INTERNATIONAL JOURNAL FOR INNOVATIVE RESEARCH IN MULTIDISCIPLINARY FIELD ISSN: 2455-0620 Volume - 6, Issue - 10, Oct – 2020
Monthly, Peer-Reviewed, Refereed, Indexed Journal with IC Value: 86.87 Impact Factor: 6.719
Received Date: 27/09/2020 Acceptance Date: 14/10/2020 Publication Date: 31/10/2020

Analysis of microbes in marine Prawn, *Penaeus monodon*, from Satpati fish market of Palghar District, Maharashtra, India.

[1]Kini Pooja H. , [2]Singh R. B.
[1]Research student [2]Research guide
Department of Zoology S. D. S. M. College Palghar (M.S.) India
Email - poojakini92@gmail.com

Abstract: *A study was undertaken to analyze the microbial quality of marine prawns, P. monodon, from Satpati fish market, from December 2016 to 2019 Palghar. The total viable count (TVC) of the 16 prawn samples collected and processed ranged from 2 X 10^{-7} to 173 X10^{-7} cfu/g. A total of 60 isolates were isolated which were represented by 18 varieties of microbes. Bacillus spp. was dominant one followed by Staphylococcus Spp., pseudomonas spp., Escherichia coli the Vibrio spp. In the present paper an attempt was made to study the pathogenicity of microbes from Penaeus monodon from satpati fish market.*

Key word*: Penaeus monodon. Fish market, Microbes, Satpati.*

1. INTRODUCTION:

Crustaceans, such as shrimps, lobsters and crabs, provide high-quality protein, and marine species also contain omega-3 fatty acids that afford potential health benefits. Therefore, some of these animals have been increasingly subject to farming to supplement those caught by fishing (Wickins and Lee, 2002).

Being aquatic species, shrimp are constantly exposed to a variety of bacteria and viruses, and some can be pathogenic. Shrimp pathogens can orally enter, invade digestive tract system and cause infection (Soonthornchai *et. al*, 2010). Although shrimp has innate immune system to battle against the pathogen's invasion, it has been speculated that bacterial community in shrimp intestines may play protective roles as natural barriers.

Many studies in crustaceans, including the black tiger shrimp, focused on the development of probiotic applications to enhance disease resistance and growth (Rengpipat *et. al.,* 1998; Rengpipat *et. al.,* 2003 and Browdy *et. al.*, 1998) However, a probiotic approach for the black tiger shrimp is challenging due to the lack of understanding of natural microflora and factors contributing to the diversity of the bacterial population. The life cycle of penaeid shrimp includes egg, larval (nauplius, protozoa and Mysis), post larval, juvenile and adult stages. Shrimp from the post-larval through juvenile stages have been reported to have higher bacterial disease susceptibility than those at the later stages (Lavilla-Pitogo *et. al.*,1998, Soto-Rodriguez *et.al.*, 2012, Jiravanichpaisal *et. al.*, 1994 and Manefield *et. al.*, 2000).

The bacteria causing the most serious diseases of the post larval and adult stages of *P. Monodon* and of the genus *Vibrio, Bacillus*, *Pseudomonas* and *Aeromonas* (Lightner and Redman, 1998).Detection of microbial pathogens in food is the solution to the prevention and recognition of problems related to health and safety, Since the food safety is becoming a global health concern and the foodborne diseases take a major crisis on health (Zhao *et. al.*, 2001).

As a highly prized seafood delicacy, shrimps and prawns are cash crop grown mainly for the affluent export (and urban) markets. From farms in Southeast Asia, East Asia, South Asia and South America, shrimps are exported to the major markets in Japan, the USA and Europe; while domestic consumption accounts for only 5-20 %, foreign markets absorb 80-95 % of total farmed production. Often, products rejected for the export market due to small size, bacterial load, or chemical residue levels are shunted to local markets (Primavera, 1994).

This investigation aimed to evaluate the incidence of bacterial load and pathogens in locally available shrimp and prawn species with a view to provide potential approach for improving the quality assurance and create awareness among the consumers.

2. MATERIALS AND METHODS :

Satpati is one of the biggest fishing village on the west coast of India. It is about 90 km north of Mumbai, located in the Palghar Taluka of Palghar district in Maharashtra. The main industry in Satpati is fishing, with large exports abroad. were collected from Satpati fish market.

