Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.4, No.4, 2014

Antibiogram of Bacteria Isolated from Locally Processed Cow Milk Products Sold in Keffi Metropolis, Nasarawa State, Nigeria

Makwin Danladi Makut[1*], Mary Azumi Nyam[2], Tarfena Yoila Amapu[3] and Abbul-Mutalib Ahmed[1]
1. Microbiology Unit, Dept. of Biological Sciences, Nasarawa State University, P.M.B. 1022, Keffi, Nigeria
2. Applied Microbiology Unit, Department of Plant Science and Technology, University of Jos, P.M.B. 2084, Jos, Nigeria
3. Department of Science Laboratory Technology, University of Jos, P.M.B. 2084, Jos, Nigeria
[*]E-mail of the corresponding author: makmakwin@yahoo.com

Abstract
An investigation was conducted to determine the antibiogram of bacterial species isolated from locally processed cow milk products sold in Keffi metropolis, Nasarawa state, Nigeria. Samples of the cow milk products, Raw milk, Nono, Kindrimo, and Manshanu, were each collected in triplicates from ten different sales locations in Keffi, and analyzed using standard bacteriological methods. Pour plate technique was employed for the isolation of bacteria from these cow milk products. The isolates were identified using cultural, morphological and biochemical methods, and thereafter the antibiotic susceptibility of each isolate was determined by modified Kirby Bauer diffusion method. Bacterial counts for Raw milk, Nono, Kindrimo and Manshanu were respectively in the range of 3.2×10^8 - 6.9×10^8, 8.1×10^8 - 2.70×10^9, 7.3×10^8 – $9.8.\times10^9$ and 5.8×10^8- 5.2×10^9. The bacterial species isolated were *Staphylococcus aureus, Salmonella* spp, *Streptococcus* spp. and *E. coli* with overall occurrence frequencies of 50%, 20%, 17.5% and 12.5%, respectively. *E. coli* was highly resistant to Septrin (80.2%), Sarfloxacin (75.3%) and Erythromycin (90.2%). *Salmonella* species was also highly resistant to Septrin (71.4%), Chloramphenicol (71.4%), Sparfloxacin (85.7%) and Erythromycin (71.4%). Similarly, *Staphylococcus aureus* was highly resistant to Septrin (75%), Chloramphenicol (75.4%), Sparfloxacin (87.5%), Amoxacillin (75.4%) and Augmentin (87.5%), while *Streptococcus* spp was highly resistant to Chloramphenicol (80.1%), Sparfloxacin (80.1%) and Augmentin (80%). These isolates may have developed resistance due to indiscriminate and frequent use of antibiotics which has now put the consumers of these milk products at risk of being infected with antibiotic resistant strains of pathogenic bacteria.
Keywords: Antibiotic resistance, bacteria, cow milk, Keffi, Nigeria

1. Introduction

Nono, Kindrimo, Manshanu and Raw milk are local dairy products that are widely consumed as food in many African countries including Nigeria, and these have been reported to contain high nutritional values. The pH of normal cow milk range from 6.4 - 6.6 and this favours the growth of pathogenic microorganisms (Akinyele et al., 1999).

In Nigeria, locally processed cow milk products are prepared mainly by the Fulanis, where Raw milk is processed into Nono, Kindrimo and Manshanu thereby having four products (Eka and Ohaba, 1977). Raw milk is obtained from cows at homes in the Fulani hamlets and villages where shelf-life and safety of the products are not considered. The raw milk is however processed into its constituent products and sold to both rural and urban dwellers as food. Nono is produced from non-pasteurized cow milk collected in a container called calabash and allowed to ferment naturally for 24 hours (Eka and Ohaba, 1977; Olusupo *et al.*, 1996), while Kindrimo is produced from locally pasteurized cow milk which are prepared by heating to boiling point and then allowed to cool to 37^{o}C, and then milk butter from the previous day production is added to it at the rate of 0.5-1% of the amount of milk to be processed, and then left overnight to become sour until it coagulate (Odunfa, 1988). Manshanu is produced by a process of boiling of the Raw milk and then extracting the solidified milk that is in form of cream (which is the milk butter). This creamy substance is extracted from the milk in a process called churning. Manshanu consist of buttery flavor, aroma and appear yellowish in color (Olasupo *et al.*, 1996). Raw milk on the other hand is produced by collection of milk directly from cows, and the milk is not allowed to ferment or pasteurized. In other words, Raw milk is an unfermented cow milk that contain varying types and proportions of microorganism more than the locally pasteurized milk (Alan and Heather, 1990; Adesiyun, 1995). Milking is carried out in highly aseptic condition yields milk that is practically free from bacterial flora. The udder of healthy cow is often sterile, and microbial contamination does not occur in the udder, but as the raw milk leaves the udder, contamination may begin (Bramley and McKinnon, 1990). Fresh milk may contain varying numbers of microorganisms depending on the care employed during milking, cleaning and handling of utensils (Alan and Heather, 1990). *Bacillus cereus, Listeria monocytogenes, Yersinia, enterocolitica, Salmonella* spp, *E. coli* 0157:H7, *Campylobacter jejuni*, and *Staphylococcus aureus* have been implicated to be associated with milk borne diseases, and these are often isolated from fresh cow milk (Vasanvade, 1988). Gilmour *et al.*

