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ISOLATION OF SALMONELLA SPP IN 'WARA' (LOCAL CHEESE) FROM THREE DIFFERENT LOCATIONS IN ADO-EKITI, EKITI STATE, NIGERIA

ABSTRACT

Three samples of wara(local cheese) were collected from three different locations in Ado- Ekiti, Ekiti-State. Bacteria species were isolated from the samples. In the analysis, the total plate count for each of the samples was 2.67×10^6 CFU/g for sample A, 2.76×10^6 CFU/g. For sample B and 2.72×10^6 CFU/g in sample C. the total salmonella count was 2.14×10^6 CFU/g for sample A, 2.03×10^6 CFU/g for sample B and 2.11×10^6 CFU/g in sample C. also, 4 different types of bacterial were obtained from the isolates. These include Salmonella spp. With occurrence of 12 (60%), Klebsiella spp with occurrence of 2(10%), Lactobacillus spp with occurrence of 1(5%) and Escherichia coli with occurrence of 5(25%) The isolated Salmonella spp precisely was tested against eight different types of antibiotics which 9(77%) were resistance to ceftazidine, 4(34%) were resistance to cefuroxine, 7(64%) were resistance, to cefotaxine, 10(87%) were resistance to amoxicillin, 5(41%) were resistance to augmentin and gentamycin, ofloxacin and nitrofurantoin showed no resistance to the Salmonella isolates.

Key words: antibiotics, bacterial contamination, coliforms, 'wara', Salmonella.

INTRODUCTION

Milk is an extremely nutritious food. It is an aqueous colloidal suspension of proteins, fat and carbohydrates that contains numerous vitamins and minerals such as calcium, phosphorus, sodium, potassium and magnesium (Sangoyomi et al., 2010). Milk proteins are ideal in that they are complete and have high essential amino acids composition. Although milk and its various derivatives such as butter, yoghurt and cheese are vital human foods. It provides an excellent medium for the growth of many kinds of micro-organisms (Adesokan et al., 2009). Worldwide cow's milk is the most commonly used but milk from water buffalo, goats, sheep, camels and yaks is also used in various parts of the world.

Cheese as one of milk derivatives or products is the curd or hard substance formed by the coagulation of milk of certain mammals by rennet or similar enzymes in the presence of lactic acid. It is produced by added or adventitious microorganisms. When moisture has been removed then cutting and pressing, which shaped the cheese in a mould and then ripened by holding for sometime at suitable temperatures and humidity (Raheem et al., 2009).

Cheese is produced at suitable temperature and humidity (Raheem et al., 2009). Cheese is produced throughout the world in wide-ranging flavors, textures and forms. Cheese consists of proteins and fat from milk, usually the milk of cows, buffalo, goats or sheep. It is produced by coagulation of the milk protein casein. Typically, the milk is acidified and addition of the enzyme rennet causes coagulation. The solids are separated and pressed into final form. Some cheeses have molds on the rind or throughout. Most cheeses melt at cooking temperature.

Hundreds of types of cheese are produced. Their styles textures and flavors depend on the origin of the milk (including the animal's diet), whether they have been pasteurized, the butterfat content, the bacteria and mould, the processing and aging. Herbs, spices or wood smoke may be used as flavoring agents (Callon et al., 2010). Compared with the quantities produced in Europe and North America, the amount of cheese produced in Africa is quite small. Most of the cheese produced in Africa is made on a small scale and generally at farm level due to shortage in milk production (Uaboi et al., 2010).

'Wara' is a Nigeria soft white unripened cheese that originated from Fulani cattle rearers in the northern part of the country. It is commonly produced by Fulani women from unpasteurized cow milk and sold along the major streets of Nigeria. 'Wara' is a fresh cheese, that is, moist curd that has been cut and drained of the whey but never ripened and is unsalted and uncolored. The cheese is prepared by coagulating fresh cow milk with the leaf extract of the Sodom apple (Colotropis procera) or pawpaw (Carica papaya). About one kilogram of cheese will be obtained from about five liters of milk (Sangoyomi et al., 2010; Adetunji and Alonge, 2009).

