Aug 22, 2020

Isolation and Characterization of Enteric Bacteria from Oysters

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1 Works for me dx.doi.org/10.17504/protocols.io.bj6ekrbe

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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.

DOI

dx.doi.org/10.17504/protocols.io.bj6ekrbe

PROTOCOL CITATION

Sade Aisha Folashade John, Patrick E. Akpaka, Chandrashekhar Unakal, Arvind Kurhade, Angel Justiz-Vaillant 2020. Isolation and Characterization of Enteric Bacteria from Oysters. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bj6ekrbe

Citation: Sade Aisha Folashade John, Patrick E. Akpaka, Chandrashekhar Unakal, Arvind Kurhade, Angel Justiz-Vaillant (08/22/2020). Isolation and Characterization of Enteric Bacteria from Oysters. https://dx.doi.org/10.17504/protocols.io.bj6ekrbe

CREATED

Aug 22, 2020

LAST MODIFIED

Aug 22, 2020

PROTOCOL INTEGER ID

40870

1 Study Design: This cross sectional study was carried out over a five-month period during the rainy season (22 May - 09 September). The study evaluated the total and fecal coliform of the oyster cocktails including the water used by the vendors. Additionally, the enteric organisms present were isolated and characterized. The vendors situated near the Western coastal line were chosen for the study because of convenience of collection and the fact that most vendors are present on that coast of the island.

2 Study Samples: The study was performed with oyster cocktails from both prepared and unprepared samples taken from vendors near the Western coastal line. The oyster meat chosen for the study was observed to ensure that they were not discoloured or possess a foul odour, in other words, they were not spoiled or rotten.

3 Sample collection: One hundred and fifty-six samples comprising 104 oyster samples (52 unprepared and 52 prepared) and 52 water samples were bought from roadside vendors near the Western coastline of Trinidad. Twenty-nine vendors agreed to participate in the study by allowing materials to be collected from their stall or vending sites. No vendor was tested more than twice during the study period. A single order of oysters of both unprepared and prepared cocktails [10 oyster meat per cup] from each district was tested from the North comprising the areas of Curepe, Tunapuna and Piarco). The South comprises the areas of San Fernando, Debe, Penal. The West comprises the areas of Port of Spain and Aranguez, and the Central (comprises the areas of Cunupia, Chaguanas and Couva.

4 These samples were collected using sterile new zip lock bags for the oysters and sterile 25ml universal specimen containers for the preparation of water samples (1 container per vendor). The prepared oyster samples were taken with sauces based on the vendors liking, especially with respect to peppering or "hotness" intensity.

5 On collection, the oyster samples and water were kept on dry ice, in an isothermal cooler (to minimize spoilage) and immediately transported to the laboratory for analysis. All the vendors who participated were interviewed during the specimen collection. This was done so that the data collected can be used to correlate with positive results and determine poor food handling practices.

6 Laboratory Analysis: Detection of total and faecal coliforms in water. Water used from each participating vendor was tested for the total and faecal coliforms using the Spread Plate Method on Nutrient agar and EMB agar, respectively:

7 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 9 ml sterile distilled water in another sterile test-tube, vortexed for 5 seconds and so on until 10 serial dilutions were achieved.

8 10 plates were labelled according to the sample number and desired dilution.

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

Then using a sterile plastic L-shaped spreader, the sample was spread over the surface of the agar, carefully rotating

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10 the Petri dish underneath at the same time. The L-shaped spreader was then discarded.

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

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

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

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

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

16 The following equation was used: No. colonies/0,1ml plated divided by 10^-X and the results expressed as Colony Forming Units/0.1millimetre x 10^4 (CFU/0. 1ml x 10^4) in the range < or > 200 for the total and faecal coliform.

17 This method was repeated per vendor.

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