Aetiology and antibiotic resistance patterns of urinary tract infections in the elderly: a 6-month study

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Urinary tract infections (UTIs) are a common cause of bacteraemia in the elderly and are associated with a high probability of hospitalization. Despite the impact of UTIs on health status and quality of life, a limited number of studies have evaluated their aetiology in this population. This study aimed to evaluate the microbial aetiology and pattern of susceptibility of bacteria causing UTIs in the elderly. For this purpose, a retrospective cohort study of elderly residents (n=472, aged >65 years) in 14 nursing homes in Milan (Italy) and its province was performed. Globally, 393 micro-organisms from 328 samples were isolated: Escherichia coli was the most prevalent (44.8 %), followed by Proteus mirabilis (20.4 %), Providencia spp. (8.9 %), Klebsiella spp. (6.4 %) and Pseudomonas aeruginosa (4.6 %). Enterococci were the most frequently isolated Gram-positive organisms (7.4 %). Almost all Enterobacteriaceae were susceptible to nitrofurantoin, carbapenems and amikacin. Extended-spectrum β-lactamases were detected in 42.1 % of isolates. The most active antibiotics against P. aeruginosa were colistin, amikacin and piperacillin/tazobactam. All Gram-positive organisms were susceptible to glycopeptides and linezolid, and 90 % were susceptible to nitrofurantoin. Fluoroquinolones showed a limited activity against all the tested micro-organisms. Escherichia coli remains the major micro-organism responsible for UTIs in older people, although to a lesser extent than in a younger population. The high rates of resistance observed in this study make careful use of antibiotics advisable to limit further development of resistance.

INTRODUCTION

Urinary tract infections (UTIs) represent the most common infections in the elderly (aged >65 years), whether they are community dwelling, live in long-term care facilities or are hospitalized. UTIs are also recognized as the most common cause of bacteraemia in the elderly and are associated with a high probability of hospitalization (Rebelo et al., 2011; Cherubini et al., 2012). In older patients, the diagnosis and treatment of UTIs can be more complicated than in younger people, as underlying host factors, including age, diabetes, spinal cord injury, catheterization and general disability, can affect the pathogenesis of these infections (Tal et al., 2005). Moreover, ageing-related immune-system changes including decreased humoral and cellular immune reactivity may lead to a decreased ability to respond appropriately to antigen challenge and to maintain immunological memory (Castle, 2000).

To date, little has been determined about the epidemiology, pathogenesis and treatment of UTIs in the elderly. Previous studies have shown a wider aetiology among geriatric patients than in younger patients and a higher prevalence of polymicrobial infections, comprising ~30 % of UTIs (Shortliffe & McCue, 2002). Moreover, high rates of resistance have been reported in nursing homes compared with the geriatric population living in the community (Das et al., 2009).
The current treatment of UTIs is empirical, based on the limited and predictable spectrum of the causative microorganisms, but the susceptibility of bacteria shows significant geographical variations (Kahlmeter & ECO.SENS, 2003; Stratchounski & Rafalski, 2006; Schito et al., 2009).

As complications from UTIs are more likely among the elderly, ranging from bacteraemia and abscess to such non-infectious effects such as dehydration, stroke and functional losses, greater attention must be placed on the epidemiology of bacteria isolated from urine samples and on antimicrobial prescribing practices in order to optimize antimicrobial treatment.

The aim of this study was to investigate the epidemiology of UTIs and the spectrum of antimicrobial resistances of isolates from patients aged ≥65 years residing in nursing homes located in an area of north Italy.

**METHODS**

**Sample collection and analysis.** The study was conducted on samples (freshly voided midstream specimens of urine) sent to the Laboratory of Clinical Chemistry and Microbiology of IRCCS Galeazzi Orthopaedic Institute of Milan, Italy, from June to November 2010.

Samples were obtained from residents from 14 nursing homes in Lombardy ranging in size from 30 to 300 beds. Patients enrolled in the study or their representatives gave their approval to the informed consent prepared by the medical staff of any single nursing home.

The exclusion criteria were: terminally ill patients defined as having an anticipated life expectancy of <4 weeks, age <65 years, and patients with spinal cord injury, on dialysis, on antimicrobial or anti-infective therapy in the previous month, on chronic suppressive therapy or in residency for <4 weeks. Patients with permanent catheters were also excluded from the study.

