

Antibiogram of selected bacteria isolated from urine polluted environment in Benue State Campus, Makurdi

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World Journal of Advanced Research and Reviews, 2023, 20(02), 459–467

Publication history: Received on 01 October 2023; revised on 05 November 2023; accepted on 09 November 2023

Article DOI: <https://doi.org/10.30574/wjarr.2023.20.2.2275>

Abstract

Urine contaminated soil contains various potential hazardous components which create an environment for bacteria to adapt, multiply and share their resistant genes. This study aimed to determine the antibiogram of selected bacteria isolated from urine polluted environment. Urine contaminated soil were randomly sampled from four different sites in Benue State University, Makurdi metropolis. Standard microbiological cultures, biochemical tests, modified Kirby-Bauer techniques and Clinical and Laboratory Standard Institute (CLSI) were used to isolate, identify, and determine the antibiotic susceptibility profiles of the bacteria at a 0.5 scale Mcfarland's standard (1.5×10^8 cells/ml). Bacteria isolated include *Bacillus* sp. (25), *E. coli* (13), *Klebsiella* sp. (10), *Staphylococcus* sp. (8) *Salmonella* sp. (7). The colony and colony forming unit (CFU) of eastern wing campus (7.85×10^7) was slightly higher compared to western wing campus (7.05×10^7). *Escherichia coli* (100 %) was resistant to all the antibiotics (Imipinem, Oxacillin, Ciprofloxacin, and Gentamicin), *Staphylococcus* spp isolates obtained were resistant to all the antibiotics except 50 % that were susceptible to Gentamicin, while 50 % of *Bacillus* spp on the other hand was susceptible to Imipenem, all were susceptible to Gentamicin and Ciprofloxacin (100 %); and all were however resistant to Oxacillin (100.00 %). *Salmonella* spp and *Klebsiella* spp showed similar pattern of susceptibility. All the isolates of the two organisms were susceptible to Imipenem, Gentamicin and Ciprofloxacin (100 %). All the isolates were resistant to Oxacillin. All the selected bacteria showed resistance to at least one antibiotic ($P < 0.05$) Unavailability of good toilet systems has contributed to the open urination and environmental contamination. Provision of more and adequate toilet facilities within the university premises and good antibiotic misuse orientation should be giving priority to prevent excessive contamination of soil with urine and antibiotic resistance.

Keywords: Antibiotics; Antibiotic resistance; Bacterial isolates; Urine-contaminated soil; Antibiogram

1. Introduction

Soil is a natural cultural media for the growth of many types of organism. The organic and inorganic matter in the soil determines the soil fertility and aid the proliferation of various micro flora that play vital roles in maintaining the nutritional balance of the soil [4].

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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 product, it contains some dissolved substances such as ammonia, urea, uric acid, and creatinine. These constitute the organic solids in the urine. Urine also contains inorganic dissolved substances such as sodium chloride, calcium, potassium, phosphate, sulfates and also residues of partially metabolized antibiotics [4].

The dissolved substances in the urine can be utilized by microorganisms of various groups as nutrients whenever urine finds its way into the environment. The antibiotics residues can be tolerated due to constant exposure enabling the microorganism to gain resistance to those antibiotics. It 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. [6].

Urine contains micro pollutants such as synthetic hormones, pharmaceuticals and their metabolites that is mainly excreted via urine. These components of urine create a conducive environment for bacteria to breed, multiply and also share their resistant genes with other bacteria through mobile genetic elements (Deni and Pennick, 2009). The major problem associated with the antimicrobial resistant bacterial is the emergence of antimicrobial resistance among pathogenic bacteria to human and animals which makes treatment difficult for some life-threatening infections [2]. The improper disposal of urine and organisms present in the urine may pose a great risk to the environment and the general public. The resultant persistent and resistant genes that may emerge in bacteria results in high resistance to first generation and even modern-day antibiotics. This research will evaluate the susceptibility profile of bacteria isolated from urine polluted environment and permit improvement in the provision of toiletries.

1.1. Statement of the Problem

Multidrug resistance is an increasingly serious public health challenge. It is speculated that if measures are not taken properly, by 2050 AMR will cause 300 million human deaths, financial losses will be equivalent to 100 trillion USD and an 11 % fall in animal production. Urine contaminated environment is a breeding ground for multidrug resistant bacteria because of antibiotic abuse and limited metabolization of antibiotics as a result environmental bacteria and other bacteria that are passed out in the urine tends to adapt and share resistance genes with themselves leading to resistance.

