

Research Article

ANTIMICROBIAL EFFECTIVENESS OF GUT MICROBIOTA AGAINST MICROORGANISMS ASSOCIATED WITH URINARY TRACT INFECTIONS AMONG SYMPTOMATIC STUDENTS OF NIGER DELTA UNIVERSITY, NIGERIA

^{1,*} Oluwayemisi A. Olorode (PhD, MLS), ²Ofonime M. Ogba (PhD, MLS), ¹Tuwere Benedict Efe

¹Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Niger Delta University, Nigeria.

²Department of Medical Laboratory Science, Faculty of Allied Medical Sciences, University of Calabar, Nigeria.

Received 19th November 2021; Accepted 20th December 2021; Published online 30th January 2022

ABSTRACT

Urinary Tract Infections (UTIs) is one of the major causes of morbidity in patients, mostly due to resistance development among the uropathogens which frustrate chemotherapy. This study was carried out between March and October, 2021 to determine the Antimicrobial activity of GUT microbiota on bacteria causing urinary tract infection. A total of 72 Mid Stream Urine (MSU) samples, males (18/72, 25%) ; female (54/72,75%), collected at random from Niger Delta University students attending University hospital showing the symptoms of urinary tract infection, were cultured and analyzed using Cysteine Lactose Electrolyte Deficient agar (CLED) and Nutrient Agar. The standardized pure isolates with 0.5 McFarland turbidity standard were subjected to antimicrobial susceptibility testing using six (6) Gut microbiota. The findings showed 100% prevalence of UTI on the study subjects with females preponderance over males. The total number of isolates were 154 [males (36,23.4%); female (118,76.6%), comprise of *Escherichia coli* (58/154, 37.7%), *Staphylococcus saprophyticus* (42/154 , 27.3%), *Proteus mirabilis* (18/154, 11.7%), *Pseudomonas aeruginosa* (18/154, 11.7%), *Klebsiella pneumoniae* (12/154 , 7.8%), and *Staphylococcus aureus* (6/154 , 3.9%). Of 154 uropathogens subjected to gut microbiota (Gut *Escherichia coli* 1, Gut *Escherichia coli* 2, Gut *Escherichia coli* 3, Gut *Escherichia coli* 4, Gut *Escherichia coli* 5 and Gut *Salmonella* spp 6) for antimicrobial susceptibility testing, 30/154 (19.56%) were susceptible; with percentage susceptibility of 33.3, 23.8, 22.2, 17.2 and 11.1 for uropathogen *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Proteus mirabilis* respectively. All *Staphylococcus aureus* uropathogens showed resistance to all the test gut microbiota in this study. In conclusion, this study has revealed the potential antimicrobial activities of gut microbiota against uropathogens with the exception of *Staphylococcus aureus*, therefore the pharmaceutical industries are compelled to incorporate these gut microorganisms into their formulation in drug production for the treatment of urinary tract infection. Further studies are advised, however, gut microbiota are potential probiotics for the treatment of urinary tract infection.

Keywords: UroPathogenic Microorganisms; Gut Microbiota; Antimicrobial Susceptibility Testing; Probiotics.

INTRODUCTION

Urinary tract infection (UTI) is a common contagious infection among men and women though the incidence is quite high among women due to their physiology, men and children are not free from this condition. A urinary tract infection (UTI) is an infection in any part of the urinary system (the kidneys, ureters, bladder and urethra). Most infections involve the lower urinary tract (the bladder and the urethra) (Lipsky 2013; Stamm and Hooton.,2014). Generally the treatment for UTI should be based on the laboratory diagnosis of the etiological agents of the infection, followed by antimicrobial susceptibility testing to determine the drug of choice. It was observed that self medication and indiscriminate prescription of drugs without any laboratory investigation has erupted resurgence of antimicrobial resistance of uropathogens. This infection can be contacted through toilet usage and sexual intercourse (Prodigy, 2011).The human gastrointestinal tract hosts a diverse network of microorganisms, collectively known as the microbiota that plays an important role in health and disease. For instance, the intestinal microbiota can prevent invading microbes from colonizing the gastrointestinal tract, a phenomenon known as colonization resistance. Perturbations to the microbiota, such as antibiotic administration, can alter microbial composition and result in the loss of colonization resistance. In humans, the gut microbiota has the largest numbers of bacteria and the greatest number of species