(1990) reported that the bacteria associated with locally processed cow milk products include Lactic acid bacteria, *Escherichia coli* 0157:H7, coliforms, *Salmonella* spp., *Campylobacter* spp., *Listeria* spp., spoilage and other pathogenic species. Fook *et al.* (2004) reported that the presence of organisms like *Bacillus cereus, Staphylococcus aureus, E. coli,* and *Streptococcus* spp., and may render milk and milk products unsuitable for human consumption. Olasupo *et al.* (1996) asserts that it is possible that contamination by these bacteria could have occurred due to poor hygiene practice during processing, handling, preservation and storage by the Fulani women that prepare and hawk these products.

Lactic acid bacteria are known to ferment lactose to lactic acid (Gilmour *et al.*, 1990), and they are normally present in milk as starter culture organisms in the production of locally processed milk products. *Lactobacillus acidophilus, Lactobacillus lactis, Lactobacillus cremoris, Lactobacillus fermentum, Lactobacillus leichmani, Lactococcus lactis* and *Streptococcus thermophilus* are the most prominent lactic acid bacteria in locally processed cow milk fermentation (Urbach, 1995; Abd El-Aal, 2008).

In Nigeria, the traditional method of processing and selling cow milk and its products exposes these products to the danger of microbial contamination from spoilage and pathogenic microorganisms. All the milk products except raw milk processed by the local cattle handlers are often boiled before sales to the public, and this is considered as a form of pasteurization. However, the milk may be re-contaminated from the handler's, utensils and other external sources (Adesiyun, 1994). Raw milk or processed milk is a well-known good medium that supports the growth of several microorganisms with resultant spoilage of the product or infections and intoxications in consumers (Oliver *et al.*, 2005). Microorganisms found in Nono, Kindrimo, Manshanu and Raw milk mostly come from the water used in preparing the milk or handling, storage and processing activities. Hayes *et al.* (2001) reported that bacteria in milk can occur through colonization of the teat canal or an infected udder (clinical or subclinical mastitis) or get contaminated at various stages whether from the animal, milker (manual as well as automated), extraneous dirt or unclean water. Shehu and Adesiyuh (1990) had earlier reported that in order to increase the volume and improve colour of Nono, Kindrimo, Mashanu and Raw milk, the female Fulani hawkers prior to sales often do engage in fraudulent act of adding stream water and milky white supernant of water obtained from soaked baobab tree seeds. This act could further lead to the contamination and spoilage of the cow milk products.

The presence of contaminant microorganisms, especially pathogenic bacteria in milk and milk products is of serious public concern. Poor hygiene practices by handlers of these products do lead to introduction of pathogenic microorganisms into the products, and since these products do not undergo further processing before consumption, they may pose health risk to the consumers of these products (Adeyemi and Umar, 1994).

Pathogenic bacteria that have become resistant to antibiotic drug therapy have increased the problems of public health all over the world, and it is an ever-increasing global health threat (Levy, 2001). Generally, the presence of antibiotic resistant bacteria in human foods such as milk and its products has been implicated in contributing to the increasing drug resistance which often leads to failures in chemotherapy.

This investigation is therefore aimed at determining the antibiotic resistance pattern of bacteria isolated from locally processed cow milk products being sold in Keffi metropolis, Nasarawa state, Nigeria.

2. Materials and methods

2.1. Study area

This investigation was carried out in Keffi metropolis, Nasarawa State, Nigeria. Keffi is in the middle belt of Nigeria. It is located on latitude 8^0 32'N and longitude 8^0 52'E, and on an altitude of 850 meter above sea level. Keffi is 56km away from Abuja, the capital city of Nigeria (Wikipedia, 2013).

