/et.Med.J.,Giza. Vol.45, No.3. (1997): 337-343.

IICROBIOLOGICAL STATUS AND THE DEPURATION OF THE :GYPTIAN OYSTERS

IODA A. AWAD and EL-SHATER, M.A.

Animal Health Researh Institute, Agric. Genter, Dokki, Egypt.

;UMMARY

The microbiological quality of fresh Egyptian oysters collected from different markets in smailia and Cairo governorates were determined. The mean values of aerobic plate counts incubated at 35°C was 1.8 x10^7 CFU/g. The mean of the most probable numbers of coliforms and fecal coliforms was 235/g. The mean *S. aureus* count was 2.1 x 10^4/g. *E. coli* was found in 18 of 20 samples, while *S.aureus, Salmonella* and *Vibrio parahaemolyticus* were found in 14,1 and 3 of 20 samples, respectively.

The role of depuration processes on the bacterial load of oysters was studied. *E. coli* was undetected after 24 hr, while aerobic count reached the acceptable level after 48 hr. Coliforms, fecal coliforms, *S. aureus* were completely eliminated after 72hr., while *Vibrio parahaemolyticus* was still present even after three days of depuration.

INTRODUCTION

In Mediterranean countries, oysters are considered the most favourable sea food, due to it characteristic taste and nutritive value. Oysters ar harvested in esturine water and may becom contaminated with sewage, derived pathogens, a well as human pathogenic microorganisms whic are naturally present in aquatic environment suc as *Vibrios* and *Aeromonas* species (Herringto 1984; Nolan et al., 1984; and Abeyta et al., 1986 On the other hand, bivalve molluscs includin oysters were able to concentrate enteric bacter and viruses during normal filter feeding activiti from surrounding water environment (Hill et a 1976; and Katzenelson et al., 1979). Sin microorganisms may remain viable within oyst shell fish for long periods. Kaysner et al. (198 and due to the fact that oysters are traditiona eaten raw or very mildly cooked they seem being a high risk food and are widely associa with food poisoning cases (Wood, 1976). Mc microbiological studies of marine shell fish h focused on public health hazards associated v consumption of contaminated sea food.

Several outbreaks were reported world-w associated with ingestion of contaminated oys (Bryan, 1980).

On the other hand, contaminated shell fish may be rendered safe for human consumption, by a purification process, leaving the shell fish for short period of time in tanks of clean sea water just before sale. this simple depuration process is considered to be very effective for removal of microbial contaminants within 36 to 48 hr (Furfari, 1976; and Fleet, 1987).

The efficiency of purification is related to pumping rates of shell fish although other parameters of factors have been full elucidated by Richards (1988). Several studies of depuration have indicated that diverse bacterial species are eliminated at different rates by molluscan shell fish (Canzonier, 1971; Scotti et al., 1983; and Power & Collins, 1986).

This paper describes the microbioogical status of Egyptian market oysters as well as the role of depuration in elimination of accumulated bacteria in oysters.

MATERIAL AND METHODS

Sample collection:

A- Market samples:

Fresh oysters (20 smaples) were collected from retail markets in both Ismailia and Cairo governorates. The samples were transported in ice to the laboratory for analysis. The samples were subjected to the following microbiological evaluation:

a- Aerobic plate count using plate count
on nutrient agar at 35°C.

b- Coliforms count (MPN) using multi
fermentation technique in lauryl
broth.

c- *Staphylococcus aureus* count using
spread plate method on Baird Parker aga
suspected colonies were subjecte
staphylase reaction kit (Oxoid, 1990)
three forementioned methods were carrie
according to ICMSF (1978).

d- Isolation of *Salmonellae* was carried o
pre-enrichment in peptone, enrichmen
Rapapport vassiliads broth then platin
XLD Agar according to Harvey and P
(1981).

e- *Vibrio parahaemolyticus* count and isolati
The count was carried out by using multi
technique in Glucose Salt Salt Teepol Br
(GSTB) and streaked onto TCBS mediu
after identification of isolates the count w
recorded using MPN table (FAO, 1992).

B- Depurated Harvested samples:

Eighty samples of oysters were used as follows:

- Twenty samples were taken immediately aft
harvest.
- The rest of samples were collecctd at interva
of 24, 48 and 72 hrs. depuration (20 specimen
of each).

All samples were assayed for all before mentionec microbiological tests, analyses were three folc replicated.

