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OCCURRENCE AND SIGNIFICANCE OF *PSEUDOMONAS AERUGINOSA* IN SOME MEAT PRODUCTS

(With 5 tables)

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مدى تواجد وأهمية الميكروب الصديدى الأخضر "سيدوموناس إيروجينوزا" فى بعض منتجات اللحوم

الفـــونس بسطاوروس

أجريت الدراسة على ١٠٥ عينة من منتجات اللحوم بواقع (٤٥ عينة لحم مفرى طازج، ٣٠ عينة سجق طازج و ٣٠ عينة هامبورجر متجمد) جمعت عشوائيا من مصادر متعددة بأسيوط وذلك لإستبيان العدد الكلى لميكروبات السيدوموناس بإتباع وسيلة الإنتشار السطحى ولقد تبين من الدراسة أن ٧١,١١٪، ٤٣,٣٣٪، ٢٦,٧٪ من اللحم المفرى الطازج، السجق الطازج والهمبورجر المتجمد تحتوى على ميكروبات السيدوموناس بمتوسط ٣,٢ × ١٠^{٤} ، ١,٠٧×١٠^{٣}، ٢,٢×١٠^{٣} /جم على التوالى، وقد تم عزل عدد ٨ عترات من الميكروب الصديدى الأخضر من هذه العينات وأجريت الإختبارات البيوكيميائية لمعرفة الخواص الكيميائية والمنتجات الانزيمية للعترات المعزولة، وقد لوحظ فى هذه الدراسة أن نواتج نمو عصويات الصديد الأخضر كان لها تأثير قاتل على فئران التجارب المعملية التى تم حقنها بهذه النواتج وبإجراء اختبارات الحساسية بالمضادات الحيوية على هذه المعزولات أتضح أنها غير حساسه لمعظم المضادات الحيوية المستخدمة ماعدا الجنتاميسين، البولى مكسين بى، والكلورامفينيكول بفاعلية ١٠٠٪، ٨٧,٥٪، ١٢,٥٪ على الترتيب.

SUMMARY

A total of 105 random samples of meat products including 45 fresh minced meat, 30 Fresh sausage and 30 Frozen hamburger were collected from different sources in Assiut city. The samples were examined for enumeration of *Pseudomonas* organisms. The obtained results pointed out that 71.11%, 43.33% and 26.7% of the examined fresh minced meat,

fresh sausage and frozen hamburger samples were positive for *Pseudomonas* microorganisms with an average count of $3.2x10^4$, $1.07x10^3$ and $2.2x10^3$/g using surface spread plate technique respectively. Biochemical and enzymatic activities for 8 isolates of *Pseudomonas aeruginosa* were investigated. It was noticed that the exoproducts of isolates exhibited lethal effect when injected intraperitoneally into white mice. Concerning the antibiotic sensitivity of the 8 isolated strains of *Pseudomonas aeruginosa* to 8 antimicrobial agents *in vitro*, they were only sensitive to Gentamicin, polymyxin -B and Chloramphenicol with an activity of 100%, 87.5% and 12.5% respectively.

Key words: *Pseudomonas aeruginosa - Meat products*

INTRODUCTION

The genus *Pseudomonas* represents the most psychrotrophic bacteria by far. These organisms are highly proteolytic and/or strong lipolytic and lead to biological changes in the composition of meat and its products particularly at low temperature (Witter, 1961 and Gill and Newton, 1982). On this assumption, *Pseudomonas* count in food may be taken as an indicator for its keeping quality and can be used as a measure for spoilage of meat and its products.

The genus *Pseudomonas* includes more than 140 species. *Pseudomonas aeruginosa* sometimes colonizes humans and is the major human pathogen of the group. *Pseudomonas aeruginosa* produces infection of wounds and burns, giving rise to blue-green pus; meningitis and urinary tract infection. It also infects eye, which may lead to its rapid destruction.

The bacterium may invade the blood stream and result in fatal spesis; this occurs commonly in infants and/or debilitated persons (Brooks et al., 1995).

