Animal Health Research Institute
Assiut Regional Laboratory
Lab. Director Prof. Dr. S. M. Nashed

STUDIES ON CAMPYLOBACTER ORGANISMS AS FOOD-POISONING ORGANISMS IN FRESH WATER AND SALTED FISHES " MOLOHA " IN ASSIUT GOVERNORATE

(With 3 Tables)

By
AMAL A. MOHAMMED and A.F. BASTAWROWS

(Received at 21/12/1998)

دراسات على ميكروبات الكامبيلوباكتر كمسببات للتسمم الغذائى فى الأسماك الطازجة والمملحة "الملوحة" فى محافظة أسيوط

آمال أحمد محمد ، ألفونس فخرى بسطاوروس

أجريت هذه الدراسة على ١٤٠ سمكة من أسماك الماء العذب والاسماك المملحة " الملوحة " بواقع (١١٥ من أسماك البلطى + ٢٥ من الأسماك المملحة والمصنفة تحت أسم كلب البحر) جمعت عشوائيا من أسواق السمك ومحلات بيع الملوحة المتعددة بأسيوط وذلك للفحص البكتريولوجى لتحديد مدى تواجد ميكروبات الكامبيلوباكتر باستخدام المستنبتات الخاصة والجو اللازم لعزل ميكروبات الكامبيلوباكتر وقد تم تقدير كمية ملح الطعام وتقدير الأيون الأيدروجينى للاسماك المملحة " الملوحة " وأسفرت النتائج عن عزل عدد ٤ عترة من الكامبيلوباكتر جيوجينای (٣ من نوع ١ , ١ من نوع ٢) وعدد ٣ عترة من الكامبيلوباكتر كولای من أسماك البلطى التى تم فحصها بينما لم تعزل ميكروبات الكامبيلوباكتر من الأسماك المملحة " الملوحة ". وقد تم اجراء اختبار الحساسية لكل من الكامبيلوباكتر جيوجينای والكامبيلوبكتر كولای وقد وجد مدى الاستجابة الشديدة بفاعلية ١٠٠ % لكل من الايرثيرومايسين وحامض النالديكسيك وقد نوقشت الاهمية الصحية لهذه الميكروبات وكذلك الاشتراطات الصحية الواجب توافرها لدرء خطر هذه الميكروبات على صحة المستهلك.

SUMMARY

A total of 140 random samples of fresh water and salted fishes including 115 *Tilapia nilotica* and 25 *Hydrocynous forskallii* salted fishes were collected from fish markets and from different Moloha's shops in Assiut

City. Bacteriological examination was carried out using both enrichment and selective media together with microaerophilic atmosphere for isolation of different biotypes of Campylobacter microorganisms. A total of 4 isolates of *Campylobacter jejuni* (3 of biotype 1 and 1 of biotype 2) and 3 isolates of *Campylobacter coli* were recovered from the examined *Tilapia nilotica* fishes. The salt contents and pH values of salted fishes were determined, while no Campylobacter organisms were recovered from salted fishs. The isolated organisms were tested for their sensitivity to 12 chemotherapeutic agents. Campylobacter jejuni / coli proved to be highly sensitive to Erythromycin and the Nalidixic acid with an activity of 100 %. The public health significance of these microorganisms, as well as recommended sanitary measures were also discussed.

Key Words: *Campylobacter-Fresh water-Fishes-Salted fishes (Moloha).*

INTRODUCTION

The genus *Campylobacter* has been classified with the family *Campylobacteraceae* (Nachamkin, 1995). The *Campylobacter genus* contains three homolgy groups each of which represents a species (Varnam and Evans, 1991). The genus *Campylobacter* includes 20 species (Nachamkin, 1995), but the most frequently identified human pathogen in the genus is *Campylobacter jejuni*. A second species, *Campylobacter coli* is a much less common cause of human disease (N. A. C. M. C. F., 1994).

Campylobacter enteritis is caused by the two closely related species, *Campylobacter jejuni* and *Campylobacter coli* (Skirrow, 1991). The pathogenic mechanisms by which campylobacter cause diarrhea in humans seem to be adhesion of the mucous membrane, toxin production and / or invasion of the epithelial cells, since the clinical effect varies between watery diarrhea and bloody diarrhea (Lindbiom and Kaijser, 1995).

