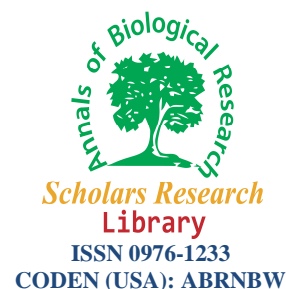




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Imprudent usage of antibiotics in dairy farms and antibiotics detection in milk

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ABSTRACT

A survey questionnaire was conducted to investigate the use of antibiotics on 136 dairy farms in Khartoum state/ Sudan. The survey revealed that 49.3% of the farms owners were illiterate and 35.3% did not complete their education. 80% of the farms lacked written records, 75.7% had no written plans for treating sick animals with antibiotics, 17.6% of the farms completed the course of treatment and only 4.4% of the farms determined the antibiotic withdrawal period. Antibiotic-treated cows were physically separated from other milking cows in 19% of the visited farms, 8.8% of the farms milked the treated cows in a separate milking units and their milk was added to the farm whole milk in 86% of the farms. Mastitis (89%) followed by pneumonia (79%), enteritis (53%), metritis (46%), foot rot (41%) and eye infection (22%) were the most prominent diseases in dairy farms. Penicillins (83%) and Tetracyclines (78%) were mostly used for the treatment of the cases specially mastitis and in dry cows therapy. Tylosine (65%), Gentamycin (62%) and Sulpha drugs (43%) were mostly used in the treatment of other diseases. These antibiotics were obtained from veterinary pharmacies (89.7%) and venders (10.3%) without prescription and they were used for therapeutic purposes in 81% of the farms. Only 31.6% of the farms allowed veterinarians to administer these antibiotics. Also 78.7% of the antibiotic users and 56% of the milk consumers lack knowledge about the emerging threat of antimicrobial resistance in bacteria and/ or antibiotic residues in animal products. Milk samples were collected and investigated for the presence of penicillins. 37% of the samples were found positive for Penicillin. Taken together, these results obviously demonstrated that antibiotics were extensively misused in the dairy farms. These findings may be useful in preventing the antibiotic resistance and developing new strategies for prudent usage of antibiotics.

Key words: Antibiotics, Dairy farms, Milk, Questionnaire, Khartoum state

INTRODUCTION

Antibiotics are used in the dairy farms for multiple purposes such as growth promoters, prophylaxis and for therapeutic purposes [1, 2]. These veterinary drugs include a large number of different types of compounds that can be administrated in the feed or in the drinking water. However the imprudent use of these drugs may exert adverse effects due to the presence of antibiotics residues in the animal products, such as milk and meat, which can resulted in allergic reactions [1, 3, 4]. Moreover there are multiple scientific evidences that demonstrated the relationship between the use of antibiotics in food producing animals and the emergence and selection of antibiotics resistance bacteria [2, 4, 5].

Some preservatives such as formaldehyde, hydrogen peroxide, boric acid, antibiotics are intentionally added to the milk to inhibit the growth of microorganism and subsequent spoilage including fungus, mould and rope inhibitors [6, 7]. These substances could extend the shelf life and the quality of milk during transportation to the market and sales points. However addition of these preservatives to the milk could be associated with a potential hazard to the consumer's health [4, 8]. Also the presences of antibiotics in milk have an inhibitory effect on culturing processes of fermented milk products [9-11].

To understand the public health risks associated with antibiotic usage within the dairy industry, it is important to define the type and the specific use of antibiotics that are associated with on-farm management practices. Most importantly, the dairy farms should maintain complete records on individual animal health and antibiotic usage. These records help in analyzing and determining the type of antibiotics used, the purpose of their administration, the withdrawal period of the drug [12, 13].

Despite multiple reports that were conducted to observe and monitor the antibiotic residues in milk in Sudan [7, 10, 11, 14, 15] but these reports did not heavily investigate the status or the condition of the farms and the purpose of using antibiotics in the farms. Moreover it is important to investigate if the antibiotics in these dairy farms are prudently used through keeping records in the farms and how are antibiotics treated animals are kept within the farms.

