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INCIDENCE OF PSEUDOMONAS ORGANISMS IN CLINICAL, SUBCLINICAL MASTITIS AND RAW MILK OF CATTLE IN ASSIUT GOVERNORATE

(With 3 Tables and 1 Figure)

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مدى انتشار ميكروبات السودوموناس في ألبان الضرع الملتهب إكلينيكيا وتحت الإكلينيكي ولبن الأبقار الخام في محافظة أسيوط

صديق رشوان صديق ، حسين على عبد القادر ، الفونس فخري بسطاوروس

أجريت هذه الدراسة على عدد ١٥٠ عينة من لبن الابقار (٥٠ عينة لبن من أبقار تعانى من التهاب الضرع الاكلينيكى ، ٤٥ عينة لبن من أبقار تعانى من التهاب الضرع تحت الإكلينيكي، ٥٥ عينة من لبن الابقار المجمع في أقساط) وقد أوضحت النتائج أن نسبة الإصابة بميكروب السيدوموناس كانت ٢١،٣٣ % وقد تم عزل ٣٢ عترة من هذه العينات الإيجابية وتم تصنيف هذه العترات كالتالى : ٤ عزلات من ميكروب السيدوموناس ايروجينوزا بنسبة ٨% ، وعترة واحدة من ميكروب السيدوموناس فلورسنس بنسبة ٢% وذلك من الحالات التي تعانى من التهاب الضرع الإكلينيكى ، وقد تم عزل ٧ عترات من ميكروب السيدوموناس ايرجينوزا بنسبة ١٥،٦% من عينات لبن الابقار التي تعانى من التهاب الضرع تحت الإكلينيكى ، وتم عزل ١٥ عترة من ميكروب السيوموناس ايروجينوزا بنسبة ٢٧،٣% ، ٣ عترات من ميكروب السيدوموناس فلورسنس بنسبة ٥،٥ % وعزل عترتين من ميكروب السيدوموناس الكالجينز بنسبة ٣،٦٣% من عينات اللبن المجمع من الابقار في أقساط. ،قد تم إجراء اختبار حساسية هذه العترات لعدد من المضادات الحيوية (٩ مضاد حيوي) هي الجنتاميسين، الكلورامفينيكول، البنسلين، اوكسىتتراسيكلين، التتراسيكلين، سيفالوكسين، اموكسيسلين، اللينكوسين واللينكوسبكتين، وقد أوضحت النتائج أن معظم العترات من ميكروب السيدوموناس كانت شديدة الحساسية لكل من الكلورامفينيكول ، جنتاميسين واللينكوسين وقليلة الحساسية لكل من اوكسيتتراسيكلين وكانت مقاومة لكل من البنسلين واللينكوسبكتين والسيفالوكسين، وقد تمت مناقشة الخطورة الصحية لهذا الميكروب على الصحة العامة وكذلك طرق الحد من انتشاره.

SUMMARY

The present study was carried out on 150 milk samples (50 milk samples were collected from dairy cows suffering from clinical mastitis, 45 milk samples were collected from subclinical mastitic cow milk and the rest 55 milk samples were collected from raw bulk milk collected in cans. The total incidence of positive samples out of 150 samples were 32 (21.33%). The isolated strains were 32 (5 strains from mastitic milk, 7 strains from subclinical mastitic milk and 20 strains were from collected cans milk). The incidence rate of isolated strains from mastitic Friezian Cow milk was *P.aeruginosa* 4 (8%) and *Ps. Fluorescens* 1 (2%), in subclinical mastitic milk P. aeruginosa was 7 (15.6%) and in raw collected milk *Ps.aeruginosa* was 15 (27.3%), *Ps.fluorscens* 3 (5.5%) and *Ps.alcaligens* 2 (3.63%). Antibiotic sensitivity test was performed using Gentamycin, Chloramphenicol, Pencillin, Oxytetracycline, Cephaloxine, Amoxicillin, Tetracycline, Lincocine and Lincospectine. It was found that the most isolated strains were sensitive to Chloramphenicol, Gentamycin, and resistant to Penicillin, Tetracycline, Lincospectine, Cephaloxine and Amoxicillin. Public health significance was discussed.

