Microbiological Analysis of Sediment and Water–Column Samples

from St. John's Harbour

And

Antibiotic Resistance of the Isolates

By

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Introduction

Urban and industrial sewage discharges into harbour or marine environments can cause contamination of waters and aquatic life forms. An earlier study, "A Bacteriological Investigation of Selected Flounder, Crab and Lobster Collected from St. John's Harbour" (June 2001) prepared by Deborah Squires-Parsons, MUN, in June 2002 revealed the presence of various human pathogens of bacterial origin. Consumption of harvested organisms from contaminated areas therefore presents a risk to public health. A further study, "Analysis of Steroid Hormones as Endocrine Disruptors in Sewage, Seawater and Mussels Using GC.MS techniques" by Gurusankar Saravanabhavan, M.Sc. thesis, Memorial U., June 2003 provides additional evidence of marine contamination in St. John's Harbour from the discharge of untreated municipal wastewater.

Many bacterial species of enteric origin (animal faecal matter) can be isolated from harbours with human /or animal habitation. Marine sediments have been shown to harbour large numbers of pathogenic bacteria with less numbers being present in the water column and surface films.

St. John's Harbour receives substantial quantities (120 million litres) of untreated sewage and stormwater run-off per day. Rapid multiplication of microbes is expected in such waste with high organic content. As a part of a large study to examine safety of marine environment undertaken by ACAP the present study describes the prevalence of selected bacterial species in the sediment and water column samples collected from selected designated sites in St. John's Harbour.

Objectives

The intent of this study is to:

i) Collect sediment and water-column samples from five different locations in the St. John's Harbour.
ii) To isolate enteric pathogens using selective microbiological media.
iii) To identify the isolated bacterial species.
iv) To determine antibiotic resistance or sensitivity of the isolates to various antibiotics.

Material and Methods

1. *Sediment / Water column samples.*

Sediment and water-column samples were collected by the Department of Fisheries and Oceans, St. John's. They consisted of five sediment (G1-G5) and five water-column (W1-W5) samples collected from the same corresponding location as the sediment samples. Sample collection began near the Chain Rock location in The Narrows for Samples G1/W1; south of Pier 17 for Samples

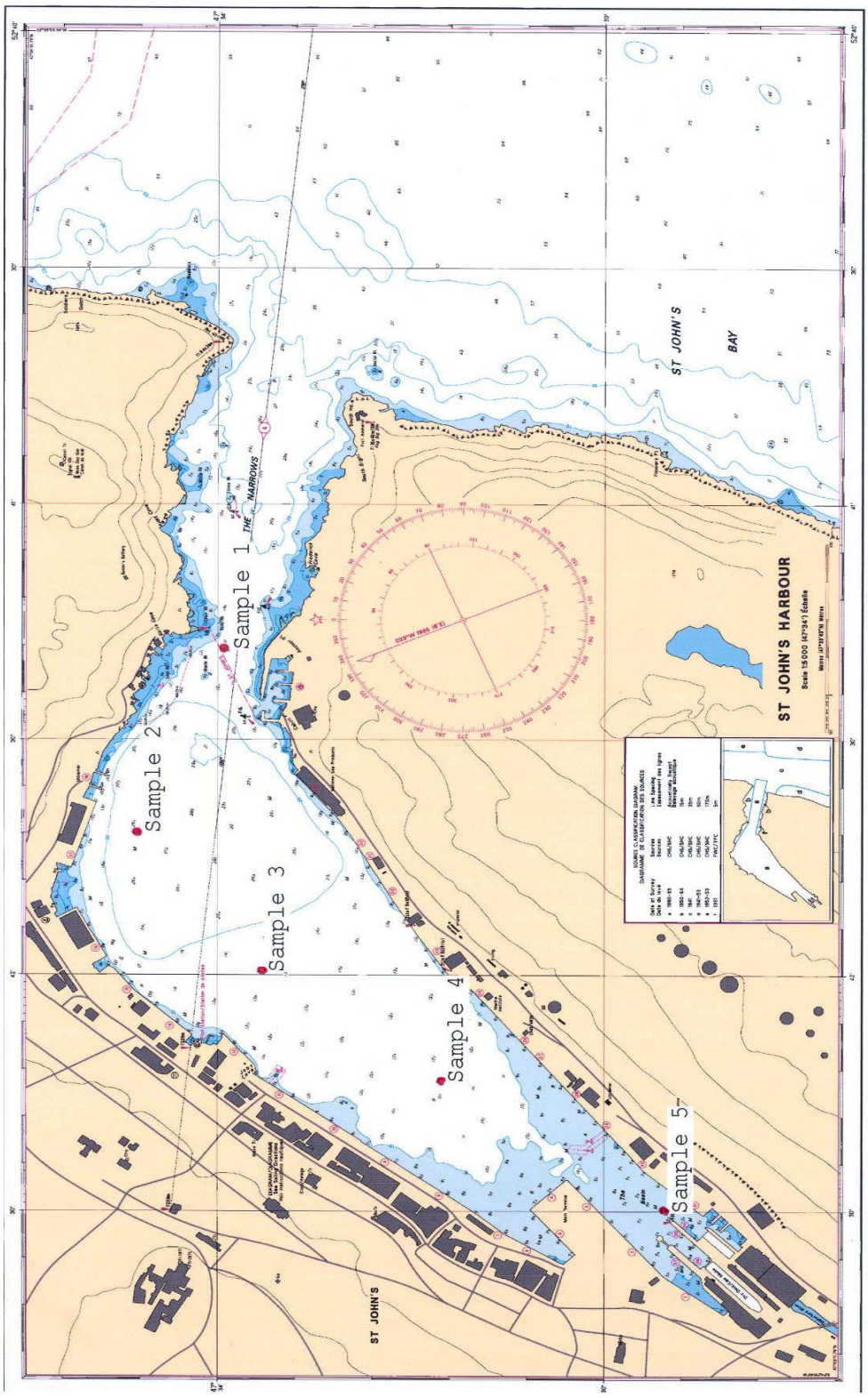

Figure 1: Location of Sample Sites

G2/W2; east of Pier 12 for Samples G3/W3; equi-distant between Pier 9 (adjacent to Atlantic Place) and Pier 25 (Marine Institute) for Samples G4/W4; and just north-east of Pier34/35 in The Basin for Samples G5/W5 (Figure 1). The appropriate latitude and longitude coordinates are as follows for these sample locations:

Sample 1 (G1 & W1):	N 47° 33.986'	W 052° 41.312'
Sample 2 (G2 & W2):	N 47° 34.100'	W 052° 41.702'
Sample 3 (G3 & W3):	N 47° 33.940'	W 052° 41.997'
Sample 4 (G4 & W4):	N 47° 33.708'	W 052° 42.230'
Sample 5 (G5 & W5):	N 47° 33.417'	W 052° 42.505'

Sediment samples in plastic containers (buckets) and water-column samples in plastic bottles were transported to a lab at MUN (Dr. Thakor Patel), on ice in large ice coolers. These samples were processed within 24 hr after arrival.