Prawns (*Penaeus monodon*) sample were collected from December 2016 to May 2019. To avoid further contamination, during transportation from the source to laboratory, Samples were carried in special sterile bags packed in insulated box with ice to maintain the temperature around 5 to 6 ^{0}C.

2.1 STANDARD PLATE COUNT (SPC)

The whole body of prawn was used as sample and around 10 grams was transferred to a sterile beaker to which 90 ml of sterile normal saline solution (NSS) was added. The samples were serially diluted by 10-fold serial dilution method in the normal saline solution up to 10^{-7}. The 10^{-7} dilution were used in 0.1 ml quantities for the SPC on plate count agar (PCA). The agar plates were inoculated by pour plate method and incubated at 37 ^{0}C for 24 hrs. The 10^{-4} dilutions of each sample were taken for plating following differential media simultaneously during processing of the samples, Baird Parker agar, Slanetz and Bartley agar, MacConkey agar, Violet Red Bile agar, Salmonella Shigella agar, TCBS agar and Bacillus cereus agar 0.1 ml of the 10^{-4} dilutions were inoculated by the spread plate method on the above media plates and were incubated at 37 ^{0}C / 44 ^{0}C for 24 / 48 hrs. 1 ml of Prawn sample was taken in 10 ml of Selenite cystine broth which was incubated at 37 ^{0}C for 18 hrs. It was then streaked on Salmonella Shigella agar and incubated at 37 ^{0}C for 24 hrs. For *Salmonella* sp. The colonies from the differential media plates were transferred in sterile peptone water and the same were identified based on morphology Gram's staining. Some biochemical tests. The preparation was carried out according to Cowan and Steel (1970, 1993), Diliello (1982) and Hi-media (2013, 2015).

3. RESULT AND DISCUSSION:

A total of 60 isolates were isolated from 16 sample of *P. monodon* collected from Satpati fish market. *Bacillus* spp. was the predominant spp. followed by *Staphylococcus* spp., *Pseudomonas* spp., *E. coli* and *vibrio* spp. TVC ranged from 02 x10^{-7} cfu/g to 173.x 10^{-7} cfu/g. Table 1 and figure 1.

During this study the number of *Bacillus* spp. were 19 which constituted 31.65 % of the total isolates. As per the Claus and Berkeley, (1986), some spp. can be pathogenic to humans and other animals and can cause food-borne gastro-enteritis. So, precautions are necessary while handling and processing of fisheries. During present study *Bacillus oceanisediminis, Bacillus paramycoides, Bacillus tequilensis, Bacillus wudalianchiensis* and *Lysinibacillus baronitolerans + R* are found to be novel spp. These species are used as bioremediate and good decomposers (liu *et. al*., 2017).

An overall percentage of *Staphylococcus sp.* was 16.66 %. Albuquerque *et. al*., 2007; Ayulo *et. al*., 1994 and Leung *et. al*., 1992, reported that, *Staphylococcus* species are one of the most important food borne opportunistic bacteria in fishes and some are potential pathogens and high population of these bacteria indicates the degree of the spoilage. It can be spread through skin contact. It was observed during this study that seller women periodically dipping their hands, cutting instruments and the fishes in the same basket water and the water almost become black in color. Hands of those people were continually communicating with all those things which may cause skin infection.

Pseudomonas spp. was third highest in number (9) and was represented by *Pseudomonas sihuiensis* a novel sp. from family *Pseudomonace.* It was isolated from forest soil in sihui city, south china (*Min Wu et al 2014).* The other species was *Pseudomonas stutzeri, an* opportunistic pathogen for humans. It can cause osteomyelitis, arthritis, endocarditis, meningitis, pneumonia, empyema, skin infections, eye infections, urinary tract infections, and diverticulitis.

Occurrence of *E. coli* in the present finding was 13.33%. Kumar *et al*., 2005, reported that estuaries and coastal water bodies, which are the major sources of seafood in India, are often contaminated by human activities and are associated with the widespread occurrence of *E. coli* in seafood. *Escherichia coli* is often used as an indicator for fecal contamination; it can cause human illness, some strains of E. coli are capable of causing food-borne disease, ranging from mild enteritis to serious illness and death. It can cause a variety

of diseases, including diarrhoea, dysentery, hemolytic uremic syndrome, and bladder and kidney infections. Different strains are usually associated with different diseases; this versatility of *E. coli* strains is due to the fact that different strains have acquired different sets of virulence genes (Teophilo *et. al*., 2002).

