Isolation and Identification of Staphyococci from Raw Milk of Cows Infected with Mastitis

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Summary

Twenty-six isolates of staphylococci were recovered from of 100 raw milk samples collected from one hundred cows infected with mastitis. For the isolation of bacteria, Mannitol salt agar was used as a selective medium. Staphylococcal species were identified by API –STAPH system. The identified species were: - *Staphy aureus* [13], *Staph. epidermidis*[9], *Staph. haemolyticus*[2], *Staph. lugdunensis*[1], and *Staph. hominis*[1].

Staphylococcus aureus was the most prevalent pathogen among other Staphyloccocal species isolated in this study. *Staph. aureus* isolates revealed positive results for the defection of capsule structure and for production of some essential enzymes such as coagulase, phosphatase, DNA ase and haemolysin which are highly associated with the virulance of bacteria. *Staph. aureus* isolates were tested for their sensitivity to the antibiotics and the results were: 13(100%) isolates were sensitive to Gentamycin and Tetracyclene, 11 (84.6%) isolates were sensitive to Oxacillin, Penicillin, Ampicillin, Erythromycin and Cephalexin, where as only 2 (15.4%) isolates were Methicillin Resistant *Staph. aureus* (MRSA) which showed multi –resistant towards many antibiotics used in this work.

عزل وتشخيص المكورات العنقودية من الحليب الخام لأبقار مصابة بالتهاب الضرع

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الخلاصة

تم عزل26 عزلة من المكورات العنقودية من مجموع 100 عينة من الحليب الخام جمعت من مئة بقرة مصابة بألتهاب الضرع حيث أستخدم Mannitoal salt agar كوسط زرعي أنتقائي لعزل البكتريا ولقد تم تشخيص أنواع المكورات العنقودية بواسطة نظام API-STAPH والتي كانت : 13 عزلة من *Staph aureus* ، 9 عزلات من *Staph. Epidermidis* ، 2 عزلة من *Staph.haemolyticus* ، عزلة واحدة من *Staph. Lugdunensis*، وعزلة واحدة من *Staph. hominis* .

المكورات العنقودية الذهبية كانت أكثر الأنواع المرضية انتشاراً من الأنواع الأخرى

للمكورات العنقودية التي عزلت في هذه الدراسة حيث أظهرت عـــزلات *Staph. aureus* نتـائج ايجابية في تشخيص تركيب المحفظة وفي انتاج بعض الأنزيمات الأساسية مثـــل أنزيـم التجلـط ، الفوسفاتاز ، الديناز ، والأنزيم الحال للدم والتي لها علاقة كبيرة بضراوة البكتريا . وقد تم أختبـــار حساسيةعزلات المكورات العنقودية الذهبية للمضادات الحياتية وكانت النتائج هـــي : 13 (100%) عزلة حساسة للجنتامابيسين والتتراسايكلين ، 11 (84,6 %) عزلة حساسة للأوكزاسيلين، البنسـلين ، الأمبســـلين ، الأرثرومايســـين والسفاليكســـين. فقـــط 2 (15,4 %) عزلـــة كـــــانت Methicillin_Resistant Staph. Aureus (MRSA)حيث أظهرت مقاومة متعددة تجاه عـــدد من المضادات الحياتية التي استخدمت في هذه العمل.

Introduction

Mastitis is probably more wide spread than and disease common in dairy herds, many cows have mastitis without showing abnormal milk. Unless active mastitis is checked, the disease may spread to most of the cows in the herd.[1]

Staph. aureus is a common mastitis pathogen world wide. the main source of infection is the milk secreted by adult cows with infected udder quarters. Contaminated milking equipment and the hands of milkers are common sources of transmission.

