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🌐 Detection of total and faecal coliforms in the oysters

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Abstract

Enterobacteriaceae the leading cause of gastroenteritis. These gram- negative bacteria from species like Escherichia, Shigella, Salmonella, Vibrio and Helicobacter etc are among those that cause severe disease in consumers, especially those that indulge in uncooked seafood. Oysters have been shown to harbour these pathogenic organisms, which pose a major health risk. Numerous outbreaks of gastroenteritis due to oyster consumption have been reported worldwide because of the enteric bacteria as well as viral pathogen contamination

This research was conducted to identify the Enterobacteriaceae present in oysters sold by vendors in Trinidad. During a five-month period (the rainy season, May - September), a total of 156 oyster samples (comprising 104 oysters samples and 52 water samples of the prepared, unprepared oyster cocktails plus the water used by vendors) were analysed. These samples were collected from roadside vendors operating near to the coastal line of the Western part of Trinidad These were processed at the microbiology unit of the Department of Para-clinical Sciences of the University of the West Indies, St. Augustine using standard microbiology techniques. The antibiotic susceptibility profiles of the recovered organisms were performed using the Kirby Bauer method for the following antibiotics- Tetracycline, Ampicillin, Trimethoprim-Sulfamethoxazole, Cefuroxime and Ceftazidime. The SPSS 21 programme was used to analyse the biometric data and a chi-square test was used to determine if there was any significant difference between the kinds of preparation cocktails with respect to the coliform and organisms found.

Results of the 104 oyster samples tested, 112 isolates were obtained, 13.4% (15/112) were E.coli of which 0.1% (1/15) was the 0157:H7 strain; 72.3% (81/112) were Shigella species of which 27.2% (22/81) were Shigella dysenteriae; 14.3% (16/112) were Salmonella species of which 25% (4/16) were Salmonella cubana. The susceptibility profiles of the organisms revealed that 86.5% of Shigella, 100% of E.coli and 93.7% of the Salmonella were resistant to multiple antibiotics. With the recovery of these organisms from these samples, the health of the Trinidadian and foreign consumers of mangrove oysters is at risk particularly from Shigella, E.coli and Salmonella species. As a result, there should be an increased need for public awareness as well as regulations by the Ministry of Health to be made so that illness from these hazardous Enterobacteriaceae can be prevented.

1   The meat (10 oysters per cup) of the unprepared oysters was blended with 25ml of sterile distilled water and 25ml of sterile peptone water using a sterilized blender (the blender was washed with antibacterial soap as well as bleach and rinsed with sterile distilled water along with 70% ethanol before autoclaving prior to each use). This was repeated for the meat from the prepared oysters. Each homogenate was then poured into a sterile 100ml flask using a sterilized funnel and filter paper 205µm thick and then poured into a sterile 100ml beaker. The filtered homogenate was then used to perform the Spread Plate Method for the total and fecal coliform on Nutrient agar and EMB agar respectively:

2   A sterile graduated Pasteur pipette was used to dilute 1ml of sample water in a sterile test-tube of 9 ml sterile distilled water and vortexed for 5 seconds. Then, using another sterile graduated Pasteur pipette, 1ml of this diluted sample was further diluted in 9ml sterile distilled water in another sterile test-tube, vortexed for 5 seconds and so on until 10 serial dilutions were achieved.

3   ·Ten (10) plates were labelled according to the sample number and desired dilution.

4   ·Using a new sterile graduated Pasteur pipette 0.1ml drop of the desired serial dilution was placed onto the centre of the surface of the agar plate.

5   ·Then using a sterile plastic L-shaped spreader, the sample was spread over the surface of the agar, carefully rotating the Petri Dish underneath at the same time. The L-shaped spreader was then discarded.

6   ·The plate was then inverted and incubated overnight at 34°C.

7   ·This was done in duplicate and plates with the best/significant single colonies were chosen, ie; those that grew 30-200 single colonies.

8   ·The procedure was repeated for each serial dilution, so that each plate had a different dilution.

9   ·After incubation, in order to easily count the colonies, the plates were divided into quadrants with a marker on the underside of the plate.

10 ·The colonies grown were then counted manually. The dilution that showed to have the best countable colonies was 10^-4.

11 ·The following equation was used: No. colonies/0.1ml plated divided by 10^-X and the results expressed as Colony Forming Units/0.1 millimetre x 10 (CFU/0.1ml x 10^4) in the range < or > 200 for the total and faecal coliform.

12 ·This method was repeated per vendor.