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https://dx.doi.org/10.4314/nvj.v38i4.7 **ORIGINAL ARTICLE**

Evaluation of Bacteria and Antibiotic Resistance Profiling of *Wara* (White Soft Cheese) in Oyo-State, South-West, Nigeria

Amosun, E. A.[1]; Agbato, A. O.[1]; Daodu, O. B.[2]* and Ojo, O. E.[3]

[1]. Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria. [2]. Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ilorin, Ilorin, K*wara* State, Nigeria. [3]Department of Veterinary Microbiology and Parasitology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun state, Nigeria. *Corresponding author: Email: daodu.ob@unilorin.edu.ng ; Tel No:+2348130821559.

SUMMARY

Fresh milk from healthy cows contains few bacteria but contamination during handling and processing can rapidly increase bacterial population and subsequently depreciate it quality. This study evaluated the presence of bacteria and their antibiotic resistance profiles in white soft cheese (*Wara*) in three major producing local Government areas in Oyo-State, Nigeria. The cheese samples were collected in sterile universal bottles immediately after production. Samples were immediately transported to the laboratory for bacteria isolation, identification and antibiotic susceptibility test of the isolates. A total of one hundred and sixty-five (165) bacterial isolates of ten different bacterial genera were identified from 102 cheese samples. The isolation rates of different species/genera were *Lactobacillus* spp (39.8%), *E. coli* (32.5%), *Klebsiella* spp (9%), *Citrobacter* spp (2.4%), *Acinetobacter* spp (3.6%), *Pseudomonas* spp (6.6%), *Bordetella* spp (0.6%), *Proteus* spp (1.2%), *Staphylococcus* spp (3.6%) and *Streptococcus agalactiae* (0.6%). Results demonstrated substantial differences in their resistance patterns to various antibiotics. The isolates were tested against ten different antibiotics. The Gram negative isolates showed twenty-five resistance patterns while Gram positive isolates revealed fifty-four distinct resistance groups. In all, Gram positive isolates showed resistance to ceftriaxone (61.1%), gentamicin (51.4%), chloramphenicol (51.1%), amoxicillin (48.6%), ciprofloxacin (44.4%), cotrimoxazole (43.1%), perfloxacin (40.3%), streptomycin (40.3%) Erythromycin (31.9) and ofloxacin (30.6%) while Gram positive showed resistance to chloramphenicol (22.6%), trimethoprim /sulfamethoxazole (20.4%), amoxillin/clavulanic acid. (10.8%), streptomycin (9.7%), gentamicin (8.6%), sparfloxacin (6.5%), amoxicilin (5.4%), Perfloxacin (5.4%), ofloxacin (4.3%) and ciprofloxacin (3.2%). Multi-drug resistance to three or more antibiotics was observed in 69 isolates, 56 Gram positive and 13 Gram negative bacteria. This study confirmed that white soft cheese "*wara*" can be a source of bacteria with multidrug resistant phenotypes.

Key words: Bacteria, Antibiotic resistance, White soft cheese, Oyo-State, Nigeria.

INTRODUCTION

Production of milk and milk products is in short supply in Africa owing to a lot of factors. In Nigeria, dairy farms and industries are concentrated in the northern part of the country where milk is produced mostly by small-scale holders and also by semi-nomadic livestock owners.

Cheese is one of the milk products which is made up of 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 (Raheem *et al.,* 2009). The preparation of cheese is probably dated back many centuries to the time when nomadic tribes of Eastern Mediterranean countries carried milk of domesticated mammals in sack made from animal skin (Helen and Elisabeth,1990; Sanni *et al*., 1999). When milk is kept warm, it rapidly became sour and separated into curds and whey. Sanni *et al* (1999) reported that curds are supplied as supplement in the absence of liquid milk since it retains much of the milk value.

"*Wara*kashi" popularly called '*Wara*' is a white soft non-ripened cheese made by the addition of a plant extract (*Calotropis procera*) to the non- pasteurized whole milk from cattle (Adeyemi and Umar, 1994). *Wara*kashi originated from Fulani cattle rearers in the northern part of the country and it is an excellent source of protein, fats and minerals. Generally, coagulating agents for cheese production have been drawn from many avenues including animal, plant, bacterial and fungal sources (Alves *et al.,* 2013; Mahajan and Chaudhari, 2014).

