



ISOLATION, CHARACTERIZATION AND ANTIBIOGRAM OF BACTERIAL PATHOGENS ISOLATED FROM MILK OF COW, GOAT AND SHEEP.

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ABSTRACT

Bacteriological analyses of the milks of cow, sheep and goat were conducted in Keffi, North-Central Nigeria. The bacteriological analyses revealed the presence of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella* spp. This study also revealed a total heterotrophic count, total faecal count and coliform count ($\times 10^4$ cfu/ml) in the cow, sheep and goats milk to be 4.8, 1.0 and 2.9; 4.5, 0.6 and 2.5; 3.6, 0.4 and 1.6 respectively. However, rate of occurrences of the respective isolates indicate varying degree of occurrences. *E. coli* and *Salmonella* spp. had the highest occurrences of 86.7% each. Percentage occurrence in descending order is in the order: *Staphylococcus aureus* (80.0%), followed by *Klebsiella* spp. (73.3%), *P. aeruginosa* (66.7%), then *Proteus vulgaris* (33.3%). Antibioqram of the bacterial isolates revealed a varying level of resistance and susceptibility to the antibiotics tested. All the bacterial isolates were susceptible to Septrin. Similarly, all the isolates were very susceptible to Chloramphenicol, Sparfloxacin, Ciprofloxacin, Augmentin, Perfloxacin, Erythromycin and Streptomycin. Gentamicin has effect on all the isolates, except *S. aureus* that had slight effect at 12mm. Nevertheless, all the bacterial isolates were resistant to Amoxicillin, except *E. coli* and *Proteus vulgaris* that were only slightly susceptible with zones of 12mm each. Also, *Salmonella* spp. was found to be highly resistant at 2mm to Ciprofloxacin. The isolates are highly susceptible to Gentamicin and Chloramphenicol due to their requirement for parenteral administration that discourage abuse and misuse. However, the increased susceptibility of the isolates to the antibiotics tested perhaps can assist in the design of treatment regimen due to these organisms.

Keywords: Isolation, antibiogram, bacteria, milk, Keffi

INTRODUCTION

Milk is an extremely nutritious food. It is an aqueous colloidal suspension of proteins, fat and carbohydrates that contains numerous vitamins and minerals such as calcium, phosphorus, sodium, potassium and magnesium (Falegan and Akele, 2014; Sangoyomi *et al.*, 2010). Milk proteins are ideal in that they are complete and have high essential amino acids composition. Although milk and its various derivatives such as butter, yoghurt and cheese are vital human foods. It provides an excellent medium for the growth of many kinds of micro-organisms (Adesokan *et al.*, 2009). Worldwide cow's milk is the most commonly used but milk from water buffalo, goats, sheep, camels and yaks is also used in various parts of the world (Falegan and Akele, 2014).

The livestock industry in Nigeria centers mainly on the production of cattle, sheep, goats, pigs and poultry. The estimated population for the resources includes: 19,830,000 cattle, 20,500,000 sheep, 24,300,000 goats, 4,855,000 pigs and 130 million poultry. It is obvious that Nigeria has a great potential in animal resources. It accounts for about 5% of the nations' GDP and plays a vital role in meeting the national requirement for protein uptake, internal and external trade (Otoikhian, 2012).

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 (Makut *et al.*, 2014; 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 (Makut *et al.*, 2014).