Introduction of mastitis due to Staph. spp. into non infected herd generally occurs through the aquisition of infected animals or handling by attendants who have had prior contact with infected cows[2]

Staphylococcal mastitis must be considered a herd problem, ignoring the infection will damage the productive capacity of the herd. The high incidence of treatment failures prevent the procedure from forming the basis for an effective control programme since coloniasation and infection with Methicillin. Resistant *Staphylococcus aureus* (MRSA) can extremely difficult to treat, the rapid detection of (MRSA) is very helpful for the control and prevention of their spread.[3]

The present study aimed to identify staphylococcal species isolated from raw mastitic milk using API-STAPH system and to identify the most prevalent pathogenic species of staphylococci by traditional bacteriological methods. This study also aimed to investigate the sensitivity of the main pathogens to different types of antibiotics.

Materials and methods

One hundred raw milk samples were collected from one hundred mastitic cows from different herds in Baghdad city from January to

September, 2002. Each samples was collected by sterile tubes and centrifuged,

the preciptate was taken and inoculated onto mannitol salt agar (MSA) as a selective medium for staphylococci. 26 isolates of staphylococci were detected out of 26 positive cultures and checked microscopically using Gram's stain technique. Capsule structure was detected by the use of wet India ink method [4]. The species of isolated bacteria of staphylococci were identified by API – STAPH system (biomerieux) [5]. The most prevalent pathogens isolated in this work were staph. Aureus isolates which were further examined for their essential enzymes by inoculating the isolated colonies onto phosphate agar, DNA Agar and Blood Agar respectively. Coagulase enzyme was tested by human plasma using tube method. Pigment production was checked using milk agar medium [6].

Antibiotics sensitivity test was conducted for Staph. aureus isolates using disc diffusion method according to kirby Bauer technique.[7] For this purpose, seven different types of antimicrobial agents were used as shown in (Table-1).

Results

Among 100 milk samples collected from 100 mastitic cows mastitis, 26 samples were positive for staphylococci isolates. The isolates were identified microscopically as grams positive cocci. The identified species were: *Staph aureus*[13], *Staph.epidermidis*[9], *Staph.haemolyticus*[2], *Staph.lugdunensis*[1], and *Staph.hominis*[1].

All the isolates of *Staph.aureus* were positive in the tests of capsule formation, pigment production, mannitol fermentation, and production of some enzymes like phosphatase, coagulase, DNAase and haemolysin.

The result of antibiotic sensitivity tests for *Staph. aureus* isolates are presented in (Table -2), it was shown that:

13(100%) isolates were highly sensitive to Gentamycin and Tetracycline, 11(84.6%) isolates were sensitive to Oxacillin, Penicillin, Ampicillin, Erythromycin and Cephalexin. Only 2 (15.4%) isolates were resistant to Oxacillin Cephalexin, Ampicillin and Erythromycin which are considered as Methicillin Resistant Staph. aureus (MRSA) [3]. An isolate was considered to be resistant to a given antibiotic according to the interpretation of zone of inhibition (Biomerieux; Becton Dickinson, Microbiology system, 1996).

Table. (1): Standard antibiotic discs their code and concentration (gs/disc).

	Antibiotic	Code	Concentration
1	Gentamycin	GM	10 ugs/disc
2	Tetracycline	TE	30 ugs/disc
3	Oxacillin	OX	1 ugs/disc
4	Penicillin	P	10 ugs/disc
5	Ampicillin	AM	10 ugs/disc
6	Erythromycin	ER	15 ugs/disc
7	Cephalexin	CK	30 ugs/disc
Ugs: micrograms			

Table (2): Readings of sensitivity test of *Staph.aureus* isolates

Isolate No.	CK	GM	TE	OX	P	AM	ER	Nature of isolate
SA-1	S	S	S	S	S	S	S	Sensitive
SA-2	S	S	S	S	S	S	S	Sensitive
SA-3	S	S	S	S	S	S	S	Sensitive
SA-4	R	S	S	R	R	R	R	MRSA
SA-5	S	S	S	S	S	S	S	Sensitive
SA-6	S	S	S	S	S	S	S	Sensitive
SA-7	R	S	S	R	R	R	R	MRSA
SA-8	S	S	S	S	S	S	S	Sensitive
SA-9	S	S	S	S	S	S	S	Sensitive
SA-10	S	S	S	S	S	S	S	Sensitive
SA-11	S	S	S	S	S	S	S	Sensitive
SA-12	S	S	S	S	S	S	S	Sensitive
SA-13	S	S	S	S	S	S	S	Sensitive
SA: *Staph.aureus* S: Sensitive R: Resistant MRSA: Methicillin Resistant *Staph. Aureus.*								