Fresh milk from a healthy cow contains few bacteria, but poor hygiene during handling and processing can rapidly increase bacterial contaminants and load. Milk is an ideal food and many bacteria grow readily in it. Although, some bacteria are useful in milk processing causing milk to sour naturally, yet pathogenic bacteria such as *Salmonella* species, *Mycobacterium bovis, Brucella abortus, Listeria* spp and *coli* form bacteria have been isolated from milk (Amosun *et al*., 2005). Amosun *et al* (2005) explained that poor hygiene practiced by handlers of these products (immediately after pasteurization to consumption) may lead to introduction of pathogenic microorganisms since it does not undergo further heat processing before consumption. In the same vein, contamination of milk can come from infected animals. The occurrence of *coli* form bacteria (*Escherichia coli* , *Klebsiella* species and *Enterobacter* species) in milk suggested faecal contamination (Todar, 2008) which is an indication of potential human health risk (Ogunbanwo *et al.*, 2011). The consumption of an unpasteurized milk is said to be unsafe (Ogunbanwo *et al.*, 2011), but the consequence of consuming bacterial contaminated milk and milk products after pasteurization is detrimental to unsuspecting public (Fox, 2000; Adetunji *et al.*, 2008; Vahid *et al*., 2009). Ivbade *et al* (2014) isolated *Escherichia coli* 0157: H7 from white soft cheese '*wara*' that were sold in Ogun state, Nigeria. Some of the bacterial contaminants have been reported to be multidrug resistant (Makut *et al.*, 2014). The unrestricted extensive use of antimicrobial agents has resulted in the emergence of resistant strains of bacteria.

This study investigated occurrence and antibiotic susceptibility of bacteria isolated from white soft cheese "*wara*" which were ready for sale for human consumption in Oyo state. It aimed to know the hygienic state of this milk product and aid government in its policy formulation to ensuring the safety of the public.

MATERIALS AND METHODS

Collection of samples

A total of one hundred and two locally made cheese (*wara*) samples were collected from different local producers immediately after production from three towns namely:

Igboora (15), Eruwa (29), Ilora (58) in Oyo State, South west, Nigeria.

and were ready for sale. Samples were collected into sterile container and labeled; only one sample was collected per producer. The samples collected were put in a cold box with ice packs and transported to the microbiology research laboratory, Department of Veterinary Microbiology, University of Ibadan for bacteriological analysis.

Isolation and identification of bacteria
Each cheese (*wara*) samples (10g) was inoculated into ninety milliliters of sterile Tryptic soy broth (TSB) and sterile selenite F broth (Oxoid, Basingstoke, UK) in universal bottles. The broth cultures were incubated at 37°C for 18 to 24 hours. After incubation, a loopful of the TSB culture was inoculated onto MacConkey agar, 7% sheep blood agar, Eosin Methylene Blue (EMB) (Oxoid, Basingstoke, UK) while selenite F culture was sub-cultured on Xylose lysine deoxycholate agar (XLD Agar). These inoculated media were incubated at 37°C for 18 to 24 hours. Colonial morphology and Gram staining of all the isolates on the plates were carried out; all the isolates were selected for oxidase and catalase production. Coagulase test was carried out for all the Gram positive cocci in clusters. Other biochemical and sugar utilization tests were performed. Results of biochemical tests were interpreted using Cowan and Steel's manual for the identification of medical bacteria Third edition (Barrow and Feitham *et al.*, 2003).

Antimicrobial susceptibility test
The susceptibility of identified bacterial isolates to antimicrobial agents was determined by the standard Kirby-Bauer disk diffusion method. A single colony of the isolates under test was inoculated unto TSB and incubated for 8 to 12 hours. After incubation, the turbidity of the TSB culture was adjusted to 0.5 McFarland standards. A sterile swab was dipped into the adjusted

About 200g of the locally made cheese (*wara*) samples were collected from each of the producers immediately after production TSB culture and inoculated onto Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) plate by swabbing the entire surface of the MHA. The antimicrobial disks were individually placed firm on the inoculated MHA plate. The plates were incubated at 37°C for 18 to 24 hours. After incubation, the diameter of the clear zone of inhibition around each antimicrobial disk was measured (in millimeters) and the result was interpreted in accordance with the recommendation of Clinical and Laboratory Standards Institute (CLSI), (2008). Susceptibility to the following antimicrobials (10 antibiotics were selected for Gram positive and negative each) was determined for the one hundred and two bacterial isolates: amoxicillin (30μg), ciprofloxacin (10 μg), perfloxacin (10μg), erythromycin (10μg), gentamicin (10μg), streptomycin (10μg), ofloxacin (5μg), cotrimoxazole (25μg), chloramphenicol (30μg), ceftriaxone (25μg), Sulfamethoxazole / trimethoprim (30 μg), Augmentin (30 μg), Sparfloxacin (10 μg) and Tarivid (Ofloxacin) (30μg). Oxford *Staphylococcus aureus* NCTC 6571 was used as Gram positive control organism while *E. coli* American Type Culture Collection (ATCC) 25922 was included for Gram negative quality control.

