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Susceptibility of Gram-negative pathogens isolated from patients with community-acquired urinary tract infections in three European Countries

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Introduction and Purpose

Ceftibuten, an oral third-generation cephalosporin (3GC) licensed in the 1990ies, has been shown to possess *in vitro* activity against Gram-positive and Gram-negative pathogens including *Escherichia coli* and other Enterobacteriaceae [1-3]. Since the introduction of ceftibuten into the market, increased rates of acquired resistance to a variety of antibiotics (including 3GC) have complicated the management of community-acquired UTI (CA-UTI) [4]. It is therefore of interest to investigate the current prevalence of resistance to ceftibuten among Gram-negative pathogens causing CA-UTI.

As the resistance epidemiology may differ between regions and countries, the study aimed to investigate the occurrence of resistant isolates in three European countries with the focus on *E. coli, Klebsiella pneumoniae* and *Proteus mirabilis* isolates.

Methods

Six laboratories, two each in Belgium, Germany and Spain, participated in the study. Each site was requested to collect 200 clinical isolates. These were 90 *E. coli*, 40 *Klebsiella* spp., 40 *Proteus* spp., and 30 other Enterobacteriaceae. Only first isolates obtained from patients with CA-UTI were included.

Susceptibility of the strains for a panel of antibiotics was determined in a central laboratory (Antiinfectives Intelligence) according to the international standard ISO 20776-1 by the help of industrially manufactured trays (Micronaut-S ES-AII-UTI; Merlin Diagnostika GmbH, Bornheim-Hersel, Germany), [5]. Minimal inhibitory concentrations (MIC) were interpreted by EUCAST criteria (v 5.0) [6]. ESBL-producing *E. coli* were confirmed according to the broth dilution procedure as described by the CLSI [7]. Statistical significance of differences in resistance rates was judged by comparing 95%, 99% and 99.9% confidence intervals (CI) using the Newcombe-Wilson method without continuity correction. If neither rate was contained in the CI of the other one, corresponding significance was assumed.

Results

From August 2013 to January 2014 a total of 1,190 isolates were collected, including 538 *E. coli*, 234 *P. mirabilis* and 196 *K. pneumoniae*. 96% of all isolates were obtained from patients in ambulatory care. Patients ranged in age from <1–96 years (median 64 years). 937 (78.7%) isolates were obtained from females.

Highest resistance rates in *E. coli* were observed for amoxicillin-clavulanic acid (46.8%; 28.1% if the breakpoint for uncomplicated UTI was applied), ciprofloxacin (23.4%) and trimethoprim-sulfamethoxazole (21.4%), while resistance to fosfomycin and nitrofurantoin was rare (<1.5%). Resistance to cephalosporins varied between 6.3% (ceftibuten) and 11.7% (cefuroxime oral). Based on $MIC_{50/90}$ values, ceftibuten (≤0.25/0.5 mg/L) showed comparable activity to ceftriaxone ($MIC_{50/90}$ ≤0.25/≤0.25 mg/L), (**Table 1**). The cephalosporins, including ceftibuten (MIC_{90} ≤0.25 mg/L), provided also good activity against *P. mirabilis* (n=234) and *K. pneumoniae* (n=196).

7.1%, 5.6% and 0.4% of the *E. coli, K. pneumoniae* and *P. mirabilis* isolates, respectively, showed an ESBL phenotype (**Table 1**). *E. coli* isolates with an ESBL phenotype were more common at the study sites in Spain (9.4%) and Germany (8.9%) than in Belgium (2.8%), while ESBL-producing isolates of *K. pneumoniae* were more widely disseminated in Belgium (8.6%) than in Germany (4.4%) and Spain (4.3%), respectively. All ESBL-positive *E. coli* isolates were susceptible to nitrofurantoin and 92.1% were susceptible to fosfomycin, whereas resistance rates of 47.4–100% were recorded for the remaining drugs. Highest susceptibility rates in ESBL-producing isolates of *K. pneumoniae* were determined for fosfomycin, followed by trimethoprim-sulfamethoxazole and ciprofloxacin.

In general, resistance was most common in *E. coli* isolates from men and elderly women, and ESBL-producers were more frequently found among isolates from men (17.4%) than women (5.5%), (**Table 2**). Resistance to ciprofloxacin in *E. coli* isolates from women gradually increased with age. In all age groups, resistance in *E. coli* to ceftibuten (3.5-6.6%) was less frequent than resistance to the other cephalosporins tested.

Resistance to cephalosporins, ciprofloxacin and trimethoprim-sulfamethoxazole in *E. coli* were lowest among isolates from Belgium (**Table 3**), while geographical variations in the prevalence of resistance among *K. pneumoniae* were noted only for fosfomycin (Spain: 50%; Belgium: 25.9%; Germany: 22.1%). Resistance to ciprofloxacin in *P. mirabilis* was more frequently distributed among isolates from Belgium (18.1%) than Germany (12.7%) or Spain (16.3%).

