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CATHETER-ASSOCIATED URINARY TRACT INFECTIONS CAUSED BY QUINOLONE-RESISTANT BACTERIA ISOLATED FROM ASSIUT UNIVERSITY HOSPITALS

Osama F. E. Hassanein*	Pharmacist at Drug Research Center; Assuit University, Egypt *Corresponding Author
Khaled M. Hassanein	Professor, Medical Microbiology & Immunology Department Faculty Of Medicine, Assuit University, Egypt
Ahmed S. Ahmed	Professor, Medical Microbiology & Immunology Department Faculty Of Medicine, Assuit University, Egypt
Wafaa E. Soliman	Lecturer, Microbiology Department; Faculty Of Pharmacy, Delta University For Science And Technology, Egypt
Gamal F. M. Gad	Professor, Microbiology Department; Faculty of Pharmacy, Minia University, Egypt

ABSTRACT The aim of the study was investigation of ciprofloxacin-resistant bacterial isolates in cases with catheter-associated urinary tract infections (CAUTIs). Study objectives included isolation and identification of the bacterial isolates and determination of their antibiogram.

For ciprofloxacin-resistant strains, the minimal inhibitory concentration was determined; host characteristics were also studied. The causative bacterial uropathogens detected in all the studied cases were headed by *Klebsiella spp*. 44.9% followed by *Pseudomonas aeruginosa* 18.1%, *Escherichia coli* 17.3% and *Enterococcus* species (14.4%). Quinolone resistance was found in 30 out of 100 patients studied (30%) and in 39 out of 127 bacterial isolates 30.7%. The highest rates of quinolone resistance were shown by *Pseudomonas* and *Klebsiella* spp. then *E. coli.* (39.1%, 32.1% and 31.8% respectively). It is noted that quinolone resistance was higher in patients with mixed bacterial infections (55.6%) than in patients with mono-bacterial infection (19.2%), that may suggest that mixed bacterial infection is a risk factors for development of quinolone resistance

KEYWORDS : Catheter, urinary, quinolone, resistance, Assuit.

INTRODUCTION

Urinary tract infections are common nosocomial infections, which usually follows catheterization ***(Patel &Arya, 2000; Johansen, et al., 2007; Nicolle, 2014).***

It is expected that 100% of patients will acquire CAUTI by the fourth day of indwelling catheter insertion. Among the host characteristics that make patient liable to such infection are advanced age and debilitation. CAUTI are commonly caused by a variety of Gram positive and Gram negative bacteria ***(Rao, et al., 2011).***

In this study isolation of causative agents of CAUTIs and determination of their sensitivity pattern to commonly used antibiotics for a proper selection of empirical antimicrobial therapy were performed.

SUBJECTS & METHODS

This study is cross sectional study involving 100 male patients attending the Urology Department, Assiut University Hospitals. They all had indwelling urethral catheters inserted under aseptic condition for various medical and surgical indications. For those attending the urology clinic, catheters are usually inserted and routinely changed after 2 or more weeks or changed when there are clinical complaints of fever, dysuria, cloudy urine, catheter blockage or other symptoms of urinary tract infections. For those on admission, catheters are removed when they are deemed no longer necessary or when there are symptoms of infections.

Specimen collection

Prior to catheter change or removal from each patient, 10 ml of urine was obtained from the distal end of the catheter tube (after cleaning with an antiseptic) using a sterile needle and syringe into sterile universal container ***(Kunin and McCormack, 1966; Kunin, 1979)*** and transported to the medical microbiology laboratory for analysis.

