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INCIDENCE AND SIGNIFICANCE OF AEROMONAS HYDROPHILA GROUP IN MEAT AND SOME MEAT PRODUCTS IN ASSIUT

(With 4 tables)

By

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"أهمية ومدى تواجد مجموعة ميكروب *أيروموناس هيدروفيليا*
في اللحوم وبعض منتجاتها بأسسيوط"

سهام محمد على

تضمنت الدراسة فحص عدد ٧٥ عينة من قطع اللحم البقري الطازج والمبرد معمليا، اللحم المفري المجمد والسجق المجمد بواقع ٢٥ عينة من كل منهم وذلك بعد تجميعها من محلات الجزارة ومنافذ البيع المحلية المختلفة بأسسيوط بهدف التعرف على تواجد مجموعة ميكروب *أيروموناس هيدروفيليا* بواسطة طريقة الأغناء والاختصاص ثم الزرع باتباع وسيلة الانتشار السطحي. لقد تبين من الدراسة أن ٤٤٪، ٥٦٪، ٢٨٪ من قطع اللحم البقري الطازج الذى برد معمليا، اللحم المفري المجمد والسجق المجمد تحتوى على مجموعة ميكروب *أيروموناس هيدروفيليا*، بينما لم يعزل الميكروب من اللحم الطازج عند وقت الشراء وقت الصفر وقد تم عزل ٥٢ عترة ميزت إلى مستوى الرتبة كمالى: ٣٣ عترة *أيروموناس هيدروفيليا*، ١٢ *أيروموناس كافي*، ٧ *أيروموناس سوبريا*. ولقد تم دراسة الخواص المرضية والنشاط الانزيمى للمعزولات ونوقشت الأهمية الصحية والاقتصادية للميكروب.

SUMMARY

75 beef cuts (chilled in the lab.), Frozen minced meat and Frozen sausage (25 each) were collected from different butcher's shops and different localities of different sanitation levels at Assiut. These samples were examined for the presence of *Aeromonas hydrophila* group, using enrichment procedure and surface spread plate technique. The obtained results pointed out that 44%,

56% and 28% of the examined beefcuts meat "chilled in the lab.", frozen minced meat and frozen sausage respectively, while no *Aeromonas hydrophila* group was recovered from beef cuts meat at "zero time". 52 *Aeromonas hydrophila* strains isolated in this study were characterized according to species level as follows: 33 *Aeromonas hydrophila*, 12 *Aeromonas caviae* and 7 as *Aeromonas sobria*. All strains were examined for their ability to produce haemolysin as a virulence factor. Strains identified as *Aeromonas hydrophila* were the strongest producers of haemolysin. Concerning Gelatinase and DNase, the majority of the 52 strains had DNase and Gelatinase activities. The public health and economic significance of the obtained results were discussed.

Key words: Meat and meat products - Aeromonas hydrophila - Assiut.

INTRODUCTION

The genus *Aeromonas* has been classified with the family *Vibrionaceae* (Baumann and Schubert, 1984), but more recently Colwell et al. (1986), noting the low level of DNA relatedness of *Vibrio* and *Aeromonas*, have proposed a new family, the *Aeromonadaceae*. The genus *Aeromonas* consists of 10 named species (Koneman et al., 1994). The three most important motile species in human are *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* (Brooks et al., 1995). Many laboratories continue to group all motile aeromonads in the general category, *Aeromonas hydrophila* group or complex (Hickman - Brenner et al., 1987).

Aeromonas hydrophila group could be readily isolated in considerable numbers from meat and its products (Osuchowska et al., 1989, Fathi and Moustafa, 1991 and Sallam, 1993). Furthermore, *Aeromonas* is well established as a component of the spoilage microflora of raw meat (Dainty et al., 1983). Intestinal carriage can not explain the incidence of *Aeromonas* on meats, and water used in washing has been considered the most likely source (Stern et al., 1987).

In recent years *Aeromonas* group has received increasing attention as an agent of food-borne diarrhoeal disease in human. Foods including ground beef and sausage may play an important role in the etiology of human *Aeromonas hydrophila* gastroenteritis outbreaks. (Palumbo et al., 1985a).

