, Strict Anaerobes,

Voges Proskauer, Methyl Red, Indole, Lysine Decarboxylase, Ziel-Nelseen, Motility, and Coagulase tests)

LIMITATIONS

Seqeuel to microbial load determination, the aflatoxin levels (AFM1, AFG1, AFG2, and AFB2) could have been determined by immunoaffinity column extraction and high-performance liquid chromatography (HPLC) with detection of fluorescence. However, these could nor be done due to non availability of the laboratory equipment required for such high-level analysis. Also, it was a bit difficult to convince mothers to participate in the research; due to cultural bias or other unknown reseans. During the initial sample survey, it could be inferred that some mothers withheld some important information or gave wrong information especially concerning the incidence of diarrhea in their children and the treament employed. Some even denied commencement of weaning even when it was obviously they had started. These impediments notwithstanding, the results obtained are viable and can be generalised.

Table 1: Areas Samples Were Collected from, the Time and Date, the Type of Weaning Food and their Components

Samples	Areas	Date of Collection	Time of Collection	Type of Food	Food Component
A	Isale-osun	25th September, 2013	8 : 30 am	Fermented Corn Pap (ogi)	Corn
B	Oke-ayepe	25th September	9 : 20 am	Fermented guinea corn gruel (ogi oka baba)	Millet
C	Oke-Baale	25th september	10 : 45 am	Fermented Corn pap (ogi agbado)	Corn
D	Kola-balogun	25th september	9 : 50 am	Fermented corn gruel (ogi)	Corn
E	Ogo-oluwa	25th september	7 : 30am	Cereal (comercially marketed as Golden morn)	Maize flour, soya bean flour(dehulled), salts, acidity regulators, vitamin A, milk

RESULTS

In the laboratory analysis of the five samples, a total of 30 microorganisms comprising 16 bacterial species, and 14 fungal species (4 yeasts species and 10 mould species) were isolated. The organisms includes bacteria, moulds as well as yeasts. The bacterial species are *Acinetobacter calcaecelicus, Bacillus coagulans, Bacillus substilis, Micrococcus luteus Aerococcus viridans, Branhamella catarrhalis, Staphylococcus aureus, Corynebacterium xerosis, Bacillus cereus, Lactobacillus lactis, Streptococcus lactis, Lactobacillus ferrentium, Lactobacills plantarum, Zymomonas mobilis, Thiocapsa rosea and Thiocapsa roscopersicina*. The yeasts species isolated are *Candia vini, Saccharomyces cerevisiae and kleokella apiculata*, while the moulds species found are *Candida albiclum, Penicillium italicum, Apergillus falvus, Apergillus repens, Articulospora inflata, Pleurothecium recurvatum, Pseudotorula hoterospora, varicosporium inflata and*

*Gonatobotryum sapiculatum.*The results shows the bacterial count ranged from 11 x 10^2 cfu/mlto 1.42 x 10^4cfu/ml (mean 3.5x 10^3 ± 5 x 10^2cfu/ml) and the fungal count ranged from 5 X 10^2 to 0.42 X 10^4 cfu/ml (mean 1.3 X 10^3 ± 1.2 x 102cfu/ml). These values exceed the standards for food safety limits as set by the Food and Agricultural Organisation (of bacterial load of≤ 10,000cfu/ml and fungal loadof ≤700 cfu/ml). Table 2 and 3 show the list of bacteria isolated from the samples, their number of occurences and percentages.

Table 2: Percentage of Occurrence of Bacteria Isolated From the Weaning Foods

S/N	Lists of Bacteria (First 10)	Number of Occurence	Percentage (%)
1	*Acinetobacter calcaocelicus* (Gram positive)	1	4
2	*Bacillus coagulans* (Gram positive)	1	4
3	*Bacillus subtilis* (Gram positive)	1	4
4	*Bacillus cereus* (Gram positive)	1	4
5	*Aerococcus viridans* (Gram negative)	2	8
6	*Branhamella catarrhalis* (Gram negative)	2	8
7	*Staphylocoocus aureus* (Gram positive)	3	12
8	*Corynebacterium xerosis* (Gram positive)	1	4
9	*Lactobacillus lactis* (gram positive)	1	4
10	*Lactobacillus plantarum (gram positive)*	2	8

