The Iraqi J. Vet. Med. 19 & 20 , No.(1), 1995-1996.

ISOLATION AND IDENTIFICATION OF SOME BACTERIA FROM COW'S MILK IN NORTHERN IRAQ

Abdul-Karim Fattah Omar

Dep. of Microbiology, College of Medicine,
University of Tikrit, Iraq.

SUMMARY

A total of 190 bacterial strains were isolated from both apparently healthy cows and those suffering from mastitis. The isolates were identified and classified according to their cultural, morphological , and biochemical characteristics. Staph. aureus was dominant bacterium (44.2%), being followed by E.coli (21%), P.aeruginosa (11.5%), and Strept.agalactiae (7.4)%.

The Staph. aureus strains were studied most intensively, during the course of the investigation. The toxic properties of the strains were compared with other physiological properties and with their susceptibility to antibiotics. Resistance to antibiotics was slightly more widespread among the toxigenic than the nontoxigenic strains as indicated by the in vitro sensitivity test.

INTRODUCTION

Milk and its products are important food items of people throughout the world. It is also an important source of transmission of some infectious disease to man, especially if the dairy animals were infected with mastitis.

It has been observed that the incidence of staphylococcal mastitis is fairly high in cows in different countries in the world.

Aungier and Austin (3) In Ireland, isolated Staph. aureus , Strept.uberis and E.coli at a ratio of 18% , 17% and 12% respectively from cow's milk with clinical mastitis . In Denmark, Klastrup and Halliwell (11) found that more than 80% of subclinical mastitis cases were due to Staph. aureus and Strept.agalactiae whereas other types of streptococci and aerobic Gram negative bacilli occurred in low ratios.

In Iraq, some workers (1,2,10) found that staphylococci were the predominant causative bacteria for mastitis in cows. The aim of this study is to isolates and identify bacteria from milk of apparently healthy cows as well as those suffering from mastitis in a location near Arbil city.

MATERIAL AND METHODS

95 milk samples were collected in 1989 from 55 cows (10 of them were suffering from mastitis and 45 cows were apparently healthy) in sterile test tubes under aseptic precautions from Koshtaba village which is about 20 kms far from Arbil city. Aloopful of milk from each samples was streaked on 10% blood agar, crystal violet tetrazolium agar, MacConkey's agar, nutrient broth and agar with 10% sterile serum, mannitol salt agar, Staphylococcal medium No. 110. Edwards medium (modified), and Sod. hipurate broth (9). Identification was done according to Cowan (6)

The enterotoxigenicities of different Staph. aureus strains were conducted as described by Robbins et al., (13). The susceptibilities of the strains to antibiotics were carried out by using Antibiotic sensitivity discs (Oxoid) as described by Bauer et al. (4).

RESULTS

The bacteriological examination of 20 milk samples which were collected from mastitic cows revealed the presence of 70

strains of Staph. aureus , 7 strains of Staph. epidermidis , 9 strains of Strept. dysagalactiae, 12 strains of Strept.agalactiae, 30 strains of E.coli , 19 strains of Pseudomonas aeruginosa , and 8 strains of now: Actinomyces pyogenes (Total 155). But 75 milk samples taken from apparently healthy cows showed lower frequency of occurrence of each of the above-named strains of bacteria with numbers of 14 ,1,2,3,10,3 and 2 respectively (Total 35). Table (1) shows all the isolated bacteria where Staph. aureus was the most abundant organism (44.2 %) followed by E.coli (21%) , P. aeruginosa (11.5%) , and Strept.agalactiae (7.4%).

Table (2) shows some physiological properties of the isolated Staph. aureus strains. These strains were classified into three biotypes namely, 1S, 2S and 3S. Strains of biotype 1S were characterized by their ability to produce coagulase and thermonculease (TNase) enzymes and may be considered as a typical Staph. aureus . However, strains of biotypes 2S were TNase producers, while those of 3S were not TNase producers and also differ from 2S by their ability to produce coagulase.

