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ISOLATION AND CHARACTERIZATION OF BACTERIA FROM PATIENTS WITH URINARY TRACT INFECTION ATTENDING DUTSIN-MA GENERAL HOSPITAL, KATSINA STATE.

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ABSTRACT

Urinary Tract infections (UTIs) are one of the major causes of morbidity and co morbidities in patients with underlying conditions, and it accounts for the majority of the reasons for hospital visit globally. Sound knowledge of factors associated with UTIs may allow timely intervention that can easily bring the disease under control. This study is aimed to isolate and characterize the different bacterial etiological agents that cause UTIs in patients attending General Hospital Dutsin-ma, Katsina State. A total number of sixty (60) urine samples were collected aseptically and analyzed using standard microbiology methods. Out of the total samples examined 35 (58.3%) were infected. Female patients have highest percentage of infection compare to that of male patients. The prevalence rate for urinary tract infections in this study was 35(58.3%). Escherichia coli was the major cause of urinary tract infections with (45.7%). Females had the highest prevalence rate of (77.8%) while male had (22.8%) prevalence rate. Prevalence rates for other bacterial species isolated for the cause of urinary tract infections were Staphylococcus saprophyticus (28.6%), Staphylococcus aureus (11.4%) and Klebsiella pneumoniae (14.3%). Appropriate measures may help reduce urinary tract infections through knowing the factors that cause high prevalence rate of urinary tract infections in females.

***KEYWORDS**: Urinary tract infections, Dutsin-Ma, Escherichia coli and urine samples.*

INTRODUCTION

Urinary Tract Infections are the inflammatory disorder of the urinary tract caused by the abnormal growth of pathogens (Prakash et al., 2013). Urinary tract infection is known to cause long term morbidity in terms of fever, dysuria and lower abdominal pain (LAP) and may result in permanent scarring of the kidney (Amali et al.,

2003).Urinary tract infections can either be community acquired or nosocomial. Community acquired urinary tract infection is defined as the infection of the urinary system that takes place in one's life in the community setting or in the hospital environment with less than 48 hours of admission and also is the second most commonly encountered microbial infection in the community setting (Sabrina.,2010).While Nosocomial urinary tract infection (N-UTIs) are the infection of the urinary tract that occurs after 48 hours of hospital admission and the patient was not incubating at the time of admission or within 3 days after discharge (Lacovelli et al., 2014).Urinary tract infection is characterized by bacterial invasion and multiplication involving the kidneys and urinary tract pathways.
Urinary Tract Infection represents to one of the most frequent infectious pathogens, it affect predominantly female patients .Approximately 30% of women will have at least one episode of UTI throughout their live (Toporovski., 1991).UTIs may be asymptomatic, acute, chronic and complicated or uncomplicated and clinical manifestations of UTI depend on the portion of the urinary tract involved, the etiologic organisms, the severity of infection and the patient's ability to mount an immune response to public health care, hence reducing the quality of life and resulting in to work absenteeism. 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. The prevalence in females and the prevalence in males were14.6% and 7.4% respectively (Jombo et al., 2006).UTIs are caused by various gram negative bacteria, Escherichia coli is the most common causative agent in all groups of patients. Staphylococcus saprophyticus is now recognized as a common cause of UTI in adolescents and young adult women.
Urinary tract are among the most common bacterial infections in human both in the community and hospital settings, it occur in all age groups and required urgent treatment (Orrette et al., 2003). UTI remains a leading cause of health care expenditure for people of all age groups with an overview annual cost of 1.6 billion dollars in the United States of American (Vasquez, 2004). Malnutrition, poor hygiene, low socio economic status are associated with UTIs and these factors are rife in rural setting (Ahmed et al., 2008). Although Escherichia coli has been reported as the predominant isolate causing UTI (Omoregie et al., 2008 & Eghafona et al., 2009).Urinary pathogens especially from community patients have been known to include strains that are resistant to many of the commonly used antibiotics (Orrette, 2003).Therefore there is need for periodic monitoring of etiologic agents of UTI and their susceptibility pattern especially in a rural setting. UTI is challenging not only because the diagnosis of UTI is not always straight forward (Kolawole et al.,2009). Criteria for the diagnosis of UTI vary greatly depending on the patients and context. Urinary catheters: Any catheterization of the bladder increases the risk of infection, but indwelling has a higher risk than intermitted catheterization. Treatment of asymptomatic bacteriuria in patients with chronic indwelling catheters has shown no benefit (Nicolle, 2003). It is known that the infection targets the different part of the urinary tract and as a consequence results in the contagion of the lower and upper urinary tracts. The infection is named based on the site of infection, the infection of urethra and urethra are referred to as urethritis and urethritis respectively whereas cystitis and pyelonephritis corresponds to bladder and kidney infections. Cystitis is a common type of injection whereas the infection associated with the renal damage is an issue of serious concern. Therefore the infection of bladder and urethra are referred as the infection of lower urinary tract whereas the kidney and urethra infection is an indication of upper tract infection.
Generally UTIs are classified based on the factors that trigger the infection (Uncomplicated or complicated) and nature of occurrence (Primary and recurrent).The bacterial count is an important parameter to signify the presence of symptomatic and asymptomatic urinary tract infections, bacteria is the means of this infection predominately. Many researcher have investigated the significance of associated risk factors and its role in affecting thousands of people annually due to the infection of gram negative pathogen leading to bacteremia. The significance of risk factors like anaemia, low income level, past history of UTI and sexual activity in causing UTI among women