RESULTS

A total of 165 bacterial isolates of 10 different bacterial species were obtained from 102 *wara* (locally made cheese) samples collected from three major *wara* producing areas in Oyo State (Table I and II). The most frequently isolated bacteria was *Lactobacillus* species 66 (40.0%) followed by *Escherichia coli* 54 (32.7%), *Klebsiella* species 15 (9.1%), *Pseudomonas* species 11 (6.7%), *Acinetobacter* species 6 (3.6%), *Staphylococcus aureus* 5 (3.3%), *Citrobacter* species 4 (2.4%), *Proteus* species 2 (1.2%), *Bordetella* species 1 (0.6%) and *Streptococcus agalactiae* 1

(0.6%). *Lactobacillus* species and *Escherichia coli* ranked highest for Gram positive and Gram negative bacteria respectively (Table I).

Based on location, in Igboora (n= 29), *Escherichia coli* 13 (48.1%) was the highest isolated bacteria followed by *Lactobacillus* species 7 (25.9%), *Staphylococcus aureus* 5 (18.5%), *Streptococcus agalactiae* 1 (3.7%) and *Acinetobacter* species 1 (3.7%). In Eruwa (n=15), *Lactobacillus* species 18 (48.6%) ranked highest followed by *Escherichia coli* 11 (29.7%), *Klebsiella* species 3 (8.1%), *Acinetobacter* species 3 (8.1%), *Citrobacter* species 1 (2.7%) and *Proteus* species 1 (2.7%). In Ilora (n=58), *Lactobacillus* species 41 (40.6%) had the highest frequency of isolation followed by *Escherichia coli* 30 (29.7%), *Klebsiella* species 12 (11.9%), *Pseudomonas* species 11 (10.9%), *Citrobacter* species 3 (3.0%), *Acinetobacter* species 2 (2.0%), *Proteus* species 1 (1.0%) and *Bordetella* species 1 (1.0%) (Table II).

Table I: Frequency of isolation of various bacterial genera and species

Bacteria	Frequency (%)
Gram positive bacteria	72 (43.6%)
Lactobacillus species	66 (40.0%)
Staphylococcus species	5 (3.3%)
β haemolytic *Streptococcus* species	1 (0.6%)
Gram negative bacteria	93 (56.4%)
Escherichia coli	54 (32.7%)
Klebsiella species	15 (9.1%)
Pseudomonas species	11 (6.7%)
Acinetobacter species	6 (3.6%)
Citrobacter species	4 (2.4%)
Proteus species	2 (1.2%)
Bordetella species	1 (0.6%)
Total number of isolate	165 (100%)

In decreasing order, antibiotic susceptibility testing showed that *Lactobacillus* species (n= 66) had 39 (59.1%), 33 (50.0%), 32 (48.5%), 31 (47.0%), 29 (43.9%), 27 (40.9%), 26 (39.4%), 25 (37.9%), 20 (30.3%) and 19 (28.8%) resistance to ceftriaxone, gentamycin, chloramphenicol, amoxicillin, cotrimoxazole, ciprofloxacin, perfloxacin, streptomycin, erythromycin and ofloxacin respectively. Also, *Staphylococcus aureus* (n= 5) showed 4 (80.0%), 4 (80.0%), 4 (80.0%), 3 (60.0%), 3 (60.0%), 3 (60.0%), 2 (40.0%), 2 (40.0%), 2 (40.0%) and 1 (20.0%) resistance to ceftriaxone, ciprofloxacin, chlopamphenicol, gentamicin, streptomycin, amoxicillin, perfloxacin, erythromycin, ofloxacin and cotrimoxazole respectively. The only *Streptococcus agalactiae* (n=1) isolated was resistant to the entire antibiotics used including ceftriaxone, gentamicin, chlopamphenicol, amoxicillin, cotrimoxazole, ciprofloxacin, perfloxacin, streptomycin, erythromycin and ofloxacin (Table III). Generally, the resistance pattern of Gram positive bacteria ranges between 30.6% and 61.1% for all the ten (10) antibiotics used (Table IV). *Escherichia coli* (n= 54) displayed 6 (11.1%), 5 (9.3%), 2 (3.7%), 2 (3.7%), 2 (3.7%), 2 (3.7%), 0 (0.0%), 0 (0.0%), 0 (0.0%) and 0 (0.0%) resistance to Chloranphenicol, Sulfamethoxazole / trimethoprim, ofloxacin, sparfloxacin, Ciprofloxacin, pefloxacin, amoxacilin, amoxillin/clavulanic acid, gentamicin and streptomycin. *Klebsiella* species (n= 15) had resistance 5 (33.3%), 4 (26.7%), 4 (26.7%), 3 (20.0%), 3 (20.0%), 2 (13.3%), 1 (6.7%), 1 (6.7%), 1 (6.7%) and 0 (0.0%) to chloramphenicol, streptomycin, trimethoprim/sulfamethoxazole, sparfloxacin, gentamicin, amoxacilin, ciprofloxacin, amoxillin/clavulanic acid, ofloxacin and pefloxacin respectively.

