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HUMAN ENTERIC BACTERIA IN DISH WASHING WATER USED BY STREET FOOD VENDORS IN OTUOKE, BAYELSA STATE OF NIGERIA

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ABSTRACT

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Microbiological assessment of water emphasizes estimation of the hygiene quality of the water with reference to community health significance. The present study was undertaken to investigate the presence of some enteric bacteria in dish washing water used by different food vendors in Otuoke community in Bayelsa State of Nigeria. A total number of 14 water samples were collected with sterile bottles from street vendors at Apolota, Ebele, Apoloogbo, Eluan, Obebhegbolom, Asuka, Okporain zones of Otuoke community. The samples were immediately taken to the laboratory for analysis. Samples were analyzed for the presence of some human enteric bacteria. Samples were inoculated into various selective media such as MacConkey agar, Salmonella-shigella agar (SSA), and Thiosulfate citrate bile sucrose salt (TCBS) for the detection of E. coli, Salmonella, Shigella and Vibrio spp respectively. Klebsiella spp and Pseudomonas spp were isolated from SSA and MacConkey agar. All the samples were found to be contaminated with organisms in the range of 42 to 197cfu per ml of water sample. Out of 14 water samples, the occurrence of E. coli was 100%, Salmonella 87.5%, Shigella 64.3%, Vibrio 28.5%, Klebsiella 50% and Pseudomonas 42.8% as were found contaminated with the organisms respectively. The numbers of coliform bacteria and presence of some common enteric bacteria suggested that the water used by street food vendors in Otuoke does not satisfy the minimum quality requirement specified in the WHO standard for potable use, with implications for health and livelihood.

Keywords*: enteric bacteria, food vendors, waterborne diseases, dish washing waters, water quality*

INTRODUCTION

Street food is ready-to-eat food or drink sold in a street or other public place, such as a market or fair provided by hawkers or vendors, often from portable food booths, carts or trucks (Simopoulos *et al.*, 2011). The street vended foods are prepared under unhygienic conditions and unprotected from the immediate environment leading to a high degree of contamination. Thus, the microbiological quality of street vended foods becomes important as these could act as a major source for transmission of food borne infections and intoxications (Sharma and Mazumdar, 2014). Water is a critical material in many street-vended operations. Contaminated water can create a public health risk when it is used for drinking, washing of foods, incorporated in the food as an ingredient, used in the processing of food or used for washing equipment, utensils and hands. It is a well-known vehicle for enteropathogens such as *Esherichia coli*, *Salmonella spp*. and *Campylobacter spp*. amongst others (Sharmila, 2011). Safe water for human consumption (drinking and cooking) should be free from pathogens such as bacteria, protozoan parasites, viruses and meet the standard guidelines (WHO, 2004). However, lack of access to safe water, together with inadequate sanitation and hygiene are implicated in 88% of diarrheal diseases in both developed and developing countries and 2.2 million people die annually of diarrhea in developing countries (WHO, 2004; Tumwine, 2005). In many developing countries, people living in rural areas collect water from communal sources which are either exposed (e.g. unprotected wells, unprotected springs, and rivers) or improved (e.g. protected wells, boreholes and public standpipes) (Sobsey, 2002). The microbiological contamination of water may occur during collection, transportation and storage at the point-of-use due to secondary contamination factors (Sobsey, 2002; Gundry *et al.*, 2004).

Studies carried out in different regions of Asia, Africa and South America has pointed the unavailability of potable water for various activities at the vending site as a major concern. Due to the shortage of clean potable water, many vendors tend to re-use the water, especially for cleaning utensils and washing dishes (Sharmila, 2011). The water for washing and rinsing the utensils were rarely renewed and generally was observed to be dirty. In some others, the dishes washing places were characterized by the presence of small puddles, insects and animals. Unrenewal of dish washing waters explains their poor quality (Muinde and Kuria, 2005). In the present study, water samples used for washing dishes, equipment and utensils were collected from street vendors, from different location within Otuoke community in Bayelsa State. This research work was carried out to determine the health implication of feeding from such vendors through the investigation for presence of some pathogenic organisms such as enteric bacteria in water samples and subsequently quality of the water.

MATERIALS AND METHODS

Study site and samples collection chores

The current study was carried out to examine the quality of dish washing water used by food vendors in Otuoke community. Otuoke is a community in Ogbia local government area of Bayelsa State in the Niger Delta region of Nigeria. It is a rural community majority of its inhabitants are farmers and fishermen. The community depends on river water both for household usage and other domestic chores. But now all the rivers are polluted with petroleum oil spillage and sand dredging activities, it is difficult to get portable water which is a major problem in the community. The investigations were done during the period from March to July 2016. The study was conducted in Apolota, Ebele, Apoloogbo, Eluan, Obebhegbolom, Asuka, Okporain zones of Otuoke community from fourteen vending site. These water samples were collected in a sterile bottle directly from the water used by these food vendors for washing and rinsing of cooking utensils and equipment and immediately taken to the laboratory and analyzed.

