ORIGINAL ARTICLE

RESISTANCE TO FLUOROQUINOLONES AMONG MULTIDRUG RESISTANT ESCHERICHIA COLI FROM URINARY TRACT SPECIMENS OF DIABETIC PATIENTS

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ABSTRACT

Objective: To analyze bacterial susceptibility of Escherichia coli isolated from urine samples of hospitalized diabetic patients to fluoroquinolones.

Material and Methods: A total of four hundred and ten (410) isolates of Escherichia coli were studied from May-October 2018 at two teaching hospitals of Peshawar. Urine samples from Diabetic patients were generally enlist from internal medicine, surgical wards and intensive care divisions. Primary susceptibility testing was performed in accordance with Clinical and Laboratory Standard Institute (CLSI) guidelines. Results were interpreted as norfloxacin resistant if zone diameter is ≤ 12 mm andciprofloxacin resistant for zone diameter ≤ 15 mm.

Results: Out of 410 isolates of Escherichia coli, 373(91%) were fluoroquinolones resistant and 37(9%) were fluoroquinolones sensitive.

Conclusions: Fluoroquinolones resistance in Escherichia coli causing UTI is prevalent in our setup due to irrational use of antibiotics. Appropriate antibiotic management strategies are needed to curtail the spread of this form of resistance.

Keywords: Fluoroquinolones, Antibioticresistance, Escherichia coli, Urinary Tract Infection

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INTRODUCTION

Urinary tract infection (UTI) are coarsely classified anatomically as upper and lower UTI, or both[1]. It is established when urine culture results were affirmative and pyuria (10 WBC/hpf) in urine sediment[2]. Bacterial UTI cases are prevalent in medical practice, where around 80 out of 1000 clinical examinations direct to its finding[3]. Aerobic gram negative microbes of the intestinal microflora are the prevailing cause of UTI. The prominent species being Escherichia coli that is usually linked to acute illness[4].

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Among the common risk factors of UTI are female sex, sexual activities, urinary tract anomalies like vesicoureteral reflux, blockage, catheter use, and an immuno suppressed status such as Diabetes mellitus (DM)[5]. It is a metabolic disorders described as high blood sugar levels over a long period[6]. Predisposition to UTIs in diabetes mellitus is associated with several aspects, like susceptibility upsurges with extended duration and greater austerity of the disease[7]. Increased urine glucose content and malfunctioned host immune factors influence the course of infection. Hyperglycemia accounts for dysfunctional neutrophil by growing intracellular calcium levels and impeding with act in leading to phagocytos is and diapedesis[8]. Patients with diabetes may progressto complications like nephropathy, cystopathy, and renal papillary necrosis, predisposing to UTIs. The microorganisms involved most frequently are Klebsiella pneumoniae, Escherichia coli and Candi-

da spp[9]. A study in United Kingdom noticed in general consultation research data bank that the prevalence of UTI was higher i.e. 46.9 per 1,000 person years among patients with diabetes as opposed to 29.9 for patients with no DM[10]. An American database during 2014 established that UTI diagnosis was more prevalent in subjects with diabetes mellitus compared to those with no disease(9.4% versus 5.7%)[11]. Another study in Americastated, over 70,000 patients (8.2%) with Diabetes were suffering from UTI in first year (12.9% female and 3.9% of male, and increase in incidence with age)[12].

Antimicrobial management of infections from Escherichia coli needs to be applicably addressed, for this reason, the medical fraternity avail varied of drugs unaided or in combo. Among the broadly used antibiotics in Pakistan remain ciprofloxacin, amoxicillin-clavulanate, norfloxacin and nitrofurantoin. The indiscriminate, empirical, protracted, or incorrect usage of antibiotics addsto the emergence of novel infections, promotingresistant strains selection[13]. Antibiotic resistant microorganisms are pervasive, affecting both nosocomial settings and community, highlighting the adversity and probable genetic mechanisms of resistance, in addition tosignifying an added selection to fluoroquinolones, sulphonamides and aminoglycosides[14].