The objective of this study was to investigate the condition of the farms and the extent of use of antibiotics by farmers. The investigation was mainly performed through a survey questionnaire for collecting information on antibiotic usage. Also the study aimed to screen for antibiotics in milk samples collected from farms and milk vendors

MATERIALS AND METHODS

Questionnaire survey

A questionnaire survey on antibiotic usage was developed for collecting information on the antibiotic usage in Khartoum state. The questionnaire survey was administered to 136 dairy farm producers or managers. The questionnaire included dairy farms from the three localities of Khartoum state (Khartoum, Khartoum North and Omdurman). The survey was conducted from July 2014 to July 2015. Also the milk consumers (milk buyers) participated on the questionnaire through their knowledge about the presence of the antibiotics in milk and the emergence of antibiotics resistance.

Milk samples collection and detection of antibiotics

A total of 122 milk samples were randomly collected and examined for detecting antibiotic residues in milk from the farms during milking process and from milk vendors. Samples were transported to the Laboratory of the College of Veterinary Medicine, University of Bahri on the same day and were analyzed for the presence of antibiotics, mainly penicillin. For analysis of antibiotic residues, two tests were used the SNAP Beta-Lactam ST test and the disc diffusion test. The formers was done using IDEXX SNAP* Beta-Lactam ST Test- kit- USA according to the instructions of the manufacturer. The disc assay was carried as previously described [16]. Briefly, sterile filter paper discs were immersed in the milk sample and then placed in agar media previously inoculated with *E. coli* and incubated overnight. Inhibition zone around the discs indicate presence of antibiotics in the sample.

Data Analyses

The respond of the farm managers or farms owners to the questionnaire were analyzed according to the percentage of the response to the questions (e.g. yes or no).

RESULTS AND DISCUSSION

In this study, a survey questionnaire was conducted containing questions that reflect the real situation in the dairy farms concerning antibiotic usage. One important observation of this study was the management of the farms which was directed mostly by illiterate owners or illiterate antibiotic users (15% educated vs 85% illiterate or did not complete their education) (table-1). The inquiry of the knowledge and practices among farmers concerning antibiotics usage and antibiotic resistance is cornerstone in investigating antibiotic resistance and antibiotics residues in animal product. Some reports demonstrated that farmers misused antibiotics in animal dairy farms due to their ignorance of the importance of optimal use of antibiotics, the potential health hazards and the economical waste

associated with antibiotic misuse [5, 17]. Moreover one report revealed that 52% of the farms owners are uneducated [17].

Table (1) showed the total number of the visited farms and the number of the visited farms in each locality. The numbers in the brackets showed the number of the people responded to the questions with yes or no or others and their percentages

State	Farm location			Total
	Khartoum state	Khartoum north	Khartoum	
Number of the visited farms	41	51	44	136
Survey questions				
1- What is the educational status of the owner or of the antibiotics user?				
	Illiterate (67) (49.3%)			
	Intermediate or higher secondary school (48) (35.3%)			
	University graduated (21) (15.4%)			
2- Does the farm maintain written records for antibiotic treatments including medicated feeds?				
	Yes (27) (20%)			
	No (109) (80%)			
3- Does the farm have written plans for treating sick animals with antibiotics?				
	Yes (33) (24.3%)			
	No (103) (75.7%)			
4- Following administration of an antibiotic, is the course of treatment completed?				
	Always (24) (17.6%)			
	Sometimes (43) (31.6%)			
	No (69) (50.7%)			
5- Does drug residues and withdrawal periods determined?				
	Yes (6) (4.4%)			
	No (130) (95.6%)			
6- Are antibiotics treated cows physically separated from other milking cows?				
	Yes (26) (19%)			
	No (110) (81%)			
7- Are antibiotic treated cows milked with a separate milking unit?				
	Yes (12) (8.8%)			
	No (124) (91.2%)			
8-Does the milk of antibiotic-treated cow thrown away or added to the farm whole milk?				
	Thrown away (19) (14%)			
	Added to the whole milk (117) (86%)			
9-What are the main diseases that required antibiotic treatment in the farm? See table 2				
10- Types of antibiotics used in the farm? See table 2				
11- From where are the antibiotics agents obtained?				
	Companies or veterinary pharmacies (122) (89.7%)			
	Other sources (venders) (14) (10.3%)			
12- For which of the following purposes are antibiotics used?				
	Growth promotion (12) (8.8%)			
	Prophylaxis (14) (10.2%)			
	Therapeutic (110) (81%)			
13- Are veterinarians allowed to administer antibiotics to the sick animals or others?				
	Always veterinarian (43) (31.6%)			
	Sometimes veterinarians (12) (8.8%)			
	Owner or Farm workers (81) (59.6%)			
14- Is there awareness from the antibiotic users about the emerging threat of antimicrobial resistance in bacteria and/ or antibiotic residues in animal products?				
	Yes (29) (21.3%)			
	No (107) (78.7%)			
15- Is there awareness from the consumers about the presence of drug residues in the animal's milk?				
	Yes (36) (26.4%)			
	Hear about it (24) (17.6%)			
	No (76) (56%)			

