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Isolation and Susceptibility to Antibiotics of Bacterial Strains From Burkina Faso Fermented Milk Samples

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Abstract: The aim of this study was carried out to determine the antibiotics resistance of aerobic flora strain isolated from fermented milk in Burkina Faso. Nineteen representative strains of aerobic flora were concerned by this study. Antibiotic susceptibility and resistance of nineteen (19) representative aerobic microorganisms strains to six (06) antibiotics was investigated using disc diffusion method. The antibioctic used for the tests included Gentamycin, Penicillin, Ampicillin, Tetracyclin, Trimethoprime sulfamethoxazole, Erythromycin. The inhibition zone diameters of all antibiotics were between 0 mm and 48 mm for all the strains isolated and tested. The strains were highly resistant to Penicillin (78.94%), to Trimethoprime sulfamethoxazole (73.68%), to Gentamycin (63.15%) but had low resistance to Tetracyclin (57.89%), to Erythromycin (52.63%) and to Ampicillin (42.10%). The higher inhibition zone diameter was obtained with Ampicillin (48 mm). The lower one was more obtained with Trimethoprime sulfamethoxazole (73.68 % of 0 mm inhibition zone diameter) and Penicillin (68.42 % of 0 mm inhibition zone diameter). The high percentage of resistance to the antibiotics studied could be attributed to their usage and abuse in the animals' sanitation.

Key words: Antibiotics, bacteria, fermented milk, susceptibility

INTRODUCTION

Antibiotics have mainly been used to cure infections caused by pathogenic bacteria in human and animals. Antibiotics use is suggested to be a major risk factor for development of antibiotics resistance (de Man *et al*., 2000; Neu, 1992; Witte, 1998).

The widespread use of antibiotics has been responsible for the development of numerous problems including the emergence of multidrug resistant bacteria. The level of antibiotic resistance is a given community or hospital can be predicted by these important measure; the proportion of resistant organisms introduced from outside the population, the extensive use of antimicrobial agents and the proportion that is spread from animals medication.

It is reported that every year more than 3 millions children die of diarrhoeal diseases including bacterial infections (Murray and Lopes, 1997). Antimicrobial agent resistance has been recognized as an emerging worldwide problem in both human and animals.

The prevalence of antibiotic resistance among food-borne pathogens has increased during recent decades (Davison, 1999).

High incidence of antibiotic resistance among staphylococcal strains in the Ile Ife (Nigeria) community in the neonate and the adolescent was reported by Ako-Nai *et al.* (2002).

Several work were done on *E. coli* resistance (Bonten *et al*.,1992; Barrett *et al*., 2000; Stümer *et al*., 2004; Reynolds *et al*., 2004; Zhanel *et al*., 2006).

Several studies (Neu, 1992; Witte, 1998; Davison, 1999; de Man *et al*., 2000) have demonstrated that the patterns of antibiotic usage may greatly affect the number of resistant microorganisms which occur in an environment.

An earlier study in Kenya (Shitandi and Sternesjö, 2001) found betalactam antibiotics residues to be prevalent in milk.

The rural people in Burkina Faso still produce unpasteurized fermented milk (from gost's and cow's which can be traited with antibiotics) by traditional methods, since such milk products still enjoy loyal following in rural communities. The antimicrobial agents used in animal care are also significant, not only in increasing the resistance in animal pathogens, but also in bacteria transmitted from animals to human. Lactic acid bacteria present in milk from treated animals with antibiotics can carry resistance bacteria to the population. Fermented milk play important role in the diet of low income and the majority of Fulani living in the rural areas of Burkina Faso. Fulani of Burkina Faso ferment their milk in calabashes, gourds and claypots (Savadogo *et al*., 2004).

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Evidence has been found which indicates that resistance strains of pathogens can be transmitted to humans through food (Oosterom, 1991; Khachatourians, 1998).

The nature of fermented products is different from one region to another; this depends on the local indigenous microflora, which in turn reflected the climatic conditions of the area.

This work deal with antibiotics resistance of aerobic microorganisms isolated from fermented milk product tradionally and consumed in Burkina. The aim of this study was to assess the *in vitro* antimicrobial resistance or susceptibility of aerobic microorganisms from fermented milk focusing on Gentamicin, Penicillin, Ampicillin, Tetracyclin, Trimethoprime sulfamethoxazole and Erythromycin used in human clinical medicine.

