

## Study About Pathogenic Bacteria Associated With Bovine Mastitis.

Safana A. S. AL-Ta'an    Asmaa H. AL-Jobori    Anton S. AL-Bana.

Department of Microbiology, College of Veterinary Medicine,  
University of Baghdad.

### Summary

Out of 100 raw milk samples collected from one hundred cows infected with mastitis, thirty three isolates of different types of pathogenic bacteria were isolated using different types of selective media.

On the basis of traditional bacteriological tests, these isolates were identified as the following: 13 isolates of *Staphylococcus aureus*, 9 isolates of B-haemolytic Streptococci, 6 isolates of Coliform bacteria, 4 isolates of *Corynebacterium pyogenes*, and only one isolate *Bacillus cereus*.

In addition to these isolates, a number of non-pathogenic bacteria were recovered during the isolation and they were considered as contaminants of milk such as *S. epidermidis*, *C. bovis* and *B. subtilis*.

The total number of living bacteria present in milk samples was measured and it was ranged from (1400 to 1800) bact./ml.

The isolates of pathogenic bacteria were tested for their sensitivity to different types of antibiotics and the results were:

100% of these isolates were sensitive to Gentamycin and Tetracycline, 11 (84.6%) isolates of *S. aureus*, 6(66.2%) isolates of B-haemolytic Streptococci, 4(66.2%) isolates of Coliform bacteria and 3(75%) isolates of *C. pyogenes* were sensitive to Ampicillin, Penicillin, Erythromycin and Cephalosporin. While *Bacillus cereus* isolate was resistant to all these antibiotics.

As a result of this work, generally the bacteriological quality of this milk is acceptable due to the low number of viable count of bacteria in

milk and the isolates showed high level of sensitivity to the antibiotics used in this study.

## دراسة حول البكتريا المرضية المتعلقة بالتهاب الضرع البقري

سفانة عبد الستار الطعان أسماء حمودي الجبوري انطوان صبري البنا  
قسم الاحياء المجهرية - كلية الطب البيطري - جامعة بغداد

### الخلاصة

تم عزل 33 عزلة لأنواع مختلفة من البكتريا المرضية من مئة عينة من الحليب الخام جمعت من مئة بقرة مصابة بالتهاب الضرع و ذلك باستخدام أوساط زرعية انتقائية مختلفة . و على أساس الفحوصات البكتريولوجية التقليدية تم تشخيص هذه العزلات كالاتي : 13 عزلة من *Stapylococcus aureus* ، 9 عزلات من B-haemolytic Streptococci ، 6 عزلات من Coliform bacteria ، 4 عزلات من *Corynebacterium pyogenes* و عزلة واحدة فقط من *Bacillus cereus* . إضافة إلى هذه العزلات كان هناك نمو لعدد من البكتريا الغير مرضية أثناء العزل حيث اعتبرت من ملوثات الحليب مثل *S. C. bovis* ، *B. subtilis* ، & *epidermidis* وقد تم حساب العدد الكلي للبكتريا الحية الموجودة في عينات الحليب و الذي كان يتراوح بين ( 1400 - 1800 ) بكتريا/مل . تم اختبار عزلات البكتريا المرضية من حيث حساسيتها لمختلف أنواع المضادات الحيوية وكانت النتائج هي:

100% من العزلات حساسة لكل من Gentamycin و Tetracycline و 11(84.6%) عزلة من *S. aureus* ، 6 (66.2%) عزلة من B-haemolytic Streptococci ، 4(66.2%) عزلات من coliform bacteria و 3(75%) عزلات من *C. pyogenes* حساسة لكل من Ampicillin ، Penicillin ، Ertythromcin و Cephalexin . بينما كانت عزلة *B. cereus* مقاومة لهذه المضادات الحياتية . ونستنتج من هذا العمل ، إن هذا الحليب يعتبر ذو نوعية بكتريولوجية مقبولة عموما حيث انخفاض العد الحيوي للبكتريا في الحليب . وكما أظهرت العزلات مستوى عال من الحساسية للمضادات الحياتية التي استخدمت في هذه الدراسة .

## Introduction

Mastitis is more widely spread than any disease common in dairy herds. Bovine mastitis is the most important source of loss for the dairy industry<sup>(1)</sup>. A rapid and specific test for the detection of the main pathogens of bovine mastitis is not actually available, but molecular probes reacting in PCR with bacterial DNA from bovine milk, providing direct and rapid detection of *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus disagalctia*, *Streptococcus parauberis* and *Streptococcus uberis*, have been developed<sup>(2)</sup>.