Key words: Pseudomonas, mastitis, cattle.

INTRODUCTION

Pseudomonas species is ubiquitous in nature. It is the most important psychrotrophic microflora in cold strored raw milk not only because it predimoninate (Muir et al., 1979), but also because many strains elaborate extracellular enzymes such as proteases and lipases which are extremely thermostable (Cogan, 1977). These enzymes are able to withstand pasteurization causing orange of flavour defects in milk and diversity of dairy products as rancid fruity, bitter, putrid, cheesy, unclean, soapy, musty and fishy (Bassette et el., 1986). In addition slime and pigment production were reported, (Walker, 1988). *Ps. aeruginosa* is normal inhabitant of human and animal intestinal tract (Merchant and Packer, 1961). The organism is sometimes found on normal skin of human being and animals (Ruzicka, 1898). *Ps. aeruginosa* was reported as a cusative organism of conjunctivitis, corneal ulceration, mastitis, otitis media, various respiratory infections, infections of bones, joints, urinary tract, skin, wound, severe gastroenteritis and calf diarrhea (Mottelib, 1973 and Tood, 1981). Also *Ps.aeruginosa* is the most frequent pathogen causing nercotizing

enterocolitis in infancy and early childhood alone or incombination with other organisms, it shows diffuse abdominal distension and blood diarrhea which is watery and has a foul smell (Perena et el., 1977 and Sutter et el., 1966).

In Egypt several cases of food poisoning due to *Ps. aeruginosa* have been reported which have been traced to the consumption of contaminated dairy products (Ahmed et el., 1989).

In recognition of the economic and public health significance of pseudomonas spp. this study was planned to investigate the incidence of pseudomonas spp. in clinical, subclinical mastitic milk and raw milk in dairy farms and application of antibiotic sensitivity of isolated strains in Assiut Governorate.

MATERIAL and METHODS

Milk samples:

Samples of milk (mastitis, subclinical mastitis milk and raw milk) 25 ml each were collected under aseptic conditions in sterile MacCarteny tubes from different dairy Friesian farms in Assiut Governorate. All those collected samples were submitted to the laboratory for examination immediatly or were kept in refrigerator untill they are examined.

Isolation of pseudomonas:

The milk sample either clinical, subclinical mastitis milk or raw milk were centrifuged at 1500 rpm for 20 min., a loopful from the sediment of each sample was inoculated into Nutrient agar media and pseudomonas selective agar base media. The inoculated plates were incubated at 42°C for 48 hrs. The presence of pseudomonas was detected by the production of grape like and rancid fruits odour, greenish, blue pigment in case of *Ps.aeruginosa* (Brown and Lowbury 1965) and yellow green in case of *Ps.floursence* or colourless in some other species, (Merchant and Packer, 1961and Wilson and Miles, 1983).

Identification of isolates:

The organisms were identified by their motility, reduction of nitrate, production of oxidase, ability of gelatin liquification, catalasae reaction, citrate utilization, urease activity, the production of water soluble pigment (green pigment) soluble in water and chloroform in aeruginosa and yellow pigment called fluorscience soluble in water but not chloroform in case of *Ps. flourscience* (Merchant and Packer 1961).

Antibiotic sensitivity:

Different types of sensitivity discs obtained from Bio Merieux were used. The antibiogram of isolated strains were applied on several types of antibiotics. Used disc diffusion method described by Stokys (1968) was performed using Chloramphenicol (30 μg), Gentamycin (10 μg), Lincocine (20 μg), Oxytetracycline (30 μg), Tetracycline (30 μg), Lincospectine (20 μg), Pencillin (10 IU), Amoxicilline (30 μg) and Cephaloxine (30 μg).

RESULTS

Results are shown in Tables 1-3 and Fig. 1

DISCUSSION

As shown in Table (1), *Pseudomonas spp.* were most frequently isolated from raw bulk milk samples (36.36%) followed by subclinical mastitic milk samples (15.56%) and lastly from clinical mastitis milk samples (10%).