2. *Preparation of sediment samples for microbiological analysis.*

The following methods are based on the analysis of 25g analytical unit at 1: 9 sample dilution ratio.

25g of sediment were suspended in 225 ml of 0.1% peptone water. Five such 250ml (total) suspensions were prepared and used for detection and quantification of selected bacterial species described below.

3. *Preparation of marine water-column samples for microbiological analysis.*

25 ml water sample was added to 225 ml of 0.1% peptone water and mixed thoroughly. Five such suspensions representing samples W1 - W5 were prepared and used for detection and enumeration of selected bacterial species.

4. *Enumeration of Escherichia coli and Coliform Bacteria.*

To test the presence of sanitary index bacteria (the coliforms, faecal coliforms and ***E. coli*** as coliforms) the method described in Bacteriological Analytical Manual, 7th Edition, 1992, Association of Official Analytical Chemists (AOAC) International, was used. This method also detects virulent strains of ***E. coli***, of which there are several major groups.

E. coli and the coliforms are Gram-negative, rod shaped bacteria. Identification criteria used are production of a gas from glucose (and other sugars) and fermentation of lactose within 48 hr at 35^{o}C (coliforms) and at 45.5^{o}C (faecal

coliforms and ***E. coli*** as coliforms). It is common to use 44.5^{o}C for detection of ***E. coli*** (1, 2).

The method used in the present study corresponds to AOAC's Official Method of Analysis (6).

Presumptive and confirmed tests for coliform bacteria were carried out using the procedure of Hutchins, et al. (1992) while EC broth method was used for the Most Probable Number for ***E.Coli*** (1).

E coli counts were also obtained by plating serial dilutions of the samples on a selective medium – Eosine Methylene Blue (EMB) agar plates. On this medium E. coli gives blue-black colonies with dark centres and green metallic sheen. ***Enterococcus faecalis*** and ***Salmonella typhimurium*** give colorless colonies.

5. ***Detection of Salmonella organisms.***

Procedure of Andews, et al. (1992) was used for detection and isolation of ***Salmonella*** organisms. Bacto selenite cystin broth (SC-broth) is used for selectively enriching ***Salmonella*** in an unknown sample. Selenite broth favours the growth of ***Salmonella*** while reducing growth of faecal coliforms and enterococci.

The growth and recovery of ***Salmonella*** in food samples can be hindered by non-Salmonella bacteria, substances indigenous to the sediment samples or water samples, and low numbers of ***Salmonella*** which may be partially injured. Using protocols that involve pre-enrichment, selective enrichment and selective plating increases the likelihood of recovering ***Salmonella***. In most standard method procedures selenite - cystine broth is recommended in the selective enrichment step. As a selective enrichment medium selenite - cystine broth is formulated to allow proliferation of ***Salmonella*** while inhibiting the growth of competing non-Salmonella bacteria.

Tetra-thionate broth is used for enriching ***Salmonella*** from samples prior to isolation procedures. TT – broth is very useful in detecting ***Salmonella*** that can be injured in the environment or during processing procedures. Factors such as temperatures, drying, radiation, preservatives and sanitizers can injure ***Salmonella*** cells. Although injured cells may not form colonies on selective media, they can cause diseases if ingested. ***Salmonella species***, in particular, can cause many types of infections from mild self-limiting gastroenteritis to life-threatening typhoid fever.

Selectivity in TT-broth is accomplished by the combination of sodium thiosulfate and tetra-thionate, suppressing coliform organisms. Organisms containing

tetrathionate reductase will proliferate in this medium. Sodium deoxycholate and brilliant green in TT-broth are selective agents that suppress coliform bacteria.

Bismuth sulfite indicator and brilliant green in Bismuth Sulfite Agar, are complementary in inhibiting gram positive bacteria and members of coliform group while allowing ***Salmonella*** to grow luxuriantly. Dark brown or black colonies result from ferrous sulphate precipitating iron.

Hektoen Enteric Agar was also used for the detection and isolation of ***Salmonella*** colonies. On this selective medium, ***Salmonella typhimurium*** gives black colonies while ***Shigella flexneri*** yields greenish-blue. ***E. coli*** and ***Enterococci*** are inhibited on this medium.

To facilitate detection and isolation of Salmonella, two other selective media were used, namely, XLD (Difco, xylose,lysine & deoxycholate) agar and XLT4 (difco, xylose, lysine & sodium thiosulfate) agar.

On XLD plates ***Entercocci*** and ***E. coli*** give yellow colonies while ***Salmonella*** and ***Shigella*** give red/black and red colonies, respectively. Lysine is added to differentiate ***Salmonella*** organisms carrying lysine decarboxylase which acts on lysine to create alkaline conditions. High acid production by fermentation of sugars (xylose, lactose, and saccharose) in the medium inhibit other lysine – positive organisms.

On the XLT4 agar plates both ***Staphylococci*** and ***Escherichia & Enterococci*** are inhibited while ***Salmonella*** grow into yellow to red colonies with black centers.

6. ***Isolation of specific organisms in pure cultures***.

a) ***Detection and isolation of Salmonella and Shigella organisms using selective media***

From Bismuth Sulfite Agar plates 12 colonies of Salmonella were obtained for further testing.

From XLT4 Agar plates 18 colonies representing Salmonella were isolated.

Thus, a total of 30 Salmonella colonies were obtained for testing of their antibiotic resistance.

For the isolation of Shigella organisms, HEK and XLD agar plates were used. A total of 30 colonies were picked at random from HEK agar plates (12 colonies) and XLD agar plates (18 colonies).

b) ***Detection and isolation of Staphylococci organisms using selective medium***.

Procedures outlined in the Manual of Health Protection Branch (MFHPB-21) were used for the detection and isolation of staphylococci. Baird Parker Agar contains glycine and sodium pyruvate which stimulate growth of ***Staphylococci***. Lithium chloride and potassium tellurite in the medium suppress the growth of organisms other than ***Staphylococci***. Coagulase positive ***Staphylococci*** yield clear zone around colonies while opaque zone of precipitation may form due to lipase activity. Reduction of potassium ellurite causes blackening of the colonies.

c) ***Detection and isolation of Listeria organisms using selective media.***

Isolation and detection protocols of Hitchins (1992a) were used for isolation of ***Listeria*** organisms. BactoOxford agar medium containing oxford antimicrobic supplement is highly selective for isolating and differentiating ***Listeria*** organisms. This organism can cause human illness and death, particularly in immuno-compromised individuals and pregnant women.

