

**ECO-DIVERSITY OF AQUATIC BACTERIA AND
VIRUSES ISOLATED FROM RIVER NILE
AND DRAINAGE WATER IN EGYPT**

By

MOHAMED IBRAHIM HASAN AZZAM

B.Sc. Agric. Cooperative Sc., Higher Institute for Agric. Cooperation, 2003

M.Sc. Agric. Sc. (Agricultural Viruses), Ain Shams University, 2010

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ABSTRACT

Mohamed Ibrahim Hasan Azzam: Eco-diversity of Aquatic Bacteria and Viruses Isolated from River Nile and Drainage Water in Egypt. Unpublished Ph.D. Thesis, Department of Agricultural Microbiology, Faculty of Agriculture, Ain Shams University, 2015.

This study aims to determine the impact of five main drains as sources of pollution on the water quality of River Nile at Rosetta branch, concerning physicochemical and microbiological characteristics. Eco-diversity studies of bacterial isolates were carried out through: antibiotic sensitivity, virulence and genetic variability. Eco-diversity studies of viral isolates were throughout: plaque morphology, host range, particle size, shape and molecular weight of genome.

The results of physicochemical, bacteriological and virological analyses revealed that, all drains selected in the study were suffering from varying levels of pollution. There was a gradual decrease in pollution levels along Rosetta branch (from downstream El-Rahawy to downstream Tala drain). The pollution impact caused by drains on Rosetta branch was remarkable from El-Rahawy drain which was considered the main point source of pollution. Results of water quality index was very bad in El-Rahawy and Sabal drains outlet and bad for all sites taken in this study except for upstream El-Rahawy drain, it was evaluated as being of medium quality.

Identification of bacterial isolates was carried out according to Bergey's Manual of Systematic Bacteriology and confirmed using the analytical profile index 20 E strip system. Results revealed that, out of 225 isolates, 212 were belonging to four main bacterial families. The results of antibiotics resistance patterns for different bacterial isolates showed that *E. coli* isolates were resistant to about (75%), *Citrobacter freundii* isolates (45%), *Salmonella sp.* isolates (85%), *Proteus vulgaris* isolates (87.5%), *Pseudomonas aeruginosa* isolates (100%), *Enterococcus faecalis* isolates (82.5%) and *Staphylococcus aureus* isolates (77.5%) of the tested antibiotics. Most of isolates were multiple antibiotic resistant (MAR). Methicillin resistant *Staphylococcus aureus* (MRSA) was reported in four isolates from drains and thirteen isolates from Rosetta branch. While, vancomycin resistant isolates (VRSA) were recorded, three isolates from drains and four isolates from Rosetta branch. Results of virulence test for all isolated bacteria showed positive congo red test (100%) for *C. freundii* and *Salmonella sp.*, followed by *P. aeruginosa* (98%), *P. vulgaris* (91%), *E. coli* (60%), *E. faecalis* (40%) and *S. aureus* (72.3%).

The three *P. aeruginosa* isolates selected from different sites were successfully amplified and sequenced using 16s rDNA gene. Data showed that partial nucleotide sequences were 1274, 1280 and 1286bp, respectively

and compared with species recorded on Genbank and identified as *P.aeruginosa_1*, *P.aeruginosa_2* and *P.aeruginosa_3*, respectively.

Eight coliphage and *P.aeruginosa* phage isolates were isolated from water samples. The maximum of phage counts were recorded in River Nile and the minimum were detected in drainage water samples. Electron microscopy of the isolated *E.coli* and *P.aeruginosa* phages particles revealed that, phage particles had an isometric head and long-contractile tail and some particles appeared containing short tail with full heads.

Biological characteristics for isolated phages were determined by the spot test method. It was found that phages specific for *E.coli* could lyse *E.coli* strains 1, 3, B (ATCC) but failed to lyse *E.coli* strain 2. While, phages specific for *P.aeruginosa* could lyse *P.aeruginosa* strains B2 and 101 but failed to lyse *P.aeruginosa* strain 1, 2. On the other hand, coliphages had activity and were able to lyse their host at pH ranged from 6 to 10 and *P.aeruginosa* phages had activity and able to lyse their host at pH ranged from 6 to 9. Viral stability to acidity and alkalinity was also different from type of phage to another. Data of turbidity test showed that phage infection produced a drastic decrease of *E.coli* and *P.aeruginosa* cultures as compared to control and a constant increase in O.D₆₀₀ was seen after 16 and 18 h.