1.2. Justification of the Study

This research will evaluate the antibiotics susceptibility profile of bacteria isolated from urine polluted environment in Benue State University, Makurdi, metropolis and provide improvement, insights on the misuse of antibiotics and also enable proper and improved toilets systems availability in Benue State University, Makurdi.

Aim

To determine the antibiogram of selected bacteria isolated from urine polluted environment.

Objectives

- To isolate and identify selected bacteria in urine contaminated soil
- To determine the antimicrobial response of the isolates to some antibiotics
- To compare the responses of the bacteria to the selected antibiotics

2. Materials and methods

2.1. Study Area

Makurdi is the capital of Benue State; it was established in the early twenties and gained prominence in 1927 when it became the headquarters of the Benue province. Makurdi being a river port attracted the establishment of trading depots by companies such as UAC and John Holt Limited. Its commercial status was further enhanced when the Railway Bridge was completed and opens in 1932. Makurdi lies between latitude 7° 43' 50" N and longitude 8° 32' 10" E. it shares boundaries with Guma Local Government North East, Gwer to the South, Gwer-West to the west and Doma Local Government Area of Nasarawa State to the North-West. The town is divided into two major blocks by River Benue, hence, the North and South bank. Makurdi comprises of eleven council wards and inhabited predominantly by the indigenes of Makurdi; Tiv and Jukun people but Makurdi being the State capital, has become a cosmopolitan city with people of different tribes as Idoma, Igede, Agatu, Etulo, Igbo, Hausa and Igala with an estimated population of 500,797

with a landmass of 16km radius. The town has two universities which are, Federal University of Agriculture and Benue State University.

Benue State University is located near southern bridgehead of the Benue River on sandy alluvial formation. It occupies 6 square kilometres piece of land between Gboko Road and River Benue approximately 1.5km wide and 4km long. It is bounded to the West by the Benue Links Headquarters and to the East by Tilley-Gyado House. It embraces the premises of the Government Technical Training School, the Government Day Secondary School, and the Government Girls Secondary School.

The land slopes gently from Gboko road towards River Benue with variable gradients ranging from 0-50. As a consequence, much of the area is well-drained though some portions experience hydromorphic conditions. Being part of Makurdi town, the University experiences sticky debilitation conditions, both in the wet and dry seasons, a characteristic of the Benue trough the Benue State University is located between the south bank of River Benue and the Makurdi Gboko highway. The University's gates are located along a busy motor way that leads up to the Makurdi Airport. The North and South Bank of divided by the river Benue, is connected by two bridges, one of which carries a railway line across the river.

