American Journal of Infection Control 42 (2014) 1033-8

Contents lists available at ScienceDirect

American Journal of Infection Control

journal homepage: www.ajicjournal.org

Major article

Evolution of the resistance to antibiotics of bacteria involved in urinary tract infections: A 7-year surveillance study

Antonio Sorlozano PhD [a], Antonio Jimenez-Pacheco PhD [b], Juan de Dios Luna del Castillo PhD [c], Antonio Sampedro PhD [d], Antonio Martinez-Brocal PhD [d], Consuelo Miranda-Casas PhD [d], José María Navarro-Marí PhD [d], José Gutiérrez-Fernández PhD [a,d,*]

[a] Department of Microbiology, School of Medicine, University of Granada, Granada, Spain
[b] Department of Urology, Santa Ana Hospital, Motril, Spain
[c] Department of Statistics and Operation Research, University of Granada, Granada, Spain
[d] Department of Microbiology, Virgen de las Nieves University Hospital, Granada, Spain

Key Words:
Antimicrobial resistance
Antibiotic susceptibility
Urine analysis
Infection control

Background: We conducted a retrospective analysis on the identification and antibiogram of all bacteria isolated from urine samples with microbiological confirmation of urinary tract infection (UTI) in a Spanish reference hospital over a 7-year period.
Methods: A retrospective analysis was performed of the identification and antibiogram data.
Results: A total of 31,758 uropathogens were isolated. *Escherichia coli* accounted for the majority (55.2%) of these, followed by *Enterococcus faecalis* (18.0%) and *Klebsiella* spp (10.3%). The highest *E coli* susceptibility rates were to imipenem (93.0%-99.8%), amikacin (97.3%-99.5%), nitrofurantoin (96.7%-98.9%), and fosfomycin (95.3%-100%), and the lowest were to cefuroxime (67.8%-86.4%), ciprofloxacin (61.2%-69.8%), and co-trimoxazole (55.0%-65.5%). We highlight the overall high activity of imipenem, piperacillin-tazobactam, nitrofurantoin, and fosfomycin on isolates versus the low activity of fluoroquinolones, co-trimoxazole, or cephalosporins. The activity of amoxicillin-clavulanic acid and fosfomycin decreased significantly over the 7-year study period.
Conclusions: Imipenem and piperacillin-tazobactam appear to be good options for the empiric treatment of UTI acquired in hospital or requiring hospitalization, whereas nitrofurantoin and fosfomycin can be first-choice antibiotics for the treatment of uncomplicated community-acquired cystitis. However, surveillance studies are required to detect resistance to these antibiotics, given that an increase in uropathogen resistance rates may contraindicate its future use in empiric UTI therapy.

Urinary tract infections (UTIs) are caused by a limited number of bacterial species, more than 95% of which are monomicrobial. *Escherichia coli* is the most frequently implicated uropathogen reported by virtually all epidemiologic studies worldwide. Other pathogens of the genera *Enterococcus*, *Klebsiella*, *Enterobacter*, *Proteus*, *Morganella*, *Citrobacter*, *Serratia*, *Pseudomonas*, *Streptococcus*, and *Staphylococcus*, and fungi, such as *Candida* spp, are also isolated with variable frequency.[1]

The high incidence of UTIs and their usually mild character call for empiric antibiotic treatment in most cases.[2] Providing rational empiric treatment requires identifying the microorganisms involved and establishing their antibiotic susceptibility patterns to the largest possible number of agents; this is especially important for *E coli*, the most frequently isolated uropathogen.[1,3]

The objectives of the present study were to identify the bacteria most frequently responsible for UTIs in our area and their susceptibility profiles, using a standardized work procedure, and to evaluate the activity on all isolates of antibiotics widely used for the treatment of community- and hospital-acquired UTIs.

MATERIALS AND METHODS

A retrospective analysis was performed of the identification and antibiogram data for all consecutive bacteria isolated from urine samples with microbiological confirmation of UTI at the

* Address correspondence to Dr José Gutiérrez-Fernández, PhD, Laboratorio de Microbiología, Av de las Fuerzas Armadas 2, 18014 Granada, Spain.
E-mail address: josegf@ugr.es (J. Gutiérrez-Fernández).
Conflict of interest: None to report.

