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OCCURENCE, DETECTION AND SIGNIFICANCE OF Pseudomonas aeruginosa IN RAW MILK

(With 2 Tables)

By

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مدى تواجد وأهمية الميكروب الصديدي الأخضر في اللبن

نوال غبريال

تم فحص عدد ١٣٥ عينه من اللبن الخام لمعرفة مدى تواجد ميكروب الصديدي الأخضر بها . وقد تم عزل عدد ٢٢ عتره من هذه العينات وأجريت الاختبارات الكيميائيه لمعرفة الخواص الكيميائيه والمنتجات الأنزيميه لهذه العترات المعزوله وكذلك تم دراسة حساسية هذه العترات للمضادات الحيويه . وقد نوقشت الخطوره الصحيه لهذا الميكروب لدرء خطر التسمم الغذائي بهذا الميكروب .

SUMMARY

135 samples of raw milk were studied for the incidence of Ps.aeruginosa. Several enzymatic activities such as DNAs, gelatinase, Lecithinase, Lipase, caseinase and haemolysine for 22 isolates of Ps.aeruginosa were investigated. Also, antibiotic susceptibility using Polymyxine, Gentamycine, Tetracycline, Neomycine, Nalidoxic acid, Pencilline, Ampicilline, chloramphenical, Streptomycine and Erythromycine were studied. Public health significance was discussed.

INTRODUCTION

Pseudomonas aeruginosa was reported as a cusative organism of conjunctivitis, corneal ulceration, endocarditis, meningitis, mastitis, otitis media, various respiratory infections, infections of bones and joints, infections

of urinary tract, infection of skin and wounds and sever forms of acute gastroentritis (LUSIS and SOLTYS, 1971 and TOOD, 1981).

Ps.aeruginosa is the most frequent pathogen causing necrotising enterocolitis alone or in combination with other organisms, it shows deffuse abdomenal distention and blood diarrhea which is watery and has a foul smell (PERENA, et al. 1977; SUTTER, et al. 1966).

Several cases of Ps.aeruginosa food poisoning have been reported in Egypt due to consumption of contaminated dairy products (ABDEL-AZIZ, 1979; AHMED, 1980 and AHMED, et al. 1989).

The organism is mainly found in the intestine of animals and human being (BERGAN, 1975). So its presence in food items could be taken as an indication of faecal contamination and an inadequate treated of water supplies (AHMED, 1980).

Due to the public health significance of Ps.aeruginosa, the present study was undertaken to study the incidence of this organism in raw milk in Assiut city, the biochemical reactions and antibiotic sensitivity of the isolates were studied.

MATERIAL and METHODS

Samples of milk:

135 samples of raw milk (50 ml each) were collected under aseptic precautions in sterile MaCcarteny bottles from street vendors and dairy shops. All samples were submitted to the laboratory with minimum of delay and were held in refrigerater until they were examined.

Isolation of Ps.aeruginosa:

The samples were centrifuged, a loopfull from the sediment of each sample were inoculated into Pseudomonas Selective Agar base (citrimde agar) plates, and incubated at 42 C for 48 hrs. The presence of Ps.aeruginosa was detected by the production of greenish-blue pigment (BROWN and LOWBURY, 1965). The isolated colonies were tested for purity by Gram's stain.

Identification of isolates:

The organisms were identified by their motility, reduction of nitrate, production of oxidase, ability to liquefy gelatin, catalase reaction, citrate utilization, haemolysis of sheep blood, urease activity (CRUICKSHANK, et al. 1975).

The identified strains were evaluated for the production of enzymes DNAs test, protease (gelatinase gelatin charcoal disks), Lecithinase and Lipase

(egg yolk reaction), haemolysin (haemolytic activity) and caseinase activity (digestion of milk medium) (CRUICKSHANK, et al. 1975 and WILSON and MILES, 1983).

