ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 11, Issue, 08, pp.6210-6213, August, 2019

DOI: https://doi.org/10.24941/ijcr.36248.08.2019

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

BACTERIOLOGICAL EVALUATION OF SOFT CHEESE SOLD FOR HUMAN CONSUMPTION IN ADO-EKITI, NIGERIA

[1,*]Oluwapelumi B. Oluboyo, Ph.D., [1]Oghenekevwe E. Etso, B.MLS., [1]Funmilayo J. Akinseye, Ph.D., [1]Yomi R. Akele, Ph.D., [1]Adeola O. Oluboyo, Ph.D., [2]Funmilayo A. Adewumi, M.Sc.

[1]Department of Medical Laboratory Science, College of Medicine and Health Sciences, Afe Babalola University, Ado- Ekiti, Ekiti State, Nigeria
[2]Department of Medical Microbiology and Parasitology, College of Medicine, Ekiti State University, Ado-Ekiti, Nigeria

ARTICLE INFO

Article History:
Received 20th May, 2019
Received in revised form
14th June, 2019
Accepted 18th July, 2019
Published online 31st August, 2019

Key Words
Local Cheese,
Bacterial Contamination,
Susceptibility, Resistance.

Corresponding author:
Oluwapelumi B. Oluboyo

ABSTRACT

Background: Dairy products strongly enhance the growth of microorganisms. Soft cheese sold in Ado-Ekiti are mostly prepared locally and hawked by women who are mostly illiterates. The paucity of information on health safety of consumers of the local cheese necessitates this study. The prevalence, microbial load and antibiotic sensitivity pattern of isolated bacteria were investigated. **Methods:** Twenty samples of soft cheese randomly collected from various hawkers were processed. One gram of the curd was homogenized in 9 ml of peptone water and 0.1 ml of various dilutions from 10^{-1} to 10^{-6} was plated on standard media. Aerobic plate count, total coliform count and other bacteria count were enumerated after culture. The antibiotic susceptibility pattern of isolated organisms was evaluated using disk diffusion method. **Results:** The mean aerobic plate count of bacteria and the total coliform count were 6.8 x 10^7 and $3.7x10^7$ respectively. The most prevalent organism was *Escherichia coli* 18 (90%) followed by *Klebsiella pneumoniae* 14 (70%), *Pseudomonas aeruginosa* 12 (60%), *Salmonella typhi* 6 (30%), *Staphylococcus aureus* 5 (25%) and *Bacillus cereus* 5 (25%). The mean colony forming unit/g of cheese was highest in *Escherichia coli* ($8.2x10^6$), followed by *Klebsiella pneumoniae* ($6.4x10^6$), *Pseudomonas aeruginosa* ($9.3x10^5$), *Staphylococcus aureus* ($6.9x10^4$) and *Bacillus cereus* ($4.6x10^4$). *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa*, and *Bacillus cereus* were susceptible to ofloxacin and ciprofloxacin. *Salmonella typhi* and *Staphylococcus aureus* were susceptible to ceftaxidime, cefuroxime, gentamycin, ofloxacin and ciprofloxacin. **Conclusion:** Local cheese sold in Ado-Ekiti may not be safe for human consumption.

Citation: Oluwapelumi B. Oluboyo, Oghenekevwe E. Etso, B.MLS., Funmilayo J. Akinseye, Ph.D., Yomi R. Akele, Ph.D., Adeola O. Oluboyo, Ph.D., Funmilayo A. Adewumi, M.Sc.. **2019.** "Bacteriological evaluation of soft cheese sold for human consumption in Ado-Ekiti, Nigeria", *International Journal of Current Research*, 11, (08), 6210-6213.

INTRODUCTION

The soft cheese called "wara" (a Yoruba cheese) is a dairy product consumed in the western part of Nigeria. It is mainly prepared and sold by the Fulani women living among the Yoruba ethnic group. The preparation of this local cheese is based on heating milk to coagulate the protein in it. Both the mass of coagulated milk (curd) and the liquid part (whey) contain nutrients suitable for body development. The Curd is mainly made up of milk proteins (casein) and milk fat; while whey mainly contains water, milk sugar (lactose), protein (serum proteins) and B-vitamins (Ayodeji, 2006; Ebing, 2006). Yoruba cheese is a ready-to-eat (RTE) food product; that do not undergo any further treatment to ensure their safety before consumption. Bacterial contamination of the product with food-borne pathogens may happen either from infected animal or during processing or post processing.

