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Antibiotic Resistance patterns

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Abstract- Worldwide data show that there is increasing resistance among urinary tract pathogens to conventional drugs. Urinary tract infections are one of the most common infectious diseases diagnosed in outpatients as well in hospitalized infections. Since over the last few decades the resistance pattern of urinary isolates has been showing dramatic changes all over the world, it was felt clinically useful to study the microbiological pattern of urinary tract infections.. Antimicrobial sensitivity was performed by standard disk diffusion method. The retrospective analysis of 100 such samples which were found positive for pathological bacteria revealed that all 100 UTI isolates were resistant to cefixime (100%). Multidrug resistance was defined as resistance to two or more classes of antibiotics. Multidrug resistance was detected in all the UTI isolates. These findings call for wiser use of antimicrobial agents and their continuous *in vitro* monitoring.

Introduction- Urinary tract infection is prevalent and disruptive disease and is the most common bacterial infection seen by physicians. Urinary tract infection represents one of the most common diseases today occurring from neonate to the geriatric age groups encountered in medical practice today. Urinary tract infection is the condition where infection caused by bacteria occurred anywhere in the urinary system. An infection occurs when microorganisms cling to the opening of urethra and begin to multiply (Schaeffer *et al.,* 2001). From there, some microorganisms often move on to the bladder and if the infection is not treated properly, microorganisms may then colonise to the kidneys. From the skin around the rectum and genitals, bacteria may get into the urinary tract and spread further (Hotoon *et al.*, 2005). Among the bacteria which are responsible for UTI, *Escherichia coli* is the most common. Many other UTI causing genera are also isolated from patients with variable degree of infection such as *Klebsiella*, *Enterobacter*, *Proteus*, *Serratia and Pseudomonas aeruginosa* and *Staphylococcus aureus* (Ronald, 1989).

Although, many drugs have been introduced for UTI such as norfloxacin, ciprofloxacin, gentamycin, etc., the problem of drug resistance and toxic manifestations of long term use of drugs are common. The increasing prevalence of antimicrobial resistance is a major health problem and is associated with high morbidity and mortality. Area specific monitoring studies aimed to gain knowledge about the type of pathogens responsible for UTIs and their resistance patterns may help the clinician to chose the right empirical treatment. The aim of this study was to obtain data on susceptibility patterns of major pathogens for both community and hospital UTIs to antimicrobial agents currently used in the treatment of UTIs.

Materials and methods-

The chemicals used during the experiment were purchased from Hi Media Laboratories, India; Qualigens Fine Chemicals, India; Sisco Research Laboratories (SRL), India; Merck, India.

Chemicals used included: ethanol, acetone, amyl alcohol, glycerol, DMSO (Dimethyl sulphoxide), sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl), hydrogen peroxide (H_2O_2), barium chloride, ammonium oxalate, potassium iodide, H_2SO_4, potassium hydroxide (KOH), tetramethyl-p-phenylenediamine dihydrochloride, p-dimethyl aminobenzaldehyde, α-napthol, various dyes viz., crystal violet, methylene blue, iodine, safranine, methyl red, etc.

During the lab work, different types of media were used for culturing bacteria and for performing biochemical tests. The media were purchased from Hi Media Laboratories, India

All Glasswares and plasticwares used during the experiment were purchased from Borosil, India; Tarson, India respectively. Glasswares and plasticwares used included: Sample containers, plastic vials, petriplates, petri plate stand, flasks, glass spreader, beakers, micropipettes, test tubes, culture bottles.

Antibiotics- All antibiotics used during the study were purchased from HiMedia, India. The antibiotic discs and their concentrations were: amikacin (AK, 30µg), cefixime (SF, 5µg), ceftazidime (FG, 30 µg), ciprofloxacin (RC, 5 µg), netilmicin (NT, 30 µg), nitrofurantoin (FD, 300 µg) , norfloxacin (NX, 10 µg), ofloxacin (ZN, 5 µg).

Bacterial isolates- Bacterial isolates of UTI were isolated from urine samples obtained from patients suffering from UTI. The samples were collected from Subharti Medical College and Lokpriya Nursing Home, Meerut. Among 200 samples collected, 120 belong to female patients and 80 belong to male patients.