Microscopy and culture isolation

Urine microscopy was performed on un-centrifuged catheter urine specimen to detect the presence of leukocytes, erythrocytes and other cells. Significant growth of $\geq 10^3$ bacteria/ml of catheter urine was interpreted as a urinary tract infection ***(Hooton, et al., 2010 & Hannan, 2010).*** Centrifugation was applied to the obtained specimens and sediment was cultured onto each of blood, MacConkey and nutrient agar plates and incubated aerobically at 37C° for 24-48 hours. Pure colonies of isolated organism on the culture plates were biochemically characterized to identify the species using recommended guidelines ***(Colle, et al., 1996***; ***Tang, et al., 1998; Gephart,& Murray, 1994; Farmer, et al., 1981).*** Antibiotic susceptibility was performed on pure colonies of each species to commonly used antimicrobial agents using the disc diffusion method ***(Bauer et al, 1966).***

On Mueller-Hinton agar, the zone diameter of inhibition for each antimicrobial agent was compared with the NCCLS interpretive table ***(CLSI, 2016)*** to determine sensitivity or resistance.

RESULTS

A total of 100 male patients with indwelling urinary catheters were studied. The age range is 5 – 81 years with a mean age of 43.5 years. Over 60% of patients were above 40 years of age. All the patients had indwelling urethral catheter inserted for a period ranging from 7 – 21 days before change or removal of catheter and all were routinely placed on prophylactic systemic antibiotic following catheterization.

The common indications for catheterization were bladder outflow obstruction due to renal stone formation in 40% of patients, urethral stricture in 32% and hydronephrosis in 7%.

A total of 127 microbial isolates were recovered from the 100 patients with significant bacteriuria. The rates of causative agents are shown in **tables 1 and 2.**

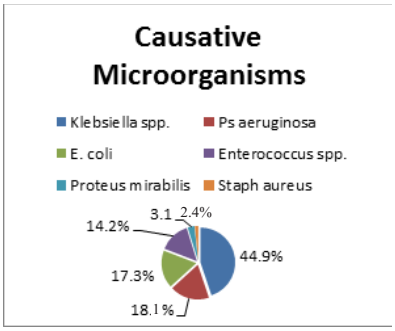
Table. 1 Rate of Causative agents in 127 bacterial isolates from 100 patients with CAUTI

Causative agent	Rate
Klebsiella spp.	57 (44.9%)
Pseudomonas aureginosa	23(18.1%)
Escherichia coli.	22 (17.3%)
Enterococcus fecalis	18 (14.2%)
Proteus mirabilis	4(3.1%)
Staphylococcus aureus	3 (2.4%)
Total No.	127 (100%)

Table 2 Species distribution among the isolated Klebsiella strains

Microorganism	Prevalence	API 20 E code
Kl. Pneumonia	44 (77.2%)	5215773
Kl. ozaenae	13 (22.8%)	5004773
Total No.	57	

Figure.1 The relative frequency of causative organisms



The *in vitro* antibiotic susceptibility pattern of the 106 Gram negative isolates showed high resistance to commonly used antibiotics such as cefotaxime (78.3%), cefazoline (81.3%), amoxacillin-clavulanic acid (88.1%) and gentamycin (54.2%).

Among 106 Gram negative isolates, quinolone resistance was detected in 31 isolates (24.4%). The ciprofloxacin-resistant Gram-negative isolates were 15 *Klebsiella spp.* isolates, 9 *Pseudomonas aeruginosa* isolates, and 7 *Escherichia coli* isolates.

From one hundred patients with CAUTI studied; 73 patients showed mono-bacterial infections and 27 patients showed mixed bacterial infections with two strains in the same patient. The common mixed bacterial strains were *Pseudomonas aeruginosa* and *E. coli* in **12** cases, *Klebsiella* species and *E. coli* in **4** cases, *Klebsiella* species and *Pseudomonas aeruginosa* in **4** cases, *Klebsiella* spp. and *Enerococcus* species in **3** cases, *Pseudomonas aeruginosa* and *Enterococcus* species in 2 cases and *E. coli* and *Enterococcus* species in **2** cases**.**

It is noted that quinolone resistance was higher in patients with mixed bacterial infections (55.6%) than patients with mono-bacterial infection (19.2%) **(Table. 3),** and the rate of mixed bacterial infection was higher in patients with quinolone resistance (46.6%) than in patients with quinolone sensitive infection (14.2%) **(Table. 4)**that may suggest that mixed bacterial infection is a risk factor for development of quinolone resistance.

