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PREVALENCE OF URINARY TRACT INFECTION AMONG SCIENCE LABORATORY TECHNOLOGY STUDENTS IN FEDERAL POLYTECHNIC OKO, ANAMBRA STATE.

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Abstract

Prevalence of Urinary Tract Infection among Science Laboratory Technology student's in Federal Polytechnic Oko, Anambra State was investigated. 5 samples of urine were analyzed for urinary tract infection associated organisms. Mac-Conkey agar medium and manitol salt agar were used to culture the urine samples by streak method and pure cultures obtained were indentified by various standard biochemical tests. The result showed Escherichia coli, and Staphylococcus aureus. Escherichia coli was isolated in all samples, followed by Staphylococcus aureus. The isolated bacterial pathogens were screened for their susceptibilities to antibiotics using disk diffusion method. Result showed the average zone of inhibition to be the widest for ampiclox and ciprofloxacin at 15.0mm while amoxaccilin had the least at 6.0mm for Escherichia coli while the average diameter of inhibition showed gentamycin to be widest at 15.0mm for Staphylococcus aureus.

KEYWORDS:
Urinary, Laboratory, Prevelence, Infection, Science

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INTRODUCTION

Urinary tracts infection (UTI) is the colonization of the urinary tract by pathogenic microorganism. Infection is usually caused by fungi, bacteria and viruses. The infection can result to prolonged admissions in hospitals with high morbidity in general population along with high population, financial cost implications to the patients. Majority of UTIs are caused by bacteria that are found in bowel and live as normal flora and often result from faecal and perineal areas. These organisms are capable of invading the tissues of the urinary tract and adjacent tissues causing lower urinary tracts infections and upper tract infections. UTI is a common condition that is found in very young children as well as older people. In general population and hospital set up, UTI is a common infection although there are new and more powerful antibiotics in use but bacteria resistance persists (Patel, 2012). The spectrum of causative agents and their antimicrobial resistance pattern has been dynamic worldwide.

Urinary tract infection may lead to life threatening complications and death. Urine culture is the most effective diagnosis of UTI. Lower UTI (cystitis) and upper UTI (Pyelonephritis) are the two clinical entities mostly found in patients with symptomatic UTI. Lesions caused by UTI are severe and contribute to morbidity in the population resulting in loss of renal function, which leads to long term illness.

Urine passing through the urethra allows the entry of uropathogens into the urinary tracts initiating an inflammatory response. Due to their anatomical orientation, that is the short distance between the anus and vagina women are at higher risks of getting UTIs,(Foxman, 2010). A second re-infection occurs in about 50% of all women with a first UTI within six months. Bacteria establish infection in the urinary tract only after overcoming possible elimination by normal flora during micturation and innate host defense mechanism in the bladder .

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Only about 2.5% of documented UTIs are acquired hematogenously and usually results from bacteremia caused by relatively virulent organisms such as Salmonella spp. and *Staphylococcus aureus*. Common symptoms f UTIs include, burning sensation during urination, loss of bladder control, increased frequency of urination especially in small amount, low back pains, cloudy and bloody or foul smelling urine.

Multidrug resistance should be monitored worldwide and surveillance systems should be used to determine the aetiology for UTI. There is a worldwide setback in management of many bacterial infectious diseases due to antibiotic resistance. It is estimated that globally 26% of deaths are due to infectious diseases such as UTIs of which 98% occur in low income countries.

Statement of problem

Being the most common infectious disease in the community and hospitalized patients, UTI has globally affected over 150 million people per year which costs global economy more than 6 billion US dollars. Worldwide, infectious diseases cause a significant amount of financial burden and morbidity. In the USA, about 7 million patients who visits the clinicians are diagnosed with UTI while more than 100,000 are hospitalized annually .In community and hospitals, acquired bacterial infections has been a growing concern worldwide due to UTIs caused by multidrugs resistant Uropathogens. There is pressure resulting from intensive and indiscriminate use of antibiotics in treatments leading to a rapid spread of antimicrobial agent resistance genes to uropathogens. A global concern is on the rise over rapid dissemination of drug-resistant bacteria creating serious complications on the treatment of infectious diseases. A major concern to clinicians is the increase in number of resistant and multi-resistant strains of bacteria

and the decline in the number of new antibiotics available for treatments of UTIs (Annapurna and Lakshmi, 2013).
In Nigeria, in a study conducted among 12, 458 urine samples, reported prevalence of community acquired and nosocomial UTIs were 12.3% and 9.3% respectively. In a prevalence in males were 14.6% and 7.4% respectively.