Antibiotic sensitivity of Ps.aeruginosa:

Different types of sensitivity discs obtained from Bio M'erieux were used, Diffusion method described by STOKYS (1968) was performed using Gentamycin (10 ug) Polymyxine (300 U), tetracycline (30 ug), Neomycine (30 ug), Nalidoxic acid (30 ug), Pencilline (10 lu), Ampicilline (10 ug) Chloramphenical (30 ug) Streptomycine (10 ug) and Erythromycin (10 ug).

RESULTS

The results were tabulated in tables 1 and 2.

DISCUSSION

This study was conducted to investigate the incidence of Ps.aeruginosa in raw milk. The incidence was found to be 17.03% (Table 1).

The results were nearly similary to those obtained by AMEMIYA, et al. (1976 & 1978) who reported an incidence of 22.8% and 20.9% respectively in raw milk. On the other hand this incidence of isolation was significantly high when compared with the results obtained by MICOVA, et al. (1989) who reported isolation rate of 8.87% of Ps.aeruginosa in raw milk. The results could be considered low when compared with that reported by SUNTA GROVER and SRINIVSAN (1988) and ERGULLU (1982) who reported 90.4% and 79% respectively.

The variation between the obtained results and those 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 milking utensils.

The ability to produce extracellular hydrolytic enzymes (protases, Lecithinases, lipases) is common to Ps.aeruginosa (STANIRE, et al. 1966). Those products play some role in its pathogenicity (LIU, et al. 1961 and SEDDIK, et al. 1987).

In this study it was noticed that all isolates produce haemolytic products, also they produce protease which liquefy gelatin (Table 1) and these results were similar to those reported by JOHNSON, et al. (1967); SEDDIK, et al. (1987) and SUNITA GROVER and SRINIVSAN (1988). Twenty isolates

produced lipase and sixteen produced lecithinase (Table 1) while COLWELL (1964) cited that all isolates produce lipase and lecithinase. Twenty two isolates showed DNAs activity while seventeen showed caseinase activity (Table 1).

No unusual pattern of antibiotic susceptibility was observed among the isolates, All isolates (Table 2) were sensitive to Polymyxine, and 90.9% were senstive to Gentamycin. The same results were obtained by LUSIS and SOLTYS (1971), DWIGTH, et al. (1979) and SUNTA GROVER and SRINIVSAN (1988) who reported that over 95% of the isolates were sensitive to Polymyxine and Gentamycin. There was little sensitivity to Neomycine and Nalidoxic acid.

Ps.aeruginosa was found to be resistant to Pencilline, Ampicilline, Chloramphenicol, Streptomycine and Erythromycin (Table 2). The same results were reported by LUSIS and SOLTYS (1971) and DWIGHT, et al. (1979) and SUNTA GROVER and SRINIVSAN (1988).

On conclusion, milk is liable to contamination by Ps.aeruginosa from infected feaces from animal or from the use of pollouted water or dairy equipiments during milking and preparation. Dairymen and other workemen on the farm may be responsible for contamination of milk and the organism could grow to numbers sufficient to induce food poisoning. Therefore, governmental regulation should be imposed for those who are concerned with dairy industry. Also stringent hygienic measures must be followed during all steps of milk production.

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Table I: Bacteriological study of 22 isolates of Ps.aerugonase from raw milk.

Raw milk samples		Production of pyocinin	Production of oxidase	Production of urease	Nitrate reaction	Motility	Catalase reaction	Growth in citrate medium	Methyle red test	Voges - Proskour test.	Indol test	Production of DNAs	Production of protease liquefication of gelatin	Production of lipase	Production of lecithinase	Production of haemolysine	Production of caseinase
135																	
Positive	Negative																
22	113	22	22	22	22	22	22	22	-	-	-	22	22	20	16	22	17

Table 2: Results of antibiotic sensitivity of 22 isolates of Ps.aeruginosa

Sensitivity disc	No. of resistant	No. of sensitive
Gentamycin	2	20
Polymyxine	0	22
Tetracycline	7	15
Neomycine	19	3
Nalidoxic acid	21	1
Pencilline	22	0
Chloramphenical	22	0
Streptomycine	22	0
Erythromycine	22	0
Ampicilline	22	0