Conclusions

- **Resistance to oral antibiotics that are commonly used as first-line treatment of UTI in the ambulatory sector seems to be widespread in Gram-negative pathogens from patients with CA-UTI.**
- **However, regional differences as well as variations in the prevalence of resistance between patient subgroups must be considered when empirical treatment of UTI is prescribed.**
- **Ceftibuten retained excellent activity against *E. coli, K. pneumoniae* and *P. mirabilis* isolates with resistance rates below 7%.**

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Conflict of Interests

MK is a partner and CEO of Antiinfectives Intelligence GmbH, a research organization providing services to pharmaceutical companies and BK-I is an employee of Antiinfectives Intelligence GmbH. HS is supported by research grants from Basilea, Novartis and Pfizer, has received speaking fees from Bayer, Gilead, Novartis, Oxoid, and Pfizer, and is a consultant to Astellas, Astra-Zeneca, and Novartis. AP has received speaking fees from Astra-Zeneca, Merck, and Novartis. RC has participated in educational programs sponsored by MSD and Novartis. All other authors declare there is no conflict of interest in this investigation.

Table 1: In vitro activity of oral antibiotics against *E. coli, K. pneumoniae* and *P. mirabilis* isolates

Organism / phenotype (no. tested)	Drug	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Proportion of isolates (%)		
				S	I	R
E. coli, all isolates (538)	Amoxicillin-clavulanic acid	4	≥128	53.2 / 71.9[a]	–	46.8 / 28.1[a]
	Cefuroxime oral	4	16	88.3	–	11.7
	Cefixime	≤0.25	1	90.9	–	9.1
	Cefpodoxime	≤0.25	1	90.5	–	9.5
	Ceftibuten	≤0.25	0.5	93.7	–	6.3
	Ceftriaxone	≤0.25	≤0.25	92.2	0	7.8
	Ciprofloxacin	≤0.063	≥16	76.4	0.2	23.4
	Fosfomycin	2	4	98.7	–	1.3
	Nitrofurantoin	16	32	99.6	–	0.4
	Trimethoprim-sulfamethoxazole	≤0.125	≥16	78.1	0.6	21.4
E. coli, non-ESBL phenotype (500)	Amoxicillin-clavulanic acid	4	≥128	56.2 / 75.0[a]	–	43.8 / 25.0[a]
	Cefuroxime oral	4	8	95.0	–	5.0
	Cefixime	≤0.25	0.5	97.4	–	2.6
	Cefpodoxime	≤0.25	0.5	97.4	–	2.6
	Ceftibuten	≤0.25	0.5	97.8	–	2.2
	Ceftriaxone	≤0.25	≤0.25	99.2	0	0.8
	Ciprofloxacin	≤0.063	≥16	80.4	0.2	19.4
	Fosfomycin	2	4	99.2	–	0.8
	Nitrofurantoin	16	32	99.6	–	0.4
	Trimethoprim-sulfamethoxazole	≤0.125	≥16	80.4	0.2	19.4
E. coli, ESBL phenotype (38)	Amoxicillin-clavulanic acid	64	≥128	13.2 / 31.6[a]	–	86.8 / 68.4[a]
	Cefuroxime oral	≥64	≥64	0	–	100
	Cefixime	16	≥64	5.3	–	94.7
	Cefpodoxime	≥64	≥64	0	–	100
	Ceftibuten	2	16	39.5	–	60.5
	Ceftriaxone	≥64	≥64	0	0	100
	Ciprofloxacin	≥16	≥16	23.7	0	76.3
	Fosfomycin	2	32	92.1	–	7.9
	Nitrofurantoin	16	32	100	–	0
	Trimethoprim-sulfamethoxazole	4	≥16	47.4	5.3	47.4
K. pneumoniae, all isolates (196)	Amoxicillin-clavulanic acid	2	≥128	75.5 / 82.1[a]	–	24.5 / 17.9[a]
	Cefuroxime oral	2	8	90.8	–	9.2
	Cefixime	≤0.25	≤0.25	94.4	–	5.6
	Cefpodoxime	≤0.25	0.5	94.4	–	5.6
	Ceftibuten	≤0.25	≤0.25	94.4	–	5.6
	Ceftriaxone	≤0.25	≤0.25	94.4	0	5.6
	Ciprofloxacin	≤0.063	0.5	91.8	1.5	6.6
	Fosfomycin	32	128	66.8	–	33.2
	Nitrofurantoin	64	128	–	–	–
	Trimethoprim-sulfamethoxazole	≤0.125	2	90.3	0.5	9.2
K. pneumoniae, non-ESBL phenotype (185)	Amoxicillin-clavulanic acid	2	64	80.0 / 86.5[a]	–	20.0 / 13.5[a]
	Cefuroxime oral	2	4	96.2	–	3.8
	Cefixime	≤0.25	≤0.25	100	–	0
	Cefpodoxime	≤0.25	≤0.25	100	–	0
	Ceftibuten	≤0.25	≤0.25	100	–	0
	Ceftriaxone	≤0.25	≤0.25	100	0	0
	Ciprofloxacin	≤0.063	0.25	95.1	1.1	3.8
	Fosfomycin	32	128	67.0	–	33.0
	Nitrofurantoin	64	128	–	–	–
	Trimethoprim-sulfamethoxazole	≤0.125	1	93.0	0.5	6.5
K. pneumoniae, ESBL phenotype (11)	Amoxicillin-clavulanic acid	≥128	≥128	0 / 9.1[a]	–	100 / 90.9[a]
	Cefuroxime oral	≥64	≥64	0	–	100
	Cefixime	≥64	≥64	0	–	100
	Cefpodoxime	≥64	≥64	0	–	100
	Ceftibuten	4	16	0	–	100
	Ceftriaxone	≥64	≥64	0	0	100
	Ciprofloxacin	2	≥16	36.4	9.1	54.5
	Fosfomycin	32	64	63.6	–	36.4
	Nitrofurantoin	128	128	–	–	–
	Trimethoprim-sulfamethoxazole	≥16	≥16	45.5	0	54.5
P. mirabilis, all isolates* (234)	Amoxicillin-clavulanic acid	1	64	79.1 / 85.0[a]	–	20.9 / 15.0[a]
	Cefuroxime oral	1	2	97.4	–	2.6
	Cefixime	≤0.25	≤0.25	97.9	–	2.1
	Cefpodoxime	≤0.25	≤0.25	97.4	–	2.6
	Ceftibuten	≤0.25	≤0.25	97.9	–	2.1
	Ceftriaxone	≤0.25	≤0.25	99.1	0.4	0.4
	Ciprofloxacin	≤0.063	4	72.6	11.5	15.8
	Fosfomycin	8	≥256	73.9	–	26.1
	Nitrofurantoin	128	128	–	–	–
	Trimethoprim-sulfamethoxazole	0.25	≥16	65.0	4.7	30.3