Objective of the study

The main objectives of this study was to investigate the antibacterial susceptibility patterns of bacteria isolated from urine of students in Science Laboratory Technology Department, Federal Polytechnic Oko, Anambra State.

Significance of the study

The result of this study will assist clinicians to administer antibiotics after culture and therefore reduce multi drug resistance.

MATERIALS AND METHOD

Study population

Five (5) students of Science Laboratory Technology Oko, Anambra state were involved in this study. They comprise 3 females and 2 males.

Sample collection

Informed consents of the students were also obtained for sample collection. Sterile, leak-proof, universal, plastic containers were used for collection of freshly voided midstream urine from the students and transported to the laboratory for cultural analysis.

Isolation of bacterial pathogens

A sterile loop was dipped vertically just below the surface of a well-mixed urine sample and a loop full of the urine sample was taken

avoiding air bubbles. The sample was then inoculated onto duplicate sterile agar plates of chocolate and MacConkey media by streaking techniques for the isolation of the bacterial etiologic agents. Thereafter, inoculated plates were incubated at a temperature of 37c for 18-24hours.

Identification of bacterial isolates

All cultured plates with bacterial growths were selected and examined macroscopically. Cultured characteristics of discrete bacterial colonies on the plates with significant bacterial growths were noted and subcultured on nutrient agar for purification. Phenotypic identification of the isolates were confirmed based on selected biochemical reactions using the standard procedures of Barrow and Felltham (1995) and Cheesebrough, (2000).

Antibiotic susceptability pattern

The isolated bacterial pathogens were screened for their invitro susceptibilities to antibiotics using the disk diffusion method as described by the clinical and laboratory standard institute (CLSI; formally NCCLS) guidelines (NCCL 1999) on Muller-Hinton agar plates. Overnight pure cultures of the isolates were employed for this purpose. Few colonies of each isolate were suspended in 5ml sterile normal saline (0.85%w/v.NaCl) and mixed gently to obtain a uniform suspension. The suspension to the optical density of 0.5 McFarland standards.

Each bacterial isolates was then be seeded into the Muller-Hinton agar medium using a sterile cotton swab dipped into the standardized suspension, drained, and used for inoculating the medium. Inoculated plates are allowed to stand for about 10minutes before sterile forceps were used to aseptically transfer some antibiotic sensitivity disks onto the surface of the cultured plates. The plates were incubated

aerobically at 370c for 18-24 hours. After incubation, the diameters of the zones of inhibition were observed and measured (Zinnah, 2008) with a ruler and compared with a Zone-interpretation chart (Bauer, 1966). *Escherichia coli* ATCC 25922 were used as control for Gram negative, while *Staphylococcus aureus* (NCTC 6571) was used as control for Gram positive bacteria.

The following antibiotics disc from Oxoid were used for the susceptibility test: Ceftazidime (Caz 30µg), Cefuroxime (Crx 30µg), Oxacillin (Oxc 10µg), Lincomycin (Lin 2µg), Cloxacillin (Cxc 20µg), Ofloxacin (Ofl 5µg), Gentamicin (Gen 10µg), Nitrofurantoin (Nit 200µg), Nalidixic acid (Nal 30µg), Augmentin (Aug 30µg), Cotrimoxazole (Cot 25µg), Amoxicillin (Amox 25µg), and Tetracycline (Tet 25µg).

Urinalysis

5mls each of the urine samples was centrifuged at 2,500g for 5 minutes. The supernatant was discarded and the deposit re-suspended with the small amount of urine left in the tube by tapping the base of the centrifuge tube. A drop was placed on cleaned grease free slide, covered with cover slip and examined under miscroscope using 10x and 40x objective lenses for the presence of white blood cells, red blood cells, epithelia cells, casts, crystals, bacteria, yeast cells and Trichomonas.

Culture and sensitivity

A calibrated standard wire loop was used for inoculating culture plate. A loopful (0.002ml) of well mixed un-centrifuged urine was plated on a dried CLED and MacConkey agar media. These plates were incubated aerobically at 370c for 24 hours. Colony counts were determined at the end of incubation period. Each urine sample with over 105 CFU per milliliterwas followed up as significant bacteria growth and isolated

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colonies were sub-culture, Subculture of isolated colony. To obtain pure isolates, discrete colonies of pathogens isolated were inoculated in a well dried MacConkey agar media. The plates were incubated aerobically at 370c for 24hours. After overnight incubation, colonies were subjected to biochemical tests for identification.

