Poster No.

P0077

Multidrug resistance to oral antibiotics in *Escherichia coli* urine isolates from outpatients in Germany and *in vitro* activity of nitroxoline: results of the PEG 2013 study

26th ECCMID
Amsterdam, Netherlands
9–12 April 2016

Michael Kresken[1,2*], Barbara Körber-Irrgang[1], Dieter Hafner[3], and Working Party "Antimicrobial Resistance" of the Paul-Ehrlich-Society for Chemotherapy[§]

[1] Antiinfectives Intelligence GmbH, Rheinbach, Germany; [2] University of Applied Sciences, Cologne, Germany; [3] Institute of Pharmacology and Clinical Pharmacology, Heinrich-Heine-University, Düsseldorf, Germany

*Contact information (presenting author)

Michael Kresken, PhD
Antiinfectives Intelligence GmbH
Campus of the University of Applied Sciences
Von-Liebig-Straße 20
53359 Rheinbach
Germany

Telefon: + 49-2226-908-912
Fax: +49-2226-908-918

E-mail: michael.kresken@antiinfectives-intelligence.de

Background

Escherichia coli (ECO) is the leading pathogen responsible for community-acquired urinary tract infections (UTI) (1). Resistance to a variety of orally administered antibiotics in ECO has complicated the management of UTIs in the community setting (2-4). Nitroxoline exhibits activity against a broad spectrum of bacteria including ECO (5, 6). The use of nitroxoline for first-line therapy of uncomplicated UTI has attracted attention as there is a lack of cross resistance between nitroxoline and other classes of antibiotics (7, 8). The objectives of this study were i) to evaluate the occurrence of multidrug-resistant (MDR) strains among uropathogenic ECO from patients in outpatient departments and ii) to determine the susceptibility of these isolates to nitroxoline.

Material/methods

In a surveillance study conducted by the Paul-Ehrlich-Society (PEG) in 2013, 25 laboratories across Germany were requested each to collect 20 non-duplicate ECO urine isolates. Isolates were identified by MALDI-TOF. MICs were determined by the broth microdilution procedure (BMD) according to the standard ISO 20776-1 at a central laboratory (9). Breakpoints approved by the German breakpoint committee NAK (nitroxoline) or EUCAST (all other drugs) were applied (10, 11). MDR was defined as resistance to at least three of the following eight antibacterial drug classes/subclasses: penicillins (amoxicillin), penicillin + β-lactamase inhibitors (amoxicillin/clavulanic acid), second-generation cephalosporins (2GC – cefuroxime), third-generation cephalosporins (3GC – cefixime, cefpodoxime), fluoroquinolones (FQ – ciprofloxacin), folate pathway inhibitors (trimethoprim/sulfamethoxazole), phosphonic acids (fosfomycin), and nitrofurans (nitrofurantoin), which are hereafter referred to as 'standard' drugs. Isolates with resistance to cefpodoxime were tested for ESBL production according to the BMD procedure described by the CLSI (12). PCR methods used for molecular detection of ESBLs and isolates belonging to the clonal group O25b-ST131 have been described elsewhere (13).

Results

494 isolates were collected. Isolates were obtained from 431 (87.2%) female and 63 (12.8%) male patients. Patients ranged in age from <1 to 100 (median 61) years. $MIC_{50/90}$ values as well as the susceptibility and resistance rates obtained with the 'standard' drugs are shown in **Table 1**. 261 (52.8%) isolates did not show resistance to any 'standard' drug class/subclass, 37 (7.5%) were resistant to one drug class/subclass, 67 (13.6%) to two drug classes/subclasses and 129 isolates (26.1%) met the criterion of MDR **(Table 2)**.

An ESBL phenotype was confirmed for 23 isolates (4.7%) **(Table 2)**. The most prevalent ESBL type was CTX-M-15 (n=9), followed by CTX-M-1 (n=6), CTX-M-14 (n=4) and CTX-M-27 (n=3). One isolate did not possess a CTX-M, SHV or TEM ESBL. Four isolates producing CTX-M-15 and two isolates each of which produced CTX-M-14 and CTX-M-27 belonged to clonal group O25b-H4-ST131 known to possess numerous virulence factors (14).