Pseudomonas species (n=11) had resistance 6 (54.5%), 6 (54.5%), 5 (45.5%), 4 (36.4%), 3 (27.3%), 3 (27.3%), 2 (18.2%), 0 (0.0%), 0 (0.0%) and 0 (0.0%) to Sulfamethoxazole / trimethoprim, chloranphenicol, amoxillin/clavulanic acid, streptomycin, pefloxacin, gentamicin, amoxacilin, ciprofloxacin, sparfloxacin and ofloxacin respectively. *Acinetobacter* species (n= 6) displayed 3 (50.0%), 2 (33.3%), 1 (16.7%),

Table II: Frequencies of isolation of various bacterial species with respect to study locations

Location	Sample distribution and isolate frequency	Gram positive bacteria			Gram negative bacteria						
		L. spp.	*Staphy.* spp.	β haemolytic *Strep.* spp.	*E. coli*	*Kleb.* spp.	*Pseud.* spp.	*Acine.* spp.	*Citro.* spp.	*Prot.* spp.	*Bord.* spp.
Igboora	Location A (n=12)	7	4	1	12	0	0	0	0	0	0
	Location B (n=2)	0	1	0	1	0	0	1	0	0	0
	Isolate subtotal (n= 27)	7 (25.9%)	5 (18.5%)	1 (3.7%)	13 (48.1%)	0 (0.0%)	0 (0.0%)	1 (3.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Eruwa	Location A (n= 9)	7	0	0	7	1	0	2	1	1	0
	Location B (n= 7)	6	0	0	2	1	0	1	0	0	0
	Location C (n= 7)	5	0	0	2	1	0	0	0	0	0
	Isolate subtotal (n= 37)	18 (48.6%)	0 (0.0%)	0 (0.0%)	11 (29.7%)	3 (8.1%)	0 (0.0%)	3 (8.1%)	1 (2.7%)	1 (2.7%)	0 (0.0%)
Ilora	Location A (n= 12)	10	0	0	2	3	3	0	0	0	0
	Location B (n= 14)	13	0	0	11	3	0	1	0	0	0
	Location C (n= 17)	12	0	0	11	6	2	0	1	0	1
	Location D (n= 12)	6	0	0	6	0	6	1	2	1	0
	Isolate subtotal (n= 101)	41 (40.6%)	0 (0.0%)	0 (0.0%)	30 (29.7%)	12 (11.9%)	11 (10.9%)	2 (2.0%)	3 (3.0%)	1 (1.0%)	1 (1.0%)
Total number of bacteria isolates (n= 165)		66	5	1	54	15	11	6	4	2	1

Legend:
L. spp- *Lactobacillus* species
Staphy. spp- *Staphylococcus* species
E. coli - *Escherichia coli*
Kleb. Spp- *Klebsiella* species
Acine. spp.- *Acinetobacter* species
Citro. spp.- *Citrobacter* species
Prot. spp- *Proteus* species
Bord. spp- *Bordetella* species
β haemolytic *Strep.* spp- β haemolytic *Streptococcus* species
Pseud. spp.- *Pseudomonas* species

1 (16.7%), 1 (16.7%), 0 (0.0%), 0 (0.0%), 0 (0.0%), 0 (0.0%) and 0 (0.0%) to amoxillin/clavulanic acid, chloramphenicol, Sulfamethoxazole / trimethoprim, gentamicin, ofloxacin, sparfloxacin, ciprofloxacin, amoxacilin, pefloxacin, and streptomycin respectively. *Citrobacter* species (n= 4) showed 1 (25.0%) resistance to amoxillin/clavulanic acid, chloramphenicol, Sulfamethoxazole / trimethoprim, amoxacilin, streptomycin, gentamycin and sparfloxacin while no resistance was shown to ofloxacin, ciprofloxacin and pefloxacin. *Proteus*

Table III: Percentage susceptibilities of various Gram positive bacteria to 10 different antibiotics