It was mentioned that *Pseudomonas aeruginosa* has the ability to cause spoilage of meat and its products and leads to several outbreaks of food poisoning (Pererra et al., 1977). It was responsible for several cases of mastitis and remains in the udder for number of years (Howell, 1972). Moreover, many strains of *Pseudomonas aeruginosa* produce exotoxin A, which causes tissue necrosis and is lethal for animals when injected in the purified form (Brooks et al., 1995). Exotoxin A inhibits intracellular

protein synthesis and it is toxic for human blood macrophages (Wilson and Miles, 1983). Antibodies to exotoxin A were found in some human sera (Brooks et al., 1995).

Pseudomonas aeruginosa produces a variety of extracellular enzymes, including proteases, lipases and lecithinase (Wilson and Miles, 1983). The pathogenesis of *Pseudomonas aeruginosa* is mostly due to its extracellular products which are responsible for most of the histopathological effects of the infection (Blackweed et al., 1983).

Reviewing the available literature concerning antimicrobial sensitivity of *Pseudomonas aeruginosa.* It is shown that the organism was refractory to most chemotherapeutic agents (Blair et al., 1970; Ziv and Risenberg, 1970; Richmond et al., 1971; pajnoo et al., 1994 and Brooks, et al., 1995). Such phenomenon is still a point of argument and its investigation is the request of many bacteriologists and specialists of infectious diseases in both medical and veterinary practice. Such resistance might be due to gene transfer between strains of *Pseudomonas aeruginosa* that can occur through conjugation and transduction (Joklik, et. al., 1984).

Due to the public health significance of *Pseudomonas aeruginosa* and little information regarding its incidence in meat products, the present study was undertaken to study the presence of *Pseudomonas aeruginosa* in some meat products (fresh minced meat, frozen hamburger and fresh sausage). The biochemical reactions of the isolates, the production of enzymes and exotoxin as well as the antibiotic sensitivity of the isolates were also studied.

MATERIAL and METHODS

A total of 105 random samples of some selected meat products was examined. The samples included fresh minced meat (45), frozen hamburger (30) and fresh sausage (30) which were collected from different local retailers and was supermarkets of different sanitation levels at Assiut city. Each sample weighed 250 gms approximately and was transferred to the laboratory under aseptic conditions with a minimum of delay where it was bacteriologically examined.

I- Preparation of samples.

Fresh samples (minced meat and sausage) were prepared once it arrived to the laboratory, while frozen samples (hamburger) were allowed to thaw in its original containers in a refrigerator at 5 °C for 10 hours.

Ten grams of each sample were transferred under aseptic condition to a sterile blender jar to which 90 ml of sterile peptone water 0.1% were added to provide a dilution of 10^{-1}. The blender was operated to give 3000 r.p.m for not more than 2.5 minutes, then the mixture was allowed to stand for 15 minutes at room temperature. The contents of the jar were mixed by shaking before tenfold serial dilutions were prepared up to 10^{-6} using sterile peptone water 0.1%.

II Experimental techniques.

(A) Determination of *Pseudomonas* organisms count.

The count was determined by using surface spread plate technique as recommended by Anon (1978). From each of the prepared dilutions, 0.1 ml was carefully transferred and evenly spread by a sterile bent glass rod over a dry surface of *Pseudomonas* selective agar medium "Cetrimide agar" (Anon, 1986); duplicate plates were used. The inoculated plates were incubated at 25°C for 24 hours. The number of suspected colonies which showed fluorescent, blue green or brown pigmentation in countable plates were enumerated as *Pseudomonas* organisms and calculated as follows: *Pseudomonas* count/g=Number of colonies X dilution/10.

(B) Isolation and identification of *Pseudomonas aeruginosa*.

The suspected blue-green or brown colonies with characteristic grape-like odour of O-aminoacetophenone were picked up and subjected to further identification based on colonial and cellular morphology, and the different biochemical tests as recommended by Quinn, *et al.* (1994).

(C) Production of extracellular enzymes.