Resistance to FQ (ciprofloxacin) and folate pathway inhibitors (trimethoprim/sulfamethoxazole) was found in 22.2% and 23.8% of the isolates from men, 10.3% and 21.6% from pre-menopausal (18–45 years) women as well as in 17.9% and 25%, respectively, from post-menopausal (>45 years) women, whilst rates of ESBL producing isolates were fairly similar between the three patient groups **(Table 3)**.

Nitroxoline was tested against a subset of 263 isolates comprising all isolates from female patients aged 18–45 years as well as the resistant isolates from the remaining patient groups. Nitroxoline MICs showed a normal (Gaussian) distribution, with $MIC_{50/90}$ values of 2/4 mg/L **(Figure)**. All tested isolates were nitroxoline-susceptible **(Tables 1 and 3)**.

Conclusions

In comparing the results from the present surveillance with those from the previous study conducted in 2010 (13), resistance to second and third-generation cephalosporins as well as ciprofloxacin and trimethoprim/sulfamethoxazole decreased in urinary ECO from outpatient departments in Germany. Nitroxoline showed promising *in vitro* activity against ECO isolates irrespective of patients' age and resistance phenotype.

§ Members of the Working Party

The list of members is shown on the website of the Paul-Ehrlich-Society for Chemotherapy (http://www.p-e-g.org/econtext/resistenzdaten).

References

1. Stamm WE and Norrby SR, 2001, J Infect Dis, 183 (Suppl 1):1-4.
2. Kahlmeter G and Poulsen HO. 2012. Int. J. Antimicrob. Agents. 39: 45-51.
3. Lee SJ et al. 2011. J. Infect. Chemother. 17: 440-446.
4. Maraki S et al. 2013. J Microbiol. Immunol. 46:202-209.
5. Fachinformation Nitroxolin forte 250 mg.
6. Jacobs MR et al. 1978. S Afr Med J. 54:959-962.
7. Naber KG et al. 2014. BMC Infect Dis. 14:628. doi: 10.1186/s12879-014-0628-7.
8. Kresken M and Körber-Irrgang B. 2014. Antimicrob Agents Chemother. 58:7019-20.
9. ISO 20776-1: 2006. German version EN ISO 20776-1:2006. Beuth-Verlag, Berlin.
10. Nationales-Antibiotika-Sensitivitätstest-Komitee (NAK). 2016. Nitroxoline rationale v 1.2.
11. European Committee on Antimicrobial Susceptibility Testing (EUCAST). 2015. Breakpoint table v 6.0.
12. Clinical and Laboratory Standards Institute (CLSI). 2015. CLSI document M100-S25. Wayne, PA, USA.
13. Kresken M et al. 2014. Int J Antimicrob Agents. 44:295-300.
14. Petty NK et al. 2014. Proc Natl Acad Sci U S A. 111:5694-9.

Table 1: *In vitro* activity of ten antibiotics against urinary *E. coli* isolates (n=494)

Antibiotic	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Percent of isolates		
			S	I	R
Amoxicillin	4	≥ 64	57.3	–	42.7
Amoxicillin/ clavulanic acid[a)]	4	32	65.0 / 90.7[c)]	–	35.0 / 9.3[c)]
Cefuroxime	4	8	93.3	–	6.7
Cefixime	0.25	0.5	93.9	–	6.1
Cefpodoxime	0.25	0.5	94.1	–	5.9
Ciprofloxacin	≤ 0.063	≥ 16	83.6	0.4	16.0
Trimethoprim/ sulfamethoxazole[b)]	≤ 0.25	≥ 32	75.3	1.0	23.7
Fosfomycin	≤ 1	4	99.2	–	0.8
Nitrofurantoin	≤ 16	32	99.0	–	1.0
Nitroxoline[d)]	2	4	100	–	0

S, susceptible; I, intermediate; R, resistant; [a)] Concentration of clavulanic acid was constant 4 mg/L; [b)] Trimethoprim/sulfamethoxazole ratio was 1:19, MIC values refer to the trimethoprim concentration; [c)] Susceptibility and resistance rate if breakpoint for uncomplicated UTI was applied (S: ≤ 32, R > 32); [d)] Nitroxoline was tested against 263 isolates.