Wadstrom and Ljungh (1991) found that *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* were recovered in 36 out of 50 patients (72%) where abdominal cramps were the predominant symptoms. In addition to gastroenteritis *Aeromonas hydrophila* group infects human causing four types of infections: Cellulitis and wound infection, acute diarrheal disease of short duration, septicemia and miscellaneous infections such as urinary tract infection, hepatobiliary, meningeal infection, endocarditis and ear infection (Koneman *et al.*, 1994).

On the other hand *Aeromonas hydrophila* produces a number of potential virulence factors, including, enterotoxins, cytotoxins, haemolysins and proteases (Trust and Chipman, 1979 and Ljungh and Wadstrom, 1983). Relatively little is known, however, about the occurrence, growth behaviour and significance of these organisms in foods.

The aim of this study was to determine the incidence and level of *Aeromonas* group in meat and some meat products (frozen minced meat and frozen sausage), and to investigate the ability of the isolated strains to produce enzymes such as DNase, gelatinase and haemolysin.

MATERIAL and METHODS

A total of 75 random samples of meat and meat products were obtained from different butcher's shops and groceries in Assiut city. The samples comprised 25 each of fresh beef cuts, frozen minced meat and frozen sausage. Each sample was 250 grams approximately and was transferred to the laboratory under aseptic conditions without undue delay where they were bacteriologically examined.

Preparation of samples:-

Fresh beef cuts were sampled on the day of purchase (Zero time) and after 7 days of storage at 5°C. While frozen samples (minced meat and sausage) were allowed to thaw in its original containers in a refrigerator at 5°C for 10 hours.

Enrichment procedure:-

20 grams of each sample were aseptically transferred to 180 ml of Trypticase Soy broth containing 10 ug Ampicillin/ml and blended for 2 minutes, then incubated at 28 °C for 24 hrs.

Isolation of *Aeromonas* spp.:-

After incubation of enrichment broth 0.1 ml of each was streaked on

the surface of MacConkey Mannitol Ampicillin medium and incubated at 28°C for 24 hrs. (Fathi and Moustafa, 1991). Loopfuls from suspected colonies which showing typical red pigment were picked up onto nutrient agar slants and incubated at 28°C for 24 hrs. for further identification.

Identification and characterization of *Aeromonas* Spp.

Presumptive aeromonads (typical red colonies) were identified to genus and species level after Gram staining (The cells were straight-sided, medium-sized nonpleomorphic Gram-negative bacilli), Oxidase reaction by using few drops of a 1% solution of N.N - dimethyl-*p* phenylenediamine monohydrochloride on the growth of the nutrient agar slants and observed for the evolution of a black discoloration characteristic of the colonies of *Aeromonas* species, their ability to ferment glucose in triple sugar iron agar (TSIA) and other tests according to the schedule of biochemical reaction provided by Koneman *et al.*, (1994). In addition, the identified strains were evaluated for the haemolytic activity of 5% sheep blood agar and gelatinase production on agar with 15% gelatin (Rogulska *et al.*, 1994). While DNase production was evaluated by streaking on DNase agar medium (Palumbo *et al.*, 1985a).

RESULTS

The Results are tabulated in Tables (1, 2, 3 and 4).

DISCUSSION

This study was conducted to investigate the incidence of *Aeromonas hydrophila* group in meat and some meat products. The incidence was found to be 42.67% (Table, 1). *Aeromonas hydrophila* microorganisms were detected in all types of samples examined except in beef cut samples at the time of purchase (zero time) but was observed after 7 days of refrigerated storage (5°C) in 11 (44%) samples.

This suggests that the organisms were initially present at levels below the limits of detection. Clinical isolates of *Aeromonas hydrophila* have been shown to be capable of significant growth at 4 °C (Palumbo *et al.*, 1985b), while strains isolated from foods have been shown to have even lower

minimum growth temperature of -0.1 to 1.2 °C (Walker and Stringer, 1987).

From the results achieved, one can easily conclude that *Aeromonas* species multiplied at 5°C which is significant risk in food storage, These findings substantiate those reported by Wadstrom and Ljungh (1991).