0196-6553/$36.00 - Copyright © 2014 by the Association for Professionals in Infection Control and Epidemiology, Inc. Published by Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.ajic.2014.06.013

Microbiology Department of Virgen de las Nieves Hospital Complex, Granada, in southern Spain, between January 2006 and December 2012.

All urine cultures were analyzed by calibrated loop seeding in usual media. A count $\geq 10^5$ colony-forming units (CFU)/mL was considered to indicate significant bacteriuria. A count of $>10^4$ CFU/mL of a single microorganism was considered positive in the presence of >40 leukocytes/μL in noncentrifuged urine. Urine cultures with growth of more than 2 microorganisms were considered contaminated. The WIDER system (Francisco Soria Melguizo, Madrid, Spain) was used for identification and antibiogram profiling of all bacteria from positive urine cultures up to March 2012, when it was replaced with the MicroScan system (Siemens Healthcare Diagnostics, Spain). Duplicate positive urine cultures (ie, of the same genus or species obtained sequentially from the same UTI episode) were excluded. Furthermore, if a subject contributed more than 1 urine sample, regardless of the origin (community or hospital), then an interval of at least 30 days had to pass before the second sample was considered a significant finding and included in the report. This was done to avoid duplicate isolates from a single UTI episode.

Amikacin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, cefuroxime, ciprofloxacin, fosfomycin, gentamicin, imipenem, nitrofurantoin, piperacillin-tazobactam, tobramycin, and co-trimoxazole were tested against Enterobacteriaceae. The presence of extended-spectrum beta-lactamase (ESBL) producers among enterobacteria was considered only from January 2010, when the Clinical and Laboratory Standards Institute (CLSI) modified the susceptibility cutoff points to cephalosporins. Amikacin, cefepime, ceftazidime, ciprofloxacin, gentamicin, imipenem, piperacillin-tazobactam, tobramycin, and co-trimoxazole (not for *Pseudomonas* spp) were tested against *Pseudomonas* spp and *Acinetobacter* spp. Ampicillin, levofloxacin, fosfomycin, nitrofurantoin, and co-trimoxazole (only against *Streptococcus agalactiae*) were tested against *Enterococcus* spp and *S agalactiae*; and amikacin, levofloxacin, fosfomycin, gentamicin, nitrofurantoin, oxacillin, tobramycin, and co-trimoxazole against *Staphylococcus* spp.

Isolates were classified as susceptible, intermediate, or resistant to each antibiotic according to CLSI recommendations.[4] The clinical categorization of all isolates against nitrofurantoin and of *S agalactiae* against co-trimoxazole followed the recommendations of the European Committee on Antimicrobial Susceptibility Testing.[5] For each study year and each bacterial species identified in ≥5 isolates/year, the proportion of susceptible organisms was calculated by dividing the number of urinary isolates susceptible to each antibiotic by the number of organisms tested against that antimicrobial agent. (Intermediately resistant and resistant organisms were grouped together.)

The activity of each antibiotic was evaluated on all bacteria isolated during the study period. The following assumptions were made: (1) Each of the aforementioned antibiotics is potentially active against enterobacteria; (2) fosfomycin, nitrofurantoin, amoxicillin-clavulanic acid, cefotaxime, and cefuroxime have no activity against nonfermenting gram-negative bacilli, and *Pseudomonas aeruginosa* is intrinsically resistant to co-trimoxazole; (3) among staphylococci, oxacillin predicts the response to all beta-lactam antibiotics indicated for ITUs; (4) among enterococci, ampicillin predicts the response to amoxicillin-clavulanic acid, piperacillin-tazobactam, and imipenem, and cefepime, cefotaxime, ceftazidime, cefuroxime, amikacin, gentamicin, tobramycin, and co-trimoxazole are not clinically active against these microorganisms[6]; (5) fluoroquinolone activity can be determined from the activity of ciprofloxacin on gram-negative bacilli and of levofloxacin on gram-positive cocci; (6) the activity of fosfomycin on enterobacteria and gram-positive cocci can be assessed using the cutoff points recommended by the CLSI for this antibiotic against *E coli* and *Enterococcus faecalis*, respectively[7,8]; and (7) the activity of nitrofurantoin on enterobacteria can be assessed using the cutoff points recommended by the European Committee on Antimicrobial Susceptibility Testing for this antibiotic in *E coli.* Data for *S agalactiae* were excluded owing to the absence of antibiograms for this microorganism between 2006 and 2010.