Therefore there is a clear relationship between low education and poor knowledge of farmers towards antibiotic use and the emergence and dissemination of antibiotic resistant bacteria on one side and the presence of the antibiotic residues in the animal products.

Another remarkable finding of this study is that only 20% of the visited farms maintain written records for antibiotic treatments. Also 24.3% of the farms have written plans for treating sick animals with antibiotics. Moreover 50.7% of the visited farms did not complete the course of the treatment and about 31.6% sometimes completed the course of the treatment. Also 95.6% of the visited farms did not determine the withdrawal periods of antibiotics in the treated

cows (table-1). The absence or incomplete records concerning antibiotic treatment is associated with poor knowledge about the antibiotics usage such as when the antibiotics treatment is initiated, which antibiotic is administered, which cow is treated and when the antibiotic withdrawal period is [13]. Maintaining proper and complete records and the presence of written plans records for antibiotic treatment is considered as an important factor that prevents the presence of the drug residues in the animal products [13].

The investigation of the physical contact between the antibiotic treated cows and none treated cows is an important factor that prevent the dissemination of antibiotic resistant factors. The results of this study clearly showed that there was direct contact between the two categories. For instance the antibiotic treated cows were physically separated from none treated cows in only 19% of the visited farms. Moreover the treated cows were milked together with the other cows in 91.2% of the visited farms. Noticeably, the milk of the treated cows was not thrown away, instead was added to the whole milk of the farm in 86% of the visited farms. Since antibiotics are improperly used in the dairy farms this poses the risk of milk to be easily contaminated with antibiotics. One of the most important practices in the farms that prevent the contamination of milk with antibiotics is to separate the sick or the treated animals from the health ones. Moreover the antibiotics treated animals should have special marks and milking them last in separate milking units. In addition to that the milking machines that are used in the antibiotic treated animals should not be used at the same time in milking health animals otherwise should be well cleaned and disinfected. Such practices are effective in preventing the contamination of milk with the drug residues [12, 18, 19]. In this study the majority of the visited farms were extensively used antibiotics in the dry cows, which were not separated from the other milking cows, to aid in mastitis control. These antibiotics vary between Penicillins, Oxytetracycline and Gentamycin that were given intra-mammary or systemically.

Table (2): showed the most frequently used antibiotics in the visited farms and their percentages. Sometime more than one or all of these medicines were used in one farm. Also the table showed the main diseases that occur in the visited farms and their percentages. Sometime more than one disease occurred in one farm. (*) indicates the presence of other medicine rather than antibiotics. () indicates the occurrence of other diseases that might not required antibiotic treatment**

The frequently used antibiotics	%	The main diseases in the farms	%
Penicillin	83%	Mastitis	89%
Tetracycline	78%	Pneumonia	79%
Tylosine	65%	Enteritis	53%
Gentamycine	62%	Metritis	46%
Sulpha drugs	43%	Foot rot	41%
Other drugs*	-----	Eye infection	22%
Parvacon	36%	Other diseases**	-----
Albendazol	33%	Theileria	39%
Dexamethazone	26%	Internal parasites	37%
Calcium supplements	22%	Abortion	29%
Ivermectin	13%	FMD	23%
		Bloat	14%

Moreover the use of these antibiotics continued to be a predominant practice for the treatment and control of mastitis in the visited farms. It was reported that these antibiotics were administered intramammary and systemically for the control of mastitis [12].