Sample analysis

The pour plate method was employed; one ml of each sample was take aseptically with sterile pipette and transferred into a sterile petri dish (duplicates) before aseptically pouring the media in the plate. For each water sample, there was a plate for maconkey agar, SSA, and TCBS agar. After pouring, the plates were incubated for 24hours at 37 °C. After 24hours incubation, colonies were counted using colony counter. The average number of colonies in particular dilution was multiplied by the dilution factor to obtain the total viable count. The results of the total bacterial count were expressed as the number of colony forming units (CFU) per gram of water samples (IS 5402, 2002).Organisms from the original plates were sub-cultured onto a fresh media-MacConkey agar, Salmonella-Shigella (SSA) agar and Thiosulfate citrate bile sucrose salt (TCBS) to obtain pure culture which was used for Gram staining and biochemical test.Enteric bacteria isolated on respective selective or differential media were identified on the basis of their colonial, morphological and Biochemical properties following Bergey's Manual of Determinative Bacteriology(Holt *et al.*,1994).

RESULTS AND DISCUSSION

The plate counts of the three media were as presented in Table 1: It was observed that there were heavy contamination of the samples with the highest count recorded was 197 and lowest was 42 CFU per ml. There were bacterial growths in all the macConkey plates as well as the Salmonella-Shigella plates. The thiosulfate citrate bile sucrose salt plate had growth only in samples from Ebele 1 (44 CFU per ml), Ebele 2(57 CFU per ml), ApoloOgbo 1 (53 CFU per ml) Asuka 2 (42 CFU per ml), Okporain 1(69 CFU per ml), Okporain 2 (88 CFU per ml) were observed. This is in line with the work of Mahejabin and Sayyad (2015) who reported large number of bacterial growth in 11 water Samples, which indicates heavy contamination of the water sample which is one of the important source of microorganisms in street vended food.

Table 1: Total Plate Count (PCA) in CFU per ml

Sample	MAC	SSA	TCBS
Apolotama 1	101	96	nil
Apolotama 2	95	82	nil
Ebele 1	85	97	44
Ebele 2	72	98	57
ApoloOgbo 1	86	67	53
ApoloOgbo 2	92	51	nil
Eluan 1	65	64	nil
Eluan 2	72	92	nil
Obegbegbolom 1	60	77	nil
Obegbegbolom 2	78	105	nil
Asuka 1	92	89	nil
Asuka 2	105	49	42
Okporain 1	195	95	69
Okporain 2	142	197	88

MAC = MacConkey agar**,** SSA = Salmonella Shigella Agar, TCBS = Thiosulfate Citrate Bile Sucrose Salt

Cultural characteristics, morphological and staining characteristics of bacteria recorded from water samples by gram staining are presented in Table 2 and Table 3 respectively. All the isolated organisms appeared to be pink in colour at the end of Gram's procedure, this shows that they picked up the colour of the counter stain safranin indicating negative Gram staining result. Another common feature observed was that they appeared in rod forms. The bacteria that were identified in the present study include *E. coli, Salmonella, Shigella, Vibrio, klebsiella and pseudomonas*. This result is in line with the findings of Tambekar *et al.*(2011) they reported 93% pathogenic bacteria contamination with *Escherichia coli, Staphylococcus aureus, Klebsiella species Pseudomonas species* indicating poor bacteriological quality of the water sample of *Panipuri*. Saxena *et al.* (2013) documented *E. coli, Staphylococcus aureus, Bacillus cereus, Shigella* and *Salmonella* from street vended foods in *Jaipur* city of *Rajasthan* and concluded that, isolation of these organisms from street foods is as a result of contaminated water

used in food vending activities. Similarly, Barro *et al.* (2006) found out that pathogens like *Salmonella* and *Shigella* have been detected in the water used by vendors for dishwashing. In most cases, running water is not available at vending sites; hands and utensils washing are done sometimes without soap, providing nutrients for insects and rodents, which may carry food borne pathogens (Tambekar *et al*. 2009)

Table 2: Cultural characteristics of the bacterial isolates of water samples

Media	Bacteria	Colony characteristics
Maconkey agar	*E.coli*	Pink colonies
SSA	*Salmonella*spp	Colonies with blank centres
SSA	*Shigella*spp	Circular, clear, colourless transparent colonies
TCBS agar	*Vibrio*spp	yellow colonies
Maconkey agar	Pseudomonas	colourless or white colonies
SSA	Klebsiella	mucord/green colonies

SSA = Salmonella Shigella Agar, TCBS= Thiosulfate Citrate Bile Sucrose Salt

Table 3: Morphological and staining properties of the bacterial isolates of water samples by gram's staining

Shape	Arrangement	Gram 'S Staining Reaction	Bacterial Isolates
short plump rods	single, paired or in short chains	(-) ve	*E.coli*
rod shape	single, some are together.	(-) ve	*Salmonella spp*
short rods	single straight rod	(-) ve	*Shigella*
curved rods	single, and slim	(-) ve	*Vibrio*
rod shape	short rod	(-) ve	*Pseudomonas*
shorter rods	single, some are in cluster	(-) ve	*Klebsiella*