compared to other areas of the body (Quigley., 2013). In humans, the gut flora is established at one to two years after birth, by which is seen in the intestinal epithelium and the intestinal mucosal barrier (Sommer and Bäckhed., 2013) that also provides a barrier to pathogenic organisms (Faderl et al., 2015) . Probiotics are live microorganisms promoted with claims that they provide health benefit when consumed, they can be found in Yogurt, Sauerkraut, Tempeh, Pickles. Probiotics are made up of good bacteria that helps keep body in healthy condition. These good Bacteria serve as immune systems which fight harmful bacteria from the body system and help maintain healthy condition. Commonly used probiotics are Lactobacillus and Bifidobacterium (*Saccharomyces boulardii*). Probiotics are live Micro-organisms that may be able to help prevent and treat some illness. According to the Food and Agriculture Organisation of the United Nations (FAO) and the World Health Organisation (WHO), probiotics are defined as live microorganisms, which when administered in adequate amounts, confer a health benefit on the host (Gould et al., 2011). Probiotics are GUT micro-organism that help with food digestion, immunity and inflammation . The non pathogenic strain of *Escherichia coli* Nissle (ECN) is one of the most used probiotics strain in GUT microbiota homeostasis (Andrew et al., 2014). It has been shown that ECN can stimulate the production of human β -defensin 2, which can protect the mucosa barrier adhesion and invasion by pathogenic commensals (Andrew et al., 2014). GUT microbiota can be potential probiotics in the treatment of infection caused by micro-organism of the urinary tract. Most commonly used probiotics micro-organisms against these pathogens are *Lactobacillus acidophilus*, *Lactobacillus rhamnosus*, *Saccharomyces boulardii*, *Bifidobacterium bifidum* and *Bacillus*

*Corresponding Author: DR OLUWAYEMISI AGNES OLORODE,

1Department of Medical Laboratory Science, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island. Bayelsa State, Nigeria.

coagulans. The GUT microbiota have important role in nutrient and mineral absorption, enzymes, vitamins, amino acids synthesis with production of short-chain fatty acids (SCFAs) (Arellano *et al.*, 2011). The infection can occur more frequently with increasing age, due to a blockage in the urinary tract, having a bladder catheter, or with a decreased immune system.

The infection is named after the location in the urinary tract, where it occurs:

- Infection of the ureter is urethritis
- Infection of the bladder is cystitis
- Infection of the prostate is prostatitis
- Infection of the testicles is orchitis
- Infection of the epididymis is epididymitis
- Infection of the kidneys is pyelonephritis. This study aims to determine the antimicrobial effectiveness of GUT microbiota on bacteria associated with Urinary Tract Infection (UTI).

MATERIALS AND METHODS

Study Area

The study was carried out in the Niger Delta University School Hospital in southern ijaw Local Government Area in Amassoma community, Bayelsa State. Amassoma is geographically located at lat 4°58'13.15"N, Long 6°06'32.94"E. It has an area of 4km² and populated by different ethnic groups across the country. Niger Delta University School Hospital is located at Wilberforce island, PMP 071 Bayelsa NG, 560103, Amassoma.

Ethical Clearance

Ethical clearance was obtained from the ethical committee of the hospital

Sample Size

To calculate the minimum sample size, the Taro Yamane formula was used.

Sample Collection

Seventy-two (72) mid stream urine samples were obtained from the department of Medical Laboratory Science in Niger Delta University School Hospital, Amassoma, Bayelsa Sate.

