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Bacterial Analysis of Urine Polluted Environments in Federal University of Agriculture, Makurdi

Aernan Tracy Paulyn [1], Odo Joel Inya [2], Ukahi Philip Onaah [1]

[1]Department of Microbiology, University of Agriculture, Makurdi, Nigeria
[*2]Departments of Fisheries and Aquaculture University of Agriculture, Makurdi, Nigeria
[*]Corresponding author E-mail: odojoel@gmail.com

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Abstract

Urine remains sterile in the bladder. To carry out bacterial analysis of urine polluted environments in Federal University of Agriculture, Makurdi, soil samples and bathrooms floor swabs were collected from different sites within Federal University of Agriculture, Makurdi. They samples were taken to the Department of Microbiology Laboratory where they were analyzed using standard microbiological techniques which includes culture, microscopy and biochemical tests. The bacteria isolates from urine contaminated soil were identified to be Escherichia coli. Enterobacter spp, Pseudomonas spp, Proteus spp and Staphylococcus spp while isolates from bathroom swabs were identified to be Salmonella spp, E. coli, Enterobacter spp, Klebsiella spp and Proteus spp. Salmonella spp, Staphylococcus spp and E. coli were also isolated from non urine contaminated (cleaned) bathrooms. E. coli was the most common organism. Pathognicity test was carried out to determine the disease causing ability of the isolates in humans. All isolates from the urine contaminated bathrooms except E. coli (isolated from urine contaminated bathroom A) were pathogenic. Salmonella spp and E. coli isolated from non urine contaminated (cleaned) bathroom A were also found to be pathogenic while E. coli (isolated from urine contaminated soil A), Enterobacter spp, Proteus spp and Staphylococcus spp were the pathogenic organisms amongst the isolates from the urine contaminated soils. From the analysis of the pathogenicity test using analysis of variance (ANOVA), there was significant difference in the pathogenicity test as the significant values (P-values) of the zones of haemolysis ranged between 0.51(8.3±2.08) to 1.00(12.00±2.00) which is beyond the level of significance where P<0.05. The study showed the diversity of pathogens in the urine polluted environments. However, this can be controlled through proper disinfection and avoiding indiscriminate urination.

1. Introduction

Soil is a natural cultural medium that support the growth of different types of microorganisms. Soil consists of organic and inorganic matters which determine the soil fertility and the proliferation of various micro flora and air that helps in maintaining the nutritional of the soil. The topsoil is known to have the highest concentration of organic matter and microorganism (Chukwu et al., 2018). Urine is a liquid waste product from the kidney of both animals and humans. It is collected in the bladder and excreted through the urethra. As a waste liquid (> 95% water) product, it contains some dissolved substances such as ammonia, urea, uric acid, and creatinine. Urine also contains inorganic dissolved substances such as sodium chloride, calcium, potassium, phosphate and sulfates (Cobire and Wewedo 2002). The dissolved substances in the urine can be utilized by microorganisms of various groups as nutrients whenever urine finds its way into the environment. This is evidenced by the fact that urine polluted environments usually have very strong odour, signifying that the biological oxygen demand (BOD) is high. This phenomenon is observed in toilets, bathrooms, street corners and fallow grounds (Den, and Pennick, 1999). The different groups of microorganisms can represent different microbial functions and activities. Some can be harmful relating to public health risk, or beneficial relating to

positive economic value. Urine leached into ground and surface water is often with much of the nitrogen intact. When microorganisms in lakes and other surface water consume the nitrogen, it results into a great bloom of growth. When this dies and decomposes, it pulls oxygen from the water or eutrophiles, which can suffocate fish and other aquatic life. Underground nitrogen can seep into drinking water, posing a potential health hazard.Urine contains micro pollutants such as synthetic hormones, pharmaceuticals and their metabolites that is mainly excreted via urine (Alder, 2002). Excreted urine may be harmful to the ecosystems and human health (Daughton, and Ternes, 1999). Indiscriminate urination is often observed in the hostels. In a healthy individual, urine is sterile in the bladder but when transported out of the body, different types of bacteria are picked up and freshly excreted. Urinary infections which in more than 80% of cases are caused by E.coli (Murray et al., 1990).Today, many micro pollutants reach the aquatic environments because their degradation in waste water treatment plant is poor (Barker, and Jones 2005).The objective of this research is to evaluate bacteria in urine polluted environments in Federal University of Agriculture, Makurdi. Benue state. Nigeria.

