Microbiology and Drug Resistance of Pathogens in Patients Hospitalized at the Nephrology Department in the South of Poland

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Abstract

A retrospective study was conducted among 498 patients with urinary tract infections (UTI) referred to our department from January 2013 to December 2015. This study was performed to evaluate the etiology of UTI and the antibiotic susceptibility profile of Escherichia coli (E. coli) as the main etiological factor in different age groups. Urine samples were examined using standard microbiological methods. Three hundred sixty-three samples (72.9%) were identified as E. coli, of which 29 (8.0%) can produce extended-spectrum β-lactamases (ESBL). E. coli was highly sensitive to imipenem (100.0%), gentamicin (91.0%), nitrofurantoin (89.4%), amikacin (88.2%), piperacillin/tazobactam (87.0%) and cephalosporins (87.0–79.7%). Low sensitivity was found in relation to fluoroquinolones (60.3–70.4%). E. coli was least sensitive to ampicillin (30.2%) and amoxicillin/clavulanic acid (49.9%). We observed a significant fall in susceptibility level to piperacillin/tazobactam (68.4% vs. 88.8%; p = 0.017), amikacin (61.1% vs. 90.7%; p = 0.001), gentamicin (70.0% vs. 93.2%; p = 0.002), cefalexin (41.2% vs. 83.3%; p < 0.001), cefotaxime (63.6% vs. 89.4%; p = 0.002), cefazidine (61.9% vs. 85.6%; p = 0.008), ceftazidime (73.7% vs. 91.1%; p = 0.025), ciprofloxacin (54.1% vs. 72.2%; p = 0.024) and norfloxacin (40.5% vs. 62.5%; p = 0.011) among patients with catheter-associated UTI (CAUTI) compared to those with non-CAUTI. A similar susceptibility profile was observed between different age groups. In the longevity, E. coli showed a higher sensitivity to cephalosporins than in the young-old group. E. coli susceptibility to fluoroquinolones was low, which excludes them as a first-line drug in our department. Nitrofurantoin may be used as an alternative drug to carbapenems. Monitoring of susceptibility pattern is of great importance.

Key words: urinary tract infection (UTI), Escherichia coli, antimicrobial susceptibility testing (AST)

Introduction

Urinary tract infections (UTI) are among most common bacterial diseases both in community and hospital settings. Due to their high rate of frequency, recurrence, complications as well as increasing antimicrobial resistance, they pose a real challenge to medical professionals. Older people are more susceptible to UTI because their immune system is weaker, and comorbidities are often present (Aplay et al. 2018). Escherichia coli (E. coli) remains the predominant isolated uropathogen in Poland accounting for 80% of all uncomplicated infections (Stefaniuk et al. 2016). However, in the presence of a urinary catheter, the spectrum of E. coli accounts for approximately 50% of total catheter-associated UTI (CAUTI), and other uropathogens become more prevalent (Flores-Mireles et al. 2015).

Of all hospital-associated UTI, 70–80% result from an indwelling urinary catheter, especially in older people, after surgical procedures and among patients staying in intensive care units (Temiz et al. 2012; Wójkowska-Mach et al. 2013; Piechota 2016). Catheter use is also associated with noninfectious outcomes, including mechanical trauma, mobility impairment as well as urethral strictures (Esposito et al. 2011; Hollingsworth et al. 2013). Additionally, prolonged catheterization increases the risk of biofilm formation in which uropathogens are difficult to treat with antimicrobial...
agents (Zalewska-Piatek et al. 2009). Therefore, before deciding to catheterize the bladder it is important to estimate indications individually.

An optimal empirical therapy for UTI requires understanding of local epidemiology and antimicrobial susceptibility profiles. Uropathogens have developed resistance mechanisms to commonly prescribed antibiotics what limits treatment options of effective therapies. So, we conducted a study to describe characteristics of patients with UTI, including CAUTI, as well as to determine etiology and susceptibility profiles of most common urinary isolates in different age groups.