The milking process is such that contamination of milk with bacteria is almost inevitable. Therefore milk and milk products need to be evaluated for microbial content to determine its quality as worthy food. Milk has been referred to as an excellent culture medium for the growth and multiplication of many kinds of microorganisms (Singh, 2011; Melese, 2015). Microbial load of ready-to-eat foods between 0 -10^3 are acceptable, 10^4-10^5 (Melese, 2015; ICMSF, 1996) is tolerable and 10^6 and above is unacceptable (ICMSF, 1996). Fresh milk collected from a healthy cow is reported to have less than 1000 CFU/ml, but this may increase up to 100 fold or more upon storage at normal temperature (Alebel *et al.,* 2014). Therefore, storage of milk in hygienic containers at low temperatures shortly after milking will certainly delay the proliferation of microorganisms in milk between the time of collection in the firm and transportation to the place of processing (Chye *et al.,* 2004).

Thus, total viable bacterial counting has become one of the accepted criteria for evaluating milk for human consumption and processing for dairy products. Coliform bacteria are always found in raw milk but with good sanitary methods of production the number can be kept very low. A specific subgroup of coliform is the faecal coliform bacteria, the most common member being *Escherichia. coli*. This bacterium is associated with the faecal material of humans and animals. The presence of faecal material is an indicator for a potential health risk (Ogunbanwo *et al.,* 2004). The processed Yoruba cheese is mostly hawked about in sizeable containers and dispensed to customers according to quantity demanded in polythene bags tied manually. The infection of these products by pathogenic organisms may be mainly after production as the heating process may have destroyed the vegetative cells of bacteria with the exception of some heat resistant organisms in the fresh milk. Therefore it is very pertinent that this product be tested for the presence of pathogenic organisms and the viable microbial load of the product (Hayes, 2001) to ensure safety of consumers. The paucity of information on health safety of consumers of local cheese in Ado-Ekiti necessitates this study. This study investigated the prevalence, microbial load and antibiotic sensitivity pattern of bacteria found in local cheese sold in Ado-Ekiti.

MATERIALS AND METHODS

The research was carried out in Ado-Ekiti, Ekiti State, Nigeria. The city is located between latitude 7^o 34' and 7^o 44' north of the equator and longitude 5^o 11' and 5^o 18' east of the Greenwich Meridian and 36.7 Km^2 land area (Olusegun, 2013). It has population of 424,340 (WPR, 2018). Twenty (20) samples of soft cheese (Yoruba cheese) were randomly collected from various vendors into sterile universal containers and transported in Ice Park to the Medical Microbiology Laboratory Unit of the Department of Medical Laboratory Science, Afe Babalola University, for immediate processing.

Bacterial count, isolation, characterization and identification: One gram of the curd was added to 9 ml peptone water and homogenized in stomacher bag. Dilutions of 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6} of the samples were prepared; and 0.1 ml of each dilution were plated using spread and pour plate technique in duplicates on standard media for bacteria.[12,13] The aerobic plate count (APC) was enumerated in pour-plate of plate count agar (OXOID, UK) after incubation at 30^oC for 72 hours. Plates for the estimation of total coliform count, *Escherichia coli, S. aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* were incubated at 37^oC for 48 hours. Numbers of colonies were counted and colony forming units (CFUs) were obtained by the product of the number of colony and its serial dilution factors. Selenite F broth (LIOFILCHEM, Italy) was inoculated with the marched soft cheese aseptically and incubated at 37^oC for 48 hours. It was then sub cultured on xylose-lysin dextrose (XLD agar (HIMEDIA) and incubated at 37^oC overnight for the isolation of enteric pathogens. All isolates were characterized using standard microbiology and biochemical tests (Barrow, 2004; Cheesbrough, 2006) Characterized bacterial isolates were identified according to standard procedures (Barrow, 1993; Garrity *et al.,* 2005).