during pregnancy has been explored and validating by several researchers (Beyene, 2013).The prevalence of asmptomatic UTI among women varies from 2% -5% compared symptomatic UTI during pregnancy. This in turn is a consequence of microbial colonization of the urinary tract.

Owning to the fact that anybody can get UTI, but some people are more likely than others to get, like People who have diabetes may have changes in their body's immune system, making it easier for them to get UTIs. Also People with blockages in their urinary tract, such as a kidney stone, are more likely to get UTIs. An enlarged prostate gland in a man can also block the flow of urine and cause a UTI. Infants who are born with an abnormality of their urinary tract have an increased chance of getting a UTI. Surgery is sometimes needed to correct the problem. People who have a catheter, or tube, placed in their bladder for a long time are more prone to UTIs. This is because bacteria on the catheter can infect the bladder. Women get more UTIs than men. This may be because women have a shorter urethra, which makes it easier for bacteria to travel up to the bladder. UTIs may be more serious during pregnancy because the bacteria are more likely to travel to the kidneys. A pregnant woman may be more careful with UTIs because it may even result to problems like high blood pressure or premature delivery of her baby. About 80 to 90 percent of UTIs are caused by a single type of bacteria. Therefore this study will identify thus bacteria causing UTIs in human being.

The aim of this study is to isolate and characterize bacteria from patients with Urinary tract infection attending Dutsin-Ma General Hospital, Katsina State.

STUDY AREA

This study was conducted in Dutsin-ma Local Government Area, Katsina State, North-Western Nigeria. This area is located between latitude of 12○ 27'18"N and longitude 7○ 29'29"E and has its headquarters in the town of Dutsin-ma. It has an estimated area of 527km2 (203sqkm) and a population of 169,671 as at 2006 census (NPCC, 2006). The population and activities in the Local Government Area have increased in the last 3 years which may be due to the establishment of the new Federal University. The Local government is bounded by kurfi and Charanchi Local Government to the North, Kankia Local Government to the East, Safana and Dan-musa Local Government to the West, and Matazu Local to the Southeast. There are two main source of water i.e Zobe Dam (that lies to the south of the town) and Dutsin-ma Dam. The people are predominatly farmers, cattle rearers, fishers and traders.

SAMPLE COLLECTION

A total of Sixty (60) Urine samples were collected in a labeled sterile containers from patients in General Hospital Dutsin-ma. The samples were collected aseptically and transported to the Department of Microbiology Laboratory for analysis.

