

PREVALENCE OF BACTERURIA LINKED GRAM-NEGATIVE STRAINS AND PHENOTYPIC VIRULENCE DYNAMICS IN APPARENTLY HEALTHY STUDENTS OF WESTERN DELTA UNIVERSITY AT SCHOOL RESIDENCE

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Abstract

Bacteria in urine of apparently healthy human subjects which is also known or referred to as asymptomatic bacteriuria has been receiving public health attention in recent times especially as there are increasing reports of clinical asymptomatic infection cases amongst individuals. As a strategy towards public awareness campaign within the University accommodation environment and assessment of microbiological wellness of students, the prevalence of Gram negative asymptomatic bacteriuria and phenotypic virulence determinants were determined amongst asymptomatic or apparently healthy students in Western Delta University at student Residential area. Eighty-five freshly void urine specimen were collected from students in WDU student residential area. Applying standard microbiological techniques and characterisation, retrieved isolates were presumptively identified. Our result revealed that 60% (48/85) of the examined samples show positive presence of pus cell using microscope and a simultaneous occurrence of diverse organisms ranging from *Escherichia coli* (37.1%), *Staphylococcus aureus* (25.8%), *Pseudomonas aeruginosa* (9.7%), *Serratia* species (4.8%), *Proteus* species (6.5%), *Bacillus* species (12.9%), and *Staphylococcus epidermis* (3.2%). Very importantly, the observed organisms are potential urinary tract infections (UTIs) pathogens as were previously reported by diverse investigators of related studies. The antibiotic susceptibility testing (AST), the antibiogram and phenotypic virulence tests shows that the observed organisms are both potential pathogens and harbour multiple antibiotic resistant (MAR) determining factors including resistance to piperacilin and tozobactam (PIT-110C; 100%), Cefotaxin (CTX-30C; 100%), Clindamycin (CD-2C; 100%); Ceftriaxon-sulbactam (CRO-45C; 100%), Cefuroxime (CXM--30C; 100%), Ampiclox ACX-10C. The need for regular evaluation of apparently healthy individuals (especially the students) for urinary tract infections is suggestive. It is also important to apply standard antibiotics and specific treatment processes which are adequate for the treatment of infection cases to avoid the continuous occurrence/reporting of resistant strains amongst human subjects.

Keyword: Asymptomatic bacteriuria; WDU students; antibiotic resistance; Gram-negative organisms

INTRODUCTION

Homeostasis and the regular passage of waste in the form of fluid from the human system has been a normal characteristic of the usual livelihood of man. However, the unhealthy activities by various individuals as well as environmental dynamic has resulted the reporting of some infection related cases which affects the lining of fluid passage. Such fluids are referred to as

sweat and urine. Urine is a waste product release from the human system as fluid from the ureter and urethra. Over the years, there had been an observed failure in application of appropriate health and hygienic practices by some individuals who regularly passes out urine (Chatterjee *et al.*, 2009). These unhealthy practices have resulted in urinary bacterial infections associated with both Gram positive and Gram negative (Mezue *et al.*, 2006). Such infections are prevalent amongst pregnant

women attending antenatal clinics and reports have shown that Gram-negative bacteria are most frequently associated in addition to immuno-compromised patients in the hospitals or health systems (Okaijahet *al.*, 2019). Diseases that affects the urinary tubules are usually called urinary tract infections (UTI's), which affects the bladder, ureter, kidney and urethra (Kriplainet *al.*, 1993). These diseases are variously complicated when it ascends the upper urinary tract or genitourinary tracts which may be symptomatic and/or asymptomatic (Mezueet *al.*, 2006; Akinloyeet *al.*, 2006). The asymptomatic bacterial associated urinary tract infection is a condition whereby freshly void urine culture reveals growth of bacterial pathogens numerical density with number greater than 10^5 bacteria colonies per millilitre (ml) in the absence of notable symptoms (Lavanya and Jogalakshmi, 2008). Urinary Tract Infections affects all age, but common amongst female subjects than males due to their anatomically short urethra which may easily be contaminated with faecal flora. Asymptomatic bacteriuria (ASB or ABU) has been shown to be implicated with most Gram negative bacteria which belong to the Enterobacteriaceae family. These Gram negative bacteria include: *Escherichia coli*, *Proteus mirabilis*, *Klebsiella* species, *Pseudomonas aeruginosa* etc. *Escherichia coli* (*E. coli*) is a Gram-negative and rod-shaped bacteria belonging to the phylum *Proteobacteria* and family *Enterobacteriaceae* (Okaijahet *al.*, 2019; Mezueet *al.*, 2006; Akinloyeet *al.*, 2006). Such Gram-negative bacteria are also reported to be resistant to multiple antibacterial agents and are increasingly