Characterization of Isolates

The various isolates obtained were subjected to morphological, physiological, and biochemical tests. Morphological characterization, the isolated colonies were examined and recorded base on the type of growth, elevation, size, colour, margin, edge, consistency, opacity, and change in medium.

Gram staining

A thin smear of each isolate was made on clean grease free glass slide, air dried and heat fixed by passing it gently over flame and then gram stained. Gram positive cells stained purple while the gram negative cells stained pink.

Biochemical Tests

The *Candida albicans* were identified by performing Germ Tube Test (GTT) on any isolate whose Gram result shows yeasts. Catalase test will be done on the Gram positive cocci to differentiate *Staphylococcus spp.* from *Streptococcus spp.* Coagulase test was done to identify *Staphylococcuss aureus* which produces the enzyme coagulase.
Oxidase test was done on the Gram negative bacilli (GNB) to identify *Psuedomonas spp* from other Gram Negative bacilli. MICROBACT (API) identification system was used to identify the species of the oxidase negative GNB.

3Antibiotic susceptibility test

Antibiotic susceptibility of pure culture of confirmed isolate was performed on diagnostic sensitivity test agar (Mueller Hinton agar) by

the Kirby Bauer disc diffusion method, using the appropriate Gram positive and negative disc. Isolates were considered sensitive after incubation for 24hours at 370c by measuring zone diameter interpretative to National committee for clinical laboratory standard (CLSI chart) for different organisms and different antibiotics.

To guarantee precision and reliability of antibiogram data, quality control strains of *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853 supplied by department of Science Laboratory Technology Polytechnic Oko, Anambra, Nigeria were used.

Isolation and identification of microorganisms

The urine specimens from all the patient were cultured on CLED agar (Oxoid LTD, UK) and identified to determine the microorganisms involved. Inoculation of urine specimen was done using sterile calibrated wire loop inoculating 0.001ml of urine specimen onto CLED agar (Oxoid LTD, UK). The cultured media was then incubated at 35c for 24 hours. For the media which had no growth after 24 hours incubation were further incubated up to 48 hours before declaring absence of bacterial growth/negative. The numbers of isolated bacterial colonies were enumerated and were multiplied by dilution factor for the estimation of bacterial load per milliliter (ml) of urine sample. Urine samples with colony $\geq$ 105 CFU/ml were taken as significant growth (positive urine culture = 105 CFU/ml). The significant growth was identified further using biochemical reaction.

Biochemical identification of isolates

Catalase test

This is an enzymatic test that breaks down hydrogen peroxide into water and oxygen which is indicated by production of bubbles of air and it was used to identify *staphylococcus* from other Gram-positive

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cocci. Hydrogen peroxide forms an oxidative end product of aerobic carbohydrate metabolism. Staphylococci produce catalase enzyme which reacted with hydrogen peroxide thereby producing bubbles of oxygen.

Coagulase test

Coagulase is an enzymatic test which catalyzes the formation of fibrin clot in blood plasma inoculated with test organism. This differentiates *Staphylococcus aureus* from other coagulase negative staphylococci. The fibrin in the blood plasma appears within 2-3 hours but if not fibrin it is further incubated for 24 hours. If no fibrin clots after this period the test was declared as negative (Cheesbrough, 2007).

Oxidase test

This is an enzymatic test used in microbiology in the identification of enterobacteria which are non lactose fermenting Gram negative rods. In this analysis it was used to differentiate between *Pseudomonas* from *Proteus.* The enzyme will oxidize a redox dye such as tetramethyl paraphenylene diamine dihydrochloride to give deep purple colour which is a positive test. This enzyme is produced by some aerobic microorganisms as part of their respiratory oxidation.

Triple sugar iron (TSI) agar test

This media will be used for detecting enterobacteria especially non lactose fermenting organisms. This will be achieved when sugars are fermented with production of gas and hydrogen sulfide. This media combines multiple biochemical test in a single medium. It assists in separation of the enterobacteria from other non-lactose Gram negative bacteria. In this case it was used in identification of *Escherichia coli, Proteus and Klebsiella*.