The serum creatinine level was significantly higher in patients with quinolone-resistant infection than in patients with quinolone sensitive infection **(Table.4)** and it was significantly higher in patients with mixed bacterial CAUTI (77.8%) than in patients with mono-bacterial-infection(49.3%)**(Table. 3).**

These data suggest that diabetes mellitus and renal insufficiency increase the risk of quinolone resistance and the risk of mixed bacterial CAUTI.

Diabetes (hyperglycemia) was observed in 22% of all cases studied. Its rate was significantly higher in cases with mixed-bacterial infections (48.1%) compared to those with mono-bacterial infections (8.2%) and similarly it was significantly higher in patients with mixed infections compared to those with mono-bacterial infection.

Table. 3 Comparison of host and laboratory data between 27 mono-infection and 73 mixed-infection catheter-associated UTI patients

Patient data	Mixed infection (27)	Monoinfection (73)	p value
Age (years)	46.2	42.8	0.761
Age ≥40 years	14 (52%)	35 (62%)	0.264
Duration of Catheterization (days)	15.4	13.9	0.662
Duration of Catheterization ≥14 days	19 (70.3%)	27 (50.6%)	0.085
Quinolone Resistance	15 (55.6%)	14 (19.2%)	0.001 *
Serum Creatinine (umol/L)	203.7	140.9	0.001**
Blood glucose (mmol/l)	7.5	5.9	0.001
Hyperglycemia (> 7.8 mmol/l)	13 (48.1%)	6 (8.2%)	0.001*

* Statistically significant (p value <0.05) using chi square test
** Statistically significant (p value <0.05) using independent sample t test

Table. 4 Comparison of host and laboratory data between 30 quinolone-resistant and 70 quinolone-sensitive catheter-associated UTI patients

Patient data	Q. R. (30)	Q. S. (70)	p value
Mean age (years)	43.3	43.6	0.472
Age ≥40 years rate	15 (50%)	45 (64.2%)	0.147
Duration of catheterization (days)	14.8	13.7	0.513
Duration of catheterization ≥14 days	19 (63.33%)	27 (51%)	0.175
Mixed infection	14 (46.6%)	10 (14.2%)	0.001 *
Serum creatinine (umol/l)	195.8	139.2	0.008 **
Renal insufficiency rate (> 106 umol/l)	25 (83.3%)	32 (45.7%)	0.011 *
Hyperglycemia (> 7.8 mmol/l)	12 (40%)	6 (8.6%)	0.001*

Q. R. = Quinolone-resistant
Q. S. = Quinolone-sensitive
* Statistically significant (p value <0.05) using chi square test
** Statistically significant (p value <0.05) using independent sample t test

DISCUSSION

In a hospital based descriptive cross-sectional study at Assuit University Hospitals Urology Department, one hundred patients were included over one year from June 2015 to June 2016. Urine specimens from 100 patients revealed 127 bacterial isolates.

The duration of indwelling the duration urinary catheterization ranged from 7 to 21 days. The mean S. D. of duration was 14.1 ± 3.1 days for all cases, 14.8 ±2.8 days for quinolone-resistant cases and 15.4 ± 2.9 days for cases with mixed bacterial CAUTI.

Taiwo and Aderounmu (2006) observed an increased rate of UTI with increasing the duration of indwelling catheterization

The causative bacterial uropathogens detected in all the studied cases of CAUTI was headed by *Klebsiella spp.* (44.9) followed by *Pseudomonas aeruginosa* (18.1%), *Escherichia coli* (17.3%) and *Enterococcus* species (14.2). Quinolone resistance was found in 30 out of 100 patients studied (30%) and in 39 out of 127 bacterial isolates (30.7%).

The highest rates of quinolone resistance were shown by *Pseudomonas* and *Klebsiella* spp. then *E. coli*. (39.1%, 32.1% and 31.8% respectively). The overall rate of resistance to ciprofloxacin was much lower than rates of resistance to many other tested antibiotics including cefotaxime, ceftriaxone, cefazoline, ampicillin-clavulanic acid and gentamicin. Amikacin yielded resistance rates comparable to that of ciprofloxacin, and imipenem were even better than ciprofloxacin.