2.2. Sample collection

Ten different locations where cow milk and its products are sold in Keffi metropolis were randomly selected for the purposes of sample collection. The locations selected were Angwan Lambu, Angwan Kare, Angwan Jaba, Angwan Tivi, Angwan Waje, Angwan Nepa, Old Barracks, Area Commander, Old Market and Main Market. Samples of Raw milk, Nono, Kindirmo and Manshanu were aseptically collected into sterile MacCarthny bottles in triplicates from each of the ten (10) locations, and these were immediately transported to the laboratory in an ice-packed container (cooler) maintained at a temperature of 4^oC.

2.3. Determination of Total Aerobic Microbial Counts in Milk and Milk products

Standard plate count method was used to determine the Total Aerobic Colony Counts of microorganisms in the samples of the four different cow milk products: Raw milk, Nono, Kindirmo and Manshanu (Sanders, 2012). A seven-fold serial dilution of the samples from each of the different locations was performed and plated out on Aerobic Plate Agar using pour plate technique. The plates were incubated at 37^oC for 24 h. The average microbial loads of each of the four cow milk products from the ten different locations were obtained and expressed as Colony Forming Units per milliliter [CFU/ml] (Harrigan and McCance, 1976).

2.4. Isolation and identification of bacterial isolates

Standard bacteriological methods were employed for the isolation of bacteria as recommended by Cheesbrough (2006). Serially diluted samples were also inoculated by pour plate technique in MacConkey Agar, Chocolate Agar, Eosin Methylene Blue (EMB) Agar, Mannitol Salt Agar (MSA) and Salmonella-Shigella Agar (SSA) in order to commence the preliminary process of identification of the isolates. McConkey Agar was used to isolate lactose fermenting gram negative bacteria, Chocolate agar was used to isolate fastidious bacteria, Eosin Methylene Blue was used for the selective isolation of enteric coliforms, Manitol Salt agar was for the selective isolation of salt-tolerant bactria and Salmonella-Shigella agar was used for the isolation of enteric bacilli. All plates were incubated at 37^{o}C for 24 h, and identification was based on cultural, morphological and biochemical characteristics as recommended by Holt (1994).

2.5. Antibiotic Susceptibility Test

The isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disk diffusion method (CLSI, 2009). A suspension of each isolate was prepared in peptone water to match 0.5 McFarland turbidity standards in order to standardize the inoculum. The standardized inoculum of each isolate was inoculated in triplicates onto the surfaces of plain Mueller-Hinton agar plates and Septrin (30µg), Chloramphenicol (30 µg), Sparfloxacin (5 µg), Amoxycillin (30 µg), Ciprofloxacin (5 µg) Augmentin (30µg), Gentamicin (10 µg), Pefloxacin (10 µg), Erythromycin (15µg) and Streptomycin (10 µg) discs were placed and incubated at 37°C for 24 h. The zones of inhibition were measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009).

2.6. Statistical Analyses

The data obtained were analysed using Statistical Package for Social Sciences (SPSS) version 20.0 software (SPSS, 2012). The analyses involved computation of averages, proportions (percentages), Two-way analysis of variance as well as the test for Least Significant Difference to separate means.