Experimental

Materials and Methods

A retrospective study was conducted on hospitalized adults from 1st January 2013 to 31st December 2015 in a Department of Internal Medicine and Nephrology with Dialysis Centre of Regional Hospital in southern Poland. During this period 4512 patients (2452 women and 2060 men) were hospitalized in our department in whom we diagnosed 498 cases of UTI. Among these cases, a group with CAUTI was distinguished. In the study, we made a systematic analysis of medical records of patients with diagnosed UTI.

UTI was diagnosed based on a positive result of a urine culture test with significant bacterial growth ≥ 10^5 CFU/ml and a presence of at least one of the following symptoms: body temperature ≥ 37.5°C, dysuria, perineal pain, suprapubic pain or flank pain. Laboratory tests usually showed the elevated level of inflammatory markers and leucocyturia in urinalysis. According to the European Centre for Disease Prevention and Control (ECDC 2015), diagnostic criteria for CAUTI included the maintenance of a catheter in the bladder for at least 7 days. The patients were also categorized by age as follows: 19–74 years (young old), 75–85 years (old old) and >85 years (longevity).

Urine for bacteriological examination was obtained from the middle stream but in catheterized patients after a catheter replacement with the hygiene and sterility principles preserved. All urine samples were inoculated in a Microbiology Department on Columbia agar with 5% sheep blood, Sabouarud agar and Chromogenic media. Inoculated agar plates were incubated aerobically at 35–37°C for 18–24 hours. Colonies were counted on the inoculated medium and multiplied by the loop volume to determine bacterial count. Bacterial identification was done by standard biochemical procedures, including Vitek 2 Compact system.

Antibiotic susceptibility testing (AST) as an in vitro assessment of activity of the antimicrobial drug was determined using the disc-diffusion and diffusion-gradient (E-test) methods. Zone diameters for individual antimicrobial agents were translated into susceptible, intermediate and resistant categories. According to the European Committee on Antimicrobial Susceptibility Testing (EUCAST), intermediate sensitivity strains were classified as resistant. A susceptibility level was calculated by dividing the number of sensitive strains to the antimicrobial agent by the total number of strains in relation to which the sensitivity to a particular antimicrobial agent was determined. Strains were tested against the following antibiotics: ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, amikacin, gentamicin, cefalexin, cefuroxime, cefotaxime, ceftazidime, cefepime, ciprofloxacin, norfloxacin, cotrimoxazole, imipenem and nitrofurantoin.

Because of E. coli predominance in the studied population, we analyzed susceptibility pattern only in reference to this uropathogen, including also division into age groups. The remaining microbes were not representative enough to be analyzed in this issue.

We accepted p < 0.05 as the cut-off value for the level of statistical significance. The logistic regression test and chi-square test were used to compare independent variables. The calculations were carried out by the SPSS software (Statistical Package for the Social Sciences, STATISTICS 20, Armonk, NY, USA).

Results

Among 4512 hospitalized patients, we recognized 498 cases of UTI (11.0%), of which 60 were CAUTI (1.3%). The mean age was 74.8 ± 14.6 years (ranging from 19 to 101 years) without differences between CAUTI and non-CAUTI population (76.2 ± 10.4 vs. 74.6 ± 15.0 years; p = 0.988). Hospitalization time was significantly longer in patients with CAUTI compared to those with non-CAUTI (13.63 ± 10.47 vs. 9.58 ± 6.66 days; p = 0.002).

Table I presents the demographic and clinical characteristics of the studied population. Females showed much higher prevalence of the total UTI as 319 (64.1%), and in the non-CAUTI group than in the CAUTI group (67.6% vs. 38.3%; p < 0.001). However, males were more common in the CAUTI group (61.7% vs. 32.4%; p < 0.001). Patients who developed catheter-associated infections had more frequently genitourinary tumors (15.0% vs. 7.3%; p = 0.047) and urine stasis in kidneys (8.3% vs. 4.1%; p = 0.001). In addition, 10 men were after prostatectomy. No significance was found for other diseases and risk factors.

