

BACTERIOLOGICAL STUDY ON OVINE RAW MILK IN BAQUBAH CITY

Shaymaa Jabbar Hassoon^{1,2}
Heba Ibrahim Abaas¹

Noor Their Talib¹
Zainab Fadle Rahman¹

Mohammed Abed Mahmood¹
Heba Ayad Ismail¹

¹College of Vet. Med., Diyala University, Iraq.

²Corresponding author: sheimaajabbar@gmail.com

ABSTRACT

This study aimed to detect the behavior of bacterial pathogens in raw apparently normal milk. For this purpose 85 milk samples were collected from 85 ewes in different areas in Baqubah city. Study was carried from the period 1/4/2017 to 15/8/2017. The results showed that only 73 samples showed growth of more than one species of bacteria, while 12 samples gave negative bacterial growth on different media. The isolated bacteria were identified by studying cultural properties on different media, Gram staining and biochemical tests. Bacterial isolates included *Escherichia coli* (64.7%), *Staphylococcus aureus* (45.8%), *Pseudomonas aeruginosa* (32.9%), *Streptococcus spp.* (24.7%), *Klebsiella spp.* (22.3%), *Proteus mirabilis* (18.8%), *bacillus spp* (11.7%), and *serratia marcesences* (2.3%). The *S. aureua* and *E. coli* were the most predominant bacterial isolates. The results of antibiotic sensitivity test showed variable results in different isolates (Ampicilin, tetracyclin, Amoxcilin, Gentamicin and Vancomycin).

Key words: Raw milk, Baqubah city, antibiotics susceptibility.

INTRODUCTION

Milk is an important source of nutrients to humans and nursing animals and it is a source of food that is widely available, inexpensive and safe if boiled or pasteurized. Two main factors contribute to the microbiological quality of milk: (a) the existence of organisms in secreted milk (pre harvest) and (b) the contamination of milk at the time of collection, processing, packaging, transport, distribution and storage (post-harvest). If pathogenic bacteria are among the contaminants, the product will pose a food safety threat to consumers Although milk was once considered to be an important vehicle for foodborne diseases, but processes such as pasteurization and ultra-high-temperature treatments (UHT) have and are being used to produce safe and clean milk (LeJeune *et al.*, 2009). The consumption of raw milk poses a risk to public health via its potential contamination with pathogenic microorganism as *Brucella* and *Mycobacteria Spp.* Milk and milk products may carry toxic metabolites of different pathogenic

organisms growing in it. Ingestion of such products contaminated with these metabolites cause food poisoning for consumers. On the other hand the ingestion of viable pathogenic bacteria along with the food product leads to food borne infection (Aneja *et al.*, 2002).

In recent years, there has been a growing interest in local food production and consumption, and consumers are looking for foodstuffs that have undergone the least processing. As a result, there is an increased tendency to consume raw milk (Claeys *et al.*, 2013). Milk has a complex biochemical constituent and its high water activity and nutritional value serves as a good medium for growth and multiplication of many types of microorganisms when suitable conditions exists (4). This study aimed to identify bacteria present in sheep`s raw milk in Baqubah city and study its susceptibility to certain antibiotic.

MATERIALS AND METHODS

Samples

A total of 85 sheep`s raw milk samples were collected from different areas in Baqubah city in the period from the period 1/4/2017 to 15/8/2017. Processing was carried out immediately following delivery to the laboratory collection. The collected milk samples were immediately cooled and transported to the Microbiology of Laboratory at the College of Veterinary Medicine, Diyala University for bacteriological analysis.