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Indole test

This test is used for indole production of Gram negative bacilli. The enterobacteria produce aromatic amino acid tryptophan which was present in the medium. A bright red ring with formation indicating that bacteria had broken the amino group and indole was produced. After an overnight growth Kovacs reagent will be added to the broth culture which reacted with indole to form a bright red ring colour at the surface of a positive test. The test was negative when Kovacs reagent will be added and no colour change occurred. Indole test was used to differentiate between *Escherichia coli* from *Klebsiella pneumonia* (Cheesbrough, 2007).

Motility test

The test was used to determine if an organism will be motility. The motility will be demonstrated by growth and spread of an organism throughout the medium from the stab. Non-motile microorganisms will be seen to grow only in stabbed area of the medium. In this case it will be used in identification of *Escherichia coli, Proteus* and *Klebsiella*.

Citrate test

The citrate agar (green) slants and butt was streaked with test organisms containing citric acid, which was a tricarboxylic acid and Bram Cresol agar. The citrate was metabolized to acetoin and carbon dioxide. The isolates with citrate permease allowed intake of citric acid, causing alkaline end products that change pH indicator from green to blue. The isolate was identified by the ability to utilize citrate as the source of carbon, and ammonium as its source of nitrogen. Uropathogens that change slant to blue will be considered as a positive test. Those organisms turned slant green will be negative. The test was used in identification of enterobacteria such as *Escherichia coli* and *Klebsiella* (Cheesbrough, 2007).

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Urease test

The isolates were inoculated into urea broth medium, which contain phenol red indicator. Microorganisms contained urease enzyme decomposed urea to form carbon dioxide and ammonia. The ammonia produced reacted with water to form ammonium hydroxide causing a change in pH which will be indicated by phenol red indicator. Broyh becomes red-purple color when the test will be positive due to production of ammonium hydroxide. If the test was negative, the broth remains orange. The test were used for differentiating enterobacteria such *Escherichia coli, Proteus* and *Kledsiella*.

Antibiotic susceptibility testing

This test was performed using disc diffusion method as described by Cavalieri, (2005). In this technique organisms isolated were inoculated in normal saline with the help of sterile wire loop. Briefly, colonies will be taken from 24 hours culture plates into nutrient broth. The turbidity formed will be adjusted to an equivalent of 0. 5 McFarland. The test organism will be streaked over the surface of Muller Hinton agar plates using sterile cotton swabs. Disc impregnated antibiotics which were commercially available was placed on plates firmly by means of sterile forceps aseptically and the inoculated plates were incubated for 24 hours at 37c. Afterwards diameters of zone of inhibition were measured in mm. The antibiotics used and their zones of sensitivity were measured using veneer caliber and graded according to sensitive, intermediate or resistant. The inoculated plates will be air dried, and antibiotic discs (ABTEK BIOLOGICAL LTD., UK) will be placed on Muller-Hinton agar using sterile forceps and gently pressed down to ensure contact. The following 8 antibiotic discs will be used; Nitrofurantoin (NIT, 300µg), Cefotaxime (CEF, 10µg), Amoxicillin-clavulinic acid (AMC, 10µg), Gentamicin (GET, 10µg), Nalidixic acid (NA, 30µg), Ampicillin (AMP, 10µg), Ciprofloxacin (CIP, 25µg), and

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Cotrimoxazole (SXT, 25µg). Standard strains of *Escherichia coli* ATCC 25922 and *S. aureus* 25923 will be used as control during antimicrobial susceptibility testing.

RESULT

Five samples of urine were used for the project and the result showed growth of two species of bacterial namely S*taphylococcus aureus* and *Escherichia coli*

The two bacteria's found on the urine sample was identified by inoculation of urine sample unto the Manitol salt agar and Mac-Conkey agar and was incubated at 35c for 24hrs and bacterial growths were seen and viewed under the microscope which was noted down as *Staphylococcus aureus* and *Escherichia coli* and it was further subcultured on nutrient agar for purification. The significant growth where identified further using biochemical reaction as seen in table 4.1

Table1: Result of biochemical test

Isolates	***Staphylococcus aureus***	***Escherichia coli***
Gram staining	+R	-R
Catalase test	+	+
Coagulase test	+	-
Oxidase test	+	-
TSI agar test	-	+
Indole test	-	+
Motility test	-M	+M
Citrate test	+	-
Urease test	+	-