Antibiotic	Organisms	*Lactobacillus* species n= 66	*Staphylococcus* species *n=5*	*β- haemolytic Streptococcus* spp. n= 1	TOTAL n= 72
Ceftriaxone	Sensitive	27 (40.9%)	1 (20.0%)	0 (0.0%)	28 (38.9%)
	Resistant	39 (59.1%)	4 (80.0%)	1 (100.0%)	44 (61.1%)
Gentamycin	Sensitive	33 (50.0%)	2 (40.0%)	0 (0.0%)	35 (48.6%)
	Resistant	33 (50.0%)	3 (60.0%)	1 (100.0%)	37 (51.4%)
Chlopamphenicol	Sensitive	34 (51.5%)	1 (20.0%)	0 (0.0%)	35 (48.6%)
	Resistant	32 (48.5%)	4 (80.0%)	1 (100.0%)	37 (51.4%)
Amoxicillin	Sensitive	35 (53.0%)	2 (40.0%)	0 (0.0%)	37 (51.4%)
	Resistant	31 (47.0%)	3 (60.0%)	1 (100.0%)	35 (48.6%)
Cotrimoxazole	Sensitive	37 (56.1%)	4 (80.0%)	0 (0.0%)	41 (56.9%)
	Resistant	29 (43.9%)	1 (20.0%)	1 (100.0%)	31 (43.1%)
Ciprofloxacin	Sensitive	39 (59.1%)	1 (20.0%)	0 (0.0%)	40 (55.6%)
	Resistant	27 (40.9%)	4 (80.0%)	1 (100.0%)	32 (44.4%)
Perfloxacin	Sensitive	40 (60.6%)	3 (60.0%)	0 (0.0%)	43 (59.7%)
	Resistant	26 (39.4%)	2 (40.0%)	1 (100.0%)	29 (40.3%)
Streptomycin	Sensitive	41 (62.1%)	2 (40.0%)	0 (0.0%)	43 (59.7%)
	Resistant	25 (37.9%)	3 (60.0%)	1 (100.0%)	29 (40.3%)
Erythromycin	Sensitive	46 (69.7%)	3 (60.0%)	0 (0.0%)	49 (68.1%)
	Resistant	20 (30.3%)	2 (40.0%)	1 (100.0%)	23 (31.9%)
Ofloxacin	Sensitive	47 (71.2%)	3 (60.0%)	0 (0.0%)	50 (69.4%)
	Resistant	19 (28.8%)	2 (40.0%)	1 (100.0%)	22 (30.6%)

Table IV: Percentage antibiotic susceptibility of Gram positive bacteria

Antibiotics	**Amount**	**Sensitive**	**Resistant**
Ceftriaxone	25	28 (38.9%)	44 (61.1%)
Gentamicin	10	35 (48.6%)	37 (51.4%)
Chloramphenicol	30	35 (48.6%)	37 (51.4%)
Amoxicillin	30	37 (51.4%)	35 (48.6%)
Ciprofloxacin	10	40 (55.6%)	32 (44.4%)
Cotrimoxazole	25	41 (56.9%)	31 (43.1%)
Perfloxacin	10	43 (59.7%)	29 (40.3%)
Streptomycin	10	43 (59.7%)	29 (40.3%)
Erythromycin	10	49 (68.1%)	23 (31.9%)
Ofloxacin	5	50 (69.4%)	22 (30.6%)

species (n= 2) had resistance 1 (50.0%) resistance to Sulfamethoxazole / Sulfamethoxazole / trimethoprim (Table V). Generally, the resistance pattern of Gram negative bacteria ranges between 3.2% and 22.6% for all the ten (10) antibiotics used (Table VI).

trimethoprim and chloramphenicol while *Bordetella* species (n=1) was resistant to

DISCUSSION

Soft cheese has high nutritive value, however its quality can be reduced with the presence of microbial contaminants. The production of "*wara*", a white soft cheese, is traditionally carried out at the household

Table V: Percentage susceptibilities of various Gram negative bacteria to 10 different antibiotics