resistant to most available antibiotics. These bacteria have built-in abilities to find new ways to be resistant and can pass along genetic materials that allow other bacteria to become drug-resistant as well (Akinloyeet *al.*, 2006). In addition, some of such Gram-negative infections may include those caused by *Klebsiella* species, *Acinetobacter* species, *Pseudomonas aeruginosa* as well as many other less common bacteria. Bacteria are present both *in vivo* and on the surface of the human body, especially on the skin and the mucous membranes. Most of these bacteria are innocuous, many are beneficial, and some are even necessary flora. Virulence is the pathogenic ability to cause disease, and the pathogenic bacteria possess several factors that enable them to elicit their virulence dynamics (i.e., the degree of pathogenicity). Most Gram-negative organisms make use of a combination of two properties to cause disease; such as toxicity (the degree to which a substance causes harm) and invasiveness (the ability to penetrate into the host and spread) (Lavanya and Jogalakshmi, 2008). The final balance of an infectious disease process will depend on the virulence or pathogenicity of the microbe as well as the host status in relation to risk factors such as immune status, age, diet, and stress, which determine the host susceptibility to infection (Akinloyeet *al.*, 2006). Employing the Center for Disease Control's (CDCs) aggressive recommendations may encourage prevention and spread of such Gram-negative pathogens (Lavanya and Jogalakshmi, 2008; Mezueet *al.*, 2006; Akinloyeet *al.*, 2006). One of such step employed in the control of such organisms is the use of pharmaceutical agents and

antibiotics. However most of these organisms have become resistant to various groups of antibiotics making the problem of controlling Asymptomatic bacteriuria to persist amongst the populace. It is important to note that asymptomatic bacteriuria as well as urinary tract infections (UTIs) is among common infectious diseases suspected to occur amongst the general public especially as the clinical symptoms are not revealed. This case is not far from what is presumed to be happening in the Western Delta University (WDU) Hostel as the student does not employ proper hygienic practices in their use of public toiletries. As a strategy towards public awareness campaign within the University accommodation environment and assessment of microbiological wellness of students, the prevalence of Gram negative bacteriuria and phenotypic virulence determinants was determined amongst asymptomatic or apparently healthy student in Western Delta University Students Residential area.

Materials and methods

A total of eighty-five freshly void mid stream urine samples were collected from apparently healthy students both females and males who are resident in WDU student residential area. All the students were provided with wide mouth, red cap tightly closed sterilized collection container/bottle or urinalysis containers. The students were taught the technique to apply (to collect the clean catch midstream urine). The ethical clearance was approved on the 7th October 2019 by the Department of Microbiology and Biotechnology Research Ethics Board with reference number WDUMCB/2020/ECCvol/140. Samples

were transported to the microbiology laboratory immediately for analysis. Samples were inoculated onto MacConkey agar, Cystine lactose electrolyte deficient agar (CLED) for *E. coli*, Methyl violet agar for *Klebsiella spp.*, Heart infusion agar for *Proteus* and on Citrimide agar for *Pseudomonas spp.* Plates were incubated in an incubator at 37 °C for 24 hours. An aliquote of the urine samples was centrifuged and deposit or sediment was examined macroscopically for epithelial/pus cells, crystals, *Trichomonas vaginalis* etc (Shadracket *al.*, 2019; Lavanya SV, Jogalakshmi D. 2008). Diverse biochemical and phenotypic virulence test including Methyl red, Voges Proskauer medium, Cimmmons citrate agar, Oxidase, Urea agar, Indole test, Catalase, Coagulase, protease were conducted on presumptive isolates (Igereeet *al.*, 2021).