E. coli was the predominant isolated uropathogen in the studied population (72.9%). However, its occurrence in the CAUTI group was significantly smaller com-
Drug resistance of pathogens in hospitalized patients

Table I
Characteristic of patients with UTI enrolled in the study.

| Characteristic          | Total UTI n = 498 (100%) | CAUTI n = 60 (100%) | non-CAUTI n = 438 (100%) | OR (95% CI) | p-value |
|-------------------------|--------------------------|---------------------|--------------------------|-------------|---------|
|                         | n | %         | n | %         | n | %         |             |            |
| Gender: Male            | 179 | 35.9% | 37 | 61.7% | 142 | 32.4% | 3.35 (1.92–5.86) | <0.001 |
| Female                  | 319 | 64.1% | 23 | 38.3% | 296 | 67.6% | 0.30 (0.17–0.52) | <0.001 |
| Hypertension            | 263 | 52.8% | 37 | 61.7% | 226 | 51.6% | 1.51 (0.87–2.62) | 0.145 |
| Heart failure           | 174 | 34.9% | 23 | 38.3% | 141 | 32.2% | 1.31 (0.75–2.29) | 0.344 |
| Diabetes mellitus       | 190 | 38.2% | 18 | 30.0% | 172 | 39.3% | 0.72 (0.40–1.28) | 0.258 |
| Urolithiasis            | 22  | 4.4%  | 1  | 1.7%   | 21  | 4.8%   | 0.34 (0.04–2.55) | 0.292 |
| Bronchopneumonia        | 9   | 1.8%  | 1  | 1.7%   | 8   | 1.8%   | 0.91 (0.11–7.41) | 0.931 |
| Malignancy (total)      | 80  | 16.1% | 11 | 18.3%  | 69  | 15.8%  | 1.34 (0.68–2.65) | 0.405 |
| Genitourinary malignancy| 41  | 8.2%  | 9  | 15.0%  | 32  | 7.3%   | 2.24 (1.01–4.96) | 0.047 |
| Prostatic hyperplasia   | 57  | 11.4% | 10 | 16.7%  | 47  | 10.7%  | 1.66 (0.79–3.50) | 0.180 |
| Urine stasis in kidneys | 23  | 4.6%  | 5  | 8.3%   | 18  | 4.1%   | 4.34 (1.76–10.73) | 0.001 |
| Percutaneous nephrostomy| 8   | 1.6%  | 1  | 1.7%   | 7   | 1.6%   | 1.04 (0.13–8.63) | 0.968 |
| Hemodialysis            | 20  | 4.0%  | 2  | 3.3%   | 18  | 4.1%   | 0.80 (0.18–3.56) | 0.774 |
| Immunotherapy           | 26  | 5.2%  | 4  | 6.7%   | 22  | 5.0%   | 1.35 (0.45–4.06) | 0.593 |

Data are expressed as number and percentage.
One patient may have several co-existing diseases.

Table II
Microbial uropathogens isolated from the urine samples.

| Pathogen                  | Total UTI | CAUTI | non-CAUTI | OR (95% CI) | p-value |
|---------------------------|-----------|-------|-----------|-------------|---------|
| E. coli                   | n | %         | n | %         | n | %         |             |            |
| 363 | 72.9% | 37 | 61.7% | 326 | 74.4% | 0.55 (0.31–0.97) | 0.039 |
| Proteus sp.               | 30 | 6.0%  | 6  | 10.0%  | 24  | 5.5%  | 1.92 (1.05–3.52) | 0.047 |
| Staphylococcus sp.        | 12 | 2.4%  | 5  | 8.3%   | 7   | 1.6%   | 5.60 (1.72–18.24) | 0.004 |
| Enterobacter sp.          | 20 | 4.0%  | 4  | 6.7%   | 16  | 3.7%   | 1.88 (0.61–5.84) | 0.272 |
| Klebsiella sp.            | 40 | 8.0%  | 2  | 3.3%   | 38  | 8.7%   | 0.36 (0.09–1.54) | 0.170 |
| Enterococcus sp.          | 11 | 2.2%  | 2  | 3.3%   | 9   | 2.0%   | 1.64 (0.35–7.80) | 0.332 |
| Other                     | 22 | 4.4%  | 4  | 6.7%   | 18  | 4.1%   | 1.67 (0.54–5.10) | 0.371 |
| Total                     | 498 | 100.0% | 60 | 100.0% | 438 | 100.0% | –             | –         |

