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Prevalence of antibiotic resistant bacteria in milk sold in Accra

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Antimicrobial resistance is said to currently be the greatest challenge to the effective treatment of infections globally. This study evaluated the risks of antimicrobial resistant microbes associated with 8 types of branded and unbranded milk sold in Accra, Ghana. The study revealed that 6 categories of milk sampled were all contaminated whiles 2 categories were not. Common isolates identified were *E.coli, Klebsiella spp , Enterobacter spp, Proteus vulgaris, Salmonella tyhpi, Enterococcus faecalis, Staphylococccus aureus* and *Staphylococcus epidermidis.* Antibiotic susceptibility tests indicated that all (100%) isolates were multi-resistant to Ampicillin, Tetracycline, Chloramphenicol, Gentamycin, Cotrimoxazole, Ceturoxime and cefotaxime. Ceftriaxime was the most effective antimicrobial but even then, 90.57% of isolates were resistant to it. Antimicrobial resistant isolates were found in all types of milk sampled. Unpasteurized locally produced cow milk accounted for the highest (26.42%) of resistant microbes and imported skimmed milk the least (3.77%). The others were locally-produced pasteurized cow milk (20.75%**), imported whole milk (**11.32%**), soya milk (**22.64%**) and powdered milk (15.09%). Even though the risk level varied from different types of milk, it cut across almost all types of milk. The study demonstrated that milk sold in Accra is a potential hazard of pathogenic food borne bacteria as well as antimicrobial resistant bacteria that may have public health implications. There is the need for some additional food safety measures to be applied before the consumption of milk.**

Key words; Antimicrobial, resistance, bacteria, isolates, milk, contamination, pasteurization

INTRODUCTION

Sufficient evidence exists to the effect that milk is an excellent source of nutrients for both man (Sharm and Joshi, 1992) and bacteria (Henry and Newlander, 1997)). It is therefore required that milk be consumed only after it is pasteurized to make it safe. Unfortunately, recent evidence has revealed post-pasteurization contamination of milk with pathogenic bacteria (Oliver *et al*., 2005; Brisabois *et al*.1997) and antibiotic resistant bacteria. According to (Hawkey 2008) antimicrobial resistance is currently the greatest challenge to the effective treatment of infections globally. For example, more than 70 percent of the bacteria that cause infections acquired in hospitals are resistant to at least one of the drugs most commonly used to treat them and more than 80% of food poisoning bacteria such as *Salmonella* are reported as antibiotic-resistant to at least one type of antibiotic, and more than 50% as resistant to two or more (Hitoshi, 2006). Contamination of milk with microbes therefore results in rapid multiplication of the microbes and this may deteriorate the quality of milk leading to issues of food safety (Frazier and Westhoff, 1986) and/or public health concerns when the microbes are antibiotic resistant (Guerra et al., 2003).

Antibiotic-resistant strains of bacteria cause humans

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to become more virulently ill for longer periods of time than their antibiotic-susceptible counterparts. Treatment failure occurs as a consequence and there is the need for expensive and/or toxic alternative drugs which in most cases are more expensive (WHO, 2007). Furthermore, morbidity and mortality rates are increased with longer durations of hospitalization which leads to increased health care costs and the need for changes in empirical therapy (Hunter and Reeves 2002; Dudley 1995). Evidence indicate that the global rise of antibiotic resistance is mainly due to the exposure of bacteria in human and veterinary medicine(Levy, 1997;WHO, 1997), in crop protection(Falkiner, 1998), aquaculture(Reilly and Kaferstein, 1997), industry and the environment coupled with the increasing mobility across the globe of people, food and animals (Moellering JR., 2007;. Alekshun and Levy 2007).

Contamination of milk and dairy products by micro-organisms can be of endogenous origin, following excretion from the udder of an infected animal and /or exogenous origin, through direct contact with infected herds or through the environment. During and after milking, the milk is susceptible to contamination from the vessels and equipment used for milking and storage of milk. The air and the milking surroundings can also lead to bacterial contamination of milk. In order to produce milk of good hygienic quality it is therefore important to have clean healthy cows and clean utensils for milking and storage of the milk. Unfortunately, the consumption of unpasteurized milk in most developing countries including Ghana has not attracted the desired attention. Furthermore, antibiotic use in both humans and the veterinary sector is unregulated.

