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ORIGINAL ARTICLE

DATA ANALYSIS AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF BACTERIAL PATHOGENS CAUSING URINARY TRACT INFECTION

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INTRODUCTION

Urinary tract infection (UTI) is the commonest bacterial infectious disease in community practice with a high rate of morbidity and financial cost. It has been estimated that 150 million people were infected with UTI per annum worldwide which costing global economy more than 6 billion US dollars [1]. UTIs is described as a bacteriuria with urinary symptoms [2]. UTI can affect lower and sometimes both lower and upper urinary tracts. The term cystitis has been used to define the lower UTI infection and is characterized by symp- toms such as dysuria, frequency, urgency, and suprapubic tenderness. The presence of the lower UTI symptoms does not exclude the upper UTI which is often present in most UTI cases [3]. The treatment of UTI can be classified into uncomplicated and complicated on the basis of their choice of treatment [4]. UTI is more common in females than in males as female urethra structurally found less effective for preventing the bacterial entry [5]. It may be due to the proximity of the genital tract and urethra [6] and adherence of urothelial mucosa to the mucopolysaccharide lining [7]. The other

main factors which make females more prone to UTI are pregnancy and sexual activity [8]. In pregnancy, the physiological increase in plasma volume and decrease in urine concentration develop glycosuria in up to 70% women which ultimately leads to bacterial growth in urine [9]. Also, in the nonpregnant state the uterus is situated over the bladder whereas in the pregnant state the enlarged uterus affects the urinary tract [10]. Sexual activity in females also increases the risk of urethra contamination as the bacteria could be pushed into the urethra during sexual intercourse as well as bacteria being massaged up the urethra into the bladder during child birth [11, 12]. Using a diaphragm also causes UTI as it pushes against the urethra and makes the urethra unable to empty the bladder completely and the small concentration of urine left in the bladder leads to the growth of bacteria which ultimately causes UTI [13].The spectrum of bacteria causing complicated UTI is much broader than of those causing uncomplicated UTI. However, the most commonly encountered microorganisms are Gram negative bacteria including Escherichia coli, Cit- robacter spp., Enterobacter aerogenes, Pseudomonas aerugi- nosa, and Proteus vulgaris whereas Klebsiella spp., Staphylo- coccus aureus, and Salmonella spp. are found rarely [14].

Increasing multidrug resistance in bacterial uropatho- gens is an important and emerging public health prob- lem. The Infectious Disease Society of America (IDSA) identified some microorganisms for new effective therapies. Those microorganisms were called “ESKAPE pathogens” which include Enterococcus faecium, S. aureus, Klebsiella spp., Acinetobacter spp., Pseudomonas spp., and Enterobacter spp. Increasing drug resistance in UTI needs regular mon- itoring of the antibiotic susceptibility of uropathogens in a particular area. Various factors such as the type of UTI (complicated or uncomplicated), gender, age, and previous history of antibiotic therapy of each UTI patient should also be considered to find out the correct global data on susceptibility [15]. The distribution of antimicrobial suscep- tibility data of UTI-causing microorganisms changes from time to time and from place to place [13]. The susceptibility data provided by regional microbiology laboratories helps to choose the empirical choice of antimicrobials to treat UTI; however, these conditions are limited to complicated UTI as the samples of uncomplicated UTI are rarely sent to laboratories [16, 17]. Generally, the antimicrobial treatment is initiated before the laboratories results which may lead to the frequent misuse of antibiotics [18]. The

resistance pattern of community acquired uropathogens has not been extensively studied in India [19–21]. To the best of our knowledge, no data regarding the bacterial resistance in UTIs from Meerut District (Uttar Pradesh), India, has been documented. Since most UTIs are treated empirically, the criteria for the selection of antimicrobial agents should be determined on the basis of the most likely pathogen and its expected resistance pattern in a geographic area. Therefore, there is a need for periodic monitoring of etiologic agents of UTI and their resistance pattern in the community.

This study was undertaken in view of paucity of reports of UTIs in patients of Meerut District (Uttar Pradesh), India. The aim of the study is to determine the prevalence of UTI in male and female patients as well as the effect of gender and age on its prevalence. The UTI-causing microorganisms, their distribution among different ages and genders, and their antimicrobial susceptibility will also be determined.

Urine culture

The urine samples were inoculated in different culture media. A calibrated loop of 1 μL was dipped in vertical position in the urine sample and the loop was used to inoculate the plates using the streak plate method. Gram negative bacilli, were detected using the Levine medium. For Gram positive cocci, the urine samples were spread in Mannitol Salt Agar for the detection of Staphylococcus spp, in Bile Esculin Agar for the detection of E. faecalis and in Blood Agar for the detection of Streptococcus spp. The Petri plates were incubated at 37°C during 24–72 hours, depending on the microorganism. The plates of Blood Agar were incubated in 5-10% CO2 atmosphere. After incubation, the urine cultures were classified as negative, positive and contaminated. The samples were classified as contaminated when polymorphic bacterial growth (growth of two or more bacterial species) was observed (exclusion criterium). The urine cultures were classified as negative when bacterial growth was lower than 103 CFU/mL (exclusion criterium). When monomorphic bacterial growth was higher than 105 CFU/mL the culture was classified as positive (inclusion criterium) and, for these cases, the AST was performed. The AST was also performed when the result of urine culture was between 104 and 105 CFU/mL.

Identification of bacterial isolates

Additional biochemical tests were done when the urine culture was positive. These tests were performed based on the morphology of the isolated bacteria and on

the results of the microscopic examination of the Gram-stained smear. The Enterobacteriaceae were differentiated using the the Kligler, Tryptone, Simmons Citrate and Urea media. Proteus mirabilis was distin- guished from the Proteus vulgaris by the indol test. The coagulase test was used to differentiate Staphylococcus aureus from the other Staphylococcus. Staphylococcus epidermidis (novobiocin- sensitive) was differentiated from Staphylococcus saprophyticus (novobiocin-resistant) using the novobiocin susceptibility test. The catalase test was used to distinguish Staphylococcus spp from Enterococcus faecalis and Streptococcus spp. The oxidase test was used to identify Pseudomonaceae. The uro-pathogen Pseudomonas aeruginosa was identified by production of diffusible pigments on Mueller-Hinton Agar and for a grape-like odour released [50].

Antimicrobial Susceptibility

Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive to. Most often, this testing complements a Gram stain and culture, the results of which are obtained much sooner. Antimicrobial susceptibility tests can guide the physician in drug choice and dosage for difficult-to-treat infections.

The antimicrobials misuse in clinical medicine has led to an increase of the microbial resistance and the consequent spread of bacterial resistant strains is a serious public health problem. Urinary tract infection (UTI) is one the most common infectious diseases of the community and also of the hospital settings, resulting in high rates of mor- bidity and high economic costs associated with its treat- ment [22-24]. Uncomplicated UTI occurs in patients without any anatomic or functional abnormality in the urinary tract and may reach, on average, 6.1 days of symptoms, 2.4 days of restricted activity and 0.4 bed days [25-27]. Uncom- plicated cystitis (infection of bladder) is the most common UTI and is responsible for 95% of all symptomatic urinary tract infection [28].

Some studies carried out in the community have shown that uropathogens such as Escherichia coli (46.4 - 74.2%), Klebsiella spp (6.0 - 13.45%), Proteus spp (4.7 - 11.9%) and Enterococcus spp (5.3 - 9.54%) represent the main causes of UTI [223,29-37]. E. coli has been indicated as the most fre- quent uropathogen involved in the community-acquired UTI [31,34,37,38] due to the fact of belonging to the nor- mal flora of the human intestine and therefore easily colon- izing the urinary tract. Some strains of E. coli

isolated from sexually active patients matched with faecal isolates from their partners, which indicate that the ITU can be sexually transmitted [39]. Community-acquired urinary tract infec- tions are mainly uncomplicated, colonizing preferably the bladder and causing cystitis. However, E. coli may ascend through the ureters to the kidneys and cause more severe infections such as pyelonephritis [39,40]. The bacter- ium Pseudomonas aeruginosa is emerging as an opportun-istic pathogen of UTI in the community and has been associated to 10.7 - 25% of cases [24,31,32,41-43].

The early treatment of UTI decreases the rate of morbid- ity, implying that in most cases antimicrobial therapy be prescribed empirically [31]. In order to administer an appropriate empirical therapy, it is crucial to know the main bacteria usually involved in the urinary tract infection as well as their respective antimicrobial resistance pattern [36,44]. This procedure allows controlling the increase of antimicrobial resistance and the spread of resistant bacterial strains that represent a public health problem worldwide.