Milk is considered as good nutrient for micro-organisms as well as for humans, animals hence most types of organisms grow and multiply in it if other conditions for growth are favorable wherever milk is produced and processed, certain types of organisms are found in such large numbers that it is impossible to exclude them entirely, even when the most sanitary conditions are maintained. Since the bacteria causing mastitis may be present in barns, on feed on the hands of milkers on teat skin and on milking machines. It is not difficult to understand how readily such a sensitive organ as the cows udder and teat if bruised or injured in any way, may become infected if exposed to these bacteria. Flaky milk, clotes of casein in the yellowish watery fluid will be observed in extreme cases of mastitis<sup>(1)</sup>. Mastitis may spread to most of the cows in the herd. The physical inspection of infected and non-infected cattle eliminates the source and spread of infection. Such isolation procedures can be accomplished either by culling infected cattle or by segregation the herd into culture- positive and culture -negative groups following culture of composite milk samples from all cows<sup>(3)</sup>.

Since pathogenic microorganisms are readily isolated from raw milk, many health centers strongly recommend that unpasteurized milk should not be drunk or used in the manufacture of any dairy product<sup>(4)</sup>.

A numbers of pathogenic microorganisms are transmissible to man through unpasteurised milk, i.e. *Salmonella*, pathogenic *E.coli*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *S. aureus*.

Disease out breaks from raw milk are usually associated with children visiting a dairy farm and drinking the raw milk. Pasteurized milk is usually considered pathogen free with the exception of the spores of *Bacillus cereus*, if present in large numbers<sup>(4)</sup>.

In the present study, we aimed to determine the type and number of pathogenic bacteria present in raw mastitic milk.

The sensitivity of these bacteria to different antibiotics was also investigated. Testing of milk samples from cows with clinical or sub clinical mastitis helps to minimize the risk of such infection and to improve milk quality.

### **Materials and Methods**

A total of 100 samples of raw milk were collected from one hundred cows infected with mastitis. The samples obtained from two different herds around Baghdad City (from January to September, 2002). Milk samples were preincubated at 21 C° for 24 hours in order to be examined for viable count test using pour-plate method<sup>(5)</sup>. Then milk samples were centrifuged and cultured onto Nutrient Agar and blood Agar for primary isolation. The bacteria which were recovered were subcultured onto different types of selective media such as Blood Azide Agar, MacConkey Agar and Mannitol Salt Agar.

For primary identification, cultures reading were performed as well as microscopic examination using Gram's stain to enhance the identification. The isolates were further checked by some typical biochemical tests such as catalase, oxidase, gelatinase, coagulase, urease, Starch hydrolysis, Sugar fermentation and hemolysin production test<sup>(6),(7)</sup>.

The isolates were also tested for their sensitivity to different types of antibiotics such as: Tetracycline, Gentamycin Cephalexin. Disc diffusion method was used for this purpose<sup>(5)</sup>.

## **Results**

Among 100 samples of raw mastitic milk, 33 isolates of different pathogenic bacteria were obtained and identified according to the some traditional bacteriological tests as the following: (see the table) 13 isolates of *S.aureus*, 9 isolates of B-hemolytic streptococci. 6 isolates of coliform bacteria , 4 isolates of *C. Pyogenes* and only one isolate of *B. cereus*. In addition to these isolates, a number of non-pathogenic bacteria were recovered during the primary isolation and they were considered as contaminants of milk such as *S. epidermidis*, *C. bovis* and *B. subtilus*.

The total number of living bacteria present in milk samples was measured and it was ranged from (1400 to 1800) bact./ml. Generally it is acceptable and indicated to good quality of milk with the exception of some samples of milk which resulted the isolation of coliform bacteria, >100 bact/ml<sup>(8)</sup>.

The sensitivity tests of pathogenic isolates against 6 types of antibiotic showed that 100% of the isolates were sensitive to Gentamycin and Tetracycline, 11(84%) isolates of *S. aureus*, 6(66.2%) isolates of B-hemolytic Streptococci, 4(66.2) isolates of coliform bacteria and 3(75%) isolates of *C. Pyogenes* were sensitive to (Ampiciline, Penicillin, Erythromycin and Cephalexin) while *B. cereus* isolate was resistant to these antibiotics. The isolates, as showed, were highly sensitive to the most antibiotic used in this work.