In the present study the results (in Table 2) showed that the incidence of *Ps.aeruginosa*, *Ps.fluorescens* and *Ps. alcaligens* in mastitis milk were (8%), 2% and (0.0%) respectively.

These results are in accordance with those reported by Fabre et el. (1992) and Nawal et el. (1996) who isolated *Ps. aeruginosa* from mastitic cow milk with an incidence (8.8%, 7.14%) respectively and failed to isolate any other species of pseudomonas species.

On the other hand the obtained results in Table (2) were more higher than that recorded by Melennan et el. (1997) who isolated *Ps. aeruginosa* from 6 cows (mixed breed herds) out of 215 (2.8%) suffering from clinical mastitis in Australia, while in an outbreak of mastitis in Athames Valley dairy herd NewZealand *Ps. aeruginosa* was identified by Coats (1998) with a higher incidence in 22 cows out of 102 (21.6%) than that recorded in our investigation. Also Sabah and Elmer (1994) recorded a higher incidence of *Ps.aeruginosa* (15.38%) from cow mastitis milk than that reported in our studies, these differences in incidence percent may be attributed to different endemic status of *Pseudomonas spp.*, herd management and resistance of tha animals (Wilesmith et el., 1986).

The obtained results in this subject in Table (2) and Fig.1 revealed that the incidence of *Ps.aeruginosa* in subclinical mastitis milk collected

from some cow dairy farms was (15.6%), this incidence of *Ps.aeruginosa* was in agreement to that reported by Nawal et el. (1996) whose mentioned that the incidence of *Ps. aeruginosa* isolated from subclinical mastitic cow milk was (14.29%) which was nearly similar to our recorded results.

The high incidence of the *Ps.aeruginosa* obtained by the same authors insured that hygienic measures in this farm was not well controled and bad handling of milker dairy men during milking may be shared in spreading of Ps.aeruginosa inbetween dairy farm animals.

The recorded results in Table (2) & Fig. 1 explained that the incidence of *Ps. aeruginosa*, *Ps.fluorescens* and *Ps.alcaligens* isolated from raw milk collected in cans from some dairy farm cow in Assiut Governorate were 27.3%, 5.5% and 3.63 respectively. These results were nearly similar to those obtained by Amemiya et el. (1976 & 1978) who found that incidence of *Ps. aeruginosa* was 22.8% and 20.9% respectively in raw milk while the same authors failed to detect any other species of *Pseudomonas species*. On the other hand our incidence was significantly higher when compared with the results obtained by Micova et el. (1989) who reported isolation rate 8.87% of *Ps.aeruginosa* in raw collected milk.

The obtained results are much more lower than that recorded by Al-Ashmawy et el. (1991) who isolated *Ps.aeruginosa* and *Ps. fluorscens* from milk collected in cans and tanks from some dairy farm cow animals with an incidence (76%, 20%) and (60%, 28%) respectively. Also Al-Ashmawy et el. (1997) mentioned that the incidence of *Ps.aeruginosa, Ps.fluorescens* and *Ps.alcaligens* were 61.33%, 67.33% and 24% from farm raw bulk milk collected in tanks at different and private governmental dairy farms at El-Mansoura city, Egypt. Those incidences were more higher than that recorded in this study and that may be attributed to that this milk exposed to high degree of contamination with faecal matter or polluted water. The finding in table (1) and Fig. (1) showed that the incidence of *Ps.aeruginosa* (27.3%) was more higher than incidence of *Ps.fluorescens* (5.5%) and *Ps.alcaligens* (3.63%), this indicates that *Ps.aeruginosa* is widely distributed in nature and the most common microorganism contaminate the milk, these supported by Al-Ashmawy et el. (1991).