RESULTS AND DISCUSSION

Microbial quality of fresh oysters collected from retail markets is expressed in Table (1), the mean value of aerobic plate count per gram (APC/gm) was 1.8 x 10^7, the most probable number of coliforms count was 235, faecal coliforms 235 and S. aureus was 2.1 x 10^4. Microbial criteria for satisfactory oysters at wholesale level have been set at a faecal coliforms density of < 235/gm and an APC/gm of < 5x105 CFU. The recommended wholesale standards are not directly applicable to examined samples at retail level;' however, APC, total and faecal coliforms in present study exceeded the recommended limits adopted by ICMSF (1974). Also, they were generally higher than results achieved by Wentz et al. (1983). This increase may be attributed to the warm water where oysters had been harvested; also oysters may by stored for an excessive period of time between harvest and sampling as explained by Thompson et al. (1976).

The mean value of *S. aureus* was 2.1 x 10^4, similar results were reported by Mousa (1986). ICMSF (1974) recommended *S. aureus* count limit < 10^2/g. The increase may be attributed to the absence of acceptable means of harvesting, handling and storage.

E. coli was isolated from 18 samples (90%) of market oysters, while *S. aureus* was isolated from 14 sampels (70%). The *Salmonella* and *Vibrio parahaemolyticus* were isolated from one sample (5%) and 3 samples (15%) respectively. The occurrence of these microorganisms in oysters

Table (1) : Microbiological state of fresh Egyptian market oysters.

	Min	Max	Mean	+/tested	%
APC(35°C)	$6x10^6$	$9x10^7$	$1.8x10^7$		
Coliforms(MPN)	42	$1x10^3$	235		
Faecal coliforms (MPN)	42	$1x10^3$	235		
Staph.aureus count	$2x10^2$	$9x10^5$	$2.1x10^4$		
E.coli				18/20	90
S.aureus				14/20	70
Salmonella				1/20	5
Vibrio parahaemolyticus				3/20	15

APC : Aerobic plate count
MPN : Most probable number
S. aureus : Staphylococcus aureus
E.coli : Escherichia coli

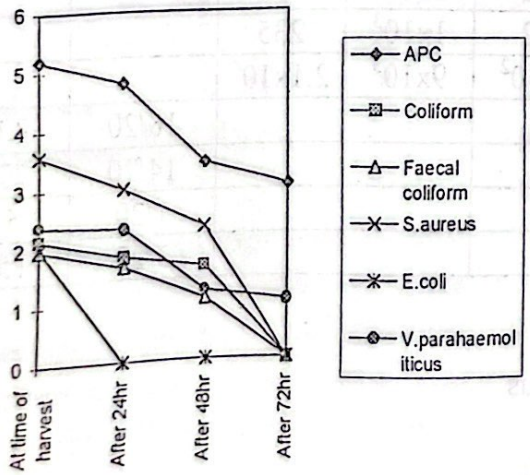
l their public health importance had been fully cussed by several authors (Sobsey et al., 1980; & Fleet, 1980, Nolan et al., 1984; and West, 9).

e role of depuration process in elimination of umulated bacteria in oysters is shown in table and Figure (1). The mean APC/g was reduced 7%, 98.5% and 99.3% of the original count during 72hr. The efficiency of shell purification is measured by the extent to which indicator bacteria have been cleansed (Borrego et al., 1991). In this study, the depuration process was efficient after 24hr to make the oysters on acceptable market level but it seemed better to recommend 48 hr depuration to reach the safe APC count limit.

Table (2) : Efficiency of Depuration on the bacterial load of oysters.

Depuration Time	At time of harvest	After 24hr	% of bacterial load elimination	After 48hr	% of bacterial load elimination	After 72hr	% of bacterial load elimination
APC	$1.7x10^5$	$7.3x10^4$	57	$2.6x10^3$	98.5	$1x10^3$	99.3
Coliform (MPN)	138	64	53.6	42	69.6	<3	100
Faecal coliform	93	42	54.8	11	88.2	<3	100
S.aureus	$3.8x10^3$	$1x10^3$	73.6	$2x10^2$	94.8	U.D.	100
E.coli	$1x10^2$	U.D.	100				
Vibrio parahaemolyticus	$2.3x10^2$	$2x10^2$	13	70	69.5	10	95.6

Efficiency of Depuration on the bacterial load of Oysters



Concerning the coliforms and faecal coliforms depuration processes, the mean MPN of coliforms was reduced from 138/g 64/g to and faecal coliforms from 93/9 to 42/g. Similar results were obtained by Devlin and Neufeld (1971) and Matcalf et al. (1973). Accordingly coliforms and faecal coliforms needed 72 hrs. to be totally eliminated from examined samples. The same results were recorded by Son and Fleet (1980).