Raw meats, including fish and shellfish, have been implicated as a source of campylobacter enteritis. The infectious dose can be as low as a few hundred bacteria (Skirrow, 1990 and Fang et al 1991).

Raw fish has been identified as carrying an increased risk of campylobacter enteritis in case-control study (Skirrow, 1998). Unlike

salmonella, campylobacters do not multiply in food, so they seldom cause explosive outbreaks of food poisoning (Varnam and Evans, 1991).

"Moloha" is a quite Egyptian popular salted fish which are consumed during certain occasions of the year. The uneviscerated fresh water fish "*Hydrocynous forskallii*" is usually salted by spreading the salt among the fish layers, upon the bottom of the tin, as well as on the surface of the upper fish layer before the lid was tightly closed. The tins were stored at room temperature for at least one month before use (Abdel-Rahman et al., 1988).

The purpose of our study was to determine if fresh water fishes *(Tilapia nilotica* and *Hydrocynous forskallii)* are indeed as a source of *Campylobacter jejuni* and *Campylobacter coli* and the response of the campylobacter isolates to various chemotherapeutic agents.

MATERIAL and METHODS

A total of 140 random samples of fresh water and salted fishes were used for this study. The samples included fresh water fish " *Tilapia nilotica* " (115) and salted fishes "Moloha" (25). The samples were collected from different fish markets and Molohas'shops of different sanitation levels at Assiut City. Each sample was obtained in sterile polyethylene bag and the collected samples were transferred directly to the laboratory in sampling cases with crushed ice (Temp. $\leq 4.4°c$). In the laboratory flesh including skin, liver, kidneys were taken under sterile conditions from every fresh water fish, but only muscle samples were taken from salted fish after disinfection by flaming.

1- Determination of pH in Moloha samples:

The pH of the Moloha water mixture was measured by using Ionalyzer digital, 701 A, Orion research Inc. U. S. A. Each pH measurement was read and recorded to the nearest 0.01 pH units. (Anon, 1977).

2- Determination of sodium chloride % in Moloha samples:

Sodium chloride percentage was carried out as described by A. O. A. C., (1980)

3- Isolation and identification of Campylobacter species:

A 20 grams quantity of each sample were added to 50 ml of Preston campylobacter enrichment broth comprised brucella broth plus 5% lysed horse blood, preston campylobacter selective supplement and campylobacter growth supplement. The mixture was shaken and inoculated broth was incubated at 42°c for 48 h in a gas-pak jar containing a gas generating kit (Oxoid, BR56) for campylobacters, which produce approximately 85 % Nitrogen, 10 % Co_2 and 5 % O_2 (Bolton and Robertson, 1982). A loopful was taken from each tube and placed onto a clean dry slide, covered and examined by dark field microscopy for motile bacteria (Skirrow, 1977). Preston broth containing motile bacteria having the characteristic cork-screw motility of campylobacter were subcultured onto Preston agar (brucella agar base supplemented with Preston's selective agents). The plates were incubated for 48 h at 42°c under appropriate microaerophilic conditions. The plates were examined for growth and characteristics of Campylobacter colonies (smooth, convex, slightly raised, translucent, non haemolytic, pin-point, 2 to 4 mm in diameter and colourless to cream coloured colonies). Further identification of these colonies was carried out following the techniques of Barret et al, (1988).

4- 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) and Sicinschi (1996) using the following: Nitrofurantion (300 µg/disc), Ampicillin (10 µg / disc), Polymyxin-B (300 U /disc), Chloramphenicol (30 µg /disc), Trimethoprim-Sulphamethoxazol (1.25 + 23.75 µg / disc), Erythromycin (15 µg / disc), Nalidixic acid (30 µg / disc), Gentamycin (10 µg / disc), Tetracyclin (30 µg / disc), Cephalothin (10 µg / disc), Streptomycin (10 µg / disc) and Penicillin G (10 IU / disc).

RESULTS

The results are recorded in tables 1-3

DISCUSSION

Since Campylobacter organisms were recognized as major human pathogens more than two decades ago, there have been significant improvements in bacterial isolation, taxonomy, pathogenesis and epidemiology (Penner, 1988).