'Wara' processing involves the use of rudimentary equipment, in many cases, starter cultures are not used as processing conditions are not normally standardized or optimized. Although, very recently an alternative coagulant "lemon juice" was introduced to the processing of 'Wara' soft cheese to reduce the microbial load. 'Wara' is often eaten in various forms either as normal cheese, a flavored snack and meat substitute in sauces or as fried cake or sandwich filling

Organisms associated with milk and milk products include Streptococcus spp, Lactococcus spp, coliform bacteria especially E. coli, Micrococcus spp, Clostridium butyricum, Bacillus spp, Brucella abortus, Salmonella spp, Pseudomonas spp and some fungi. All these organisms are from various sources and they act on different substrates in milk, thereby producing various products.

Cheeses are ready-to-eat (RTE) food products that do not undergo any further treatment to ensure their safety before consumption. Contamination of 'Wara' with food borne pathogens may occur at several stages.Microbial contamination of wara cheese may originate from various sources. As contamination may occur from the equipment used in the production of the wara and from the environment where it is being produced. Bacteria can gain access to the products from which infected animal through milk secretions or by secondary contamination occurring during post processing. Coliform bacteria, Salmonella, Mycobacterium bovis, Brucella and Listeria may contaminate the products Coliform are facultative-anaerobic, Gram negative, non-sporulating and rod-shaped bacteria which include: Escherichia, Enterobacter, Klebsiella, and Citrobacter. These micro-organisms are considered as a collection of relatively harmless bacteria that live in large numbers in intestinal tract of humans and warm blooded animals. Coliform are always found in raw milk but with sanitary methods of production the number can be kept very low. A specific subgroup of coliform is the faecal coliforms bacteria, the most common member being E.coli. These bacteria may be separated from coliform by their ability to make infection and are associated with the faecal material of humans and animals. The presence of faecal material and is an indicator for a potential health risk (Ogunbanwo et al., 2011). Although cheese is generally considered to be relatively safe food, if consumed unpasteurized can be health hazard due to contamination with coliform and other pathogenic bacteria (Ogunbanwo et al., 2011). Previous laboratory studies have confirmed the presence of Listeria monocytogene in milk and milk products produced in Nigeria include; ice cream, fermented milk, the local butter (Adetunji et al., 2008). Aspergillus and Mucor spp. were also detected in the cheese (Vahid et al., 2009).

Moreover, human have been found to serve as a contamination source for cheese with pathogenic bacteria like Staphylococcus aureus (Callon et al., 2010). Salmonella bacteria when found in food can pose a very big problem an example is Salmonellosis. Food borne infection that may result when unpasteurized dairy products are consumed. Salmonella can be referred to as one of the most common enteric (internal) infection. It is a type of bacteria that causes typhoid fever and many other infections of internal organs. Salmonella bacteria multiply in the small intestine and invade the gut lining.

This study is to determine the incidence of Salmonella spp in 'Wara' (local cheese) and evaluate the hygiene quality of 'Wara' cheese in three locations in Ado-Ekiti, Ekiti State. This is to enlighten people about the risk involved in consumption of contaminated 'Wara' without frying.Since it is usually consumed cold and at room temperature ($27^{o}C$).
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MATERIALS AND METHOD

Collection Of Samples

Three samples of 'Wara' were bought from different retailers at 3 different highly populated locations within Ado-Ekiti metropolis, Ekiti-state, Nigeria. The locations are University School gate, Mary Immaculate (Old Garage) and Olorunda, all in Ado-Ekiti. The sampling were collected aseptically immediately after production. They were collected in a sterilized McCartney bottles and kept inside a food flask containing ice cubes and conveyed to the laboratory for immediate analysis.

Microbial Isolation

One gram of 'Wara' is homogenized in 9ml sterile diluents (5g peptone (oxoid) and 1000ml of distilled water, pH 4.2) with a sterile blender, and serially diluted. One ml each from dilution 10^{-3} and 10^{-4} of the homogenized samples was placed on nutrient agar to determine total aerobic count; on salmonella-shigella agar for salmonella count using spread plate method. Pure strains of the microbes were gotten by successive streaking on the same agar substrate. Under daylight observation, some microbial colonies have metallic sheen appearance while some possessed grayish black colouration on Bismuth sulphide agar. Further identification were done using biochemical procedures.

Antibiotic Sensitivity Test

The antibiotics susceptibility of the organisms was performed using Gram negative and the third generation multiple disc. The antibiotics multiple discs used include; ceftazidime (30µg), cefotaxine (30µg), ofloxacin (5µg), amoxicillin (30µg), augmentin (30µg), nitrofurantoin (30µg), gentamycin (10µg) and cefaoxime (30µg).