Table 2: Prevalence of resistance to eight antibacterial drug classes/ subclasses[a)] among *E. coli* isolates (n=494)

Phenotype / resistance phenotype	Percent (n)
Isolates with no resistance	52.8 (261)
Resistance to 1 drug class/subclass	7.5 (37)
Resistance to 2 drug classes/subclasses	13.6 (67)
MDR[b)]	26.1 (129)
Resistance to 3 drug classes/subclasses	14.8 (73)
Resistance to 4 drug classes/subclasses	6.3 (31)
Resistance to 5 drug classes/subclasses	2.6 (13)
Resistance to 6 drug classes/subclasses	2.2 (11)
Resistance to 7 or 8 drug classes/subclasses	0.2 (1)
ESBL-positive	4.7 (23)
E. coli 025b-H4-ST131	0.8 (4)

[a)] Penicillins (amoxicillin), Penicillin + β-lactamase inhibitors (amoxicillin/clavulanic acid), 2GC (cefuroxime), 3GC (cefixime and/or cefpodoxime), FQ (ciprofloxacin), folat pathway inhibitors (trimethoprim/sulfamethoxazole), phosphonic acids (fosfomycin) and nitrofurans (nitrofurantoin)
[b)] MDR was defined as resistance to at least three drug classes/subclasses.

Disclosures

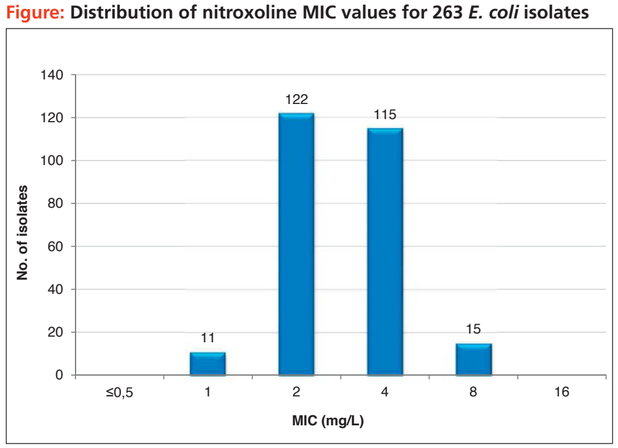
MK is a partner and CEO of Antiinfectives Intelligence GmbH (AI), a research organization providing services to pharmaceutical companies. BK-I is head of laboratory of AI. DH declares no competing interests.

Table 3: Prevalence (%) of drug resistance among *E. coli* isolates (n=494) of different subgroups of patients

Antibiotic	Patient subgroup (n)			
	Men (63)	Women total (431)	Women 18–45 years (97)	Women > 45 years (268)
Amoxicillin	39.7	43.2	34.0	46.3
Amoxicillin/ clavulanic acid[a)]	33.3/ 11.1[c)]	35.3/ 9.0[c)]	25.8/ 5.2[c)]	39.9/ 10.8[c)]
Cefuroxime	9.5	6.3	4.1	7.1
Cefixime	7.9	5.8	4.1	6.7
Cefpodoxime	7.9	5.6	3.1	6.7
Ciprofloxacin	22.2	15.1	10.3	17.9
Trimethoprim/ sulfamethoxazole[b)]	23.8	23.7	21.6	25.0
Fosfomycin	0	0.9	1.0	1.1
Nitrofurantoin	3.2	0.7	0	1.1
Nitroxoline	0[d)]	0[e)]	0	0[f)]

[a)] Concentration of clavulanic acid was constant 4 mg/L; [b)] Trimethoprim/sulfamethoxazole ratio was 1:19, MIC values refer to the trimethoprim concentration; [c)] Susceptibility and resistance rate if breakpoint for uncomplicated UTI was applied (S: ≤ 32, R > 32); [d)] Nitroxoline was tested against 31 isolates; [e)] Nitroxoline was tested against 232 isolates; [f)] Nitroxoline was tested against 135 isolates.

Figure: Distribution of nitroxoline MIC values for 263 *E. coli* isolates



Acknowledgements

The authors are grateful to Rosen Pharma GmbH for supporting the study by a grant. We would particularly like to thank Yvonne Pfeifer (Robert Koch-Institute, Wernigerode, Germany) for her active support in the molecular characterization of the ESBLs.