The identified strains were tested for the production of enzymes, DNAs test. gelatinase, lecithinase and lipase, haemolysin and caseinase as described by Cruickshank et al. (1975) and Wilson and Miles (1983)

(D) Toxigenicity test:

The eight identified strains were also examined for the production of the exotoxin, using white mice (each of average weight 20 gm). The animals were obtained from the Animal Health Research Laboratory. The filtrate of the organism was first prepared according to the method described by Seddik et al. (1987). A total of 64 male white mice was inoculated intraperitoneally. The inoculum was the centrifuged supernatent of 48 hours nutrient broth culture of each strain. Each supernatent was filtrated through Seitz filter and divided into two portions. The first was used without treatment while the second portion was heated at 60C° for 10 minutes. For each strain 8 mice were used:

each two mice received either 0.5 or 1 ml of either untreated or the treated filtrate. A group of 8 mice served as control where the animals were inoculated by sterile broth. All the inoculated mice were kept under observation. The number of dead mice was recorded.

(E) Antimicrobial susceptibility testing.

All isolates obtained in this study were tested for antimicrobial susceptibility by disc diffusion method as described by Finegold and Martin, (1982) using the following antibiotics: Neomycin (30 mcg/disc), Ampicillin (10 mcg/disc), Polymyxin-B (300 U/disc), Chloramphenicol (30 mcg/disc), Gentamicin (10 mcg/disc), Nalidixic acid (30 mcg/disc), Erythromycin (15 mcg/disc) and Penicillin G (10 IU/disc).

RESULTS

The results are tabulated in Tables 1, 2, 3, 4 and 5.

DISCUSSION

The results of the present study revealed that *Pseudomonas* spp. were detected in 32 (71.11 %), 13 (43.33 %) and 8 (26.7 %) with an average count of $3.2x10^4$, $1.07x10^3$ and $2.2x10^3$ of the investigated minced meat, sausage and frozen hamburger samples respectively (Table 1). Higher results were reported by EL- Nawawi and Nouman (1981), Nortje et al. (1990) and EL-Kateib and Fathi (1992). Such variation may be attributed either to the variations in media used for isolation and enumeration of the *Pseudomonas* organisms, differences in quality and sanitation level of meat or the ingredients used for manufacturing. The hygienic standards during processing or time and temperature of storage and retailing of products, also play a role.

In this study the incidence of *Pseudomonas aeruginosa* in meat products was found to be 7.62% (Table 2). From the examined 45 fresh minced meat samples, 5 (11.11%) contained *Pseudomonas aeruginosa*. The present results is somewhat similar to those reported by Lefebvre et al. (1992) who found that 10% of 10 minced meat samples were contaminated with *Pseudomonas aeruginosa*, although a higher incidence (15%) was reported by Sallam (1993). On the other hand, Youssef et al., (1984) and Hefnawy et al., (1985) reported lower incidences 1.7% and 6.47% respectively.

In this study, two of the fresh Sausage samples (6.67%) were contaminated with *Pseudomonas aeruginosa*. This finding agrees to a certain extent, with that reported by Abd-EL- Rahman and Ahmed (1988) who isolated *Pseudomonas aeruginosa* from sausage samples in a rate of 7.4%,. On the other hand it disagreed with that reported by Ahmed et al. (1988) who failed to isolate the organism from 20 fresh sausage samples, while Sallam (1993) recorded a higher figure (10%).

Concerning frozen hamburger samples, the organism was isolated from one (3.33%) of samples. A contradictory finding was given by Abd-EL Rahman and Ahmed (1988) and Ahmed et al., (1988) who failed to isolate *Pseudomonas aeruginosa* from 25 and 20 frozen hamburger samples respectively. Therefore, one can assume that *Pseudomonas aeruginosa* existed in low percentage in hamburger. This can be attributed to the bacteriostatic or germicidal effect of Nacl and spices including garlic and onion (Fraizer and Westhoff, 1986), and/or the effect of freezing storage which would destructed some of the initial microbial load. Hanna et. al. (1982) and Lukasova and Mraz (1986) found that 3% Nacl reduced the growth of *Pseudomonas aeruginosa*, while freezing at -18 °C reduced *Pseudomonas aeruginosa* count but did not result in complete destruction of the organism. Furthermore, Nagah and Fathi (1992) showed that *Pseudomonas aeruginosa* decreased in number from 10^6/g to $1x10^3$/g at deep freezing by the end of 47th day.