These animals serve as the major sources of milks for human consumption. However, there are preponderance of microorganisms, some pathogenic and some normal flora that colonize many parts of the animal's body. Momin *et al.* (2011) have observed that Pneumonia in goat is an infection of the lungs characterized by fever (40-41°C), anorexia, painful coughing, dyspnea, mucopurulent nasal discharge and depression. It is one of the most common respiratory illnesses in goats throughout the world (Ackermann and Brogden, 2000). Although pneumonia is more frequently seen in kids but it also infects adult goats. Both infectious and non infectious agents are responsible for lung diseases. Among the infectious agents *Pasteurella maltocida* and *Pasteurella haemolytica* are more frequently associated with the outbreak of acute pneumonia and death of goats in all ages (Falade, 2002). These bacteria are commonly found in the upper respiratory tract of healthy goats. Poor management condition, transportation stress, overcrowding pens, sudden environmental changes, poor housing conditions, viral infection

(e.g. parainfluenza-3 virus), lung parasites and other stressful conditions increase goats' susceptibility to pneumonias. Pneumonia caused by *P. multocida* and *P. haemolytica* can lead to wide spread financial losses because of death, reduced live weight, delayed marketing, treatment cost and unthriftiness among survivors (Momin *et al.*, 2011; Daniel *et al.*, 2006; Davies *et al.*, 1997).

Many of the common enteric pathogens such as *Salmonella*, *Escherichia coli* O157: H7 and *Campylobacter* are carried in the intestinal tract of ruminants, including domestic animals used in milk production, e.g. cows, sheep and goats. Preventing faecal material contaminating the milk is an important step in reducing the prevalence of pathogens entering raw milk. Effective cleaning procedures, including removing faecal material from udders prior to milking, can reduce the risk, although heat treatment of raw milk is the most important process used to eliminate the risk from viable vegetative pathogenic bacteria and provide safe products (Baylis, 2009). Animal milk are the main sources of nutrition for infants whose vulnerability due to undeveloped immune system is obvious therefore contaminated cow milk products pose serious health concern as such they can no longer be ignored as they are among the main entry routes of microbial contamination into the human dietary system in Africa (Susan *et al.*, 2014; Okeke *et al.*, 2012).

Raw milk can be a significant source of food-borne pathogens, and there have been numerous food-poisoning outbreaks associated with direct consumption of raw milk. The presence of this pathogenic bacteria in raw milk and its products have been reported to be a major threat to human health especially those who still drink raw milk and also reduces the keeping quality of milk (i.e. its shelf life). It is a common experience that in Northern part of Nigeria direct consumption of locally processed raw milk in both cities and rural areas is much frequent and more popular than consumption of pasteurized milk because it is believed, especially in rural areas, that locally processed raw milk and its by-products have nutritional advantages over the pasteurized one. However, consumption of raw milk and its by-products is considered potentially hazardous and has been associated with several types of infections including brucellosis, tuberculosis, salmonellosis, yersiniosis, *Escherichia coli* O157 and Staphylococcal enterotoxin poisoning. This constitutes a serious public health problem considering the wide acceptability and consumption of milks from these animals in Nigeria. This work therefore is aimed at isolating, characterizing and investigating the antibiogram of bacterial pathogens from the milks of cow, goat and sheep sold within Keffi metropolis.

MATERIALS AND METHODS

This work was carried out in Keffi metropolis and the microbiological analyses were conducted in the quality control laboratory of Nagari Integrated Dairy Farms and Microbiology Laboratory of the Department of Biological Sciences, Nasarawa State University, Keffi. Keffi is geographically situated on a latitude 8°50' N and longitude 7°52' E. Keffi town is about 850m above sea level and it is the North-West of Lafia, the state capital. It is 53km away from Abuja (Capital of Nigeria) in the Guinea Savannah region of Nigeria (Akwa *et al.*, 2007).

Five samples each of cow, goat and sheep milks was obtained from local sellers in Keffi main market. The samples were then collected in sterile sampling bottles and were immediately transported in an ice-packed box within temperature range of 4-6°C to the laboratory for analyses (Obiekezie *et al.*, 2012). Ten (10) millilitres out of each sample (raw milk of cow, sheep and goat) were aseptically transferred by means of sterile pipette into 90ml of sterile diluents (0.1% peptone water). Serial dilutions were prepared up to 10⁻⁴ for total bacterial count. Standard bacteriological methods were employed for the isolation of bacteria as recommended by Cheesbrough (2006).