A total of 84 Staph. aureus strains were tested for enterotoxigenicity and an attempt was made to connect this property with some physiological properties (Table 3). Of these 48(57%) produced one or more of enterotoxin A,B,C and D. Most toxigenic isolates (83.3%) produced one toxin only , while the rest (16.7%) produced more than one toxin-producing strains. 50% produced A, 31.3% each of C and D. None of the isolated strains showed any tendency to produce enterotoxin E either alone or in conjunction with other toxins.

As shown in Table (3), all of the strains tested utilised glucose anaerobically. Of the 48 toxigenic strains, examined 100% produced coagulase, 98% haemolysin, and 92% produce yellow or orange pigment when grown on Staphylococcus medium No. 110. It was found that 83.3% of non-toxigenic strains produced coagulase, 75% phosphatase, 83.3 % haemolysins, and 80.6 % with yellow or orange pigment.

The in vitro sensitivities of the toxigenic and non-toxigenic strains of isolated Staph. aureus to antibiotics are shown in Table (4) . It was found that 87.5% of the Txigenic strains were resistant to Nalidixic acid , 67.5% to Colistin and Pencillin G, 24% to Ampicillin, 20.5% to Tetracycline, 7.5 % to Chloramphenicol, 5% to Gentamycin and Kanamycine, and 2% to Neomycin. None of the toxigenic strains were resistant to Erythromycine, Methicillin, and Rifampicin. The non-toxigenic strains exhibited a lower degrees of resistance.

DISCUSSION

The results presented in this study showed that the high proportion of Staph. aureus (44.2%) and to a lower extent that of E.coli (21%) are somewhat similar with the findings of Aungier and Austin (3) who reported that these bacteria were the major pathogenic agents associated with udder infection in diary cattle. Also similar findings were recorded for cows in this country (1,2,10).

In the present work it was found that Staph. aureus to be the sole cause of disease in 50.9% of mastitic milk. In early 1950, Staph. aureus as compared to Strept. agalactiae , was not a major cause of mastitis in dairy animals (15). With the introduction of antibiotic therapy in treatment of mastitis the incidence of Staphylococcal mastitis increased many folds which may be due to the ability of this organism to develop antibiotic resistant strains as compared to other mastitis causing bacteria which failed to do so.

As indicated from Table (2), isolated Staph. aureus of different biotypes reacted differently in the other tests indicated. Similarly , Ryman et al.(12) reported very few coagulase positive but TNase negative strains of Staph. aureus . Also Hall (8) has reported that some Staph. aureus strains became coagulase negative when stored for long periods of time . In the light of these

interpretations the biotypes (1S, 2S , and 3S) of Staph. aureus which were identified in the present work, might be one strain with very little variable reactions.

The finding that isolated Staph. aureus strains examined most often produced enterotoxin A, was similar to those previously documented (5). It should , however, be borne in mind that there is evidence for the existence of still unidentified enterotoxins. Begdoll (5) has reported that during an examination of 61 strains with monkey feeding test only four nontoxigenic strains were found. Thus, it is most likely that at least some of the strains classified in the present work as non-toxigenic on the bases of negative results obtained were in fact toxic.

The phosphatase test was positive for 98% of toxigenic strains and about 92% of them produced yellow or orange pigment. However, 75% of the non-toxigenic strains produced phosphatase and 80% with yellow or orange pigment. Thatcher and Simmon (14) have reported that these correlations cannot be reliable indicators of toxin production.

The isolated strains of Staph. aureus , in the present study, were found to be quite sensitive to Erythromycine, Gentamycin, Methicillin, Neomycin and Rifampicin, somewhat less to Kanamycine, Chloramphenicol and Tetracycline and much less so to Ampicillin. These findings are in general agreement, with findings of Al-Izzi et al.(1) for cows in this country. Although, they didn't study the effect of Methicillin against their Staph. aureus strains.