Antibiotic	**Organisms**	*E. coli n=54*	*Klebsiella* species *n=15*	*Pseudomonas* species *n=11*	*Acinetobacter* species *n=6*	*Citrobacter* species *n=4*	*Proteus* species *n= 2*	*Bordetella* species *n= 1*	*Total n= 93*
Sparfloxacin	Sensitive	52(96.3%)	12(80.0%)	11 (100.0%)	6 (100.0%)	3 (75.0%)	2(100.0%)	1(100.0%)	87(93.5%)
	Resistant	2 (3.7%)	3 (20.0%)	0 (0.0%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	6 6.5%)
Ciprofloxacin	Sensitive	52(96.3%)	14(93.3%)	11 (100.0%)	6 (100.0%)	4(100.0%)	2(100.0%)	1(100.0%)	90(96.8%)
	Resistant	2 (3.7%)	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (3.2%)
Amoxacilin	Sensitive	54(100.0%)	13(86.7%)	9 (81.8%)	6 (100.0%)	3 (75.0%)	2(100.0%)	1(100.0%)	88(94.6%)
	Resistant	0 (0.0%)	2 (13.3%)	2 (18.2%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	5 (5.4%)
Augumentin	Sensitive	54(100.0%)	14(93.3%)	6 (54.5%)	3 (50.0%)	3 (75.0%)	(100.0%)	1(100.0%)	83(89.2%)
	Resistant	0 (0.0%)	1 (6.7%)	5 (45.5%)	3 (50.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	10(10.8%)
Gentamycin	Sensitive	54(100.0%)	12(80.0%)	8 (72.7%)	5 (83.3%)	3 (75.0%)	2(100.0%)	1(100.0%)	85(91.4%)
	Resistant	0 (0.0%)	3 (20.0%)	3 (27.3%)	1 (16.7%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	8 (8.6%)
Pefloxacin	Sensitive	52 (96.3%)	15(100.0%)	8 (72.7%)	6 (100.0%)	4(100.0%)	2(100.0%)	1(100.0%)	88(94.6%)
	Resistant	2 (3.7%)	0 (0.0%)	3 (27.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (5.4%)
Ofloxacin	Sensitive	52 (96.3%)	14 (93.3%)	11 (100.0%)	5 (83.3%)	4(100.0%)	2(100.0%)	1(100.0%)	89(95.7%)
	Resistant	2 (3.7%)	1 (6.7%)	0 (0.0%)	1 (16.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (4.3%)
Streptomycin	Sensitive	54(100.0%)	11 (73.3%)	7 (63.6%)	6 (100.0%)	3 (75.0%)	2(100.0%)	1(100.0%)	84(90.3%)
	Resistant	0 (0.0%)	4 (26.7%)	4 (36.4%)	0 (0.0%)	1 (25.0%)	0 (0.0%)	0 (0.0%)	9 (9.7%)
Sulfamethoxazole /trimethoprim	Sensitive	49 (90.7%)	11 (73.3%)	5 (45.5%)	5 (83.3%)	3 (75.0%)	1 (50.0%)	0 (0.0%)	74(79.6%)
	Resistant	5 (9.3%)	4 (26.7%)	6 (54.5%)	1 (16.7%)	1 (25.0%)	1 (50.0%)	1(100.0%)	19(20.4%)
Chloranphenicol	Sensitive	48 (88.9%)	10 (66.7%)	5 (45.5%)	4 (66.7%)	3 (75.0%)	1 (50.0%)	1(100.0%)	72(77.4%)
	Resistant	6 (11.1%)	5 (33.3%)	6 (54.5%)	2 (33.3%)	1 (25.0%)	1 (50.0%)	0 (0.0%)	21(22.6%)

level by women. There exist varying poor to good hygienic practices during *wara* production leading to varying bacterial contaminations. This study reported the presence of 10 different bacterial genera/species (some of which are potentially pathogenic) from 102 *wara* samples. These include *Lactobacillus* species 66(40.0%), *Escherichia coli* 54 (32.7%), *Klebsiella* species 15 (9.1%), *Pseudomonas* species 11(6.7%), *Acinetobacter* species 6 (3.6%), *Staphylococcus aureus* 5 (3.3%), *Citrobacter*

Table VI: Percentage antibiotic susceptibility of Gram negative bacteria

Antibiotics	Amount(μg)	Sensitive	Resistant
Chloramphenicol	30	72 (77.4%)	21 (22.6%)
Sulfamethoxazole / trimethoprim	30	74 (79.6%)	19 (20.4%)
Augumentin	30	83 (89.2%)	10 (10.8%)
Streptomycin	10	84 (90.3%)	9 (9.7%)
Gentamycin	10	85 (91.4%)	8 (8.6%)
Sparfloxacin	10	87 (93.5%)	6 (6.5%)
Amoxicilin	30	88 (94.6%)	5 (5.4%)
Pefloxacin	10	88 (94.6%)	5 (5.4%)
Ofloxacin	30	89 (95.7%)	4 (4.3%)
Ciprofloxacin	10	90 (96.8%)	3 (3.2%)