The treatment of acute uncomplicated cystitis recom- mended by the guidelines of the European Association of Urology (EAU) include fosfomycin, trometamol, pivmecilli- nam (a penicillin), nitrofurantoin (a nitrofuran) as first-line therapy and, as an alternative therapy, fluoroquinolones, cepodoxime proxetil, the sulfonamides SXT and trimetho- prim, if the local resistance is less than 20% [45,46]. These recommendations for UTI empiric treatment should be adjusted taking into account the geographical location of the patient, age, sex and other diseases [47]. According the ARESC, an international survey on the antimicrobial resistance of pathogens implicated in uncomplicated UTIs [48], E. coli showed high resistance to the sulfonamide SXT (29.4%) and reduce resistance to nitrofurantoin (1.6%) and to fluoroquinolone ciprofloxacin (8.1%) in nine European countries and in Brazil.

Unfortunately, there are few publications about the main uropathogens implicated in community-acquired UTI and their antimicrobial resistance pattern, when compared with UTI acquired at hospital level. This information is very important and reflects changes over the years, which implies a periodic monitorization in order to decrease the number of therapeutic failures [36,47,49].

The main objective of this study was to evaluate the prevalence and the antimicrobial resistance pattern of the main bacteria responsible for urinary tract infection in the community of Aveiro

District (Portugal), throughout a ten-year period, in order to establish an appropriate empirical therapy.

EMERGENCE OF ANTIMICROBIAL RESISTANCE AND THE RATIONALE FOR PERFORMING SUSCEPTIBILITY TESTING

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. Empirical therapy continues to be effective for some bacterial pathogens because resistance mechanisms have not been observed e.g., continued penicillin susceptibility of Streptococcus pyogenes. Susceptibility testing of individual isolates is important with species that may possess acquired resistance mechanisms (e.g., members of the Enterobacteriaceae, Pseudomonas species, Staphylococcus species, Enterococcus species, and Streptococcus pneumoniae).

OVERVIEW OF COMMONLY USED SUSCEPTIBILITY TESTING METHODS

Broth dilution tests. One of the earliest antimicrobial sus- ceptibility testing methods was the macrobroth or tube-dilution method [51]. This procedure involved preparing two-fold di- lutions of antibiotics (eg, 1, 2, 4, 8, and 16 mg/mL) in a liquid growth medium dispensed in test tubes [51, 52]. The antibiotic-containing tubes were inoculated with a standardized bacterial suspension of 1–5 × 105 CFU/mL. Following overnight incu-bation at 35°C, the tubes were examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of antibiotic that prevented growth represented the minimal in- hibitory concentration (MIC). The precision of this method was considered to be plus or minus 1 two-fold concentration, due in large part to the practice of manually preparing serial dilutions of the antibiotics [53]. The advantage of this technique was the generation of a quantitative result (ie, the MIC). The principal disadvantages of the macrodilution method were the tedious, manual task of preparing the antibiotic solutions for each test, the possibility of errors in preparation of the antibiotic solutions, and the relatively large number of reagents and space required for each test.

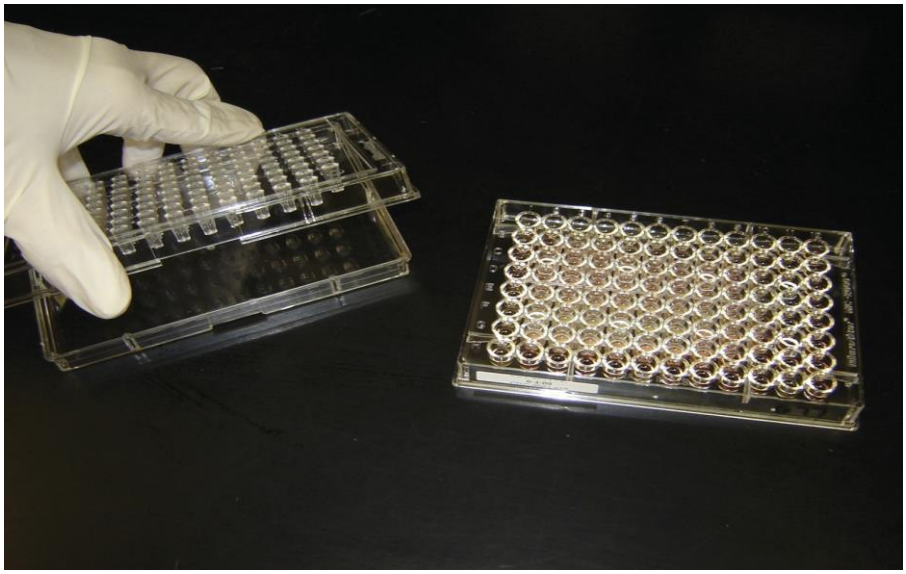

Figure 1. A broth microdilution susceptibility panel containing 98 reagent wells and a disposable tray inoculator

The miniaturization and mechanization of the test by use of small, disposable, plastic "microdilution" trays (Figure 1) have made broth dilution testing practical and popular. Standard trays contain 96 wells, each containing a volume of 0.1 mL that allows approximately 12 antibiotics to be tested in a range of 8 two-fold dilutions in a single tray [52, 54]. Microdilution panels are typically prepared using dispensing instruments that aliquot precise volumes of preweighed and diluted antibiotics in broth into the individual wells of trays from large volume vessels. Hundreds of identical trays can be prepared from a single mas- ter set of dilutions in a relatively brief period. Few clinical microbiology laboratories prepare their own panels; instead, fro- zen or dried microdilution panels are purchased from one of several commercial suppliers. The cost of the preprepared pan- els range from approximately $10 to $22 each. Inoculation of panels with the standard 5 × 105 CFU/mL is accomplished us- ing a disposable device that transfers 0.01 to 0.05 mL of stan- dardized bacterial suspension into each well of the microdi- lution tray or by use of a mechanized dispenser. Following incubation, MICs are determined using a manual or automated viewing device for inspection of each of the panel wells for growth [52].

The advantages of the microdilution procedure include the generation of MICs, the reproducibility and convenience of having preprepared panels, and the economy of reagents and space that occurs due to the miniaturization of the test. There is also assistance in generating computerized reports if an au- tomated panel reader is used. The main disadvantage of the microdilution method is some inflexibility of drug selections available in standard commercial panels.

Antimicrobial gradient method

The antimicrobial gradient diffusion method uses the principle of establishment of an antimicrobial concentration gradient in an agar medium as a means of determining susceptibility. The Etest (bioMe´rieux AB BIODISK) (Figure 2) is a commercial version available in the

United States. It employs thin plastic test strips that are im- pregnated on the underside with a dried antibiotic concentra- tion gradient and are marked on the upper surface with a concentration scale. As many as 5 or 6 strips may be placed in a radial fashion on the surface of an appropriate 150-mm agar plate that has been inoculated with a standardized organism suspension like that used for a disk diffusion test. After over- night incubation, the tests are read by viewing the strips from the top of the plate. The MIC is determined by the intersection of the lower part of the ellipse shaped growth inhibition area with the test strip.

The gradient diffusion method has intrinsic flexibility by being able to test the drugs the laboratory chooses. Etest strips cost approximately $2–$3 each and can represent an expensive approach if more than a few drugs are tested. This method is best suited to situations in which an MIC for only 1 or 2 drugs is needed or when a fastidious organism requiring enriched medium or special incubation atmosphere is to be tested (eg, penicillin and ceftriaxone with pneumococci) [55–57]. Generally, Etest results have correlated well with MICs generated by broth or agar dilution methods [55–59].

Figure 2. A Staphylococcus aureus isolate tested by the Etest gradient diffusion method with vancomycin (VA), daptomycin (DM), and linezolid (LZ) on Mueller-Hinton agar. The minimum inhibitory concentration of each agent is determined by the intersection of the organism growth with the strip as measured using the scale inscribed on the strip.

However, there are some sys- tematic biases toward higher or lower MICs determined by the Etest when testing certain organism-antimicrobial agent com- binations [56, 60]. This can represent a potential shortcoming when standard MIC interpretive criteria derived from brothdilution testing [60] are applied to Etest MICs that may not be identical.

Disk diffusion test.