Nandini. M. S. &Madhusudan. K. (2016) reported that the commonest bacterial uropathogens were *E. coli* (34.61%), *Klebsiella* species (21.15%) and *Pseudomonas* species (17.3%). Similarly ***Dund J. V. &Ninama R., et al (2015)*** reported that in CAUTI cases common uropathogens were *E. coli* (40.06%) and *Klebsiella* (21.8%). ***Khelkal I. N. (2015)*** in Iraq found that the most common was *E. coli* which showed multi-drug resistance. ***Patil T. (2014)*** found that common bacterial isolates were *E. coli* (30.76%), *Pseudomonas aeruginosa* (26.15%) and *Klebsiella*species (23.07%).

Taiwo&Aderounmu (2006) reported that *Escherichia coli* was found in 20%, *Ps. aeruginosa*in 20.6% and *Klebsiellaspp*in 17.5% of CAUITs.

Savas et al. (2006) reported that in cases with CAUTIs. *E. coli* was found in 24.5%, *Klebsiella spp.* in 8.3% and *Pseudomonas spp* in 6.5%. ***Khan, et al. (2010)*** reported that in nosocomial UTI, *E. coli* was isolated from 60.9%, *Klebsiella Pneumoniae* from 18.69%, *Proteus*

mirabilis from 4.06% and *Ps. aeruginosa from* 5.46% of cases. ***Rao, et al. (2011)*** in a retrospective study of CAUTI cases over 5 years, found that *E. coli* was isolated at a rate of 35.1%, *Staphylococcus aureus*14%, *Enterococcus fecalis*12.5%, *Klebsiellaspp*10.1%, *Proteus mirabilis* 4.3% and *Ps. aeruginosa*3.2%.

Bouza, et al. (2001) in Europe found that CAUTI was caused by *E. coli* (25.1%), *Candida spp*(16.4%), *Enterococcus spp*(13.2%), *Ps. aeruginosa*(10.5%), *Klebsiella spp*(10%) and *Proteus spp*(7.3%).
Milan & Ivan (2009) reported that *E. coli* was found among CAUTI patients at a rate of 30%, *Klebsiellaspp*19%, *Pseudomonas spp*18%, *Enterococcus fecalis*11% and *Proteus mirabilis* 8%.

Quinolone resistance was found in 39 out of 127 bacterial isolates from 100 patients with CAUTI. The rate of quinolone-resistant isolates was 30.7% (39/127). Among 106 Gram negative isolates, quinolone resistance was detected in 31 isolates (24.4%). The resistant Gram-negative isolates were 15 *Klebsiella spp.*, 9 *Pseudomonas aeruginosa*, and 7 *Escherichia coli.*

From one hundred patients with CAUTI studied; 73 patients showed mono-bacterial infections and 27 patients showed mixed bacterial infections with two strains in the same patient. The common mixed bacterial strains were *Pseudomonas aeruginosa and E. coli* in 6 cases, combined infection with *Klebsiella* species and *E. coli* in 5 cases, *Klebsiella* species and *Pseudomonas aeruginosa* in 4 cases and *E. coli* and *Enerococcus* species in 5 cases.

Croxall G. & Weston V., et al. (2011) from United Kingdom found that *E. coli* was the most common uropathogen in patients with mono-bacterial urinary tract infection. *Escherichia coli* strains that shared in polymicrobial urinary infection were more resistant to antibiotics compared with those causing mono-bacterial urinary tract infection. ***Taiwo & Aadernounmu (2006)*** reported that in CAUTI mono-bacterial infection was found in 85.1% and poly-microbial infection in 14.9% of cases.

It is noted that quinolone resistance was higher in patients with mixed bacterial infections (55.6%) than patients with mono-bacterial infection (19.2%) and the rate of mixed bacterial infection was higher in patients with quinolone resistance (46.6%) than in patients with quinolone sensitive infection (14.2%) that may suggest that mixed bacterial infection is a risk factors for development of quinolone resistance.