Implicated vehicle of transmission include food items and water. These organisms are ubiquitous in nature, being present in a wide range of unprocessed foods, and in soil, sewage, silage, and river water.

Oxford agar containing esculin make ***Listeria*** colony and surrounding medium black due to hydrolysis of esculin and formation of hydroxyl-coumarine which reacts with ferric chloride ions in the medium. Lithium chloride and high salt content in the medium are selective for ***Listeria. Enterococci*** are sensitive to high salt content. Selectivity is increased by various antimicrobial agents present in the antimicrobic supplement.

Bacto PALCAM medium base with antimicrobic supplement is used in isolating and cultivating ***Listeria*** from various types of samples. This medium is recommended by Health Canada for the detection of ***Listeria monocytogene***s in food and environmental samples. Selectivity of the complete medium is achieved by incorporating antimicrobic supplement in the agar medium. The antimicrobic agents effectively suppress growth of most commonly occurring ***non-Listeria*** species of bacteria. Esculin functions in the same way as it does in the oxford medium.

7. ***Identification of Enterobacteriaceae and other non-fastidious, Gram-negative rods.***

API-20 E is a standardized identification system for Enterobacteriaceae and other non-fastidious Gram-negative rods which use 21 miniaturized biochemical tests and a database. The complete list of those organisms that is possible to identify with this system is given in the Identification Table and includes, amongst them, organisms included in the present study.

The API20-E strip consists of 20 micro-tubes containing dehydrated substrates. These micro-tubes are inoculated with bacterial suspensions that reconstitutes the media. During incubation, metabolism produces colour changes that are either spontaneous or revealed by the addition of reagents.

The reactions are read according to the Reading Table and the identification is obtained by referring to the Analytical Profile index or using the identification software. In the current study Analytical Profile index was used.

Table 27 lists selected isolates (Gram-negative) that were confirmed using API system.

8. ***Gram Staining:***

The Gram staining is a very useful technique for identifying and classifying bacteria. The Gram stain is a differential stain that allows one to classify bacteria as either gram-positive (stained blue or purple) or gram negative (stained red or pink). It is one of the first procedures to be performed for the identification of bacteria. Bacterial classification schemes are based on the gram-nature of bacterial cells.

Isolates were randomly picked from each group to confirm their Gram-nature, and are listed in Table 28.

Results

A. *Coliforms:*

Table 1 shows the presence of ***E. coli*** in both types of samples, i.e. sediment samples G1 – G5 as well as water column samples W1 – W5. ***E. coli*** numbers were higher in the sediment samples when compared to water column samples.

Sediment sample G5 showed the highest counts with 13,500 colony forming units per gm. (cfu/g) while sample G2 had a lower value of 2,400 per gm. High counts of 3700 per ml and a low of 100 colony forming units per ml were detected in samples W3 and W2, respectively.

Coliform content of the samples showed higher counts when samples were analysed using the MPN-method. Table 2 shows the coliform MPN per 100 ml or gm. of the samples. Sediment samples (G1 – G5) in general yielded higher MPN values than the water column samples (W1 – W5). Samples G4 and G5 showed the highest counts of 110,000 MPN per 100g. In contrast, the water column samples gave an elevated value of 46,000 MPN per 100 ml (W5), which is less than 50% of the value detected for G4 or G5.

B. *E. coli:*

Estimation of ***E. coli*** MPN revealed that with EC broth tubes incubated at 45^{o}C, the values doubled (Table 3) compared to similar values for coliforms (Table 2). A high MPN value of 240,000 per 100 g sediment samples was obtained with G4 and G5. MPN values for water-column samples under similar conditions ranged between 700 and 21,000 (Table 3). Thus, there was a ten-fold difference in the values for sediment and water column samples.

C. *Listeria:*

No ***Listeria*** organisms were detected in the majority of water- column samples (W1, W3, W4, and W5) when plating was performed on selective medium such as PALCAM (Table 4). Only one sample (W2) showed the presence of ***Listeria.*** In contrast, the majority of sediment samples (G2 – G5) revealed the presence of this organism. A high count of 10,400 cfu / g was obtained for G2 while a low count of 100 cfu / g was estimated for samples W2.

Surprisingly, W1 was the only sample that detected the presence of ***Listeria*** when plating was performed on Oxford agar plates (Table 5). On the same medium, the sediment samples (G2, G3, and G5) gave positive results. No growth was detected with samples G1 and G4. Sample G2 showed the highest count of 18,300 cfu / g while counts in samples, W1, G3, and G5 was about 3,700 cfus.

D. ***E. coli and Enterococci:***

Selective medium such as XLD agar enhances the growth of ***E. coli-Enterococci***, and ***Shigella*** (Table 6) with each of the organisms displaying characteristic colony morphology. On this selective medium both ***E. coli-Enterococci*** group and ***Shigella*** organisms were detected. Only one sample, G5, showed the presence of ***Shigella*** while evidence of the ***E. coli-Enterococci*** group could be detected in samples G1, G3, G4, and G5. Only water column samples W1, W2, and W5, as well as sediment sample G5, revealed the presence of low counts (100 – 300 cfu / gm or ml) of ***Shigella*** (Table 6).

E. ***E. coli and Salmonella*****:**

On selective medium like XLT4 agar, it is possible to detect the growth of ***E.coli*** and ***Salmonella*** simultaneously. ***E. coli*** was detected using this medium in samples G1, G2, G4, and G5, as well as samples W2, W4, and W5. Thus, seven out of ten samples showed the presence of ***E. coli***. In contrast, only four samples (G2, G5, W4, and W5) revealed the presence of ***Salmonella*** (Table 7). Highest counts of 3,400 cfu / g were estimated for ***Salmonell***a (G5).

F. ***Staphylococci*****:**

Baird Parker agar plates are suitable for detecting ***Staphylococcus*** in unknown samples. This selective medium enhances the proliferation of these organisms which form black colonies that are easily visualised on BP agar plates.

Two sediment samples (G2 and G5) revealed the presence of staphylococci organisms. These organisms were not detected in water column samples W1-W5 (Table 8). Sediment sample G2 revealed a high count of 14,700 cfu / g while the numbers decreased by ten-fold in G5.