2. Materials and Methods

2.1 Study Area

This research was carried out within the South core region of the Federal University of Agriculture, Makurdi. The sample collection sites were Urine and Non-Urine polluted sites behind Academic Block A (GAIUS IGBOELI), Science Lecture Theatre (SLT) and University hostel bathrooms.

2.2 Materials

Nutrient Agar (N.A), Salmonella Shigella Agar (SSA), Mannitol Salt Agar (MSA), Eosin Methylene Blue Agar (EMBA), Cystine Lactose Electrolyte Deficient Agar (CLED), Petri dishes, wire loop, measuring cylinder, conical flasks, test tubes, test tube racks and spatula. The following equipments were used during the course of the laboratory analysis of the samples; microscope, autoclave, refrigerator, incubator and weighing balance. The study also used Gram Reagents, Oxidase reagents, Urease reagents, Citrate and Coagulase reagents, Catalase reagents, Kovac's reagent, Oil immersion and distilled water.

2.3 Sample Collection

A total of 20 samples were collected; 5 urine contaminated soil samples, 5 non-urine contaminated soil samples, 5 urine contaminated bathroom floor swab samples and 5non-urine contaminated (cleaned) bathroom floor swab samples. Urine polluted soil samples were collected from urine polluted areas behind Block A and SLT (Science Lecture Theatre) in the campus of Federal University of Agriculture, Makurdi. Unpolluted soil samples were also collected from areas located within the University premises and the soil samples were immediately transferred into sterile sample bottles and wrapped with aluminum foil. Samples were also collected from the University hostel bathrooms using swab sticks. This was done by swabbing the floor of both urine contaminated hostel bathrooms and non-urine contaminated (cleaned) hostel bathrooms. All the samples were immediately taken to the microbiology laboratory for analysis.

2.4 Sterilization and Disinfection of Materials.

Standard methods as described by (Cheesborough, 2000). were adapted throughout this research Work. Benches were properly disinfected with sodium hypochlorite. All glass wares (Petri dishes, test tubes, conical flasks) were washed during the bench work with detergents, rinsed with clean water and sterilized at 121°C for 15 minutes.

2.5 Preparation of Media for Bacterial Isolation

The nutrient agar was prepared according to the manufacturer's recommendation and allowed to cool. On cooling, 20ml were aseptically dispensed into sterile Petri dishes and allowed to solidify. Nutrient agar is a general purpose medium suitable for the cultivation of non-fastidious organisms. In this work, the medium was used for mixed culture and as slants for preparing isolates. Media such as CLED, EMBA, SSA and MSA are indicator media that distinguish one organism type from another growing on the same media. CLED, EMBA, SSA and MSA were prepared according to the manufacturer's recommendation, allowed to cool and were poured into petri dishes aseptically. The differentiations of the organisms by the media were based on their ability to ferment the indicator media with characteristic colour changes. For the Isolation of Total Heterotrophic Bacteria Count One gram each of the soil samples (urine contaminated and non-urine contaminated) were weighed out using weighing balance and aseptically added into 9ml of sterile water contained in the test tube and serially diluted to five dilution factors using ten –fold serial dilution technique. Aliquots (0.1ml) of 10-2 and 10-4 dilution factors were appropriately dispensed into Petri dishes and nutrient agar was poured into the plates using pour plate technique. The plates were incubated at 37°C for 24hrs. Ten-fold serial dilutions of the bathroom samples

were aliquots (0.1ml) of 10-1 and 10-3 dilution factors were appropriately dispensed into plates and nutrient agar was poured into the plates using the pour plate technique. The plates were incubated at 37°C for 24hrs. For the Identification of Bacteria Isolates, Methods described by (Odo et al., 2022) were adapted in the identification of isolates. This was done by the morphological appearance, Gram reaction and biochemical characteristics of the isolates. For biochemical tests, standard inocula were prepared in stocks and used when needed and this was done by aseptically sub- culturing from the stock culture with freshly prepared nutrient agar and incubated at 37°C for 18-24hrs.

2.6 Gram Reaction

Discrete colonies which developed after 24hrs were picked with a sterile wire loop onto a clean (grease-free) glass slide. A drop of distilled water added to the slide to make a thin film of smear. The smear was allowed to dry and then heat fixed by passing it thrice over the Bunsen blue flame. The fixed smear was then stained for 60 seconds with crystal violet solution. The stain was washed off by gently running tap water and then flooded with Lugol's iodine solution (a mordant) for 60 seconds. The iodine was drained and the slide rinsed in running tap water. The stained film was then decolorized with 95% (v/v) ethanol until the entire violet colour disappeared. The smear was counterstained with safrainn solution for 60 seconds. After the slide was washed and blotted dry, it was observed under oil immersion (x100) objective of the light microscope.