Dornbusch et al.(7) found a high level of correlation between Methicillin resistance and enterotoxin B production and it has been proposed that they are transferred within the same plasmid. In the present work, however, none of toxigenic strains of Staph. aureus examined so far were resistant to Methicillin.

This study serves the public health by the determination of the pathogenic micro-organisms responsible for contamination of milk and its products. As well as, the epidemiology of the

causative micro-organisms of bovine mastitis and to aid in making preventative measures for veterinary and public health programmes. In addition this study was undertaken with a view to promoting the efforts in understanding and eradicating mastitis in Iraq.

Table 1 : Distribution of the isolated bacteria from cow's milk.

Isolates	No. of Samples Examined	No. of Samples	No. of Isolates
Staph. aureus	95	39	84(44.2)
Staph. epidermidis	95	5	8(4.2)
Strept. dysagalactiae	95	7	11(5.8)
Staph. aureus	95	9	15(7.4)
E.coli	95	18	40(21.0)
P.aeruginosa	95	11	22(11.5)
Actinom. pyogenes	95	5	10(5.3)

Table 2 Biotyping of isolate Staph. aureus

Properties	Biotyping		
	1S	2S	3S
Coagulase	+	-	+
Therminuclease	+	+	-
Alkaline Phosphotase	+	+1	+
Mannitol ferm. (anaerobic)	+	+1	V
Haemolysin	+	+1	+1
Acetoin	+	+	+
Colony pigment	+	+	+
D-Xylose	-	+2	+2

1 = 90% or more strains positive.
2 = 90% or more strains negative.
3 = variable.

Table 3 : Some properties of staphylococcal isolates compared to their toxigenicity

Toxin type	n[1]	Biochemical Parameters										
		Coagulase		Phosphatase		Ferm.glucose anaerobiocally		Haemolysin		Pigment		
		+[2]	-[3]	+	-	+	-	+	-	W	Y	Or
Nontoxigenic	36	30	6	27	9	36		30	6	7	22	7
Toxigenic	48	48	0	47	1	48		46	2	4	38	6
A	17	17		17		17		17		1	14	2
B	2	2		1	1	2		2			2	
C	12	12		11	1	12		11	1		11	1
D	9	9		9		9		9	1	1	6	2
AC	2	2		2		2		2		1	1	
AD	5	5		5		5		5		1	3	1
CD	1	1		1		1		1			1	

Table 4 : Susceptibility of staphylococcal isolates to antibiotics

Antibiotics	Disc contents (µg)	% of strains in different sensitivity groups					
		(36 Nontoxigenic strain)			(48 Toxigenic strain)		
		R%	I%	S%	R%	I%	S%
Ampicilline	10	8.0	14.0	78.0	24.0	30.0	46.0
Chloramphenicol	30	4.5	9.5	86.0	7.5	8.5	84.0
Colistin	10	66.0	23.5	10.5	67.5	25.0	7.5
Erythromycin	15		1.0	99.0		2.0	98.0
Gentamycin	10	1.5	3.5	95.0	5.0	7.5	87.5
Kanamycin	30	4.0	6.0	90.0	5.0	5.0	90.0
Methicillin	5		1.0	99.0		2.0	98.0
Nalidixic acid	30	81.0	16.5	2.5	87.5	11.0	1.5
Neomycin	30	1.0	2.0	97.0	2.0	2.0	96.0
Penicillin G	10	60.5	4.5	35.0	67.5	5.0	27.5
Rifampicin	30		1.0	99.0		4.0	96.0
Tetracycline	30	18.5	1.5	80.0	20.5	2.5	77.0

REFERENCES

1- Al-Izzi ,S.A.; Abo,N.K. and Al-Azawi,W.A.(1989). Bovine subclinical mastitis: Bacterial isolation and phage typing of Staph. aureus isolates.J.Iraqi.Vet.Med.,13:161-169.