G. ***Salmonella and Shigella:***

Hektoen agar plates are commonly used to detect these organisms which form characteristic colonies. ***Salmonella*** was detected in samples G1, G5, W1, W2, W4 and W5 when HEK plates were inoculated with a loopful of samples from selenite-cystin broth cultures. Thus, four out of five water column samples were positive for ***Salmonella,*** whereas only two sediment samples (G1 and G5) were positive. In contrast, nine out of ten samples revealed the presence of ***Shigella*** (Table 9). The detection of ***Salmonella*** on HEK plates inoculated with loopfuls of innocula from tetrathinonate broth cultures revealed the distribution shown in Table 10. All sediment samples were positive for ***Salmonella*** together with sample W1. Samples G2, G4, G5 and W1 only showed the presence of ***Shigella*** organisms (Table 10).

On selective XLD agar plates inoculated with loopfuls of inocula from selenite cystin broth cultures, the distribution of ***Salmonella*** and ***Shigella*** is depicted in Table 11. All sediment samples (G1 – G5) were positive for ***Salmonella*** but all water column samples (W1 – W5) were negative. ***Shigella*** organisms were detected in sediment samples G1, G5, and water column sample W1 only. All samples were tested positive for E. coli (Table 11). On repeating the tests using inoculum from tetrathionate broth cultures, the results changed drastically (Table 12). Four sediment samples (G1, G3, G4, and G5) tested positive for ***Shigella***. All water column samples (W1 - W5) tested negative for ***Shigella***. ***E. coli*** was tested positive in only three samples (G1,G2 and G5). The remaining seven samples tested negative on XLD agar plates (Table12).

Bismuth sulfite agar is also selective medium that allows the growth of ***Salmonella*** and ***E. coli***. When samples from selenite – cystin broth cultures were used as inocula and inoculated on BS agar plates, the growth pattern observed is shown in Table 13. All the samples tested positive for ***Salmonella*** and ***E. coli*** (Table 13). All ten samples tested positive for ***Salmonella*** and ***E. coli.***

H. ***Purification of isolates:***

Tables 14, 15, 16, 17 and 18 list 30 isolates each of ***Listeria, E. coli***, ***Staphylococci, Salmonella***, and ***Shigella***, respectively. Single colonies were sub-cultured to purify the isolates. From 30 colonies of each genera about 15 – 20 were tested for their sensitivity to about 15 different antibiotics.

I. ***Antibiotic sensitivity of selected isolates:***

Table 19 and 20 list ***Listeria*** isolates and their response to 15 different antibiotics. It is evident from these Tables that these isolates vary in their response to these antibiotics. Some isolates are resistant to specific antibiotics in varying degrees.

It is evident that some isolates listed in Table 19 are resistant to nalidixic acid (NA), gentamycin (G), and erythromycin (E). The results in Table 20 indicate that almost all of the Listeria isolates tested were resistant to bacitracin (B) while others were resistant to streptomycin (S) and oxacillin (OX).

All twenty one (21) isolates of ***E. coli*** were resistant to OX, B, and penicillin (P) while EMB -18, 19, 21, and 22 were resistant to ampicillin (AM). Isolate EMB-21 was also resistant to carbecillin (CB) (Table 21). Table 21 also shows that isolate EMB-21 was resistant to NA, G, and trimethoprim (TMP) and slightly resistant to oxytetracycline (OT). Isolate EMB-26 showed resistance to G and E to a lesser degree.

Table 23 shows that some ***Staphylococcus*** isolates exhibited slight resistance to B and P. In Table 24, isolates BP-13, 15, 17, 18, 20, and 26 were resistant to E while isolates BP-12 and BP-20 were resistant to G.

The majority of the ***Salmonella*** isolates were resistant to OX, B, and P and slightly resistant to S. Isolate XLT4 – 23 was found to be resistant to PB (polymyxin B) and slightly resistant to AM (Table 25). Table 26 indicates that all ***Salmonella*** isolates tested were resistant to E and slightly to G.

Table 27 gives a summary of ***Salmonella, E. coli***, and ***Shigella*** species that were identified using the API system. For practical reasons only a limited number of isolates from each genus were tested.

Table 28 lists isolates that were subjected to Gram staining procedures. Their response to this staining method is given in this table.

J. ***Observed drug resistance in the isolates:***

Listeria *species*.

Isolate 02 resistant to NA, G, B, & S
Isolate 07 resistant to E, Ox, & B

E. coli.

Nineteen (19) isolates were resistant of OX, B, & P
Five (5) isolates were resistant to OX, B, P, & AM
One (1) resistant to NA, C, & TMP
One (1) resistant to NA
One (1) resistant to G

Staphylococci.

Two (2) resistant to G & E
Six (6) resistant to E
One (1) resistant to G & E

Salmonella *species*

Fifteen (15) resistant to OX, B, P, E
One (1) resistant to OX, B, P, & AM
One (1) resistant to OX, B, P, & PB

Thus, it is evident that some of the isolates carry multiple drug resistance and can pose a potential health hazard to the public if contaminated marine life forms are consumed and originate in the

vicinity of the harbour.

Discussion

In interpreting the results of this research study, a number of questions were raised which deserve further exploration. While recognizing the limitations that exist within a research project of this size, these questions are identified below in an effort to provide the best possible answers with the information available at the present time.

Q1. Do any of the species identified have natural resistance to antibiotics?

Natural resistance is due to genetic make up of an organism. Micro-organisms may spontaneously mutate against a given trait in their environment once every 100,000 to1,000,000 cell divisions. Because of their rapid multiplication rates, the chance of microbial mutations against a given antimicrobial agent is quite probable. In this situation, the mutant may rapidly multiply in the presence of antibiotic and produce many resistant progeny.

In addition to spontaneous mutations genetic resistance may be passed from one bacterium to another by small circular extra-chromosomal DNA fragments called 'resistant plasmids' or 'R factors'. A resistance plasmid may contain the genetic information that codes for resistance to one or several antibacterial agents(multiple drug resistance); when the plasmid is passed to a new cell, the trait of antibiotic resistance is also passed.

The accumulation of antibiotics in the environment speeds up the evolution of drug resistant bacteria which begin to dominate since the sensitive ones are destroyed.

There are three mechanisms by which drug resistance can be transferred amongst bacterial populations:

a. Conjugating cells transfer plasmid from a donor cell to a recipient cell. Thus a resistant bacterium can transfer plasmid to a sensitive bacterial cell which now becomes resistant having acquired the R factor.

b. Transformation is a process in which a bacterial cell is capable of taking up (absorbing) fragments of DNA into its cytoplasm where a recombination event takes place and the acquired DNA fragment is integrated into the host chromosome. This integrated fragment encodes genetic information for drug resistance.

c. Bacterial viruses (bacteriophages, phages) play a role in transferring genetic information from one bacterium to another by a process called

transduction. If the fragment or gene transferred is related to the drug resistance, then the recipient cell becomes resistant as well.