Phenotypic Detection of virulence characteristic

Haemolysin detection

Hemolysin which is a cytolytic toxin that may occur either as alpha and/or beta-hemolysin was included as virulence test was conducted using blood agar as described by Igbinosaet *al.*, 2017. Pure presumptive isolates were cultured onto blood agar. After about 18-24hours incubation, an observation of lysis of the red pigment around the region surrounding the growth of organism indicates positive haemolysis. This technique involves inoculating a loopful of an overnight test organism onto blood agar by streaking and incubated at 37 °C for 24 hr. Zone of haemolysis (β - haemolysis) appearing

around colonies indicates a positive test (Elliot *et al.*, 2001; Miyagi *et al.*, 2016).

Antibiotic Susceptibility Testing

Antimicrobial susceptibility test of the various isolates was performed using the standard disk diffusion test also commonly known as Kirby-Bauer test. This technique uses Mueller-Hinton agar plate and a paper disk which contains a single concentration of each antimicrobial agent. It is placed centrally on the surface of the agar plate containing the isolate and incubated for 18 – 24 hours in an incubator (37⁰C). Antibiotics used for both Gram positive cocci (GPC) and Gram negative bacilli (GNB) are as follows; (Clindamycin (CD); Ofloxacin (OFX); Nalidixic acid (NA); Furadantin (F); Cefexime (CFX); Levofloxacin (LBC); Azithromycin (AZN); Erythromycin (ERY); Ciprofloxacin (CIP); Gentamicin (CN); Amoxicillin (PIT); Cefuroxime (CRO); Amoxi-clavulanic acid (Augmentin) (ZEM); etc). For Quality Control of antibiotic susceptibility test, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 were used. MDR, XDR, and PDR strains were detected as per criteria described by CLSI and EUCAST (CLSI, 2015; Matuscheket *al.*, 2014; Magiorakoset *al.*, 2012).

Results are interpreted using a standard method published by National Committee for Clinical Laboratory Standards (NCCLS) now known as Clinical Laboratory Standards Institute (CLSI, 2015; Matuscheket *al.*, 2014; Magiorakoset *al.*, 2012).

RESULTS

In the present study, Urine samples from apparently healthy students were analyzed for presence of microorganisms and bacterial infection. It was observed that the standard microbiological techniques applied for characterisation of retrieved isolates identified the following isolates *Escherichia coli* (37.1%), *Staphylococcus aureus* (25.8%), *Pseudomonas aeruginosa* (9.7%), *Serratia* species (4.8%), *Proteus* species (6.5%), *Bacillus* species (12.9%), and *Staphylococcus epidermis* (3.2%) as shown in Table 1 and figure 1

Table 1: Prevalence of retrieved isolates

Presumptive Organisms Retrieved	Numbers retrieved	Percentage occurrence
<i>Escherichia coli</i>	23	37.1%
<i>Staphylococcus aureus</i>	16	25.80%
<i>Pseudomonas aeruginosa</i>	6	9.70%
<i>Serratia specie</i>	3	4.80%
<i>Proteus specie</i>	4	6.50%
<i>Bacillus specie</i>	8	12.90%
<i>Staphylococcus epidemidis</i>	2	3.20%