Other pathogens:
Pseudomonas sp. (n = 6), Morganella morganii (n = 4), Acinetobacter baumannii (n = 3), Serratia sp. (n = 3), Citrobacter sp. (n = 2), Raoultella planticola (n = 1), Salmonella (n = 1), Stenotrophomonas maltophilia (n = 1), Streptococcus agalactiae (n = 1)
ESBL-producing pathogens responsible for CAUTI: E. coli (n = 6), Klebsiella sp. (n = 2), Enterobacter sp. (n = 1)
ESBL-producing pathogens responsible for non-CAUTI: E. coli (n = 23), Klebsiella sp. (n = 12), Enterobacter sp. (n = 11), Proteus sp. (n = 4)

pared to non-CAUTI group (61.7% vs. 74.4%; p = 0.039) (Table II). The occurrence of other microbes in the CAUTI and non-CAUTI groups were much lower: Proteus sp. (10.0% vs. 5.5%; p = 0.174), Staphylococcus sp. (8.3% vs. 1.6%; p = 0.004), Enterobacter sp. (6.7% vs. 3.7%; p = 0.272), Klebsiella sp. (3.3% vs. 8.7%; p = 0.170) and Enterococcus sp. (3.3% vs. 2.0%; p = 0.532).

Production of extended-spectrum β-lactamases (ESBL) was found in 11.8% of total strains with the prevalence as follows: in the CAUTI (9/60; 15.0%) group and in the non-CAUTI (50/438; 11.4%) group. Enterobacter sp. were most frequently associated with ESBL production as 60.0% of all Enterobacter sp. isolates was positive, followed by Klebsiella sp. (35.0%).

Table III presents susceptibility profile of E. coli in the studied population to various antimicrobial agents. E. coli showed the highest prevalence of susceptibility to imipenem (100.0%), followed by gentamicin (91.0%), nitrofurantoin (89.4%), amikacin (88.2%), piperacillin/tazobactam (87.0%) and cephalosporins (79.7–89.5%). The prevalence of susceptibility was least to amoxicillin (30.2%) and amoxicillin/clavulanic acid (49.9%).
also observed a fall in sensitivity level of *E. coli* to antibiotics among CAUTI patients compared to non-CAUTI individuals, with the significant differences in relation to piperacillin/tazobactam (68.4% vs. 88.8%; *p* = 0.017), amikacin (61.1% vs. 90.7%; *p* = 0.001), gentamicin (70.0% vs. 93.2%; *p* = 0.002), cefazidime (61.9% vs. 85.6%; *p* < 0.001), cefotaxime (63.6% vs. 89.4%; *p* = 0.002), cefepime (73.7% vs. 91.1%; *p* = 0.025), ciprofloxacin (54.1% vs. 72.2%; *p* = 0.024) and norfloxacin (40.5% vs. 62.5%; *p* = 0.011).

An antimicrobial susceptibility profile of *E. coli* depending on age groups was summarized in Table IV. The youngest group constituted the reference for other groups. *E. coli* showed a similar susceptibility level to most antimicrobial agents between different age groups. In the longevity, *E. coli* demonstrated higher sensitivity to cephalosporins than in the young-old group, with significant differences relating to cefazidime (61.9% vs. 85.6%; *p* = 0.008), cefepime (73.7% vs. 91.1%; *p* = 0.025), ciprofloxacin (54.1% vs. 72.2%; *p* = 0.024) and norfloxacin (40.5% vs. 62.5%; *p* = 0.011).

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Urinary tract catheterization is one of the most common reason of bacteriuria caused by tendency of bacteria to adhere to artificial materials. It is believed that CAUTI occurs at a rate of 3–7% per day of catheterization and the incidences approach 100% within 30 days (Lo et al. 2014). Among patients hospitalized in our department, CAUTI were diagnosed in 1.3% cases that was a slightly higher than in the study carried out in Australia – 0.9% (Gardner et al. 2014). In another study, CAUTI were reported in 2.2% of hospitalized patients of the urological and orthopedic department (Giles et al. 2015).