Most importantly, this study investigated the most common diseases that required antibiotic therapy in the visited farms. As shown in table-2, mastitis was the most prominent disease in the visited farms (in 89% of the visited farms). Previously it was reported that 80% of conventional dairy herds used antibiotics for treatment of mastitis in lactating cattle [20]. Moreover mastitis was the first disease of dairy cattle to be treated with antibiotics and the most common reason for administering antibiotics [21]. For instance a study of dairy herds in the Netherlands reported the use of 1.9 antibiotic treatments per case of clinical mastitis [22]. Other important diseases that occurred in the visited farms in this study were pneumonia (79%) and enteritis (53%). Enteritis and pneumonia were the diseases that required antibiotic therapy that mostly recognized in calves [23]. Other commonly observed diseases in the visited farms in this study that required antibiotic therapy were metritis (46%), foot rot (41%) and eye infections (22%). Other common diseases such as theileria infection, internal parasites, abortion, FMD and bloat were remarkably present despite they may required no antibiotic therapy. In this study 8.8% of the antibiotics were used as growth promoter, 10.2% for prophylaxis and 81% therapeutic agents. It is well known that antibiotics are used on dairy farms as therapeutics and prophylactics. Therapeutic usage is intended to treat bacterial infection associated with disease such as pneumonia, metritis, and mastitis [24]. The use of prophylactic antimicrobials is for treating

healthy animals to prevent a disease during periods of increase susceptibility. In specific cases, antimicrobials may be used for therapeutic and prophylactic [2, 24].

In this study the questionnaire clearly demonstrated the specific antimicrobial classes that were utilized in the visited farms. As shown in table-2, the Penicillin and Tetracycline drugs (83% and 78%, respectively) were the most preferred drugs for the treatment of the diseases in the farms. Previous studies showed that Penicillin G procaine, Pirlimycin, and Amoxicillin could be used effectively to eliminate intra-mammary infections caused by environmental *Streptococci* and *Staphylococcus aureus* [25- 28]. Other common antibiotics reported in this study were Tylosine (65%) Gentamycine (62%) Sulpha drugs (43%) and they were mostly used to treat pneumonia. Other drugs rather than antibiotics that used in the visited farms were Parvacon, Albendazol, Dexamethazone, Calcium supplements, Ivermectin. It is noteworthy that these drugs were obtained from the veterinary pharmacies (89.7%) and venders (10.3%) without any prescription; instead they were bought according to the owner diagnosis in most cases and they have no knowledge about the indication, dosing intervals, duration and exposure of the drug. Moreover the veterinarians were allowed to administer these medicines in only 31.6% of the visited farms compared to 59.6% by the farm owner or farm workers.