3. Results

Table 1 shows the bacterial countS (cfu/ml) of the four milk products sold in Keffi metropolis. The highest bacterial count of 6.9 x 10^9 cfu/ml for Raw milk was recorded in Angwan Kare, while the lowest count of 3.3 x 10^8 cfu/ml was recorded at Angwan Jaba and Area Commander, respectively. The highest count for Nono was recorded at Angwan Nepa (2.70 x 10^9 cfu/ml) while the lowest count was recorded at the Main Market (8.1 x 10^8 cfu/ml). The highest count for Kindirmo was recorded at Angwan Tiv (9.8 x 10^9 cfu/ml) while the lowest count was recorded at Angwan Lambu (7.3 x 10^8 cfu/ml). The highest counts for Manshanu were recorded at Angwan Tiv (5.2 x 10^9 cfu/ml) and Main Market(5.2 x 10^9 cfu/ml) respectively, while the lowest count was recorded at Angwan Lambu (5.6 x 10^8 cfu/ml). Table 2 shows the percentage occurrence frequencies of bacterial isolates from the four different cow milk products. *E. coli* was the most predominant bacterial isolate with percentage frequencies of 60%, 80%, 30% and 30% for Raw milk, Nono, Kindirmo and Manshanu respectively, followed by *Staphylococcus aureus* which had percentage frequencies of 30%, 20%, 30% and 0%, respectively, and then *Salmonella* spp which had 20%, 30%, 10% and 10% for Raw milk, Nono, Kindirmo and Manshanu respectively. *Streptococcus* spp. had the least percentage frequencies of 10% , 20% , 10% and 10% , respectively.The overall occurrence frequencies for *E. coli*, *Staphylococcus aureus*, *Salmonella* spp. and *Streptococcus* spp. were 50.0%, 20.0%, 17.5% and 12.5%, respectively. Table 3 shows the antibiogram of the six different bacterial species isolated with respect to the ten antibiotics tested. The results showed that *E. coli* was highly resistant to Septrin (80.2%), Sarfloxacin (75.3%) and Erythromycin (90.2%). *Salmonella* spp. was also highly resistant to Septrin (71.4%), Chloramphenicol (71.4%), Sparfloxacin (85.7%) and Erythromycin (71.4%). Similarly, *Staphylococcus aureus* was highly resistant to Septrin (75%), Chloramphenicol (75.4%), Sparfloxacin (87.5%), Amoxacillin (75.4%) and Augmentin (87.5%), while *Streptococcus* spp. was highly resistant to Chloramphenicol (80.1%), Sparfloxacin (80.1%) and Augmentin (80%).

Table 1: Bacterial counts (cfu/ml) in the four different milk products sold in different locations

Location	Raw Milk	Nono	Kindrimo	Manshanu
Angwan Lambu	3.8×10^8	9.5×10^8	7.3×10^8	5.6×10^8
Angwan Kare	6.9×10^9	2.4×10^9	1.5×10^9	9.4×10^8
Angwan Jaba	3.3×10^8	1.8×10^9	1.1×10^9	5.8×10^8
Angwan Tivi	3.9×10^8	1.8×10^9	9.8×10^9	5.2×10^9
Angwan Waje	4.7×10^8	9.2×10^8	1.5×10^9	1.0×10^9
Angwan Nepa	5.1×10^8	2.7×10^9	1.8×10^9	1.1×10^9
Old Barrack	6.6×10^8	2.2×10^9	2.0×10^9	9.3×10^8
Area Commander	3.3×10^8	1.4×10^9	1.1×10^9	9.4×10^8
Old Market	4.7×10^8	1.9×10^9	9.2×10^8	6.9×10^8
Main Market	3.2×10^8	8.1×10^8	7.9×10^8	5.2×10^9

Table 2: Occurrence frequencies of bacterial isolates from the four different milk products sold

Bacterial isolates	Percentage occurence of isolates in milk products				Overall occurrence frequency(%)
	Raw milk	Nono	Kindirmo	Manshanu	
Escherichia coli	60	80	30	30	**50.0**
Staphylococcus aureus	30	20	30	0	**20.0**
Salmonella spp	20	30	10	10	**17.5**
Streptococcus spp	10	20	10	10	**12.5**

Table 3: Antibiotic resistance pattern of bacterial species isolated from the four different milk products sold

Antibiotics	Concentration (µg)	Percentage of resistance of isolates			
		E. coli	*Salmonella* spp	*Staphylococcus* spp	*Streptococcus* spp
Septrin	30	80.2	71.4	75.0	40.2
Chloramphenical	30	10.2	71.4	75.4	80.1
Sparfloxaciin	5	75.3	85.7	87.5	80.1
Ciprfloxacin	5	5.2	28.6	25.3	20.4
Amoxacillin	30	15.3	14.2	75.4	60.2
Augmentin	30	10.2	14.4	87.5	80.0
Gentamycin	10	10.1	14.1	12.5	20.3
Pefloxacin	10	20.2	14.3	25.3	20.4
Erythromycin	15	90.2	71.4	12.5	20.2
Streptomycin	10	10.3	14.2	37.5	20.1

4. Discussion

The bacterial loads of four different cow milk products sold in Keffi were relatively high. Milk and milk products are nutrient rich foods that can serve as very good media for the growth of all kinds of microorganisms. This corroborate the earlier report by Ruegg and Reinemann (2002) that milk is a highly nutritious food that serves as an excellent growth medium for wide range of microorganisms.