The antibiogram study conducted on the isolated strains (Table 4) showed that Gentamicin was the most effective antibiotic against *Pseudomonas aeruginosa isolated* from meat products at a rate of 100%. A finding that simulate those reported by Chakrabarty et al. (1980), kheir EL-Din and Awaad (1985), Enany et, al. (1986), Hamouda et al. (1987) and Hariharan et al. (1995). While a lower figure was recorded by El-Jakee et al. (1995) who found that the *Pseudomonas aeruginosa* isolates were sensitive to Gentamicin at a rate of 75%. *Pseudomonas aeruginosa* isolates afforded a significantly high degree of sensitivity against Polymyxin-B at a rate of 87.5%. This finding agrees to a certain extent with that reported by Hariharan et al. (1995) who found that of 16 antibiotics tested against *Pseudomonas aeruginosa*, Polymyxin-B, Tobramycin, and Netilmicin were the only drugs to which a 100% susceptibility was noticed. A reverse result was reported by Pajnoo et al. (1994) who repored that a total of 103 isolates was resistant to Polymyxin-B. As regards chloramphenicol, 12.5% of the isolates were sensitive, while the remaining 87.5% of isolates were resistant. Nearly

similar results were reported by Enany et al. (1986) who found that two isolates (6.7%) from thirty strains of *Pseudomonas aeruginosa* were sensitive to chloramphenicol. A contradictory result was reported by kheir El- Din and Awaad (1985), Hamouda et al. (1987) and El-Jakee *et.al.* (1995) who found that the isolated strains of *Pseudomonas aeruginosa* were resistant to chloramphenicol. A high degree of resistant (100%) *Pseudomons aeruginosa* isolates were detected in the present work against 5 out of 8 tested antibiotics. These findings agree to a certain extent with those reported by Blair et.al. (1970), Richmond et al. (1971), Brooks et al. (1995) and El-Jakee et al. (1995) who indicated that *Pseudomonas aeruginosa* strains were highly resistant to many antibiotics and such phenomenon might be due to R-factor.

Information derived from Table (4) and the findings of El-Jakee et al. (1995) revealed that *Pseudomonas aeruginosa* was 100% resistant to Penicillin G. Such phenomenon might be due to B-lactamase enzyme which is normally present in *Pseudomonas aeruginosa*. This conclusion substantiates what have been reported by Wilson and Miles (1983).

The aforementioned results either of the current study or those demonstrated in the available literatures showed marked variations in the behaviour of the tested *Pseudomonas aeruginosa* microorganisms. These variations are most probable due to biological alterations which took place as a result of the miss use of different antibiotics by both medical and veterinary practice.

All *Pseudomonas aeruginosa* strains isolated in our present work were able to produce protease which liquefy gelatin, while five isolates showed caseinase activity. All isolates produced lipase and lecithinase (Table, 3). These results substantiate those reported by Colwell (1964), Seddik et al. (1987) and Sunita and Srinivasan (1988). Six of our isolates produced haemolytic and DNAs activities; these results contradict those reported by Johnson et al. (1967).

It is evident from table (5) that intraperitoneal inoculation of 1mL untreated filtrate broth culture of each strain produced death of all inoculated experimental mice. This result goes hand in hand with those reported by Seddik et al. (1987). However, the smaller dose of the same filtrate had a mortality rate ranging from 40 to 50% of the inoculated mice. A contradictory finding was given by Seddik et al. (1987) who found that untreated crude products of *Pseudomonas aeruginosa* killed all the injected mice at a dose of 0.5mL.