A suspension of the isolate is prepared to 0.5 McFarland standards in trypton soy broth and then swab with sterile cotton swab evenly on Mueller Hinton agar in a Petri-dish. The antibiotic Gram negative multiple discs were placed aseptically on the surface of the inoculated agar and incubated aerobically at 37^0C for 24 hours.

RESULTS AND DISCUSSION

Twenty microbes were isolated from three different 'wara' samples at various locations in Ado-Ekiti, Ekiti State. Table 1 shows the total plate count and total salmonella count. Sample A total plate count was 2.67 x10^6 CFU/g and salmonella count was 2.14 x10^6 CFU/g, sample B total plate count was 2.76 x10^6 CFU/g and total salmonella count was 2.03 x 10^6 CFU/g while sample C total plate count was 2.72 x 10^6 CFU/g and its total salmonella count was 2.11 x10^6cfu/g. During the course of this work, it was observed that wara was contaminated because; it was not produce under sterile or standard hygienic conditions. Also because the producer did not practice standard sanitary procedures such as disinfecting of hands and sterilization of equipment and materials (Adetunji et al., 2010) .Also in addition crude implements were used in production. Since milk is a balanced culture for most microorganisms hence, contamination easily takes place. (Ibrahim and Falegan, 2013).

In the biochemical table for the bacteria isolated in table 2, only one gram- positive bacteria organism was found which is Lactobacillus spp. Salmonella spp was found to be the highest and most occurrence bacteria isolated and Escherichia coli was also isolated. Klebsiella spp were another gram negative bacteria organism isolated from the samples. Different bacteria vary in biochemical characteristic. The biochemical test done for the isolates include citrate test, urease, coagulate test, catalase test, Voges Proskauer test, Gram staining, and sugar fermentation test such as (Glucose, sucrose, lactose, xylose, and maltose).

Table 3 shows the occurrence of each of the bacteria isolated and the percentage distribution. Four (4) different bacterial isolates were obtained, theses includes Salmonella spp. which are the most occurring bacteria isolates of 12 (60%) Klebsiella spp 2(10%), Lactobacillus spp 1(5%), and Escherichia coli 5(25%).

'Wara' was being made by Fulani women who prepared it themselves using traditional methods and basic equipments. There is a need for further study on standardizing the production methods and prevalence of these pathogenic bacteria in this food commodity (wara) as also suggested by Maria et al. (2011), This results and data from the current study on wara cheese will be helpful to public Health professionals to identify this a high risk food item and to identify that pathogenic bacteria species are food borne and their presence in ready to eat food illustrates the need to keep a careful studies over this pathogens.

The antibiotics susceptibility pattern of Salmonella spp isolated from the 'wara' samples (Table 5). In the antibiotics susceptibility test, eight different types of antibiotics were used and they are ceftazidine with 9 resistance (77%) of the isolates, cefuroxine with 4 (84%) resistance, gentamycin which no organism was resistance to it, cefotaxine with 7(64%) resistance, amoxicillin with 10(87%) resistance, ofloxacin which no organism was also resistance to, augmentin with 5(41%) resistance and nitrofurantoin with no resistance. The effects of this resistance have been discussed and demonstrated by Beaumont et al. (2011).

CONCLUSION

The result of this study demonstrate that consumers of the 'wara' cheese in Ado–Ekiti, Ekiti State Nigeria were at a serious risks of salmonella infection since Salmonella spp was isolated from the finished product (ready to eat food).

RECOMMENDATION

Microbiological analysis of the cheese product revealed that the sample was contaminated by Salmonella and hence not safe for consumption without frying. Therefore, regular sterilization of dairy equipment, washing of utensils and proper hygiene during production of 'wara' were some of preventive measures that could be applied to check contamination of Salmonella. This can be achieved by educating the major distributor of milk products and 'wara' cheese producers on the benefit of disinfection and sterilization of the equipments. The health of the producer is also important.