Table 4: Biochemical characteristic of bacterial isolates

Catalase	Oxidase	Indole	Coagulase	Motility	Bacteria
+ve	-ve	+ve	-ve	non motile	E.coli
+ve	-ve	-ve	-ve	motile	Salmonella
+ve	-ve	-ve	-ve	non motile	Shigella
+ve	+ve	+ve	-ve	motile	Vibrio
+ve	+ve	-ve	-ve	motile	Pseudomonas
+ve	-ve	-ve	-ve	non motile	Klebsiella

+ve = Positive -ve = Negative

Results of Biochemical Tests are presented in Table 4. All isolated organisms produced bubbles in the presence of hydrogen peroxide indicating positive catalase result. In addition, all the detected organisms exhibited no coagulation during coagulase test indicating negative coagulase result. Salmonella shows negative oxidase and indole results while vibrio shows positive oxidase and indole result. Out of 14 water samples, *E. coli* were present in 100% of the samples, *Salmonella spp* were present in 85.7%, *Shigella*64.3%, *Vibrio spp* 28.5% whereas *Klebsiella* and *Pseudomonas* were found in 50% and 42.8% of samples, in other words, these organisms appeared in samples from Okporain 2, Asuka 2, Obegbegbolom 1, Ebele 2, Ogbo 2 out of Eluan 1 and Apolo 14 samples respectively. These reports were similar to the findings of Agard *et al.*, (2002) who reported that 35% of street foods in *Trinidad* and *Tobago* were contaminated by *E. coli* while 57.5% of water used by vendors was contaminated by coliforms. These reports were similar to the findings that the stored water used by consumers and vendors, at the vending site, showed heavy bacteriological contamination of faecal origin (Dawson and Canet, 1991). Presence of coliform bacteria such as *E.coli* and *klebsiella* indicates high degree of pollution and low sanitary quality of water. Bhat and Waghray, (2000) studied water samples from storage tanks used by some food vendors at different localities in *Pune*, India, it was revealed that 29.6% of the water samples were not conforming to the WHO standards of portability and had coliform counts of more than 16 per 100 ml, while fecal coliform counts were more than 16/100 ml in 15.5% of water samples, 4.5% of samples were positive for *E. coli* and 2.7% for enteropathogenic *E. coli.*

The isolation of coliform bacteria from water samples indicates fecal contamination. Studies done to find out the bacteriological quality of the water used by some street vendors have revealed frequent contamination with coliforms and fecal coliforms. Such heavily contaminated water is a primary source of diarrheal diseases to the street food consumers (Dawson and Canet, 1991). The presence of *E.coli* and other common human pathogenic enteric bacteria indicates that the water used by street food vendors in Otuoke has poor quality. According to WHO (2000) guidelines, coliform may be used as water quality indicator, and if such bacteria are not detectable, the water can be said to be potable water. The presence of these enteric bacteria in all samples varied insignificantly except for Okporain water samples. *Vibrio* spp were majorly found in Okporain water samples and

the numbers of enteric bacteria in the samples (Okporain) were relatively high indicating massive faecal contamination. This was almost related to the findings of Abhishek *et al.* (2015) who reported that out of 36 water samples, the occurrence of *E. coli* was present in 61%, *Salmonella* (25%) *Staphylococcus aureus* (14%) and *P. aeruginosa* (53%). Several authors have observed those bacteria from dirty dish washing waters and other sources can adhere to utensil surfaces and constitute a risk for contamination during food vending (Bhaskar *et al.,* 2004; Mosupye *et al..*, 2000).

Trabulsi *et al*. (2002) found out that typical enteropathogenic *E. coli* strain is a leading cause of infantile diarrhoea in developing countries, whereas they are rare in industrialized countries. Unlike *E.coli*, humans infected with *salmonella* can carry the bacteria in the gut without signs of the disease. Infected humans can harbour the bacteria for considerable periods of time. About 5% of patients clinically cured from typhoid fever remain carriers for months or even years (Trabulsi *et al*. 2002). These people can be chronic holders of the bacterium in the gut, and constitute the main reservoir of the bacteria in the environment. *Staphylococcus aureus* was also identified in the water samples. It is a pathogenic bacterium responsible for several severe health problems. According to Pujari *et al.* (2007) the onsite unsanitation that is increasingly adopted in various developing countries is possibly responsible for high levels of these bacterial contaminations in water sources. Water for street food preparation and dish washing was not always enough. This resulted in vendors using little water for washing utensils hence hygiene was compromised. This study is in agreement with a study done in Accra on the safety of street food, which found out that running water was not available (Mensah *et al*., 2002). Without enough water, hygiene and sanitary practices cannot be met. Latham (1997) emphasises that personal hygiene can only be achieved if adequate water is available. Therefore, vendors should have sufficient potable water for drinking, preparation of all kinds of foods and sufficient running water for all washing operations.

CONCLUSION

Based on the results it can be concluded that the street vendors in Otuoke community were using contaminated water in their dish washing. Quality of water is essential for all human beings. There is an urgent need that potable water is ensured in all means to reduce risk in the area. Government authorized body should provide potable water to the community because safe water is an essential pillar for health.

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