The samples were collected in wide mouthed plastic universal containers (350 ml) by clean catch midstream urine method. Before collecting the specimen, the periurethral area was properly cleaned to avoid mild contamination. Once collected, the specimens were transported to the lab (Department of Microbiology, C.C.S. University, Meerut) without any delay and preserved in refrigerator at 4 ^{0}C. Microbial counts in a urine sample remain same at 4 ^{0}C in refrigerator for as long as 24 h. Besides the organisms isolated, reference strains which were procured from IMTECH, Chandigarh were used as positive control.

Results and discussion-

Isolation of pure cultures from urine samples

All specimens received from hospitals may or may not contain urinary tract pathogens. Therefore, they were cultured to test whether the urine sample contained the infectious UTI pathogens. So, the bacterial count was done using plate count method. The serial dilutions of 12-24 h old urine samples were done upto 10^{-6} and that dilution was spread on the nutrient agar plate. The plates were incubated overnight and the colony count was performed next day. The samples found positive (microbial count = or > 10^5 CFU/ml of urine) were further tested and those found negative were discarded. For enumeration of colony forming units (CFUs), the following formula was applied:

CFU per ml = Volume of sample × No. of colonies on plate × Dilution factor

The standard protocol for isolation of microorganisms included blending in sterile normal saline (0.85% sodium chloride) and suitable serial dilutions were made of the sample before plating them onto suitable culture media (nutrient agar medium, eosine methylene blue agar, Mac-Conkey agar, mannitol salt agar). Pure cultures were made by quadrant streaking method and spread plate method. The plates were then incubated at 37 ^{0}C for 24 h.

Identification of UTI isolates The cultures were identified on the basis of colony morphology, colour of pigmentation, variations in the colony size, microscopic characters, biochemical tests and other standard characters using standard reference books (Collins *et al.*, 1995; MacCarty *et al.*, 2000).

Antibiotic susceptibility testing- Discs procured from Hi Media were used for sensitivity tests. These are sterile filter paper discs. Each disc is impregnated with the specified amount of antibiotic and dried at low temperature under vacuum.

Antimicrobial susceptibility testing was performed on all 100 UTI isolates against 8 antibiotics by following the CLSL disc diffusion method. Sterile Mueller Hinton agar was poured into plates kept on level surface. The depth of the medium was approximately 4 mm. After solidification of the medium, plates were dried for 30 minutes in an incubator (37 ^{0}C) to remove excess moisture from surface. 4-5 similar colonies from a pure culture were selected and transferred into tube containing nutrient broth. Broth culture was incubated at 37 ^{0}C for 24 h to obtain turbidity equivalent of 0.5 McFarland standards. Sterile cotton swab was dipped into the culture inoculums and was further used to spread the agar surface of the plate. One disc at a time was taken with the help of flamed forcep and was carefully placed on the surface of the medium. The plates were kept in the incubator at 37 ^{0}C for 24 h. At the end of incubation, the diameters of the zones of inhibition were measured in mm using a ruler and compared to the zone diameter interpretive standards (Performance standards for antimicrobial susceptibility confirmed as having UTI, of which, 75 (75%) were from female patients and 25 (25%) from male patients . This is similar to the findings of Obi *et al*. (1996) who found that *E. coli* was more common in females (69%) as compared to males (37%). Mohammad Tariq (2010), Ravikumar (2010), got similar results. Uzunovic (2006) also found that most UTI isolates were obtained from female patients (77.2%). The samples showing > 10^5 CFUs/ml of urine were further subcultured on nutrient agar media and maintained for identification of bacteria causing UTI.

Prevalence of various bacteria causing UTI

Based on microscopic examination and different biochemical tests, following bacterial isolates were identified from positive urine samples investigated in present study : (i) *Escherichia coli* (70%), (ii) *Klebsiella pneumoniae* (10%), (iii) *Staphylococcus aureus* (10%), (iv) *Pseudomonas aeruginosa* (6%), and (v) *Proteus vulgaris* (4%). These results agree with the observations reported by Manzoor Kadri (2004) who also found that vast majority of UTI isolates were *E. coli* (90%), *Klebsiella* (8%), *and Staphylococcus aureus* (2%). These findings are also in congruence of reported by Inabo and Obanibi (2006) and Patton (1991). It can also be concluded that in the present study, Gram-negative bacteria (*E. coli, Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Proteus vulgaris*) were the most common of uropathogens responsible for UTI with 90% in comparison to 10% of Gram-positive bacteria (*Staphylococcus aureus*), and that *E. coli* dominated the group of UTI causing organisms .