The disk diffusion susceptibility method [52, 61, 62] is simple and practical and has been well- standardized. The test is performed by applying a bacterial inoculum of approximately 1–2 × 108 CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Up to 12 commercially-prepared, fixed concentration, paper an- tibiotic disks are placed on the inoculated agar surface

(Figure 3). Plates are incubated for 16–24 h at 35°C prior to deter- mination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest mil- limeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) [63] or those included in the US Food and Drug Administration (FDA)– approved product inserts for the disks. The results of the disk diffusion test are "qualitative," in that a category of suscepti- bility (ie, susceptible, intermediate, or resistant) is derived from the test rather than an MIC. However, some commercially- available zone reader systems claim to calculate an approximate MIC with some organisms and antibiotics by comparing zone sizes with standard curves of that species and drug stored in an algorithm [64, 65].

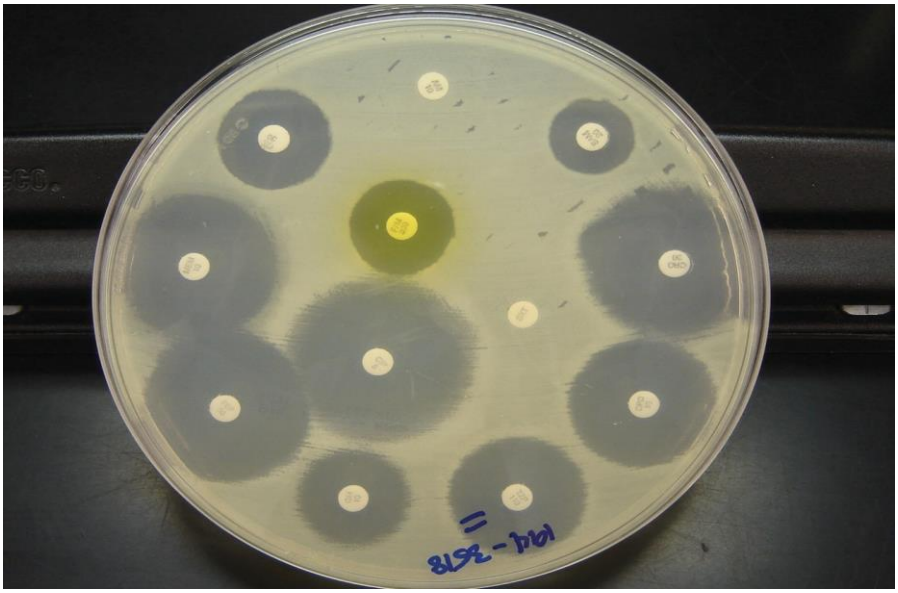

Figure 3. A disk diffusion test with an isolate of Escherichia coli from a urine culture. The diameters of all zones of inhibition are measured and those values translated to categories of susceptible, intermediate, or resistant using the latest tables published by the CLSI.

The advantages of the disk method are the test simplicity that does not require any special equipment, the provision of categorical results easily interpreted by all clinicians, and flex- ibility in selection of disks for testing. It is the least costly of all susceptibility methods (approximately $2.50–$5 per test for materials). The disadvantages of the disk test are the lack of mechanization or automation of the test. Although not all fas- tidious or slow growing bacteria can be accurately tested by this method, the disk test has been standardized for testing streptococci, Haemophilus influenzae, and N. meningitidis through use of specialized media, incubation conditions, and specific zone size interpretive criteria [62].

Automated instrument systems. Use of instrumentation can standardize the reading of end points and often produce susceptibility test results in a shorter period than manual read- ings because sensitive optical detection systems allow detection of subtle changes in bacterial growth. There are 4 automated instruments presently cleared by the FDA for use in the United States. Three of these can generate rapid (3.5–16 h) suscepti- bility test results, while the fourth is an overnight system [66]. The MicroScan WalkAway (Siemens Healthcare Diagnostics) is a large self-contained incubator/reader device that can incubate and analyze 40–96 microdilution trays. The WalkAway utilizes standard size microdilution trays that are hydrated and inoc- ulated manually and then placed in one of the incubator slots in the instrument. The instrument incubates the trays for the appropriate period, examining them periodically with either aphotometer or fluorometer to determine growth development. Gram-negative susceptibility test panels containing fluorogenic substrates can be read in 3.5–7 h. Separate gram-positive and gram-negative panels read using turbidimetric end points are ready in 4.5–18 hours.

The BD Phoenix Automated Microbiology System (BD Di- agnostics) has a large incubator reader with a capacity to process 99 test panels that contain 84 wells devoted to antibiotic dou- bling dilutions and are inoculated manually. The Phoenix mon- itors each panel every 20 min using both turbidometric and colorimetric (oxidation-reduction indicator) growth detection. Test panels for gram-negative, gram-positive, S. pneumoniae, b-hemolytic, and viridans group streptococci are available. MIC results are generated in 6–16 h.

The Vitek 2 System (bioMe´rieux) is highly automated and uses very compact plastic reagent cards (credit card size) that contain microliter quantities of antibiotics and test media in a 64-well format. The Vitek 2 employs repetitive turbidimetric monitoring of bacterial growth during an abbreviated incu- bation period. The instrument can be configured to accom-modate 30–240 simultaneous tests. The susceptibility cards al- low testing of common, rapidly growing gram-positive, and gram-negative aerobic bacteria, and S. pneumoniae in a period of 4–10 h. An older, less automated, Vitek 1 System is still used in some laboratories. The system is more limited with a 45- well card and does not include S. pneumoniae.

The Sensititre ARIS 2X (Trek Diagnostic Systems) is an au- tomated, overnight, incubation and reading system with a 64-panel capacity. The test panels are standard 96-well microdilution plates that can be inoculated with a Sensititre Autoinculator. Growth is determined by fluorescence mea- surement after 18–24 h of incubation. Test panels are available for gram-positive and gram-negative bacteria, S. pneumoniae, Haemophilus species, and nonfermentative gram-negative bacilli.

The Phoenix, Sensititre ARIS 2X, Vitek 1 and 2, and WalkAway instruments have enhanced computer software used to interpret susceptibility results including “expert systems” for analyzing test results for atypical patterns and unusual resis-tance phenotypes [66]. Two studies [67, 68] have shown that providing rapid susceptibility test results can lead to more timely changes to appropriate

antimicrobial therapy, substantial direct cost savings attributable to ordering of fewer additional laboratory tests, performance of fewer invasive procedures, and a shortened length of stay. These benefits are best realized when coupled with extended laboratory staffing schedules, and real- time, electronic transmission of verified results. One of the early shortcomings of rapid susceptibility testing methods was a less- ened ability to detect some types of antimicrobial resistance including inducible b-lactamases and vancomycin resistance. However, the recently FDA-cleared instruments have made sig- nificant improvements in large part through modifications of the instruments' computer software to either provide extended incubation for problematic organism-drug combinations, or by editing of susceptibility results using expert software to prevent unlikely results from being reported. In some cases, these mod-ifications result in prolonged incubation (ie, 110 h) of test panels to assure accurate results, thus rendering them less "rapid."

SELECTION OF DRUGS FOR ROUTINE TESTING

The laboratory must test and report the antimicrobial agents that are most appropriate for the organism isolated, for the site of the infection, and the institution's formulary [63, 69]. The CLSI provides tables that list the antimicrobial agents appro- priate for testing members of the Enterobacteriaceae, Pseudo- monas, and other gram-negative glucose nonfermenters, staph- ylococci, enterococci, streptococci, Haemophilus species, etc. [63]. The listings include recommendations for agents that are important to test routinely, and those that may be tested or reported selectively based on the institution's formulary.

The availability of antimicrobial agents for testing by the laboratory's routine testing methodology must next be deter- mined. The disk diffusion and gradient diffusion procedures offer the greatest flexibility including testing of newly available drugs. Most broth microdilution or automated test panels con- tain "96 wells, effectively limiting the number of agents tested or the range of dilutions of each drug that can be included. Manufacturers of commercially prepared panels have attempted to deal with this problem by offering a number of different standard panel configurations, or by including fewer dilutions of each drug in a single panel [69]. Another solution to this problem is testing antimicrobial agents that have activities that are essentially the same as the desired formulary drugs. The CLSI susceptibility testing document [63] lists groups of some antimicrobial agents with nearly identical activities that can provide practical alternatives for testing.

