THE PREVALENCE OF *STAPHYLOCOCCUS AUREUS* IN URINE POLLUTED ENVIRONMENT WITHIN DELTA STATE POLYTECHNIC OZORO

Okobia B. U*. and Orogu J.O.

Department of Science Laboratory Technology, Delta State Polytechnic Ozoro, Delta State, Nigeria

Email- joshuaorogu4@gmail.com

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ABSTRACT

The prevalence of *Staphylococcus aureus* in urine polluted environment was carried out to evaluate bacteria in urine in polluted environment in Delta State Polytechnic Ozoro, Delta State. Sixteen (16) different sites in the institution were used as the sampling points. The sites used are the undesignated areas for urine discharge. All samples were analyzed in the laboratory. Six (6) bacterial species were obtained; *Staphylococcus aureus, Corynebacterium spp., Bacillus spp, Streptococcus spp., E. coli, and Klebsiella spp*. The total heterotrophic plate count ranges from 1.8 X 10^3CFU/ML to 2.13 X 10^4CFU/ML. *Staphylococcus aureus* have the highest percentage occurrence of 27.27% while *Corynebacterium spp. Klebsiella spp*. has the least percentage occurrence of 9.09%. The public health implications of persistence urination in public soils as potential source of pollution to man and plants cannot be overemphasized.

INTRODUCTION

The soil environment includes viable plants (roots), animal and microorganism that in the pores spaces and are attached to the geological materials. (Dada and Aruwa 2014) opined that soil organic and inorganic matter is determinants of soil fertility and they also aid the proliferation of various soil micro flora which in turn play vital roles in the maintenance of the nutritional balance and geochemical cycles of the soil. Some species of bacteria thrive on different food sources and in different micro-environment in the soil (Dada and Aruwa 2014).

Despite soil being the habitat for the majority of earth's terrestrial species, far less attention has been paid to understanding maintenance of soil biodiversity until recently as pointed out by (Wardle 2002). Now, there is a growing interest in the below ground biodiversity, largely as a rescue of advances in technologies that enable more ready characterization of these below ground diversity (Blaxter and Floyd, 2003; Young and Crawford, 2004) and also because of the increasing recognition among ecophysiologists that soil biota play key roles in eco system functioning, especially organic matter turnover, nutrient mineralization (Horper *et al*., 2000; Wardle, 2002: Heimsbergen *et al*., 2004) and material flow through the ecosystem.

Urine is the pale yellow fluid produce by the kidneys and it contain urea, uric acid, minerals, chloride, nitrogen, sulphur, ammonia, copper, iron, phosphate, sodium, potassium, manganese, carbolic acid, calcium, sails; vitamins A, B,C and E; Enzymes hipphric acid, creatinine, as well as kctose. Other sugars are sometimes excreted in urine, if their concentration in their body is high. Urea is abundant in the urine of humans and other mammals (Dranget, 2000). The PH of urine range between 4 - 8.

Urine is a filtered produce of kidney which contains only low molecular weight substances and at excretion the PH is normally around 6 but can vary between 4.5 and 8.2 (lentner *et al*., 2011). It was further shown by lentner that of the Nitrogen constituent of urine 75 -90% is excreted as urea and the remainder as ammonium and creatinine. In the presence of increase, urea is quickly degraded to ammonium and carbon dioxide and the hydroxide ions produces will invariably increase soil PH from 9 – 9.3 and this usually occur within hours of deposition (Jonssen *et al*. 2000). But the continual deposition of the soil because the conversion of ammonium (NH_4) to nitrate (NO_3) involves release of protons, thereby promoting acidity.

In Nigeria, urine deposition in public places go unchecked and has become a menace, a close examination of such soil macrocosm reveals patchiness of soil, obvious discoloration, purget ammonical smell (per comm.). There is therefore the need to establish the impact of human urine deposition on soil biota.Earlier report had describes human urine as non-toxic except when mixed with faeces and other organic compounds in septic tank (Dedeke, 2011). There the need to establish the impact of human urine

deposition on soil and soil biota cannot be over emphasis.

