ISOLATION, IDENTIFICATION AND BIOTYPING OF *BRUCELLA* SPP. FROM MILK PRODUCT AT BASRAH PROVINCE

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ABSTRACT

Three hundred milk product samples were collected from different locations of Basrah province. By using enrichment broth technique and Brucella selective medium nine isolates (8 from soft cheese and one from cream) where isolated. No Brucella strain was isolated from ice-cream. The species and biotypes of these isolates were determined and it was found that 4 isolates of *Brucella abortus* biotype 4 and 5 isolates of *B. melitensis* biotype 2. Antibiotic sensitivity test showed that all isolates were sensitive to streptomycin, gentamycin, kanamycin, rifampicin, trimethoprim, trimethoprim with sulfamethoxazole and tetracycline, some strains showed resistance to doxycyclin, cephalexin, ampicillin and erythromycin. *Brucella* isolates also showed growth in different range of temperature and pH.

INTRODUCTION

Brucellosis is known as contagious abortion or bang's disease in cattle. In human, the disease called undulant fever or Malta fever that was first recognized as human disease in Malta Island. Brucellosis transmitted as chronic contagious disease and persists for all animals' life and infection occurs in cattle of all ages but persists most commonly in sexually mature animal. Congenital infection may occur in calves borne from infected dams (1). It may cause lesion in male reproductive tract in cattle sheep, goat, dogs as well as bursitis in horse (2). People infected with *Brucella* organism as a result of taking non pasteurized raw milk or milk products or come into direct contact with infected animal specially farmers, ranchers, veterinarians and backing plant workers (3).

The disease is caused by a group of bacteria known scientifically as the genus *Brucella*. This genus include six major species namely, *B. abortus, B. melitansis, B. suis, B. canis, B. ovis*

B. neotomae. Recently new species was discovered namely *B. marine mammals* (4). Each species of *Brucella* divided into some of biotypes like *B. abortus* include 6 biotypes, *B. melitensis* include 3 biotypes. *B. suis* include 5 biotypes. These biotypes can be recognized by biochemical test and the ability of growth in the presence of dyes, agglutination with monospecific antisera for *B. abortus* (A) and *B. melitansis* (M) and DNA hybridization (5 ; 6; 7).

Brucella are sensitive to direct sunlight, disinfectant (8). Pasteurization temperatures of 62.7C˚ for 30 min, or 71.6C˚ for 15 min, are adequate to destroy the organism in milk. *Brucella* survive for several months at 4C˚ to 8C˚ in tap water and other liquid and are killed under freezing and thawing condition. Acidic condition are detrimental to *Brucella*, and they do not survive in sour milk or other low pH media (9).

MATERIALS AND METHODS

Collection of samples

A total of 300 samples were collected from different locations of Basrah province. Out of these 100 samples of locally produced cheese, 100 samples locally produced cream and 100 samples of Ice-cream. About twenty grams from each sample were collected in sterilized polyethylene bag and transmitted to laboratory by cooling box (10).

Bacterial Isolation and Diagnosis

Cheese, cream and Ice-cream samples, were treated as described by Marth (10). 10g from each sample were added to 90ml of sterilized 2% sodium citrate and mixed well by stomacher for about 5min. Two tubes containing 4ml of brain heart infusion broth (Difco) was inoculated with 1ml of each above mixture, and incubated for 48hrs at 37˚C. 0.1 of each tube was streaked on *Brucella* selective agar (Himedia) plates and incubated at 37˚C for 3 – 5 days. Plates were observed daily for bacterial growth. Colonies having a characteristic of *Brucella* were subcultured for purification and identification.

Identification of *Brucella* isolates and biotyping

The bacterial isolates were identified as *Brucella* species using the following methods: Colony morphology and staining; motility test and Oxidase test (11); Blood haemolysis; Lactose fermentation; Urease test; Catalase test (12); Gelatin analysis; Indol test (13).