INTERPRETATION OF SUSCEPTIBILITY TEST RESULTS

The results of a susceptibility test must be interpreted by the laboratory prior to communicating a report to a patient's phy-sician. Optimal interpretation of MICs requires knowledge of the pharmacokinetics of the drug in humans, and information on the likely success of a particular drug in eradicating bacteria at various body sites [70]. This is best accomplished by referring to an expert source such as the CLSI, which publishes inter- pretive criteria for MICs of all relevant antibiotics for most bacterial genera [63]. Indeed, both MIC values and disk dif- fusion zone diameters must be

interpreted using a table of values that relate to proven clinical efficacy of each antibiotic and for various bacterial species [62]. The CLSI zone size and MIC interpretive criteria are established by analysis of 3 kinds of data:

(1.) microbiologic data, including a comparison of MICs and zone sizes on a large number of bacterial strains, including those with known mechanisms of resistance that have been defined either phenotypically or genotypically;

(2) phar- macokinetic and pharmacodynamic data; and

(3) clinical studies results (including comparisons of MIC and zone diameter with microbiological eradication and clinical efficacy) obtained during studies prior to FDA approval and marketing of an antibiotic [70].

A "susceptible" result indicates that the patient's organism should respond to therapy with that antibiotic using the dosage recommended normally for that type of infection and species [63, 70]. Conversely, an organism with a MIC or zone size interpreted as "resistant" should not be inhibited by the con- centrations of the antibiotic achieved with the dosages normally used with that drug [63, 70]. An "intermediate" result indicates that a microorganism falls into a range of susceptibility in which the MIC approaches or exceeds the level of antibiotic that can ordinarily be achieved and for which clinical response is likely to be less than with a susceptible strain. Exceptions can occur if the antibiotic is highly concentrated in a body fluid such as urine, or if higher than normal dosages of the antibiotic can be safely administered (eg, some penicillins and cephalospo- rins). At times, the "intermediate" result can also mean that certain variables in the susceptibility test may not have been properly controlled, and that the values have fallen into a "buffer zone" separating susceptible from resistant strains [63, 70]. Generally, reporting of a category result of susceptible, intermediate, or resistant provides the clinician with the in- formation necessary to select appropriate therapy. Reporting of MICs could aid a physician is selecting from among a group of similar drugs for therapy of infective endocarditis or oste- omyelitis, in which therapy is likely to be protracted.

It is important that the tables used for susceptibility test interpretations represent the most current criteria. Indeed, the CLSI documents are reviewed and updated frequently, usually once per year. Use of old or outdated information from the original editions of FDA-approved drug labels or older CLSI tables could represent a serious shortcoming in the reporting of patients' results[70]..

WHAT IS THE ACCEPTABLE ACCURACY OF A SUSCEPTIBILITY TEST METHOD?

When assessing the accuracy of various susceptibility testing methods as compared to standard reference methods, the terms very major and major errors have been used to describe false- susceptible or false-resistant results, respectively. In evaluations of new susceptibility testing methods, it is important to examine a representative number of strains that are resistant to various drugs to verify the ability of the new test to detect resistance and to test a number of susceptible strains to determine the rate of major errors that might be expected in a typical clinical

laboratory setting [66, 71]. To be cleared for marketing in the United States, the FDA requires that very major errors attrib- utable to a test device should be !1.5% for individual species/ drug comparisons, major errors should not exceed 3%, and an overall essential MIC agreement of 190% of device MICs within one doubling dilution of a CLSI reference MIC [72]. A recent, international standard on susceptibility test device evaluation proposes similar but not identical criteria for acceptable ac- curacy [73]. The emergence of new antimicrobial resistance mechanisms, including some that may be difficult to detect (eg, vancomycin intermediate susceptibility in S. aureus and car- bapenemase production in some gram-negative organisms) re- quires that the performance of susceptibility devices be con- stantly reassessed and updated when needed. In some cases, it has been necessary to employ special ancillary testing methods (eg, single concentration screening agars, modified Hodge test for carbapenemase production) [63] to supplement routine testing by a commercial instrument system[64].

LITERATURE REVIEW

Teshome Belachew et. al. 2020,

In the present study, the prevalence of urinary tract infection among children was high and considerably a high proportion of multidrug resistance was observed. This result will have a significant impact on the selection of appropriate antimicrobial agents for the treatment of urinary tract infection[65].

Shaper Mirza et. al. 2021,

In this study we set out to determine resistance patterns in pathogens isolated from blood and CSF cultures. We found that resistance has been at rise for several of these pathogens. Highest resistance rates were observed in Acinetobacter species against all tested antimicrobials including carbapenems. Resistance against 3rd and 4th generation cephalosporins has been reported in S. Typhi during the study period. Policy makers should prioritize and expedite implementation of infection control practices and antimicrobial stewardship in the country to control the emerging threat of AMR to public health[66].

Weldegebreal F et. al. 2015,

The overall prevalence of UTI was 14% among pregnant women. It was higher among pregnant women with symptoms than those without symptoms. E. coli (34.6%), CONS (19.2%), P. aeruginosa (15.4%), and Klebsiella spp. (11.5%) were common bacterial isolates. Low-income level, past history of UTI, educational status, and age of pregnant women with 25–34 years were highly likely to be affected by UTI. Gram-negative isolates showed a high level of sensitivity to CRO, GEN, and CIP. However, Gram-positive isolates were highly sensitive to GEN, ERY, CRO, CIP, and F. Most of the bacterial isolates are resistant against the available commonly used antibiotics such as AMP, AMO, TTC, SXT, and C. MDR was seen in 100% of the isolated bacteria. The majority of bacterial isolates were sensitive to CIP, CRO, ERY, and GEN. Therefore, the empirical antibiotic selection should be based on the knowledge of the local prevalence of bacterial organisms and antibiotic sensitivities rather than on universal

guidelines. This study recommends that the early detection of causative agent of UTI and determining their drug susceptibility pattern in pregnant women will help to ensure adequate treatment of UTI and to prevent its further complication in mother and fetus. Health information dissemination about causes of UTI and drug use should be given to pregnant women. CIP, CRO, GEN, and ERY can be used for the empirical treatment of UTI when there is no facility of taking culture and drug susceptibility tests in their areas. However, it should be used with great care to reduce further emergence of drug resistance [67].

Saad Alhumaid et. al. 2021,
The observed increase in susceptibility of gram-positive and gram-negative bacteria to studied antimicrobials is important; however, reduced sensitivity of MRSA, CoNS and Enterococcus species to gentamicin; and increased resistance of MRSA to linezolid and vancomycin is a serious threat and calls for effective antimicrobial stewardship programs[68].

Chanu Rhee et. al. 2020,
In this study of a large US cohort, we found that most patients with culture-positive community-onset sepsis did not have resistant organisms; however, empiric, broad-spectrum antibiotics targeting these organisms were frequently prescribed. Both inadequate and unnecessarily broad empiric therapy were associated with higher mortality. These findings underscore the need for better diagnostic tests to rapidly identify resistant pathogens and an increased focus on judicious use of broad-spectrum antibiotics for the empiric treatment of sepsis[69].

Anwar Ullah et. al. 2018,
Urinary tract infections are a one of commonly distributed infection. Recently the emergence of drugs resistance has been observed among urinary isolates. In this study, it was concluded that the resistance of antimicrobial agent among uropathogens was increased and there is marked variation in the antibiotic's susceptibility patterns of uropathogens. Meropenem is appropriate antibiotic to treated UTIs causes by Gram positive bacteria. These data demonstrate that future studies should be focused on the causes of antibiotics resistance to find the solves for this problem; and in implementation of health education to prevent drugs abuse in communities[70].

Muktikesh Dash et. al. 2013,
The worldwide trend of empirically treating CA-UTI may not apply for specific geographical regions, where decreased susceptibility rates are documented for common uropathogens. As more than two thirds of all pathogens are E. coli, local antimicrobial susceptibility patterns of E. coli in particular should be considered in antimicrobial selection for CA-UTIs. In the Indian setting, routine urine cultures may be advisable, since treatment failure likely to occur with commonly used antimicrobials. Therefore, development of regional surveillance programs is necessary for implementation of Indian CA-UTI guidelines[71].

Kalsoom BANO et. al. 2012,
The appropriate treatment for UTI has been a subject of recent research. After statistical analysis it was concluded that the incidence of disease is higher in females than males. The study found that E. coli, Klebsiella spp. and S. aureus are

the more common isolates in female subjects and in case of male patients E. coli also is principal etiological agent of UTI. The occurrence of UTI is significantly related to age in female patients, that is, the disease incidence increases with increasing age and vice versa for male patients. Identi-fication of the causative organisms and its susceptibility to antimicrobials is important, so that proper drug is chosen to treat the patient in early stages of UTI. It is therefore recommended that routine microbiological analysis and antibiotic sensitivity test of midstream urine samples of patients be carried out before the treatment in the management of UTIs. Our results suggest that the following antibiotics, amikacin, cefapime, norfloxacin,ciprofloxacin, nalidixic acid, imipenem, oxacillin, erythromycin, nitrofurantoin, vancomycin, augmentin and trimethoprim can be chosen in management of UTIs by the clinicians after having the culture sensitivity results. Over and above for prevention of UTIs implementation of strict infection control guidelines, effective hand washing and judicious use of antimicrobials is mandatory which goes a long way to cope up, with the emergence of drug resistance among uropathogens[72].