Our results simulate those reported by Sallam (1993) who could isolate *Aeromonas* species from 9 (45%) out of 20 cattle meat samples. Higher results were reported by Osuchowska *et al.*, (1989) and Ibrahim and MacRae (1991) who isolated *Aeromonas* species from beef meat samples at a rate of 60%. On the other hand, Goda *et al.*, (1981) reported lower incidence (1.57%).

The incidence of *Aeromonas hydrophila* species in frozen minced meat samples is 14 (56%). A higher incidence (100%) was recorded by Fathi and Moustafa (1991).

Concerning frozen sausage samples *Aeromonas hydrophila* group was isolated from 7 (28%) of the examined samples. Lower findings (23%) were reported by Hudson and Delacy (1991). Fathi and Moustafa (1991) reported a higher incidence (100%).

From the results achieved, one can easily conclude that *Aeromonas* group existed in low percentage (28%) in frozen sausage samples. This could be attributed to the bacteriostatic or germicidal effect of NaCl and some other formulation parameter (Palumbo *et al.*, 1985 b). In contrast to much of the literatures in which this organism is considered an undesirable of the microflora of food. Buttiaux (1959) has reported that *Aeromonas* plays an important role in the biological processes involved in the manufacture, of a French raw sausage.

These variations in results may be attributed either to the variation in media and the enrichment method used for isolation of *Aeromonas hydrophila*, differences in quality and sanitation level of meat and / or time and temperature of storage.

Identification of isolated *Aeromonas hydrophila* isolates:

Aeromonas hydrophila microorganisms amounting 52 strains were isolated from 75 samples of meat and its products. All of the 52 strains were belonging to the motile *Aeromonas* species (table, 2). These strains included 21 (40.38%) from samples of cattle meat cuts, 18 (34.62%) from frozen minced meat and 13 (25%) from frozen sausage samples. Thirty- three strains (63.46%) were identified as *Aeromonas hydrophila*, 12 (23.08%) as *Aeromonas caviae* and only 7 (13.46%) as *Aeromonas sobria*.

From the results achieved, one can easily conclude that the isolation of *Aeromonas* species from all kind of samples examined establishes these organisms as omnipresent in meat and its products and this confirms the findings of Okrend *et al.*, (1987), Fathi and Moustafa (1991) and Sallam (1993).

Although *Aeromonas* is a cosmopolitan genus, we found in this study that the species distribution was not homogeneous but depended rather on the type of meat samples. *Aeromonas hydrophila* was the predominated followed by *Aeromonas caviae* and to less extent *Aeromonas sobria*. These results substantiate what have been reported by Okrend *et al.*, (1987), Osuchowska *et al.*, (1989) and Fathi and Moustafa (1991). A contradictory findings were given by Nishikawa and Kishi (1988) who found that *Aeromonas hydrophila* and *Aeromonas sobria* were the most common species isolated from meat products, whereas *Aeromonas caviae* was common in sea- foods, and vegetables.

The allocation of *Aeromonas* strains to species based on the method of Koneman *et al.*, (1994) showed that all the 52 strains isolated gave the typical biochemical reactions (table, 3). A finding that simulate those reported by Okrend *et al.*, (1987) and Fathi and Moustafa (1991). While lower figures were reported by Majeed *et al.*, (1989) who found that 77% of *Aeromonas* strains had given the same reactions as the typical cultures, whilst the remainder (23%) showed different biochemical reactions and designated as "atypical" of the particular species of *Aeromonas*.

Since *Aeromonas hydrophila* group is potential opportunistic agents of gastroenteritis, bacteraemias and other diseases in man and animals, the distribution of virulence-related characters was studied among the isolated strains.