Sample Processing

Isolation, identification and characterization of bacteria associated with Urinary Tract Infection (UTI)

The samples were cultured into Cystine-Lactose-Electrolyte-Deficient agar (CLED) at 37°C for 24hrs. The colonies in the CLED were identified through their colonial morphology. The colonies were subcultured into nutrient agar and incubated at 37°C for 24hrs. The isolates were identified using Standard Microbiological Methods

Antimicrobial Susceptibility Testing Using Gut Microbiota MATERIALS:

GUT microbiota (from faecal samples of healthy subjects) labelled 1,2,3,4,5 and 6; Microbial isolates (Uropathogens); Muller Hinton; Sterilized disc; forceps; bunsen burner

METHOD:

- 1) Muller hinton agar was prepared, dried in the hot air oven and labelled according to the microbial isolates
- 2) Each microbial isolate was inoculated in corresponding plate of muller hinton agar by spread plate method
- 3) GUT Microbiota (isolated from the faecal samples of healthy individuals) suspension compared with Mac Farland Standard
- 4) Sterilized perforated disc was impregnated with the GUT microbiota labelled 1 and placed into the muller hinton plate, This was repeated with second gut microorganism (2), third (3), fourth (4), fifth (5) and the sixth (6) GUT Microbiota
- 5) The plate was labelled according to the GUT microbiota and placed in the incubator for 24 hours

RESULTS

Table 1.0: Demographic frequency distribution table

	FREQ.	MALE(%)	FEMALE(%)	AV.AGE	MIN AGE	MAX.AGE
NDU SCH. HOSP.	72	18(25%)	54(75%)	25 YRS	17YRS	34 YRS

72 samples were collected, 18 (25%) males and 54 (75%) females, with the minimum and maximum age of 17years and 34years respectively

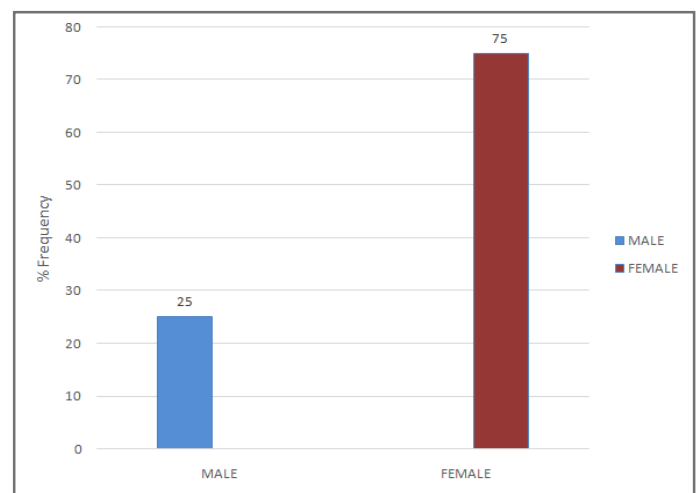


Fig 1: Percentage frequency distribution of urinary tract infected patients attending Niger Delta University School Hospital based on Gender. Females (75%) having the highest percentage frequency than males (25%)

Table 2.0: Frequency distribution of microorganisms associated with urinary tract infection from Niger Delta University School Hospital based on Gender.

Microbial isolates	FREQUENCY		PERCENTAGE	FREQUENCY%	
	Total	Male Female		Total	Male Female
<i>E. coli</i>	58	10 48	37	18	82
<i>P. mirabilis</i>	18	4 14	11.7	21	79
<i>P. aeruginosa</i>	18	3 15	11.7	16	84
<i>S. saprophyticus</i>	42	13 29	27.3	32	68
<i>K. pneumoniae</i>	12	3 9	7.8	29	71
<i>S. aureus</i>	6	1 5	3.9	25	75

58/154,37% *Escherichia coli* [males (10/58,18%), females (48/58,82%)], 18/154,11.7% *Proteus mirabilis* [males (4/18,21%), females (14/18,79%)], 18/154,11.7% *Pseudomonas aeruginosa* [males (3/18,16%), females (15/18 ,84%)] 42/154,27.3%

Staphylococcus saprophyticus [males (13/42,32%), females (29/42,68%)], (12/154,7.8%) *Klebsiella pneumoniae* [males (3/12,29%), females (9/12,71%)] and 6/154,3.9% *Staphylococcus aureus* [males (1/6,25%), females (5/6,75%)].