Table 3: Percentage of Occurrence of Bacteria Isolated From the Weaning Foods

S/N	Lists of Bacteria (Rest 6)	Number of Occurence	Percentage (%)
11	*Lactobacillus fermentum* (Gram positive)	2	8
12	*Streptoccocus lactis* (Gram positive)	1	4
13	*Mirococus luteus* (Gram positive)	3	12
14	*Zymomonas mobiilis* (Gram negative)	1	4
15	*Thiocapsa roscopersicina* (Gram negative)	1	4
16	*Thiocapsa rosea* (Gram negative)	2	8

Table 4 shows the nature of occurence of bacteria isolated and identified from different locations within Osogbo local government. From sample A (collected from Isale-Osun), the following seven spieces of bacteria were isolated and identified:*Thiocapsa roscopersicina, Thioscapsa rosea, Lactobacillus plantarum, Bacillus subtilis, Aerococcus viridans, Brahamella carttarhalis* and *Mirococus luteus.* Four species of bacteria were isolated from sample B (collected from Oke-Ayepe area) and these include *Aerococcus viridans*, *Brahamela carharhalis*, *Mirococus luteus* and *Bacillus coagulans.* Sample C (sourced from Oke-Baale) containedthe following five spieces: *Zymomonas motilis,Lactobacillus lactis*, *Corynebacterium xerosis*, *Staphylocoocus aureus* and *Mirococus luteus* while Sample D (taken from Kola-Balogun area) had five species of bacteria isolated and identified as *Lactobacillus plantarum, Lactobacillus ferrentium,Thiocapsa rosea,Bacillus cereus* and *Staphylocoocus aureus.* The last sample -C (collected from Ogo-Oluwa area) contained *Actinobacter calcaocelicus, Staphylocoocus aureus, Streptoccocus lactis* and *Lactobacillus ferrentium*as the five bacteria isolated and identified. See plate 1 for the grown culture on petri dishes.

Table 4: Occurrence of Bacteria from Weaning Meals in Osogbo Local Government

Isolated Organisms	Sample A (From Isale Osun Area)	Sample B (Oke Ayepe)	Sample C (Oke- Baale)	Sample D (Kola Balogun)	Sample E (Ogo-Oluwa)
Acinetobacter calcaocelicus	-	-	-	-	+
Bacillus coagulans	-	+	-	-	-
Bacillus subtilis	+	-	-	-	-

Table 4: Contd.,

Micrococus luteus	+	+	+	-	-
Aerococcus viridans	+	+	-	-	-
Branhamella catarrhalis	+	+	-	-	-
Staphylocoocus aureus	-	-	+	+	+
Corynebacterium xerosis	-	-	+	-	-
Bacillus cereus	-	-	-	+	-
Lactobacillus lactis	-	-	+	-	-
Streptoccocus lactis	-	-	-	-	+
Lactobacillus fermetium	-	-	-	+	+
Lactobacillus plantarum	+	-	-	+	-
Zymomonas mobilis	-	-	+	-	-
Thiocapsa rosea	+	-	-	+	-
Thiocapsa roscopersicina	+	-	-	-	-

KEY: + means Presence of organism; - meansAbsence

Plate 1: Showing the Grown Cultures on Petri Dishes

The mean bacteria count of the five weaning foods sourced from Osogbo local government shows that the dilutions used were dilution 2 and dilution 4. It was observed that sample A sourced from Isale-Osun area has the highest microbial loads while sample E sourced from Ogo-Oluwa area has the lowest microbial load. The microbial load of all the samples in colony forming units per ml decreased in the following order: Sample A (Isale-Osun) > Sample C (Kola-Balogun) > Sample D (Oke-Baale) > Sample B (Oke-Ayepe) > Sample E (Ogo-Oluwa) with respect to dilution factor 4. Sample A sourced from Isale-Osun had the highest microbial loads (microbial loads1.12 x 10^4cfu/ml cfu/ml and 1.42 x 10^4cfu/ml with respect to dilution factors 2 and 4 respectively. Sample B Sourced from Oke-Ayepe with respect to dilutions 10^2 and 10^4respectively also has its microbial load 15 x 10^2 (cfu/ml) and 0.60 x 10^4 (cfu/ml). Sample C from Kola-Balogun had loads 22 x 10^2 and 0.73 x 10^4 cfu/ml with respect to dilution factors 2 and 4. With respect to dilution factors 2 and 4, the microbial loads of sample D from Oke-Baale were 17 x 10^2 cfu/mland 0.68 x 10^4 respectively. Sample E from Ogo-Oluwa with the lowest microbial loads has its loads recorded as 11 x 10^2 cfu/ml and 0.54 x 10^4 (cfu/ml) with respect to dilution factors 2 and 4. See table 5.