From Table (2) and Fig. (1), in general the incidence of *Ps.aeruginosa* in three groups (mastitic milk, subclinical mastitic and raw milk) were 8%, 15.6% and 27.3% while the total incidence of *Ps. aeruginosa* clinical mastitis (M/M), subclinical mastitis (S/C/M) and raw milk was 17.33%. These explained that *Ps.aeruginosa* incidence was the

highest % and most common pathogenic microorganism than *Ps.fluorescens* and *Ps. alcaligens* in M/M, S/C/M and raw milk, also the total incidences of *Pseudomonas spp.* were higher in raw milk (36.4%), than M/M and S/C/M (10%), (15.6%) these indicate that the contamination in these farms of several levels and bad hygienic conditions of these farms and the collected milk exposed to severe degree of contamination during milking steps.

The variation between the obtained results and these reported by other investigators may be due to the variation in media used for isolation of the organism, difference in area of isolation, atmospheric temperature and hygienic condition of milking and milk utensils (Nawal, 1992).

In referring to antibiogram sensitivity as explained in Table (3) the results showed that the most isolated strains of *Ps.aeruginosa, Ps.fluorescens* and *Ps. alcaligens* from some dairy farm animals in Assiut Governorate were highly sensitive to Chloramphenicol (30 μg), Gentamycin (10μg) and Lincocine (20 μg), less sensitive to Oxytetracycline (30 μg) and Tetracycline and resistant to penicillin (10 IU), Lincospectine (20 μg) and cephaloxine (30 μg). These findings are in agreement with those reported by Vasil (1994); Nawal et al. (1996); Islam and Chowdhury (1997) and Coats (1998) while Nawal (1992) reported that the most isolated strains of *Ps. aeroginosa* isolated from raw milk are sensitive to Gentamycin (30 μg) and polymyxin and resistant to penicillin, Chloramphenicol and Nalidixic acid, Streptomycin and Erythromycine.

On conclusion, the milk is liable to be contaminated by *Ps.aeruginosa, Ps. fluorescens* and *Ps. alcaligens* from infected feacal matter of farm animals or from the use of polluted water, soil or dairy equipments during milking so the presence of *pseudomonas* spp. in clinical, subclinical mastitis and raw collected bulk milk. Dairy men and other workmen on the farm may be responsible for contamination of milk and the organism could grow to numbers sufficient for inducing food poisoning, so governmental control should be imposed for those who are contact with dairy industry and strict hygienic measures must be followed during steps of milking or milk production. Temperature storage (below 4°C) improving the quality of farm milk and eliminate spreading microorganism infection and avoid food poisoning of human being through consumption of contaminated milk. Also identification of microorganism and sensitivity testing are important in the treatment of clinical and subclinical mastitis to minimize the spreading of microorganisms besides culling of untreatable animals.

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Table (2): Incidence of isolated pseudomonas strains from clinical, subclinical mastitic and raw collected farm milk in some governomental dairy farms in Assiut Governorate.

Isolated strains	Mastitic milk samples (50)		Subclinical mastitic milk samples (45)		Raw milk samples (55)		Total milk samples (150)	
	No	%	No	%	No	%	No	%
Ps. aeruginosa	4	8	7	15.6	15	27.3	26	17.33
Ps. fluorescens	1	2	0	0	3	5.5	4	8.88
Ps. alcaligens	0	0	0	0	2	3.63	2	3.64
Total	5	10	7	15.6	20	36.4	32	21.33

Table (3): Results of antibiotic sensitivity tests against 32 strains of *Ps. aeruginosa* , *Ps. fluorescens* and *Ps. alcaligenes*

Isolated strains	C (30 µg)	CM (10 µg)	Lin (20 µg)	Ot (30 µg)	TE (30 µg)	Lin-sp (20 µg)	P (10 IU)	AML (10 µg)	CN (30 µg)
Ps. aeruginosa	++++	++++	+++	+++	++	-	-	-	-
Ps. fluorescens	++++	++++	++	++	+	-	-	-	-
Ps. alcaligenes	++++	++++	++	+	+	-	-	-	-

C = Chloramphenicol CM = Gentamycin Lin = Lincocine Ot = Oxytetracyclin TE = Tetracycline

Lin-sp = Lincospectine P = Pencillin-G AML = Amoxycilline CN = Cephaloxin