Sample sources	Total plate count	Total salmonella count
A	2.67	2.14
B	2.76	2.03
C	2.72	2.11

Table 1: Bacterial count (x 10^6 CFU/g) of 'wara' samples

Isolates	Gram staining	Coagulase	Catalase	Citrate	Urease	Oxidase	V.P	Maltose	Xylose	Sucrose	Cellulose	Lactose	Probable bacteria
1	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
2	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
3	-	-	-	+	-	-	+	-	-	-	+	-	*E. coli*
4	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
5	-	-	+	+	-	-	-	-	+	+	+	-	*Salmonella* spp
6	-	-	+	+	+	-	+	-	-	-	+	-	*Klebsiella* spp
7	-	-	-	+	-	-	-	-	--	+	+	-	*Salmonella* spp
8	-	-	+	+	-	-	+	-	-	-	+	+	*E. coli*
9	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
10	-	-	+	+	-	-	+	-	-	-	+	+	*E. coli*
11	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
12	-	-	-	+	-	-	+	-	-	-	+	+	*E. coli*
13	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
14	+	+	+	-	+	-	-	+	-	+	-	+	*Lactobacillus* spp
15	--	-	+-	+	-	-	-	-	-	+	+	-	*Salmonella* spp
16	-	-	-	+	-	-	-	-	-	+	+	-	*Salmonella* spp
17	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
18	-	-	+	+	-	-	+	-	-	-	++	+	*E. coli*
19	-	-	+	+	-	-	-	-	-	+	+	-	*Salmonella* spp
20	-	-	+	+	-	-	-	-	+	-	+	+	*Klebsiella* spp

KEY: + = Positve, - = Negative, V.P = Voges Proskauer.

Table 2: Biochemical characterization of the isolated bacteria

Isolated bacteria	Frequency of occurrence	Percentage distribution
Salmonella spp	12	60%
Klebsiella spp	2	10%
Lactobacillus spp	1	5%
Escherichia coli	5	25%

Table 3: Percentage distribution of isolated bacteria

Antibiotics	Resistance (%)	Intermediate (%)	Susceptible n (%)
CAZ	9(77%)	3(25%)	-
CRX	4(34%)	6(50%)	2(12.5%)
GEN	-	1(6%)	11(95%)
CTX	7(64%)	4(35%)	1(6%)
AMX	10(87%)	2(12.5%)	-
OFL	-	2(12.5%)	10(87%)
AUG	5(41%)	6(50%)	1(6%)
NIT	-	1(6%)	11(95%)

KEY:
CAZ- Ceftazidine   CRX- Cefuroxine   CTX- Cefotaxine,
GEN- Gentamyxine   OFL - Ofloxacin   AMX - Amoxicillin
AUG- Augmentin   NIT- Nitrofurantoin

Table 4: Susceptibility pattern of Salmonella isolated from WARA (cheese)

REFERENCES

1. Adebiyi, A.O., Adebiyi, A.P. and Olaniyi, E.O. (2008). Nutritional composition of sorghumbicolour starch hydrolysed with analyses from Rhizopus spp. African J. of Biotech; 4(10):1089-1094.

2. Adesokan, I.A., Odetombo, B. B., and Olubamiwa, A.O. (2009). Bio preservative activity of Lactic acid bacteria on suya produced from poultry meat. African Journal of Biotechnology 7: 3796-3800

3. Adetunji, V.O; Ikheloa, J.O, Adedeji, A,M; &Alonge, D.O. (2009). Evaluation of the bacterial in milk products sold in south – western Nigeria. Nigeria Veterinary Journal, 24(3): 92-96.

4. Adetunji, V.O, Alonge, D.O, Singh, R.K. and Chen, J. (2007). Production of wara, a West African soft cheese using lemon juice as coagulant (LWT). J. Chem. Swiss soc. Food Sci. Technol; 41:331-336.

5. Alalade, O.A. and Adeneye, J. A. (2010). The effect of storage period on the chemical composition and coliform microflora of wara cheese. International Journal of Dairy Science, 1:126-130.

6. Ashaye OA, Taiwo OO, Adegoke GO,(2009). Effect of local preservative (Afremoniumdanielli) on the chemical and sensory properties of stored warankasi.African Journal of Agricultural Research 1:010-016.

7. Brito, J.R.F., Santos, E.M.P., E.F., Lange, C.C., Brito, M.A.V.P., SOUZA, G. N. (2008). Retail survey of Brazilian milk and mina"sfrescal cheese and a contaminated dairy plant to establish prevalence, relatedness, and sources of Listeria monocytogenes isolates, Applied and Environmental Microbiology. 74:4954-4961.

8. Callon, C., Gilbert, F. B., Cremoux, R. D; & Montel, M.C. (2010). Application of variable number of tandem repeat analysis to determine the origin of S.aureus contamination from milk to cheese in goat cheese farms. Food control. 19:143-150.

