

## Study of cattle milk contamination with antibiotic residues in Afak city, Al-Diwaniya

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### Abstract

The present study aimed to determine range of contamination of cow milk with antibiotic residues in number of villages of Afak city using microbiological analysis. For mentioned purpose (145) samples of milk were collected randomly from three villages: Al-Juruf, Al-Bunashi, Shat- Hussein with 55, 45, 45 milk samples, respectively. The results showed that numbers of samples and their percentage ratio which positive for antibiotic residues test were 11 (20%), 5 (11.1%), 6 (13.3%) for: Al-Juruf, Al-Bunashi, Shat- Hussein villages, respectively. The total positive samples for antibiotic residues test were 22 samples of 145 Samples for all three villages with ratio 15.1%.

The results also showed a significant increase ( $P < 0.05$ ) in numbers of positive samples for antibiotic residues in the village of Al-Juruf as compared with other villages.

### دراسة تلوث حليب الأبقار بمتبقيات المضادات الحيوية في مدينة عفك / الديوانية

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### الخلاصة

هدفت الدراسة الحالية إلى تحديد مدى تلوث حليب الأبقار بمتبقيات المضادات الحيوية في عدد من قرى مدينة عفك باستخدام طريقة التحليل المايكروبيولوجي. استخدم لهذا الغرض 145 عينة حليب جمعت عشوائياً من قرى الجرف، البو ناشي وشط حسين وبواقع 55، 45، 45 عينة حليب على التوالي. بينت النتائج بان أعداد العينات الموجبة لاختبار متبقيات المضادات الحيوية ونسبها المئوية كانت 11 (20%)، 5 (11.1%)، 6 (13.3%) لكل من قرية الجرف، البو ناشي و شط حسين على التوالي، وكانت عدد العينات الكلية الموجبة لاختبار متبقيات المضادات الحيوية 22 عينة من مجموع 145 عينة للقرى الثلاثة وبنسبة مئوية 15.1%. كما بينت النتائج وجود ارتفاع معنوي وتحت مستوى احتمالية ( $P < 0.05$ ) في عدد العينات الموجبة لاختبار متبقيات المضادات الحيوية في قرية الجرف مقارنة مع قريتي البو ناشي وشط حسين.

### Introduction

Milk is consider as a first food which has been known by human since his presence on the earth surface and he depended on it mainly in his nutrition and realized since many centuries ago, milk plays an important role in growth and maturation of new borns (1).

Many studies have taken different sites of milk contamination, starting from its presence inside udder of animal until reaching consumer (2, 3, 4, 5). Milk can be contaminated with different forms, some of them are endogenous source as bacterial infection and contamination with toxic materials which enter animal body through contaminated food and water, the others are exogenous as bacterial contamination, antiseptics, chemical materials and soils (6). Antibiotics contamination is considered as one form of endogenous contaminations due to their use for treatment of mastitis through intramammary or intravenous infusion and for disease therapy by intramuscular or intravenous injections, also oral administration (7). As well as antibiotics use as feed additives for increasing growth and preventing from diseases, Van (8) referred that which probably excreted with milk causing risks on human and animal health and increasing resistance of pathogenic bacteria to antibiotics with time (9).

FDA surveys indicated that improper use of drugs in the control of mastitis is the major source of residues found in milk supply (7).

Antibiotics residues in milk cause great problems due to their effects on consumer health causing allergy which may some times be dangerous to persons who have allergy after milk ingestion. This allergy appears as rashes and hypersensitivity and may lead to death sometimes (10, 11). So, this study aimed to determine range of contamination of raw cow milk with antibiotics residues in number of villages of Afak city.

## **Materials and Methods**

- **Samples collections:** Samples of raw milk has been collected from cattle of three villages lying within the geographical area of Afak city, Diwaniya province; they are Al-Juruf, Al-Bunashi, Shat- Hussein with 55, 45, 45 milk samples respectively, randomly collected and put in clean containers, sterilized and tightly closed, transferred to laboratory and preserved in 4 C until using.
- **Testing bacteria:** *Bacillus subtilis* was used, got from microbiology laboratory-veterinary medicine college, transferred to laboratory and cultured on brain - heart broth and preserved in fridge with 4C until using.
- **Preparation of bacterial suspension:** Bacteria have recaptured on slop of nutrient agar, then bacterial growth washed using 10 ml nutrient broth and the suspension shaken well, transferred to clean test tube, to determine the total count of bacteria by using spectrophotometer, where the ratio of light passing 20% with wave – length 580 nanometer, after adjusting the previously mentioned apparatus for passing 100% of light bundle on the same wave length through its passing with bacteria – free nutrient broth.
- **Sensitivity test:** Sensitivity test has been done to know sensitivity range of *Bacillus subtilis* for different antibiotics using discs saturated with antibiotics which added to the culture media including bacteria and the diameter of inhibition zone has been detected.
- **Test of presence of antibiotics in milk:** Nutrient agar has prepared after sterilization by autoclave, then left to decrease its temperature to 48c, 0.1 ml of already prepared bacterial suspension has added for each sterilized dish, then put in fridge for helping hardness of media, 4 pores were made in each dish then filled with milk to test, put in incubator with 32C for 18-20 hours, inhibition zone was measured from the end of pore margin to beginning of bacterial growth, the result was considered positive if the diameter of inhibition zone more than 1 mm according to (10).