Generally, food has been identified to be a very efficient vehicle for bringing a large number of people into contact with a potential hazard (Jordan 2007*)* . Thus, from a population perspective, food-borne exposure and milk in particular may be the most critical pathway for transfer of antibiotic resistant microbes to humans. Yet, data of antibiotic resistant microbes from milk is scanty and scatted. To be able to reduce considerably the problem of antibiotic resistance in the country, there is the need to assess the risk of antibiotic resistance microbes associated generally with food sources and milk in particular. The objective of the current study was to evaluate the risks of antimicrobial resistant microbes associated with milk sold in Accra.

MATERIALS AND METHODS

Sample collection

Branded and Unbranded milk samples were purchased for this work from super markets, milk vendors and farm gates randomly selected. In all 10 fresh pasteurized cow milk (locally-produced), 10 unpasteurized cow milk (locally produced), 10 pasteurized skimmed milk (imported), 10 pasteurized whole milk (imported), 10 soya milk (imported and local), 10 powdered infant milk formula and 10 powdered milk samples were used for this work. Milk samples were transported on ice to the laboratory and stored at 4^0C if analysis was not to be done immediately.

Culture, isolation and identification of isolates

Milk samples were serially diluted in sterile 1% peptone water before plating onto Mannitol Salt Agar (MSA, Oxoid) to select for *Staphylococcus aureus*, Violet Red Bile Agar (VRBA, Oxoid) for coliforms, Plate Count Agar (PCA, Oxoid) for aerobic spore formers and Oxytetracycline Glucose Yeast Extract Agar (OGYE) for yeast and moulds. Inoculated plates were incubated at 37^0C for a maximum period of 48h. Conventional biochemical tests were applied to isolates leading to identification. Methods described by the American Public Health Association (APHA) (1976) were modified and applied to canned milk samples. In this study anaerobes were not incubated for.

Antibiotic sensitivity testing

Inocula of isolated strains were prepared in 1% peptone water and adjusted to turbidity equal to 0.5 McFarland standards and applied onto Mueller-Hinton sensitivity agar using a wire loop. A sterile swab was then used to spread the culture on the media. The inoculated plate was allowed to dry for some few minutes after which sensitivity disks were applied to it using a sterile forceps. Zones of inhibition around sensitivity disks were measured after 18-24hr of incubation at 37℃.The sensitivity of all isolates was tested against: ampicillin (AMP) 10 µg/disk, tetracycline (TET) 10 µg/disk, cotrimoxazole (COT) 25 µg/disk, ceturoxime (CRX) 30 µg/disk, gentamycin (GEN) 10 µg/disk, cefotaxime (CTX) 30 µg/disk, chloramphenicol (CHL) 10 µg/disk and ceftriaxime (CTR) 30 µg/disk according to the CLSI requirements) using the disk diffusion method. The interpretation of zones of inhibition around the disks was according to CLSI (2006).

RESULTS

The study revealed that except evaporated canned milk and infant powdered milk formula all milk samples were contaminated and in certain cases with pathogens of public heath importance. Percentage occurrence of the samples is presented in Table 1.

Table 1. Occurrence of bacteria isolates identified in milk samples

Type of milk sample	No. of samples	Bacteria isolated/occurrence (%)
1	10	*Klebsiella spp* (40%), *Ecoli* (50%),
		Proteus vulgaris (20%)
2	10	*Klebsiella spp* (40%), *Ecoli* (50%)
		Salmonella typhi (10%), *Enterococcus. faecalis* (10%), *Staph. edidermidis* (20%),
		Staph aureus (10%)
3	10	*E.coli* (10%), *Enterococcus. faecalis* (10%)
4	10	*E.coli* (20%), *Enterobacter spp* (10%),
		Staph. aureus (10%), *Enterococcus faecalis* (10%)
		Staph. epidermidis (10%)
5	10	*E.coli* (30%), *Klebsiella spp* (40%),
		Enterobacter spp (20%), *Staph aureus* (10%)
		Proteus vulgaris (20%)
6	10	ND
7	10	*Staph aureus* (30%), *Staph epidermidis* (40%),
		Enterobacter spp(10)
8	10	ND