MATERIALS AND METHODS

Fermented milk sampling: Fermented milk from thirty goat's and cow's were collected from individual households of rural areas in southern Burkina Faso from May 2008. Twenty (20) samples were collected in sterile small bottles and stored in laboratory under refrigeration at 4ºC until they were used in experiments.

Microorganisms numeration and isolation: Ten milliliters of fermented milk were aseptically added into 90 ml of sterile 0.9 % NaCl solution and mixed thoroughly. Serial dilutions (10^{-1} to 10^{-9}) were performed and 1 ml aliquots of the appropriate dilutions were directly inoculated in triplicate on the following media:

Plate count agar (Fluka Biochemika 70152) incubated at 30ºC for 72 h for enumeration of total aerobic mesophilic bacteria. Developed colonies were counted.

Colonies developing on plate were regrouped by their macroscopic and microscopic morphotypes.

The representative colonies in each sample were picked randomly from Plate count agar and selected. Nineteen colonies were obtained after the selection and these colonies (19) were cultivated in nutrient broth. Purity was checked by streaking on Plate count agar. These pure isolates were cultivated in Nutritive broth at 30ºC for 18 h.

Microorganisms characterization: All isolates were examinated for cell morphology, Gram staining, catalase test (Harrigan and McCance, 1976). Cell Morphology was observed by phase contrast microscopy. Colonies were subsequently identified using standard methods.

Antibiotics susceptibility testing: The antibiotics susceptibility pattern of all isolates to 10 UI Gentamicin (Wuham, China), 6 µg Penicillin (Becton Dickinson, USA) , 10 µg Ampicillin (Medreich, India), 30 UI Tetracyclin (BIO-RAD, France), 1.25/23.75 Trimethoprime sulfamethoxazole (Oxoid, England) , 15 UI Erythromycin (BIO-RAD,France) were evaluated by the modified Kirby-Bauer diffusion technique (Cheesbrough, 2002). The antibiotics were chosen based on their importance in treating human or animal infections and their ability to provide diversity for representation of different antibiotics classes.

Standardised overnight culture of each isolate was used to flood the surface of Mueller Hinton (Oxoid, England) agar plates and excess drained off and dried while the petri dish (90 mm diameter) lid was in place. Antibiotics discs were directly placed onto the bacterial culture with turbidity of 0.5 Mac Farland standards (10^8 cfu/ml). The plates were incubated at 37ºC for 24 h (Ehinmidu, 2003).

Tests were performed using the standard disc diffusion method (NCCLS, 1993). The diameter of the zone of inhibition produced by each antibiotic disc was measured (Rammelsberg and Radler, 1990), recorded and isolates (strains) were classified as "resistant", "intermediate sensitive", or "sensitive" based on the standard interpretative chart updated according to the current NCCLS standard (Cheesbrough, 2002; NCCLS, 2002; NCCLS, 2003) and Fluka zone interpretative chart in accordance WHO requirements. The diameters of antibiotic inhibition of growth were measured and recorded as susceptible (S), intermediary (I) or resistant (R). Zone sizes were interpreted by using standard recommendations.

RESULTS

The values of aerobic microbial count for all the fermented milk samples were ranged from 2×10^5 to 8×10^7 cfu ml^{-1}.

Among isolated strains fourteen (14) are Gram positive and five (5) are Gram negative (Table 1). One strain is catalase positive, eighteen (18 strains) are catalase negative (Table 1).

Eighteen (18) strains are Rods and one have Spherical morphology (Table 1). All of the strains showed resistance to one or more antibiotics and a pattern of multiple drug resistance was observed (Table 2).

The inhibition zone diameters of all antibiotics were between 0 mm and 48 mm for all the strains isolated and tested (Table 2). The higher inhibition zone diameter was obtained with Ampicillin (48 mm). The lower one was more obtained with Trimethoprime sulfamethoxazole (73.68 % of 0 mm inhibition zone diameter) and Penicillin (68.42 % of 0 mm inhibition zone diameter).