Materials and Methods

Study Area

This research was conducted in Ozoro, Delta State of Nigeria. Ozoro is the local Government headquarters of Isoko North Local Government of Area Delta State. The people are Isoko speaking and hospitable. Their main activities are food crop, farming accompanied by some hunting. They are also engaged in trade of food crop for cash to meet the other basic house hold needs. The region experience higher rainfall and humidity most of the year.

Sample Collection

Urine contaminated soil sample used in this study were collected from sixteen different sites in the institution. The sites used are the undesignated areas for urine discharge. The sites are the soil area noted for frequent urine discharge. The samples were dispensed in an EDTA container and then brought to the laboratory.

Materials

Commercially available nutrient agar was used in the study. The media was prepared according to manufacturer's instruction. The microbial loads of various groups of bacterial species were determined using the culture techniques involving different cultural media. The different soil samples were analyzed for the microbial diversity as described earlier in the sample collection. Bioloads were determined after decimal serial dilutions.

Method

Pure bacterial isolates were identified based on their characteristics such as morphology, microscopy, staining and capsule staining methods. The samples were analyzed for fungal isolates or sabouraud Dextrose Agar on establishment of growth after 4 days of incubation at room temperature, the plates were carefully examined and distinct growths were sub-cultured on fresh medium for purity. The fungal were indentified on the basis of their cultural characteristics and microscopy with reference to the methods describes by Barnett *et al* (2000).

Characterization and Identification of Isolates

Discrete colonies that developed after incubation were subcultures to obtain pure culture which were stored at 4^0c and used subsequently for microscopic characterization and biochemical analysis. The distinct colonies that develop in the pure cultured plates were observed for the morphological and cultural characteristics, shapes, color of bacterial on the media were observed and recorded.

Gram Stain

The gram stain was carried out on 24hours culture. A bearer of each of the bacterial isolates was made on clean grease free slide and heat fixed using flame. Crystal violent stain (0.3% W/O) was added and allowed to stand for 1minute. The stain was washed off with distilled water. Iodine (0.4% W/O), mordant was added and allow to stand for 50 seconds before rinsed off with distilled water. Ethanol (95% W/O), a decolourizer was then added and allow to stand for 30 seconds before being rinsed off with distilled water and then counter stained with the secondary stain, safranin (0.4% W/O) was allowed to stand for 1

minute. This was then washed off with distilled water and allowed to dry. The stained smear was then observed under the microscope using oil immersion lens magnification (X100).

Biochemical Test

I. Indole Test, Motility Test

The test organism was inoculated with broth that contains tryptophan and incubates at 37^0c for 48hours. The 2ml of the broth suspension was transferred to another test tube under aseptic condition. About 0.5ml of kovac's reagent was added to the broth. The mixture was shaken properly to ensure a thoroughly mixing and then observed for colour reaction. A positive result was indicated by a pink coloured ring round the interface between the broth suspension and alcohol reagent which rose to the surface.

II. Oxidaze Test

A piece of glass rod was used to remove a colony of the test organism and was smear on the filter paper which was placed on a petri dish and 3 drop oxidize reagent was added. A positive result was indicated by a**VI.** blue-purple colour, while no blue-purple indicated negative.

III. Catalase Test.

Three (3) ml of the hydrogen peroxide solution was pour into a test tube then colonies were removed and immerse in the hydrogen peroxide solution using a sterile stick. Active babbling shows positive catalyst test. While non active babbling shows negative catalyst.

IV. Citrate Test

Simmons agar was prepared in a test tube and sterile straight wire was used to break the slope with a saline suspension of the test organism. It was incubated at 37^0c for 48 hours.13yardbluecolour show positive citrate while no change in colour shows negative citrate test.

V. Glucose Test

The test organism was inoculated with broth medium that contain bromocresol contain purple. An invents Durham tube is kept submerged in it. A color change to yellow and gas accumulates in Durham tube indicate fermentation for the glucose and acopogenic whole colour changes to yellow bur no gas accumulate in Durham tube indicate fermentation for glucose and anaerogenic. And colour of broth does not change indicated non fermentation for the glucose.