A wide range of media is available for the recovery of *Campylobacter jejuni* and *Campylobacter coli* from foods. In this study, Preston's medium is the medium of choice because it does not contain Cephalothin and is therefore suitable for the isolation of strains sensitive to that antibiotic, is highly sensitive and selective (Varnam and Evans, 1991).

As shown in table (2) out of 115 examined *Tilapia nilotica* fish, 7 (6.08 %) revealed Campylobacter organisms. Moreover 4 out of the 7 isolates were identified as *Campylobacter jejuni* and 3 as *Campylobacter coli*. Furthermore, 3 out of the four isolates of *Campylobacter jejuni* were identified as being *Campylobacter jejuni* biotype 1 and the remaining one was belonging to *Campylobacter jejuni* biotype 2 (table 2). The low isolation rate of Campylobacter species in this study could be attributed to the low contamination level, method of isolation and presence of competitive bacteria which reduce the survival of Campylobacter and this may be attributed to a reduction of the pH value and the production of organic acid and other metabolites toxic to Campylobacter organisms.

It was hard to discuss the aforementioned incidence as scanty informations about such subject were available, but generally these findings agree, to a certain extent, with those reported abroad by Skirrow, (1990) Fang et al, (1991) and Skirrow, (1998) who reported that fishes have been implicated as a source of campylobacter enteritis in humans.

It is worth mentioning that the presence of Campylobacter organisms in fresh water fishes in the present study is not surprising since the natural habitat of these organisms is untreated water of lakes, rivers and streams polluted by faecal material shed from wild and domesticated animals (Varnam and Evans 1991 and Adak et al, 1995), from which the organisms might have gained enterence into fishes, other possible source of contamination, fish monger and fish wife who catch and sell the fishes by hand contact , who may lack good personal hygiene and sanitary

practices are very likely sources of the organisms, and these individuals may contaminate fishes that are hand held during sorting and selling.

Fish may become actively infected with many pathogens of epidemiologically importance when caught in contaminated water of rivers and lakes, constituting a potential health hazard to both handlers and consumers (Janssen and Meyers, 1968).

There are three ways in which campylobacter infection can be acquired from raw meats including fish 1) Campylobacter organisms may be transferred unwittingly from fingers to mouth when handling the raw product in the kitchen, 2). The product may be consumed raw or undercooked. Conventional cooking readily kills campylobacters, but fondue and barbecue cooking may not.

3) Bacteria may be transferred from raw meats to " innocent " ready-to eat foods on fingers and utensils (Skirrow, 1998).

From the data achieved in table (1) it is obvious that the mean sodium chloride content of the examined salted fish samples was 14.45 % ± 0.06, while the mean pH value was 5.80 ± 0.04.

The incidence of Campylobacter species in salted fish (Moloha) samples was zero (Table 2). This can be attributed to the bacteriostatic or germicidal effect of NaCl (Fraizer and Westhoff, 1986).

The relation between the incidence of Campylobacters and sodium chloride concentration was studied by some investigators. Hanninen (1981) found that sodium chloride concentration over 1.5 % inhibited growth or had bactericidal effect on the growth of *Campylobacter jejuni / coli* strains. Also, results obtained by Skirrow and Benjamin (1980) showed that most of *Campylobacter jejuni / coli* strains could not grow on 1.5 % sodium chloride agar medium at 37°c. Furthermore, Doyle and Roman (1982) reported that at room temperature and in the presence of 4.5 % NaCl, a comparable (10^6 to 10^7 / ml) population of *Campylobacter jejuni* may survive for 3 to 5 days only.

The effect of pH on the survival of *Campylobacter jejuni/ coli* will undoubtedly depend upon the characteristics of individual food (Christopher et al, 1982).

In vitro antibiotic sensitivity testing indicated that Erythromycin and Nalidixic acid were the most effective antibiotics, while Penicillin G, Cephalothin and Polymyxin-B were not effective (table 3). Therefore, selected antibiotic to be used in treatment of campylobacteriosis should rbe based on sensitivity test (D'Amato and Hochstein, 1982). These

findings agree to a certain extent with those reported by Stephens et al., (1984); Varnam and Evans (1991); Quinn et al. (1994) and El-Gohary (1998).