9. Cetinkaya, F. and Soyutemiz, E. (2007). A study on survival of Listeria monocytogenesduring manufacture and ripening of kashar cheese.Turkish Journal of Veterinary and Animal Science 28:927-932.

10. CENTRE for Disease Control and Prevention. (2004). Preliminary food net data on the incidence of food borne illnesses – selected sites, United States, 2002. MMWR Morb.Mortal.Wkly. Rep. 52:340-3.

11. Centers for Disease Control and Prevention (2005). Summary of notifiable disease, United State. MMWR, 48:1-104

12. FAO.(2008). Milk for health and wealth.FAO, Rome, Italy.

13. Gilbert, D. N., Moellering R. C., Sande, M. A. (2007). The Sanford guide to antimicrobial therapy 2007. Antimicrobial therapy inc; hyde park VT.

14. Glynn, M. K., Bopp, C., Dewitt, W. K., Dabney, P., Mokhtar, M., Angulo, F. J. (2006). Emergence of multi drug- resistance Salmonella enterica serotype typhimurium DT104 infection in the united states. N Engl j Med 338:1333-8.

15. Humphrey, T. J., Greenwood, M., Gilbert, R. J., et al. (2010). The survival of Salmonella in shell eggs cooked under simulated domestic conditions. Epidem. 103:35-45.

16. Ibrahim, T.A. and Falegan,C.R. (2013) Anti-bacterial activities of crude cell free supernatants of Lactic acid bacteria from wara (Nigeria soft cheese). Research and Reviews.Journal of food and Dairy Technology. Vol.1 (2) pp.1-4.

17. Maria, kousta, Mario, Mataragas, PanagiotisSkandaus, Eleftherios, Drosinos. (2009). Laboratory of food quality control and hygiene.Agricultural University of Athens.Department of Food Science and Technology.

18. Ogunbanwo, S. T., Sanni, A. I., Onilude, A. A. (2011). Effect of bacteriocinogenic Lactobacillus spp. On the shelf life of fufu, a traditional fermented cassava product. World Journal of Microbiology and Biotechnology 20:57-63.

19. Raheem, V.D and saris, D.E.J. (2009). Characterization and Application of Calotropisprocera, a coagulant in Nigeria wara cheese.International Journal of Food Science and Technology, in press.

20. Raheem, V.D. and Saris, D.E.J. (2009). Inhibition of toxicogenic Bacillus licheniformis553/1 in Nigeria wara soft cheese by nisin producing LactococcuslactisLAC 309, manuiscript.

21. Rodiriques, E, Arques, J. L., Nuez. M., Gaya, P., Medina, M. (2008). Combined effect of high pressure treatments and Bacteriocin – producing lactic acid bacteria on inactivation of Escherichia coli 0157:H7 in raw milk cheese. Applied and Environmental Microbiology 7:3399-3404.

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22. Sangoyomi, T.E., Owoseni A.A. and Okerokun, O. (2010). Prevalence of enteropathogenic and Lactic Acid Bacteria species in wara. A local cheese from Nigeria African journal of microbiology research vol. 4(15), pp. 1624-1630, 4 Specialist cheese makers association, (2009). The specialist cheese makers code of the best practices. Available from http:llwww.specialist cheese makers.

23. Threlfall, E. J., Skimner, J. A., Ward, L. R. (2010). Detection of decrease in vitro susceptibility to ciprofloxacin in Salmonella enteric serotypes Typhi and paratyphi A. J AntimicrobChemother. 48(5):740-1.

24. Uaboi – Egbmni, P.O, P.N. Okolie, T.L. Akintunde, O., Bisi – Johnson, L. Enwe, N. and P.O. Bessong. (2010). Pakistian Journal of nutrition 9(9):920-25,ISSN 1680-5194@Asian network for scientific information. 2U.S Department of Agriculture (USDA) USDA: APHIS: US: CEAH, 2005. NRRC Building B, M.S 2E7 2150 Centre Avenue fort Collins, Co 80526-8117 970.494.7000.

25. Vahid, M., Aissi, Mohammed, M., Soumanous, HonoreBankole, Fatiou Toukourou, comalan A. De Souza. (2009). Evaluation of hygienic and mycological quality ofm local cheese marked in Benin. Australian journal of basic and applied science, 3(3):2397-2404. ISSN 1991-8178.

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