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PREVALENCE OF MOTILE AEROMONADS IN CHICKEN MEAT AND THEIR GROWTH DURING REFRIGERATION STORAGE

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SUMMARY

The presence of motile aeromonads in chicken meat from the local wet markets was studied. The finding showed that 83.3% of the meat was positive for motile *Aeromonas*. The most frequently isolated species was *Aeromonas sobria* (57.0%), followed by *A. hydrophila* (23.0%) and *A. caviae* (20.0%). The *Aeromonas* sp. count at time of purchase which was in the range of 13 x 10^2 /g to 23 x 10^3/g, was found to increase about 10 to 100- fold after seven days of refrigeration at 5°C.

Keywords: motile *Aeromonas*, chicken meat, refrigeration growth

INTRODUCTION

Aeromonas species are associated with marine and fresh water environments, including streams, lakes, natural mineral springs, polluted rivers and estuaries, untreated and chlorinated drinking water and even bottled water (Barnhart and Pancorba, 1992; Abeyta and Wekell, 1988). The organisms have been isolated from aquatic animals, including fish, shellfish, crustaceans and amphibians and are pathogenic to many of these aquatic species (Abeyta and Wekell, 1988). Under the classification based on the classic biochemical tests and DNA hybridisation tests, 15 species of *Aeromonas* are recognised. Among these species, *Aeromonas hydrophila, A. sobria* and *A. caviae*, are mesophilic and motile, thus are known as motile aeromonads (Kirov, 1993) and categorised as *A. hydrophila* group; another group, the *A. salmonicida* group, are psychrophilic and are known as nonmotile aeromonads.

Motile aeromonads were considered as infrequent human pathogen (Agger *et al.*, 1985); however, recent studies have associated them with three types of human illness – extraintestinal infection (hepatobiliary infection, septicaemia), wound infection and cellulites, and gastroenteritis (Abeyta and Wekell, 1988; Agger *et al.*, 1985). In the last decade, these motile aeromonads have received considerable recognition as a human enteric pathogen.

Aeromonas sobria appears to be most commonly associated with dairrhoea, followed by *A. hydrophila* as enterotoxigenic whereas *A. caviae* does not produce enterotoxin and commonly found in asymptomatic patients (Kirov *et al.*, 1990). A variety of foods were shown to harbour *Aeromonas* sp., including vegetables, fresh beef, chicken, pork, lamb, raw milk and beef products (Hudson and De Lacy, 1991). It has also been reported that motile *Aeromonas* are pychrotrophic, capable of growth at 5°C in foods (Palumbo *et al.*, 1985; Palumbo and Buchanan, 1988). Questions are then posed as to whether these aeromonads are capable of causing gastroenteritis after consumption of contaminated foods that have been refrigerated for a period of time. At the same time, the study by Okrend *et al.* (1987) and Fricker and Tompsett (1989) found many of the *Aeromonas* isolates were cytotoxin and haemolysin producers; as such these foods are a potential source of virulent aeromonads for man. The involvement of these organisms in food borne gastroenteritis is unclear as to this date; however, there are few cases reported in which *Aeromonas* has been strongly implicated as the causative agent.

The studies on motile *Aeromonas* in Malaysia mainly focused on fish and in waters, lacking on their occurrence in foods such as meat. The present study was undertaken to determine the occurrence of motile aeromonads in chicken meat, to determine the species of the *Aeromonas* isolated and to enumerate *Aeromonas* sp. on freshly purchased meat after seven days of refrigeration at 5°C .

MATERIALS AND METHODS

Collection of samples

Chicken parts, namely breasts, thighs and wings, were purchased from 12 vendors at wet markets situated in four different localities – Sri Serdang, Seri Kembangan, Kajang and Petaling Jaya. The chicken parts were placed in plastic bags provided by the vendors and were then brought to the Veterinary Public Health laboratory in Faculty of Veterinary Medicine, Serdang as soon as possible, within 2 – 4 hours.