Preparation of inoculums: Discrete colonies of the identified isolate was inoculated into peptone water and incubated overnight. The turbidity was compared with 0.5 McFarland turbidity standard equivalents to bacteria concentration of 10^7 CFU/ml (Mohammed *et al.,* 2014).

Antibiotic sensitivity testing: The disk diffusion method was used for antibiotic sensitivity testing. Using a sterile swab, the surface of Mueller Hinton agar was inoculated with the bacterial suspension by streaking the surface of the agar. The plates were allowed to dry for 10 minutes before antibiotic discs were aseptically applied to the surface of the agar. They were allowed a further drying period of 30 minutes and then incubated at 37°C for 18 hours. The of zones of inhibition produced by each antibiotic disc was measured and the isolates were classified as resistant, intermediate, and sensitive based on Kirby-Bauer National Committee for Clinical Laboratory Standards (NCCLS) modified disc diffusion technique (Cheesbrough, 2006).

Statistical analysis: The statistical analysis was performed using the Statistical Package for Social Science (SPSS) version 20. The prevalence of the organisms was recorded in percentages and the bacteria load recorded in mean and standard error of mean.

RESULTS

The mean aerobic plate count (APC) of bacteria on the 20 samples of "wara" and the total coliform count were 6.8 x 10^7 and 3.7x10^7 respectively. The most prevalent organism was *Escherichia coli* where 18 (90%) growth of the organism was recorded out of 20 samples. This was followed by *Klebsiella pneumoniae* 14 (70%), *Pseudomonas aeruginosa* 12 (60), *Salmonella typhi* 6 (30%), *Staphylococcus aureus* 5 (25%) and *Bacillus cereus* 5 (25%) (Table 1). The mean CFU/g of cheese was highest in *Escherichia coli*, followed by *Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Bacillus cereus* (Table 1). *Escherichia coli* was generally sensitive to only ofloxacin and ciprofloxacin but resistant to ceftazidime, cefuroxime, cefoxime, gentamicin, augmentin and nitrofurantoin (Table 2).

Klebsiella pneumoniae was only sensitive to ofloxacin and ciprofloxacin but resistant to all other tested antibiotics (Table 2). *Pseudomonas aeruginosa* recorded sensitivity to only ciprofloxacin, intermediate sensitivity to oflxacin and resistance to all other antibiotics (Table 2). *Salmonella typhi* recorded sensitivity against five antibiotics: ceftaxidime, cefuroxime, gentamycin, ofloxacin and ciprofloxacin, intermediate sensitivity against cefixime but resistant to augmentin and nitrofurantoin (Table 3). In similar manner, *Staphylococcus aureus* recorded sensitivity to six antibiotics: ceftaxidime, cefuroxime, gentamycin, ofloxacin, nitrofurantoin and ciprofloxacin but resistant to cefixime and augmentin (Table 3). *Bacillus cereus* recorded sensitivity to ofloxacin and ciprofloxacin, but resistant to ceftaxidime, gentamycin, nitrofurantoin, cefuroxime, cefixime, and augmentin (Table 3).

DISCUSSION

The aerobic plate count and total coliform count on the soft cheese hawked in Ado-Ekiti are higher than the international recommendation of microbial load (x 10^5) for ready-to-eat foods (ICMSF, 1996; IFCN, 2006).

Table 1. The prevalence of isolated bacteria on soft cheese (n=20) sold in Ado-Ekiti.