ESBL, extended-spectrum-β-lactamase; S, susceptible; I, intermediate; R, resistant; *Including one isolate showing the ESBL phenotype;
[a]Rates of susceptible or resistant isolates if the EUCAST breakpoint for uncomplicated UTI was applied

Table 2: In vitro activity of oral antibiotics against *E. coli* isolates (n=538) in different subgroups of patients

Patient group (no. of isolates)	Drug	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Proportion of isolates (%)		
				S	I	R
Men (69)	Amoxicillin-clavulanic acid	16	≥128	37.7 / 66.7[a]	–	62.3 / 33.3[a]
	Cefuroxime oral	4	≥64	73.9	–	26.1
	Cefixime	0.5	32	76.8	–	23.2
	Cefpodoxime	0.5	≥64	76.8	–	23.2
	Ceftibuten	≤0.25	4	84.1	–	15.9
	Ceftriaxone	≤0.25	≥64	81.2	0	18.8
	Ciprofloxacin	0.25	≥16	63.8	0	36.2
	Fosfomycin	2	8	97.1	–	2.9
	Nitrofurantoin	16	32	100	–	0
	Trimethoprim-sulfamethoxazole	≤0.125	≥16	66.7	2.9	30.4
	No. of isolates with an ESBL phenotype	12 (17.4%)				
Women (469)	Amoxicillin-clavulanic acid	4	≥128	55.4 / 72.7[a]	–	44.6 / 27.3[a]
	Cefuroxime oral	4	8	90.4	–	9.6
	Cefixime	≤0.25	0,5	93.0	–	7.0
	Cefpodoxime	≤0.25	1	92.5	–	7.5
	Ceftibuten	≤0.25	0.5	95.1	–	4.9
	Ceftriaxone	≤0.25	≤0.25	93.8	0	6.2
	Ciprofloxacin	≤0.063	≥16	78.3	0.2	21.5
	Fosfomycin	2	4	98.9	–	1.1
	Nitrofurantoin	16	32	99.6	–	0.4
	Trimethoprim-sulfamethoxazole	≤0.125	≥16	79.7	0.2	20.0
	No. of isolates with an ESBL phenotype	26 (5.5%)				
Women aged <18 years (58)	Amoxicillin-clavulanic acid	4	≥128	62.1 / 77.6[a]	–	37.9 / 22.4[a]
	Cefuroxime oral	4	8	91.4	–	8.6
	Cefixime	≤0.25	0.5	93.1	–	6.9
	Cefpodoxime	≤0.25	0.5	93.1	–	6.9
	Ceftibuten	≤0.25	0.5	94.8	–	5.2
	Ceftriaxone	≤0.25	≤0.25	93.1	0	6.9
	Ciprofloxacin	≤0.063	0.25	94.8	0	5.2
	Fosfomycin	≤1	2	100	–	0
	Nitrofurantoin	16	32	100	–	0
	Trimethoprim-sulfamethoxazole	≤0.125	≥16	86.2	0	13.8
	No. of isolates with an ESBL phenotype	4 (6.9%)				
Women aged 18-65 years (229)	Amoxicillin-clavulanic acid	4	≥128	59.0 / 76.9[a]	–	41.0 / 23.1[a]
	Cefuroxime oral	4	8	93.4	–	6.6
	Cefixime	≤0.25	0.5	95.2	–	4.8
	Cefpodoxime	≤0.25	0.5	94.8	–	5.2
	Ceftibuten	≤0.25	0.5	96.5	–	3.5
	Ceftriaxone	≤0.25	≤0.25	96.1	0	3.9
	Ciprofloxacin	≤0.063	8	86.9	0	13.1
	Fosfomycin	2	4	98.7	–	1.3