Antimicrobial susceptibility testing-

Antimicrobial susceptibility testing was performed on all 100 bacterial isolates against eight commonly used antibiotics using disc diffusion method. These antibiotics are used especially for bacteria causing urinary tract infections and these are the drugs prescribed by doctors in case of UTI's.

Antibiotics profiling of *E. coli* isolates

Disk diffusion test of all 70 *E. coli* isolates showed susceptibility against netilmicin, ciprofloxacin, ceftazidime, norfloxacin, amikacin, ofloxacin (6 antibiotics) with mean inhibition zones of 16 mm, 22 mm, 19 mm, 23 mm, 18 mm, 17 mm, respectively. However, all isolates were resistant against cefixime and nitrofurantoin.

Antibiotics profiling of *Klebsiella pneumoniae* isolates

Disc diffusion test of all 10 *Klebsiella pneumoniae* isolates showed sensitivity against three antibiotics, namely netilmicin, ceftazidime, amikacin with mean inhibition zones of 16 mm, 8 mm, 13 mm diameter and were found resistant against five antibiotics, such as cefixime, ciprofloxacin, norfloxacin, nitrofurantoin and ofloxacin

Antibiotics profiling of *Pseudomonas aeruginosa* isolates

Disc diffusion test of six isolates of *Pseudomonas aeruginosa* showed sensitivity of these isolates against netilmicin, ciprofloxacin, norfloxacin, amikacin and ofloxacin (5 antibiotics) with mean inhibition zones of 16 mm, 23 mm, 22 mm, 17.5 mm, 16.5 mm diameter, respectively. However, these isolates were found resistant against cefixime, ceftazidime and nitrofurantoin

Antibiotics profiling of *Proteus vulgaris* isolates

Disc diffusion test of four isolates of *Proteus vulgaris* showed sensitivity of these isolates against netilmicin, ciprofloxacin, norfloxacin, and ofloxacin with mean inhibition zones of 23 mm, 22 mm, 22.5 mm, 17 mm diameter, respectively, whilst these isolates were resistant against cefixime, ceftazidime, amikacin, nitrofurantoin

Antibiotics profiling of *Staphylococcus aureus* isolates

Disc diffusion test of all 10 isolates of *Staphylococcus aureus* showed susceptibility against netilmicin, amikacin, and nitrofurantoin with mean inhibition zones of diameter 9 mm, 8 mm, 15 mm, respectively and resistant against cefixime, ciprofloxacin, ceftazidime, norfloxacin, and ofloxacin

From the above results of antibiotics profiling, it can be concluded that all 100 UTI isolates were resistant to cefixime (100%). These results are in accordance with the findings reported from Chandigarh by Gupta *et al. (*2002). However, all isolates were sensitivity (100%) to netilmicin. Hence, cefixime is not at all effective drug for UTI treatment while netilmicin is most effective.

E. coli was found to be highly sensitive for ciprofloxacin and norfloxacin, less sensitive for netilmicin, ceftazidime, amikacin, least sensitive for ofloxacin, and resistant for cefixime and nitrofurantoin. Similar to our results, Yismaw (2010) has also reported resistance of *E. coli* to cefixime and nitrofurantoin, and Inabo and Obanibi (2006) found that *E. coli* was most sensitive to ciprofloxacin followed by *Klebsiella pneumoniae, Proteus vulgaris, Staphylococcus aureus.* However, the results of present study are in contrary to the findings of Sharmeen *et al.* (2009) who reported that *E. coli* was less sensitive (40%) to ciprofloxacin and highly sensitive (80%) to ceftazidime. Resistance of *E. coli* strains to these antibiotics is mainly due to the production of β-lactamases. According to Namboodiri *et al.* (2011), these antibiotics have been subjected to widespread abuse resulting in the high rates of resistance.

Klebsiella pneumoniae was moderately sensitive for netilmicin producing mean inhibition zone of 14 mm, less sensitive for ceftazidime, amikacin, nitrofurantoin producing mean inhibition zone 9.4 mm, 11.8 mm, 8.3 mm, respectively and resistant for cefixime, ciprofloxacin, norfloxacin, ofloxacin. However, Sharmeen *et al.* (2009) observed that *Klebsiella pneumoniae* was 100% sensitive to amikacin. Akteruzzaman (2007) found in his study sensitivity of 65% to nitrofurantoin, 83% to ceftazidime, 83% to ciprofloxacin and 100% to norfloxacin. But in our study, *Klebsiella pneumoniae* showed resistance against ciprofloxacin, ceftazidime and norfloxacin.