Ashley Bryce et. al. 2016,

Prevalence of resistance to commonly prescribed antibiotics in primary care in children with urinary tract infections caused by E coli is high, particularly in countries outside the OECD, where one possible explanation is the availability of antibiotics over the counter. This could render some antibiotics ineffective as first line treatments for urinary tract infection. Routine use of antibiotics in primary care contributes to antimicrobial resistance in children, which can persist for up to six months after treatment[73].

James J. Yahaya et. al. 2022,

The prevalence of UTI confirmed by urine culture among neonates that were included in the present study indicates that this problem is common in the population where the study was conducted. Klebsiella pneumoniae and Enterobacter spp. were the uropathogens which were isolated. Ciprofloxacin, nitrofurantoin, and amikacin were sensitive to the isolated uropathogens[74]..

Michel Kengne et. al. 2022,

E. coli remains the most common bacterial uropathogen responsible for UTIs in Ndjamena. This study confirms the presence of antibiotic-resistant uropathogens in this study area. As drug resistance is an evolving process, routine surveillance and monitoring studies should be conducted to provide physicians with knowledge about the most effective empirical treatment of UTIs[75]..

Doua Saad et. al. 2020,

Gram-negative organisms were the main cause of UTI. Bacteria causing UTI in Sudan frequently develop resistance against 17 antimicrobial drugs, and thus, we emphasize a serious dilemma in front of the health system. Given rising antimicrobial resistance trends, appropriate use of antibiotics and the development of novel agents are important to face this problem. To invent novel antibiotics, it is vital to study antimicrobial resistance on a molecular basis so that we can avoid and defeat mechanisms of resistance.

For all suspected cases, a culture and sensitivity test has to be conducted before the initiation of empirical antibiotics

therapy and then drugs should be adapted according to the results. Personal hygiene, as prevention and hydration, may replace the use of antibiotics in many cases. Current empirical antibiotic therapy for UTI should be modified, and new guidelines should be established based on local resistance rates. This study comes up with precious regional data for evidence-based empirical antibiotic treatment, but a national sentinel surveillance system and regional antibiograms should be established to track the bacterial susceptibility profiles in Sudan. As well, antimicrobial stewardship programs are essential to provide educational activities and issue the announcement of bacterial susceptibility rates to antibiotics with the ultimate goal of appropriate and cost-effective prescription behaviour[76]..

Seyed Abdol Reza Mortazavi-Tabatabaei et. al. 2019,

According to the present study, E. coli was the most common cause of UTI, and after that, Klebsiella, Staphylococcus aureus, and Enterobacter rank the next category. The results of this study showed that resistance is likely to be against the most common used antibiotics. The most effective antibiotics for E. coli are imipenem, nitrofurantoin, amikacin, chloramphenicol, and ciprofloxacin. By considering the results of this study, less use of gentamicin, the second generation of cephalosporins and nalidixic acid recommended, on the other hand, consuming of the penicillin, tetracycline, trimethoprim-sulfamethoxazole and the first generation of cephalosporins prescribed in the initial treatment of infections caused by E. coli. For Klebsiella isolates that separate from urine samples, effective antibiotics are imipenem, ciprofloxacin, amikacin, and nalidixic acid. Similarly, the use of ampicillin and cephalexin is not recommended in this case. In the treatment of UTIs that caused by Staphylococcus, ciprofloxacin is prescribed and consumed. It is obvious that due to the more use of antibiotics, uncontrolled use, and antibiotics misuse, antibiotic resistance emerging control is essential and this is one of the most important factors affecting these phenomena and attempts should be made for proper use of antibiotics[77]..

Ibssa Ibrahim Abdullahi et. al. 2018,

Significant bacteriuria was detected from 88 symptomatic UTI patients resulting in the overall prevalence of 25.3 %. However, a total of 97 different bacterial uropathogens were isolated making the isolation rate of bacteria from urine 27.9 %. E. coli was the dominating bacterial isolate. The results of this study also showed that the etiologic agents of UTIs mainly belonged to Gram-negative enteric bacteria. More than one type of organisms was isolated in 2.6 % of urine specimens cultured. Significant bacteriuria was significantly associated with patient settings, previous history of hospitalization, pregnancy and diabetes.

Even though amoxicillin-clavulanic acid and nitrofurantoin were reported to have very effective activity against urinary isolates in previous studies (Assefa et al., 2008; Moges et al, 2002) they were not available in the study area which limited the present study to assess their effectiveness. However, single and multiple drug resistance to the available commonly used antibiotics in the study area was found to be very high leaving clinicians with a very few choices of drugs for the treatment of UTIs. Therefore, it is

critical that use of antimicrobial agents with in hospitals, public healthcare providers as well as private ones should be reviewed and further studies to find out the overall resistance patterns and their possible causes and associated factors in the region at large need to be carried out. In the present study, it is indicated that the majority of bacterial isolates were sensitive to ciprofloxacin, ceftriaxone, nalidixic acid and gentamicin.

Thus, these drugs appear to be effective against uropathogens in the study area. These antibiotics should however be used with caution because of the emerging low level of resistance which may portent great danger for their future use[78]..

METHOD AND MATERIAL

Study Area.

The study was carried out in the micro-biology laboratory of the Department of Botany, India. The urine samples were collected from the OPDs (outpatients departments) section of three major hospitals. These sample collection sites were chosen as they mostly covered the urban area of the city. The duration of the study was one and a half year.

Study Population.

The urine samples of 288 patients, comprised of 148 males and 140 females, who attended the outpatient departments (OPDs) of three hospitals and had clinical evidence of urinary tract infection, determined by treating physicians, were included in this study. The age of patients included in the study ranged from 15 to ≥48 years. Patients with history of hospital admission a week before their presentation in OPDs were excluded from the study to rule out hospital-acquired infections. The patients on antibiotic therapy were also excluded from the study.

Sample Collection.

Clean catch midstream urine was collected from each patient into a 20 mL calibrated sterile screw-capped universal container which was distributed to the patients. The specimens were labeled, transported to the laboratory, and analyzed within 6 hours. In each container boric acid (0.2 mg) was added to prevent the growth of bacteria in urine samples. All patients were well instructed on how to collect sample aseptically prior to sample collection to avoid contaminations from urethra. Verbal informed consent was obtained from all patients prior to specimen collection. The study was conducted after due ethical approval which was subjected to the hospital administrations.

Sample Processing.

A calibrated loop method was used for the isolation of bacterial pathogens from urinary samples. A sterile 4.0 mm platinum wired calibrated loop was used which delivered 0.001 mL of urine. A loopful urine sample was plated on Cystine-Lactose-Electrolyte Deficient (CLED) agar, MacConkey agar, and blood agar medium. The inoculated plates were incubated at 37∘C for 24 h and for 48 h in negative cases. The number of isolated bacterial colonies was multiplied by 1000 for the estimation of bacterial load/mL of the urine sample. A specimen was considered positive for UTI if an organism was cultured at a concentration of ≥105 cfu/mL or when an organism was cultured at a concentration of 104 cfu/mL and >5 pus cells per high-power field were observed on microscopic examination of the urine.

Identification and Maintenance of Pure Bacterial Isolates.

Identification of bacterial isolates was done on the basis of their cultural and biochemical characteristics. Gram negative bacteria were identified by the standard biochemical tests [64, 73] and Gram-positive Bauer's disc diffusion method [75]. Standard inoculums adjusted to 0.5 McFarland was swabbed on Mueller Hinton agar and was allowed to soak for 2 to 5 minutes. After that antibiotic disk were placed on the surface of media and pressed gently. Mueller Hinton agar plates were then incubated at 37∘C for 24 h. After 24 h the inhibition zones were measured and interpreted by the recommendations of clinical and laboratory standards [76]. The following standard antibiotic discs were used for the isolates, ciprofloxacin (CIP), moxifloxacin (MOX), ofloxacin (OFL), sparfloxacin (SPR), levofloxacin (LEV), nalidixic acid (NAL), gatifloxacin (GTX), tobramycin (TOB), amikacin (AMK), gentamycin (GET), ceftazidime (CTZ), cefotaxime (CTX), ceftriaxone (CFX), imipenem (IMP), meropenem (MRP), nitrofurantoin (NTF), netillin (NTL) and co-trimoxazole (COT). Standard strains microorganisms were identified with the corresponding laboratory tests: catalase, coagulase, and mannitol test for Staphylococcus aureus [74]. Identified and pure isolates were maintained in nutrient agar slants and incubated at 37∘C for 24 hrs. The isolates were subcultured periodically.