Table 6 shows the lists of fungi isolated from the weaning foods, their number of ooccurence and percentages. 19 isolates of fungi with 13 species of fungi were isolated and identified from the weaning foods. The fungi species with the highest level of occuerrence is penicillium italicum (15.8%) and was found in three out of the five samples.

Table 5:Mean Microbial Count of Isolated Bacteria

Samples	Dilution Factor 2 (cfu/ml)	Dilution Factor 4 (cfu/ml)
Sample A	1.12 x 10^2	1.42 x 10^4
Sample B	15 x 10^2	0.60 x 10^4
Sample C	22 x 10^2	0.73 x 10^4
Sample D	17 x 10^2	0.68 x 10^4
Sample E	11 x 10^2	0.54 x 10^4

Table 6: Occurrence of Fungi from Weaning Meals in Osogbo Local Government

S/N	Lists of Fungi Isolated	Number of Occurence	Percentage (%)
1	*Kleokella apiculata*	1	5.3
2	*Saccharomyces cerevisae*	2	10.5
3	*Candida vini*	1	5.3
4	*Penicillium italicum*	3	15.8
5	*Apergillus flavus*	2	10.5
6	*Apergillus repens*	2	10.5
7	*Articulospora inflata*	1	5.3
8	*Pleurothecium recurvatum*	1	5.3
9	*varicosporium elodeae*	1	5.3
10	*Pseudotorula hoterospora*	1	5.3
11	*Gonatobotryum apiculatum*	1	5.3
12	*Candida albicum*	2	10.5
13	*Aspergillus fumigatus*	1	5.3

Two species of fungi were isolated and identified from Sample A which include *Penicillium italicum, Aspergillus flavus* and *Varicosporium elodea*.No yeasts was identified from this sample. Sample B has *Aspergillus repens,Varicosporium elodea, Saccharomyces cerevisae* and *Penicillium italicum.*while sample C was observed to harbour the spores of *Articulospora inflata, Aspergillus repens, Saccharomyces cerevisae, pleurothecium reccrratum* and *Candia*

albidum. Sample D contains five species of fungi isolated and identified. They are *Aspergillus fumigatus, Klekella apiculata, Penicillium italicum. Gonatobotryum apiculatum* and P*seudotorula hoterospora*, while Candia albidum, Candia vini and *Aspergillus flavus, Saccharomyces cerevisae* exist in sample E. See table 7.

Table 7: Occurence of Fungi Isolated from the Weaning Foods Sourced within Osogbo Local Government

Isolated Organism	Sample A	Sample B	Sample C	Sample D	Sample E
Kleokella apiculata	+	-	-	+	-
Saccharomyces cerevisae	-	+	+	-	-
candida vini	-	-	-	-	-
Penicillium italicum	-	+	-	-	-
Apergillus flavus	+	-	-	-	+
Apergillus repens	-	+	+	-	-
Articulospora inflata,	-	-	+	-	-
Pleurothecium recurvatum	-	-	+	-	-
varicosporium elodeae	-	+	-	-	-
Pseudotorula hoterospora	-	-	-	+	-
Gonatobotryum sapiculatum	-	-	-	+	-
Candia albiclum	-	-	-	-	+