Lactose Test

Sterile inoculating tool is used to pick colonies of the test organisms and was immersed into a test tube that contains phenol. Colour changes to yellow indicate a positive test, while whole colour changes to pink indicate a negative test.

Results and Discussion

Results

Table 1: The identification of Isolates

Cultural morphology	Gram Lecture	catalyst	Oxidase	Mutility	Glucose	Lactose	H_2S	Acid	Citrate	Indose	Name of organism
Cocci	+	+	+	+	-	-	-	+	+	-	*Staphylococcus aureus*
Rod	+	+	-	+	+	-	-	+	+	-	*Corynebacterium spp.*
Rod	+	+	+	-	-	-	-	+	+	-	*Bacillus spp*
Cocci	+	+	-	-	+	-	-	+	+	-	*Streptococcuss pp*
Rod	-	+	-	-	-	-	-	-	-	+	*E. coli*
Rod	-	-	-	+	-	-	-	+	+	-	*Klebsiella spp*

KEY + **=Positive**
- **=Negative**

Table 2: The Heterotrophic Bacterial Counts of Urine Samples from Different Sites.

Samples	Bacterial Count
A	1.8×10^4
B	1.00×10^4
C	1.19×10^4
D	1.28×10^4
E	2.13×10^4
F	1.45×10^4
G	1.27×10^4
H	1.71×10^4

I	1.18×10^4
J	1.60×10^4
K	1.50×10^4
L	1.37×10^4
M	1.8×10^3
N	2.5×10^3
O	2.2×10^3
P	9.6×10^3

Table 3: Percentage Occurrence of Bacteria Isolates

Isolates	% Occurrence of Bacteria
Staphylococcus aureus	27.28
Corynebacterium spp.	9.09
Bacillus spp.	18.18
E. coli	18.18
Streptococcus spp.	18.18
Klebsiella spp.	9.09
Total	100

Discussion

Different colonies were observed at the end of the procedure necessary for the isolation and identification of bacterial associated with urine. The bacterial present in samples of urine were identifies bases on their culture, morphological and biochemical characteristics. The characterization and the identification of the bacterial isolates are shown in table 1.

The occurrence of the bacterial isolate from the urine samples obtained from different sites in the institution is shown in table 1. From the entire urine sample obtained from sixteen different spots, *Staphylococcus aureus* was the most prevalent recording with 27.28% while *Klebsiella spp and Corynebacterium spp.* was the least prevalent recording with 9.09%. The value of heterotrophic bacteria counts of the urine sample from the samples in Ozoro Delta during the period of study is presented in table 3.

The majority of microbial population is found in the upper 6-12 inches of soil and the number decrease with depth (Bridge and Spooner, 2001). The number and kinds of organism found in soil, matter, temperature, moisture and aeration. The presence of bacteria in the urine without the symptoms of an infection is quite common and has been identified as a contributor to antibiotic misuse, which promotes resistance. Between 65% and 90% of urine tract information's in children are caused by *Escherichia coli*. Other pathogens include *Klebsiella species*,

Proteus species, *Pseudomonas aeruginosa* and *Enterococcus species* (Nir, 2005).

Conclusion and Recommendations

Conclusion

Urine contaminates soils are public health hazards which are avenues for transmission of infection from one person to another. Poor hygienic habits, overpopulation and overstretched facilities encourage indiscriminate urination in public places. The practice can either increase or decrease the microflora of urine contaminates soils. An increase in microbial load in such environments may result in increases probability of contracting opportunistic infections. Available toilet facilities within the study area fell far below that requires for the ever growing population of students.

Recommendations

1. The provision of more and adequate toilet facilities should be provided by the government.
2. The practice and enforcement of basis sanitary rules would help prevent unnecessary deaths and protects the health of millions of persons.
3. Water closet toilet type and/or mobile toilet should be made available.

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