Isolation and enumeration methods

Twenty gram (20 g) of sample from each chicken parts was aseptically transferred to a sterile plastic bag. A hundred and eighty (180) ml of sterile 0.1% peptone water was added and the sample was homogenised using Stomacher 400 for two minutes. Several dilutions of each homogenate were prepared using 0.1% peptone water. The diluents (0.1 ml) were surface plated on *Aeromonas* agar plates (Oxoid). The plates were incubated at 37°C for 24 h.

After removing aseptically 20g of meat from the chicken part for isolation and enumeration of *Aeromonas* on the day of purchase, (designated as Day 0), the remaining portions were kept cold in a refrigerator at 5°C for seven days. On Day 7, 20 g of the meat was removed aseptically from each chicken part, and the same procedure as described above was carried out.

Identification Method

Colonies characteristic of *Aeromonas* sp. have a typical flat dark green centre surrounded by a yellow green zone (Oxoid). At least two suspected colonies from each plate were purified by plating on the same medium and incubated at 37°C for 24 h. Presumptive *Aeromonas* isolates were confirmed using the following tests: Gram stain, oxidase, catalase, motility (after 4 – 7 h at 30°C in alkaline peptone water) and sensitivity to vibriostatic agent 0/129 (Oxoid). These colonies were then counted.

The presumptive *Aeromonas* isolates were classified into species using API 20E kit system (Analytab Products) and Popoff (1984) criteria (Table 1).

RESULTS AND DISCUSSION

A total of 36 chicken parts were examined during the study and 30 (83.3%) of the chicken parts were positive for *Aeromonas* sp. Only one colony was selected for each sample and when subjected to the API 20E system and Popoff criteria, the 30 isolates (100%) were motile aeromonads - seven (23.0%) were *A. hydrophila,* 17 (57.0%) *A. sobria* and six (20.0%) *A. caviae.*

A number of studies have reported that aeromonads were readily isolated from poultry meat and meat products (Palumbo *et al.*, 1985; Kirov *et al.*, 1991; Hudson and De Lacy, 1991). Fricker and Tompsett (1989) and Barnhart *et al.* (1989) found that 79.3% and 98% of poultry meat were positive for *Aeromonas,* respectively. Okrend *et al.* (1987) found that 100% of chicken meat were positive with levels of *Aeromonas* ranging from 4.44 x 10 to > 4.44 x 10^3 /g. In another study, Akan *et al.* (1998) isolated motile aeromonads in 90.5% of the chicken carcasses. The finding of this study was in agreement with these studies which found a large percentage of raw chicken meat frequently contaminated with high numbers of *Aeromonas* sp.

According to Barnhart *et al.* (1989) the isolates recovered from the carcasses may likely be of intestinal origin and that the defeathering and evisceration procedures were the probable causes of contamination. Chickens were found to shed the organisms in the faeces. Akan *et al.* (1998) isolated *Aeromonas* in 14.8% of faecal samples from broiler chickens. Stern *et al.* (1987) found similar low prevalence (14.3%) of *Aeromonas* in the faeces of turkey. Hence, during poultry processing, the faeces may contaminate the carcasses through common water rinses and as a result, a few contaminated carcasses further contaminate a large number of uncontaminated carcasses (Stern *et al.*, 1987; Akan *et al.*, 1998). Barnhart *et al.* (1989) detected *A. hydrophila* in 92% of chilled water samples. They also found that the number of *A. hydrophila* increased rapidly in the chilled water and remained constant during the 8 h processing shift. Thus, contaminated water has been suggested to contribute to the high prevalence of contamination with *A. hydrophila* in chicken carcasses (Fricker and Tompsett,1989; Akan *et al.*,1998).

In this study, it was found that *A. sobria* was the most frequently isolated motile aeromonads compared to *A. hydrophila* and *A. caviae.* A similar finding was reported by Kirov *et al.* (1990) in which 63.6% and 36.4% of motile aeromonads isolated were *A. sobria* and *A. hydrophila,* respectively while *A. caviae* was not isolated. However, the studies by Okrend et al (1987) and Akan *et al.* (1998) found otherwise – in their former study, *A. hydrophila* was isolated in all (100%) while *A. sobria* and *A. caviae* in 60% of chicken samples; in the latter study, 66.9% were *A. hydrophila,* followed by *A. caviae* (21.3%) and *A. sobria* (11.6%). Although only one colony was selected for speciation in this study, it should be known that cross-contamination is most likely to occur and hence chicken carcasses can be contaminated by more than one *Aeromonas* sp. (Kirov *et al.*, 1990).