Nutrient Agar was used as a general medium for total viable count, MacConkey Agar was used to enumerate for total coliform counts and to isolate lactose gram negative bacteria, EosineMethylene Blue was used for the total faecal coliform count and selective isolation of enteric coliforms. All plates were incubated at 37°C for 24hrs (Makut *et al.*, 2014; Odu *et al.*, 2011). Identification of the isolates was based on cultural, morphological and biochemical characteristics as recommended by Holt (1994).

The bacterial isolates were screened for antimicrobial susceptibility using the Kirby-Bauer agar disk diffusion method as described by 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 then inoculated in triplicates onto the surfaces of plain Mueller-Hinton agar plates and Septrin (30µg), Chloramphenicol (30 µg), Sparfloxacin (5 µg), Amoxicillin (30 µg), Ciprofloxacin (5 µg) Augmentin (30µg), Gentamicin (10 µg), Pefloxacin (10 µg), Erythromycin (15µg) and Streptomycin (10 µg) discs were placed aseptically and incubated at 37°C for 24 h. The zones of inhibition was measured and compared with the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2009).

RESULTS AND DISCUSSION

This study revealed a total heterotrophic count, total faecal count and coliform count ($\times 10^4$ cfu/ml) in the cow, sheep and goats milk to be 4.8, 1.0 and 2.9; 4.5, 0.6 and 2.5; 3.6, 0.4 and 1.6 respectively (Table 1). However, the rate of occurrences of the respective isolates indicates varying degree of occurrences. Cultural, morphological and biochemical tests indicates the presence of the following bacteria; *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella* spp. (Table 2).

E. coli and *Salmonella* spp. had the highest occurrences of 86.7% each respectively. Percentage occurrence in descending order is in the order: *Staphylococcus aureus* (80.0%), followed by *Klebsiella* spp. (73.3%), *P. aeruginosa* (66.7%) and finally *Proteus vulgaris* (33.3%) as shown in Table 3. Antibiogram of the bacterial isolates revealed a varying level of resistance and susceptibility to the antibiotics tested. The diameter of zone of inhibition used for this study were those defined by Johnson and Case (1995), where <10mm was considered as resistant, 11–15 was considered intermediate and >16mm was considered as susceptible. All the bacterial isolates were susceptible to Septrin that was found to be resistant at 8mm. Similarly, all the isolates were very susceptible to Chloramphenicol. Also, *E. coli* and *Proteus vulgaris* were slightly susceptible to Sparfloxacin, but all the other bacterial isolates were found to be very susceptible to the antibiotics tested. Ciprofloxacin had 100% effect on virtually all the bacterial isolates, except *Salmonella* spp., which is highly resistant at 2mm.

Nevertheless, all the bacterial isolates were resistant to Amoxycillin, except *E. coli* and *Proteus vulgaris* that were only slightly susceptible with zones of 12mm each. On the other hand *Staphylococcus aureus* (20mm), *E. coli* (16mm), *P. aeruginosa* (18mm), *Proteus vulgaris* (22mm) were highly susceptible to Augmentin; while *Salmonella* spp. showed some degree of susceptibility (14mm). Gentamicin has effect on all the isolates, except *S. aureus* that had slight effect at 12mm each. Pefloxacin on the other hand, was only slightly susceptible against the isolates, except *Klebsiella* spp. (18mm) and *P. vulgaris* (20mm) that were readily susceptible. Also, Erythromycin has efficacy against all the bacteria; only *S. aureus* (14mm) has slightly susceptibility. Similarly, Streptomycin was effective against most of the isolates and only slightly susceptible to *P. aeruginosa* (14mm), *P. vulgaris* (14mm); but *E. coli* was completely resistant to Streptomycin as shown in Table 4.