Restriction enzyme pattern of the eight isolated coliphages (C1 to C8) by *EcoRI*, *HindIII* and *BamHI* showed the presence of dsDNA as well as heterogeneity among these phages. The results showed that *EcoRI* produced 8, 7, 5, 4, 4, 7, 9 and 5 fragments and *HindIII* produced 4, 3, 0, 0, 1, 6, 5 and 4 fragments while *BamHI* produced only 1, 1, 1, 0, 0, 3, 0 and 1 unique fragment, for the eight phage isolates, respectively. Restriction enzyme pattern showed the DNA phage genomes diversity among eight phage isolates. Sixty four fragments appear specific amplified fragments represented (81%), one fragment appear common amplified fragment represented (10.13%). Also, the restriction enzyme pattern appear eight unique (genetic marker fragment) represented (10.13%).

Out of fifteen sites, two only (El-Rahawy and Sabal drains outlet) were found to be polluted with enteroviruses with rate of 3.6×10^4 and 3.4×10^4 genome copies per microliter, respectively using real time - quantitative reverse transcriptase – polymerase chain reaction (rt-qRT-PCR).

Key Words: River Nile, Drainage water, Pollution, Bacteria, Viruses, Bacteriophages, 16s rRNA gene, Restriction enzyme, rt-qRT-PCR.

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CONTENTS

	Page
LIST OF TABLES.....	IV
LIST OF FIGURES.....	VI
LIST OF ABBREVIATIONS.....	IX
I. INTRODUCTION.....	1
II. REVIEW OF LITERATURE.....	4
III. MATERIALS AND METHODS.....	38
1. Sampling sites and study area.....	38
2. Sampling procedures.....	40
3. Media and solutions used in this study.....	41
4. Physico-chemical analyses of water samples.....	49
4.1. Temperature.....	49
4.2. Values of pH.....	49
4.3. Electric conductivity (EC).....	50
4.4. Biochemical oxygen demand (BOD).....	50
4.5. Chemical oxygen demand (COD).....	50
4.6. Dissolved oxygen (DO).....	50
4.7. Total dissolved solids (TDS).....	50
4.8. Turbidity.....	50
4.9. Ammonia (NH ₃).....	50
4.10. Nitrate and phosphate.....	50
5. Bacteriological analyses of water samples.....	51
5.1. Enumeration of standard plate count bacteria (SPC).....	51
5.2. Total coliforms (TC) count.....	51
5.3. Fecal coliforms (FC) count.....	52
5.4. Fecal streptococci (FS) count.....	52
5.5. Detection and isolation of <i>Escherichia coli</i>	52
5.6. Detection and isolation of <i>Salmonella</i> and other Gram-negative enteric bacteria.....	52
5.7. Detection and isolation of <i>P.aeruginosa</i>	53
5.8. Detection and isolation of <i>Staphylococcus aureus</i>	53
5.9. Detection and isolation of <i>Enterococcus faecalis</i>	54
5.10. Purification and identification of bacterial isolates.....	54
5.10.1. Gram reaction.....	54
5.10.2. Motility test.....	54

II

	Page
5.10.3. Sporulation.....	54
5.10.4. Capsule stain.....	54
5.10.5. Catalase test.....	55
5.10.6. Coagulase test.....	55
5.10.7. Oxidase test.....	55
5.10.8. Urease test.....	55
5.10.9. Phenylalanine deaminase test.....	55
5.10.10. Starch hydrolysis test.....	55
5.10.11. Gelatin liquefaction.....	55
5.10.12. Hydrogen sulphide production.....	55
5.10.13. Tween 80 hydrolysis.....	56
5.10.14. Indole production.....	56
5.10.15. Methyl red test.....	56
5.10.16. Voges-Proskauer test.....	56
5.10.17. Citrate test.....	56
5.10.18. Nitrate reduction.....	56
5.10.19. Haemolysis on blood agar medium.....	57
5.10.20. Sugar fermentation test.....	57
5.11. Identification by Analytical Profile Index (API) 20E strips.....	57
6. Antibiotic sensitivity test for bacterial isolates.....	57
7. In vitro pathogenicity test for bacterial isolates.....	59
8. 16s rDNA gene sequencing of <i>Pseudomonas aeruginosa</i>	59
8.1. Isolation of bacterial genomic DNA.....	59
8.2. Amplifying of 16s rDNA gene.....	59
8.3. PCR product for cycle sequencing.....	61
8.3.1. Performing cycle sequencing.....	61
8.3.2. Purifying extension products.....	61
8.3.3. Electrophoresis and sequencing extension products	61
8.4. Analyzing data.....	61
8.5. Sequence analyses.....	62
9. Detection of bacteriophages.....	62
9.1. Preparation of viruses lysate.....	62
9.2. Assaying of bacteriophages.....	62
9.3. Preparation of high titer phage stock.....	62
9.4. Preparation of high titre phage lysates.....	63