Bacteriological study

Culture media used for isolation and purification of bacteria included: Nutrient agar (NA), Blood agar, MacConkey agar, Mannitol salt agar (MSA), Salmonella –Shigella (SS) agar medium and Brain heart infusion agar (BHI), Media were prepared according to the manufacturer's instructions. Inoculated media were incubated aerobically at 37 °C for 24 hours. All the isolates were stored in brain heart infusion broth with 15% glycerol at deep freeze until further use. Different colony types were seen in the initial cultures, each colony type was sub cultured on sheep blood agar for purification. The bacterial isolates were identified according to colony morphology on different media, biochemical characters and Gram`s reaction for Gram`s negative rods. The biochemical tests included: oxidase, IMVC test (indol production, methyl red, vogas-proskauer and citrate utilization), TSI (triple sugar iron), urease production, gelatin liquefaction, hemolysis on sheep blood agar and different carbohydrates utilization. For *staphylococcus* Spp., hemolysis on sheep blood agar, Catalase and Coagulas tests were applied (Sneath *et al*, 1986).

Antibiotics susceptibility

Antimicrobial susceptibility tests was determined by the disc diffusion technique on Muller Hinton agar according to (Bauer *et al.*, 1996). Results were recorded as sensitive or resistant, based on the diameter of inhibition zone of each antimicrobial disc used.

RESULTS AND DISCUSSION

Bacterial growth was obtained from 73/85 (85.88%) of milk sample. Most samples showed growth of more than one species of bacteria on different media. Bacterial isolates included *Escherichia coli* (64.7%), *Staphylococcus aureus* (45.8%), *Pseudomonas aeruginosa* (32.9%), *Streptococcus spp.* (24.7%), *Klebsiella spp.* (22.3%), *Proteus mirabilis* (18.8%), *Bacillus spp.* (11.7%), and *serratia marcesences* (2.3%) table 1.

Table 1. Bacterial species which isolated from ewes raw milk and its Percentage

Bacterial species	No.	Percentage (%)
<i>Escherichia coli</i>	55	64.7
<i>Staphylococcus aureus</i>	39	45.8
<i>Pseudomonas aeruginosa</i>	28	32.9
<i>Streptococcus spp</i>	21	24.7
<i>Klebsiella spp.</i>	19	22.3
<i>Proteus mirabilis</i>	16	18.8
<i>bacillus spp</i>	10	11.7
<i>serratia marcesences</i>	2	2.3

These results are in agreement with (Melese *et al.*, 2016), who reported 84.1% of milk samples contained at least one bacterium including *E. coli*, *Salmonella sp.*, *Staph. aureus*, and *Proteus Sp.*, with isolation rates of 70 (58 %), 4 (3.3%), 29 (24.2%), and 9 (7.5%), respectively and also it agreed with (Abbas, 2011) that isolated different types of bacteria from 100 milk samples collected from Abu–Ghraib district in Baghdad, including *Staphylococcus aureus*, (%10) *Streptococcus* group D (%51), *Escherichia coli* (%10) *Klebsiella Sp.* (%1). The increased incidence of *E. coli* may be attributed fecal contamination of samples *E. coli* opportunistic bacteria, when they move to location other than their natural habitat, may become pathogenic (Azher, 2013). Presence of other bacterial species may be due to several factors (Sligh and Timbury, 1994), including their being a cause of subclinical mastitis as *staph. aureas* which is well known as udder pathogen (Tenhagen *et al.*, 2009). A number of special

kind of *Serratia* can act as a reason of mastitis, including *S. marcescens*, *S. rubidaea* and *S. liquefaciens* (Hogan, 1997 ; Asmaa, 2017).

The antibiotics susceptibility test reveal that *S. aureus* was highly resistance to ampicillin, intermediate to amoxicillin and gentamycin, highly sensitive to tetracycline as showed in table 2 *E. coli* showed sensitivity to ampicillin, *vancomycin* and resistance to amoxicillin, *P. aeruginosa* sensitive to tetracycline, and intermediate to vancomycin and Amoxicillin while was resistant to ampicillin, the *Bacillus* spp was sensitive to tetracycline, Amoxicillin, gentamycin and ampicillin, The other species included *Klebsiella* spp. were resist to Amoxicillin and vancomycin while they were sensitivity to tetracycline. *Streptococcus* spp. showed sensitivity to gentamycin and amoxicillin but resistant to ampicillin figure (1, 2, 3 and 4). Bacteria detected in ovine raw milks may be present as saprophytes opportunistic pathogen under conditions of stress may result in disease, (Bukar *et al.*, 2007).