1-Pasteurized milk (local) , 2- Unpasteurized milk (local), 3- Pasteurized skimmed milk (imported), 4- Pasteurized whole milk (imported), 5- Soya milk (imported & local), 6- Powdered infant milk formula (imported), 7- Powdered milk (Imported & local), 8-Evaporated canned milk (Local), ND- No bacteria detected

The results in Table 1 showed that whereas *E. coli* was the most common (50%) bacterial in both pasteurized and unpasteurized locally produced milk samples, the most prevalent isolate in powdered milk was *Staph epidermidis* (40%)(Table 1) , Whiles *E.coli* (10%) and *Enterococcus faecalis* (10%) were the most prevalent isolates obtained from imported pasteurized skimmed milk, *E.coli* (20%) was the most common isolate in imported pasteurized unskimmed milk (Table 1). In the case of soya milk, the commonest isolate was *Klebsiella spp* (40%). Cultures from evaporated canned milk and powdered infant milk formula did not yield any growth (Table 1).). It is worth noting that *Salmonella typhi* which is an organism of public health importance was isolated from only locally produced unpasteurized milk and that *E.coli* occurred in all types of milk except powdered milk (Table 1)

Antimicrobial sensitivity tests revealed that most isolates were resistant to all antimicrobials tested. Results of susceptibility tests on identified isolates are presented in Table 2.

The results revealed that isolates exhibited high resistance to all the antimicrobials tested (Table 2). For example (100%) of all identified isolates showed resistance to all antibiotics except ceftriaxone suggesting multiple resistance (Table 2) 100% of all identified isolates except *E.coli* and *Klebsiella spp* were resistant to ceftriaxone (Table 2). Resistance of ceftriaxone was exhibited by 87.5% of *E.coli* and 75% of *Klebsiella spp* (Table 2). The most effective antibiotic against the isolates tested was therefore ceftriaxone with only 12.5% of *E.coli* and 25% of *Klebsiella spp* being susceptible to it (Table 2). No isolate was susceptible to all 8 antimicrobials tested (Table 2)

Antibiotic resistant bacteria were isolated from all 6 categories of milk sampled. Occurrence of resistant bacteria in categories of milk samples is summarized in Table 3.

Table 3. Occurrence of antibiotic resistant bacteria in milk samples

1-Pasteurized milk (local) , 2- Unpasteurized milk (local), 3- Pasteurized skimmed milk (imported), 4- Pasteurized whole milk (imported), 5- Soya milk (imported & local), 6- Infant milk formula (imported), 7-

Table 2. Antibiotic resistance of identified isolates to 8 antimicrobial agents.

Bacteria species	No. of isolates	Antibiotic resistance (%)							
		AMP	TET	CHL	GEN	COT	CRX	CTX	CTR
E.coli	16	16(100)	16(100)	16(100)	16(100)	16(100)	16(100)	16(100)	14(87.5)
Klebsiella sp	12	12(100)	12(100)	12(100)	12(100)	12(100)	12(100)	12(100)	9(75)
Enterobacter sp	4	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)
P. vulgaris	4	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)
S. tyhpi	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
E. faecalis	3	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)
S. aureus	6	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)
S. epidermidis	6	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)
Total	53	53(100)	53(100)	53(100)	53(100)	53(100)	53(100)	53(100)	48(90.57)

AMP= Ampicillin, TET=Tetracycline, CHL=Chloramphenicol, GEN=Gentamycin,Cotrimoxazole, CRX= Cefuroxime, CTX= Cefotaxime, CTR= Ceftriaxone

Table 3. Occurrence of antibiotic resistant bacteria in milk samples

Type of milk sample	Number of bacteria isolated/Percentage				
	Tota l	Resistant bacteria		Susceptible bacteria	
1	11	11(20.75%)		0	
2	14	14(26.42%)		0	
3	2	2(3.77%)		0	
4	6	6(11.32%)		0	
5	12	12(22.64%)		0	
6	-	-		-	
7	8	8(15.09%)		0	
8	-	-		-	

1-Pasteurized milk (local) , 2- Unpasteurized milk (local), 3- Pasteurized skimmed milk (imported), 4- Pasteurized whole milk (imported), 5- Soya milk (imported & local), 6- Infant milk formula (imported), 7- Powdered milk (Imported & local), 8-Evaporated tin milk (Local)

Powdered milk (Imported & local), 8-Evaporated tin milk (Local)