In the present study *Vibrio* spp., *Acinetobacter baumannii, Aeromonas taiwanesis and Shigella* spp. were isolated. Incident of these microbes can be responsible to human infections. *Vibrio* spp. capable to causing gastrointestinal illness (*gastroenteritis*), *Acinetobacter baumannii* is pathogenic red alert pathogen to cause nosocomial and community-acquired infections, *Aeromonas taiwanesis* can cause gastrointestinal infections and *Shigella* sp. can cause mild watery diarrhea to severe inflammatory bacillary dysentery or shigellosis, manifested by severe abdominal cramps, nausea and vomiting, fever, tenesmus, anorexia, and stool containing blood and mucus.

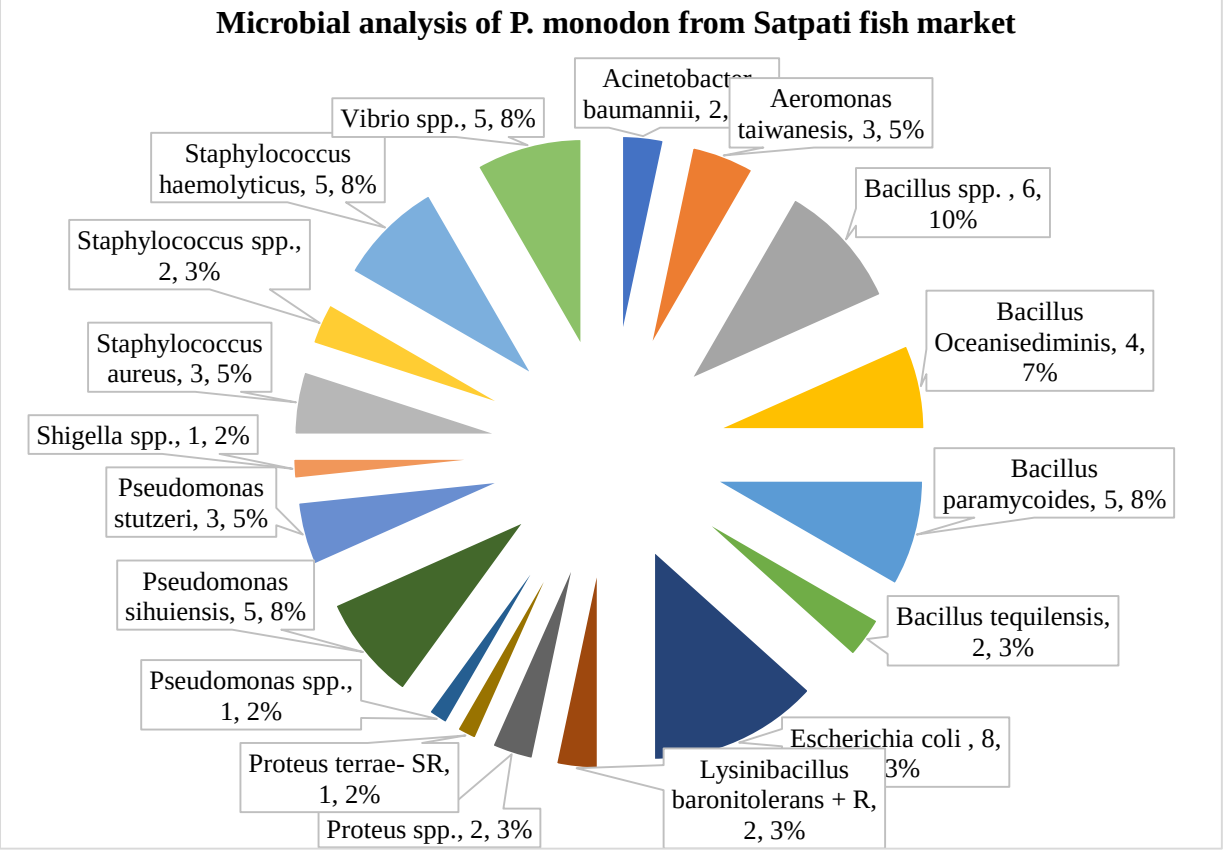
Anderson *et. al.*, 1989; Prem Anand *et. al.*,1996; Costa *et. al.*,1998; Jayakumar *et. al*., 1999; Mukherjee, 2002 and Oxley *et. al*., 2002 reported that the most common pathogenic bacteria identified from penaeid shrimps include *Vibrio alginolyticus, V. parahaemolyticus, V. anguillarum* and other species and strains of the genus *Vibrio, Pseudomonas, Flavobacterium, Moraxella, Aeromonas, Leucothrix, Mycobacterium marinum, Micrococcus sp., Cytophaga sp., Acinetobacter sp., Arthrobacter sp., Alteromonas sp., Bacillus sp., Alcaligenes sp., Plesiomonas sp., Pasteurella sp., Photobacterium sp., Flexibacter sp., Staphylococcus sp., Enterococcus sp.,* and *Nocardia sp.*