TABLE VIIa: Resistance patterns of Gram positive bacteria isolates

Antibiotic	***Lactobacillus* species**	***Staphylococcus* species**	***β-haemolytic Streptococcus spp.***
COT	1	0	0
ERY	1	0	0
CEF, GEN	0	1	0
OFL,COT	1	0	0
CPX, CEF, GEN	1	0	0
CPX,ERY,AMX	1	0	0
GEN, PEF,COT	1	0	0
ERY,AMX, OFL	1	0	0
CHL, CEF,COT	1	0	0
ERY,AMX, STR	1	0	0
CPX, CEF, GEN	1	0	0
ERY,AMX, OFL	1	0	0
CPX,AMX, CHL	1	0	0
CHL, CEF, PEF	1	0	0
STR, CHL, CEF,COT	1	0	0
CPX, AMX, OFL, CHL	0	1	0
CPX,AMX, CEF, GEN	1	0	0
ERY, CHL, CEF,COT	1	0	0
CPX, AMX, CEF, GEN	1	0	0
AMX, OFL, STR, PEF	1	0	0
AMX, CHL, CEF, PEF	1	0	0
CPX,ERY, CHL,COT	1	0	0
CPX,ERY,AMX, OFL	1	0	0
OFL, STR, CHL, CEF	1	0	0
CPX,AMX, CEF, GEN	1	0	0
CPX, CHL, CEF, GEN	1	0	0
CPX,AMX, CEF, GEN	1	0	0
CPX, OFL, CHL, PEF,COT	1	0	0
CPX,AMX, STR, CEF, GEN	1	0	0
OFL, CHL, GEN, PEF,COT	1	0	0

TABLE VIIb: Resistance patterns of Gram positive bacteria isolates

Antibiotic	*Lactobacillus* species	*Staphylococcus* species	*β-haemolytic Streptococcus spp.*
CPX,ERY,AMX, CEF,COT	1	0	0
CHL, CEF, GEN, PEF,COT	1	0	0
AMX, STR, CHL, CEF, GEN	1	0	0
AMX, STR, CEF, GEN, PEF	1	0	0
OFL, STR, CHL, CEF, GEN	1	0	0
ERY, OFL, CHL, GEN,COT	1	0	0
OFL, STR, CHL, PEF,COT	1	0	0
CHL, CEF, GEN, PEF,COT	1	0	0
CPX, STR, CEF, PEF,COT	1	0	0
CPX,ERY, STR, CEF,PEF,COT	1	0	0
CPX,ERY,AMX, CHL, CEF,COT	1	0	0
ERY,AMX, OFL, CHL, PEF,COT	1	0	0
CPX,AMX, CHL, CEF, GEN, PEF	1	0	0
ERY,AMX, STR, CEF, GEN, PEF	1	0	0
CPX,ERY, OFL, STR, GEN, PEF	1	0	0
CPX,ERY,AMX, STR, CHL, GEN	1	0	0
CPX, STR, CHL, CEF, GEN,COT	1	0	0
CPX,AMX, CHL, CEF, GEN, PEF	1	0	0
ERY,AMX, OFL, CEF, GEN, PEF,COT	1	0	0
CPX, STR, CHL, CEF, GEN, PEF,COT	1	0	0
CPX, AMX, STR,CHL, CEF, GEN, PEF	0	1	0
ERY, OFL, CHL, CEF, GEN, PEF,COT	1	0	0
CPX,AMX, CHL, CEF, GEN, PEF,COT	1	0	0
AMX, OFL, STR, CHL, CEF, GEN,COT	1	0	0
CPX,ERY, OFL, STR, CEF, GEN, PEF,COT	1	0	0
CPX,ERY, STR, CHL, CEF, GEN, PEF,COT	1	0	0
CPX, ERY, AMX, OFL, STR, CHL, CEF, CEF, GEN, PEF, COT	1	0	1

species 4 (2.4%), *Proteus* species 2 (1.2%), *Bordetella* species 1 (0.6%) and *Streptococcus agalactiae* 1 (0.6%). *Lactobacillus* species and *Escherichia coli* ranked highest for Gram positive and Gram negative bacteria respectively. This is in agreement with Sangoyomi *et al.* (2010) who reported high prevalences of these two bacteria in *wara*. Although, *Lactobacillus* species belongs to Lactic acid producing bacteria (LAPB) which are known to improve food quality and also play an important role in preventing the growth of undesirable bacteria (Steinkraus, 1996; Lu *et al.*, 2008), the presence of members of *Enterobacteriaceae* in this study deny the hygienic quality of the *wara* sampled. The occurrence of *coli* form bacteria in milk has