## Results and Discussion

The problem of presence of antibiotics residues in milk became the most and large problem especially resisting owners of dairy cattle from selling milk which produced from cattle which are previously treated with antibiotics until withdrawing of residues from body completely in adding of antibiotics to milk in order to delay of damage or spoiling (3); therefore, this study aimed for detection about the range of contamination of milk which produced from dairy cattle in many villages which associated with afak city and showing range of its suitability for human consumption.

The results in Table (1) revealed high susceptibility of *Bacillus subtilis* for most antibiotics which frequently used in veterinary treatment, and this agreed with what mentioned by (12) who preferred using of *Bacillus subtilis* because it is sensitive for most antibiotics as compared with other bacteria as well as this bacteria non- pathogenic , growth rapidly and easy to preserve. All these factors explained why we selected this bacteria for detection about antibiotics residues in milk. This bacteria revealed high sensitivity for Amoxicillin, Oxytetracycline and Tetracycline and gave inhibition diameters of growth (31,28,28) mm, respectively, and moderate sensitivity for Ampicillin, Gentamicin, Neomycin and Chloramphenicol (23,22,21,21) mm respectively and less sensitivity for Lincomycin and Cloxacillin (18,16) mm respectively. These findings pointed to efficiency of this bacteria for detection of antibiotics residues in milk even under low concentration.

The results show there were antibiotics residues in milk samples which collected from three villages as in (Table 2). The samples were 11 from 55 samples collected from Al-Juruf village with ratio 20%, 5 from 45 samples collected from Al-Bunashi village with ratio 11.1% and 6 from 45 samples collected from Shat-Hussin with ratio 13.3%. The sum of positive samples were 22 from 145 samples for three villages with ratio 15.1% and the average of inhibition diameters for three villages were 2.03 mm.

The results mentioned above explained the presence of antibiotics residues in relatively high population in milk of cattle for three villages which revealed highly use for antibiotics from owners of animals in these villages and this reflex bad sanitation and public health for local breed cows from different bacterial diseases especially diseases of genital system from site and ignorance of owners with risks of using antibiotics and drugs for animals and then to consumers in other site, which need owner attention for risks which produced from highly using antibiotics and educating owners to apply veterinarians advice. In the same time that need to take veterinarians attention for decreasing use antibiotics in the milk by typical using of drugs especially in dairy cattle (13).

Also there were significant variations under probability level ( $p < 0.05$ ) in milk samples contained antibiotics residues which has taken from cows of Al-Juruf village comparing with cows of Al-Bunashi and Shat-Hussein villages. The region may belonged to the cows of Al-juruf village shuttered from genital tract infections with high proportions more than other villages; as metritis, retained placenta & mastitis which need usually local and systemic treatment with antibiotics ,so owners used antibiotics with high proportions. Due to high level of contamination, milk produced from this area is not suitable for human consumption or manufacturing as limited by WHO (World Health Organization and Food Health Organization (14).

**Table (1) Sensitivity of *Bacillus subtilis* for number of antibiotics which commonly used in veterinary treatment**

N	Type of Antibiotics	Symbol	Concentration	Diameter inhibition zone
1	Ampicillin	Amp	10	23
2	Amoxicillin	Amo	25	31
3	Chloramphenicol	C	30	21
4	Colistin	CT	10	16
5	tetracycline	DO	30	28
6	Gentamicin	GN	10	22
7	Neomycin	(N)	30	21
8	Lincomycin	(LI)	15	18
9	Oxytetracyclin	(TE)	30	28

**Table (2) Positive milk samples for test of antibiotics residues and their percentages in village of afak city**

Name of village	Number of samples tested	Number of positive samples	Percentage	Average of diameter inhibition zone (mm)
Al-Juruf	55	11	20%	2.3
Al-Bunashi	45	5	11.1%	2
Shat-Hussein	45	6	13.3%	1.8
Total samples	145	22	15.1%	2.03

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