Information derived from table (3) revealed that no much difference was noticed between the behaviour of the Campylobacter species as regard to their susceptiblity to various drugs. These results substantiate those reported by Henin and Kaldas (1996).

Several noteworthy observations have been made from this study they includes (i) *Tilapia nilotica* fishes can become carrier of campylobacters and therefore under certain circumstances can be of epidemiological significance for campylobacter enteritis in man. (ii) Fish do not normally suffer from campylobacter infection. However if they are harvested in polluted waters they may mechanically carry these organisms. (iii) It is rare for species of bacteria including campylobacter which cause diseases in man and animals to be pathogenic for fish. (iv) This and an earlier study (Skirrow, 1990) support the theory that fish is one of the important sources of human symptomatic infection.

In conclusion, the information given by the achieved results revealed that campylobacters existed in the examined fish samples and therefore there is a risk associated with consuming under cooked or improperly handled fishes. The low infectious dose of campylobacters and their prevalence in fishes indicate that great care must be taken to avoid cross-contamination of ready-to-eat foods. This risk can be avoided by consuming thoroughly cooked fishes, good food handling practices at home may reduce the risk of illness. In addition, wash and sanitize hands, cutting boards, utensils and containers before and after contact with raw fishes and other raw foods of animal origin to prevent cross-contamination to ready-to-eat foods.

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Table 1: Statistical analysis of pH and sodium chloride percentage in the examined samples of salted fishes "Moloha"

No. of examined samples		pH	Sodium chloride % (Na Cl %)
25	Minimum	5.12	9.81
	Maximum	6.59	18.69
	Mean	5.80	14.45
	S. E.	± 0.04	±0.06

Table 2: Frequency of isolation of Campylobacters from the examined fish samples

Type of fish samples	No. of examineds amples	Positive samples		Isolated biotypes			
				Campylobacter jejuni		total	*Campylobacter coli*
		No.	%	*C. jejuni biotype i*	*C.jejuni biotype 2*		
Tilapia nilotica	115	7	6.08	3	1	4	3
Hydrocynous forskallii " Salted Fish "	25	0	0.00	0	0	0	0

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Tilapia nilotica	115	7	6.08	3	1	4	3
Hydrocynous forskallii " Salted Fish "	25	0	0.00	0	0	0	0

Table 3: Antimicrobial sensitivity test of Campylobacter species isolated from fish

Chemotherapeutic agents and disc potency	*Campylobacter jejuni* (4)		*Campylobacter coli* (3)	
	Sensitive	Resistant	sensitive	Resistant
Nitrofurantion (300 µg)	1 (25%)	3 (75 %)	0 (0.00 %)	3 (100 %)
Ampicillin (10 µg)	1 (25%)	3 (75 %)	1 (33.33 %)	2 (66.67 %)
Polymyxin - B (300 U)	0 (0.00 %)	4 (100 %)	0 (0.00 %)	3 (100 %)
Chloramphenicol (30 µg)	2 (50 %)	2 (50 %)	2 (66.67 %)	1 (33.33 %)
Trimethoprim sulfamethoxazol (1.25 + 23.75 µg)	1 (25 %)	3 (75 %)	1 (33.33 %)	2 (66.67 %)
Erythromycin (15 µg)	4 (100 %)	0 (0.00 %)	3 (100 %)	0 (0.00 %)
Nalidxic acid (30 µg)	4 (100 %)	0 (0.00 %)	3 (100 %)	0 (0.00 %)
Gentaamycin (10 µg)	3 (75 %)	1 (25 %)	2 (66.67 %)	1 (33.33 %)
Tetracyclin (30 µg)	3 (75 %)	1 (25 %)	2 (66.67 %)	1 (33.33 %)
Cephalothin (10 µg)	0 (0.00 %)	4 (100 %)	0 (0.00 %)	3 (100 %)
Streptomycin (10 µg)	2 (50 %)	2 (50 %)	1 (33.33 %)	2 (66.67)
Penicillin G (10 IU)	0 (0.00 %)	4 (100 %)	0 (0.00 %)	3 (100 %)