Multiple Antibiotic Resistance (MAR) Indexing.

The multiple antibiotic resistance indices (MARI) were calculated by the method described by Tambekar et al. [68]. The following formula was used for the calculation of MAR index of antibiotics:

MAR index for an antibiotic = [number of antibiotics resistant to the isolates/ (number of antibiotics × Number of isolates)]. The number of MAR index for an antibiotic indicates its sensitivity and resistance. Antibiotic resistance increases with the increasing MAR values.

Statistical Analysis.

The data were analyzed using Chi- square ($\chi 2$) test, confidence interval (CI), odds ratio (OR) analysis, and student's t-test for paired samples. Relative risk and odds ratio were performed to compare the risk factors in the different groups of interest (male and female patients), and the Chi square test was conducted to find out the significant difference between the isolated uro pathogens, infected male and female patients related to different age groups, and statistical comparisons for the MAR indices group; however,

$\chi 2$ test for trend was conducted for antimicrobial resistance and sensitivity variables among all isolated uropathogens. The paired t-test was used to compare resistance versus sensitivity against isolates. A P value of <0.05 was considered as statistically significant for all tests and at 95% level of confidence interval. All statistical tests were performed by Statistical Package for Social Sciences (SPSS) software, Inc. 233 South Wacker Drive, 11th Floor Chicago, IL 60606-6412, USA, for Windows, version 20. The $\chi 2$ test for trend and graphs were prepared by GraphPad PRISM software (version 5.03), Inc. 2236 Avenida de la Playa La Jolla, CA 92037, USA.

RESULT

The overall prevalence of UTI in both male and female patients was found to be 53.82%. Total 155 urine samples showed the significant bacterial growth which were com- prised of 52 (35.14%) samples from males and 103 (73.57%) from females. These results indicated that the prevalence of UTI was higher in female patients than in males. The *P* value and the odds ratio showed the significant variation between male and female patients (Table 1).

The highest susceptible age group of patients to UTI was ≥48 years (63.51%) followed by 26–36 years (58.11%), 15–25 years (54.55%), and 37–47 years (39.19%). Comparatively, however, more cases of UTI were observed in females than in males in all age groups. The highest prevalence of UTI in females was found in the age group of 26–36 years (90.69%); however, in males the highest susceptible age group to UTI was ≥48 years (71.15%). The Chi square test showed statistically significant variations ($P < 0.05$) at 95% level of confidence interval for the infected and not infected male and female patients variables among all age groups. For the infected and not infected male patient's variable the Chi-square test values were $\chi 2 = 13.081$; degree of freedom = 1; $P = 0.000$ and the values for infected and not infected female patients were $\chi 2 = 31.114$; degree of freedom = 1;

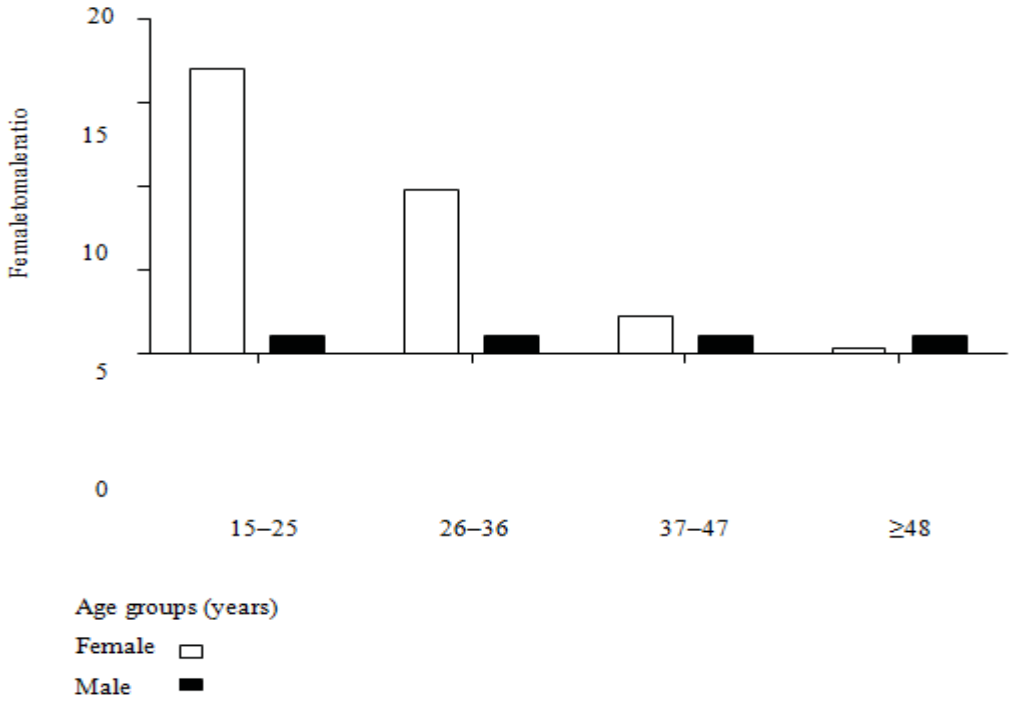

FIGURE 4: Female to male ratio for the occurrence of UTI.

$P = 0.000$ (Table 2). The highest female to male ratio for the occurrence of UTI was found in the age group of 15– 25 years (17: 1) followed by 26–36 years (9.75: 1), 37–47 years (2.22: 1), and ≥48 years (0.27: 1). The $\chi 2$ test for trend results showed significant variations ($P < 0.05$) between the female to male ratio variables in all age

groups at 95% confidence interval level ($\chi 2$ = 5.228; degree of freedom = 1; P = 0.0222) (Figure 4).

A total of 155 bacterial uropathogens comprised of 140 (90.32%) Gram negative and 15 (9.68%) Gram positive were isolated from positive urine samples. Escherichia coli was found the dominant bacteria among all isolated uropathogens with the prevalence rate of 42.58%. The second most prevalent isolate was Klebsiella pneumoniae (18.71%) followed by Pseudomonas aeruginosa (12.90%), Staphylococcus aureus (9.68%), Proteus spp. (9.03%), and Enterobacter spp. (7.10%). There was no statistically significant variation (P> 0.05) was found among the isolates (Table 3). Out of 140 Gram negative bacteria 50 (35.71%) were isolated from males and 90 (64.29%) were from female patients. Only 2 (13.33%) gram positive bacteria were isolated from male and 13 (86.67%)

Uropathogens	Number	15–25		26–36		37–47		≥48	
		Male	Female	Male	Female	Male	Female	Male	Female
E.coli	66	2(3.03%)	14(21.21%)	3(4.55%)	11(16.67%)	7(10.61%)	5(7.57%)	20(30.30%)	4(6.06%)
K.pneumoniae	29	—	11(37.93%)	1(3.45%)	7(24.14%)	1(3.45%)	2(6.89%)	6(20.69%)	1(3.45%)
P.aeruginosa	20	—	2(10.00%)	—	7(35.00%)	1(5.00%)	4(20.00%)	4(20.00%)	2(10.00%)
*Proteus*spp.	14	—	1(7.14%)	—	4(28.57%)	—	5(35.71%)	3(21.43%)	1(7.14%)
*Enterobacter*spp.	11	—	3(27.27%)	—	5(45.45%)	—	—	2(18.18%)	1(9.09%)
S.aureus	15	—	3(20.00%)	—	5(33.33%)	—	4 (26.67%)	2(13.33%)	1(6.67%)
Total	155	2	34	4	39	9	20	37	10

were isolated from female patients. The highest number of gram positive and negative uropathogens (39) was found in the female patients of the age group 26–36 years followed by 37 uropathogens which were isolated from the male patients with the age group of ≥48 years (Table 4).

