

**BOVINE SUBCLINICAL MASTITIS:
BACTERIAL ISOLATION AND PHAGE
TYPING OF *STAPHYLOCOCCUS AUREUS*
ISOLATES**

S.A.AL-Izzi¹, N.K. Abo² and W.A. AL-Azawi¹

Department of Medicine and therapeutical and Department of Microbiology², College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

SUMMARY

Out of 107 cow milk samples, 44(41.12%) yielded bacterial growth. The isolates were identified according to their cultural, morphological and biochemical characteristics as *Staphylococcus aureus* (40.91%), *Escherichia coli* (29.55%), *Staphylococcus epidermidis* (18.18%), *Streptococcus uberis* (9.09%) and *Corynebacterium pyogenes* (2.29%). Out of 18 *Staphylococcus aureus* isolates, ten (55.56%) were typable with a set of 23 human phages. These isolates were lysed by three or more phages. The most effective antimicrobial agents against the majority of *Staphylococcus aureus* isolates were: erythromycin, rifampicin, gentamicin, chloramphenicol, carbenicillin, cloxacillin, ampicillin, cephalothin and tetracycline as indicated by *in vitro* sensitivity test.

INTRODUCTION

Mastitis is a disease characterized by the presence of high leukocyte count (>500,000/ml) in milk from affected mammary gland. The most important clinical signs include swelling, heat, pain and induration in the mammary gland (1). Bovin mastitis is caused by several pathogenic

microorganisms such as *Staphylococcus aureus* (2,3,4,5,6) *Streptococcus agalactiae* (2,4), *Streptococcus dysgalactiae* (3,4), *Streptococcus uberis* (2,5), *Corynebacterium pyogenes* (2,7) and *Escherichia coli* (2,3,4). Clinical diagnosis of acute bovine mastitis is usually an easy task for practicing veterinarian. However, detection of subclinical mastitis may be more difficult because of the absence of symptoms. For this reason, laboratory examination of herd milk is recommended to detect infected animal. The present study was designed to isolate and identify the bacteria from cows milk and to test the susceptibility of *Staph. aureus* isolates to both a set of 23 human staphylococcal international phages and 11 antimicrobial agents.

MATERIALS AND METHODS

The present study was conducted on 107 clinically normal cows. Milk samples were collected from the four quarters of each cow in steril test tube aseptically. A loopful of milk from each sample was inoculated on 5% sheep blood agar and MacConkey agar. Cultures were incubated at 37 °C for three days. The growth was examined macroscopically and microscopically. The isolates were identified according to their cultural, morphological and biochemical characteristics as suggested by Carter (8). All milk samples were tested using the California mastitis test (CMT)(9).

Phage typing of *Staph. aureus* was determined using a set of 23 human international phages following the technique recommended by the Staphylococcus Reference Laboratory, Colindale, London, England. *Staphylococcus aureus* isolates were subjected to *in vitro* antimicrobial sensitivity test according to Bauer *et al* (10) using 11 different antibiotic discs. The following antibiotic discs were used: erythromycin, rifampicin, gentamicin, chloramphenicol, carbenicillin, cloxacillin, ampicillin, cephalothin, tetracycline, lincomycin and penicillin (Biomerieux, Charbonieres les Bains, France).

RESULTS

Cultural examination of 107 cow milk samples revealed that 44 (41.12%) were having bacteria. *Staphylococcus aureus*, *E. coli*, *Staph. epidermidis*, *Strep. uberis* and *C. pyogenes* were isolated from 18, 13, 8, 4 and 1 milk samples respectively. The results of the CMT indicated that 38 of the apparently healthy cows examined were affected with subclinical mastitis (Table 1).

Out of 18 coagulase positive staphylococci isolated from cow milk, ten (55.56%) were typable with human phages used in this study. Five isolates were typable with three phages only, while the other five were typable with more than three phages as shown in table 2.

The in vitro sensitivity test indicated that all *Staph. aureus* isolates were sensitive to erythromycin, rifampicin, gentamicin, chloramphenicol and carbenicillin while most of them were susceptible to cloxacillin, ampicillin, cephalothin and tetracycline (Table 3).

Table 1: Distribution of bacteria isolated from the subclinical cases of bovine mastitis.

Bacteria	No. of isolate (%)	No. of positive CMT samples
<i>Staphylococcus aureus</i>	18(40.91%)	18
<i>E. coli</i>	13(29.55%)	13
<i>Staphylococcus epidermidis</i>	8(18.18%)	2
<i>Streptococcus uberis</i>	4(9.09%)	4
<i>Corynebacterium pyogenes</i>	1(2.29%)	1

Table 2: Phage typing of *Staphylococcus aureus* isolated from cow milk.