Table 1: Enumeration of Bacterial Load from Cow, Sheep and Goat Milks ($\times 10^4$ cfu/ml)

Milk sample	Total heterotrophic count	Total faecal count	Total coliform
Cow milk	4.8	1.0	2.9
Sheep milk	4.5	0.6	2.5
Goat milk	3.6	0.4	1.6

Table 2: Cultural, morphological and biochemical characteristic of bacterial isolates from cow, sheep and goat milks in Keffi metropolis

Cultural Shape	PIG Size	M.P	G.S	Biochemical test						Probable isolates
				CAT	IN	V.P	MR	OX	CT	
Circular	0.4mm	yellowish on MSA	cocci	+	+	-	-	+	+	<i>S. aureus</i>
Circular	1mm	greenish on EMB	slightly	-	+	+	-	+	-	<i>E. coli</i>
pink on MAC		curved rod								
Circular	0.4mm	pinkish on MAC	straight rod	-	+	+	-	-	+	<i>Klebsiella</i> spp.
purple on EMB										
Circular	0.3mm	whitish on NA	curved rod	-	+	-	-	-	+	<i>Pseudomonas</i> spp.
Irregular	1mm	whitish in CLED								
pairs colourless on MAC			curved rod	-		+	+	-	+	<i>Proteus vulgaris</i>
Circular	1-2mm	red, black centre								
in SSA, colourless & transparent in MAC			straight rod	-		+	-	-	+	<i>Salmonella</i> spp.

Where: MP= Morphology, GS= Grams staining, CAT= Catalase, COA= Coagulase, IN= Indole, MR= Methylene red, OX= Oxidase, VP= VogesProskauer, CT= Citrate test, + = positive, - = negative, MSA = Mannitol salt agar, EMB = Eosin methylene blue agar, NA= Nutrient agar, MAC = MacConkey agar.

Table 3: Bacteria Isolates and Percentage Occurrences from Cow, Sheep and Goat Milks

Sample	Number of samples (n)	% Occurrence					
		<i>E. coli</i>	<i>Staph. aureus</i>	<i>Salmonella</i> spp.	<i>Proteus</i> spp.	<i>P. aeruginosa</i>	<i>Klebsiella</i> spp.
Cow	5	5(100)	5(100)	5(100)	2(40)	4(80)	4(80)
Sheep	5	3(60)	4(80)	5(100)	2(40)	4(80)	3(60)
Goat	5	5(100)	3(60)	3(100)	1(20)	2(40)	4(80)
Total	15	13(86.7)	12(80.0)	13(86.7)	5(33.3)	10(66.7)	11(73.3)

Table 4: Antibio gram of the bacterial isolates from milks in Keffi metropolis

Antibiotics	Concentration (μ g)						Susceptibility of Isolates (mm)
	<i>S. aureus</i>	<i>E. coli</i>	<i>Klebsiella</i> spp.	<i>P. aeruginosa</i>	<i>P. vulgaris</i>	<i>Salmonella</i> spp.	
Septin	30	16	22	22	20	20	20
Chloramphenicol	30	18	18	22	36	24	20
Sparfloxacin	5	20	12	16	18	14	18
Ciprofloxacin	5	24	16	28	24	14	2
Amoxicillin	30	8	12	-	-	12	-
Augmentin	30	20	16	-	18	22	14
Gentamycin	10	12	28	16	18	22	22
Perfloxacin	10	14	14	18	14	20	12
Erythromycin	15	14	26	24	24	16	32
Streptomycin	10	20	-	18	14	14	18

Key: 1–10mm= Resistant, 11–15mm= Intermediately susceptible, ≥ 16 mm= Susceptible, – = No Inhibition
A bacteriological investigation of the milks of cows, sheep and goats was conducted in Keffi metropolis, North-Central Nigeria. Makut *et al.* (2014) and Ruegg and Reinemann (2002) described milk as a highly nutritional food that serve as an excellent growth medium for wide range of microorganisms. Presumptive isolation and identification of bacterial isolates indicate the presence of *Staphylococcus aureus*, *E. coli*, *Klebsiella* spp., *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Salmonella* spp. Most of the bacterial contaminants were earlier reported by some researchers; Eka and Ohaba (1977), Da Silva *et al.* (1998), Wouters *et al.*