Q2. Are isolates picking up this resistance from the other bacteria in the harbour or because of treatment of human hosts with antibiotics?

Transfer of drug resistance (R factor) amongst bacterial communities is a common phenomenon. More work is needed to confirm if the drug resistant bacteria isolated carry plasmids and if conjugation is occurring between different species of the same genus, or whether there is also inter-generic transfer as well.

Flushing of antibiotics into the sewer system creates an ideal environment for rapid evolution of drug resistant bacteria. The presence of antibiotics in the environment allows the selection of resistant variety which begin to rapidly multiply without competition.

Q3. Comparison of sampling sites to show relative abundance of drug resistant populations.

There is not enough data to enable us to draw any conclusions with respect to the abundance of resistant populations at various sites sampled.

Q4. Are all of the antibiotics used broad spectrum antibiotics?

The majority of the fifteen (15) antibiotics used in the tests were broad Spectrum, i.e. effective against a wide variety of bacteria including gram positive and gram negative cells. Five (nalidixic, polymyxin B, erythromycin, bacitracin and oxacillin) are considered narrow spectrum i.e. effective against a limited type of bacteria. Please refer to Tables 20 & 21 of the report.

Conclusions

1. Enteric pathogens together with ***Listeria*** and ***Staphylococcus*** were present in sediment and water column samples from St. John's Harbour.

2. Five different types of bacterial species were confirmed in ten (10) samples that were analysed for their microbial contents. These included ***E. coli***, ***Salmonella***, ***Listeria***, ***Staphylococcus*** and ***Shigella.***

3. Sediment samples showed higher bacterial numbers than water column samples.

4. About 140 isolates representing five different genera were characterized with respect to their gram nature and sensitivity to fifteen different antibiotics.

5. Selected isolates were further confirmed using an API identification system.

6. Exposure of isolates to 15 different antibiotics suggests there is a potential for accumulation of drug resistant bacteria in the harbour.

Table 1

Enumeration of ***E. coli*** on selective medium, Eosine Methylene Blue, EMB, Agar Plates

Sample	cfu / ml or gm
G1	7700
G2	2400
G3	3700
G4	12500
G5	13500
W1	800
W2	100
W3	3700
W4	800
W5	1100

Cfu – colony forming units
G1 – G5 - Sediment samples
W1 – W5 - Water column samples

Table 2

Determination of ***Coliform*** MPN using 3-Tube Series of BGB-broth tubes

Sample	MPN / 100gm or 100 ml
G1	9,300
G2	4,300
G3	9300
G4	110,000
G5	110,000
W1	4,300
W2	2,300
W3	21,000
W4	24,000
W5	46,000

MPN – Most Probable Number
G-1 - G-5 - Sediment samples
W-1 - W-5 - Water column samples
Incubation Temperature, 27^{o}C

Table 3

Determination of ***E.coli*** MPN using 3-Tube Series and EC broth tubes

Sample	***E. coli*** MPN / 100g or 100ml
G-1	2,800
G-2	110,000
G-3	15,000
G-4	240,000
G-5	240,000
W-1	700
W-2	6,400
W-3	6,400
W-4	1,400
W-5	21,000

MPN - Most Probable Number
G1- G5 - Sediment samples
W1-W5 - Water column samples
Incubation temperature, 45^{o}C
EC - ***E. coli*** broth

Table 4

Enumeration of ***Listeria*** organisms on a selective medium - PALCAM supplemented with Antimicrobial Supplement

Sample	cfu / ml or gm
G1	nil, no growth
G2	10,400
G3	1,700
G4	5,000
G5	3,600
W1	nil, no growth
W2	100
W3	nil, no growth
W4	nil, no growth
W5	nil, no growth

Cfu - colony forming unit
G1 – G5 - Sediment samples
W1- W5 - Water column samples

Table 5

Enumeration of ***Listeria*** organisms on a selective medium Bacto Oxford medium containing Bacto Oxford Antimicrobic Supplement

Sample	cfu / ml or gm
G1	nil, no growth
G2	18,300
G3	3,600
G4	nil, no growth
G5	3,700
W1	3,700
W2	nil, no growth
W3	nil, no growth
W4	nil, no growth
W5	nil, no growth

Cfu – colony forming unit
G1 - G5 - Sediment samples
W1 - W5 - Water column samples

Table 6

Estimation of ***E. coli*** and ***Enterococci*** numbers on a selective medium, Xylose, Lysine, Deoxycholate Agar (XLD) Plates

Sample	cfu / ml or gm	
	E.coli/Enterococci gr.	***Shigella*** organisms
G1	200	nil
G2	nil	nil
G3	200	nil
G4	1800	nil
G5	2000	300
W1	100	100
W2	100	100
W3	100	nil
W4	200	nil
W5	400	100

Cfu - colony forming units

G1- G5 - Sediment samples
W1 – W5 – Water column samples
E. coli and ***Enterococci*** colonies can not be distinguished on this medium and hence are presented as a group

Table 7

Detection of ***E. coli*** and ***Salmonella*** organisms on a selective medium, XLT4 Agar Plates

Sample	cfu / ml or gm	
	E. coli	***Salmonella***
G1	100	nil
G2	100	1200
G3	nil	nil
G4	1200	nil
G5	4100	3400
W1	nil	nil
W2	100	nil
W3	nil	nil
W4	200	1500
W5	300	500

XLT - xylose, lysine, sodium thiosulfate agar medium

Table 8

Detection of ***Staphylococci*** organisms on a selective medium, Baird Parker Agar Plates

Sample	cfu/ gm or ml
G1	nil
G2	14,700
G3	nil
G4	nil
G5	1,000
W1	nil
W2	nil
W3	nil
W4	nil
W5	nil

Cfu - colony forming units
Baird Parker Agar is selective for staphylococci

Table 9

Detection of ***Salmonella*** and ***Shigella*** on selective medium, Hektoen Agar Plates.

