

## Full Length Research Paper

# Assessment of Antimicrobial Resistance Risks in Branded and Unbranded Milk Products Available in Accra, Ghana

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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 while 2 categories were not. Common isolates identified were *E.coli*, *Klebsiella spp*, *Enterobacter spp*, *Proteus vulgaris*, *Salmonella typhi*, *Enterococcus faecalis*, *Staphylococcus 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 scattered. 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<sup>0</sup>C 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<sup>0</sup>C 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°C. 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 health 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	<i>Klebsiella spp</i> ( 40%), <i>Ecoli</i> (50% ), <i>Proteus vulgaris</i> ( 20% )
2	10	<i>Klebsiella spp</i> ( 40%), <i>Ecoli</i> (50% ) <i>Salmonella typhi</i> ( 10% ), <i>Enterococcus. faecalis</i> (10%), <i>Staph. edidermidis</i> (20% ), <i>Staph aureus</i> ( 10% )
3	10	<i>E.coli</i> (10% ), <i>Enterococcus. faecalis</i> (10%)
4	10	<i>E.coli</i> (20% ), <i>Enterobacter spp</i> (10%), <i>Staph. aureus</i> (10% ), <i>Enterococcus faecalis</i> ( 10%) <i>Staph. epidermidis</i> ( 10% )
5	10	<i>E.coli</i> (30% ), <i>Klebsiella spp</i> ( 40% ), <i>Enterobacter spp</i> (20% ), <i>Staph aureus</i> (10%) <i>Proteus vulgaris</i> (20% )
6	10	ND
7	10	<i>Staph aureus</i> (30%), <i>Staph epidermidis</i> ( 40%), <i>Enterobacter spp</i> (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
<i>E.coli</i>	16	16(100)	16(100)	16(100)	16(100)	16(100)	16(100)	16(100)	14(87.5)
<i>Klebsiella sp</i>	12	12(100)	12(100)	12(100)	12(100)	12(100)	12(100)	12(100)	9(75)
<i>Enterobacter sp</i>	4	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)
<i>P. vulgaris</i>	4	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)	4(100)
<i>S. typhi</i>	1	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)	1(100)
<i>E. faecalis</i>	3	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)	3(100)
<i>S. aureus</i>	6	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)	6(100)
<i>S. epidermidis</i>	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		
	Total	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 while 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 *Staphylococci* 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 *E.coli* (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, chloramphenicol 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, ceftriaxime 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 were 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:
  - o improving the management of drugs used in both veterinary and human medicine
  - o Restricting access to antibiotics by enforcing medication based on prescription
  - o Applying antibiotics to targeted organisms after laboratory findings instead of the often use of broad spectrum antibiotics without prior laboratory tests.
  - o Using antibiotics only when clinically relevant.

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