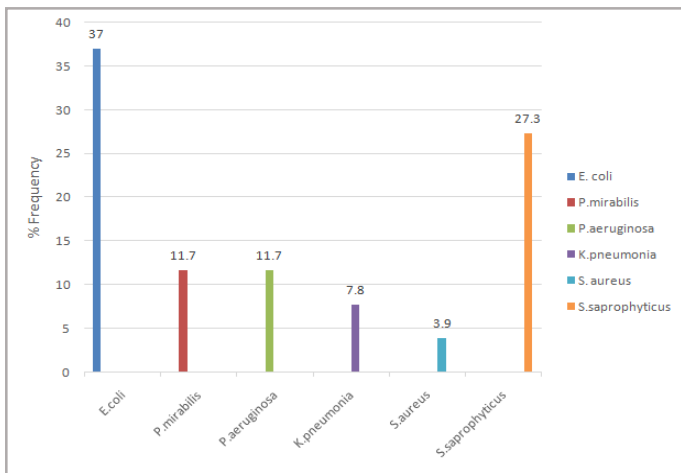


Fig 2.0 Barchart showing percentage Frequency distribution of bacteria associated with urinary tract infection among patients visiting Niger Delta University School Hospital.

Escherichia coli (37%) had the highest percentage frequency, followed by *Staphylococcus saprophyticus* (27.3%), *Proteus mirabilis* and *Pseudomonas aeruginosa* having same frequency of 11.7%, *Klebsiella pneumoniae* (7.8%) and *Staphylococcus aureus* having the lowest percentage of 3.9%.

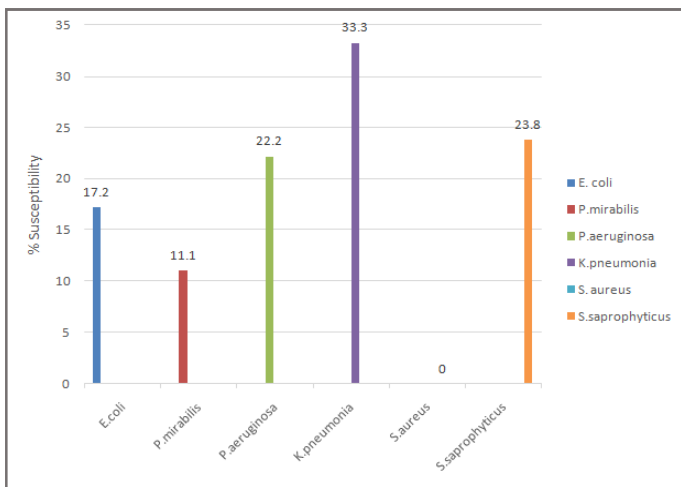


Figure 3.0 Bar Chart showing the percentage susceptibility patterns of uropathogens associated with urinary tract infection among patients visiting Niger Delta University School Hospital against Gut microbiota.

Out of fifty eight (58) *Escherichia coli* isolates, 10 (17.2%) were susceptible to Gut microbiota. Out of eighteen (18) *Proteus mirabilis* isolates, 2 (11.1%) were susceptible to Gut microbiota. Of 18 *Pseudomonas aeruginosa*, 4 (22.2%) were susceptible to Gut microbiota. Out of forty two (42) *Staphylococcus saprophyticus*, 10 (23.8%) were susceptible to Gut microbiota. Out of 12 *Klebsiella pneumoniae*, 4 (33.3%) were susceptible to Gut microbiota. Out of 6 *Staphylococcus aureus*, none were susceptible to Gut microbiota. *Klebsiella pneumoniae* (33.3%) having the highest percentage susceptibility, followed by *Staphylococcus saprophyticus* (23.8%), *Pseudomonas aeruginosa* (22.2%), *Proteus mirabilis* (11.1%) and *Escherichia coli* (17.2%). *Staphylococcus aureus* shows 100% resistance.