2.7 Biochemical Characterization of the Isolates

Catalase test demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide. The glass slide technique was used. Drop of hydrogen peroxide was placed on a clean grease-free glass slide and smeared with a loopful of isolate collected from a 24hrs fresh culture. The production of gas bubbles was an indication of a positive test. Oxidase Test test was adopted from (Cheesebrough, 2006). to differentiate between bacterial groups through the production of oxidase. A few drops of freshly prepared oxidase reagent (1% solution of tetramethyl-phenylene diamine dihydrochloride) were placed on a piece of filter paper and allowed to dry. A small quantity of growth from a fresh culture was smeared across the filter paper. A positive result was indicated by a purple-blue coloration on the filter paper within 10 seconds. Indole Testing for indole production is important in the identification of enterobacteria. Most strain of E. coli and Providencia species of bacteria breakdown the amino acid tryptophan with the release of indole. The test organism was cultured in a medium which contains tryptophan. Indole production was detected by kovac's reagent which contained 4-(P) dimethyl-aminobenzldehdyde. Production of red colour indicated positive result while no red colour was negative result. Citrate Test test was one of several techniques used to assist in the identification of Enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon and NH3 as its only source of nitrogen. The test organism was cultured in a medium which contains sodium citrate, an ammonium salt with the indicator bromothymol blue (simmon's citrate agar). Growth in the medium was shown by turbidity and a change in colour of the indicator from light green to blue, due to the alkaline reaction following citrate utilization. Coagulase test was used to differentiate Staphylococcus spp which produce the enzyme coagulase, a drop of saline water was placed on a clean slide. With a sterile loop, a trace of undiluted plasma was stirred with the bacteria suspension on the slide. Coarse clumping within 5-10 seconds indicated a positive result. Urease Testing for urease enzyme activity was important in differentiating enterobacteriaecae. Proteus strains are urease producers. Shigella and Salmonella do not produce urease. The test organism was cultured in medium which contained urea and the indicator, pheonol red. Production of red-pink colour in the medium indicated a positive urease result while no pink colour indicated negative urease result.

2.8 Microbiological Analysis

The microbiological assay carried out in this study was pathogenicity test. Pathogenicity Test was carried out to determine whether the isolated organisms are pathogenic to humans or not. Some bacteria produce exoenzymes that lyse red blood cells and degrade haemoglobin; these are called hemolysins. Bacteria can produce different types of hemolysins. Beta-hemolysin break down the red blood cells and haemoglobin completely, this leaves a clear zone around the bacterial growth. Such results are referred to as β-haemolysis (Beta-haemolysis). Alpha-hemolysins partially breakdown the red blood cells and leaves a green colour behind; this is refer to as α-haemolysis (Alpha-haemolysis). The greenish colour is caused by the presence of biliverdin which is a by-product of the breakdown of haemoglobin. If the organism does not produce hemolysins and does not breakdown the blood cells, no clearing will occur. This is called γ-haemolysis (Gamma haemolysis). The pathogenicity test was done using blood agar (Nutrient agar enriched with human blood). The nutrient agar was prepared according to the manufacturer's instruction and allowed to cool but not solidify. The nutrient agar cooled to a certain temperature and the blood was gently mixed to the nutrient agar as caution was taken to

avoid air bubbles. This was dispensed to sterile Petri dishes while liquid and isolates were inoculated and incubated at 37°C for 24hrs after which the diameter of haemolyzed zones were measured in millimeter (mm).

2.9 Data Analysis

Data collected from the pathogenicity test were statistically analyzed and expressed as mean ± standard deviation and the significant difference were analyzed using analysis of variance (ANOVA) at $P<0.05$.