The Table 2 show all result of susceptibility and resistance; the highest rates of resistance were against Penicillin (78.94%), Trimethoprime sulfamethoxazole (73.68%), Gentamycin (63.15%) but low resistance were against Tetracyclin (57.89%), Erythromycin (52.63%) and Ampicillin (42.10%).

DISCUSSION

Earliest study (Savadogo *et al.,*2004) on Burkina Faso fermented milk samples has reported that in

Table 1: Characteristics of 19 representative strains selected and isolated from Burkina Faso fermented milk

Bacteria strains	Cell form	Gram staining	Catalase
GN 1	Rods	+	-
GN 2	Rods	-	-
GN 3	Rods	+	-
GN 4	Rods	+	-
GN 6	Rods	+	-
GN 7	Rods	+	-
GN 8	Rods	-	-
GN 9	Rods	+	+
GN 10	Rods	+	-
GN 11	Rods	-	-
GN 12	Rods	+	-
GN 13	Rods	-	-
GN 14	Rods	+	-
GN 15	Rods	-	-
GN 16	Rods	+	-
GN 17	Rods	+	-
GN 18	spherical	+	-
GN 19	Rods	+	-
GN 20	Rods	+	-

+ = Positive reaction
- = Negative reaction
Gram positive = 14
Gram negative = 5

Table 2: Diameters (mm) of inhibition zone and resistance percentage with six antibiotics of 19 representative strains selected from Burkina Faso fermented milk.

Bacteria	Antibiotics					
strains	GEN	PEN	AMP	TET	TRI	ERY
GN 1	0	0	0	0	0	0
GN 2	10	0	0	0	0	0
GN 3	18	2	2	2	0	0
GN 4	1	1	4	0	0	6
GN 6	7	32	20	28	14	8
GN 7	8	0	10	8	8	12
GN 8	11	40	36	14	4	16
GN 9	0	22	12	0	0	10
GN 10	10	34	30	20	4	26
GN 11	0	0	10	10	0	0
GN 12	0	0	6	4	0	4
GN 13	0	0	0	0	0	0
GN 14	13	0	4	6	0	2
GN 15	0	0	48	26	20	20
GN 16	0	0	0	0	0	0
GN 17	0	0	4	0	0	0
GN 18	0	0	0	0	0	0
GN 19	0	0	2	0	0	2
GN 20	0	0	2	2	0	8
R (%)	63.15	78.94	57.89	63.15	84.21	57.89
0 mm (%)	57.89	68.42	26.31	47.36	73.68	42.1

GEN: 10 UI Gentamicin
PEN: 6 µg Penicillin
AMP: 10 µg Ampicillin
TET: 30 UI Tetracyclin
TRI: 1.25/23.75 Trimethoprime sulfamethoxazole
ERY: 15 UI Erythromycin
R (%) : percentage of Resistance

traditional fermented milk aerobic mesophilic bacteria are predominant. Among these bacteria lactic acid bacteria are predominant and *Lactococcus*, *Leuconostoc*, *Lactobacillus* were identified.

Strains diversity was determined in fermented milk through the antibiotic susceptibility disc diffusion method. Pattern of multiple resistances was observed among of all the strains used in this study.

Our bacteria strains gave of multiple resistance to the antibiotics used in this study, this results were in accord with those obtained by Kaspar and Burges (1990); Mckeon *et al.* (1995); Al Haj *et al.* (2007). Lactic acid bacteria are normally resistant to the principal types of antibioties, such as β-lactam, cephalosporins, aminoglycosides, quinine, imidazole, nitrofurantoin and fluoroquinolines (Halami *et al.*, 2000)

Al Haj *et al.* (2007) observed high resistance to tetracycline (81.4 %) than our result (57,89%) with tetracycline. Shitandi and Sternesjö (2001) obtained also high resistance to penicillin (72%), but low resistance to Tetracyclin (57.9%) as our result. O'Brien (1987) reported high resitance to ampicillin (85%) and tetracylin (72%). Resistance to several antibiotic has been noted in strains isolated from craft-made spanish cheeses (Herrero *et al.*, 1996).