In addition to spoilage organisms, various pathogenic organisms are associated with prawns. Like other seafood products, prawns are high on the list of foods transmitting disease (Huss *et al.*2000).

Table -01: Qualitative microbial analysis from *P. monodon* from Satpati fish market

Sr. no.	Name of Isolates	No. of isolates	Total %
1.	*Acinetobacter baumannii*	02	3.3
2.	*Aeromonas taiwanesis*	03	05
3.	*Bacillus spp.*	06	10
4.	*Bacillus Oceanisediminis*	04	6.66
5.	*Bacillus paramycoides*	05	8.33
6.	*Bacillus tequilensis*	02	3.33
7.	*Escherichia coli*	08	13.33
8.	*Lysinibacillus baronitolerans + R*	02	3.33
9.	*Proteus spp.*	02	3.33
10.	*Proteus terrae- SR*	01	1.66
11.	*Pseudomonas spp.*	01	1.66
12.	*Pseudomonas sihuiensis*	05	8.33
13.	*Pseudomonas stutzeri*	03	05
14.	*Shigella* spp.	01	1.66
15.	*Staphylococcus aureus*	03	05
16.	*Staphylococcus spp.*	02	3.33
17.	*Staphylococcus haemolyticus*	05	8.33
18.	*Vibrio spp.*	05	8.33
	Total	60	100

Figure.1 Graphical representation of microbial analysis of *P. monodon* from Satpati fish market.



4. CONCLUSION:

This study provides information on the occurrence of bacterial pathogens in marketed prawns *P. monodon*. The samples from local Satpati market were contaminated with bacteria. From 18 varieties of microbes a total of 5 (*Bacillus oceanisediminis, Bacillus paramycoides, Bacillus tequilensis, Bacillus wudalianchiensis* and *Lysinibacillus baronitolerans + R and Pseudomonas sihuiensis)* species are novel and the rest are human pathogenic in nature.

The total viable count was found higher which indicate the unhygienic condition of the processing and marketing area. It is clear from the present study that the occurrence of different pathogenic microbes is the cause of concern. Therefore, it is at most important to improve handling, sorting and sanitation at different stages starting from landing centers to consumers.

5. ACKNOWLEDGEMENT:

The authors are thankful to Dr. Kiran Save, I/C Principal and Management of S.D.S.M. College, Palghar-401404 (M.S.) India, for motivation and help during this study. We sincerely record the timely help provided by Aarti drugs Pvt. Ltd. in the form of chemicals, Hi media (agars) and glass ware. The first author is also thankful to her research colleague Swapnil Keni for the technical help in the preparation of this manuscript.

REFERENCES:

1. Albuquerque, W.F., Macrae, A., Sousa, O.V., Vierira, G.H.F., Vieira, R.H.S.F. (2007): Multiple drug resistant Staphylococcus aureus strain isolated from a fish market and from fish handlers. *Braz. J. Microbiol*., **38**: 131 134.
2. Anderson, G., Shamsudin M. N. and Nash G. (1989): A preliminary study on the aerobic heterotrophic bacterial flora in giant freshwater prawn, *Macrohurachium rosenbergii*, hatcheries in Malaysia, Aquaculture. **81**(3-4) :213-223.
3. Ayulo, A.M., Machado R.A. and Scussel V.M. (1994): Enterotoxigenic *Escherichia coli* and *Staphylococcus aureus* in fish and seafood from the southern region of Brazil. *Int. J. Food Microbiol*., 24,