3. Results

Table 1 show different bacteria isolated from different samples (urine contaminated soils, non-urine contaminated soils, urine contaminated and non-urine contaminated bathrooms). Escherichia coli were the most isolated organism occurring 6 times while the least occurring organism was Klebsiella spp. The result of the total bacterial load per ml of the samples is shown on table 2. The urine contaminated soil samples (A and B) significantly have the highest number of bacterial load while non-urine contaminated (cleaned) bathrooms had the least bacterial load. The result of the pathogenicity test reveals that, Salmonella spp isolated from Urine Contaminated Bathroom A (U.C.B.A) has the highest zone of haemolysis with 40.67±1.16 followed by E. coli isolated from Urine Contaminated Bathroom B (U.C.B.B) with 30.33±0.58. E. coli isolated from Non- Urine Contaminated Soil A (N.U.C.S.A) has the least haemolytic presentation as shown in table 3.Table 4 show the prevalence/percentage frequency of the pathogenic isolates. E. coli presented the highest prevalence/percentage frequency of 4(33.3%) while Staphylococcus spp and Klebsiella spp showed the least prevalence/percentage frequency of 1(8.33%).

Table 1: Bacterial isolates from the university hostel bathrooms and areas within Federal University of Agriculture, Makurdi (FUAM)

Samples	Organisms
U.C.S.A	*Escherichia coli* *Enterobacter spp*
U.C.S.B	*Escherichia coli* *Pseudomonas spp* *Proteus spp* *Staphylococcus spp*
N.U.C.S.A	*Pseudomonas spp* *Escherichia coli*
N.U.C.S.B	*Pseudomonas spp*
U.C.B.A	*Salmonella spp* *Escherichia coli*
U.C.B.B	*Enterobacter spp* *Escherichia coli* *Klebsiella spp* *Proteus spp*
N.U.C.B.A	*Salmonella spp*
N.U.C.B.B	*Staphylococcus spp* *Escherichia coli*

KEY: U.C.S.A = Urine Contaminated Soil A; U.C.S.B = Urine Contaminated Soil B; U.C.B.A = Urine Contaminated Bathroom A; U.C.B.B = Urine Contaminated Bathroom B; N.U.C.S.A = Non-Urine Contaminated Soil A; N.U.C.S.B = Non-Urine Contaminated Soil B; N.U.C.B.A = Non-Urine Contaminated Bathroom A; N.U.C.B.B = Non-Urine Contaminated Bathroom B

Table 2: Colony count of bacteria isolated from the different sample sites in the University hostel bathrooms and areas within the school environment

Samples	Number of colonies on nutrient agar/(CfU/ml)
U.C.S.A	2.16×10^5
U.C.S.B	1.95×10^5
U.C.B.A	1.60×10^4
U.C.B.B	1.90×10^4
N.U.C.S.A	1.50×10^5
N.U.C.S.B	1.62×10^5
N.U.C.B.A	1.18×10^4

N.U.C.B.B	1.27×10^4

KEY: U.C.S.A = Urine Contaminated Soil A; U.C.S.B = Urine Contaminated Soil B; U.C.B.A = Urine Contaminated Bathroom A; U.C.B.B = Urine Contaminated Bathroom B; N.U.C.S.A = Non-Urine Contaminated Soil A; N.U.C.S.B = Non-Urine Contaminated Soil B; N.U.C.B.A = Non-Urine Contaminated Bathroom A; N.U.C.B.B = Non-Urine Contaminated Bathroom B

Table 3: Pathogenicity test of bacteria isolates from sampling sites in the University hostel bathrooms and areas within the school environment. P<0.05

Isolates	Location	Zone of Haemolysis(mm)	Sig.	Result
Escherichia coli	Urine Contaminated Soil A	12.00 ± 2.00	1.00	++
Enterobacter spp	Urine Contaminated Soil B	14.00 ± 1.73	0.67	++
Escherichia coli	Urine Contaminated Soil B	0.00 ± 0.00	0.00	_
Pseudomonas spp	Urine Contaminated Soil B	0.00 ± 0.00	0.00	_
Proteus spp	Urine Contaminated Soil B	8.33 ± 2.08	0.51	+
Staphylococcus spp	Urine Contaminated Soil B	9.67 ± 0.58	0.67	+
Pseudomonas spp	Non-Urine Contaminated Soil A	0.00 ± 0.00	0.00	_
Escherichia coli	Non-Urine Contaminated Soil A	5.67 ± 1.16	0.67	+
Pseudomonas spp	Non-Urine Contaminated Soil B	0.00 ± 0.00	0.00	_
Salmonella spp	Urine Contaminated Bathroom A	40.67 ± 1.16	0.67	+++
Escherichia coli	Urine Contaminated Bathroom A	0.00 ± 0.00	0.00	_
Enterobacter spp	Urine Contaminated Bathroom B	20.00 ± 0.00	0.67	++
Escherichia coli	Urine Contaminated Bathroom B	30.33 ± 0.58	0.67	+++
Klebsiella spp	Urine Contaminated Bathroom B	15.67 ± 1.16	0.67	++
Proteus spp	Urine Contaminated Bathroom B	23.67 ± 0.00	0.45	+++
Salmonella spp	Non-Urine Contaminated Bathroom A	15.33 ± 1.16	0.67	++
Staphylococcus spp	Non-Urine Contaminated Bathroom B	0.00 ± 0.00	0.00	_
Escherichia coli	Non-Urine Contaminated Bathroom B	20.67 ± 1.15	0.67	++