Table 2. Results of Antibiotic Susceptibility Test of All bacteria species Isolated from milk

Bacteria	Tetracycline	Vancomycine	Gentamicin	Amoxicillin	Ampicillin
<i>Staph.</i>	40	29	25	25	4
<i>Bacillus.</i>	42	38	44	40	39
<i>E.coli</i>	38	37	10	8	40
<i>Strept.</i>	37	36	41	38	8
<i>Pseudomonase</i>	44	26	18	24	6
<i>Klebsiella</i>	38	6	8	6	34
<i>Serratia</i>	28	6	32	6	6



Figure 1. show Antibiotic Susceptibility test of bacillus to different antibiotic

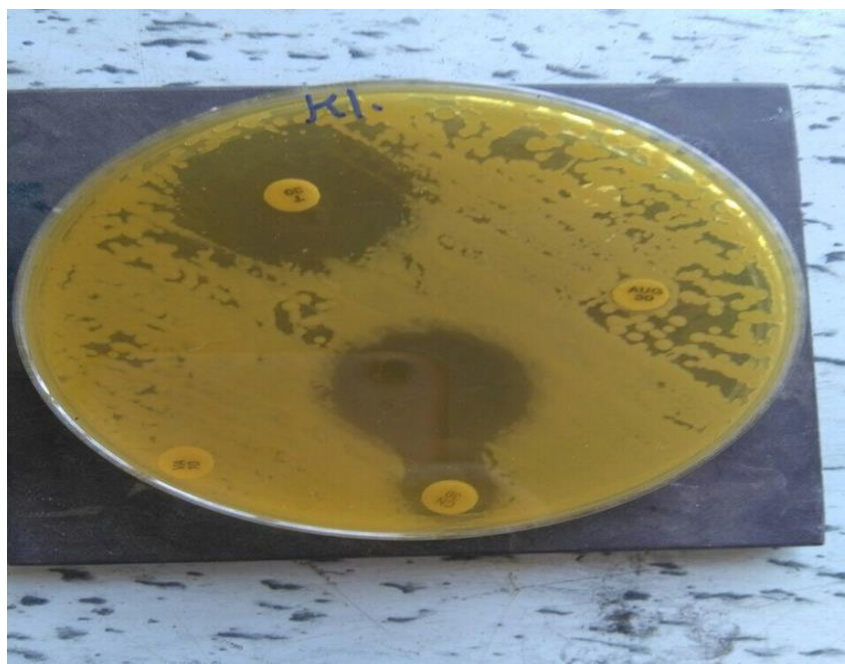


Figure 2. show Antibiotic Susceptibility test of Klebsiella to different antibiotic



Figure (3) show Antibiotic Susceptibility test of streptococcus to different antibiotic .



Figure 4. show Antibiotic Susceptibility test of *Pseudomonas* to different antibiotic

REFERENCES

- Abbas, M. S. 2011. Isolation of bacteria from milk and study the sensitivity of bacterial isolates to antibiotic and medical plants. *Al-Anbar Journal of Veterinary Science*, 4(2): 7-14.
- Aneja, R. P., B. N. Muthur, R. C. Chandan, A. K. Banerejee. 2002. Technology of Indian milk products. New Delhi: Dairy Indian Yearbook. p. 183-96.
- Asmaa, H. A., Ban N. Nadhom and Hind H. Al-Ammiri. 2017. Isolation and identification of *Serratia marcescens* from Bovine Mastitis infections in Iraq and their susceptibility to antibiotics. *Journal of Entomology and Zoology Studies*, 5(2): 489-492.
- Azher S. K. 2013. Study of drugs sensitivity against bacterial isolated from patients with urinary tract infection in Baqubah City. *Diayala journal for pure science*. 9(3).
- Bauer, A. W., W. M. Kirby, J. C. Sherris and M. Tenckhoff. 1996. Antibiotic susceptibility testing by a standard single disc method. *American Journal Clinical Pathology*, 451: 493-496.
- Bukar, K. Y. M., J. D. Amin and L. T. Zaria. 2007. Bacteria flora of the anterior genitalia of the sahelian doe in Maiduguri-borno state, Nigeria. *Nigerian Veterinary Journal*. 28(2): 60-62.
- Claeys, W. L., S. Cardon, G. Daube, J. De Block, K. Dewettinck and K. Dierick, *et al.* 2013. Raw or heated cow milk consumption: Review of risks and benefits. *Food Control*, 31: 251-262.