**Table : Biochemical tests used in identification of pathogenic bacterial isolated from bovine mastitis.**

Biochemical Tests	The isolates of pathogenic bacteria				
	<i>S. aureus</i>	B-haemolytic streptococci	Coliform bact.	<i>C. pyogenes</i>	<i>Bacillus cereus</i>
Coagulase production	+	.....	.....	.....	.....
Haemolysin production	+	+	±	+	+*
Catalase	+	-	+	-	+
Oxidase	-	-	-	-	+
Gelatinase	+	.....	-	+	+
Urease	.....	.....	±	-	-
Starch hydrolysis	.....	.....	.....	+	+
Glucose fermentation	+	+	+	+	+
Lactose fermentation	+	+	+	+	+
Mannitol fermentation	+	.....	.....	.....	.....
Mannose fermentation	.....	.....	.....	.....	-

(±) : variable

(...): not tested

\* : *B. cereus* colonies having grey-green tinge with (out growth) covering the plate (7).

## Discussion

The present study aimed to investigate the incidence of pathogenic bacteria in raw mastitic milk. Only 33 isolates of pathogenic bacteria were obtained out of 100 milk samples and *S. aureus* was the most prevalent pathogen isolated in this study. *S. aureus* is a common mastitis pathogen world wide<sup>(3)</sup>. Many samples were negative (67%) in growth of pathogenes and that because the samples may be collected from cows under treatment.

The viable count of bacteria present in milk samples was ranged from (1400 to 1800) bact. / ml. Generally this result indicates that the bacteriological quality of this milk is acceptable, with the exception of some samples which contained coliform bacteria. Standards of milk quality control differ in differing countries and the current edition of the regulations should be consulted<sup>(6)</sup>. Iraqi standards of milk quality determined the viable count of bacteria in milk which can be acceptable: Milk should contain  $\leq 500,000$  bact. / ml. with the exception of coliform which should not be more than 100 bact. / ml.

The type of bacteria present in milk is vastly more important than the number<sup>(8)</sup>. Coliform mastitis occurs on every dairy farm through out the world and it is of major significance because the condition can become a more prevalent form of clinical mastitis<sup>(3)</sup>.

Only one isolate of *B. cereus* was obtained during isolation and this type of bacteria is among a number of bacteria that can cause mastitis in abovine and other animals<sup>(9)</sup>.

The isolation of pathogenic bacteria were tested for their sensitivity to different types of antibiotics and the results showed that most of the isolates were highly sensitive to the antibiotics used in this work and it indicates that there is good, and propers use of antibiotics for treatment in these herds, which were investigated. Where the use of antibiotic is restricted to the control of serious disease which can only be treated in this manner<sup>(10)</sup>.

## **References**

1. Olson, T. M. (1956). Elements of Dairying. The Macmillan, P: 427 – 459, 347 – 365.
2. Riffon , R.; Sayasith , k. ; Khalil , H. Dubereuil , P. ; Drolet , M. ; and Lagace , J. ( 2001 ) .Development of a rapid and sensitive test for identification of major pathogens in bovine mastitis by PCR. J. clinimicro .39(7), P: 2584 –9.
3. Cullor, J. S. (1997). Risk and prevention of contamination of dairy products. Rev. Sci. tech. off. Int. Epiz. , 16(2), P: 472 – 481.
4. Holsinger, V. H.; Rajkowski, K. T.; and Stabel, J. R. (1997). Milk pasteurization and safety: brief history and update. Rev. SCI. tech. off. Int. Epiz. , 16(2), P: 441 –451.
5. Gillespie, S. H. (1994). Medical microbiology illustrated b. w. heinemann ltd. P: 234-248,263-270.
6. Duguide, j. p.; Ross, p. w.; porter, A.; Green, D. M.; and Sleigh, J. D. (1989). Mackie and Mc Crtney practical Medical Microbiology. Edrs: (Collee, J. G.; Duguid, J. P.; Fraser, A. G.; and Marmion, B.P.). 2(13), P: 300-317,374-399,432-456.
7. Oarry, J.M; Turnbull, P.C.B.; and Gibson, J.R. (1983). A color atlas of Bacillus species. Wolfe Medical Atlases-19, P: 1-6.
8. AL-Kazrajii, N.H. (1989) Evaluation of raw milk quality. Vet.jurn. 6(5), p: 90-91.
9. Jones, T.O. and Turnbull, P.C.E. (1981). Bovine mastitis caused by Bacillus Cereus. Vet. Record 108,271,P: 230.
10. Buxton, A. and Frase, G. (1977). Animal Microbiology. Black well scientific Publications. P: 157-164.