The results in Table 2 revealed that no isolate from any of the milk sampled was susceptible to all the 8 antibiotics tested. Resistant isolates occurred the most (26.42%) in unpasteurized locally produced cow milk and the least (3.77%) in imported skimmed milk (pasteurized) (Table 3). Whereas pasteurized locally produced cow milk accounted for 20.75% of resistant isolates, whole imported milk produced 11.32% of resistant isolates (Table 3). Resistant bacteria isolated from soya milk was 22.64% of all resistant isolates whiles powdered milk (imported and local) accounted for 15.09% of resistant isolates(Table 3). In the case of powdered infant milk formula (imported) and evaporated canned milk, no resistant isolates were observed (Table 3)

DISCUSSIONS

This study revealed that the microbiological quality of milk sold in Accra was unacceptable. For example, 6 categories of milk sampled including imported pasteurized and locally pasteurized samples were contaminated with bacteria and in certain cases with pathogens of public health importance. This finding is in agreement with (Schlegelová et al., 2002;Guta et al.,2002; Okpalugo et al 2008 and Adeleke et al.,2000**)** who found bulked cow milk in Czech Republic, cow foremilk in Botswana, pasteurized milk in Nigeria and soya milk in Nigeria respectively to be contaminated with bacteria pathogens. Contrary to the findings of Oladipo and Omo-Adua 1996 and Nazarowec-White 1997 however, this study did not realize any growth from evaporated canned milk and powdered infant milk formula respectively. This may be because this study did not incubate for anaerobes and viable but dormant cells or may be the sample size was too small (Warren et al.,1998). Parallel to this study Frazier and Westhoff, (1988) and Sydner et al (1978) also found soya milk, just like cow milk, to be an excellent medium for microbial growth.

The most common bacteria isolates identified in all the types of milk except powdered milk were *E. coli, Klebsiella spp*, *Enterobacter spp* all of which belong to the coliform group (Table 1). This finding might probably be due to some occurrence of faecal contamination of the samples, an indication of poor hygienic quality of milk. According to Witte, (2000) and Catry et al (2003), the presence of *E.coli* and *Enterococcus faecalis* in milk is an indication of faecal contamination from milked animals and this may occur through the hands of milk collectors or other human sources. This faecal contamination may have resulted from the faeces of milked animals through the hands of milk collectors or from human source. General coliforms (*Klesiella spp* and *Enterobacter spp*) are however, environmental microbes and may also have entered milk samples from dust, the environment, contaminated utensils or the hands of handlers. In the case of powdered milk faecal indicators were not isolated probably because of the low water activity of the product that does not favor the growth of these organisms. To a less extent, *Staphylococcus aureus, Staphylococcus epidermidis, Proteus vulgaris and Salmonella typhi* were isolated from these milk samples (Table 1). Evidence indicate that *Staphylococci spp* (Kaplan 2005) *and Salmonella typhi* (Fontaine et al.,1980) are agents for the cause of mastitis in dairy animals and may have contaminated milk from the udder of infected animals. Additionally, the nasopharyngeal cavity of human beings is the reservoir of Stahpylococci from where these bacteria get localized on the skin, especially on human hands (Kaplan 2005). Staphylococci contamination of milk could therefore also have been through the hands of milk handlers. *Salmonella spp* also reside in the intestinal tract where they cause gastro-enteritis in animals and may have occurred in milk as a result of faecal contamination.

Staphylococcal contamination of milk and milk products is associated with enterotoxicity in humans (Chao *et al*., 2007) and the presence of coliform organisms and *Enterococcus faecalis* in food is linked with a wide variety of human infections such as endocarditis, urinary and genital tract infections, meningitis and septicemia (Mannu et al., 2003). Evidence indicate that *Salmonella* spp is one of the most etiologic agents responsible for several outbreaks associated with the consumption of raw milk (De Buyser et al. 2001)