2- Al-Khatib,G. and Al-Bassam,L.(1979). Identification of some pathogenic bacteria isolated from clinical cases. J.Iraqi.Vet.Med.,3: 107-111.

3- Aungier,S.P.M. and Austin,F.M. (1987). An investigation into the nature of clinical cases. Irish.Vet.J., 41:304-310.

4- Bauer, A.W.; Kirby,W.M.M.; Sherris,J.C. and Turk,M.(1966). Antibiotic susceptibility testing by standardized single disk method. Am.J.Clin.Path.,45:493-496.

5- Begdoll,M.S.(1972). The enterotoxin, pp.301-331. In.J.D.Cohen (ed.). The staphyloccocci. Wily Inter Science, John Wily & Sons, Inc. London.

6- Cowan,S.T.(1977). Cowan and Steel's Manual for the identification of medical bacteria. Second edition. Cambridge, Cambridge Univ. Press.

7- Dornbusch, K.; Hallander,H. and Lofquist,F.(1969). Extrachromosomal control of methicillin resistance and toxin production in Staph. aureus J.Bacteriol., 98:351-358.

8- Hall,H.E.(1968). Enterotoxin and enzyme production by a selected group of Staph. aureus. Bacteriol. Proc, pp.77-78.

9- Harrigan,W.F. and McCance,M.E.(1987). Laboratory methods in food and diary microbiology. Seventh edition. London. Acad.Pre.

10-Issa,M.M.(1992). Coliform mastitis in dairy cattle.M.Sc.Thesis, College of V.M. Baghdad.

11-Klastrup,N.C. and Halliwell,R.W.(1977). Prevekence of bovine subclinical mastitis in Malawi,Nordisk.Dairy Sci.Abst. 39:864.

12-Ryman,M.K.; Park,C.K.;Philpott,J. and Todd,E.C.D.(1975). Reassessment of the coagulase and thermostable nucleasetest as means of identifying Staph. aureus. Appl.Microbiol., 29:451-454.

13-Robbins , R. and Bergdoll , S.G.M. (1974). Detecting the enteroxigenicity of Staph. aureus strains. Appl.Microbiol., 28: 946-950.

14-Thatcher, F.S. and Simmon,W.A.(1956). Comparative appriasal of the properties of staphyococci isolated from clinical sites and from dairy products. Can.J.Microbiol., 2: 713-714.

15-Wahly,A.M. and Hilms,N.(1946). Prevalence of bovine mastitis in Egyptian dairy cattle. J.Comp.Path.,56:246-250.

The Iraqi J. Vet. Med. 19 & 20 , No.(1), 1995-1996.

عزل وتشخيص بعض البكتريا من حليب الابقار في شمال العراق

عبد الكريم فتاح عمر

فرع الاحياء المجهرية- كلية الطب- جامعة تكريت - العراق.

الخـــلاصة

لقد عزلت 190 سلالة بكتيرية من حليب الابقار التي بدت سالمة ظاهريا ومن تلك التي كانت تعاني من التهاب الضرع. تم تحديد وتصنيف هذه العزلات وفقا لصفاتها الزرعية ، الشكلية ، والكيميحيوية. وجد ان Staph. aureus هي الغالبة وشكلت نسبة 44.02٪ من مجموع العزلات وتبعتها كل من Esch. coli ، P. eruglinosa و Strept. agalactiae بنسب 21٪ ، 11.5٪ و 7.4 ٪ على التوالي. درست سلالات البكتريا Staph. aureus درست مستفيضة خلال هذا البحث. وقد قورنت قابليتها السمية مع مميزاتها الفسلجية الاخرى ، وكذلك مع حساسيتها للمضادات الحيوية. ووجد بان السلالات المنتجة للسموم اكثر مقاومة للمضادات الحيوية من تلك غير المنتجة للسموم كما تم التوصل اليه من خلال فحص الحساسية الخارجي.