Four genera of bacteria were isolated from the four milk products, and these were *Escherichia coli, Staphylococcus aureus, Salmonella spp.* and *Streptococcus spp. Escherichia coli* was the most predominant bacteria isolated with 50% occurrence frequency, followed by Staphylococcus aureus which had 20% occurrence frequency. *E. coli* which is an indicator of pollution from faecal source was found present in all the four milk products sold in Keffi metropolis. This potent a risk to the health of the public, especially the consumers of these milk products. *Staphylococcus aureus* is a normal flora of the human skin, and may colonize the nasopharyngeal region. The presence of this species of bacteria in three of the milk products may have come from the human handlers. However, the absence of *Staphylococcus aureus* on Manshanu may suggest that this species probably did not survive on the Manshanu due it fatty nature. The Manshanu is the fatty component of cow milk which is extracted after heating raw milk for some time. Several strains of *Salmonella* and *Streptococcus* species are known to be pathogenic to humans, and their presence the four milk products, though at relatively small percentages, further revealed the health risks associated with the consumption of the cow milk products sold in Keffi metropolis.

Antibiogram of the bacterial isolates revealed varying levels of resistance to the antibiotic tested. *E. coli* was highly resistant to Septrin (80.2%), Sparfloxacin (75.3%) and Erythromycin (90.2%), while *Salmonella* species was highly resistant to Septrin (71.4%), Chloramphenicol (71.4%), Sparfloxacin (85.7%) and Erythromycin (71.4%). *Staphylococcus aureus* was also highly resistant to Septrin (75.0%), Chloramphenicol (75.4%), Sparfloxacin (87.5%), Amoxacillin (75.4%) and Agumentin (87.5%); whereas Streptococcus species was highly resistant to Chloramphenicol (80.1%), Sparfloxacin (80.1%) and Agumentin (80.0%). The sensitivity pattern of the bacterial species isolated to the antibiotic tested is comparable with to the reports of earlier workers (Inyang, 2009; Udo *et al*., 2001; Tagoe *et al*., 2011, Makut *et al*., 2013).

The prevalence of resistant strains of *E. coli, Salmonella* species, *Staphylococcus aureus* and *Streptococcus* species in the four cow milk products is a reflection of the use and misuse of antibiotics in the society. This is not surprising because there is indiscriminate use of antibiotics by the Nigerian public of which Keffi is a part. The public health implication of this investigation is that antimicrobial resistant strains of pathogenic bacteria may colonize the human population through consumption of contaminated cow milk products sold locally in Keffi metropolis, and this would lead to failures of chemotherapy among the human consumers of any of these products.

5. Conclusion

The presence of resistant strains of *E. coli, Salmonella* species, *Staphylococcus aureus* and *Streptococcus* species in Raw milk, Nono, Kindirmo and Manshanu sold in Keffi suggests that consumption of any of these products has potential health risk to the consumers in Keffi, Nasarawa state, Nigeria. The consumers of these cow milk products are therefore placed at health risk which may culminate into chemotherapeutic failures of commonly used clinical antibiotics.

Acknoledgement

The authors are grateful to the Microbiology Unit, Department of Biological Sciences, Nasarawa State University, Keffi, Nigeria, for providing the laboratory materials and facilities used in this investigation.

References

Abd El-Aal, A.S. (2008). Microbiological research on some dairy products. *Assiut Veterinary Medical Journal, 54*(119): 54 – 168 – 174.

Adesiyun, A.A. (1994). Bacteriological quality and associated public health risk of pre-processed bovine milk in Trinidad. *International Journal of Food Microbiology, 21*: 253 – 261.

Adesiyun, A.A., L. Webb and S. Rahman, 1995. Microbiological quality of raw cow milk at collection centers in Trinidad. *Journal of Food Production, 58*: 448.

Akinyele, B.J, M.O. Fawole & F.A. Akinyosoye (1999). Microorganisms associated with fresh cow milk, wara, nono; two local milk products hawked by Fulani women in Ilorin, Kwara state, Nigeria. *Nigerian Food Journal, 17*: 11-17.

Alan, H.L. & Heather,M.D (1990): Topley and Wilson's Principle of Bacteriology, Virology and Immunity (8th edn). New York . Pp29- 33.

Bramley, A.J. & C.H. Mckinnon (1990). The microbiology of raw milk. In R.K. Robinson (ed.)Dairy Microbiology, Vol. 1. Elsevier Science Publishers, London. P P 163 - 208

Cheesbrough, M. (2006). District Laboratory Practice in Tropical Countries, Part II. Cambridge University Press, Cambridge. Pp442.