Results of antibiotic susceptibility tests on isolates (Table 2) revealed that all the isolates were multi-resistant to more than four of the antibiotics as has been also shown in other studies on milk and milk products (Ahmed et al., 2003; Nováková et al.,2010 , Guta et al. 2002 and Okpalugo et al., 2008). For example, except *E.coli* and *Klebsiella spp* which had only 12.5% and 25% susceptible isolates respectively to Ceftriaxone, all the other isolates were 100% resistant to all antimicrobials tested (Table 2). Ceftriaxone resistance in Ecoli (87.5%) and Klebsiella spp (75%) (Table 2) was equally high. Evidence (Witte, 2000; Catry et al., 2003) indicate that *E.coli* and *Enterococcus faecalis* constitute a potential reservoir of resistance genes for pathogenic bacteria and are used internationally as indicator bacteria for antimicrobial resistance because of their high prevalence in animal faeces. The prevalence of these two organisms in the current study particularly *E.coli* which was isolated from almost every category of milk sampled (Table 1) may have enhanced the spread of resistance genes amongst bacteria isolates. Resistance by isolates to antimicrobials may however, have been acquired from over exposure to antibiotics particularly in the veterinary sector or to a lesser extent from natural sources especially when some of the antibiotics tested in this study are commonly used in both the health and veterinary sectors. Evidence (Mazel and Davies, 1999; Levy, 1992) indicate that of the more than 1 million tons of antibiotics released into the biosphere during the last 50 years approximately 50% are estimated to flow into the veterinary and agricultural channels. The application of these antibiotics at sub-therapeutical levels for increased growth and feed efficiencies in farm animals is an integrated part of modern agriculture worldwide and this leads to the emergence of antibiotic resistant microbes (Prescott 2000). When the same antibiotics are used in humans as in animals, resistant microbes passed on to humans from animal sources could then lead to treatment failure in humans with serious public health implications. This study observed 100% resistance to ampicillin, tetracycline, chloramphenicol, cefotaxime and cotrimoxazole (Table 2). A similar observation was made by Erdo and Mensah,(2007) who noted high ampicillin, tetracycline, chramphenicol and cotrimoxazole resistance in clinical isolates from some human hospitals in the western part Accra. It is important to note however that, epidemiological studies that require the use of molecular techniques will have to be carried out to confirm the molecular identity of elements found in milk and humans before any link can be drawn. The level of multi-resistance exhibited by isolates should nonetheless be a matter of concern especially the resistance to cefotaxime, ceturaxime and ceftriaxime which are all cephalosporins (Talaro and Talaro1993). According to (Jordan ,2007) the analogous drugs of third generation cephalosporins and gentamycin in human medicine are suppose to be highly valued as reserve agents and their use in healthcare should be tightly controlled.

The notable difference this study observed in the occurrence of resistant microbes in different categories of milk sampled was that, imported skimmed- milk had the lowest occurrence of resistance microbes (3.77%) (Table 3). Equally significant was the fact that powdered milk did not contain any of the indicator organisms of antibiotic resistance i.e. *E. coli* and *Enterococci faecalis*. Unpasteurized locally produced cow milk accounted for the highest (26.42%) of resistant microbes probably because it contained a lot more contaminants. Our study found an occurrence of resistance microbes of 20% in pasteurized locally produced cow milk suggesting post pasteurization contamination or in adequate pasteurization. Soya milk had resistant microbes at a level of 22.64% (Table 3) which may have been acquired probably as a result of the use of antibiotics in crop protection or through environmental contamination with resistant genes. This study noted a direct link between the percentage occurrence of resistance isolates and the percentage prevalence of bacteria isolates irrespective of whether they where pathogenic or not. It is clear from this study that even though the risk level varied for different types of milk, it cut across almost all types of milk.

In conclusion, the current study has demonstrated that milk sold in Accra is a potential hazard of pathogenic food borne bacteria as well as antimicrobial resistant bacteria that may have public health implications. Strict hygienic measures must be applied to minimize the contamination of milk in addition to education of farmers on the safe use of antibiotics. A review of the national antibiotic policy to effectively regulate the use of antibiotics in the country is necessary.

SUGGESTIONS

The following suggestions may help reduce the prevalence of antibiotic resistant microbes in milk

1. Education of farmers and milk sellers on hygienic handling of milk, pasteurization methods and proper storage conditions
2. Application of microwave, gamma radiation and heat for the pasteurization of milk before consumption.
3. Education of farmers and farm workers on the appropriate use of antibiotics and enhanced infection control measures.
4. Reviewing the antibiotic policy of the country with a view to:
   - improving the management of drugs used in both veterinary and human medicine
   - Restricting access to antibiotics by enforcing medication based on prescription
   - Applying antibiotics to targeted organisms after laboratory findings instead of the often use of broad spectrum antibiotics without prior laboratory tests.
   - Using antibiotics only when clinically relevant.