Bacteria	Occurrences (%)	Mean cfu/g of soft cheese
Escherichia coli	18 (90)	$8.2x10^6$
Klebsiella pneumoniae	14 (70)	$6.4x10^6$
Pseudomonas aerugisona	12 (60)	$9.3x10^5$
Bacillus cereus	5 (25)	$4.6x10^4$
Salmonella typhi	6 (30)	Isolated
Staphylococcu aureus	5 (25)	$6.9x10^4$

Table 2. Mean zone of inhibition and antibiotic sensitivity pattern of *Escherichia coli, Klebsiella pneumoniae and Pseudomonas aeruginosa* against some antibiotics

Antibiotics	Zone of inhibition and sensitivity of bacteria					
	Escherichia coli		*Klebsiella pneumoniae*		*P. aeruginosa*	
	Zone of inhibition (mm±Se)	Sensitivity	Zone of inhibition (mm ± Se)	Sensitivity	Zone of inhibition (mm± Se)	Sensitivity
CAZ 30µg	8.50±0.34	R	6.5±0.25	R	5.83±0.42	R
CRX 30µg	4.11±0.21	R	3.93±0.25	R	3.83±0.34	R
GEN10µg	14.00±0.34	R	7.79±0.32	R	4.92±0.29	R
CXM 5µg	4.67±0.28	R	6.21±0.24	R	0.00±0.00	R
OFL 5µg	21.56±0.65	S	15.43±0.25	S	21.58±0.63	S
AUG 5µg	5.28±0.38	R	4.14±0.25	R	3.30±0.39	R
NIT 300µg	7.39±0.36	R	4.36±0.23	R	3.50±0.29	R
CPR 5µg	21.50±0.34	S	19.64±0.63	S	23.17±0.44	S

Key: CAZ – ceftaxidime, CRX – cefuroxime, GEN – gentamicin, CXM – cefixime, OFL – ofloxacin, AUG – augmentin, NIT – nitrofurantoin, CPR – ciprofloxacin, R – resistant, S – susceptible, I – intermediate susceptibility.

Table 3: Mean zone of inhibition and antibiotic sensitivity pattern of *Salmonella typhi, Staphylococcus aureus* and *Bacillus cereus* against some antibiotics

Antibiotics	Zone of inhibition and sensitivity of bacteria					
	S. typhi		*S. aureus*		*Bacillus cereus*	
	Inhibition Diameter (mm±Se)	Sensitivity	Inhibition Diameter (mm±Se)	Sensitivity	Inhibition Diameter (mm±Se)	Sensitivity
CAZ 30µg	17.83±0.31	S	22.60±0.51	S	12.40±0.24	R
CRX 30µg	22.33±0.49	S	20.20±1.59	S	5.00±0.32	R
GEN10µg	22.67±0.33	S	23.20±0.58	S	10.20±0.73	R
CXM 5µg	12.67±0.33	I	8.60±0.24	R	6.00±0.45	R
OFL 5µg	20.83±0.54	S	24.00±0.84	S	23.00±0.45	S
AUG 5µg	6.33±0.21	R	8.20±0.37	R	3.80±0.37	R
NIT 300µg	5.17±0.60	R	17.20±2.82	S	10.00±0.55	R
CPR 5µg	23.67±0.61	S	22.40±0.51	S	25.40±0.51	S

Key: CAZ – ceftaxidime, CRX – cefuroxime, GEN – gentamicin, CXM – cefixime, OFL – ofloxacin, AUG – augmentin, NIT – nitrofurantoin, CPR – ciprofloxacin, R – resistant, S – susceptible, I – intermediate susceptibility.

Therefore, it could be said that the soft cheese ("wara") sold for consumption in Ado-Ekiti are unsafe. It is either that the heat treatment of milk during the preparation of the soft cheese did not sufficiently kill the bacteria or that there is gross contamination of the product after preparation. Information on microbial load of this product in Ado-Ekiti is scanty. The aerobic plate count (APC) recorded in this study ($6.8x\ 10^7$cfu/g of soft cheese) was higher than records in Ogbomoso ($4.4\ x\ 10^5$cfu/g) in Oyo State and in Ilorin ($2.6\ x\ 10^4$cfu/g) in Kwara State of Nigeria (Oladele, 2017) but lower than the findings in Ilorin metropolis where up to $4.0\ x\ 10^8$cfu/g was recorded (Sule, 2015).