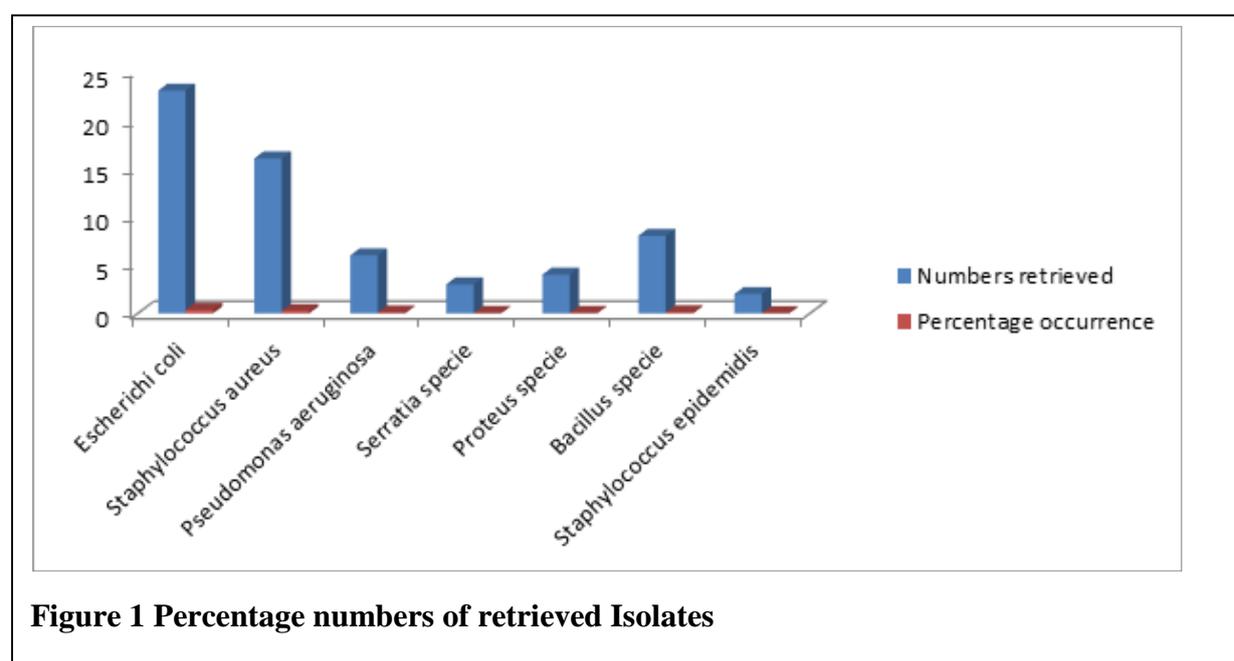


Figure 2 shows the percentage antibiotic resistant and sensitive isolates as revealed from the antibiogram of obtained organisms, Figures 3 shows the antibiogram heatmap where green represent resistant region of isolates, the yellow represent sensitivity region while gray represent intermediate as interpreted by the CLSI guidelines for antibiotic interpretation.

The figures 4 show the photo-microgram of isolates susceptibility testing to the selected antibiotics.

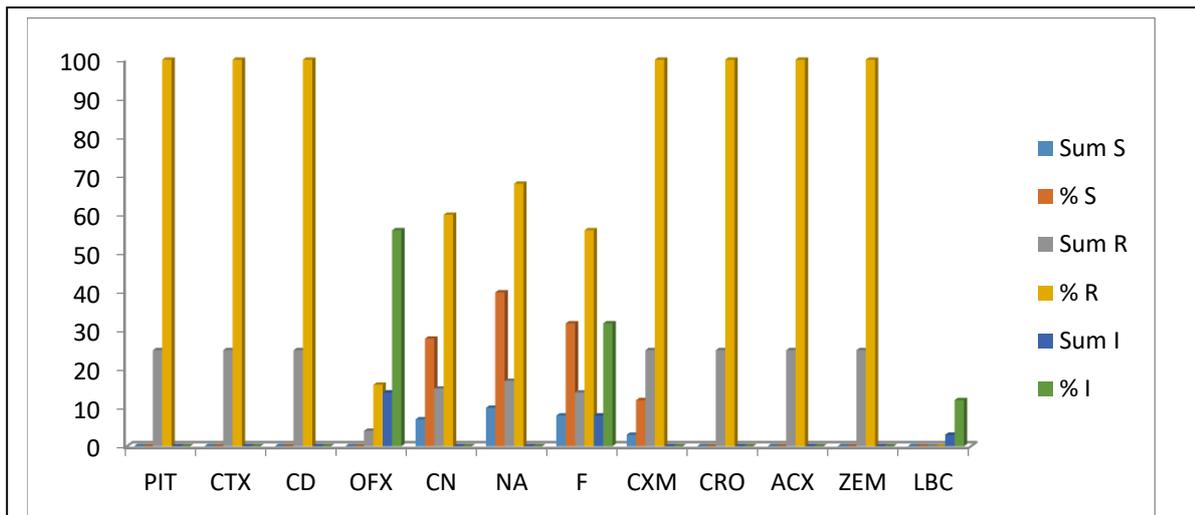


Figure 2 Shows the percentage sensitive and resistant phenotypes of retrieved isolates