In the current study, CAUTI was significantly more frequent in men that was similar to the Korean report (Kim et al. 2017). The authors, like in our study, analyzed the characteristics of CAUTI and non-CAUTI patients. They observed that CAUTI occurred significantly more often in patients with hypertension, those who used ventilators and after operations. In our analysis, CAUTI was more frequent in patients with genitourinary malignancy and urine stasis in kidneys. Patients with cancer have a greater tendency to acquire infections than general population due to cellular and humoral immune dysfunction, as well as complications of cancer therapy, including neutropenia or disruption of natural physical barriers (Thirumala et al. 2010). Urinary tract obstruction provides an opportunity for bacteria to adhere to urothelium and infect patients. In such case, effective antibiotic therapy as well as an appropriate urological intervention is necessary to prevent recurrent UTI and septic complications.

The predominant uropathogen responsible for CAUTI in our study was E. coli, followed by Proteus sp. and Staphylococcus sp. In patients with non-CAUTI, apart from E. coli, we also frequently observed infections caused by Klebsiella sp. and Proteus sp. A lot of authors indicate a very similar bacterial flora (Krygiel et al. 2012; Kalal and Nagaraj 2016; Wang et al. 2016). E. coli was the main etiological factor in both groups, however, its predominance was significantly smaller in catheter-associated infections. This is because CAUTI belong to complicated ones with higher rate of hospital-acquired infections, in which prevalence of the E. coli is not as high as in uncomplicated UTI (Holecki et al. 2012; Kalal and Nagaraj 2016; Wang et al. 2016).

In the non-CAUTI in this study. According to some
reports, *Proteus mirabilis* is also supposed to be a frequent reason of CAUTI, especially in males, with a tendency to biofilm formation accounting for 17.9% of *Enterobacteriaceae* family (Jacobsen et al. 2008; Moryl et al. 2013).

In the current study, *Klebsiella* sp. were the second common cause of non-CAUTI which is compatible with most publications (Farajnia et al. 2009; Aboudobara et al. 2010). However, another study reported that *Pseudomonas* sp. were the second frequent isolated microorganisms responsible for 8.7% of total UTI (Abejew et al. 2014).

Antimicrobial resistance is now accepted as a major problem in public health and patient care. It is mainly associated with an abuse of antimicrobial agents and makes it difficult to choose a proper empirical treatment by medical practitioners, especially in cases of multidrug resistant strains (Pobiega et al. 2015). Uropathogens developed resistance mechanisms of which the most common is ESBL. Organisms producing ESBL are clinically relevant and remain an important cause of failure of cephalosporins (Bradford 2001). In this study, *Enterobacter* sp. and *Klebsiella* sp. were the most frequently ones associated with ESBL production of all these species, followed by *Proteus* sp. and *E. coli*. Similar results were also reported by other authors from Poland (Sacha et al. 2007). *E. coli* as the predominant uropathogen was responsible for ESBL production in 8.0% of total *E. coli* isolates being positive, similarly to the previous data from southern Poland (Pobiega et al. 2013). For example, in France approximately 4.0% of *E. coli* isolates are ESBL producers, while in Mexico even 31.3% of *E. coli* isolates produce ESBL mechanism (Galindo-Méndez 2018; Zucconi et al. 2018).

Susceptibility of *E. coli* in the studied population was highest to imipenem and gentamicin (> 90.0%), and lowest to ampicillin as well as amoxicillin/clavulanic acid (< 50.0%). This is similar to the reports from other countries with the exception of gentamicin which was a less effective drug (Daoud et al. 2015; Li et al. 2017). It may be since in our country gentamicin is rarely used as the first empiric choice for UTI treatment. Although, imipenem was the most effective drug, it should not be administrated as an empirical drug unless infection is life threatening, as carbapenems are considered drugs of last resort.