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REFERENCES

Adeleke OE, Adeniyi BA, Akinrinmisi A A (2000). Microbiological quality of local soymilk: a public health appraisal. Afr.J. Biomed. Res.3: 89 - 92

Ahmed A, Jamee N, Ansari A, Khatoon H (2001). Multiple antibiotic resistance among gram negative bacteria isolated from milk in Karachi. Paki stan J. Pharm. Sci. 14(1):25-31

Alekshun MN, Levy SB (2007). Molecular mechanisms of antibacterial multidrug resistance. *Cell*; 128:1037-1050.

American Public Health Association (APHA) (1976). Compendium of methods for the microbiological examination of foods. Ed. M.L. USA Speck. Publ. By APHA Inc.

Brisabois A, Lafarge V, Brouillaud A, de Buyser ML,Collette C, Garin-Bastuji B, Thorel MF (1997). Pathogenic organisms in milk and milk products: the situation in France and in Europe. Reviews Science. and Technol., 16: 452-471

Catry B, Laevens H, Devriese LA, Opsomer G, De Kruif A (2003). Antimicrobial resistance in livestock. J. Vet. Pharmacol. Therap. 26:81-89

Chao G, Zhou X, Jiao X, Qian X, Xu L (2007). Prevalence and antimicrobial resistance of food borne pathogens isolated from food products in China. *Foodborne Pathogens and Disease*, 4: 277-284

Clinical and Laboratory Standards Institute (CLSI) (2006) CLSI/NCCLS M44S1 (2006). Zone Diameter Interpretive Standards and Corresponding Minimal Inhibitory Concentration (MIC) Interpretive Breakpoints; Informational Supplement, M44-S1,First Edition, CLSI, Wayne, USA

De Buyser ML, Dufour B, Maire M, Lafarge V (2001). Implication of milk and milk products in food-borne disease in France and different industrialized countries. – Int. J. Food Microbiol. 67: 1-17.

Dudley M (1995). Bacterial resistance mechanisms to -lactam antibiotics, assessment of management strategies. *Pharmacotherapy*; 15 Suppl.: pp 9-14

Edoh D, Mensah A (2007). Incidence of antibiotic resistant microbes in Accra west, Ghana. African Journal of Science and Technology, 8(2): 103 –109

Falkiner FR, (1998). The consequences of antibiotic use in horticulture. J. Antimicrob. Chemother. 41, 429–431.

Fontaine RE, Cohen ML, Martin WT, Vernon TM (1980). Epidemic salmonellosis from cheddar cheese-surveillance and prevention. – Am. J.Epidemiol. 111:247-254

Frazier WC, Westhoff DC (1988) Food Microbiology. 3rd edn. Tata McGraw-Hill Publishing Company Ltd., New Delhi.

Frazier WC, Westoff DC (1986), Food Microbiology. TMH Edn., Cambridge University Press, Cambridge, MA., pp 521- 540.

Guerra B, Junker E, Schroeter A, Malorny B, Lehmann S, et al. (2003). Phenotypicand genotypic characterization of antimicrobial resistance in German *Escherichia coli* isolates from cattle, swine, and poultry J. Antimicrob. Ther. 52: 489- 492.

Guta C, Sebunya TK, Gashe BA (2002). Antimicrobial Susceptibility of Staphylocacci spp from Cow Foremilk originating from dairy farms around Gaborone, Botswana. East African Medical Journal, 79 (1): 1-4

Hawkey PM (2008) .The growing burden of antimicrobial resistance. J Antimicrobial Chemotherapy 62, Suppl. 1, 11–19

Henry A, Newlander L (1997). Milk Constituents in Chemistry and Testing of Dairy Products. 5th Edn., John Wiley and Sons Inc., New York, pp: 269-273

Hitoshi I (2006) Effect of radiation decontamination on drug-resistant bacteria. Japanese J. Food Irradiation, 41;9-13.

Hunter PA, Reeves DS (2002). The current status of resistance to antimicrobial agents: report on a meeting. *J Antimicrob Chemother* ; 49:17-23.