Only samples with both pyuria (>10 white blood cells μl⁻¹) and significant bacteriuria (>10⁵ c.f.u. ml⁻¹) from subjects with at least one of the following clinical symptoms were included in the study: mild fever, increased burning pain on urination, suprapubic pain or tenderness, frequency or urgency, new or worsening of incontinence, or deterioration in mental or functional status (Arinazon et al., 2009). Leukocytes were tested for leukocyte esterase activity using a Combur Test reagent strip for urinalysis (Roche). White blood cell counts were confirmed by light microscopy (more than five cells per high power field). Samples with more than three species of organism were considered to be contaminated, whilst samples with three different bacterial species were confirmed by a second sample collected 2 days after the first. Only a single positive culture per patient was considered in the analysis, unless new pathogens from the same subject were isolated.

Pathogens were identified by Gram-staining and standard biochemical procedures (API System; bioMérieux).

**Antibiotic susceptibility testing.** Antimicrobial susceptibility testing was carried out using the disc diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI, 2009a). Antimicrobial agents were obtained from Becton Dickinson. Inhibition zone diameters were measured to the nearest millimetre with a slide gauge (CLSI, 2009a, b). Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853 and Staphylococcus aureus ATCC 25923 were used as control strains. Test results were accepted only if inhibition zone diameters were within the performance range.

Gram-negative bacteria were tested against the following antimicrobial agents: amikacin, ticarcillin/clavulanic acid, cefotaxime, chloramphenicol, gentamicin, ciprofloxacin and levofloxacin. Gram-negative isolates positive for an oxidase test (Oxoid) were also tested against ceftazidime, ceftiraxone, cefepime, piperacillin/tazobactam, colistin, pipemidic acid and meropenem. Ampicillin, cephalotin, fosfomycin, imipenem and nitrofurantoin were assayed against Gram-negative oxidase-negative isolates. The panel of antibiotics against Gram-positive bacteria comprised: penicillin, ampicillin, cefotaxin, levofloxacin, sulfamethoxazole/trimethoprim, gentamicin, amikacin, linezolid, vancomycin, teicoplanin and rifampicin.

**Extended-spectrum β-lactamase (ESBL) detection by the CLSI phenotypic method.** The CLSI ESBL confirmatory test with cefotaxime was performed for all isolates using the disc diffusion method on Mueller–Hinton agar plates. Susceptibility test results were interpreted according to criteria established by the CLSI (2009b).

**Statistical analysis.** The data collected comprised demographic characteristics, clinical presentation, urine culture results and the antimicrobial spectrum of resistance.

Variables were described by position (mean median first and third quartile) and by dispersion (SD) indexes if continuous, and by frequency and percentage if categorical.

A logistical analysis was performed to evaluate the effect of gender and class age on the type of bacteria isolated.

A multiple correspondence analysis was applied to detect qualitative association in the infectious pattern of patients; the Greenacre correction was applied to inertia (Greenacre, 1988a).

The genera of bacteria more frequently isolated (Klebsiella, Enterobacter, Morganella, Proteus and Providencia) were each introduced in the multiple correspondence analysis as a supplementary point to detect the association between the resistance pattern profile and the genus (Greenacre, 1988b).

To investigate any statistically significant association between resistance pattern identified and explanatory covariates such as gender, age class and bacterium, a logistic analysis was performed.

All analyses were performed using SAS v9.1.3 software (SAS Institute). P values <0.05 were considered significant.

**RESULTS**

**Isolated micro-organisms**

Between June and November 2010, 472 samples were analysed: 328 results were positive (69.5 %), and of these, 59 (17.9 %) were polymicrobial.

The mean age of patients with diagnosed UTIs was 86 ± 8 years (median 86 years), and 235 (71.6 %) were females and 93 (28.4 %) males.

A total of 393 micro-organisms were isolated: as reported in Table 1, the prevalent species was Escherichia coli, followed by Proteus mirabilis, Providencia spp. (mainly Providencia stuartii), Klebsiella spp. and Pseudomonas aeruginosa, whilst enterococci were the most frequent Gram-positive pathogens (Table 1).
Escherichia coli infection was reported more frequently in females than in males; the odds ratio (OR) estimate for females was 1.87 [95% confidence interval (CI) 1.143–3.06; P = 0.0127] and in older people when dichotomized by mean age (86 years) where the OR estimate was 1.54 (95% CI 1.02–2.33; P = 0.0423).

Differences in isolation rates of Escherichia coli, Proteus mirabilis and Gram-positive organisms were observed between females and males. In particular, Escherichia coli was isolated from 49.0% of urine samples in women and 32.7% in men, whilst the frequency of isolation of Proteus mirabilis was higher in men than in women (25.7 vs 18.5%). Gram-positive organisms were more frequently responsible for UTIs in males than in females (14.9 vs 8.9%, respectively) with a notable difference in isolation rates of enterococci (10.9 vs 6.2% in males and females, respectively).