- = Negative

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+ = Positive
- R= Negative Rod
+ R= Positive Rod
- M= Non-motile
+ M= Motile

The isolated bacterial pathogen's were screened for their susceptibilities to antibiotics using disk diffusion method. 1 ml of urine sample was suspended into 9ml of distilled water and mixed gently to obtain a uniform suspension. 0.5ml of the uniform suspension was suspended into the Muller-Hinton agar and were streaked over the surface of Muller-Hinton agar using sterile cotton swab. Disc impregnated antibiotics were placed on the plates by means of sterile forceps and inoculated plates were incubated for 24hr's at 37c. Diameter of zone of inhibition were measured in mm. Antibiotic's and their zone of sensitivity were measured as seen in table 4.2

Table 2: Sensitivity of *Staphylococcus aureus* and *Escherichia Coli* to antibiotic

Antibiotic	Average diameter of inhibition (mm)		Sensitivity pattern	
Name and code	***Escherichia coli***	***Staphylococcus aureus***	***Escherichia coli***	***Staphylococcus aureus***
Ampiclox (APX)	15.0	11.0	S	S
Amoxaccilin (AM)	6.0	10.0	S	R
Ciprofloxacin (CRX)	15.0	14.0	R	R
Erythromycin (ERY)	Nil	8.0	S	R

Gentamycin (GN)	Nil	15.0	S	R
Pefloracin (PEF)	13.0	10.0	R	R
Receptin (R)	10.0	11.0	R	R
Septrin (SXT)	11.0	Nil	R	S
Streptomycin (S)	Nil	13.0	S	R
Ampicillin (ANP)	7.0	Nil	R	S

R = Resistance
S = Sensitive
Nil = Not done

DISCUSSION

In this study, 3 out of 5 urine samples from the students of science laboratory technology Oko gave significant bacteria associated with urinary tract infection. The reasons for the absence of bacteria growth recorded in 2 urine samples maybe due to the fact that the students were undergoing antibiotics therapy prior to diagnosis which must have inhibited or destroyed the pathogen's.

In this study, the most predominant organism isolated out among the student's was *Escherichia coli* and *staphylococcus aureus.*(Akerele *et al*, 2009) also reported that *Escherichia coli* and *Staphylococcus aureus* are the commonest pathogen's isolated in patient's with urinary tract infections. In this study, *Escherichia coli* was found to be more prevalent, however (Ebie *et al*, 2009) reported that *staphylococcus aureus* was more prevalent than *Escherichia coli*. The prevalence of urinary tract infection was higher in females than in male's. These

results also agreed with other report's, which showed that UTI are more frequent in females than in males during adolescence and adulthood (Burbbae *et al*, 1999, and Ebie *et al*, 2010).

The most effective antibiotics in this study were ciprofloxacin and amplicox, this is because they showed higher zone of inhibition. Other antibiotics like streptomycin and erthromycin were also effective against gram positive organisms while the other antibiotics used like profloxacin, gentamycin, septrim, rocepttim and zinnacof are less effectiveness of some of these antibiotics used in the study may be as a result of indiscriminate use of these antibiotics and may have been abused. This can also be explained by the long term period for which those drug's have been available and in use for UTIs. These findings were in agreement with the work of (Obasekiebor *et al*, 2009 and Akerele *et al*, 2010).

The ciprofloxacin drug appears as promising therapeutic agents for the treatment of acute urinary tract infection. Also a contamination therapy of amino glycoside with penicillin for the treatment of acute urinary tract infection is promising as this may lead to increase in activities of the drug.

The study have showed that susceptibility testing of antibiotics is necessary to obtain sensivity reports before initiating antibiotics treatment in case of suspected urinary tract infection. However, the decision to use a particular antibiotics depends on it's toxicity, cost and mode of action.

Conclusion

Since *Escherichia coli, Staphylococcus aureus,* are organisms involved in urinary tract infection, Ciprofloxacin or the use of combined drug's is most active against these organisms, therefore ciprofloxacin or the use of combined drug's should be used for therapeutic purpose of urinary tract infection.

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Recommendation

Good personal hygiene should be practiced by the students

Female students should avoid too much use of diaphragm for contraception.

Use of cipofloxacin antibiotics or combined drug's are important for the management of UTI infection.

They should sanitize their toilets properly before use.

Giving them health education concerning the UTIs.

Policies against drug abuse must be put on antibiotics as the resistant rate in all antibiotics in this study is over 20%.

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