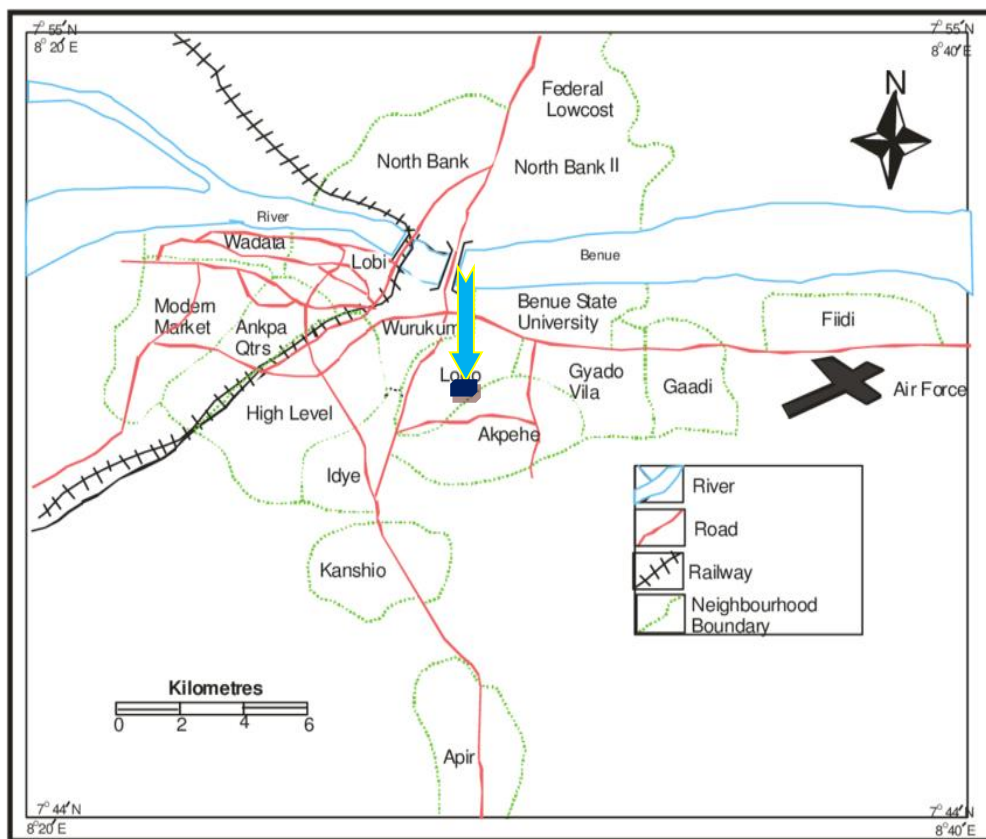


Figure 1 Map of Makurdi Showing the Study Site

Source: Benue State Ministry of Lands, Survey and Solid Minerals (2015).

2.2. Sample Source and Sample Collection

Urine contaminated soil samples that was used in this study was collected from different sites within Benue State University Campus. The sites used were undesignated areas for urine discharge. The sites was soil area noted for frequent urine discharge, while a meter away (not used for urine discharge) was chosen as the control. A total of about 50 samples was collected from the urine soil contaminated sites (10 samples each from 5 different sites) and control soil site was collected from the topsoil (between 5-20cm), respectively. Sample collection sites were Administrative Block (Faculty of Arts), Behind Auditorium, Behind Visiting Lecturer's Office, English Lecture Hall and Technical Block. The Samples from contaminated soils and control area were collected in separate sterile polythene bags, which was sealed respectively and appropriately labeled and was taken to microbiology laboratory for immediate analysis.

2.3. Determination of the Microbial Load of the Sample

The microbial loads of the Samples was determined using the Pour plate method of inoculation. The samples collected was serially diluted as follows; 9mls of sterile distilled water was pipetted into ten test tubes prepared in duplicate and labeled 10^{-1} to 10^{-10} for serial dilution. 1g of each sample was weighed using a weighing balance and was dissolved in 9ml of sterile distilled water to serve as stock sample. 1ml was taken from the stock sample and transferred into the tube labeled 10^{-1} , this was mixed properly and 1ml was taken out of the same tube (10^{-1}) and transferred into the tube labeled 10^{-2} . This was repeatedly done down to the last tube labeled 10^{-10} . 1ml was discarded from the last tube to make all equal (9ml each). 0.1ml aliquots from the 10^{-6} tubes was be aseptically inoculated onto petri dish and prepared nutrient agar medium was poured into the petri dish containing the 0.1ml of the sample. The contents of the plate were mixed together by rotating the plates gently on a flat bench surface to ensure an even distribution of the sample in the medium. The plates were aseptically left undisturbed for some minutes for the medium to solidify, and was inverted and incubated at 37°C for 24hrs. The colonies that were obtained after 24hrs of incubation, was counted using colony counter and recorded in colony forming unit per milliliter (CFU/ml).

2.4. Media Preparation

All the media to be used in this work (Nutrient agar, Manitol Salt agar, Mackoncay agar, Xylose-Lysine Deoxycholate agar, Eosin Methylene Blue agar, Peptone Water, Simmon's Citrate agar, Cystine Lactose Electrolyte-deficiency agar, Triple Sugar Iron agar, Urea Agar Base and Sulphide Indole Motility Media) were prepared according to their various manufacturer's instructions; the needed quantity of the dehydrated media was weighed and dissolved in the appropriate volume of sterile distilled water. The suspension was heated to boil and was sterilized using autoclave at 121°C for 15minutes.

2.5. Isolation Techniques

The samples were inoculated into broth medium (Peptone water) and was incubated for 18-24hrs to encourage large growth of microorganisms. The broth cultures were then be sub-cultured onto MSA, XLDA, CLEDA, EMBA and MA medium and were incubated for 24hrs. The colonies obtained were further sub-culture onto fresh agar medium as listed above and were incubated at 37°C for 24hrs to obtain a pure isolate. The growth characteristics of the isolate on their respective growth medium were observed and recorded.