In Lebanon 2013, susceptibility to ciprofloxacin was 57.4% in 2011 and 52.0% for Escherichia coli[15]. Surveys from several parts of Pakistan revealed, high rated incidence of antibiotic resistance in E. coli. The frequency of resistance among uropathogenic E. coli to different antibiotics have been stated as nalidixic acid (77.7%), fluoroquinolones (74.5%), gentamicin (58.2%), betalactam drugs (57.4%), Trimethoprim sulfamethoxazole (48.5%), amikacin (33.4%)[16,17,18].

Multidrug resistant E. coli intensify the treatmentexpense, morbidity and mortality specifically in developing countries like Pakistan. Multiresistance development to antibiotics apart from norfloxacin is another probable concern in prophylaxis of antibiotics. The antibiograms of norfloxacin resistant. E. coli displayed cross resistance concerning norfloxacin and quinolones such as ofloxacin and ciprofloxacin. This is due to mutations in the quinolone target DNA gyrase and topoisomerase IV, being the chief mechanism in quinolone resistance development[19]. Its been advocated that patients succumbed to prophylactic use of antibiotics develop infections to multiresistant bacteria which areaccompanied with greatermortality rates than in infected patients without prophylactic antibiotic use[20].

Antibiotic resistance curtails from the acquisition of resistance genes from transposons and plasmids[21]. Otherapprehension is the capability of bacteria in formations of bio films on mucosa of urinary bladder leading to resistance to conventional antimicrobial treatment and immune response of host. Dynamicslinked with the growth of biofilms are inappropriate usage of antibiotics in eliminating infections and the unsuitable medicine choices due to financial problems, particularly in developing nations[22,23].

It is substantially stated that there are not several treatment substitutes for patients affected by these resistant species. Consequently, accepting the delinquency of antibiotic resistance and formatting the preeminentanti bacterial for management or discovery of novel management substitutesem bodies one of the imperative trials of this era.

MATERIAL AND METHODS

A data collection form was used, which counted indemographic information of patient, previous medical history, antidiabetic drugs usage, and relevant laboratory investigations like glycated hemoglobin (HbA_{1C}) and fasting blood glucose. We defined hyperglycemia when fasting blood glucose levels were 140 mg/dL. All inpatients suffering from UTI at admission time or acquired during their hospital stay were enrolled in the study. Ethical clearance was acquired from the ethics committee of institution. UTI diagnosis was assumed in any patient having DM with symptoms falling under UTI, i.e. dysuria, urgency, frequency and for lower UTI were suprapubic pain. The symptoms for upper UTI were taken account of lower UTI along with chills, fever and costovertebral angle tenderness[24]. Five ml of clean voided midstream urine specimen was obtained in a leak-proof sterile container after cleansing the genital area with gauze sponges soaked in non-bacteriostatic saline. In catheterized patients, urine was collected by sterile aspiration of catheter with needle and syringe. A culture was also obtained through suprapubic aspiration in a patient for whom sample can't be obtained, such as admitted patients with derangedsensorium. Patients with indwelling catheters oflong-termplacement, the desired modefor urine specimen collection was from the recently placed catheter, since there is

development of biofilm on them[25].

Pyuria was detected by urine dipstick leukocyte esterase test, or by microscopic examination (10 or more leukocytes/mm3). The urine cultures were analyzed semi quantitatively, so that bacteria per ml of urine can be estimated. Culture of urine was performed both by standardized loop method and bacteriuritest strip (MAST DIAGNOSTICS) was dipped in the urine up to a defined mark (the strip picks 0.2μl of urine). The strip carrying urine was inoculated on Cysteine Lactose Electrolyte Deficient agar (CLED agar)(Oxoid, Basingstoke, UK) further incubated at 35-37˚C for 24 hours under aerobic conditions. Subsequently overnight incubation, the agar plates were inspected and the organisms were recognized based on colonial morphology, Gram staining and Biochemical tests. The colonies of Gram negative rods were subjected to test for catalase production, oxidase test and were examined for bacterial motility. Confirmation to the species level were carried out by using API (Analytical Profile Index) 20E (bio Mérieux). Colonies of Enterococci were identified by catalase test, gram staining, Bile esculin agar and API strep (bio Mérieux). Candida spp. was identified by gram staining, API AUX candida (bio Mérieux), Chrome agar and Germ tube test.