Discussion

Some strains of Staphylococci are pathogenic and characteristically produce coagulase such as *Staph. Aureus*, *Staph. hyicus*, *Staph. hominis* and *Staph. intermedius*[4]. In this study, only *Staph. aureus* and *Staph. hominis* were obtained from raw mastitic milk as pathogenic species of Staphylococci

(coagulase positive). Other staphylococcal isolates were non-pathogenic (coagulase negative) [8], and considered to be either normal isolates like *Staph. epidermidis* or as contaminants like the human species: *Staph. haemolyticus* and *Staph. lughdenensis* which they seemed to be particularly aggressive[9]. Staphylococci isolated from raw mastitic milk were identified for their species using API-STAPH system. This method have been applied in some other previous studies[10],[11].

Staph. aureus was the most prevalent pathogen among other staphylococcal species isolated in the present study. All these isolates were capsulated and able to produce their essential enzymes such as coagulase, phosphatase, DNAase and haemolysin which are highly correlated with the virulence of bacteria[12].

Staphylococci rapidly develop resistance to many antimicrobial agents and present difficult therapeutic problems[13]. It therefore seemed necessary to investigate the sensitivity of the isolates of Staph. aureus to different types of antibiotics. In this work, it was found that only 2 isolates were methicillin resistant Staph. aureus (MRSA) out of 13 isolates of Staph. aureus. The high level of sensitivity to the antibiotic was observed may be due to the proper use of antibiotic agents and the good conditions that were available in the herd which have been investigated.

REFERENCES

1. Olseon, T.M. (1956). Elements of Dairying. The Macmillan company, New York. P: 358 –365.
2. Cullor, J.S. (1997). Risks and prevention of contamination of diary products. Rev. Sci. Tech. Off, int. Epiz. Vol. 16, No.2. P: 472 –481.
3. Wallet, F.M.; Roussel, D.; and Courcol, R.J. (1996): Choice of a routin method for detection methicillin resistance Anti Staphylococci. Journal Anti microbiol chemother. Vol. 37, No. 5, P: 901 –909.
4. Duguid, J.P. (1989). Stephylococcus: Cluster – forming Gram positive cocci in: Mackie and McCarthey practical medical microbiology. Editor: Collee, J.G.; J.P. Duguid, A.G. Fraser, and B.P Marmion. 13th ed., Vol.2, P: 303 –326.
5. Cappuccino, J.G.; and Sherman, N. (1987). Microbiology a laboratory manual. 2nd ed., P: 361 –369.
6. Sneath, P.; Mair, N.S; Sharp, M.E. and Hott, J.G. (1986).Bergy's manual systemic of bacteriology. P:1013 –1035.

7. Connie, R.M.; and George, M.(1995): Textbook of Diagnostic microbiology. P:50 –89.
8. Buxton, A.; and Frase, G. (1977). Animal Microbiology. Black well Scientific publications. P: 157 –164.
9. Vandenesch, F.; Etienne, J.; Reverdy, M.E.; and Eykyn, S.J. (1993). Endocarditis due to staph.Lugdunensis report of 11 cases and review: clinical infectious disease. 17th ed., P:871 –876.
10. Harvey, J. ; and A. Gilmour (1985). Application of current methods for isolation and identification of Staphylococci in raw bovine milk. J. Appl. Bacteriol., 59(3): 207-221.
11. Wallet, F.; D.M. Roussal; and R.J.Courco (1996). Choice of a routine method for detecting methicillin-risistance in Staphylococci. J. Antimicrob. Chemother, 37(5): 901-909.
12. Sheagre, J. (1990). Infectious disease. P: 1634 –1638.
13. Thabaut, A.; M. Meyran; and M. Huerre (1985). Evaluation of present situation of staphylococcus aureus sensitivity to MLS in hospital (1975-83). J. Antimicrobs chemother, 16(supp.A): 205-207.