Sample	Growth	
	Salmonella	***Shigella***
G1	positive	negative
G2	negative	positive
G3	negative	positive
G4	negative	positive
G5	positive	positive
W1	positive	positive
W2	positive	positive
W3	negative	positive
W4	positive	positive
W5	positive	positive

HEK plates were inoculated with liquid cultures from selenite cystine broth.
Plates were incubated at 37^{o}C

Table 10

Detection of ***Salmonella*** and ***Shigella*** on selective HEK plates inoculated with Tetrathionate broth cultures

Sample	Growth	
	Salmonella spp	***Shigella spp***
G1	positive	negative
G2	positive	positive
G3	positive	negative
G4	positive	positive
G5	positive	positive
W1	positive	positive
W2	negative	negative
W3	negative	negative
W4	negative	negative
W5	negative	negative

Table 11

Detection of ***Salmonella*** and ***Shigella*** on XLD agar plates inoculated with selenite-cystine broth cultures

Sample	Growth		
	Salmonella spp	***E. coli***	***Shigella spp***
G1	negative	positive	positive
G2	positive	positive	negative
G3	positive	positive	negative
G4	positive	positive	negative
G5	positive	positive	positive
W1	negative	positive	positive
W2	negative	positive	negative
W3	negative	positive	negative
W4	negative	positive	negative
W5	negative	positive	negative

Table 12

Detection of ***Salmonella, Shigella and E. coli*** on XLD agar plates inoculated with Tetrathionate broth cultures

Sample	Growth		
	Salmonlla spp	***E. coli***	***Shigella***
G1	positive	positive	positive
G2	negative	positive	negative
G3	positive	negative	positive
G4	positive	negative	positive
G5	positive	positive	positive
W1	positive	negative	negative
W2	positive	negative	negative
W3	positive	negative	negative
W4	positive	negative	negative
W5	positive	negative	negative

Table 13

Detection of ***Salmonella and E.coli*** on Bismuth Sulfite Agar plates inoculated with selenite-cystine broth cultures

Sample	Growth	
	Salmonella spp	***E. coli***
G1	positive	positive
G2	positive	positive
G3	positive	positive
G4	positive	positive
G5	positive	positive
W1	positive	positive
W2	positive	positive
W3	positive	positive
W4	positive	positive
W5	positive	positive

Table 14

Isolation of ***Listeria*** organisms from selective media plates (PALCAM, And Oxford agar plates

Sample	Isolate	
	From PALCAM plates	From Oxford plates
G2	P1, P2	O10, O11, O12, O13
G4	P3, P4, P5, P6, P15	O1, O2, O3, O4,
G3	P7, P8	O4, O5
G5	P9, P10, P11, P12, P13	O7, O8. O9, O6, O15

Table 15

Isolation of ***E. coli*** from EMB plates

Sample	Isolate
W4	EMB1, EMB2
W5	EMB3, EMB4, EMB5, EMB6
G1	EMB7, EMB8
G3	EMB9, EMB10, EMB11, EMB12,
G4	EMB13, EMB14, EMB15, EMB16, EMB17, EMB18, EMB19, EMB26, EMB29
G5	EMB20, EMB21, EMB22, EMB24, EMB25, EMB30
G2	EMB27

EMB - Eosine Methylene Blue Agar

Table 16

Isolation of ***Salmonella*** organisms from selective medium, Baird Parker Plates

Sample	Isolate
G2	BP1, BP2, BP3, BP4, BP5, BP6, BP7, BP8, BP9, BP10 BP11, BP12, BP13, BP14, BP15, BP16, BP17, BP18, BP19, BP20, BP21, BP22, BP23
G3	B24
G1	BP25, BP26, BP27, BP28, BP29, BP30

Table 17

Isolation of ***Salmonella*** from Bismuth Sulfite Agar Plates

Sample	Isolate
W1	BS1, BS2, BS7
W4	BS3, BS4, BS10
W5	BS5, BS6, BS11, BS28, BS29, BS30
W2	BS8
W3	BS9
G1	BS12, BS17, BS25, BS26, BS27
G2	BS13, BS18
G3	BS14, BS19
G4	BS15, BS20
G5	BS16, BS21, BS22, BS23, BS24

Table 18

Isolation of ***Shigella*** organisms from Hektoen (HEK) Agar Plates

Sample	Isolate
G3	HEK1, HEK2, HEK3, HEK4, HEK5, HEK6, HEK7, HEK8
G5	HEK9, HEK10, HEK11, HEK12, HEK 13, HEK14 HEK15, HEK16 HEK17, HEK18, HEK 17, HEK 18, HEK 19, HEK20, HEK21, HEK-22, HEK-24, HEK25, HEK22, HEK23, HEK24, HEK25, HEK26, HEK28, HEK29, HEK30

Table 19

Sensitivity of ***Listeria*** isolates to various Antibiotics

	ANTIBIOTICS						
Isolate	NA	G	E	TMP	TE	CB	OT
P1	S(3)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
P2	S(2)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
P3	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
P4	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
P5	S(3)	S(3)	S(2)	S(3)	S(3)	S(2)	S(3)
P6	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
P7	S(3)	S(3)	S(2)	S(3)	S(3)	S(2)	S(3)
P8	S(3)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
P9	S(3)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
P10	S(3)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
P11	S(3)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
P15	S(2)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
O1	S(2)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
O2	R	R	S(3)	S(3)	S(3)	S(2)	S(3)
O4	S(2)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
O5	S(1)	S(2)	S(3)	S(2)	S(2)	S(3)	S(3)
O6	S(1)	S(2)	S(3)	S(2)	S(2)	S(3)	S(3)
O7	S(1)	S(1)	R	S(2)	S(2)	S(2)	S(2)

NA-nalidixic acid, (30ug); G –gentamycin (25ug); TMP – trimethoprim(5ug);
TE –tetrcycline(30ug); CB – carbini cillin (30ug); OT -oxytetracycline;
E – erythromycin (15ug)

Numbers in parenthesis represent diameter of zone of inhibition:
S(1) – 12mm; S(2) – 16mm; S(3) – 26 mm

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Table 20

Sensitivity of ***Listeria*** isolates to selected Antibiotics

Isolate	Antibiotic							
	OX	K	B	P	PB	AM	S	CB
P1	S(2)	S(2)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)
P2	S(1)	S(3)	R	S(1)	S(1)	S(2)	S(2)	S(3)
P3	S(2)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
P4	S(2)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
P5	S(1)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
P6	S(1)	S(3)	R	S(3)	S(3)	S(2)	S(3)	S(3)
P7	S(2)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
P8	S(2)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
P9	S(2)	S(3)	R	S(3)	S(2)	S(3)	S(2)	S(3)
P10	S(2)	S(3)	R	S(3)	S(2)	S(3)	S(2)	S(3)
P11	S(2)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
P15	S(1)	S(3)	R	S(3)	S(2)	S(3)	S(2)	S(3)
O1	S(1)	S(3)	R	S(1)	S(2)	S(2)	S(2)	S(3)
O2	S(1)	S(3)	R	S(3)	S(1)	S(3)	R	S(3)
O4	S(1)	S(3)	R	S(3)	S(2)	S(3)	S(2)	S(3)
O5	S(1)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
O6	S(1)	S(3)	R	S(3)	S(3)	S(3)	S(2)	S(3)
O7	R	S(3)	R	S(2)	S(2)	S(2)	S(1)	S(2)