The highest to lowest prevalence rate for the occurrence of different isolated uropathogens within the age groups were as follows: E. coli—≥48 years (36.36%); 15–25 years (24.24%); 26–36 years (21.21%); 37–47 years (18.18%): K.

pneumoniae—15–25 years (37.93%); 26–36 years (27.59%);≥48 years (24.14%); 37–47 years (10.34%): P. aeruginosa—26–36 years (35.00%); ≥48 years (30.00%); 37–47 years (25.00%); 15–25 years (10.00%): Proteus spp.—37–47 years (35.71%);≥48 years and 26–36 years (28.57%); 15–25 years (7.14%): Enterobacter spp.—26–36 years (45.45%); ≥48 years and 26– 36 years (27.27%); 37–47 years (0.00%): S. aureus—26–36years (33.33%); 37–47 years (26.67%); ≥48 years and 15–25 years (20.00%) (Figure 5).

Antibiotic susceptibility results showed the resistant and susceptible antibiotics for the tested uropathogens. Overall NAL was found the most resistant drug as 122 (78.71%) uropathogens were found resistant against NAL. The sec- ond most resistant drug was CTZ (71.61%) followed by CTX (67.74%); however, the most sensitive drug against all uropathogens was MRP (92.26%) followed by IMP (84.52%), LEV, and NTL each showing 74.84% sensitivity (Figure 6). The $\chi 2$ test for trend results showed a statistically significant variation ($P<$ 0.05) between the resistant and sensitive variables ($\chi 2$ = 9.152; degree of freedom = 1; P = 0.0025).

TOB was found the highest resistant drug against 96.97%

E. coli followed by NAL (90.91%) and CTX (87.88%); how- ever, both carbapenems IMP and MRP showed the highest sensitivity against 98.45% and 95.45% E. coli. 79.31% of

K. pneumoniae were resistant against CTZ and LEV was found the most susceptible drug with the rate of 89.66%. In case of P. aeruginosa the highest resistant and susceptible antibiotics were SPR (100%), and MRP (100%) respectively. 92.86% of tested Proteus spp. were resistant against CFX and 100% sensitive against both carbapenems (IMP and MRP). Enterobacter spp. showed 81.82% resistance against NTF; however, all (100%) were sensitive to OFL, SPR, LEV, IMP, and MRP. All S. aureus (100%) showed resistance against NAL and CTX; however, IMP was found 100% sensitive followed by SPR, CFX, and NTL (each showed 93.33% sensitivity against S. aureus isolates) (Table 5). The results of the paired t-test showed that there was no statistical significance between E. coli resistant versus sensitive variables (P = 0.876), K. pneumoniae resistant versus sensitive variables (P = 0.232), P. aeruginosa resistant versus sensitive variables (P = 0.950), Proteus spp. resistant versus sensitive variables (P = 0.162) and S. aureus resistant versus sensitive variables (P = 0.072), however, Enterobacter spp. showed the significant variations between resistant versus sensitive variables (P = 0.000).The highest MAR index was found for NAL (0.044) followed by CTZ (0.039) and CTX (0.038) indicating that these antibiotics were highly resistant among all tested uropathogens; however, the lowest MAR index was found for both carbapenems MRP and IMP which were 0.004.

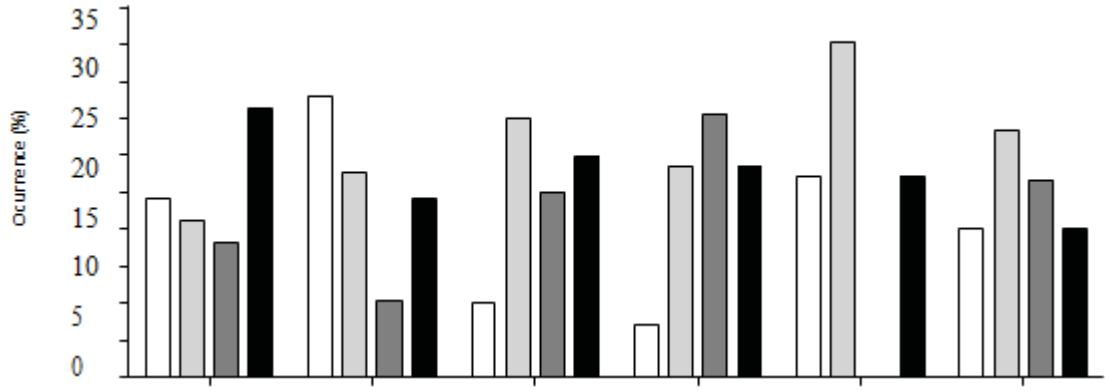

FIGURE 5:Frequency distribution of uropathogens between different age groups

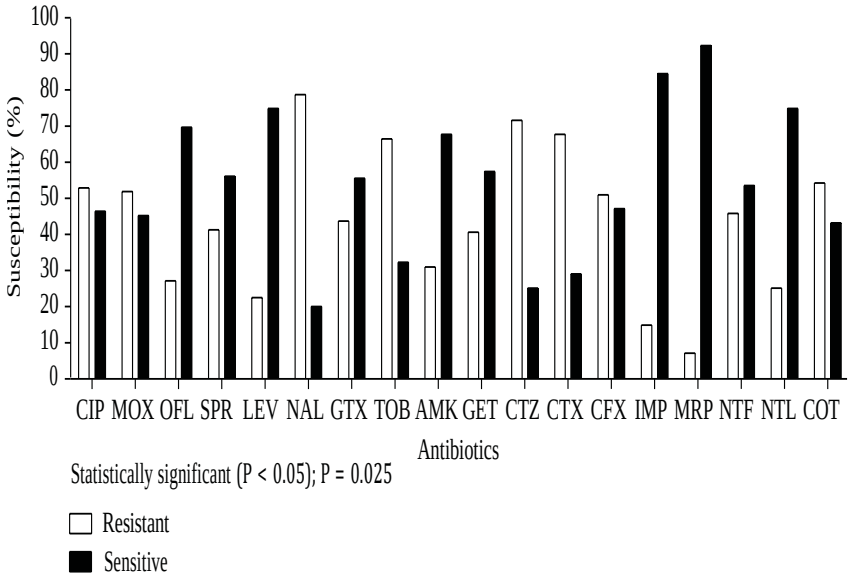

FIGURE 6:Overall resistance and sensitivity of all isolated uropathogens against tested antibiotics.

DISCUSSION

This study provides valuable data to compare and monitor the status of antimicrobial resistance among uropathogens to improve efficient empirical treatment. Increasing antimicrobial resistance has been documented globally [67–83]. The prevalence of UTI was found to be 53.82% in this study and this rate of prevalence is higher than in the other studies which accounts for 25.6% [84], 22% [85], 38.6% [86], 35.5%[61], 4.2% [87], 17.19% [60], 10.86% [61], 34.5% [88], and 36.68% [89] in India; however, the prevalence rate of UTI in our study correlates with other studies done in South Trinidad [90], and in the Mexican population [91] which showed such more highly significant uropathogens 49% and 97.3%, respectively.

Our study showed a high prevalence of UTI in females (73.57%) than in males (35.14%) which correlates with other

findings which revealed that the frequency of UTI is greater in females as compared to males [56, 80, 90–94]. The reason behind this high prevalence of UTI in females is due to close proximity of the urethral meatus to the anus, shorter urethra, sexual intercourse, incontinence, and bad toilet [95–97].

The occurrence of UTI recorded among the elderly (≥48 years, 63.51%) compared to young age patients (26–37 years, 58.11%; 15–25 years, 54.55%) and middle-age patients (37– 47 years, 39.19%) in this study differs from the other studies done in Kuwait [98] and Nigeria [99] in which the highest incidence of UTI was recorded among the age group 20 to 50 years (63.4 and 74.7%, resp.) and lowest among the age group>50 years (13.3 and 10.3%, resp.). However, our results agree with the study done in Japan with a 20-year period in which a trend of increasing complicated UTI was reported in elderly patients [100]. In our study it was found that the elderly males (≥48 years) had a higher incidence of UTI (71.15%) when compared with the elderly females (45.45%). This finding is similar to a study conducted at a tertiary care hospital in Jaipur, Rajasthan, India [94]. The main cause behind this increasing incidence of UTI with advancing age in males is due to prostate enlargement and neurogenic bladder [101]. This factor is also reported by other authors whose studies showed that the prostate disease in males is responsible for the increase in incidence of UTI and decrease in female: male ratio in patients above 50 years [102].

TABLE 5: Resistant and susceptibility rates (%) for isolated uropathogens.