(2002), Jayarao *et al.* (2006), Obi and Ikenebomeh (2007), Mohammed and El-Zubeir (2007), Ogbonna (2011), Ogbonna *et al.*, (2012), Abdulkadir and Mugadi (2012), Obiekezie *et al.*, (2012), Makut *et al.* (2013, 2014), Susan *et al.*, (2014) and Egwaikhide *et al.* (2014). The organisms detected had serious public health implication. *Staphylococcus aureus* causes diseases in humans as it is responsible for a long list of different diseases such as folliculitis, furuncles, carbuncles, erysipelas, cellulites, scalded skin syndrome, impetigo, pneumonia, osteoporosis, toxic shock syndrome (Toxemia), meningitis, and staphylococcal food poisoning (Abdulkadir and Mugadi, 2012; Marjorie and Kathleen, 2006). Similarly, Steward and Besorick (1997) opined that *Salmonella* spp. is a strict pathogen and has no habitat other than humans and animals body, and that source of human infection is therefore from human and animals as carriers; where the organism is excreted in the faeces or urine and transmitted by food or water which is ingested by another subject. *Salmonella* spp. can cause any of these three (3) types of infections: bacterial food poisoning, enteric fever and systemic fever (Septicemia) (Steward and Besorick, 1997).

Pseudomonas aeruginosa is widely distributed in soil and water and can therefore contaminate food and dairy product by the sellers (Pelczar *et al.*, 2007). Generally, Sangodoyin and Osuji (1990) described contaminating organisms as those objectionable microorganisms that can proliferate in the food adversely affecting the quality and portability of food. Unlike the presence of certain strains of *Lactobacillus* and *Streptococcus* species that may be beneficial as suggested by Perdigon *et al.* (1987). The incidence of the species of *E. coli* itself in milk and milk products, as a possible cause of food borne disease could be harmful to consumers (Hahn, 1996). The risk is magnified when the same samples of milk products are contaminated with *Salmonella*, because *Salmonella* can survive at temperatures of refrigeration where these milk products are normally stored. The bacterial load obtained from this present study ranged between 0.6–4.8 ×10⁴cfu/ml. This corroborates the finding of Ogbonna *et al.*, (2012), Abid *et al.* (2009) and Lore *et al.* (2005), but disagree with the relatively high counts reported by Egwaikhide *et al.* (2014), Susan *et al.*, (2014), Obiekezie *et al.* (2012) and Shojaei and Yadollahi (2008). Abid *et al.*, (2009) reported that counts greater than 10³cfu/ml for raw milk indicates a serious fault in hygiene during the production. Perhaps, it could be as a result of poor hygienic method of milking of the cow, sheep

and goat milks usually practiced by local producers; also, the contaminating bacteria could be introduced via microflora adhering to the calabash, spoons and bowls, and even through the use of streams or well water during production.

Nevertheless, the enteric bacteria occurred more in all the samples analyzed; *E. coli* and *Salmonella* spp. (86.7%), *Klebsiella* spp. (73.3%) are also the normal microbiota. *Staphylococcus aureus* had high percentage prevalence of 80.0%. Earlier report by Obiekezie *et al.*, (2012) reinforced the finding of this present study; the authors observed that enteric bacteria such as *E. coli* and *Salmonella* spp. among others had high occurrence in milk as a result of the unhygienic, and possibly faecally-contaminated water employed in washing utensils used in milking and further processing of the milk. The high occurrence rate of the enteric bacteria concur with the findings of Ogbonna (2011) in his microbiological analysis of Nono sold within Maiduguri, North-Eastern Nigeria, and also Soomro *et al.*, (2002) work on the presence of *E. coli* in milk and milk products with relation to public health in Pakistan. *Staphylococcus aureus* was also found to occur in high numbers. Moshood *et al.* (2013) suggested that *Staphylococcus aureus* could be much in ice cream and other milk products, and that they could be from nose, where they are commonly found, hands, skin and clothing of handlers.