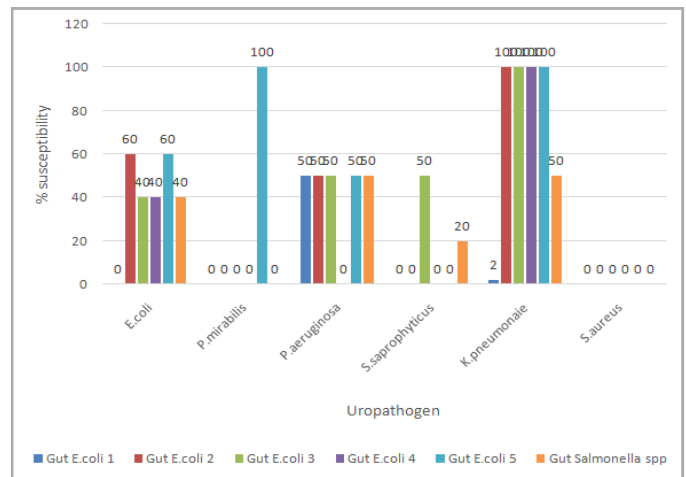


Fig 4.0 Barchart showing antimicrobial susceptibility testing of uropathogens against Gut microbiota.

Sixty percent (60%) of uropathogen *Escherichia coli* isolates were susceptible to gut *Escherichia coli* 2, 40% to gut *Escherichia coli* 3, 40% to gut *Escherichia coli* 4, 60% to Gut gut *Escherichia coli* 5 and 40% to Gut *Salmonella spp*. None was susceptible to Gut gut *Escherichia coli* 1. The percentage susceptibility of uropathogen *Proteus mirabilis* was 100 to gut *Escherichia coli* 5, with 100% resistance to gut *Escherichia coli* 1, gut *Escherichia coli* 2, gut *Escherichia coli* 3, gut *Escherichia coli* 4 and gut *Salmonella spp*. Fifty percent (50%) of uropathogen *Pseudomonas aeruginosa* isolates were susceptible to gut *Escherichia coli* 1, gut *Escherichia coli* 2, gut *Escherichia coli* 3, gut *Escherichia coli* 5 and gut *Salmonella spp*., but recorded 100% resistance to *Escherichia coli* 4. Uropathogen *Staphylococcus saprophyticus* had 50% susceptibility to gut *Escherichia coli* 3, 20% to gut *Salmonella spp*. Uropathogen *Klebsiella pneumoniae* had 100% susceptibility to gut *Escherichia coli* 2, gut *Escherichia coli* 3, gut *Escherichia coli* 4, gut *Escherichia coli* 5. 50% to gut *Escherichia coli* 1 and *Salmonella spp*. Uropathogen *Staphylococcus aureus* had 100% resistance to all the gut microbiota used as antimicrobial agents in this study.

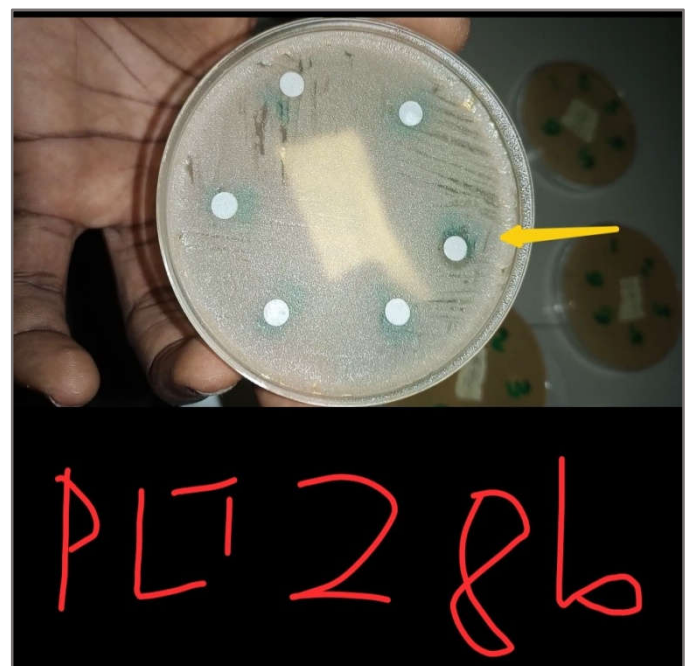


Fig 5.0 Plate 28b -As it is indicated by arrow , the zone of inhibition demonstrated by the impregnated Gut *Escherichia coli* against isolated uropathogen *Proteus mirabilis*.