Pseudomonas aeruginosa showed highest sensitivity against ciprofloxacin, high sensitivity against norfloxacin, moderate sensitivity against netilmicin and amikacin and resistant for cefixime, ceftazidime, nitrofurantoin. This is contradictory to study done by Giacometti *et al.* (2000) who found that *Pseudomonas aeroginosa* isolates were susceptible to cefixime and ceftazidime. However, Sharmeen *et al.* (2009) found 100% sensitivity of *Pseudomonas aeroginosa* to netilmicin and 67% sensitivity to nitrofurantoin. These results are similar to a study conducted by Strateva *et al*. (2007) in Europe where more than 90% of *P. aeruginosa* isolates were resistant to cefixime, ceftazidime and nitrofurantoin. Resistance to these antibiotics is largely due to the production of extended spectrum β-lactamase (ESBL) enzymes by the bacteria. *Proteus vulgaris* showed high sensitivity for netilmicin, ciprofloxacin, norfloxacin and resistant for cefixime, ceftazidime, amikacin, nitrofurantoin

Staphylococcus aureus showed moderate sensitivity for nitrofurantoin and less sensitivity against netilimicin and amikacin and resistance for cefixime, ciprofloxacin, ceftazidime, norfloxacin, and ofloxacin. Sensitivity pattern of *Staphylococcus aureus* is alarming in our study. No antimicrobial agent was moderately sensitive against it except nitrofurantoin. Netilmicin and amikacin showed less sensitivity while other five antibiotics were resistant to it. This trend of susceptibility is similar to a report by Reshma *et al.* (2007) but contrary to another report by Obiazi *et al.* (2007) who observed higher susceptibility of *S. aureus* to the antibiotics mentioned above.

The observed resistance in the present study to certain drugs is a probable indication of earlier exposure of the isolates to these drugs, which may have enhanced resistance development.

Piddock *et al.* (1989) proposed that ciprofloxacin is considered highly effective in treatment of UTI because of its concentrating ability in urine and high renal clearance. Hussain *et al.* (1996) conducted a study in pediatric population of 50 hospitalized patients with UTI and showed 100% susceptibility of *E. coli* against ciprofloxacin; however, there have been reports of evolving bacterial resistance to ciprofloxacin. Prevalence of fluoroquinolone resistance is related to the intensity of antibiotic use. In the current study, high rate of resistance to cefixime warrant special attention.

Present study showed very high resistance (90%) against nitofurantoin of all UTI isolates. However, low level (<60%) of resistance was observed by Tesfahunegn (2007). The fluoroquinolones tested in this study (ciprofloxacin and norfloxacin) showed good activity against *E. coli, Pseudomonas aeruginosa, Proteus vulgaris.*

The levels of antimicrobial resistance reported in this study, especially for cefixime and nitrofurantoin, were much higher than that in previous studies done in Ethiopia (Gedebou *et al*., 1988), but generally similar to those reported from other developing countries (Ozumba, 2005).

Thus, sensitivity pattern is changing day by day and it varies from hospital to hospital even in the same city and country to country. Palucha *et al.* (1999) reported that the prevalence of resistance to antibiotics varies greatly from one geographic area to another as well as between hospitals within community, mainly because of the differences in antmicrobial usage and infection control practices. Uropathogens are gaining resistance at an increasing rate to commonly used antimicrobials as revealed in our and other studies. Physicians should look for recent trend of sensitivity pattern especially of their hospital when choosing a treatment regimen for treating UTI.

Thus, present study suggests that almost all the test organisms exhibited multiple antibiotic (drug) resistance (MDR) in the following order: *Staphylococcus aureus* > *Klebsiella pneumoniae* > *Proteus vulgaris* > *Pseudomonas. aeruginosa* > *E. coli*. Resistance to commonly used antimicrobials in community acquired UTI represents a problem for the treatment of these infections.

Studies shows that multiple resistance is a common problem in hospital pathogens. Higher resistance to antibiotics may have accounted due to increased use of these drugs. Susceptibility patterns would be beneficial to guide empiric treatment and would help the physician in the selection of antibiotics.

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