A descriptive statistical analysis was performed, and differences in susceptibility rates were analyzed using Pearson's χ^2 test and contingency tables with Fisher's exact test. SPSS version 18.0 (SPSS, Chicago, IL) was used for all analyses. A P value <.05 was considered significant for all tests.

RESULTS

The 31,758 bacteria identified included 24,813 (78.1%) gram-negative bacilli and 6945 (21.9%) gram-positive cocci. Over the 7-year study period, there was no significant change in the frequency of the types of bacteria identified, with *E coli* the most frequently identified UTI agent in each year (55.2% of all isolates; range, 50.1%-59.4%) in both community (55.6%; range, 51.5%-59.4%) and hospital (54.2%; range, 50.1%-57.4%) isolates, followed by *E faecalis* (18.0%; range, 14.7%-22.1%) and *Klebsiella* spp (10.3%; range, 7.2%-12.9%). Together, other bacteria (*Proteus mirabilis*, *Enterobacter* spp, *Morganella morganii*, *Citrobacter koseri*, *Citrobacter freundii*, *P aeruginosa*, *Acinobacter baumannii*, *Staphylococcus aureus*, *Staphylococcus saprophyticus* , and *S agalactiae*) accounted for only 15.3% of commuity isolates and 16.8% of hospital isolates.

Antibiotic susceptibility of the isolated bacteria

E coli showed high susceptibility to imipenem, amikacin, fosfomycin, and nitrofurantoin, with annual resistance rates <5%, whereas resistance to cefuroxime, ciprofloxacin, and co-trimoxazole, key antibiotics in the treatment of community-acquired UTIs, was recorded in >20%-30% of isolates in most years. Compared with community isolates, hospital isolates of *E coli* species were significantly more resistant to amoxicillin-clavulanic acid ($P < .001$), cefepime ($P < .001$), cefotaxime ($P < .001$), ceftazidime ($P = .011$), and piperacillin-tazobactam ($P = .003$), but significantly more susceptible to cefuroxime ($P < .001$), fosfomycin ($P < .001$), and nitrofurantoin ($P = .019$). There were no significant differences between the community and hospital isolates for imipenem ($P = .520$), ciprofloxacin ($P = .152$), amikacin ($P = .054$), gentamicin ($P = .829$), tobramycin ($P = .344$), or co-trimoxazole ($P = .219$).

In 2010, the prevalence of ESBL-producing *E coli* among the community and hospital isolates was 7.5% and 5.0%, respectively. In 2011, these respective prevalences were 7.4% and 9.8%, and in 2012, they were 8.6% and 10.8%. This increase in prevalence was especially marked in isolates of hospital origin. Considered together, these bacteria had high susceptibility rates to imipenem (98%-100% susceptible isolates), piperacillin-tazobactam (87%-94%), amikacin (92%-100%), fosfomycin (88%-93%), and nitrofurantoin (93%-98%), but much lower susceptibility rates to amoxicillin-clavulanic acid (52%-68%), ciprofloxacin (13%-24%), and co-trimoxazole (39%-52%).

The community isolates of *Klebsiella pneumoniae* and *Klebsiella oxytoca* species were highly susceptible, whereas the hospital isolates were significantly more resistant to all antibiotics tested ($P \leq .001$ in all cases) except fosfomycin ($P = .872$) and imipenem ($P = .722$). The respective prevalences of ESBL-producing *Klebsiella* spp among community and hospital isolates were 4.4% and 8.6%, respectively, in 2010, 5.9% and 31.8% in 2011, and 6.6% and 7.3% in 2012. Overall, these bacteria showed high susceptibility rates only to imipenem (91%-100% susceptible isolates) and amikacin (66%-100%). Susceptibility was lower to other antibiotics, including