For the classification of *Brucella* species and biotypes the following tests were used: Carbon dioxide requirement for growth; hydrogen sulfide production; dye sensitivity test (Viz basic fuchsine 1:50.000 and 1:100.000, thionin 1:25000, 1:50.000 and 1:100.000); agglutination with monospecific antisera of *Brucella abortus* (A) and *B. melitencis* (M) were done by slide agglutination test. (5, 14; 15).

Antibiotic sensitivity test

Bacterial suspension were spreaded on nutrient agar using L-shape glass and leaved for 5 minutes then the antibiotic discs (Himedia) were fixed on the agar plate using sterilized forceps and incubated at 37C˚ for 24 hours. The zones of inhibition were measured using caliper.

Effect of pH on bacterial growth (16)

Bacterial strains were streaked on *Brucella* agar medium having different pH value (2,4,6,8,9 and 10). Plates were incubated at 37˚C for 24 – 48hrs. and the growth were observed daily. pH were adjusted using NaOH and HCl.

Effect of Temperature on bacterial growth (17)

Each strain was streaked on *Brucella* agar medium and incubated for 24 – 48hrs at different temperature degree (4, 10, 15, 18, 20, 30, 37, 40, 42, 43, 44, 45 and 50)˚C. Growth was observed daily and the result were recorded.

RESULTS AND DISCUSSION

Bacterial isolation and diagnosis

The *Brucella* colonies appear on *Brucella* agar, after 3-5 days of incubation, as pinpoint, smooth, glistening, bluish and translucent. As the age the colonies become opaque and about 2–3 mm in diameter.

The results showed that out of three hundreds milk product samples collected only 9 *Brucella* isolates were found (table 1). Cheese samples showed significant higher incidence of *Brucella* (8%) than cream samples (1%) while no *Brucella* were isolated from 100 ice-cream samples.

Eight *Brucella* isolates were isolated from cheese samples were distributed in to two species. Five isolates were *B. melitensis* and the rest were identified as *B. abortus*. Cream samples which have only one isolates identified as *B. abortus* (table 2,3)

Depending on Alton table for *Brucella* identification (14) the bacteria isolated from cheese were identified as *B. abortus* biotype 4 and *B. melitensis* biotype 2. The results also revealed that isolation of bacteria from cream was one isolate represented by *B. abortus* biotype 4 (Table 4). The low number of *Brucella* found in cream than soft cheese may be due to the way that by cream is manufactured. Boiling of the milk resulted in killing large number of bacteria. The bacteria may be appear because of contaminated instrument used post manufacturing. Also bacterial contaminated occur when the farmer's adding raw milk to cream for moist it.

The bacteria not isolated from the ice-cream as a result for using some dyes which inhibited the growth of bacteria in addition to the using pasteurized milk and powder milk.

Our results is similar to that obtained by Shanshal (18) who found 16 isolates from 600 milk and milk products sample. Seven isolates of *B. abortus* biotype 4, three isolates of *B. abortus* biotype 3 four isolates of *B. melitensis* biotype 2 and one isolates of *B. melitensis* biotype 3. Hadad (19), isolated 8 isolates of *B. abortus* from 80 buffaloes milk and 80 cream samples, 5 isolates of *B. abortus* biotype 3 and three isolates of *B. abortus* biotype 4. The result of korji (20) showed that 15 isolates of *B. abortus* biotype 1,4,5,6,7,and 7 isolates of *B. melitensis* biotype 1,3 from 304 milk samples and 96 cheese samples. Al Jobory (21) obtained 1 isolates of *B. abortus* biotype 7 and 1 isolates of *B. abortus* biotype 9 from 80 cheese sample.