CLSI (2009). Performance standards for antimicrobial susceptibility testing: Nineteenth informational supplement. M100-S19. Clinical and Laboratory Standards Institute, Wayne, Chicago, USA. Pp51.

Eka, O.U. & J.A. Ohaba (1977). Microbiological examination of Fulani milk (nono) and butter (manshanu). *Nigerian Journal of Science, 11*: 113-122.

Fook, Y.C., A. Aminah & K.A. Mohd (2004). Bacteriological quality and safety of raw milk in Malaysia. *Food Microbiology, 21*: 534 – 554

Gilmour, A. & Rowe, M. T. (1990): Microorganisms associated with milk in dairyMicrobiology. The microbiology of milk. In R.K, Robison Applied scince, Vol. 1 (2nd edn). London. Pp 37-75.

Harrigan, W.F. & McCance, M (1976): Laboratory Methods in Food and Dairy Microbiology. Academic Press, London. Pp 206-209.

Hayes, M.C., Ralyea, R.D. Murphy, S.C., Carey, N.R., Scarlett, J.M. & K.J. Boor (2001). Identification and characterization of elevated microbial counts in bulk tank raw milk. *Journal of Dairy Science, 84*: 292-298.

Holt, J.H. (1994). Bergey's Manual of Determinative Bacteriology (9th edn). Lippincott, Williams and Wilkins Co., Baltimore. Pp787.

Inyang, C.U. (2009). Antibiogram of bacteria isolated from borehole water. *Nigerian Journal of Microbiology, 23*: 1810-1816.

Levy, S. (2001). Antibiotic resistance: Consequences of inaction. *Clinical and Infectious Diseases, 33*: 124 – 129.

Makut, M.D., M.A. Nyam, S.O. Obiekezie, & A.E. Abubakar (2013). Antibiogram of bacteria isolated from Kunun-zaki drink sold in Keffi metropolis. *American Journal of Infectious Diseases, 9*(3): 71 - 76.

Odunfa, S.A. (1988). Microbiological quality of yoghurt and milk drink samples in Ibadan, a Nigerian city. Journal of Agriculture, 2: 43 -46

Olasupo, N.A., S.M., Akinsanya, O.F. Oladele & M.K. Azeez (1996). Evaluation of nisin for the preservation of nono, a Nigerian fermented milk product. *Journal of Food Processing and Preservation, 20*: 71-78.

Oliver, S.P., B.M. Jayarao, & R.A. Almedia (2005). Food borne pathogens in milk and the dairy environment, food safety and public health implication. *Food borne Pathogens and Diseases, 2*: 1115 – 1129.

Ruegg, P.L. & D.J. Reinemann (2002). Milk quality and mastitis tests. Bovine Practitioner, 36: 41 – 54

Sanders, E.R. (2012). Aseptic Laboratory Techniques: Plating Methods. *Journal of Visual Ex*periments, *63*: e3064. doi: 10.3791/3064.

Shehu, L.M. & A.A. Adesiyun (1990). Characteristics of strains of *Escherichia coli* isolatedfrom locally fermented milk (nono) in Zaria, Nigeria. *Journal of Food Protection, 53*: 574 - 577

SPSS, 2012. Statistical Package for Social Sciences for Windows (version 20.0). http://www.spss.com, 2012.

Tagoe, D.N.A., S.A. Nyako, S.A. Arthur & E. Birikorang (2011). A study of antibioticsusceptibility pattern of bacteria isolates in sachet drinking water sold in Cape Coast metropolis of Ghana. *Research Journal of Microbiology, 6*: 153-158.

Udo, S., I. Andy, A. Umo & M. Ekpo (2009). Potential human pathogens (bacteria) and their antibiogram in ready-to-eat salads sold in Calabar, South-South, Nigeria. *International Journal of Tropical Medicine, 5*(2). Doi: 10.5580/763.

Urbach, G. (1995). Contribution of lactic acid bacteria to flavour compound formation in dairy products. *International Dairy Journal, 5*: 877 – 903.

Vasavada, P.C. (1988). Pathogenic bacteria in milk – a review. *Journal of Diary Science, 71*: 2809 – 2816.

Wikipedia, 2013. The Geographical Location of Keffi. In Wikipedia, the Free Encyclopedia.en.wikipedia.org/wiki/keffi Date accessed: 30th August, 2013.