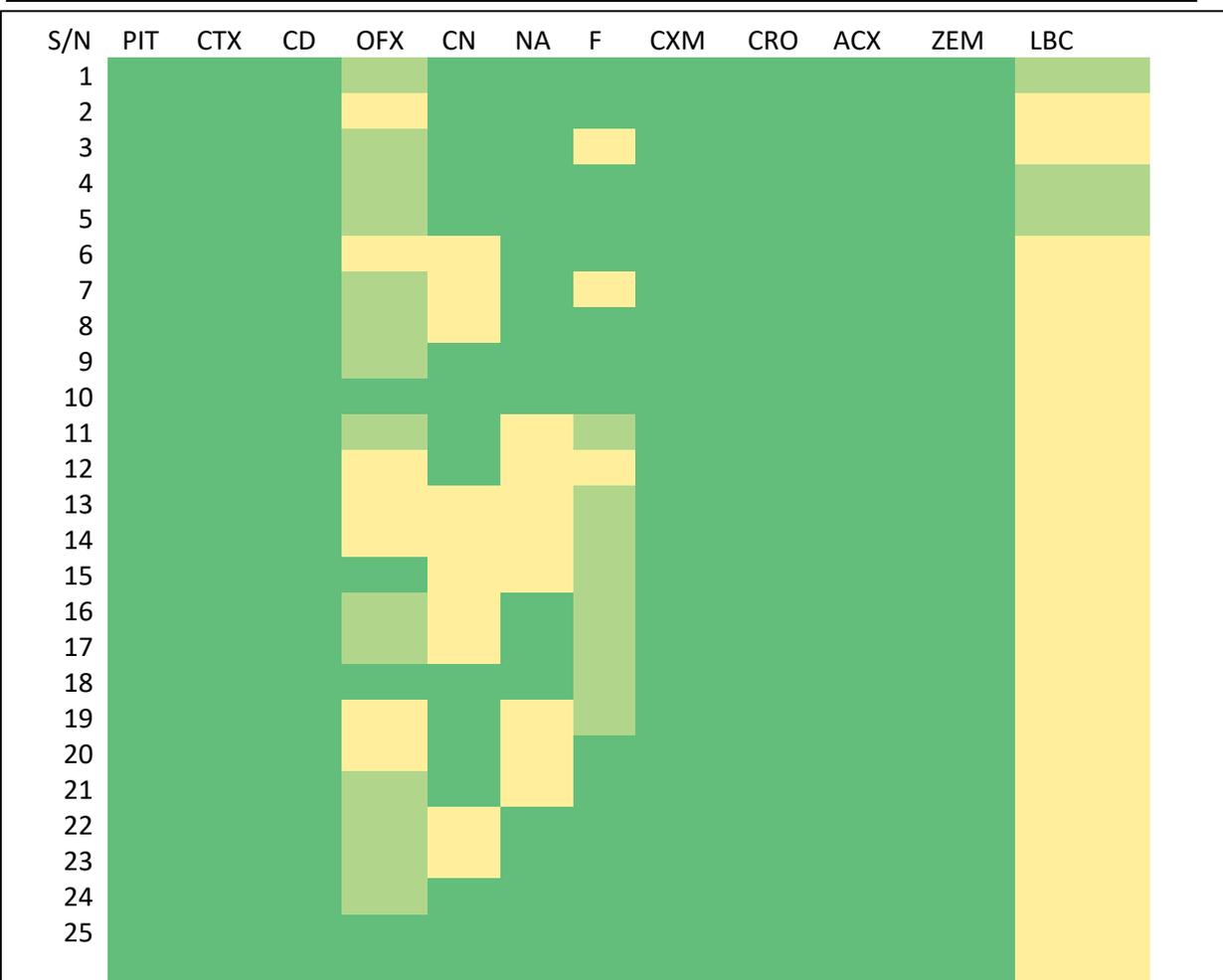


Figure 3 Shows the antibiotic profile heatmap where green represent resistant region of isolates, the yellow represent sensitivity region while gray represent intermediate as interpreted by the CLSI guidelines for antibiotic interpretation