All of the 52 *Aeromonas* strains were tested for their ability to produce haemolysin. (75.76%) of 33 *Aeromonas hydrophila* strains and 4 (57.14%) of the 7 *Aeromonas sobria* strains lysed the sheep erythrocytes with a variable halo diameter between 0.5 and 2 mm, but only 2 (16.67%) of the 12 *Aeromonas caviae* strains did so. The aforementioned results showed that the distribution of this trait "haemolytic activity" among species was distinctive and the higher concentration of haemolysins among *Aeromonas hydrophila* and of *Aeromonas sobria* in the isolated strains agrees with the results obtained by other authors (Majeed *et al.*, 1989; Krovacek *et al.*, 1991 and Krovacek *et al.*, 1992). Those authors consider that *Aeromonas*

hydrophila and *Aeromonas sobria* are more virulent than *Aeromonas caviae*. Burke *et al.*, (1981) reported that the haemolytic activity is strongly associated with enterotoxin production in members of *Aeromonas* genus. However, Rogulska *et al.*, (1994) reported that the haemolytic activity of *Aeromonas hydrophila* and *Aeromonas sobria* act as marker of pathogenicity.

Concerning Gelatinase and DNase production, the majority of the 52 *Aeromonas* strains had DNase and Gelatinase activities that were not peculiar to their species (Table 4).

The specific public health significance of these findings is unknown since clear well documented data about the role of *Aeromonas* species as food-borne pathogens is lacking. The presence of significant levels of virulent *Aeromonas* species -“*Aeromonas* organisms which had haemolytic and proteolytic activity in this study”- in meat, frozen sausage and minced meat, however, indicates that these foods may play a significant role in the epidemiology of *Aeromonas* - associated gastroenteritis.

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Table 1

Incidence and *Aeromonas* species count / g. in examined samples

Types of samples	No. of samples examined	Positive samples
Beef cuts *	25	11(44%)
Frozen minced meat	25	14(56%)
Frozen sausage	25	7(28%)
Total	75	32(42.67%)

* *Aeromonas hydrophila* species were not detected in beef meat cut sampled at (zero time).

Table 2

Distribution of motile aeromonads in samples examined

Type of samples	No. of strains isolated	<i>Aeromonas hydrophila</i> group		
		<i>Aeromonas hydrophila</i>	<i>Aeromonas caviae</i>	<i>Aeromonas sobria</i>
Beef cuts	21(40.38%)	16	3	2
Frozen minced meat	18(34.62%)	12	4	2
Frozen sausage	13(25%)	5	5	3
Total	52	33	12	7

Table (3): Differentiation of *Aeromonas* species isolated from samples examined according to the caa of Koneman et al., (1994)

Organism	No. of strains	Voges - Proskauer	Oxidase	Motility	Indole	Growth in peptone, 1% with 0% NaCl	Growth in peptone, 1% with 7% NaCl	Gas from glucose hydrolysis	Fermentation		
									L. Arabinose	Sucrose	Mannitol
<i>A. hydrophila</i>	33	+	+	+	+	+	-	-	+	+	+
<i>A. Caviae</i>	12	+	+	+	+	+	-	-	+	+	+
<i>A. Sobria</i>	7	-	+	+	+	+	-	-	V	+	+

List of abbreviations.

+, 90% or more of strains positive

V, 11% - 89% of strains positive

-, 90% or more of strains negative

Table 4
Production of haemolysin, DNase and gelatinase enzymes by *Aeromonas* species
Isolated from examined samples.

Species	No. of strains examined	Haemolysin positive strains	DNase positive strains	Gelatinase positive strains
<i>Aeromonas hydrophila</i>	33	25 (75.76%)	31(93.94)	28(84.85%)
<i>Aeromonas sobria</i>	7	4(57.14%)	6(85.71)	5(71.43%)
<i>Aeromonas caviae</i>	12	2(16.67%)	10(83.33)	8(66.67%)

1. The first part of the paper is devoted to a general discussion of the problem. It is shown that the problem is well-posed in the sense of Hadamard.

2. In the second part, the author considers the case of a linear operator. It is shown that the problem is solvable in the sense of Hadamard.

3. In the third part, the author considers the case of a nonlinear operator. It is shown that the problem is solvable in the sense of Hadamard.

4. In the fourth part, the author considers the case of a nonlinear operator. It is shown that the problem is solvable in the sense of Hadamard.

5. In the fifth part, the author considers the case of a nonlinear operator. It is shown that the problem is solvable in the sense of Hadamard.

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