Jordan D (2007). Antimicrobial resistance in animal and impacts on food safety and public health. In Focus,. 28(4):163-164

Kaplan SL (2005). Implications of methicillin-resistant *Staphylococcus aureus* as a community-acquired pathogen in pediatric patients. *Infectious. Disease. Clinics of North America,* 19: 747-757.

Levy SB (1992). *The Antibiotic Paradox: How Miracle Drugs are Destroyin the Miracle.* New York: Plenum Press

Levy SB (1997). Antibiotic resistance: an ecological imbalance. In:Chadwick, D.J., Good, J. (Eds.), Antibiotic Resistance. Origins, Evolution, Selection and Spread. John Wiley and Sons, Chichester, pp. 1– 14.

Mannu L, Paba E, Daga R, Comunian R, Zanetti S, Dupre I, Sechi LA (2003). Comparison of the incidence of virulence determinants and antibiotic resistance between Enterococcus faecium strains of

dairy, animal and clinical origin, Int. J. Food Microbiol., 88: 291-304
Mazel D, Davies J (1999). Antibiotic resistance in microbes**.** *Cell Mol. Life Sci* ., 56:742-754.
Moellering Jr RC (2007) Global antibacterial resistance issues. Australian Society For Microbiol., 28(4): 157-160
Nazarowec-White M, Farber JM (1997). Thermal resistance of *Enterobacter sakazakii* in reconstituted dried infant formula. *Lett Appl Microbiol*. 24:9-13
Nováková I, Kačániová M, Arpášová H, r Haščík P, Kunová S, Čuboň (2010). Antibiotic Resistance of Enterococci and Coliform Bacteria in Dairy Products from Commercial Farms. Scientific Papers: Animal Science and Biotechnol, 43 (1): 1-3
Okpalugo J, Ibrahim K, Izebe KS, Inyang US (2008). Aspects of microbial quality of some milk products in Abuja, Nigeria**.** Tropical Journal of Pharmaceutical Research, 7 (4): 1169-1177
Oladipo IC, Omo-Adua RO (1996). Antibiotic Resistance among bacteria isolated from evaporated milk. Asian J. Biol. Sci., 4(1):77-83
Oliver SP, Jayarao BM, Almeida RA (2005). Foodborne pathogens in milk and the dairy farm environment: food safety and public health implications*. Foodborne Pathogens and disease,* 2: 115-129
Prescott JF (2000). Antimicrobial drug resistance and its epidemiology**.** In *Antimicrobial Therapy in Veterinary Medicine*, edn 3. Edited by Prescott JF, Baggot JD, Walker RD. Ames: Iowa State University Press: pp 27-49
Reilly A, Kaferstein F, (1997). Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. Aquac. Res. 28: 735–752.
Schlegelová J, Babák V, Klímová E, Lukášová J, Navrátilová P, Šustáčková A, Šedivá Ryšánek D (2002). Prevalence of and Resistance to Anti- Microbial Drugs in Selected Microbial Species Isolated from Bulk Milk Samples. J. Vet. Med., Series B, 49: 216–225.
Sharm DK, Joshi DV (1992). Bacteriological quality of milk and milk products with special reference to Salmonella and its health significance. Journal of Science Technol, 22:100-103
Sydner IS, Johnson W, Zoltala (1978).Significant pathogen in dairy product (Marth, E.H. ad.) Public Health Association, Washington D.C. pp. 11-32.
Talaro K, Talaro A (1993). Foundations in microbiology. U.S.A: Wm. C. Brow publishers
Warren L Landry, Albert H Schwab, Gayle A Lancette (1998). Examination of Canned Foods, Bacteriological Analytical Manual, 8th Edition, Revision A, Chapt. 21A.
White DG, Zhao S, McDermott PF, Ayers S, Gaines S, et al (2002). Characterizationof antimicrobial resistance among *Escherichia coli* 0111 isolates of animal and human origin. Microbial Drug Resis. 8:139-146.
WHO (1997). The Medical Impact of The Use of Antimicrobials in Food Animals. Report of a WHO Meeting, Berlin, Germany,13–17 October. WHO, Geneva.
WHO (2007). The world health report - A safer future: global public health security in the 21st century; available at URL: http://www.who.int/whr/2007/en/index.html (accessed 25 February 2009)