Regarding to *S. aureus,* it remained detectable in oysters samples after 48 hrs., this result may be attributed to the high initial concentration; also because *S. aureus* responded differently in shell fish compared to other organisms as mentioned by Borrego et al. (1991), who detected *S. aureus* which can be used as a good indicator of the depuration process.

E. coli organisms were eliminated completely after 24 h of depuration. However, longer depuration times may be required for more heavily contaminated oysters as mentioned by Janssen (1974), and Timoney and Abston (1984).

Vibrio parahaemolyticus was detected for 4.4% even after 72 hrs depuration. Several authors have demonstrated that *V. parahaemolyticus* is not removed from filter feeding mechanism of shell fish during depuration. (Greenberg et al., 1982, Eyles & davey, 1984). On the other hand Barrow and Miller (1974) explained that the lack of nutrients in depuration tanks and the enzymatic activity of the molluscs affected the depuration of *V. parahaemolyticus.*

In conclusion, bacterial indicators of pollution might be suitable for determining the effectiveness of depuration in removal of pathogens. It can be suggested that total cliforms and fecal coliforms could be the best indicators of the presence of hygienic means during depuration process.

REFERENCES

Abeyta, C.; Kaysner, CA.; Ekell, M.M.; Sullivan, J.J. and Stelma, G.N. (1986): Recovery of *Aeromonas hydrophila* from oysters implicated in an outbreak of food borne illness. J. Food prot., 49: 643-646.

Barrow, G.I. and Miller, D.C. (1974): Microbiological and other hazards from seafood with special reference to *Vibrio parahaemolyticus.* Postgrad. Med. J., 50: 612-619.

Borrego, J.J., (corresponding author), Eduardo Martinez-Manzanares, Miguel A. Moringo, Roberto Cornax, Fernando egea (1991): Relationship between classical indicators and several pathogenic microorganisms involved in shell fish-borne diseases J. Food protection, 54 (9): 711-717.

Bryan, F.L. (1980): Epidemiology of food borne diseases transmitted by fish, shell fish and marine crustaceans in the United States, 1970-1978.

Canzonier, W.J. (1971): Accumulation and elimination of coliphage S-13 by the hard clam, *Mercenaria mercenaria.* Appl. Microbiol., 26: 1024-1031.

Delvin, I.H. and Neufeld, (1971): Oyster depuration plant. Ladysmith, B.C. (final report). Industrial Develop. Branch, fish. Ser. Dep. Environ. Ottawa, Canada.

Eyles, M.J. and Davey, G.R. (1984): Microbiology of commercial depuration of the Sydney rock oyster, *Crassostrea commercialis.* Int. J. Food Microbiol., 47: 703-706.

FAO (1992): Manual of food quality control. 4-Rev. 1- Microbiological analysis. Food and Agricultural Organization of the United Nation, Rome

Fleet, G.H. (1987): Oyster depuration: review. Food technol. Aust. 30 : 444-454.

Furfari, S.A. (1967): Shellfish purification: a review of current technology. FAO Technical conf. on Aqua culture. FIR: AG/conf., 179/RII, kyoto, Japan.

Greenberg, EP.; M. Duboise and B. Palhof. (1982): The survival of marine vibrios in *Mercenaria mercenaria*, the hard shell clam. J. food safety, 4: 113-123.

Harvey, R.W. and Price, T.H. (1981): Comparison of Selenite F, Muller Kauffmann tetrathionate and Rappaport medium for Salmonella isolation from chicken giblets and after preenrichment in buffered peptone water. J. Hyg. Camb., 87: 219-229.

Herrington, T.L. (1984): Oyster brone disease outbreaks in st. Petersburg, Florida. Dept Health Human serv./ public Health serv./ food Drug Administration, Atlanata, GA.

Hill, W.F.; W. Jakubowski; E.W. akin and N.A. Clarke (1976): Detection of virus in water: Sensitivity of the tentative standard for drinking water. Appl Environ. Microbiol., 31: 243-261.