Ko, et al. (2008) reported a rate of 22.1% of poly-microbial infection in cases with CAUTI. ***Wazait, et al. (2003)*** reported a rising rate of poly-microbial isolates in CAUTI from 6% in 1996 to 15% in 1998 and 23% in 2001. ***Bouza, et al. (2001)*** reported that in nosocomial UTI the rate of poly-microbial infection was 13% in European countries compared to 16% in non-European countries.

The present study assessed the minimal inhibitory concentration (MIC) of ciprofloxacin against resistant Gram-negative bacterial isolates.
The MICs of ciprofloxacin regarding resistant *Klebsiella spp.* isolates ranged from 4 to 256 µg/ml, modal values were 32 & 64 µg/ml, values that are ≥ 32 µg/ml were shown by 83.3% of the resistant *Klebsiella spp.* isolates.

Regarding resistant *Pseudomonas aeruginosa* isolates MICs of ciprofloxacin ranged from 32 to 256 µg/ml, the modal value was 64 µg/ml and values that are ≥ 64 µg/ml were shown by 88.9% of resistant *Pseudomonas aeruginosa* isolates. Regarding *Escherichia coli* isolates the MIC values ranged from 16 to 256 µg/ml, the modal value was 64 µg/ml and values that are ≥ 64 µg/ml were shown in 71.4% of resistant isolates. ***García& Cuevas (2008)*** reported that ciprolfoxaicn MICs regarding resistant E. coli ranged from 5 to 512 µg/ml, values that are ≥ 128 µg/ml were shown by 62.8% of the resistant *E. coli* isolates. ***Amabile-Cuevas, et al (2010)*** reported that ciprofloxacin MICs regarding *E. coli* isolated from nosocomial UTI. M ± S.D. was 180 µg/ml ± 160 µg/ml, the modal value was 256 µg/ml.

Al-Jiffri, et al (2011) reported that ciprofloxacin MICs regarding E. coli isolates from cases with UTI ranged from 15.63 to 500 µg/ml with a modal value of 500 µg/ml.

This marked resistance to ciprofloxacin, a member of the new quinolone antibiotics raises much concern about the marked development of resistance to quinolone antibiotics. These antibiotics need to be used according to antibiotic sensitivity results and there is a need to restrict the unjustified wide use of the drug on empirical basis.

Among the host characteristics considered as risk factors for CAUTI are renal insufficiency and hyperglycemia. The rates of renal insufficiency and hyperglycemia were significantly higher in patients with quinolone-resistant infection (83.3% and 40% respectively) than in patients with quinolone susceptible infection (45.7% and 8.6% respectively) and in patients with mixed bacterial CAUTI (77.8% and 48.1% respectively) than in patients with mono-bacterial infection (49.3% and 8.2%) respectively). These data suggest that diabetes mellitus and renal insufficiency increase the risk of quinolone resistance and the risk of mixed bacterial CAUTI.

Khawcharoenporn T. &Vasoo S., et al. (2013) from Chicago-USA studied the risk factors of UTI caused by multi-drug resistant enterobacteriaceae. They identified diabetes mellitus, obstructive uropathy and prior UTI among the risk factors. ***Manning S. &Lautenbach E., et al. (2015)*** from Pennsylvania University also recorded that diabetes mellitus and the presence of urinary catheter are among the risk factors of infection with flouroquinolone-resistant *E. coli.*

CONCLUSION & RECOMMENDATION

1. Urinary tract infections associating indwelling catheter is an important problem. Judicious and aseptic use of catheterization for the shortest needed period is to be stressed.
2. Causative uropathogens in CAUTI are multiple and a big portion of them is quinolone resistant. On one hand the wide use of quinolones on empirical basis needs to be restricted. On the other hand, antibiotic sensitivity testing is needed to guide the choice of suitable antibiotic and to establish basis for more rational empirical therapy when needed.
3. The high rate of quinolone resistance and the high MICs are alarming as these drugs were once established as reserve antibiotics.
4. Some host characteristics as renal insufficiency and hyperglycemia represent risk factors for CAUTI and for quinolone resistance of these infections which must be considered in managment.

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