2.6. Morphological identification

2.6.1. Gram Staining

The colony of each isolate was emulsified in a drop of normal saline on a clean microscope slide (smear). It was air dried and was heat fix by passing the slide across a flame three times. The slide was placed on the staining rack and were flooded with crystal violet for 1minute and rinsed with clean water. It was then covered with lugo's iodine (mordant) for 1minutes and rinsed with clean water. It was decolorized with acetone for few seconds and was rinsed immediately with clean water. It was lastly stained with safranin (secondary dye) for 30seconds. Back of the slide was wiped with cotton wool and was allowed to air dry; the slide was mounted on the microscope stage and a drop of immersion oil was added. The specimen were brought into focus using 100x objective with the iris diaphragm completely opened to increase the amount of light that enter the objective. Gram reaction was determined on the colour of dye retained; Gram positive organisms retained the colour of the primary dye (crystal violet) and appear purple while Gram negative organisms colour of the primary dye after decolourization and pick up the colour of the secondary dye (safranin) and appeared red in colour.

2.6.2. Motility Test

Motility of the isolates was determined using sulphide motility indole media. The colony of the 24hrs isolate were picked using a straight inoculation needle; the medium was stabbed through the center to a depth of about 3inch in the tube. The needle was removed from the medium along the same line it entered the medium. The tubes were incubated at 37°C and were examined daily for up to 7 days.

The tubes were observed for a diffuse zone of growth flaring out from the line of inoculation which is an indicative for motility positive result; inability of the organism to diffuse into the medium from the line of inoculation is an indicative of motility negative result.

2.7. Biochemical test

2.7.1. Coagulase Test

The slide method as described by Cheesbrough, [12] was used to carry out the test. A drop of distilled water was placed on a slide and colonies of the test organism was emulsified to make a thick suspension, and a loopful of plasma was added to the suspension and mixed gently and will be observed for agglutination within 10seconds. Agglutination within 10seconds indicates coagulase positive result.

2.7.2. Sugar Fermentation Test

The media (Triple Sugar Iron Agar) was prepared and aliquoted in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the isolate and was incubated at 37°C for 24hrs. The tube were be observed for lactose, sucrose and glucose fermentation on the basis of colour change as a result of acid production in the medium due to fermentation of the sugars by the test isolate.

2.7.3. Indole Test

The test organism was inoculated in a test tube containing 5ml of sterile peptone water Cheesbrough, [12].and was incubated at 37°C for 48hrs and 0.5ml of Kovac's reagent was added and shakes gently and was examined for red colour formation on the surface layer within 10mins. The formations of red colour on the surface layer indicate indole positive result.

2.7.4. Urease Test

The media (urea agar base) was prepared and aliquoted in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the isolate and was incubated at 37°C for 18hrs. The tube were observed for colour change. Red/pink colour indicates urease positive result.

2.7.5. Citrate Utilization Test

The media was prepared and aliquoted in a test tube and placed in a slant position. The butt was stabbed using a sterile straight inoculation needle and the slope was streaked with a saline suspension of the organism and incubated at 37°C for 48hrs. A bright blue colouration in the medium indicated citrate positive result.

2.7.6. Catalase Test

A drop of hydrogen peroxide was placed on a microscope slide and the isolate were picked using wire loop and was emulsified in the drop of the hydrogen peroxide and was observed for immediate bubble formation with indicate catalase positive result.

2.8. Susceptibility Testing

The susceptibility pattern of the bacterial isolates was assayed according to Kirby-Bauer disc diffusion method, on muller Hinton agar plates following the procedures described by clinical and laboratory standard institute (CLSI) [11].

2.8.1. Disc diffusion method (modified kirby-bauer technique)

Selected bacteria colonies isolated was inoculated into test tubes with peptone water. The tubes was adjusted to 0.5 McFarland standard, with the use of a sterile swab stick the inoculum was streaked on muller- Hinton agar plates. The antimicrobial impregnated discs was firmly placed on the surface of the medium and the plates was incubated at 37°C for 24 hours. The zone of inhibition was measured in millimeter, recorded and compared to Clinical Laboratory Standard Institute. This procedure was repeated for the test organisms [11].