4. Browdy, CL (1998): Recent developments in penaeid broodstock and seed production technologies: improving the outlook for superior captive stocks. Aquaculture **164**: 3–21.
5. Claus, D. & Berkeley, R. C. W. (1986): Genus *Bacillus*. In Bergey's Mantdal of Jystematic Bacteriolou, Edited by P. H. A. Sneath, N. S. Mair, M. E. Sharpe & J. G. Holt. Baltimore: K'illiams & Wilkins. vol. **2**, pp. (1104-1139).
6. Costa, R, Mermoud I. and Koblavi S. (1998): Isolation and characterization of bacteria associated with a *Penaeus* stylistics disease (Syndrome 93) in New Caledonia, Aquaculture, **164**:297-309.
7. Cowan, S. T. and Steel, K. J. (1970): Manual for the identification of Medical Bacteria. The Cambridge University Press, London, U. K.
8. Dalgaard, P., (1995): Qualitative and quantitative characterization of spoilage bacteria from packed fish. *International Journal of Food Microbiology* **26**, 319-333.
9. Diliello, L. R. (1982): Methods in food and dairy microbiology, The AVI Publishing Company, INC. Westport, Connecticut, USA.
10. Fell, G., Hamouda, O., Lindner, R., Rehmet, S., Liesegang, A., Prager, R., Gericke, B. and Petersen, L., (2000): An outbreak of Salmonella blockley infections following smoked eel consumption in Germany. Epidemiology and Infection **125**, 9-12.
11. Haley, B.J., Kokashvili, T., Tskshvediani, A., Janelidze, N., Mitaishvili, N., Grim, C.J., de Magny, G.C., Chen, A.J., Taviani, E., Eliashvili, T., Tediashvili, M., Whitehouse, C.A., Colwell, R.R. and Huq, A., (2014): Molecular diversity and predictability of Vibrio parahaemolyticus along the Georgian coastal zone of the Black Sea. Frontiers in Microbiology **5**, 1-9.
12. Huss, H.H., Reilly, A. and Embarek, P.K.B. (2000): Prevention and control of hazards in seafood. Food Control. **11**, 149-156
13. Jayakumar R. and Ramasamy P. (1999): Bacterial and protozoan diseases of prawn *Penaeus indicus* (Decapoda: Crustacea). *Indian Journal of Marine Sciences*, **28**:285-296.
14. Jiravanichpaisal, P., MIyazaki T. and Limsuwan C. (1994); Histopathology, Biochemistry, and Pathogenicity of *Vibrio harveyi* Infecting Black Tiger Prawn *Penaeus monodon*. *J Aquat Anim Health* **6**: 27–35.
15. Koonse, B., Burkhardt, W., Chirtel, S. and Hoskin, G.P., (2005): *Salmonella* and the sanitary quality of aquacultured shrimp. *Journal of Food Protection* **68**, 2527-2532.
16. Kumar, H.S., Parvathi, A., and Karunasagar, I. (2005): Prevalence and antibiotic resistance of *Escherichia coli* in tropical seafood. *World Journal of Microbiology and Biotechnology*. **21**, 619-623.
17. Kumar, R., Surendran, P.K. and Thampuran, N., (2009): Distribution and genotypic characterization of Salmonella serovars isolated from tropical seafood of Cochin, *India. Journal of Applied Microbiology* **106**, 515-524.
18. Lavilla-Pitogo, CR, Lean˜o EM, Paner MG (1998): Mortalities of pond-cultured juvenile shrimp, *Penaeus monodon*, associated with dominance of luminescent *vibrio's* in the rearing environment. Aquaculture **164**: 337–349.
19. Leung, C.K., Huang, Y.W. and Pancorbo, O.C. (1992): Bacterial pathogens and indicators in catfish and pond environment. *J. Food Protect*., **91**: 1 11.
20. Lightner, D.V., and Redman, R.M., (1998): Shrimp disease and current diagnostic methods. Aquacult.**164**: 201-220
21. Liu, Guo Hong Liu, cetin Sengonca, PeterSchumann, Jie-Ping Wang, Yu—Jing Zhu and Hai Feng Zhang (2017): *Bacillus wudalinchiensis* sp. nov., isolated forest grass soil of the wudalinchi scenic area. *International jounal of systemic and evolutionary microbiology*. **67**:2897-2902
22. Manefield, M., Harris L, Rice SA, de Nys R, Kjelleberg S (2000): Inhibition of Luminescence and Virulence in the Black Tiger Prawn (*Penaeus monodon*) Pathogen *Vibrio harveyi* by Intercellular Signal Antagonists. Appl Environ Microbiol **66**: 2079–2084.
23. Manual of hi-media, 2013,2015.
24. Martinez-Urtaza, J., Baker-Austin, C., Jones, J.L., Newton, A.E., Gonzalez-Aviles, G.D. and DePaola, A., (2013): Spread of Pacific northwest *Vibrio parahaemolyticus* strain. *New England Journal of Medicine* **369**, 1573-1574.
25. Min Wu et al. Antone Van Leeuweenhoek. (2014): pseudomonas sihuiensis sp. nov., isolated from a forest soil in south chaina. PubMed
26. Mukherjee, S. C. (2002): Fish diseases in India, their causes and control measures, Applied Fisheries and Aquaculture, **2**(2):66-71.
27. Oxley, A P., Shipton, W., Owens, L. and McKay (2002): Bacterial flora from the gut of the wild and cultured banana prawn, *Penaeus merguiensis*. *Journal of Applied Microbiology*, **93**(2) :214-223.