OX –oxacillin (1ug); K– kanamycin (30ug): B –bacitracin (10 units); P –penicillin (10 units) ; PB –polymyxin B (30 units); AM –ampicillin (10ug); S –streptomycin (10ug); CB –carbecillin (100ug)

Numbers in parenthesis represent diameter of zone of inhibition: S(1) – 12mm; S(2) – 16mm ; S(3) – 26mm

S = sensitive; R = resistant

Table 21

Sensitivity of ***E. coli*** isolates to selected antibiotics

	Antibiotic							
Isolate	OX	K	B	P	PB	AM	S	CB
EMB-1	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)
-3	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)
-4	R	S(3)	R	R	S(2)	S(1)	S(2)	S(1)
-5	R	S(3)	R	R	S(2)	S(1)	S(2)	S(1)
-6	R	S(3)	R	S(1)	S(2)	S(1)	S(2)	S(1)
-7	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)
-11	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)
-13	R	S(3)	R	R	S(2)	S(1)	S(2)	S(1)
-17	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)
-18	R	S(3)	R	R	S(2)	R	S(2)	S(1)
-18A	R	S(3)	R	R	S(2)	R	S(2)	S(1)
-19	R	S(3)	R	R	S(2)	R	S(2)	S(1)
-21	R	S3)	R	R	S(2)	R	S(2)	R
-22	R	S(3)	R	R	S(2)	R	S(2)	S(1)
-23	R	S(3)	R	R	S(2)	S(1)	S(2)	S(1)
-24	S(1)	S(3)	R	R	S(2)	S(1)	S(2)	S(1)
-25	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)
-26	R	S(3)	R	R	S(2)	S(2)	S(2)	S(3)
-28	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)
-30	R	S(2)	R	R	S(2)	S(1)	S(2)	S(1)

OX –oxacillin (1ug); K –kanamycin (30 ug); B –bacitrcin (10 units) ; P –penicillin (10 units) ; PB –polymyxin B (30 units) ; AM –ampicillin (10 ug) ; S –streptomycin (10 ug) CB – carbecillin (100 ug)

Numbers in parenthesis represent diameter of zone of inhibition:
S(1) = 12mm ; S(2) = 16 mm ; S(3) = 26 mm

S = sensitive; R = resistant

Table 22

Sensitivity of ***E. coli*** isolates to selected Antibiotics

	Antibiotics					
Isolate	NA	G	E	TMP	CB	OT
ENB-1	S(2)	S(2)	S(1)	S(3)	S(3)	S(3)
-3	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)
-4	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)
-5	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)
-6	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)
-7	S(2)	S(3)	S(1)	S(3)	S(3)	S(3)
-11	S(2)	S(3)	S(1)	S(3)	S(3)	S(3)
-13	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)
-17	S(2)	S(1)	S(1)	S(3)	S(3)	S(3)
-18	S(1)	S(3)	S(1)	S(3)	S(3)	S(3)
-18A	S(1)	S(3)	S(1)	S(3)	S(3)	S(3)
-19	S(2)	S(3)	S(1)	S(2)	S(3)	S(3)
-21	R	R	S(1)	R	S(2)	S(1)
-22	R	S(3)	S(1)	S(2)	S(3)	S(3)
-23	S(1)	S(3)	S(1)	S(3)	S(3)	S(3)
-24	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)
-25	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)
-26	S(3)	R	S(1)	S(3)	S(2)	S(3)
-28	S(3)	S(2)	S(1)	S(2)	S(2)	S(2)
-30	S(1)	S(3)	S(1)	S(3)	S(3)	S(3)

NA –nalidixic acid (30 ug); G –gentamycin (25 ug); TMP –trimethoprim (5 ug);
TE –tetracycline (30ug); CB –carbinicillin (30ug); OT –oxytetracycline (30ug);
E –erythromycin (15 ug)

Numbers in parenthesis represent diameter of zone of inhibition:
S(1) = 12 mm; S(2) = 16 mm ; S(3) = 26 mm

S = sensitive; R = resistant

Table 23

Sensitivity of ***Staphylococci*** isolates to selected Antibiotics

Isolates	Antibiotic							
	OX	K	B	P	PB	AM	S	CB
BP2	S(1)	S(3)	S(2)	S(2)	S(2)	S(3)	S(2)	S(3)
BP3	S(2)	S(3)	S(2)	S(3)	S(3)	S(3)	S(2)	S(3)
BP4	S(3)	S(3)	S(2)	S(3)	S(3)	S(3)	S(2)	S(3)
BP5	S(2)	S(3)	S(2)	S(3)	S(3)	S(3)	S(2)	S(3)
BP6	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
BP7	S(3)	S(3)	S(2)	S(2)	S(2)	S(3)	S(2)	S(3)
BP9	S(2)	S(3)	S(1)	S(1)	S(2)	S(3)	S(2)	S(3)
BP10	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(2)	S(3)
BP11	S(3)	S(3)	S(2)	S(3)	S(3)	S(3)	S(2)	S(3)
BP12	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
BP13	S(3)	S(3)	S(1)	S(2)	S(2)	S(3)	S(2)	S(3)
BP14	S(3)	S(3)	S(2)	S(3)	S(2)	S(3)	S(2)	S(3)
BP15	S(2)	S(3)	S(1)	S(1)	S(2)	S(2)	S(2)	S(3)
BP16	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)	S(3)
BP17	S(2)	S(3)	S(1)	S(2)	S(2)	S(3)	S(2)	S(3)
BP18	S(2)	S(3)	S(1)	S(3)	S(2)	S(3)	`S(2)	S(3)
BP19	S(3)	S(3)	S(2)	S(3)	S(3)	S(3)	S(3)	S(3)
BP20	S(2)	S(3)	S(1)	S(3)	S(2)	S(2)	S(3)	S(3)
BP26	S(2)	S(3)	S(1)	S(2)	S(2)	S(2)	S(2)	S(3)

OX –oxacillin (1 ug); K-kanamycin (30 ug); B-bacitracin (10 units); P-penicillin (10 units); PB-polymyxin B (30 units); AM- ampicillin (10 ug); S-streptomycin (10 ug); CB- carbecillin (100 ug).

Numbers in parenthesis represent diameter of zone of inhibition:
S(1) = 12 mm; S(2) = 16 mm: S(3) = 26 mm.