### Antimicrobial resistance

The antibiotic resistance rates of the isolates are summarized in Figs 1–3. Almost all Enterobacteriaceae were susceptible to carbapenems (>97%) and amikacin (93.3%), whilst fluoroquinolones and ampicillin showed a limited activity (Fig. 1). Resistance to imipenem was observed in three isolates of Proteus mirabilis from two different nursing homes and in one isolate of Klebsiella oxytoca. ESBLs were detected in 42.1% of isolates of Escherichia coli, Proteus mirabilis and Klebsiella spp. These bacteria showed more resistance to aminoglycosides, co-trimoxazole and fluoroquinolones compared with ESBL non-producer isolates, with susceptibility rates of 86.5, 35.3 and 14.3% compared with 98.7, 60.9 and 63.8% for amikacin, co-trimoxazole and ciprofloxacin, respectively. ESBL-positive Escherichia coli were generally more resistant to nitrofurantoin and fosfomycin (susceptibility rates of 82.6 and 69.6%) than ESBL-negative isolates (susceptibility rates of 94.3 and 92.4%).

The only isolate of Acinetobacter spp. was found to be resistant to all the tested antibiotics with the exception of the carbapenems. The most active antibiotic against Pseudomonas aeruginosa was colistin, followed by amikacin, piperacillin/tazobactam, cefepime and meropenem (Fig. 2). All Gram-positive isolates were susceptible to glycopeptides and linezolid, and 90% were susceptible to nitrofurantoin, whilst only 10% of staphylococci and <50% of enterococci were susceptible to ciprofloxacin (Fig. 3). β-Lactamase production and meticillin resistance occurred in 60 and 41.7% of staphylococci, respectively.

Four main patterns of antibiotic resistance were recognized according to point aggregation and supplementary points analysis associated with the genus of bacteria isolated. Pattern A comprised isolates resistant to chloramphenicol, fosfomycin and nitrofurantoin; bacteria resistant to gentamicin and trimethoprim/sulfamethoxazole were grouped in pattern B; pattern C was characterized by resistance to ampicillin, levofloxacin, fosfomycin and ciprofloxacin; and

### Table 1. Micro-organisms isolated from urine samples

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Females</th>
<th>Males</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>143</td>
<td>49.0</td>
<td>33</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>54</td>
<td>18.5</td>
<td>26</td>
</tr>
<tr>
<td>Providencia spp.</td>
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<td>9.2</td>
<td>8</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>18</td>
<td>6.2</td>
<td>7</td>
</tr>
<tr>
<td>Other</td>
<td>8</td>
<td>2.7</td>
<td>9</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
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<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>16</td>
<td>5.5</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>8</td>
<td>2.7</td>
<td>4</td>
</tr>
<tr>
<td>Enterococcus spp.</td>
<td>18</td>
<td>6.2</td>
<td>11</td>
</tr>
<tr>
<td>Total</td>
<td>292</td>
<td>100</td>
<td>101</td>
</tr>
</tbody>
</table>

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Fig. 1. Resistance of Enterobacteriaceae to the tested antibiotics. AM, Ampicillin; CF, cephalotin; CTX, cefotaxime; TIM, ticarcillin/clavulanic acid; IMI, imipenem; MEM, meropenem; AN, amikacin; GM, gentamicin; CIP, ciprofloxacin; LVX, levofloxacin; SXT, trimethoprim/sulfamethoxazole; FOS, fosfomycin; F/M, nitrofurantoin (only Escherichia coli).

Fig. 2. Resistance of Pseudomonas aeruginosa to the tested antibiotics. PTZ, Piperacillin/tazobactam; CAZ, ceftazidime; CRO, ceftriaxone; FEP, cefepime; PI, pipemidic acid; CL, colistin. See Fig. 1 for other abbreviations.
Pattern D comprised isolates resistant to amikacin, cefotaxime and ticarcillin/clavulanic acid.

Pattern A was more frequent in patients aged between 86 and 95 years and in females. We did not detect any difference in the prevalence of resistance in cluster B among age groups and bacterial species; however it was more frequent in females (OR 1.8, 95 % CI 1.05–3.09) than in males. Cluster C was prevalent among younger patients (65–75 years; OR 3.306, 95 % CI 1.29–8.49) and in bacterial species other than *Escherichia coli*, with no difference between genders. Resistance cluster D was not differently distributed among age groups, but showed a prevalence in females and was associated with bacterial species different from *Escherichia coli*.

**DISCUSSION**

The initial treatment of nursing-home-acquired infections is often empirical and is based on data derived from historical bacteriology and susceptibility patterns of community-acquired infections, and does not consider that nursing-home-acquired infections may differ substantially from community-acquired ones. Different studies have shown the inappropriate use of antibiotics in nursing homes, especially for the treatment of UTIs, thus underlining the need for guidelines for diagnosis and therapy of such infections (McClean *et al.*, 2011).