MEDIA PREPARATION

The media used include CLED agar, macConkey agar, Blood agar and MSA agar. They were prepared according to the manufacturer's instructions and measured separately using digital weighing balance. Each was dispensed into different sterilized conical flasks and distilled water was added to the required calibration. The mixtures in these conical flasks were swirled to ensure even dissolution of the powdered media in water to give a homogenous solution. The mouths of the conical flasks were pluged with cotton wool and aluminium foil and gently heated on a hot plate to ensure even mixture of their content. The media was sterilized in an autoclave prior to use to avoid

contamination.

Culturing and isolation of urine sample

This was carried out as described by Cheesbrough (2002, 2004) and Prescott et al. (2008). A loopful of each urine sample was streaked on CLED agar, incubated and subcultured on MacConkey agar, and Blood agar, MSA agar plate for the isolation of the bacteria present in the urine. After incubation, plates with growth were selected, the colonies were isolated using inoculating loop and subsequently subcultured on agar slants for use in further test. Bacterial colonies differing in size, shape, and color were selected from these plates and separately subcultured in Gram's reaction and in different biochemical test for further characterization and identification.

Identification of Bacterial isolates

The methods used in the identification and characterization of the bacteria isolated from the culture media include the following: Gram staining techniques followed by microscopic examination, and biochemical tests according to Cheesbrough (2002; 2004).

Gram staining techniques

The gram staining reaction was used to help identify isolates in culture (gram positive or gram negative bacteria) Using a sterile wire loop, a pure culture from a discrete colony on the culture plate was emulsified in a drop of normal saline on a grease free clean slide.

Microscopic examination

After gram staining, microscopic examination is the second step for differentiating microorganism in to either Gram positive bacteria (violet) Gram negative bacteria (pink), Shape of Bacteria; Bacteria display three basic shapes: round-cocci, rod shaped – bacilli, spiral all are showed in the microscope.

Biochemical tests

Important in the identification of a genus and species of bacteria which include the the determination of the kinds of nutrients a bacterial cell can use, the products of its metabolism, the presence of particular characteristic enzymes ,these tests include Catalase test, Coagulase test, Indole test Methyl Red/Voge's Proskauer test, Urease test, Citrate test, Motility test.

Indole test

This test was performed to help differentiate species of the family Enterobacteriaceae. Media and Reagents Used:

Tryptone broth and Kovac's reagent

Procedure Tryptone broth was inoculated with the test isolate and incubated for 18 to 24 hrs at 37°c, 4 drops of Kovac's reagent were added down to the inner wall of the tube. Formation of a red coloured ring at the top was recorded as positive for indole

Methyl Red/Voges-Proskauer (MR/VP)

Both tests are used to differentiate species of the family Enterobacteriaceae.

Media and Reagents Used:

Glucose Broth, Methyl Red indicator for MR test and Vorge's Proskauer reagent.

Procedure for MR test: Colonies from bacterial isolates cultures were inoculated into glucose phosphate broth and incubated at 37°C for 48 hours. The pH of the medium was tested. Five drops of MR reagent was subsequently added. Development of red colour was recorded as positive-MR.

Procedure for VP test: Colonies from the bacterial isolate cultures inoculated into glucose phosphate broth in test tubes and incubated for 48 hours. A 0.6 ml of alpha-naphthol and 0.2 ml of 40% KOH were added to the broth and

shaken. The preparation was allowed to stand for 15 minutes. Appearance of red colour was recorded as a positive test

Citrate Utilization test**:** This test is one of several technique used to assist in the identification of Enterobacteriaceae. The test is based on the ability of an organism to use citrate.

Procedure: The Inoculum was streaked over the slant of Simmon's citrate agar in a tube and incubated for 24-48 hrs at 37°c.Growth on the slant and a change in colour of the indicator from light green to blue was recorded as positive result

Motility Test

This test is done to help differentiate species of bacteria that are motile from non-motile. Media and Reagents Used:

Motility media contains tryptose, sodium chloride, agar, and a color indicator.