TABLE VIII: Resistance pattern of Gram negative bacteria isolates

Antibiotic	*E. coli*	*Kleb. spp.*	*Pseud. spp.*	*Acine. spp.*	*Citro. spp.*	*Prot. spp.*	*Bord. spp.*
SP	1	0	0	0	0	0	0
CPX	1	0	0	0	0	0	0
AU	0	0	0	2	0	0	0
PEF	1	0	0	0	0	0	0
OFX	1	0	0	1	0	0	0
S	0	0	1	0	0	0	0
SXT	2	0	0	0	0	0	1
CH	3	1	0	1	0	0	0
SP, CPX	1	0	0	0	0	0	0
AM,CN	0	0	1	0	0	0	0
AU, PEF	0	0	1	0	0	0	0
AU,SXT	0	0	1	0	0	0	0
PEF,OFX	1	0	0	0	0	0	0
SXT, CH	3	1	1	0	0	1	0
SP, AM,CN	0	1	0	0	0	0	0
CN,OFX, S	0	1	0	0	0	0	0
PEF, S, SXT	0	0	1	0	0	0	0
CN, S, CH	0	1	0	0	0	0	0
S, SXT, CH	0	1	1	0	0	0	0
SP, CPX, AM, AU, SXT	0	1	0	0	0	0	0
AU,CN, SXT, CH	0	0	1	1	0	0	0
SP, S, SXT, CH	0	1	0	0	0	0	0
AM, AU,CN, SXT, CH	0	0	1	0	0	0	0
AU, PEF, S, SXT, CH	0	0	1	0	0	0	0
SP, AM, AU,CN, S, SXT, CH	0	0	0	0	1	0	0

Legend: *E. coli* - *Escherichia coli* *Kleb. Spp*- *Klebsiella* species *Acine.* spp.- *Acinetobacter* species *Citro.* spp.- *Citrobacter* species *Prot.* spp- *Proteus* species *Bord.* spp- *Bordetella* species β haemolytic *Strep.* spp- β haemolytic *Streptococcus* species *Pseud.* spp.- *Pseudomonas* species

been suggested to originate from faecal contamination and carries high public risk when such milk is consumed (Wells *et al*., 1991; Todar, 2008). The presence of *E. coli* in milk products has been reported to reduce its' shelf life due to rapid spoilage resulting from fermentation of D-glucose and lactose with the production of acid and gas (Sangoyomi *et al*., 2010).

This study revealed that the bacterial contamination varied with location with *Escherichia coli* (48.7%) ranked highest in Igbo-ora while *Lactobacillus* species represented 48.6% and 40.6% of the bacteria isolated from samples obtained in Eruwa and Ilora respectively. Also, the *coli* form contamination of *wara* was highest in Igbo-ora (48.1%) as compared to Ilora (41.6%) and Eruwa (37.8%) (Table II). It is important to note that these samples were collected at the point the *wara* was ready for sale; there is high probability of further bacterial and fungal contamination of *wara* before it gets to the final consumers during which activities such as air exposure and hand-touching might have occurred. Thus, if "ready for sale" milk product (*wara*) contains these bacteria, then the bacterial contamination load would likely be higher when it gets to the final consumer (Makut *et al.,* 2014).

Among Gram positive bacteria, percentage resistant range was 30.6% - 61.1% with ceftriaxone (25µg) appearing as the most resisted antibiotic among the commonly used antibiotics (Table IV). Also, in Gram negative bacteria, percentage resistant range was 3.2% - 22.6% with Chloramphenicol emerging as the most resisted antibiotic (Table VI). The continuous build up of antibiotic-resisting bacterial contaminants in milk consumed by the public calls for public enlightenment about the risk involved. The plasmid genes coding for antibiotic resistance can be acquired by intestinal bacterial flora (Daodu *et al.,* 2017). This might make treatment difficult especially when resistant bacteria dominate in gastroenteritis and other disease conditions. Aside the public health concerns, the financial losses due to early spoilage of *wara* and other milk products should awaken the government and non-government organizations to sponsor training sessions on hygienic *wara* production since most of the *wara* processors in our study area did not have professional training on *wara* production.

Several Multidrug resistant (MDR) bacteria were also recorded in our study. MDR, defined as resistance to three or more antibiotics, was observed in 69 isolates, 56 Gram positive and 13 Gram negative. This is in agreement with reports that bacteria isolates from cow milk product have some level of antibiotic resistance which can easily be passed to unsuspected human consumers (Makut *et al.,* 2014,

This study indicated that locally made cheese "*wara*" can be a source of bacterial infection and antibiotic resistant bacteria strains could pose threats to consumers if it is not properly prepared under strict hygienic conditions. Thus, it is of great importance that *wara* producers and relevant government agencies should ensure safety status of this milk product to maintain the health status of the public.

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