For this reason, we analysed data from 14 nursing homes in the north of Italy with the aim of evaluating the most common aetiological agents and their susceptibility to antibiotics.

Our results confirmed the broad range of Gram-negative organisms isolated from the elderly population with UTIs, a finding reflected in other reports (Nicolle, 2002; Shortliffe & McCue, 2002; Das *et al.*, 2009). Nevertheless, UTIs in the institutionalized elderly are caused primarily by *Escherichia coli* both in females and males. The isolation rate of *Escherichia coli* in our population (44.8 %) was somewhat lower than that reported in a recent study by Katsarolis *et al.* (2010) in subjects >65 years, but was similar to that reported by Shortliffe & McCue (2002) and Das *et al.* (2009). This difference might be due to the fact that our study comprised data from residents in long-term facilities and not from community-acquired infections as reported by Katsarolis *et al.* (2010). In the same way, the rate of isolation of *Pseudomonas aeruginosa* was lower than might be expected in these types of subjects, but was similar to that reported by other authors in subjects of a comparable age (Linhares *et al.*, 2013).

Gram-positive organisms were responsible for only ~10 % of the UTIs, with a prevalence of enterococci, particularly *Enterococcus faecalis*, and staphylococci. Although enterococci are often considered to be colonizing agents in the elderly rather than pathogens (Shortliffe & McCue, 2002), in the present study they were mostly isolated as the single pathogen from symptomatic subjects.

Nursing-home patients may be an important reservoir of ESBL-producing multidrug-resistant *Escherichia coli* and *K. pneumoniae* (Wiener *et al.*, 1999; Das *et al.*, 2009; Nicolle, 2012). In our study, resistance to more than one antibiotic was rather common and the spread of ESBL-producing isolates was quite alarming. The resistance rate to fluoroquinolones observed in this study was quite high, particularly in *Enterobacteriaceae*, and poses some concerns about their use in empirical treatment of UTIs. Resistance to fluoroquinolones is known to be associated with the previous use of antibiotics, particularly fluoroquinolones, and previous reports have demonstrated that underlying urinary tract diseases predispose patients to repeated UTIs and, in turn, to exposure to antibiotics such as fluoroquinolones (Miliani *et al.*, 2011; Yasufuku *et al.*, 2011; Smithson *et al.*, 2012). Moreover, the high rate of resistance to fluoroquinolones was associated with ESBL production, as indicated by the marked difference in fluoroquinolone resistance observed between ESBL-producing and -non-producing isolates (63.8 vs 14.3 %).

The observed reduction in susceptibility to fosfomycin in ESBL-positive isolates was often associated with resistance to nitrofurantoin. Fosfomycin has a long history of use, in particular for the treatment of UTIs caused by *Escherichia coli* and *Enterococcus faecalis*, being generally well tolerated with a low incidence of adverse events (Michalopoulos *et al.*, 2011). For these reasons, fosfomycin has attracted renewed interest for the treatment of lower urinary tract and even systemic infections caused by Gram-negative pathogens with resistance to traditionally used agents. Similarly, nitrofurantoin has recently been suggested as an empirical treatment of nosocomial uncomplicated UTIs involving *Escherichia coli* and enterococci, comparing favourably with fluoroquinolones and co-trimoxazole (Mashouf *et al.*, 2009; McKenzie *et al.*, 2011). Our data raise some concerns on the usefulness of fosfomycin in empirical treatment against ESBL-producing *Escherichia*

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**Fig. 3.** Resistance of Gram-positive cocci to the tested antibiotics. Filled bars, *Enterococcus* spp.; open bars, *S. aureus*. P, Penicillin; AMC, amoxicillin/clavulanic acid; SXT, trimethoprim/sulfamethoxazole; VA, vancomycin; TP, teicoplanin; LNZ, linezolid. See Fig. 1 for other abbreviations.
coli – although discordance between the high frequency of mutational resistance to fosfomycin in vitro and the lower extent of this phenomenon in clinical studies has been shown (Karageorgopoulos et al., 2012) – and of nitrofurantoin, also considering the potential therapeutic failure in patients with compromised renal function, which is rather common in the elderly.

In conclusion, we found that bacterial resistance to antibiotics was rather widespread in elderly patients in nursing homes, where some antibiotics widely used to date in the treatment of UTIs have proved to be of little utility in empirical treatment of such infections. From this point of view, the increased resistance to antibacterials suggests that the empirical antibiotic regimen for nursing-home-acquired UTIs may need to be refined according to local data. This highlights the importance of urine culture and targeted therapy, when possible.

REFERENCES