Procedure: The test isolate was stabbed into the motility media using sterile inoculating needle incubated for 24hrs at 37°c. Motility of the bacterium was identified by the appearance of growth going out away from the stab line

Catalase test

Principle: This test demonstrates the presence of enzyme catalase in the organism.

Procedure: One drop of H_2O_2 solution was placed on a clean glass slide and 2-4 colonies from the bacterial isolate cultures were mixed with the H_2O_2 on the slide. Presence of gas bubbles indicates a positive test.

Urease test

Principle: To determine the ability of the organism to split urea forming 2 molecules of ammonia by the action of the enzyme Urease with resulting alkalinity

Procedure: A Urea broth was inoculated with the test isolate and incubated for 24hrs at 37°c.Liberation of red colour indicated urease-positive test while initial yellow colour indicates negative.

Coagulase test

Principle: This test is used to differentiate Staphylococcus aureus (positive) from coagulase negative Staphylococci. S. aureus produces two forms of coagulase: bound and free.

Procedure :(free coagulase),About 2 or 3 colonies were emulsified in 0.05ml of saline contained in a clean serological tube. And 1ml of citrated human plasma was added and incubation for some of the tube overnight in the incubator at 35°c and the next day increased viscosity or complete clotting indicating a positive tube coagulates test, while viscosity or clotting indicated a negative coagulates test.

RESULTS

Different bacteria were isolated and found to be associated with urinary tract infection such as Escherichia coli, Klebsiella spp, Staphylococcus saprophyticus, and Staphylococcus aureus. Cultural characteristics (like colour, shape, size of the colonies), Gram staining, Biochemical test have been used for their identification. The results of morphological characteristics of isolates from patients urine samples were presented in Table 1.

Table 1: Morphological characteristic on CLED agar and gram reaction

Bacterial isolates	Morphological characteristics On cled agar	Gram stain
Escherichia coli	Moderate, moist, yellow Lactose fermented colonies	Gram- negative bacilli
Staphylococcus saprophyticus	Small, moist, yellow Lactose fermented colonies	Gram- positive cocci
Klebsiella spp	Large, mucoid, yellow Lactose fermented colonies	Gram- negative bacilli
Staphylococcus aureus	Small, moist, yellow Lactose fermented colonies	Gram- positive cocci

Bacteria isolated from patients were characterized and identified biochemically. The isolates identified are Escherichia coli, Klebsiella spp based on Gram reaction respectively. The result were presented in Table 2 below.

Table 2*:* Biochemical tests of isolated Gram- negative bacteria

Bacterial isolates	Indole test	Urease test	Citrate test	Methyl Red test	Voges-Proskauer test
Escherichia coli	Positive	Negative	Negative	Positive	Negative
Klebsiella spp	Negative	Positive	Positive	Negative	Positive

Bacteria isolated from patients were characterized and identified biochemically. The isolates identified are Staphylococcus aureus, Staphylococcus saprophyticus based on gram reaction respectively. The results were presented in Table 3 below.

Table 3*:* Biochemical test of isolated Gram- positive bacteria

Isolates	Mannitol fermentation	Catalase test	Coagulase test
Staphylococcus saprophyticus	Negative	Positive	Negative
Staphylococcus aureus	Positive	Positive	Positive

Occurrence and prevalence of pathogenic bacteria isolated from patients samples were presented below in Table 4, in which Escherichia coli have the highest percent (45.7) while Staphylococcus aureus with the lowest percent (11.4).

Table 4: Occurrence and prevalence of pathogenic bacteria isolated from urine sample in patients.

Isolates	No. of Positive Samples (%)
Escherichia coli	16 (45.7)
Staphylococcus saprophyticus	10 (28.6)
Klebsiella spp	5 (14.3)
Staphylococcus aureus	4 (11.4)
Total	35 (100)

Table 5: Incidence and prevalence of Urinary tract infection among patients.