III

	Page
9.5. Purification and concentration of <i>E.coli</i> and <i>P. aeruginosa</i> of phages.....	63
9.6. Characterization of <i>E.coli</i> and <i>P.aeruginosa</i> phages.....	63
9.6.1. Electron microscopy examination.....	63
9.6.2. Determination of host range pattern of the phages...	64
9.6.3. Determination of pH stability.....	64
9.6.4. Determination of bacterial reduction assay.....	64
9.7. Isolation of DNA phages.....	64
9.7.1. Extraction of DNA.....	64
9.7.2. Restriction enzyme digestion.....	65
10. Detection of enteroviruses.....	65
10.1. Virus concentration.....	65
10.2. Re-concentration.....	65
10.3. Molecular detection of enteric viruses.....	66
10.3.1. Viral RNA extraction from purifies viruses.....	66
10.3.2. Real-time-RT-PCR.....	66
10.3.3. Oligonucleotide primers and TaqMan® probe for virus detection by rt-qRT-PCR.....	66
11. Calculated parameters and data analyses.....	69
11.1. Water quality index (WQI).....	69
11.2. Multiple antibiotics resistance indexing.....	70
11.3. Statistical analyses.....	70
11.4. Phylogenetic tree and similarity index.....	70
IV. RESULTS.....	71
V. DISCUSSION.....	145
VI. SUMMARY.....	167
VII. REFERENCES.....	175
ARABIC SUMMARY.....	

IV

LIST OF TABLES

Table No.	Page
1. Location of the study sites in Rosetta branch and drains.....	38
2. Antibiotic used for sensitivity test.....	58
3. Composition of MicroAmp PCR tubes for samples and controls.....	60
4. Thermal cycling conditions for samples and controls.....	60
5. Cycle sequencing conditions for PCR product.....	61
6. Standard curve dilution of enterovirus.....	68
7. Physico-chemical properties of water samples collected from Rosetta branch.....	72
8. Physico-chemical properties of water samples collected from drains.....	72
9. The standard plate count bacteria (SPC), total coliforms (TC), fecal coliforms (FC) and fecal streptococci (FS) of water samples collected from drains and Rosetta branch.....	80
10. The correlation coefficient matrix between physicochemical and bacteriological parameters.....	87
11. Calculated water quality index for collected water samples.....	88
12. Incidence of bacterial isolates recovery and identification from water samples collected from drains and Rosetta branch.....	89
13. Morphological and biochemical characteristics of identified isolates from water.....	91
14. Total number and percentages of identified bacteria in water samples collected from drains and Rosetta branch.....	95
15. Resistance profile of <i>E.coli</i> isolates against individual antibiotics...	100
16. Resistance profile of <i>C.freundii</i> isolates against individual antibiotics.....	101
17. Resistance profile of <i>Salmonella sp.</i> isolates against individual antibiotics.....	102
18. Resistance profile of <i>P.vulgaris</i> isolates against individual antibiotics.....	103
19. Resistance profile of <i>P.aeruginosa</i> isolates against individual antibiotics.....	104
20. Resistance profile of <i>E.faecalis</i> isolates against individual antibiotics.....	105
21. Resistance profile of <i>S.aureus</i> isolates against individual antibiotics.....	106
22. Total number and percentages of resistant bacterial isolates from	

Table No.	Page
collected water samples.....	111
23. Multiple antibiotic resistance (MAR) index for bacteria isolated from collected water samples.....	113
24. Percentages of pathogenic and non pathogenic bacterial isolates from water samples.....	115
25. Replacement situation of nitrogen base in nucleotide sequences of 16s rDNA gene for three <i>P.aeruginosa</i> isolates.....	126
26. Counts of nucleotides individually, in combined form, G+C/A+T and %G+C for the three isolates of <i>P.aeruginosa</i>	127
27. Incidence of <i>E.coli</i> and <i>P.aeruginosa</i> phages isolates recovery and identification from collected water samples.....	130
28. Morphology of plaques and appeared with <i>E.coli</i> after plaque assay of positive lytic area found in Rosetta and drains water samples.....	131
29. Morphology of plaques and appeared with <i>P.aeruginosa</i> after plaque assay of positive lytic area found in Rosetta and drains water samples.....	132
30. Morphological features of different phage isolates specific for <i>E.coli</i> and <i>P.aeruginosa</i> as determines by TEM.....	133
31. Lysosensibility of different <i>E.coli</i> strains to phage isolates.....	135
32. Lysosensibility of different <i>P.aeruginosa</i> strains to phage isolates....	135
33. Stability of isolated <i>E.coli</i> phages to different pH values.....	136
34. Stability of isolated <i>P.aeruginosa</i> phages to different pH values....	136
35. The reduction of the bacterial growth by isolated phages compared with control.....	137
36. Number of fragments for coliphage isolates using three types of restriction enzymes.....	139
37. Polymorphism and genetic marker of DNA genome for eight coliphages isolates by restriction fragment length polymorphism (RFLP).....	141
38. Similarity index between genome of eight coliphage isolates.....	143
39. Qualitative and quantitative assay of enteroviruses in water samples collected from drains and Rosetta branch using rt-qRT-PCR.....	144