2.9. Statistical Analyses

Data obtained was analyzed using SPSS software. The mean \pm standard deviation while analysis of chi square (χ^2) was used to analyze the extent of variation between groups and P values of less than 0.5 was considered significant while P values greater than 0.5 was considered non-significant, Fisher's least significant difference (FLSD) was used to compare several groups.

3. Results

This study investigated the antibiogram of selected bacteria isolated from urine contaminated soil on the campuses of Benue state university, Makurdi.

3.1. Bacteria isolated from urine contaminated soil in Benue State University, Makurdi.

Table 1 shows the bacteria isolated from urine contaminated soil on Benue state university campuses, Makurdi. Five bacteria isolates were observed which include: *Staphylococcus* spp., *Escherichia coli*, *Bacillus* spp., *Salmonella* spp., and *Klebsiella* spp. *Bacillus* spp was the most occurring bacterium 25(39.68 %), followed by *E coli* 13(20.63 %). The least occurring species was *Salmonella* spp with an occurrence of 7(11.11 %). A non-significant occurrence was observed with respect to the isolates ($X^2 = 1.440$, $df = 1$, $p = 0.230$).

3.2. Distribution of bacteria isolates from urine contaminated soil among sampling locations in Benue State University

Table 2 shows the distribution of the isolates in the sample locations (campuses). The western wing (first campus) was observed to have higher bacteria occurrence (55.56 %) than samples from the eastern wing (second campus) (44.14 %). There was no significant association with respect to the sample locations ($X^2 = 1.440$; $df = 1$; $p = 0.230$).

3.3. Total bacteria colony count of isolates

Table 3 shows the total bacterial colony count of the bacteria isolates from urine contaminated sites in Benue State University, Makurdi. The result showed a slightly higher colony count on the Eastern Wing Campus (7.85×10^7 cfu/mL) than the Western wing campus (7.05×10^7); no significant difference was observed between them ($p > 0.005$).

3.4. Antibiogram of selected bacteria isolated from urine contaminated soil in Benue State University.

Table 4 shows the antibiogram bacteria isolated from contaminated sites in Benue State University campuses. It was observed that all the *Staphylococcus* spp isolates obtained were resistant to all the antibiotics except 50 % were susceptible to Gentamicin. All the *E coli* isolated were resistant to all the antibiotics (100 %). While 50 % of *Bacillus* spp on the other hand was susceptible to Imipenem, all were susceptible to Gentamicin and Ciprofloxacin (100 %); and all were however resistant to Oxacillin (100.00 %). *Salmonella* spp and *Klebsiella* spp showed similar pattern of susceptibility. All the isolates of the two organisms were susceptible to Imipenem, Gentamicin and Ciprofloxacin (100 %), whole they were all resistant to Oxacillin.

Table 1 Bacteria isolated from urine contaminated soil in Benue State University, Makurdi.

Bacteria isolates	Occurrence	% occurrence
<i>Staphylococcus</i> spp	8	12.70
<i>Escherichia coli</i>	13	20.63
<i>Bacillus</i> spp	25	39.68
<i>Salmonella</i> spp	7	11.11
<i>Klebsiella</i> spp	10	15.87
Total	63	100

$X^2 = 27.069$; $df = 4$; $p = 0.000$

Table 2 Distribution of bacteria isolates from urine contaminated soil among sampling locations in Benue State University.

Bacteria isolates	sampling locations/ Western wing	Eastern wing	Total
<i>Staphylococcus</i> spp	5	3	8
<i>Escherichia coli</i>	5	8	13
<i>Bacillus</i> spp	16	9	25
<i>Salmonella</i> spp	3	4	7
<i>Klebsiella</i> spp	6	4	10
Total (%)	35(55.56)	28(44.44)	63(100)

$\chi^2 1.440$; $df = 1$; $p = 0.230$

Table 3 Total bacteria colony count of isolates

Sampling location	CFU/mL
Western Wing Campus	7.05×10^7
Eastern Wing Campus	7.85×10^7
	NS

Values are in mean duplicates; NS = Not significant

Table 4 Antibiogram of selected bacteria isolated from urine contaminated soil in Benue State University.