Furthermore, the present study recorded a lower microbial load than recorded in Hawassa town ($8.3\ x\ 10^7$cfu/g) in Ethiopia (Senbetu, 2014). The present study recorded lower bacteria load in *Escherichia coli* than in Ede (Osun State, Nigeria), Ilorin (Kwara State), Osogbo (Osun State) and Ogbomoso (Oyo State) but higher bacteria load of Klebsiella than recorded in the four cities (Ogbolu *et al.,* 2014). Another researcher (Senbetu, 2014) recorded a lower bacteria load in total coliform count and *Staphylococcus aureus* than recorded in the present study.

The APC, constituting mainly of the spoilage and lactic acid bacteria, is a reliable pointer for monitoring the sanitary conditions employed during milking and preparation of dairy products from raw milk (Chambers, 2004). Hence education of soft cheese handlers, most of who are illiterates, about proper hygiene can considerably decrease the bacterial load on the products. Unsanitary practices associated with the handling of local cheese, inadequate knowledge on the hygienic way of preparing and packaging the product coupled with lack of refrigerator for the storage of packaged products might be some of the factors contributing to high bacterial load on the hawked soft cheese. Coliforms are group of bacteria excreted in large number with human excreta and animal droppings and are therefore found on the soil. It implies that unwashed hands after defecating and soiled hands constitute a major means of contaminating soft cheese with coliform organisms especially *Escherichia coli* and enteric pathogens such as *Salmonella typhi*. The presence of *Escherichia coli* on dairy products is a measure of the level of faecal contamination of the products. Hence their presence in large number in soft cheese is an indication that the product is potentially hazardous to the consumers' health (Godefay and Molla, 2000). *Salmonella typhi* could cause food poisoning or a more serious enteric

fever. *Bacillus cereus* and *Staphylococcus aureus* are important agents of food poisoning. Infections caused by *Salmonella typhi* and *Staphylococcus aureus* as a result of consumption of soft cheese sold in Ado-Ekiti may become amenable to treatment with ceftaxidime, cefuroxime, gentamicin, ofloxacin and ciprofloxacin as the organisms were inhibited by the drugs. However, infections due to *Escherichia coli, Klebsiella pneumoniae* and *Bacillus cereus* may post treatment challenges as these organisms recorded resistance to most of the tested antibiotics except ofloxacin and ciprofloxacin. The multiresistant nature of *Pseudomonas aeruginosa* is reaffirmed by this study as only ciproflaxin recorded inhibition of the bacterium. In conclusion, this study recorded mostly enteric bacteria on local cheese sold in Ado-Ekiti. This may be reflecting poor hygiene of the product handlers. The bacterial load on the product exceeded the acceptable limit for ready to eat foods. The product may therefore not be safe for human consumption. Infections caused by consumption of the product may be difficult to treat as the organisms are resistant to most of the antibiotics tested against them.

Key points

- The mean aerobic plate count of bacteria, 6.8 x 10^7 and the total coliform count, 3.7x10^7 on local cheese sold in Ado-Ekiti exceeded the acceptable limit for ready to eat foods.
- The organisms isolated are resistant to most of the antibiotics tested against them.
- There is urgent need for enlightenment campaign about the health risk associated with consumption of local cheese in Ado-Ekiti, Nigeria.
- Education of local cheese handlers on hygienic way to produce and market their products is advocated.

Conflict of interest: Not declared.

Founding: This study was exclusively funded by the authors. No form of grant was received from any organization.

REFERENCES

Ayodeji, KR., Regina, EO. Uzeh, RE. 2006. Microbiological and nutritional qualities of dairy products: Nono and Wara. Nat Sci., 4(3): 37-40.

Ebing, P., Rutger, K., 2006. Preparation of dairy products. Agromisa foundation and CTA: Wageningen.

Singh, V., Kaushal, S., Tyagi, A., Sharma, P. 2011. Screening of bacteria responsible for the spoilage of milk. J. Chem and Pharm Res., 3 (4): 348-50.

Melese, AR., Addisu, HA. 2015. Microbiological quality assessment of raw and pasteurized milk. Int. J. Food Sci. Microbiol, 2 (6): 87-91.