Primary susceptibility testing was performed by preparing an inoculum ofbacterial suspension in normal saline, matching with 0.5 McFarland turbidity standards. This was done by touching at least five to ten colonies from a pure growth with a straight loop and mixing in normal saline. Escherichia coli ATCC 25922 was used as control strain. Inoculum were plated on Mueller-Hinton agar (Oxoid, Basingstoke, UK). Using sterile forceps/ Antibiotic disc dispenser, following antimicrobial disks (Oxoid, Basingstoke, UK) were evenly distributed on the inoculated plates. The isolated bacteria were simultaneously tested for recommended antibiotics, Ampicillin (10 μg), piperacillin-tazobactam (100 / 10 µg), cefazolin (30 μg), amoxicillin-clavulanate(20 / 10 µg), ceftriaxone (30 μg), cefepime (30 μg),ciprofloxacin (5 μg), norfloxacin disc (10 μg)nitrofurantoin (300 μg), trimethoprin-sulfamethoxazole (1.25/ 23.75 μg), tetracycline (30 μg) gentamicin (10 μg), amikacin(30 μg) imipenem(10 μg), meropenem(10μg) and fosfomycin (200 μg).The plates were incubated aerobically at 35+2°C for 16 – 18 hours. An isolated bacteria was labelled as MDR if established resistant to three or more antibiotics belonging to different classes/groups of antimicrobials. Results were interpreted as norfloxacin Sensitive for zone diameter ≥ 17 mm and Resistant for zone diameter ≤ 12 mm, ciprofloxacin Sensitive for zone diameter ≥ 21 mm and Resistant for zone diameter ≤ 15 mm in accordance with Clinical & Laboratory Standards Institute(CLSI) guidelines.

RESULTS

Over-all1520 urine specimens from individualknown Diabetic in patients already using one or more anti- diabetic drugs were received in Laboratory for culture and sensitivity during the 6 month study time (May-October 2018). Among these, 710 specimens (46.7%) yielded significant bacteriuria; 223 urine samples (14.6%) exhibited no growth and 587urine specimens (38.6%) displayed mixed growth. The different organisms isolated from urine specimen culture. (Table 1) 410 total isolates of E.coli were included.56% of isolates were recovered from female inpatients and the rest 44% were from male admitted patients. Mean age of the patients was (age range on 40-75 years)58 years.184 isolates (45%) were recovered from patients of Cardiac Care Unit (CCU), while the remaining 226 (55%) were from patients admitted in different wards including Internal Medicine Ward (31%), Urology Ward (10%), Intensive Care Unit (9%) and Surgical Ward (5%). 373 (91%) were (norfloxacin and ciprofloxacin) resistant and 37 (9%) were fluoroquinolones sensitive. Among these 85.2 % isolates of E. coli were MDR.

During investigation empirical therapy was perceived to have commenced in all cases. Among which 82% received fluoroquinolones, ceftriaxone 9% and in the remaining few amoxicillin-clavulanate, nitrofurantoin and trimethoprin-sulfamethoxazole were used.

Table 1: Microorganism isolated from urine culture of diabetic patients.

S.no	Microorganism isolated	No of Patients % age
1	Escherichia coli	410 (57.7%)
2	Klebsiella pneumoniae	108 (15.2%)
3	Pseudomonas aeruginosa	82 (11.5%)
4	Enterococcus species	44 (6.2%)
5	Proteus species	37 (5.2%)
6	Citrobacter species	19 (2.7%)
7	Candida species	10 (1.4%)

DISCUSSION

The efficacy of different antibiotics especially fluoroquinolones were tested for E. coli causing UTI in Diabetic patients. In Europe a study spanning over six years noted, the bacterial species linked to UTI in ambulatory patients had a higherincidence of infections produced by E. coli, predominantly in females. It is supported by figures on infectionsdue to E. coliglobaly[26]. A number of researches have assessed the microbicidal action of various medications used for UTI, as well as those involved in antibiotic resistance mechanism[27].