Antibiotics	*E. coli* (66)		*K. pneumonia* (29) *Enterobacter* spp. (11)		*P. aeruginosa* (20) *S. aureus* (15)		*Proteus* spp. (14)					
	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S	%R	%S
CIP	69.69	30.30	79.31	20.69	5	95	35.71	64.29	18.18	81.82	33.33	60.00
MOX	56.06	40.91	58.62	41.38	NT	NT	42.86	50.00	9.09	90.91	60.00	40.00
OFL	40.91	56.06	6.89	82.76	15	85	57.14	42.86	0	100	13.33	86.67
SPR	37.88	56.06	51.72	48.28	100	0	21.43	78.57	0	100	6.67	93.33
LEV	27.27	66.67	10.34	89.66	40	60	14.29	85.71	0	100	26.67	73.33
NAL	90.91	7.58	65.52	34.48	85	20	64.29	35.71	18.18	72.73	100	0
GTX	60.61	39.39	20.69	79.31	NT	NT	50.00	50.00	9.09	81.82	33.33	66.67
TOB	96.97	3.03	68.97	31.03	60	40	7.14	85.71	27.27	72.73	20.00	73.33
AMK	9.09	90.91	17.24	79.31	95	0	28.57	71.43	18.18	81.82	80.00	20.00
GET	68.18	30.30	34.48	65.52	10	90	21.43	71.43	9.09	90.91	13.33	80.00
CTZ	78.79	18.18	79.31	13.79	65	35	78.57	21.43	45.45	54.55	46.67	53.33
CTX	87.88	10.61	37.93	62.07	90	5	14.29	78.57	9.09	90.91	100	0
CFX	53.03	46.97	20.69	79.31	95	5	92.86	0	54.55	36.36	0	93.33
IMP	0	98.48	75.86	24.14	5	95	0	100	0	100	0	100
MRP	4.55	95.45	13.79	86.21	0	100	0	100	0	100	20.00	80.00
NTF	25.76	74.24	62.07	37.93	90	10	57.14	42.86	81.82	18.18	6.67	86.67
NTL	15.15	84.85	27.59	74.41	20	80	85.71	14.29	36.36	62.64	6.67	93.33
COT	84.85	15.15	34.48	65.52	5	85	28.57	64.29	45.45	54.55	53.33	40.00

CIP: ciprofloxocin; MOX: moxifloxacin; OFL: ofloxacin; SPR: sparfloxacin; LEV: levofloxacin; NAL: nalidixic acid; GTX: gatifloxacin; TOB: tobramycin; AMK: amikacin; GET: gentamycin; CTZ: ceftazidime; CTX: cefotaxime; CFX: ceftriaxone; IMP: imipenem; MRP: meropenem; NTF: nitrofurantoin; NTL: netillin; COT: co-trimoxazole; R: resistant; S: sensitive; NT: not Tested.

Females of the age group 26–36 years were found more susceptible (90.69%) to UTI followed by 15–25 years (82.93%), 37–47 years (58.82%), and ≥48 years (45.45%).

These findings correlate with other reports which showed that females are more prone to UTIs than males during adolescence and adulthood [62, 68, 70, 94, 103–108]. The factors of this increasing incidence of UTI in young age females are associated with high sexual activity, recent use of a diaphragm with spermicide, and a history of recurrent UTIs [109].

The highest incidence of UTIs among female to male ratio was found in the age group of 15–25 years (17: 1) followed by 26–36 years (9.75: 1), 37–47 years (2.22: 1), and ≥48 years(0.27: 1). These findings differ from other reports [107, 110] which stated a lower female to male ratio in neonates and young children. The prevalence rate of UTI in boys depends on many factors including congenital malformations and uncircumcised genitalia which are often contaminated [107].

In this study, the Gram-negative bacilli constituted 90.32% of the total bacterial isolates while Gram positive cocci constituted 9.68%. Escherichia coli (42.58%) was found the most prevalent gram-negative bacteria in the positive urine samples of UTI. This result is consistent with reports from other studies [88, 98, 99, 103, 111–113] but differs from the reports in which P. aeruginosa [114] and Klebsiella spp. [115] were recorded as the predominant bacteria in UTI. Other isolated bacteria from UTI cases in this study were K. pneumoniae (18.71%), P. aeruginosa (12.90%), S. aureus (9.68%), Proteus spp. (9.03%), and Enterobacter spp. (7.10%). These findings were not correlate with other reports in which P. aeruginosa was reported as the second most common bacterial isolate in UTI studies in India [18] and Lafia, Nigeria [62]; however, these results correlate with others in which Klebsiella spp. was reported as the second most frequently isolated organism in UTI [82, 104, 113, 116, 117].

The studies on UTI in other places of the world also showed that E. coli and Klebsiella spp. are the commonest uropathogens in UTI [70, 71, 118–120]. Higher incidence of gram-negative bacteria, related to Enterobacteriaceae, in causing UTI has many factors which are responsible for their attachment to the uroepithelium. In addition, they are able to colonize in the urogenital mucosa with adhesins, pili, fimbriae, and P-1 blood group phenotype receptor [101].In females of all age categories, E. coli is the most frequently isolated uropathogen which correlates with other studies [121–123] but not with others which found that E. coli causes most male UTIs, followed by other Enterobacteriaceae and Enterococci [124, 125] whereas Proteus mirabilis was more frequently isolated in the younger female patients of UTI and K. pneumoniae in the elderly patients [122]. Both carbepenems (MRP and IMP) used in this study were found to be the most sensitive drugs against all isolated uropathogens. The sensitivity rate of carbepenems among uropathogens was as follows: E. coli (MRP; 95.45% and IMP; 98.89%), P. aeruginosa (MRP; 100% and IMP; 95.00%),Proteus spp. (MRP; 100% and IMP; 100%), Enterobacter spp. (MRP; 100% and IMP; 100%), and S. aureus (MRP; 80% and IMP; 100%), followed by

LEV and NTL each of which showed 74.84% sensitivity, however, K. pneumonia did not show a high susceptibility to IMP (24.14%) but it was susceptible to MRP (86.21%). These antibiotic susceptibility results correlate with other studies [126, 127]. Another study conducted in India showed that meropenem was highly sensitive against Gram negative bacilli whereas cephalosporin showed highest resistance against gram negative rods [128]. In other study, meropenem and imipenem were found to be 98% and 100% sensitive, respectively, against highly resistant gram-negative bacilli [129]. A study done in King Fahd Hospital, Saudi Arabia showed that meropenem was 95.8% sensitive followed by amikacin (93.7%) and imipenem (91.71%) against extended spectrum β lactamase producing E. coli [130].

Tested fluoroquinolones in this study showed the highest resistance among uropathogens as in E. coli; NAL (90.91%): K. pneumoniae; CIP (79.31%), P. aeruginosa; SPR (100%), and S. aureus; NAL (100%); however, III generation cephalosporin showed the highest resistance in K. pneumoniae; CTZ (79.31%) the Proteus spp.; CFX (92.86%), and S. arueus; CTX (100%). This high rate of resistance against fluoroquninolones was also suggested by other studies done in Spain, Europe, and Iran [83, 131] and also by other studies done in India [71, 94, 132]. Another study done in Spain also showed the reduced susceptibility of E. coli isolates from patients with UTI to Fluoroquinolones (16%) [131]. This reduced susceptibility might be due to using antibiotics without restriction. In several studies it has been shown that the highly prescribing habits of the physicians are the driving factor for the antibiotic resistance for this group of antibiotic [133–135]. McEwen et al. [86] found that 37% of physicians actually prescribe trimethoprim-sulphamethoxazole closely followed by fluo- roquinolones (32%) and the average duration of antibiotic therapy is 8.6 days in the United States which is the best exam- ple of this problem; empiric use of fluoroquinolones should be restricted and founding the strategies against increasing resistance of pathogens to these antibiotics should be done.

Our finding about the Fluoroquinolones did not correlate with others which showed that they were highly effective (sensitive) [61, 105, 114, 137, 138]. For these organisms, drugs with inhibitors like Augmentin may be tried [139] but such drugs should be reserved for the last line of treatment. The alarming finding in this study is the resistance to third-generation cephalosporin; the highest resistance was seen against CTZ (71.61%) followed by CTX (67.74%) among all uropathogens. This is an indication that many of the organisms are ESBL producers [140]. The other possible explanation behind this situation is that the III generation cephalosporin has been in use for a long period and must have been abused and over time organisms have developed resistant mechanisms due to changing their mode of action. The inappropriate usage of wide spectrum antibiotics, insufficient hygiene, immunosuppression, and a prolonged stay in the hospital are some other major etiological factors that elevate the chances of MDR infections [139].

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