Isolate number	Phage pattern of typable isolates (RTD x 100)																						
	29	52	52A	79	80	3A	3C	55	71	6	42E	47	53	54	75	77	83A	84	85	81	94	95	96
23	++			++	++			++		+	CI	++	CI	CI	++	++	CI	CI	++	++			++
35	CI	CI		++	++	+	++			CI	CI	CI	CI	CI	CI		CI		CI	CI	++	++	++
40											+						++						+
57																+	++						+
71											+												+
72																							+
75																							-
85	++	++		++	++	+	++	++	++	++	++	CI	++	++	++	+	++						+
86	CI	CI		+	+	-	++			CI	CI	CI	CI	CI	CI				CI	CI	+	+	+
89	CI	CI		+	+	-				CI	CI	CI	CI	CI	CI	+	+	+	CI	CI	++	++	+

CI = Confluent lysis

++ = 50 or more plaques

+ = 20-49 plaques

- = 1-19 plaques

Table 3: Susceptibility of *Staphylococcus aureus* isolates to antibiotics.

Antibiotic	Disc content	No. of <i>Staphylococcus</i> isolates		
		S	I	R
Erythromycin	15 mcg	18	0	0
Rifampicin	5 mcg	18	0	0
Gentamicin	10 mcg	16	2	0
Chloramphenicol	30 mcg	16	2	0
Carbenicillin	100 mcg	15	3	0
Cloxacillin	1 mcg	17	0	1
Ampicillin	10 mcg	16	1	1
Cephalothin	30 mcg	15	2	1
Tetracycline	30 mcg	14	3	1
Lincomycin	2 mcg	10	5	3
Penicillin	10 units	11	1	6

S= Susceptible
 I= Intermediate
 R= Resistant

DISCUSSION

Pathogenic microorganisms including *Staph. aureus*, *E. coli*, *Strep. uberis* and *C. pyogenes* were isolated from the CMT positive milk samples obtained from 36 clinically normal cows indicating that these cows were affected with subclinical mastitis. *Staphylococcus epidermidis* was isolated from eight milk samples and was considered to be a normal inhabitant of the skin in six cases where the CMT showed negative reaction. These findings were in general agreement with those reported for goat and sheep mastitis in this country (11,12). Aungier and Austin (5) found that *Staph. aureus* and *E. coli* were the major pathogenic agents associated with udder infection in dairy cattle.

Human phages used in the study typed 55.56% of *Staph. aureus* isolates suggesting that these phages were of limited value for typing bovine strains of staphylococci. Similarly Jones *et al* (13) found that 46% of staphylococcal isolates were typable with human phages. The use of bovine phages in addition to human phages reduced the number of untypable isolates (13,14). Different phage types of *Staph. aureus* were isolated during the present study indicating many different strains were the cause of bovine mastitis. These findings were similar to those reported for goats and sheep in this country (11,12).

Antibiogram revealed that all of *Staph. aureus* isolates were sensitive to erythromycin, rifampicin, gentamicin, chloramphenicol, carbenicillin and most of them were sensitive to cloxacillin, ampicillin, cephalothin and tetracycline. These results were in general agreement with those reported previously (11,12).

The recovery of pathogenic microorganisms from milk of apparently healthy cows suggested that regular herd checking is necessary to prevent the transmission of the infection to another animal and man.

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التهاب الضرع تحت السريري في ابقار:
العزل البكتري والتصنيف العاشي لعزلات
المكورات العنقودية الذهبية

صلاح عبد اللطيف العزي^١ و نجلاء خالد عبو^٢
و وصال عبد الرزاق العزاوي^١

فرع الطب والعلاج البيطري^١ و فرع الاحياء المجهرية^٢. كلية
الطب البيطري جامعة بغداد، بغداد، العراق.

الخلاصة

عند زرع (١٠٧) عينة حليب ابقار اعطى ٤٤ (٤١ر١٣%) منها نمو^١
جرثومياً. تم تحديد انواع هذه العزلات وفقاً لخواصها الزرعية
والشكلية والكيميائية والتي شملت المكورات العنقودية
الذهبية (٤٠ر٩١%) الايشريشا القولونية (٢٩ر٥٥%) المكورات
العنقودية البشرية (١٨ر١٨%) المكورات السبحية (٩ر٠٩%)
والكوراني القحبية (٢٩ر٢٩%). لقد امكن تصنيف عشرة (٥٥ر٥٦%)
من عزلات المكورات العنقودية الذهبية والبالغ عددها ١٨
بأستعمال مجموعة العاشيات البشرية. وان هذه العزلات تحلت
بثلاث او اكثر من هذه العاشيات. أن المضادات الحيوية الاكثر
فعالية ضد معظم المكورات العنقودية الذهبية هي:
الارثرومايسين، الرقامبسين، الجنتاميسين الكلورمغنيكول،
الكاربنسلين، الكلوكساسلين، الامبسلين السيفالوثين
والتتراسايكلين كما تم التوصل اليه من خلال فحص الحساسية
الخارجي.