The results further showed only that 20% of the subjects got their water supply from borehole in which water ran through pipe to supply household. The rest 80% got their water from hygienically doubtful sources: 40% got water from community (open) well where only one water drawer was share by all (see plate 2 for the physical appearance of the water); 20% from personal household (covered) well; rest 20% got water from municipal water which they stored in big containers at home (to last up to 7 to 10 days). The assessment of the microbial load of the water used to prepare food were found to contain pathogenic microorganisms. The environment where food were being prepared was also found to have contributed to food contamination. From the study, 60% of the houses used pit laterines; only 20% hosehold had functional water closet system. In 40% households, there were physical presence of flies in the latrine area and visible stool around the latrine. Evidence of food contamination was revealed by the percentage of the children that had gastrointestinal ailments: 80% of the subjects said they usually visit health facilities within Osogbo four to six times per year on account of diarrheal diseases affecting their children. Similarly, 60% of them have lost babies in the past as a results of unresolved gastrointestinal ailments; and 40% still had family friends and relations having their children under hospital admission due to various childhood diseases. See table 8 for various water sources of respondents.

Table 8: Water Ources of Some Households and the Mean Bacterial Count Obtained

Samples	Area	Water Source Description	Mean Microbial Count
A	Isale-osun	open well	1.12×10^4
B	Oke-ayepe	open well	0.60×10^4
C	Oke-baale	Municipal water	0.68×10^4
D	Kola-balogun	covered well	0.73×10^4
E	Ogo-oluwa	bore-hole	0.54×10^4

Plate 2: Showing Water with Alum Added for Cooking in One of the Households

DISCUSSION

Children, according to Esrey(1990), are highly susceptible to environmental toxicants for various reasons ranging from lower detoxification capacity, immunologically immaturity and rapid growth which necessitates relatively higher intakes of air, food, and water than adults. Children are particularly vulnerable in the developing countries where environmental sanitation is not given adequate attention. Early childhood exposure to harmful bacteria and fungi, through these means, therefore contributes immensely to the immediate and future health status of children.WHO (1996) reports that mortality rates from diarrhea were expected to decrease by 30 to 50% in most areas of the world except in the Sub-Saharan Africa where the decline was estimated to be only 3%. Consequently, approximately 40% of childhood deaths from diarrhea worldwide was expected to occur annually in Sub-Saharan Africa. Though the number of death as a results of diarrhoea could not be ascertained in the current study, the large percentage of the respondents (80%), who said they usually visit health facilities 4 to 6 times per year on account of diarrhoea diseases affecting their children, points to the fact that there is no appreciable decline in diarrheal diseases among children in Africa. In this study, a total of 27 species of microorganisms were isolated. The organisms includes bacteria, moulds as well as yeasts. In the sample of weaning foods assessed, 16 bacterial species, and 14 fungal species were identified. According to Black et al. (1989) and Gomes (1991) various pathogens have been implicated in diarrheal diseases causation and most of the pathogens have been isolated from complementary foods commonly consumed in developing countries. The results of the present study corroborates this findings as all the samples (100%) collected from the five study areas had these pathogens isolated in them. These include bacteria such as *Escherichia coli*, *Shigella* spp., *Salmonella* spp., *Vibrio cholera*, *Campylobacter jejuni, Bacillus cereus*, *Staphylococcus aureus* and *Clostridium perfringens*.WHO (1993) has linked the microbial contamination of weaning food to the annual worldwide death of 13 million infants and children less than five years. The causes of diarrhoeal diseases have also been ascribed to water supply and sanitation (Oluwafemi and Ibeh, 2011). In another study, sources of water have been associated with food contamination in developing countries (Henry et. al. 1989; and Fayehun and Omololu, 2008). The results of this study show that only that 20% of the subjects got their water supply from borehole in which

water ran through pipe to supply household. The rest 80% got their water from hygienically doubtful sources: 40% got water from community (open) well where only one water drawer was share by all; 20% from personal household (covered) well; the rest 20% got water from municipal water which they stored in big containers at home (to last up to 7 to 10 days). The assessment of the microbial load of the water used to prepare food were found to contain pathogenic microorganisms. The food preparatory methods too was found to have contributed to food contamination. When children are fed with foods that have been contaminated by water or through unhygienic preparation, gastrointestinal diseases result (Motarjem et al., 1993). The results of the study is in line with this discovery as 80% of the subjects said they usually visit health facilities within Osogbo four to six times per year on account of diarrheal diseases affecting their children. Similarly, 60% of them have lost babies in the past as a results of unresolved gastrointestinal ailments; and 40% still had family friends and relations having their children under hospital admission due to various childhood diseases. It is noteworthy that 80% of the subjects observed that their babies were not having frequent diarrhoea when they were still being breasfeed exclusively.Moreover, results of several studies observed that over 50% of diarrheal cases in children eventually result in malnutrition (Henry et. al.1989; UNICEF, 2008;and Fayehun and Omololu, 2008**).** Repotorts from other studies have successfully linked the maternal and childhood under nutrition to the cause of the annual 3.5 million deaths in children.