ICMSF (1978): International Committee on Microbiological Specifications for Foods. Vol. 1.2nd Ed., Univ. of Toronto press, Toronto, Buffalo and London.

ICMSF (1974): International commission on Microbiological specifications for foods 1974 Microorganisms in foods. Vol. II. Sampling for Microbiological Analysis Principles and Specific Applications. Univ. of Toronto press, Toronto, Canada.

Janssen, W.A. (1974): Oysters: retention and excretion of three types of human water borne disease bacteria. Health Lad. Sci., 11: 20-24.

Katzenelson, E.; B. Fatatl, and T. Kostovesky. (1976): Organic flocculation: an efficient second-step concentration method for the detection of viruses in tap water Appl environ. Microbiol., 32: 638-639.

Kaysnerr, C.A.; M.L. tamplin; M.M. Wckell; R.F. [illegible] K.G. Colburn (1989): Survival of *Vibrio vulnif* shell stock and shucked oysters. (*Crassostrea gig* *Crassostrea virginica*) and effects of isolation me on recovery. Appl. Environ. Microbiol., 55: 3072-30

Metcalf, T.G.; L.W. Stanetz and C.H. Bartley (19 Enteric pathogens in esturary waters and shellfish 215-234. In: C.O. Chichester and H.D. Graham (E Microbial safety to fishshery products, Academic pr N.Y.

Mousa, M.M.I. (1986): Microbiology of some fish a shellifsh in local markets and its relation to pub health. Ph. D. thesis, Fac. Vet. Med., Alexandria Univ.

Nolan, C.M.; J. Ballard; C.A.Kaysner; J.L. Lilia; L. Williams and F.C., Tenover. (1984): *Vibr parahaeolyticus* gastroenteritis, and outbreaks associate with raw oysters in the Pacific north-west. Diagn Microbiol. Infect. Dis., 2: 119-128.

Oxoid (1990): Oxoid manual, 6th Edition, p. 2-200b.

Power, V.F. and J.K. Collins. (1986): Evaluation o depuration as a means of rendering shellfish free from viral pathogens and bacterial indicators. Ir. J. Food Sci. technol., 10:159.

Richards, G.P. (1988): Microbial purification of shell fish: a review of depuration and relaying J. Food prot., 51: 218-251.

Scotti, P.D.; G.C. Fletcher; D.H. Buisson and S. Frederickson. (1983): Viral depuration of the Pacific Oyster (*Crassostrea gigas*) in New Zealand. N. Zeal. J. Sci., 26: 9-13.

Sobsey, M.D.; C.R. Hackney; R.J. Carrick; B. Ray and M.L. Speck. (1980): Occurrence of enteric bacteria and viruses in oysters. J. Food Prot., 43: 111-113.

Son, N.t. and G.H. Fleet. (1980): Behaviour of pathogenic bacteria in the oyster, *Crassostrea commercialis*, during depuration, re-laying and storage. Appl. Environ. Microbiol., 40: 994-1092.

Thompson, C.; C. Vandderzant and S. ray. (1976): Effect of processing, distribution and storage on *Vibrio parahaemolyticus* and bacterial count of oysters. J. Food Sci., 41: 123-127.

Timoney, J.F. and A. Abson (1984): Accumulation and elimination of *Echerichia coli* and *Salmonella typhimurium* by hard clam in an in vitro system. Appl. Environ. Microbiol., 47: 986-988.

U.S. Department of Health, Education and welfare (1965): Sanitation of shell fish growing areas. Public Health Service, U.S. DHEW, Washington, DC.

Wait, D.A.; R.C. Hackney; R.J. Carrik; G. Lovelace and M. D. Sobsey. (1983): enteric bacterial and viral pathogens and indicator bacteria in hard shell clams. J. Food. Prot., 46 (5): 493-496.

Wentz, B.A.; AP.; A.P. Duran; R.A. Swartzentrube; A.H. Schwab and R.B. Read. (1983): Microbiological quality of fresh blue crabmeat, and oysters. J. Food prot, 49 (11): 478-481.

West, P.A. (1989): Human pathogens and public health indicator organisms in shell fish. pp. 273-308 In: B. Austin and D.A. Austin (ED.) Methods for the Microbiological examination of fish and shell fish. Ellis Horwood Lts., Chickester, United kingdom.

Wood, P.C. (1976): Guide to shell fish hygiene. WHO publication, No. 31, World Health Organization, Geneva, Switzerland.