Figures 4 show the photo-microgram of isolates susceptibility testing to the selected antibiotics

DISCUSSION

Several investigators have reported bacteriuria in pregnant women and immuno-compromised patient and other patients with debilitating diseases. However, much attention has not been given to the apparently healthy and Asymptomatic bacteriuria subjects. The determination of Urinary tract infections (UTIs) amongst the students with little or no clinical symptoms was conducted and our result revealed the presence of pus cell and occurrence of diverse organisms ranging from *Escherichia coli* (37.1%), *Staphylococcus aureus* (25.8%), *Pseudomonas aeruginosa* (9.7%), *Serratia* species (4.8%), *Proteus* species (6.5%), *Bacillus* species (12.9%), and *Staphylococcus epidermis*(3.2%) as shown in Table 1 and figure 1. It is pertinent to note that the observed organisms are potential urinary tract infections (UTIs) pathogens as were previously reported by diverse investigators of related studies (Mezueet *al.*, 2006; Akinloyeet *al.*, 2006). The study of **Shadrack and his colleagues also revealed the presence of similar organisms with deviation on the percentage reported for *Escherichia coli*** (Shadracket *al.*, 2019). Such observation of

high bacteria prevalence in urine is of utmost concern especially as those students do not show any clinical symptom to the urinary tract infection. There is need to sensitize the student on the need for routine screening, assessment of microbiological wellness for UTIs and a public awareness campaign within the University accommodation environment. Majority of the characterised organisms were Gram negative indicating that asymptomatic bacteriuria amongst apparently healthy student in Western Delta University Student Residence is prevalently implicated by Gram negative bacteria. This is also similar to the study of Shadrack and his group who reported high Gram negative organism implicated with asymptomatic bacteriuria (Shadracket *al.*, 2019). The phenotypic virulence indices also shows that 18% of *E.coli* were protease positive, 23% of *P aeruginosa* were positive to haemolysin positive as there was an observed clearance of the red pigment of blood cells which is peculiar to some virulent Gram-negative strains (Shadrack *et al.*, 2019; Lavanya and Jogalakshmi., 2008; Cowan and Steel, 1975). There was also an observed detection of urease production, catalase production and coagulase which are enzyme based virulent indices of some members of *P aeruginosa* and *S aureus*. The antibiotic susceptibility testing (AST), the antibiogram and virulent tests shows that the observed organisms are both potential pathogens and harbours multiple antibiotic resistant (MAR) determining factors including resistance to piperacilin and tozobactam (PIT-110C; 100%), Cefotaxin (CTX-30C; 100%), Clindamycin (CD-2C; 100%); Ceftriazon-sulbactam (CRO-45C; 100%), Cefuroxime (CXM--

30C; 100%), Ampiclox ACX-10C (figures 2,3,4). the reports from other investigators have also reported high multiple antibiotic resistant bacterial strains from asymptomatic bacteriuria (Mezueet *al.*, 2006).

CONCLUSION

The result of this study has revealed the presence of both Gram positive and negative bacteria in the urine of asymptomatic and apparently healthy students with high prevalence of Gram-negative strains. The need for a routine evaluation of such students for bacteriuria cannot be overemphasized as it would welcome a possibility for an apt management/control of related cases on or before a clinical case is registered. The observation of such clinically relevant potential pathogen amongst apparently healthy students arouse public health concern as it may affect the entire students in the WDU hostel if appropriate interpersonal hygiene is not applied by the individual students since they use the same toiletries. It is also important to apply standard antibiotics and therapeutic regimen which is adequate for the treatment of infection cases to avoid the continuous occurrence of resistant strains in the environments.

Acknowledgements

The authors wish to appreciate the students of the microbiology department WDU and other students in the University especially Dumle Gbaratee, Triumphant Onojafe. The study was funded by the WDU trust fund

Conflict of interests

The authors declare that there are no conflicting interests.

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