Antibiotic sensitivity:

The results showed that all isolated *Brucella* (100%) were sensitive to Streptomycine, Trimethoprim, Gentamycine, Rifampin, Trimethoprim 1.25 + sulamethaoxazok 23.75mg, Kanamycine and Tetracycline. Other antibiotics such as Doxycycline, Cephalxin, Cefotaxim, Ampicillin and Erythromycin have low effect on bacterial isolates (Fig. 1). This results is same to that found by Abed Mohammed (22) who revealed that all *Brucella* isolates are sensitive to Setreptomycin, Teteracycline, Gentamycin, in percentage 100%, Kanamycin, Topramycin in percentage 91.6% Chloromphinichol 82.3%, Cephalxin 72.9%, Erthromycin 50% while the isolates are resistance to pencillin, Naledixic acid, Bacetracin, polymexin B. and Linkomycin in percentage 100%. Al-Abbasi *et al.,* (23) recorded different results from ours that all *Brucella* isolates are resistance to Setreptomycine and Cotriamoxcizol in percentage of 100%.

Growth of *Brucella* at different pH value and different temperature degree:

All *Brucella* isolates cannot grow at pH less than 4 and the growth is normal at pH ranged from 6 – 9. No growth was found at pH 10 (Table 5). This indicates the ability of *Brucella* for growth in wide range of pH. Acid tolerance may relate to virulence as has been previously studied (24 ; 25) .

Brucella strains can grow at wide range of temperature from 20˚C to 40˚C (Alton 1985). At the present study (Table 6) *Brucella* strains grow at temperature range between 18-44˚C. This indicate that this bacteria can survive in and dairy products like cheese and cream for long time at different storage temperature if it not pasteurized (26 ; 27).

Table (1): *Brucella* isolates recovered from milk product samples

Sample source	No. of examined samples	+ve Culture isolation	%
Cheese	100	8	8
Cream	100	1	1
Ice-cream	100	0	0
Total	300	9	3

Table (2):- Biochemical test of the isolated *Brucella spp*.

Growth on MacConky agar	Blood heamolysis	Gelatin analysis	Catalase test	Oxidase test	Urease test	Indol	No. of Isolates	Species
–	–	–	+	+	1 – 2 h+	–	4	*B. abortus*
–	–	–	+	+	Variable	–	5	*B. melitensis*

Table (3): Distribution of *Brucella* species according to samples sources

Sample source	Total isolates	*B. abortus*	%	*B. melitensis*	%
Cheese	8	3	37.5	5	62.5
Cream	1	1	100	0	0
Ice- cream	0	0	0	0	0
Total	9	4	44.4	5	55.5

Table (4): Identification and Biotyping of *Brucalla* isolates

Sample	Isolates No.	Urease test	CO_2 require	H_2S production	Growth in present of		Agglutination with mono specific antisera		Species	biotype
					Basic fuchsine	Thionin	A	M		
Soft cheese	63	1 – 2 h+	+	+	+	–	–	+	*B. abortus*	4
Soft cheese	64	1 – 2 h+	+	+	+	–	–	+	*B. abortus*	4
Soft cheese	65	1 – 2 h+	+	+	+	–	–	+	*B. abortus*	4
Soft cheese	66	variable	–	–	+	+	+	–	*B. melitansis*	2
Soft cheese	67	variable	–	–	+	+	+	–	*B. melitansis*	2
Soft cheese	68	variable	–	–	+	+	+	–	*B. melitansis*	2
Soft cheese	69	variable	–	–	+	+	+	–	*B. melitansis*	2
Soft cheese	70	variable	–	–	+	+	+	–	*B. melitansis*	2
Cream	71	1 – 2 h+	+	+	+	–	–	+	*B. abortus*	4

Table (5): Growth of *Brucella* spp. on different pH level.

	Brucella spp. / **pH**	***B. abortus***	***B. melitansis***
1	**2**	**–**	**–**
2	**4**	**+**	**+**
3	**6**	**+**	**+**
4	**8**	**+**	**+**
5	**9**	**+**	**+**
6	**10**	**–**	**–**

Table (6): Growth of *Brucella* spp. on different temperature degree.