The early deaths were observed 10 hours after inoculation and became more prominent after 20 hours. The lethality of these products mainly was due to a heat-labile lethal exotoxin, and therefore, the virulence of *Pseudomonas aeruginosa* appears to be correlated with its ability to produce an exotoxin. This conclusion was previously reported by many authors (Liu , 1973, Pavlovaskis and Shackefford, 1974; Iglwski and kabat , 1975; Berdal et al., 1982 and Seddik et al., 1987). On the other hand, the filtrate broth culture of isolates heated at 60 C° for 10 minutes were non toxic to mice either at a dose of 0.5 mL or 1mL.

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Table (1): Total *Pseudomonas* organisms count per gram of examined meat products.

Samples	No. of samples examined	* Positive samples		Count / g.		
		No.	%	Min	Max	Average
Fresh minced meat	45	32	71.11	$5.1x10^2$	$4.3x10^5$	$3.2x10^4$
Fresh sausage	30	13	43.33	$1.2x10^2$	$5x10^3$	$1.07x10^3$
Frozen hamburger	30	8	26.67	$2x10^2$	$8x10^3$	$2.2x10^3$

* Positive samples = Samples proved to be contaminated with *Pseudomonas* organisms.

Table (2): Occurrence of *Pseudomonas aeruginosa* in meat products.

Meat product samples	No. of samples examined	No. of Positive samples	
		No.	%
Fresh minced meat	45	5	11.11
Fresh sausage	30	2	6.67
Frozen hamburger	30	1	3.33
Total	105	8	7.62

Table 3: Bacteriological study of 8 Strains of *Pseudomonas aeruginosa organisms* isolated from meat* product.

No. of isolates	Cultural screening and biochemical tests													Extracellular enzymes					
8	Production of pigment "pyocyanin"	Growth at 42 C through 3 successive subcultures	Odour "fruity" grape-like	Growth on Mac Conkey agar medium*	Growth in citrate medium	Motility	Production of oxidase	Production of urease	Reduction of nitrate to nitrite	Methyle red test	Indol test	Voges-Proskauer test	Growth on sheep blood agar**	Production of DNAs	Production of protease "liquefication of gelatin"	Production of caseinase	Production of lipase	Production of Lecithinase	Production of haemolysin
Results of reactions	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -	+ -
	8 -	8 -	8 -	8 -	8 -	8 -	8 -	8 -	8 -	- 8	- 8	- 8	8 -	6 2	8 -	5 3	8 -	8 -	6 2

+ = Positive reaction - = Negative reaction

*** = *Pseudomonas aeruginosa* had large pale colonies of a non-lactose fermenter with green-blue pyocyanin pigment superimposed**

**** = Large, flat irregular-edged colonies were shown. The pigment was most obvious in areas of heavy growth.**

Table (4): *In vitro* antimicrobial drug sensitivity of *Pseudomonas aeruginosa*.

No. of Isolates	Content/disc	No. of sensitive isolates	% of Sensitivity	No. of resistant isolates	% of Resistance
8	Neomycin (30 mcg)	-	0.00	8	100
	Ampicillin (10 mcg)	-	0.00	8	100
	Polymyxin-B (300 U)	7	87.5	1	12.5
	Chloramphenicol (30 mcg)	1	12.5	7	87.5
	Gentamicin (10mcg)	8	100	-	0.00
	Nalidixic acid (30 mcg)	-	0.00	8	100
	Erythromycin (15 mcg)	-	0.00	8	100
	Penicillin-G (10IU)	-	0.00	8	100

Table (5): Toxigenicity of culture filtrate of *Pseudomonas aeruginosa* in white mice.

Origin of strains	No. of strains	No. of mice injected	Dose of injected filtrate		Dose of injected treated filtrate	
			0.5 mL	1 mL	0.5 mL	1 mL
Minced meat	5	40	* 4/10	10/10	0/10	0/10
Fresh sausage	2	16	2/4	4/4	0/4	0/4
Frozen hamburger	1	8	1/2	2/2	0/2	0/2

* N.B: (1) The numerator gives the number of dead mice and denominator represents the number of inoculated mice.

(2) The control group of 8 mice inoculated with sterile broth survived.