Fig 6.0 Plate 19b - As it is indicated by arrow , the zone of inhibition demonstrated by the impregnated Gut *Escherichia coli* against isolated uropathogen *Staphylococcus saprophyticus*.

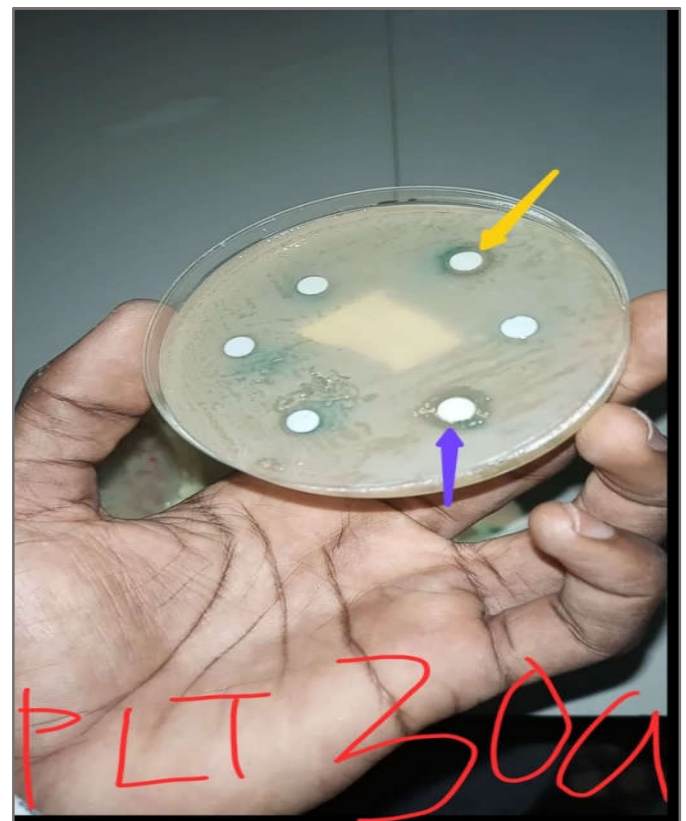


Fig 8.0 Plate 30a - As it is indicated by arrow , the zone of inhibition demonstrated by the impregnated Gut *Escherichia coli* against isolated uropathogen *Pseudomonas aeruginosa*.

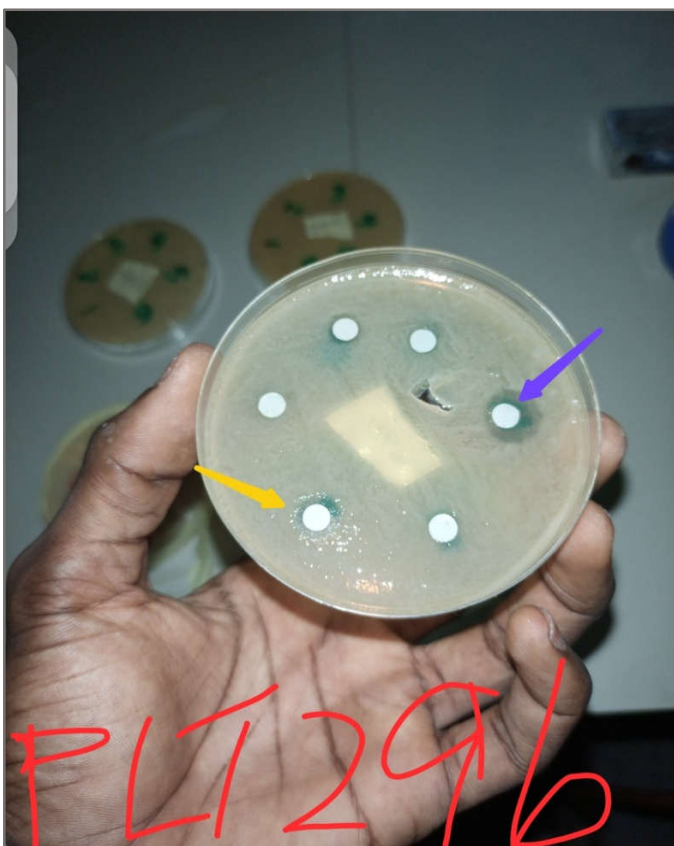


Fig 7.0 Plate 29b - As it is indicated by arrow , the zone of inhibition demonstrated by the impregnated Gut *Escherichia coli* against isolated uropathogen *Klebsiella pneumoniae*