The antimicrobial susceptibilityoutline of the E. coli isolated in present study was analogous to previous performed studies compassed in Pakistan. In our study, 85.2 % cultured E. coli were Multi-drug resistant, which is relatively on rise when equated to others study findings. Multidrug resistant E. coli analyzed by Hasan et al [17] was 52.9% in an Indian hospital setting and 7.1 percent in another research study by Sahm et al [28] in USA. Such findings demonstrate a foremost apprehension regarding the epidemiology of antibioticresistance used in UTI[29]. The risk factor most commonly related toantibiotic resistant E. coli were DM (28.7%) following renal abnormalities (21.2%) ofchronic renal disease, hydroureteronephrosis and nephrotic syndrome further risksinvolved catheterisation (13.6%) and renal calculi (9.1%)[30]. An additional risk factor to recurrent infections is formation of biofilms inside the bladder, which increases the chance of MDR strain initiating UTI[31]. Consequently, we assessed the susceptibility profiles discretely for individual antibiotic recommended for UTI. Fosfomycinwas the efficaciousoption against E. coliin urine tract specimenand conferred the nethermost resistance frequency followed by nitrofurantoin, carbapenems, cefipime and Co-trimoxazole. In evaluating antibiotic effectiveness over the span of six months study period, we perceived a substantialescalation in resistance to fluoroquinolones namely, norfloxacin and ciprofloxacin, that might be owing to empirical treatment practices for UTI. The same response was also noted in Uruguay, a state having wide spread fluoroquinolonesuse for UTI empirical management[32]. The apprehension regarding bacterial resistance necessitates modifications facilitating preventive actions or the innovation in novel therapeutic options by the scientific society. Principally owing to the findings displaying association between the mechanisms of resistance development and policies that are embraced for years to contain infectiousprocesses[33]. Patients with diabetes are more prone to have resistant pathogens as the cause of their UTI, including extended-spectrum β-lactamase-positive Enterobacteriaceae fluoroquinolone-resistant uropathogens, carbapenem-resistant Enterobacteriaceae, and vancomycin-resistant Enterococci[34].

Our statisticssubstantiate the verdicts of these study findings, as the optimal management for urinary infections in Brazilare ciprofloxacin and norfloaxacin leading to susceptibility to elements related with antibiotic resistance. Significantly, the records offluoroquinolonesresistance of six months were used in our study, to infer the bacterial resistance pattern in the prospect. By this assessment, we have construed that fluoroquinolones will no longer be adequate for useagainst UTI due to E.coli in roughly 20 years. The chief acumen for escalation in resistance is the lack of antibiotic stewardship measure, attributable to social and economic disputes in most nations[35]. As a patient with DM already takes other drugs for the chronic condition, the preference of antibiotics in such patients should also take into contemplation due to the possible drug interactions between antidiabetics and oral antihypertensive with the antimicrobials. Some antibiotics may also lead to impaired glucose homeostasis that may deteriorate the state of the patient[36].

In 43 cases nonetheless the clinical scenario did not point to Urinary tract infection, the isolateexhibited MDR properties in E. coli. Owing mostly due to inappropriate collection of mid-stream urine specimen or a deferral in sample transportation to the medical laboratory that may have disposed to a false positive reporting of culture results. The study outcomes are of grave concern for personnel involved in healthcare, therefore clinicians must be vigilant regarding their antibiotics choices bearing in mind costs and the microbial resistance manifestation. Finally, novel strategies targeting the control and treatment of bacterial infections are still desirable.

CONCLUSION

Due to irrational use of systemic antibiotics the resistance to fluoroquinolones to escheria coli in Urinary Tract Infection is more prevalent.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Khan M:	Conceived and designed the research article, data collection, performed analysis wrote article.
Zhara F:	Data Collected.
Batool Z:	Performed Analysis.
Basharat S:	wrote article.
Tariq S:	Contributed data.
Ghafoor T:	Other Contribution.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.