The incidence and prevalence *of* Urinary tract infection among patients were presented below in Table 5, in which UTI is highest in females with 65.7%as compared to 34.3% in males' patients.

Gender	No of positive samples	Percentage (%)	No of negative samples	Percentage (%)	Total
Females	23	38.3	7	11.7	30
Males	12	20.0	18	30.0	30
Total	**35**	**58.3**	**25**	**41.7**	**60**

DISCUSSION

The main objective of this research was to isolate pathogens that cause urinary tract infections (UTI) among patients. Sixty samples were collected from patients (30 females and 30 males), and were analayzed according to microbiological procedure for the isolation and identification of bacteria that causes UTI. Out of the sixty urine samples collected from these patients, 35 samples were positive for bacterial strains causing UTIs, while 25 samples were found to be negative for these bacteria. The incidence and prevalence of urinary tract infection among the patients in this study revealed (38.3%) for females and (20.0%) for males similarly reported by Toporovski (1991) which stated that UTIs affect predominantly female patients than male patients. Gasha'a et al (2020) recorded a prevalence rate UTIs in females (77.78%) and (22.22%) in males. The reason behind of the high prevalence of urinary tract infection in females is the close proximity of the urethral with anus, wider and shorter urethra, sexual behavior, incontinence and less acidic pH of vaginal surface and poor hygienic conditions (Khan et al., 2015). The highest rate among males was recorded in the age group 50-59(6.35%). The increased frequency of prostate diseases and diabetes in males may have a role in occurrence of urinary tract infection at this age of patients (Mahesh et al., 2010).

Both gram positive and gram negative bacteria commonly isolated from the patients with UTIs were also found to be associated with UTIs. Escherichia coli was the predominant bacteria (45.7%) isolated, it is the most common causative agent in all groups of patients followed by Staphylococcus saprophyticus (28.6%), Klebsiella spp. (14.3%) and Staphylococcus aureus (11.4%). The prevalence rate found in this study for Escherichia coli (45.7%) is close to the finding of Odoki et al 2018 for Escherichia coli (41.9%) while it is higher for the finding of Ochie et al 2018 (26.5%). Prevalence rates observed for Escherichia coli especially in females maybe due to the variety of virulence characteristics which allows intestinal carriage, persistence in the vagina and therefore ascending and invading the Urinary tract (Mahato et al., 2018).

Prevalence rate for this study for Klebsiella pneumoniae (14.3%) is close to the finding of Odoki et al (2018), Baguma et al in Southern Uganda and Lo et al in Sao Paulo, Brazil.

UTIs caused by Pseudomonas, Proteus and Klebsiella species are associated with hospital-acquired infections, often following catheterization or gynecological surgery. UTI by Proteus spp. are particularly associated with renal stones (Kolawole et al., 2009; Cheesbrough, 2006; Bahadin et al., 2011).

Staphylococcus aureus prevalence rate is low (11.4%) compared to some authors' findings Mugalo 2011, Awka 2016 and Bushenyi 2015 whom recorded (22.5%), (28%) and (43.7%) respectively. However, the high prevalence of Staphylococcus aureus in this study varied from other previous studies (Ochada, 2014; Bano, 2012). Previous studies have linked the increasing Staphylococcal urinary tract infections to increased use of instrumentation such as bladder catheters (Moore, 2002; Irebu, 2013).

CONCLUSION

The prevalence rate for urinary tract infections in this study was 35(58.3%). Escherichia coli was the major cause of urinary tract infections with (45.7%). Females had the highest prevalence rate of (77.8%) while male had (22.8%) prevalence rate. Prevalence rates for other bacterial species isolated for the cause of urinary tract infections were Staphylococcus saprophyticus (28.6%), Staphylococcus aureus (11.4%) and Klebsiella pneumoniae (14.3%). Appropriate measures may help reduce urinary tract infections through knowing the factors that cause high prevalence rate of urinary tract infections in females.

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