- Hogan, J. S. and K. L. Smith. 1997. Importance of dry period in *Serratia* mastitis outbreaks, *Large Animal Practice*, May/ June, 20-25.
- LeJeune, J. T. and P. J. Rajala-Schultz. 2009. Unpasteurized milk: a continued public health threat. *Clinical Infectious Diseases*. 48: 93-100.
- Melese, A. R., W. B. Tesfaye and N. A. Ayalew. 2016. Bacterial contaminations of raw cow's milk consumed at Jigjiga City of Somali Regional State, Eastern Ethiopia. *International Journal of Food Contamination* 3:4 DOI 10.1186/s40550-016-0027-5.
- Pandey, G. and G. Voskuil. 2011. Manual on Milk safety, quality and hygiene. Golden Valley Agricultural Research Trust, Zambia.
- Parekh, T. and R. Subhash. 2008. Molecular and bacteriological examination of milk from different milk animals with special reference to Coliforms. *Current Res. in Bacteriol.*, 1(2): 56-63.
- Sligh, J. D. and M. C. Timbury. 1994. Notes on medical bacteriology. Churchill livingstone Inc. New York.
- Sneath, P. A., N. S. Mair, M. E. Sharp and J. G. Hott. 1986. Bergey's Manual of systematic Bacteriology. William and Wilkinis, USA.
- Tenhagen, B. A., I. Hansen, A. Reinecke and W. Heuwieser. 2009. Prevalence of pathogens in milk samples of dairy cows with clinical mastitis and in heifers at first parturition. *Journal of Dairy Research*. 76(2): 179-87.

دراسة بكتريولوجية لحليب الاغنام في مدينة بعقوبة

شيماء جبار حسون^{2,1} نور ثاير طالب¹ محمد عبد محمود¹
هبة ابراهيم عباس¹ زينب فاضل رحيم¹ هبة اياد اسماعيل¹

¹ كلية الطب البيطري، جامعة ديالى، العراق

² المسؤول عن النشر: sheimaajabbar@gmail.com

المستخلص

هدفت الدراسة للكشف عن وجود المسببات المرضية في الحليب الظاهر طبيعياً، لذا تم جمع 85 عينة حليب من 85 نعجة من أماكن مختلفة في مناطق بعقوبة. اجريت الدراسة للمدة من 2017/4/1 وحتى 2017/8/15. اظهرت النتائج ان 73 عينة فقط اظهرت نمو اكثر من نوع واحد من البكتريا بينما 12 عينة اظهرت نتائج سالبة النمو على اوساط زرعية مختلفة. تم التعرف على البكتريا المعزولة من خلال دراسة الخصائص الزرعية على الاوساط المختلفة، وصبغة كرام والفحوصات البايوكيميائية، وشملت العزلات البكتيرية الاشريشيا القولونية (64%)، والمكورات العنقودية (45.8%) والزوائف الزنجارية (32.9%) والمكورات المسبحية (24.7%) والكليبيسيلا (22.3%) والمتقلبات الرائحة (18.8%) والبكتريا العصوية (11.7%) والسريشيا ميرسيسين (2.3%). كانت المكورات العنقودية والاشريشيا القولونية اكثر العزلات البكتيرية انتشاراً. وظهرت نتائج حساسية المضادات الحيوية نتائج مختلفة في عزلات مختلفة (امبيسيلين وتتراساكيلين واموكسيلين جنتاميسين وفانكوميسين).

الكلمات المفتاحية: الحليب الخام، الحساسية للمضادات الحيوية، مدينة بعقوبة.