Diarrhea has been reported to have serious adverse effects on nutritional status and was discovered to occur at the exact period of starting complementary foods among children (Motarjemi et al., 1993). This is not fully established in this study but the percentage of babies (60%) that had one form of diseases or the other at the time of data collection showed that there is truism in initiation of complementary feeding and occurrence of gastrointestinal infections. This is further corroborated by the results of the bacterial loads of each of the samples as presented: sample A : 1.12×10^4 ; sample B : 0.60×10^4; sample C : 0.73×10^4 ; sample D : 0.68×10^4; and sample E : 0.54×10^4 . The respective fungal loads are 0. 42 x 10^4 cfu/ml; 0.27 x 10^4 cfu/ml; 0.26 x 10^4cfu/ml; 0.24 x 10^4 cfu/ml; and0.19 X10^4 cfu/ml. The sample with the lowest fungal count which is Sample E,sourced from Ogo-Oluwa, had the counts of 0.19 X10^4 cfu/ml.The reason for Ogo-Oluwa samples having the lowest count might be due to the fact that the level of environmental sanitation in that area was much better than in the rest four study atreas. Also, the area is inhabited by elites who are mainly civil servants and bank workers. Each house in the area has its own borehole which serves as source of water for domestic use. Studies by Sheth et al., (2006) and Mannam and Rahman (2001) revealed that weaning foods prepared under unhygienic conditions are heavily contaminated with pathogenic agents and are a major risk factor in the transmission of diseases, especially diarrhea. Methods of food handling and storage, source of safe water as well as and personal hygiene contribute to the potential risk of developing acute diarrhea and subsequent malnutrition.In this study, the food samples obtained were not stored,they were collected straight from the stove, yet a sample (A) was found to harbour a colony count as high as 1.12×10^4 cfu/ml. It is plausible then to infer that there would be a possible increase in colony counts if samples had been allowed to be stored by mothers and then collected just at the temperature it is to be fed to children. According to Kung'u et al. (2009), foods kept for more than 6 hours had higher average microbial load (6.5×10^6 cfu/ml) than the foods that were kept for less than 6 hours (6×10^6 cfu/ml). From the study, 60% of the houses used pit laterines; only 20% hosehold has functional water closet system. In 20% household, there were physical presence of flies in the latrine area and visible stool around the latrine. This findings of this study is in line with the views expressed by Murray and Lopez (1998). The Report observed that improper refuse disposal was associated with an increased prevalence of diarrhea.

CONCLUSIONS

Nigeria is still faced with challenges of diarrhea among children and it contributes to more death of children under five years of age. The main causes of malnutrition are poor feeding practices, poor quality complementary foods aggravated by diarrheal diseases due to poor hygiene practice, lack of clean water and inadequate sanitation. Majority of the children who survive are often locked in a cycle of recurring illness and growth faltering. Promotion of exclusive breastfeeding for the first 6 months of life andcontinuation of breastfeeding till two years of age, together with giving of hygienically prepared food that is of high nutritional value needs to be emphasised to mothers. These impact positively on child survival, growth and development.

Recommendations

- Government should make supply of portable water on regular basis its priority in the provision of social amenities for the populace. The present situation of providing water for people in the community once every month should be discourage as stored water is bound to harbour pathogens as the days of storage increases.
- Enforcement of ventilated improved pit toilet in each house should be carried out on regular basis. Town planners and authorities in building construction should be alive to their responsibilities to punish those who build house without making provision for decent toilet/latrine. By the time every household has its toilet, the level of food contamination will be greatly reduced.
- Home gardening should be encouraged among the populace so that common foods could be grown to cater for the needs of the children and adults alike. When this is done, the level of raw food contamination will be reduced drastically.

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