ICMSF [International Commission on Microbiological Specifications for Foods] 1996. Microorganisms in foods 5: Microbiological specifications of pathogens. Blackie Academic & Professional: London.

Alebel, W., Anbessa, D., Shiferaw, D. 2014. Bacteriological quality of detection of bovine mastitis pathogens of milk sold in Jimma Town. Bacteriology Journal, 4: 12-20.

Chye, FY., Abdullah, A., Ayob, MK. 2004. Bacteriological quality and safety of raw milk in Malaysia, Food Microbiol, 21: 535-41.

Ogunbanwo, S., Sanni, T., Onilude, A., 2004. Effect of bacteriocinogenic *Lactobacillusspp.* on the shelf life of fufu, a traditional fermented cassava product. World Journalof Microbiology and Biotechnology; 20: 57-63.

Hayes, MC., Raylea, RD., Murphy, SC., Carey, NR., Scarlet, JM., Boor, K.J., 2001.Identification and characterization of elevated microbial counts in bulk tank raw milk.J Dairy Sci., 84: 292-98.

Olusegun, O. 2013. Urban expansion and urban land use in Ado Ekiti, Nigeria. American Journal of Research communication, 1 (2): 128-39.

WPR [World Population Review] (2018). Population of cities in Nigeria. Accessed online at http://worldpopulationreview.com/countries/nigeria-population/cities/ 9/06/2019 5:22PM.

APHA [American Public Health Administration]. Compendium of methods for the microbiological examination of foods, 2nd ed. APHA: Washington DC. 1984.

Richardson, GH. Standard method for analysis of dairy products, 15th edition. American Public Health Association: Washington DC, USA. 1985.

Barrow, GI., Feltham, RK. Motility test. In: Cowan and steel's manual for the identification of medical bacteria. 3rd edn. Cambridge University Press: UK. 2004 pp 26.

Cheesbrough, M. District laboratory Practice in Tropical Countries, Part 2, Cambridge University Press: New York. 2006.

Barrow, G I., Feltham, RKA. Cowan and Steel's Manual for the Identification of Medical Bacteria. Cambridge University Press: London. 1993.

Garrity, GM., Brenner, DI., Krieg, ER., Stanley, JT. Bergey's Manual of Systemic Bacteriology, 2nd edition, volume 2. Springer-Verlag: New York. 2005.

Mohammed, A., Sahile, S., Subramanian, C. 2014. Evaluation of antibacterial potential of honey against some common human pathogens in North Gondar zone of Ethiopia. Int J pure and appl zool, 2 (4): 286-95.

IFCN [International Farm Comparison Network] . Dairy report 2006: for a better understanding of milk production world-wide, Dairy Resaearch Center, IFCN: Kiel, Germany.

Oladele, DA. 2017. Comparative study of the nutritional and microbial safety of fried "wara" (local cheese) widely hawed in Ilorin and Ogbomoso towns in Nigeria. ANNALS, 18 (2): 263 -68.

Sule, IO., Agbabiaka TO., Oyeyiola, GP., Saliu, BK. and Akinyemi, TD. 2015. Microbiological assessment of some locally retailed cheeses within Ilorin metropolis, Nigeria. Nigeria J Microbiology, 27 (1): 2680 – 87.

Senbetu, DT. 2014. Comparative study on microbiological evolution of cheese collected from two different markets. American J Research communication, 2 (2): 187- 93.

Ogbolu, DO., Terry-Alli, AO., Oluremi, AS., Olanrewaju, AA. 2014. Microbial contamination of locally produced cheese and determination of their antimicrobial potential in Nigeria. Afr. J. Cln. Exper. Microbiol. 15(2): 76-83.

Chambers, JV., The Microbiology of raw milk. In: Dairy microbiology handbook: The Microbiology of milk and milk products, Robinson, R.K (Ed.). 3rd Edn. John Wiley and Sons Inc: New York, USA, 2002: 39-90.

Godefay, H., Molla, U. 2000. Identification and characterization of elevated microbial counts in bulk tank raw milk. J Dairy Sci., 84: 23-27.

****