DISCUSSION

In this cross-sectional study, a total of 72 samples of clean catch midstream urine (MSU) samples were collected aseptically from Niger Delta University School Hospital and analyzed using standard microbiology methods. One hundred and fifty-four (154) uropathogens isolated comprise of 58/154 (37.7%) *Escherichia coli*, 18/154 (11.7%) *Proteus mirabilis*, 18/154 (11.7%) *Pseudomonas aeruginosa*, 42/154 (27.3%) *Staphylococcus saprophyticus*, 12/154 (7.8%) *Klebsiella pneumoniae* and 6/154 (3.6%) *Staphylococcus aureus*. This result is in concordance with the work done by kabugo *et al.*, 2016 on patient attending Bushenyi District Hospital in Uganda and reported a lower value of 41.9% prevalence of *Escherichia coli*; while Prakash *et al.*, 2013 reported 12.44% *Proteus mirabilis*. The percentage of *Pseudomonas aeruginosa* (11.7%) reported was higher than the work done by Conwat *et al.*, 2007 and reported 10.8% for *Pseudomonas aeruginosa*. While Kabugo *et al.*, 2016 had a higher value of 29.5% *Staphylococcus saprophyticus* in place of 27.3% reported in this work. The higher percentage prevalence of urinary tract infection was observed in females (75%) than their male (25%) counterpart just like Kabugo *et al.*, 2016 observed higher values in females (79%) and males (21%) in their study carried out in Uganda, then stated that females are more preponderance to urinary tract infection due to their wider opening of the genital tract. Men have the lower value of urinary tract infection due to the little inner opening of the reproductive tract and proper awareness/precautionary measures taken to prevent the infection From the work done by kabugo *et al.*, 2016 in Uganda on patient attending Bushenyi District Hospital revealed that *Escherichia coli* (80%) was the highest prevalence organism, followed by 27/86, (31.4%) *Klebsiella pneumoniae*, 10/86 (11.66%) *Klebsiella oxytoca*, 6/86 (7%) *Proteus mirabilis*, 3/86 (3.5%) *Enterococcus faecalis* and 1/86 (1.2%) *Proteus vulgaris*. Andrew *et al.*, 2014 showed a lower prevalence of

Escherichia coli (32.7%) in their work as compared with 37.7% *Escherichia coli* gotten from this study but reported a higher prevalence of *Proteus mirabilis* (17.25%) as compared with 11.7% *Proteus mirabilis* reported in this study. Gut microbiota are micro-organisms that colonize the digestive tract of human thereby preventing the establishment of pathogenic micro-organisms in the gut. In this study, six gut microbiota (Gut *Escherichia coli* 1, Gut *Escherichia coli* 2, Gut *Escherichia coli* 3, Gut *Escherichia coli* 4, Gut *Escherichia coli* 5 and Gut *Salmonella* spp) were used as antibiotics in antimicrobial susceptibility testing against isolated uropathogen *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Staphylococcus saprophyticus* and *Staphylococcus aureus*. *Klebsiella pneumoniae* (33.3%) have the highest percentage susceptibility, followed by *Staphylococcus saprophyticus* (23.8%), *Pseudomonas aeruginosa* (22.2%), *Escherichia coli* (17.2%), and *Proteus mirabilis* (11.1%). Uropathogens susceptible to Gut *Escherichia coli* 5 include (Uropathogen *Escherichia coli*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), followed by Gut *Escherichia coli* 3 (Uropathogen *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus* and *Klebsiella pneumoniae*), Gut *Escherichia coli* 2 (Uropathogen *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), Gut *Salmonella* spp (Uropathogen *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*), Gut *Escherichia coli* 4 (Uropathogen *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*) and Gut *Escherichia coli* 1 ((Uropathogen *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*).