S = sensitive; R = resistant

Table 24

Sensitivity of ***Staphylococci*** isolates to selected Antibiotics

Isolates	Antibiotics						
	NA	G	E	TMP	TE	C	OT
BP2	S(2)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)
BP3	S(3)	S(1)	S(1)	S(20	S(3)	S(3)	S(3)
BP4	S(3)	S(2)	S(1)	S(2)	S(3)	S(3)	S(3)
BP5	S(3)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)
BP6	S(3)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)
BP7	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)
BP8	S(1)	S(1)	S(1)	S(3)	S(3)	S(3)	S(3)
BP9	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)	S(3)
BP10	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)	S(3)
BP11	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)	S(3)
BP12	S(3)	S(3)	S(1)	S(3)	S(3)	S(3)	S(3)
BP13	S(1)	R	R	S(2)	S(3)	S(3)	S(3)
BP14	S(2)	S(3)	S(1)	S(3)	S(3)	S(3)	S(3)
BP15	S(3)	S(1)	R	S(2)	S(3)	S(3)	S(3)
BP16	S(3)	S(3)	S(2)	S(3)	S(3)	S(3)	S(3)
BP17	S(1)	S(3)	R	S(2)	S(3)	S(3)	S(3)
BP18	S(1)	S(2)	R	S(2)	S(3)	S(3)	S(3)
BP19	S(3)	S(3)	S(2)	S(3)	S(3)	S(3)	S(3)
BP20	S(1)	R	R	S(1)	S(3)	S(3)	S(3)
BP26	S(1)	S(3)	R	S(3)	S(3)	S(3)	S(3)

NA-nalidixc acid (30 ug); G- gentamycin(25 ug); TMP- trimethoprim (5 ug): TE- tetracycline (30 ug); C- carbinicillin (30ug); OT- oxytetracycline (30 ug); E-erythromycin (15 ug).

Number in parenthesis represent diameter of zone of inhibition:
S(1) = 12 mm. S(2) = 16 mm; S(3) = 26 mm

S = sensitive; R = resistant

Table 25

Sensitivity of ***Salmonella*** isolates to selected Antibiotic

Isolates	Antibiotics							
	OX	K	B	P	PB	AM	S	CB
BS1	R	S(2)	R	R	S(1)	S(1)	S(1)	S(1)
BS3	R	S(2)	R	R	S(2)	S(1)	S(1)	S(2)
BS5	R	S(2)	R	R	S(2)	S(1)	S(1)	S(2)
BS7	R	S(2)	R	R	S(2)	S(1)	S(2)	S(2)
BS11	R	S(2)	R	R	S(2)	S(1)	S(2)	S(2)
BS12	R	S(2)	R	R	S(2)	S(1)	S(1)	S(1)
BS16	R	S(1)	R	R	S(1)	S(1)	S(1)	S(2)
BS17	R	S(2)	R	R	S(2)	S(1)	S(2)	S(2)
BS21	R	S(2)	R	R	S(1)	S(1)	S(1)	S(2)
BS22	R	S(1)	R	R	S(1)	R	S(1)	S(2)
BS25	R	S(2)	R	R	S(2)	S(1)	S(2)	S(2)
XLT4-23	R	S(2)	R	R	R	S(1)	S(2)	S(2)
XLT4-26	R	S(2)	R	R	S(2)	S(1)	S(1)	S(2)
XLT4-27	R	S(2)	R	R	S(1)	S(1)	S(1)	S(2)
XLT4-30	R	S(3)	R	R	S(2)	S(1)	S(2)	S(3)

OX-oxacillin (1ug); K-kanamycin(30 ug); b-bacitracin (10 units); P- penicillin (10 units); PB- polymyxin B (30 units); AM-ampicillin (10 ug); S-streptomycin (10 ug); CB-carbecillin (100ug)

Numbers in parenthesis represent diameter of zone of inhibition:
S(1) = 12 mm; S(2) = 16 mm; S(3) = 26 mm

S = sensitive to antibiotic; R = resistant to antibiotic

Table 26

Sensitivity of ***Salmonella*** isolates to selected Antibiotics

Isolates	Antibiotics					
	NA	G	E	TMP	C	OT
BS1	S(2)	S(1)	R	S(2)	S(2)	S(2)
BS3	S(1)	S(1)	R	S(2)	S(2)	S(2)
BS5	S(2)	S(2)	R	S(2)	S(2)	S(2)
BS7	S(1)	S(1)	R	S(3)	S(3)	S(3)
BS11	S(2)	S(1)	R	S(3)	S(2)	S(2)
BS12	S(2)	S(1)	R	S(2)	S(2)	S(2)
BS16	S(2)	S(1)	R	S(2)	S(2)	S(2)
BS17	S(2)	S(2)	R	S(2)	S(2)	S(2)
BS21	S(1)	S(2)	R	S(2)	S(3)	S(2)
BS22	S(2)	S(1)	R	S(2)	S(2)	S(2)
BS25	S(2)	S(2)	R	S(2)	S(2)	S(2)
XLT4-23	S(2)	S(1)	R	S(2)	S(1)	S(2)
XLT4-26	S(2)	S(2)	R	S(2)	S(3)	S(3)
XLT4-27	S(2)	S(1)	R	S(2)	S(2)	S(2)
XLT4-30	S(2)	S(1)	R	S(1)	S(2)	S(1)

NA-nalidixic acid (30ug); G-gentamycin (25 ug); TMP-trimethoprim (5 ug);
TE –tetracycline (30 ug); CB-carbinicillin (30 ug); OT-oxytetracycline (30 ug);
E- erythromycin (15 ug)

Numbers in parenthesis represent diameter of zone of inhibition:
S(1) = 12 mm; S(2) = 16 mm; S(3) = 26 mm

S = sensitive to antibiotic; R = resistant to antibiotic

Table 27

Bacterial isolates identified by the API system

Genus	Isolate #
Salmonella	**BS – 1, 5, 11, 16, 17, 22, 25, & XLT4-27**
E. coli	**EMB – 3, 5, 7, 13, 18, 21, 24, 25, 26, 30**
Shigella	**HEK – 2, 7, 9, 11, 14, 15, 15, 19, 23, 25, 30**

Table 28

List of isolates tested for their gram characteristics

Organism	Isolate
Staphylococci (Gram-positive)	**BP – 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16, 17, 18, 19, 20, 26**
Salmonella (Gram-negative)	**BS – 1, 3, 5, 7, 11, 12, 16, 17, 21, 22, 23, 25, 26, 27, 30** **XLT4 – 26, 27, 30**
E. coli (Gram-negative)	**EMB – 1, 3, 4, 5, 6, 7, 11, 13, 17, 18s, 18l, 19, 21 22, 23, 24, 25, 26, 28, 30**
Listeria (Gram-positive)	**P – 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15,** **O – 2, 4, 5,**
Shigella	**HEK – 9, 12, 15, 18, 19, 20, 24, 26, 28, 30**

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