It is important to point out that the minimum susceptibility rate to support empirical treatment of UTI is 80% of all the strains of a specific uropathogen in a given region. According to these guidelines, the European Association of Urology (EAU) recommend cotrimoxazole as a first-line drug for empirical therapy in UTI (Grabe et al. 2009). In our study, 74.6% of *E. coli* isolates were susceptible to cotrimoxazole. Despite the fact that these values may vary among reports, the resistance rate of *E. coli* to cotrimoxazole in Europe tends to be above 20%, having also been reported higher than 30% (Guneysel et al. 2009; Schito et al. 2009). *E. coli* susceptibility to fluoroquinolones in the current study ranged from 60.3% (norfloxacin) to 70.4% (ciprofloxacin) which was similar to the rates observed in Brazil and Korea (Reis et al. 2016; Park et al. 2017). Another study reported that *E. coli* was least sensitive to cephalosporins (7.0–34.0%) and fluoroquinolones (26.0–28.0%); however, a study was conducted on a population from low socioeconomic strata (Kidwai et al. 2017). A good empirical choice seems to be nitrofurantoin but a study carried out in Poland showed a high proportion of resistance to nitrofurantoin among *E. coli* isolates (Stefaniuk et al. 2016). Amikacin, as an infusion solution, may be administrated only in hospital settings. Cephalosporins also remain a good option for empirical treatment of UTI caused by *E. coli* because of relatively high sensitivity to this group of drugs and minor side effects. On the other hand, cephalosporins are commonly prescribed by medical professionals which causes a growing resistance to them among bacteria. Therefore, clinical practitioners as well as laboratory personnel should implement a program to detect and report resistant strains such as ESBL producing bacteria to control and limit the therapeutic failures.

Patients with CAUTI are a specific group in which treatment of the infection may be difficult due to a high resistance rate of uropathogens. In such a case, it is necessary to administer an adequate initial antimicrobial therapy according to local antimicrobial susceptibility situation (Saurel et al. 2006). Inadequate empirical antimicrobial therapy extends treatment contributing to various complications and transformation into a chronic illness. Our analysis confirmed that *E. coli* isolates were significantly less sensitive to most antibiotics in the CAUTI group compared to non-CAUTI group. Nitrofurantoin (83.8%) besides imipenem (100.0%), seemed to be an optimal choice in cases of catheter-associated infections caused by *E. coli*, contrary to ampicillin (19.4%). This result is compatible with another report (Albu et al. 2018). However, other authors found amikacin (100.0%) and cefepime (100.0%) to be superior to nitrofurantoin (92.8%) in susceptibility rate what is incompatible with our analysis (Piljic et al. 2013).

Regardless of the age groups among the population analyzed in this study, *E. coli* showed sensitivity to most antibiotics, including: imipenem, nitrofurantoin, amikacin, gentamicin, piperacillin/tazobactam as well as cephalosporins. Moreover, in reference to our results, it may be helpful for medical practitioners to choose cefalexin, cefotaxime or cefepime in case of UTI caused by *E. coli* in the longevity group, because of their significantly higher susceptibility rate compared to young-old
as well as old-old groups. Fluoroquinolones in our report turned out to have limited efficacy among patients with UTI, regardless of the age group. Thus, choosing them as first-line drugs in empiric therapy raises doubts and should be careful in admitted patients.

The results of this study can be used to construct a hospital formulary and internal procedure for anti-biotics usage in case of UTI in a specific department. Our results can also be helpful for comparison between departments and hospitals interested in this issue.

There are some limitations to this study. First, we retrospectively collected data through electronic medical records. It was difficult to obtain all characteristics for analyzing risk factors such as previous antibiotics use and history of recurrent UTI due to unreconciled information. Second, this study was conducted only in a single center. Therefore, it is difficult to reflect the overall characteristics of our region. The current report suggests the need for further large-scale monitoring of epidemiology and susceptibility profiles of most common uropathogens causing UTI, including CAUTI, to improve the effectiveness of empirical treatment.

Conclusions

The presented study showed considerable bacterial resistance to common empirically used antibiotics in case of CAUTI. E. coli susceptibility to fluoroquinolones was relatively low, which indicates the necessity of exclusion this group of drugs as first-line for the empirical treatment of UTI in our department. Nitrofurantoin as an alternative to carbapenems, can be used in empirical therapy of UTI, including CAUTI, regardless of the age group. Monitoring of susceptibility pattern is of great importance.

Conflict of interest

Author does not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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