	Nitrofurantoin	16	32	99.1	–	0.9
	Trimethoprim-sulfamethoxazole	≤0.125	≥16	86.0	0	14.0
	No. of isolates with an ESBL phenotype	9 (3.9%)				
Women aged >65 years (182)	Amoxicillin-clavulanic acid	16	≥128	48.9 / 65.9[a]	–	51.1 / 34.1[a]
	Cefuroxime oral	4	16	86.3	–	13.7
	Cefixime	≤0.25	1	90.1	–	9.9
	Cefpodoxime	≤0.25	2	89.6	–	10.4
	Ceftibuten	≤0.25	1	93.4	–	6.6
	Ceftriaxone	≤0.25	≤0.25	91.2	0	8.8
	Ciprofloxacin	≤0.063	≥16	62.1	0.5	37.4
	Fosfomycin	2	4	98.9	–	1.1
	Nitrofurantoin	16	32	100	–	0
	Trimethoprim-sulfamethoxazole	≤0.125	≥16	69.8	0.5	29.7
	No. of isolates with an ESBL phenotype	13 (7.1%)				

S, susceptible; I, intermediate; R, resistant; [a]Rates of susceptible or resistant isolates if the EUCAST breakpoint for uncomplicated UTI was applied

Table 3: Susceptibility of *E. coli* isolates (n=538) in three European countries to oral antibiotics

Organism (n) / Antimicrobial agent	Proportion of susceptible and resistant isolates by country (%)						Significant differences in rates between countries (p values)					
	BE (n=178)		DE (n=179)		ES (n=181)		BE vs DE		BE vs ES		DE vs ES	
	S	R	S	R	S	R	S	R	S	R	S	R
Amoxicillin-clavulanic acid	62.9	37.1	62.6	37.4	34.3	65.7	n.s.	n.s.	<0.001	<0.001	<0.001	<0.001
Amoxicillin-clavulanic acid (uncomplcated UTI)	77.0	23.0	81.6	18.4	57.5	42.5	n.s.	n.s.	<0.001	<0.001	<0.001	<0.001
Cefuroxime oral	94.4	5.6	87.2	12.8	83.4	16.6	<0.01	<0.01	<0.001	<0.001	n.s.	n.s.
Cefixime	96.1	3.9	89.9	10.1	86.7	13.3	<0.01	<0.01	<0.001	<0.001	n.s.	n.s.
Cefpodoxime	95.5	4.5	89.9	10.1	86.2	13.8	<0.05	<0.05	<0.001	<0.001	n.s.	n.s.
Ceftibuten	97.8	2.2	91.1	8.9	92.3	7.7	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.
Ceftriaxone	96.6	3.4	90.5	9.5	89.5	10.5	<0.01	<0.01	<0.01	<0.01	n.s.	n.s.
Ciprofloxacin	87.1	12.9	82.7	17.3	59.7	39.8	n.s.	n.s.	<0.001	<0.001	<0.001	<0.001
Fosfomycin	98.9	1.1	100	0	97.2	2.8	n.s.	n.s.	n.s.	n.s.	<0.05	<0.05
Nitrofurantoin	99.4	0.6	99.4	0.6	100	0	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Trimethoprim-sulfamethoxazole	85.4	14.6	80.4	18.4	68.5	30.9	n.s.	n.s.	<0.001	<0.001	<0.001	<0.001

BE, Belgium; DE, Germany; ES, Spain; n.s., not significant; S, susceptible; R, resistant