CONCLUSION

Escherichia coli was the most prevalent micro-organisms causing urinary tract infection while the least was *Staphylococcus aureus*. *Klebsiella pneumoniae* has the highest percentage susceptible to GUT microbiota while the least was *Staphylococcus aureus* having 0% susceptibility. Total percentage susceptible was 25.9% showing that Gut microbiota may be used as probiotics to bacteria causing UTI and further research need to be done.

Recommendation

The prevalence of UTI among persons has posed a life threatening infection to the health of individuals. Drug resistance has been encountered during treatment due to both intrinsic and extrinsic factors expressed by microorganisms. GUT microbiota may provide an alternative way in the treatment of UTI infection to overcome the drug resistance.

REFERENCES

- Andrews, S.J; Brooks, P. T; Hanbury, D. C. 2014. Ultra - sonography and abdom inal radiography versus intravenous urography in investigation of urinary tract infection in men: *prospective incident cohort study*. *BMJ* **324**: 454-456
- Arellano, Ronald S ., 2011. Non-vascular interventional radiology of the abdomen. New York: Springer. p. 67. ISBN **978-1-4419-7731-1**. Archived from the original on 10 June 2016.
- Conway P. H ; Cnaan A; Zaoutis T; Henry B .V; Grundmeier R.W and Keren R., 2007. "Recurrent urinary tract infections in children: risk factors and as sociation with prophylactic antimicrobials," *JAMA*, vol. 298, no. 2, pp. 179–186, 2007.
- Faderl, Martin; Noti, Mario; Corazza, Nadia; Mueller, Christoph ., 2015. "Keeping bugs in check: The mucus layer as a critical component in maintaining intestinal homeostasis". *IUBMB Life*. 67 (4): 275–85. doi:10.1002/iub.1374 PMID [25914114](#), S2CID [25878594](#).
- Gould, C. V; Umscheid, C. A; Agarwal, R. K; Kuntz, G; Pegues, D. A., (2011). "Guideline for prevention of catheter-associated urinary tract infections 2011". *Infect Control Hosp Epidemiol*. 31 (4): 319–26. doi:10.1086/651091. PMID [20156062](#)
- Kabugo D; Kizito S; Ashok D. Det al., 2016. "Factors associated with community-acquired urinary tract infections among adults attending assessment centre, Mulago Hospital Uganda," *African Health Sciences*, vol. 16, no. 4, 2016.
- Lipsky B. A.,(2013). Prostatitis and urinary tract infection in men: what's new, what's true? *Am Journal Med*106: 327-334
- Prakash and Saxena R. S., 2013. "Distribution and antimicrobial susceptibility pattern of bacterial pathogens causing urinary tract infection in Urban Community of Meerut City, India," *ISRN Microbiology*, vol. 2013, Article ID 749629, 13 page, 2013.
- PRODIGY.,(2011). Urinary tract infection (lower) – men: Sowerby Cen tre for Health Infor ma tics at Newcastle. www.prod igy.nhs.uk/guid ance.asp?gt=UTI%20 (lower %20 - %20 men. (accessed on Jan 29, 2011)
- Quigley, E. M (2013). "Gut bacteria in health and disease". *Gastroenterology & Hepatology*. 9 (9): 560–9. PMC [3983973](#). PMID [24729765](#).
- Stamm, W. E; Hooton, T. M.,(2014). Management of urinary tract infection in adults. *National England Journal Medicine* 329: 1328-1334.
- Sommer, Felix; Bäckhed, Fredrik., 2013. "The gut microbiota — masters of host development and physiology". *Nature Reviews Microbiology*. 11 (4): 227 – 38. doi:10.1038/nrmicro2974. PMID [23435359](#). S2CID [22798964](#) .
- Kabugo D; Kizito S; Ashok D. Det al., 2016. "Factors associated with community-acquired urinary tract infections among adults attending assessment centre, Mulago Hospital Uganda," *African Health Sciences*, vol. 16, no. 4, 2016.