Kaspar and Burgess (1990) observed 26-46% antibiotic-resistance in *E. coli* from urban and rural water. Mckeon *et al.* (1995) observed an 87% resistance to at least one antibiotic for the non-faecal and faecal coliforms isolated from groundwater. The important factors in the antibiotic resistance prevalence level is the antibiotic uses and dissemination of resistant bacteria or resistance genes from a resistant donor to a susceptible host.

Antimicrobial agents are also provided in water to prevent diseases in poultry flocks and in milk replaces to prevent diseases in calves. The antibiotics interfere with the normal metabolic activity of the microorganisms through inhibition or of inactivation of the enzymes. They can also act to the level of other targets that the enzymes and to disrupt (the perméation, the osmotic regulation, the interactions ribosomes-mRNA, ribosomes-tRNA, the replication, the transcription, the transduction). The antibiotics acting on the bacterial partition, on the bacterial membrane, on the DNA, on the bacterial ribosome.

Resistance acquired: The acquired resistance occurs when some stumps of a same normally sensitive bacterial species become resistant. Bacteria have the capacity to transfer the genetic information, it is an exogenous information that is recovered by the bacterium. The bacteria can transfer (the vertical transfer between bacteria of the same species, the horizontal transfer that intervenes in the exchanges between Gram positive bacteria and Gram negative bacteria) mobile elements of their genome. Most these cases of resistances meet at the hospital:

- The scrambling : Bacteria synthesize the proteins that can sequestrate the antibiotic or damage it to make he innocuous.
- Modification of the affinity of the enzyme or the biologic molecule targets as the antibiotic won't be able to set anymore. The bacteria can modify the target of the antibiotic; the one is not recognized here and becomes insensible to the antibiotic.
- Modification of the bacterial partition so that the permeability to the antibiotic is weak or hopeless.

The fermented milk is produce traditionally by women; the contaminating organisms could be through air microflora which sticks to the smoothening stick, calabash spoons and bowls used for the sale of the products and during technology process. Normal human flora of the customers or producers could also serve as contaminants. In Burkina Faso many producers sell raw unpasteurized milk directly to consumers and this fact could be a likely entry route for resistant bacteria strains to humans.

According to Aarestrup (1995) and Levin *et al.* (1997), multiple resistance capable of regional dissemination can emerge as a result of antimicrobial selection pressure in either livestock or humans. Evidence has been found which indicates that resistance strains of pathogens can be transmitted to humans through food (Oosterom, 1991; Khachatourians, 1998).

Some Conditions are needed for antibiotic resistance to develop in bacteria: the organism must come into contact with the antibiotic at levels below the strains Minimum Inhibitory Concentrations (MICs).

According to Hart and Kariuki (1998) and Okeke *et al.* (1995) Penicillin, Trimethoprim+sulfamethazine and Tetracycline are known to be extensively used in developing countries.

Then, resistance against the agent must develop, along with a mechanism to transfer it to daughter organisms or directly to other members of the same species (Noble *et al.*, 1992).

This suggests that no single drug is fully effective against the organism and it would be desirable if milk processors ensured adequate pasteurization to eliminate potential pathogens. However irrespective of the processing conditions, the miss use of antibiotics at the farm level is the heart of the resistance problem. A multidisciplinary approach is thus required to tackle the emerged problem of resistance in Burkina and reinforce milk safety efforts.

Reduction of antimicrobial resistance in the food chain is a way of reducing the effect in humans health, medical and veterinary sectors .Milk and meat obtained from animals are inadvertently contaminated with bacteria

CONCLUSION

Antibiotic resistance in food-borne pathogens is reality.

Improvement and strengthening of existing institutional guideline with regard to dispensing and use of antibiotics , establishment of a surveillance group to monitor. Scientific and political efforts must be done to eradicate the problem of antibiotic resistance.

National Research Council and Institute of Medicine (NRCIM) of USA, indicate that the use of drugs in the farm-animal production industry is not without some problems and concers, but that it does not appear to constitute an immediate public health concern (NRCIM, 1998). Antibiotics are the most important in the treatment of bacterial infections and antibiotic-resistant bacteria become major problem of health for human and animals.

Penicillin, trimethoprim+sulfamethazine, Ampicillin and Tetracycline are widely available from distributors and can be purchased easily from certain dealers without a prescription.

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