Bacteria Isolates	% Susceptibility			
	Imipenem	Oxacillin	Gentamicin	Ciprofloxacin
<i>Staphylococcus</i> spp (n = 2)	0.00	0.00	50.00	0.00
<i>Escherichia coli</i> (n = 2)	0.00	0.00	0.00	0.00
<i>Bacillus</i> spp (n = 2)	50.00	0.00	100.00	100.00
<i>Salmonella</i> spp (n = 1)	100.00	0.00	100.00	100.00
<i>Klebsiella</i> spp (n = 2)	100.00	0.00	100.00	100.00

4. Discussion

Five bacteria were isolated in this study which include: *Staphylococcus* spp., *Escherichia coli*, *Bacillus* spp., *Salmonella* spp., and *Klebsiella* spp. *Bacillus* spp was the most occurring bacterium 25(39.68 %), followed by *E coli* 13(20.63 %). The least occurring species was *Salmonella* spp with an occurrence of 7(11.11 %). A significant occurrence was observed with respect to the isolates ($\chi^2 = 27.069$; $df = 4$; $p = 0.000$). [8].had a significant occurrence of isolates which does not agree with this but their most occurring bacteria isolate was *Bacillus* spp which agree with this study. A non-significant bacteria isolate occurrence was also observed by Chukwu *et al.* (2018), the most prevalent bacteria was *Bacillus* spp which agreed with this study, similar isolates like *Klebsiella* spp, *Salmonella* spp, *Staphylococcus* spp and *Escherichia coli* were also isolated by [9].which also agreed with this study. The eastern wing has the highest colony count (7.85×10^7) which is significantly higher from sample location D (2.0×10^3) of Chukwu *et al.* (2018). These could be as a result of difference in sample sizes, 50 sample were collected for this study which is higher than the sample size used by [10].

Staphylococcus spp isolates obtained were resistant to all the antibiotics except 50 % that were susceptible to Gentamicin. All the *E coli* isolated were resistant to all the antibiotics (100 %). While 50 % of *Bacillus* spp on the other hand was susceptible to Imipenem, all were susceptible to Gentamicin and Ciprofloxacin (100 %); and all were however resistant to Oxacillin (100.00 %). *Salmonella* spp and *Klebsiella* spp showed similar pattern of susceptibility. All the isolates of the two organisms were susceptible to Imipenem, Gentamicin and Ciprofloxacin (100 %), whole they were

all resistant to Oxacillin, this disagrees with [11],[12].) as they did not assess the antimicrobial susceptibility profile of their isolates. All of the isolates except *Bacillus* spp, *Salmonella* spp and *Klebsiella* spp showed multidrug resistance to the antibiotics used for the antibiogram. Susceptibility was very high to gentamicin and ciprofloxacin. The resistant isolates will cause diseases and could also be transferred into the human body through air and runoff of the contaminated site to water bodies used for domestic purposes. The infection which might arise from the isolates that are multidrug resistant might not be treated using the antibiotics towards which they have become resistant. Choice of susceptible drugs is decreasing very fast. This may result in the death from simple diseases which will fail to resume for unavailability of appropriate susceptible drugs.

5. Conclusion

The eastern wing of the Benue State University had the highest colony count (7.85×10^7) which is not significant to the western wing (7.05×10^7). Five bacteria were isolated from the urine contaminated sites in Benue State University, this include *Staphylococcus* spp, *Escherichia coli*, *Bacillus* spp, *Salmonella* spp and *Klebsiella* spp. The most occurring bacteria isolate was *Bacillus* spp 25(39.68 %) and the bacteria that is least prevalent was *Salmonella* spp 7(11.11 %). *Staphylococcus* spp and *Escherichia coli* demonstrated multidrug resistant to all the antibiotics used for the antibiogram (IMI, OX, CN and CIP) while *Salmonella* spp and *Klebsiella* spp were only resistant to OX but susceptible to the rest antibiotics used for the antibiogram (IMI, CN and CIP).

Recommendation

- Provision of more and adequate toilet facilities within the university premises should be giving priority to prevent excessive contamination of soil with urine.
- The practice and enforcement of basic sanitation rules should be enforced by the university heads
- Awareness on the proper use of antibiotics and enlightenment on the effects of antibiotic resistance should be a priority in the university and the general public.
- Further studies should be carried out to identify specific genes responsible for resistance.
- Rapid investment in new treatment, improved infection control measures and providing more funding to develop new antibiotics.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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