KEY: (-) = Negative (No haemolysis); (+)= Positive (Weak Haemolysis; virulent); (++) = Positive (strong Haemolysis; more virulent); (+++) = Positive (Very strong Haemolysis; most virulent)

Table 4: Percentage Prevalence/ frequency of pathogenic isolates from different sampling sites within the hostel bathrooms and outside Environments.

Organisms	Total number of isolates	n (%)
E. coli	12	4 (33.33)
Enterobacter spp	12	2 (16.66)
Proteus spp	12	2 (16.66)
Staphylococcus spp	12	1 (8.33)
Salmonella spp	12	2 (16.66)
Klebsiella spp	12	1 (8.33)

4. Discussion

The microorganisms isolated in the urine polluted environments were *E. coli, Enterobacter spp, Pseudomonas spp, Proteus spp, Staphylococcus spp, Salmonella spp* and *Klebsiella spp.* Some of these microorganisms were isolated by Dada EO and Aruwa CE (2014). The incidence of a close similarity in the genera of bacteria isolated from the soils and bathrooms contaminated with urine suggests the fact that, those organisms were indeed associated with urine contaminated environments, though the biomass of the contaminated soil is higher than the contaminated bathrooms. Microorganisms are ubiquitous in nature but urine availability is an additional nutrient, hence, the cluster of these species. This agrees with the work of (Den, and Pennick, 1999). The pathogenicity test is credibly significant because the significant differences of the

analyzed data are higher than the probability value (P<0.05). The bathroom being a constructed area specific for human use is amazing to harbor such a load of pathogenic biomass even after being cleaned. This may reflect the inadequacy of daily disinfection, inefficacy of the cleaners or disinfectants used. Therefore, the urine contaminated bathrooms are threats to health. However, urine contaminated bathrooms significantly harbor more biomass and pathogens than non-urine contaminated bathrooms. The decrease in the microbial biomass of the non-urine contaminated (cleaned bathrooms) reflects the effect of disinfection which agrees with the work of (Cobire and Wewedo 2002). Most bacteria isolated from bathrooms are well established etiologic agents of human diseases. *Proteus spp* could be *Proteus vulgaris* or *Proteus mirabilis*. They cause urinary and bloodstream infections. *Klebsiella spp* cause blood, chest and urinary tract infections, *Enterobacter spp* causes lower respiratory tract infections, skin, soft-tissue infections and urinary tract infections. *Salmonella spp* causes typhoid fever and gastroenteritis, *E. coli* is implicated in many common bacterial infections including urinary tract infections (UTIs), travelers' diarrhea and other clinical infections such as neonatal meningitis. Pathogens (*E. coli*, *Enterobacter spp*, *Pseudomonas spp* and *Proteus spp*) isolated from the urine contaminated sites could be transmitted via aerosols (Gerba *et al.,* 1975).

The pathogenicity test showed a positive haemolysis by *E. coli*, *Enterobacter spp*, *Proteus spp*, *Staphylococcus spp, Salmonella spp* and *Klebsiella spp* on blood agar after 24hrs of incubation at 37°C. This reveals that, environments polluted with bacteria are potential sources of numerous diseases. This agrees with the work of (Ivanov *et al.,* 2006, Tyrrel *et al.,* 2003 and Van der Putten, and Jeffery, 2011). Most of the organisms found in the urine contaminated soils and bathrooms may be attributed to persons with UTIs. Regular visits to the public urinals may contribute to increasing microbial load above threshold levels within the body systems (Hoglund *et al.,* 2002). This would often result in an infected diseased state. Hence, persons visiting these urinals stand the risk of contracting opportunistic infections or diseases.