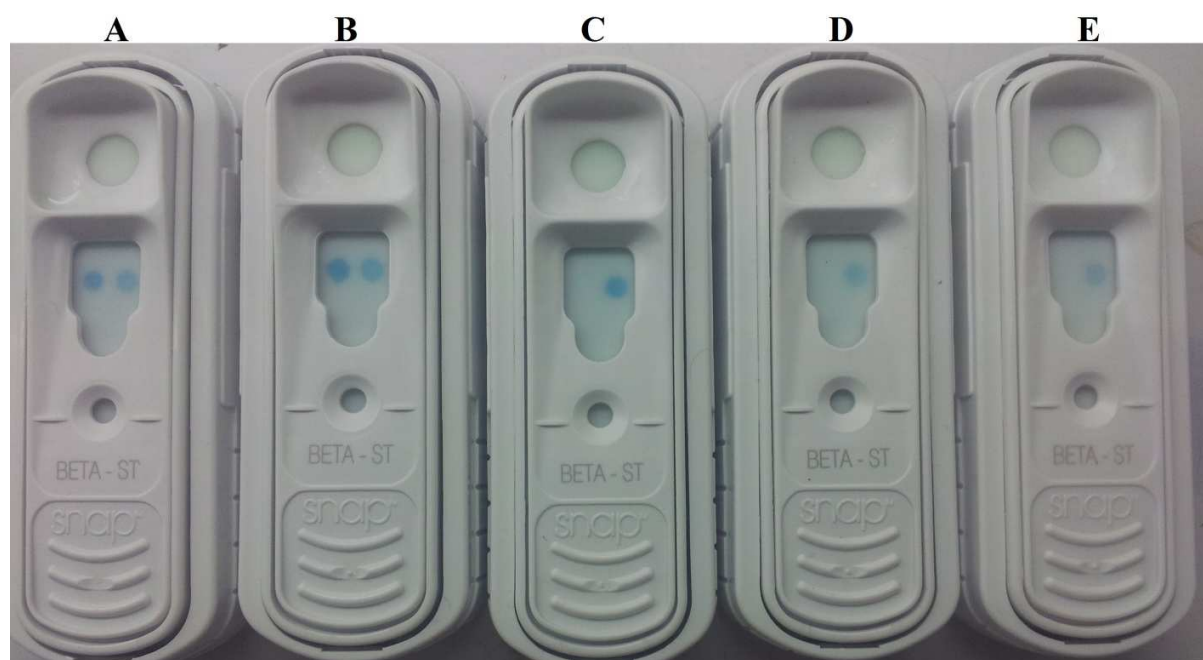


Figure-1: Antibiotic detection test: sample A and B are considered as a negative milk samples (containing no antibiotics) while sample C, D and E are considered as a positive samples (contains antibiotic, mainly penicillin). The test was performed according to the manufacturer protocol

It is noteworthy that there was no awareness from the antibiotic users about the emerging threat of antimicrobial resistance in bacteria or antibiotics residues in the animal products (78.7% of the visited farms). Also the questionnaire targeted the milk consumers about their knowledge about the presence of drug residues in the animal's milk. Unfortunately 56% of the consumers lack information about this issue and only 26.4% knew about antibiotics residues in milk.



Figure-2: Disc diffusion test. Milk samples 1, 2, 5, 8, 12 showed inhibitory zone since they contained antibiotics

To coincide the results obtained in the questionnaire and the presence of the antibiotics in the milk of the visited farms, 122 milk samples were randomly collected and investigated using SNAP* Beta-Lactam ST Test (Figure-1) and disc assay methods (Figure-2). The results showed that 37% of the samples were found to be positive for the presence of Penicillins in the milk. Despite the number of the milk samples collected was not much but clearly revealed the contamination of milk with antibiotics. This result coincided with the previously published reports concerning the presence of antibiotics in milk [7, 11, 10, 14, 15].

CONCLUSION

Multiple factors are associated with the contamination of milk with antibiotics in the dairy farms in Khartoum State. Some of these factors are related directly to the farm management. For instance lack of knowledge towards antibiotic usage, lack or improper records, bad categorization of the animals within the farm, failure to consult veterinarians, failure to complete antimicrobial treatment course were the main factors that lead to contamination of milk with antibiotics. Another important factor that leads to milk contamination with antibiotics is the addition of antibiotics (mainly Penicillin) to the milk by the milk venders to extend the shelf life of milk during transportation. Adulteration of milk supplies with antibiotics is clearly undesirable and the regulation of milk supplies to prohibit antibiotic residues is useful to protect public health. Further studies should be carried out to assess the public health hazard that associated with imprudent usage of antibiotics, mainly Penicillins and Tetracyclines as they were the most widely used antibiotics, and the emergence of antibiotic resistance in dairy farms.

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