The level of *Aeromonas* sp. detected on the chicken parts on the day of purchase or Day 0 ranged from 13 x 10^2 /g to 23 x 10^3/g. A significant increase in the number of *Aeromonas* sp. in all samples was observed after the samples were refrigerated at 5°C for 7 days. In general, the increase in the levels of *Aeromomas* sp. from Day 0 to Day 7 of cold storage was 10- to 100- fold , with counts ranging from 40 x 10^3 to 32 x 10^4 /g.

Table 1: **Biochemical tests to differentiate the three motile aeromonads sepecies**

Tests / *Aeromonas* Species	KCN	Salicin	V-P	Glucose/gas	Esculin
A. hydrophila	+[a]	+	+	+	+
A. caviae	+	+	-	-	+
A. sobria	-[b]	+	d[c]	+	-

+[a] – positive reaction for the indicated test

-[b] – negative reaction for the indicated test

d[c] – differential or inconclusive reaction for the indicated test

Source: Popoff (1984)

The refrigeration storage for seven days increased the levels of *Aeromonas* sp. in chickens. This finding was similar to other studies, including those of Palumbo *et al.* (1985) and Barnhart *et al.* (1989) – they found *Aeromonas* sp. grow readily in meat and other foods held for 7-10 days at 5°C and showed a 10 – 1000-fold increase. Hence, the refrigeration depended upon to safeguard against bacterial foodborne hazards, may not be applicable to *Aeromonas*. In their study, Barnhart *et al.* (1989) found that in iced (frozen) storage, all the broiler chicken carcasses sampled were found positive for *A. hydrophila* with mean levels at 460 ± 600 cfu/ml after 48 h storage compared to at post-chill stage which was 28 ± 28 cfu/ml. Barnhart *et al.* (1989) and Palumbo *et al.* (1985) suggested that due to the psychrotrophic nature of *A. hydrophila,* they apparently were able to survive and perhaps multiply even under such conditions.

All the three main species of motile *Aeromonas–A. hydrophila, A. sobria* and *A. caviae*–are considered human pathogens and *A. hydrophila* is ubiquitously associated with foods of animal origin (Palumbo *et al.*, 1985). Thus, chicken meat and other types of meat may play an important role as the vehicle of transmission of motile aeromonads from animals to man. Apart from ingestion of contaminated foods, one other possible food borne transmission may be due to ingestion of pre-formed exotoxins in food. According to Kirov *et al.* (1990), enterotoxin was not produced by aeromonads at temperatures below 15°C and enterotoxin is readily inactivated by heating to 60°C. *Aeromonas* sp. in foods (contamination caused by contaminated water, animal faeces and by food handlers) is readily killed by heat (Kirov *et al.*, 1990). Thus, properly stored and cooked chicken meat is unlikely to be a health risk.

In conclusion, three motile aeromonad species were isolated from chicken parts sold at wet markets in the present study. They are found to proliferate upon refrigeration storage. The sources of these organisms in chicken meat need to be determined – it may be of intestinal origin or from the environment, such as contaminated water, equipment, processing premise and retail condition. Thus, further work is needed to determine the sources of these aeromonads in meat and their significance.

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RINGKASAN

Kehadiran aeromonad yang motil pada daging ayam yang dijual di pasar telah dikaji. Hasil kajian menunjukkan bahawa 83.3% daging tersebut didapati positif. Spesis *Aeromonas* yang paling banyak dipencil adalah *Aeromonas sobria* (57.0%), diikuti dengan *A. hydrophila* (23.0%) dan *A. caviae* (20.0%). Kiraan *Aeromonas* sp. pada daging ayam semasa pembelian berkisar antara 13 x 10^2 /g to 23 x 10^3/g dan kiraan ini meningkat kepada 10- hingga 100- kali setelah disimpan dingin selama tujuh hari pada suhu 5°C.