5. Conclusion

The microorganisms isolated in the urine polluted environments which were *E. coli, Enterobacter spp, Pseudomonas spp, Proteus spp, Staphylococcus spp, Salmonella spp* and *Klebsiella spp* makes Urine contaminated soils public health hazards because the can be avenues for transmission of infection from one person to another. Pathogenicity test showed *Salmonella spp* and *E. coli* to have the highest haemolytic activity.

6. Recommendation

I. Most isolates from the urine polluted sites are pathogenic. Thus, this work recommends further identification of the isolates through molecular analysis to ascertain the variability of the pathogens.
II. The investigation of the degree of antimicrobial action of selected disinfectants against the isolates is also recommended.
III. Provision of adequate functioning toilet facilities and proper cleaning practices in disinfecting urine contaminated environments (toilets and bathrooms within the University community).

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Appendix

Appendix I: Growth morphology and microscopic examination of bacteria organisms isolated from contaminated soils, contaminated bathrooms, uncontaminated soils and uncontaminated bathroom collected university hostels bathrooms and areas within the school environment.

Characteristics / Samples	Shape of colonies	Colour of colony on N.A	Elevation of colonies	Edge of colonies	Microscopy		
					Gram reaction	Shape of cell	Colour of cell
U.C.S.A	Irregular and circular	Creamy	Flat	Smooth	_	Rod- like cells	Pink
U.C.S.B	Irregular and circular	Creamy and green	Flat	Smooth	+ _	Cluster of coccal and rod -like cells	Purple and pink
U.C.B.A	Irregular and circular	Whitish and creamy	Raised and flat	Rough and smooth	_	Rod-like	Pink
U.C.B.B	Irregular and circular	Creamy	Raised and flat	Rough	_	Cluster of rod- like cells	Pink
N.U.C.S.A	Irregular and circular	Creamy and green	Flat	Smooth	_	Rod-like cells	Pink
N.U.C.S.B	Irregular and spherical	Creamy and green	Flat	Smooth	_	Distinct rod like cells	Pink
N.U.C.B.A	Irregular and circular	Creamy	Raised	Rough and smooth	-	Rod- like cells	Pink
N.U.C.B.B	irregular	whitish	Raised	Rough	+ _	Cluster of coccal and rod-like cells	Purple and pink

KEY:

U.C.S.A = Urine Contaminated Soil A

U.C.S.B = Urine Contaminated Soil B
U.C.B.A = Urine Contaminated Bathroom A
U.C.B.B = Urine Contaminated Bathroom B
N.U.C.S.A = Non-Urine Contaminated Soil A
N.U.C.S.B = Non-Urine Contaminated Soil B
N.U.C.B.A = Non-Urine Contaminated Bathroom A
N.U.C.B.B = Non-Urine Contaminated Bathroom B

Appendix II: Biochemical tests of bacteria organisms isolated from contaminated soils, contaminated bathrooms, uncontaminated soils and uncontaminated bathroom collected university hostels bathrooms and areas within the school environment.

Test / Samples	Indole	Citrate	Catalyst	Coagulase	Urease	Oxidase	Organism
U.C.S.A1	+	-	+	-	-	-	*Escherichia coli*
U.C.S.A2	-	+	+	-	-	-	*Enterobacter spp*
U.C.S.B1	+	-	+	-	-	-	*Escherichia coli*
U.C.S.B2	-	+	+	-	-	+	*Pseudomonas spp*
U.C.S.B3	-	+	+	-	+	-	*Proteus spp*
U.C.S.B4	-	+	+	+	+	-	*Staphylococcus spp*
N.U.C.S.A1	-	+	+	-	-	+	*Pseudomonas spp*
N.U.C.S.A2	+	-	+	-	-	-	*Escherichia coli*
N.U.C.S.B	-	+	+	-	-	+	*Pseudomonas spp*
U.C.B.A1	-	-	+	-	-	-	*Salmonella spp*
U.C.B.A2	+	-	+	-	-	-	*Escherichia coli*
U.C.B.B1	-	+	+	-	-	-	*Enterobacter spp*
U.C.B.B2	+	-	+	-	-	-	*Escherichia coli*
U.C.B.B3	-	+	+	-	+	-	*Klebsiella spp*
U.C.B.B4	-	+	+	-	+	-	*Proteus spp*
N.U.C.B.A1	-	-	+	-	-	-	*Salmonella spp*
N.U.C.B.B1	-	+	+	+	+	-	*Staphylococcus spp*
N.U.C.B.B2	+	-	+	-	-	-	*Escherichia coli*

KEY:
1,2,3,4 = Number of Isolates