	Brucella spp. / **Temperature C°**	***B. abortus***	***B. melitansis***
1	**4**	**–**	**–**
2	**10**	**–**	**–**
3	**15**	**–**	**–**
4	**18**	**+**	**+**
5	**20**	**+**	**+**
6	**30**	**+**	**+**
7	**37**	**+**	**+**
8	**40**	**+**	**+**
9	**42**	**+**	**+**
10	**43**	**+**	**+**
11	**44**	**+**	**+**
12	**45**	**–**	**–**

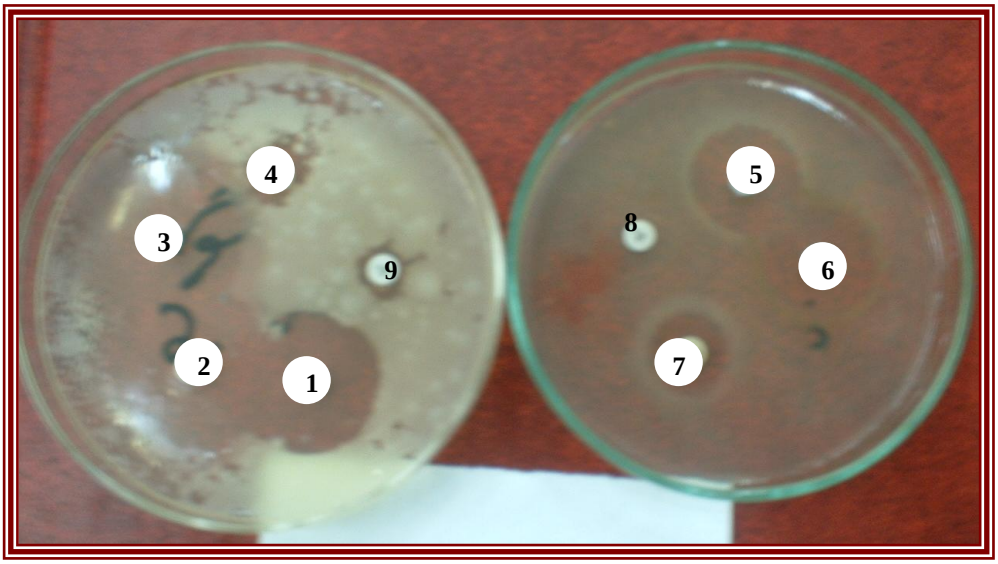

Fig 1: Antibiotic susceptibility of *Brucella* spp. to different antibiotic disc

(1) Streptomycine (2) Trimethoprim (3) Gentamycine (4) Erythromycin (5) Kanamycine (6) Tetracycline (7) Cefotaxim (8)Doxycycline, (9)Ampicillin.

عزل وتشخيص بكتريا البروسلا من منتجات الألبان في البصرة

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الخلاصة

تم جمع 300 عينة من منتجات الحليب من مناطق مختلفة من البصرة. باستخدام طريقة الاوساط الاغنائية والوسط الانتخابي للبروسيللا تم عزل تسعة عزلات من البروسيلا ثمانية منها من الجبن الطري المحلي وواحد من القيمر المحلي الا انه لم يتم عزل اي عزلة بروسيلا من مرطبات الايس كريم. تم تشخيص ه ذه العزلات ووجد ان أربعة منها من البروسيلا المجهضة وخمسة منها من البروسيلا المالطية. من خلال دراسة الحساسية الدوائية لها لوحظ ان جميع العزلات كانت حساسة للمضادات streptomycin, gentamycin, kanamycin, rifampicin, trimethoprim, trimethoprim with sulfamethoxazole and tetracycline الا ان اغلب العزلات اظهرت مقاومة للمض ادات doxycyclin, cephalexin, ampicillin and erythromycin. وعند دراسة تاثير الحرارة وال PH لوحظ نمو العزلات في مديات مختلفة من الحرارة وال PH .

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