Antibiogram of the bacterial isolates revealed varying levels of susceptibility/resistance to the antibiotics tested. Most of the bacterial isolates are highly resistant to Amoxicillin; however, *E. coli* was slightly susceptible (12mm). Gentamicin, Erythromycin and Chloramphenicol had the highest efficacy against all the bacteria tested. Similarly, *E. coli* was resistant to Streptomycin. Ciprofloxacin on the other hand had activity against all the isolates, except *Salmonella* spp. which is very resistant (2mm). More so, only *Klebsiella* spp. was entirely resistant to Augmentin, otherwise all the isolates were susceptible. The sensitivity pattern of the bacterial isolates to the antibiotics tested is comparable with reports of earlier researchers (Udo *et al.*, 2001, Inyang, 2009, Tagoe *et al.*, 2011, Makut *et al.*, 2013 and 2014). For most bacteria, there is evidence that increased usage of a particular antimicrobial correlates with increased levels of bacterial resistance (Granizo *et al.*, 2000); perhaps this explains the high resistance to Amoxicillin by the isolates because of its common and prevalent use. Resistance to Amoxicillin is not new, as Ehinmidu (2003) observed such effect earlier.

CONCLUSION

The results obtained shows that there are presence of pathogenic microorganism that may be potential source of food borne infection and some related diseases for the consumers of this product in the sampling areas. The total viable bacteria counts in all samples were above the standard. According to Nigerian Agency for Food, Drugs Administration and Control NAFDAC (2009), the microbial load limited for total liable colony count is 1.0×10^2 cfu/ml and that *Escherichia coli* should not be present in all samples. However, *E.coli* was confirmed to be present in almost all the samples analyzed. Nevertheless, the resulting microbial populations were, however at par with the Food and Agricultural Organization (FAO) recommended maximum non-pathogenic microbial population of 10^4 cells/ml for milk products (FAO, 1970). The study also demonstrate the occurrence of antimicrobial resistances among certain bacteria isolated from milks of cows, sheep and goats hawked in Keffi Local Government Area of Nasarawa State, Nigeria. The prevalence of antimicrobial resistant bacteria obtained in this study could be due to the increased use/misuse of antibiotic in human medicine, veterinary and agriculture. However, the increased susceptibility of the isolates to the antibiotics tested perhaps can assist in the design of treatment regimen due to these organisms.

The milking process, especially the equipment associated with it introduces the greatest proportion

of microorganism in cow milk (Olson and Mocquot, 1980). According to Aumaitre (1999), the health of the dairy herd, milking and pre storage conditions are also basic determinants of milk quality. Bacteria may enter milk through the udder and most of the organisms in raw milk and products are contaminants from the external surface of udder, milking utensils and handlers (Ayres *et al.*, 1980). Various types of equipment and utensils, such as milking machines, pails, cans, and milk churns are used in handling milk on the farm. In order to reduce contamination of milk, utensils used for milking should be rinsed, cleaned using detergent and disinfected immediately after use (FAO and WHO, 1997; Dodd and Phipps, 1994). More so, since the microbiological limits of raw milk and products are not established in this country: it is very likely that milk should often be tested, if found positive for pathogens then withheld from human consumption. The production of high-quality milk and products, safe milk should be of great importance to the economy of the farmer and the sustainability of the dairy industry in this country. Also, since Nono and Kindirmo serve as parts of the major food drinks for the inhabitants of Keffi metropolis, it is recommended that Nono and Kindirmo be thoroughly pasteurized and handed with utmost hygiene prior to consumption. In view of the antibiogram, it is therefore, necessary to intensify surveillance of isolates to detect emerging antimicrobial resistance phenotype especially in Keffi Nasarawa State, Nigeria.

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