28. Prem Anand T., Patterson Edward J. K. and Ayyakkannu K. (1996): Monitoring of a shrimp culture system with special reference to *Vibrio* and Fungi, *Indian Journal of Marine Sciences*, **25**:253-258.
29. Primavera, J.H. (1994): Shrimp Farming in the Asia-Pacific: Environmental and Trade Issues and Regional Cooperation In: Trade and Environment in Asia- Pacific: Prospects for Regional Cooperation. Workshop held in East-West Center, Honolulu, 23-25th September 1994. Tigbauan, Iloilo, Philippines: Aquaculture Department Southeast Asian Fisheries Development Center. http:/ /www.nautilus. org/archives /papers /enviro/trade/ shrimp. html, Accessed on 23 June 2007.
30. Reilly, P.J.A., (1987): Sources of contamination of tropical prawns and shrimps. Food Laboratory Newsletter (Sweden).
31. Reilly, P.J.A., Twiddy, D.R., (1992): *Salmonella* and *Vibrio cholera* in brackish water cultured tropical prawns. *International Journal of Food Microbiology* **16**, 293-301.
32. Rengpipat, S, Tunyanun, A., Fast, A.W., Piyatiratitivorakul, S. and Menasveta, P. (2003): Enhanced growth and resistance to *Vibrio* challenge in pond-reared black tiger shrimp *Penaeus monodon* fed a *Bacillus* probiotic. Dis Aquat Organ **55**: 169–173.
33. Rengpipat, S., Phianphak, W., Piyatiratitivoraku,l S. and Menasveta, P. (1998): Effects of a probiotic bacterium on black tiger shrimp *Penaeus monodon* survival and growth. Aquaculture **167**: 301–313.
34. Soonthornchai, W., Rungrassamee, W., Karoonuthaisiri, N., Jarayabhand, P. and Klinbunga S, (2010): Expression of immune-related genes in the digestive organ of shrimp, *Penaeus monodon*, after an oral infection by *Vibrio harveyi*. Dev Comp Immunol **34**: 19–28.
35. Soto-Rodriguez, S.A., Gomez-Gil, B., Lozano, R., del Rio-Rodriguez, R. and Dieguez, AL. (2012): Virulence of *Vibrio harveyi* responsible for the ‘‘Bright-red’’ Syndrome in the Pacific white shrimp *Litopenaeus vannamei***.** *J Invertebr Pathol* **109**: 307–317.
36. Teophilo, G.N.D., Vieira, R.H.S.D.F., Rodrigues, D.D.P. and Menezes, F.G.R. (2002): *Escherichia coli* isolated from seafood toxicity and plasmid profiles. *International Microbiology*. **5**, 11-14.
37. Wickins, J. F. and Lee, D. O’C. (2002): Crustacean Farming: Ranching and Culture, 2nd edn. Oxford, Blackwell Science.
38. Zhao, C., Ge, B., De Villena, J., Sudler, R., Yeh, E., Zhao, S., White, D. G., Wagner, D. and Meng, J. (2001): Prevalence of *Campylobacter* spp., *Escherichia coli* and *Salmonella* in retail chicken, turkey, pork, and beef from the greater Washington, D.C., area. Applied and Environmental Microbiology **67**: 5431–5436.