



2 Differential epidemiology and antibiotic resistance 3 of lactose-fermenting and non-fermenting *Escherichia coli*: Is it just 4 a matter of taste?

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8 Abstract

9 Urinary tract infections (UTIs) are some of the most common infections affecting humans worldwide. Occurrence of atypical,
10 lactose non-fermenting, biochemically “inactive” strains of *E. coli* in clinical material has been described in the literature,

AQ1 which may cause a significant diagnostic challenge. The present retrospective microbiological study was carried out using
12 isolates and data collected between January 1, 2013, and December 31, 2017, at the Institute of Clinical Microbiology. AQ2
13 $n=24,285$ positive urine samples were noted during the study period, out of which, samples positive for either *lac+* and *lac-*
14 *E. coli* were included in the analysis. *E. coli* represented $n=7075$ ($55.8\% \pm 4.6\%$) of outpatient and $n=4916$ ($42.4\% \pm 3.6\%$)
15 of inpatient isolates. $n=401$ (3.3%; 80.2 ± 14.6 /year) *lac-* *E. coli* isolates were identified from urinary tract infections. The
16 ratio of *lac-* *E. coli* isolates was significantly higher in outpatient samples (262 vs. 139). Resistance levels of *lac-* isolates for
17 antibiotics commonly used for treating UTIs were significantly higher for both inpatient and outpatient isolates: norfloxacin,
18 ciprofloxacin, fosfomycin and nitrofurantoin. It is essential to pay attention to the presence of *lac-* strains, and their omis-
19 sion from clinical material during diagnostic procedures may have significant consequences for epidemiological studies and
20 therapy.

21 **Keywords** *E. coli* · Lactose non-fermenting · Urinary tract infections · Epidemiology · Biochemical testing · Antibiotics

22 Introduction

23 Urinary tract infections (UTIs) are some of the most com-
24 mon infections affecting humans worldwide; based on their
25 prevalence, they are the third most common (following
26 respiratory tract infections and gastrointestinal infections)
27 infectious pathologies (Flores-Mireles et al. 2015; Behzadi
28 and Behzadi 2016). Women have a 50% lifetime risk of
29 developing UTIs at least once and 5% risk of having UTIs

30 more than 5 times in their lifetime; for men, this risk is
31 considerably lower (around 1–5%, especially for men aged
32 50 years or older), which may be attributed to the anatomic
33 differences of the genitourinary tract among the two
34 sexes (Stefaniuk et al. 2016; Magyar et al. 2017). UTIs are
35 an important factor of morbidity for patients visiting out-
36 patient clinics, as well as hospitalized patients (especially
37 ones undergoing urinary catheterization). In the latter group,
38 these infections may represent 25–50% of communicable
39 diseases overall (Maharjan et al. 2018). For this reason,
40 UTIs should be considered an important financial burden
41 for patients (due to the symptoms and decreased quality
42 of life), national economies (due to lost working days) and
43 healthcare institutions (due to additional costs of pharmaco-
44 therapy, hospitalization and invasive procedures) (Foxman
45 2003). The therapy of UTIs is also significantly hindered
46 by the emergence of multidrug-resistant (MDR) bacterial
47 strains, forcing clinicians to utilize drugs that are more
48 expensive, are only available to be used intravenously, or
49 that have pronounced toxicity in the patients (Milovanovic
50 et al. 2019). The increasing resistance levels are especially

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51	worrisome in drugs primarily used to treat UTIs, namely	104
52	trimethoprim/sulfamethoxazole, fosfomycin, nitrofurantoin	105
53	and the fluoroquinolones (Gajdács et al. 2019a; Jancel and	106
54	Dudas 2002; Kaskatepe et al. 2017).	107
55	Members of the Enterobacterales order (previously: the	108
56	<i>Enterobacteriaceae</i> family (Adelou et al. 2016)) are the	
57	most frequently associated with UTIs (including <i>E. coli</i> and	
58	<i>Klebsiella</i> , <i>Citrobacter</i> , <i>Enterobacter</i> , <i>Serratia</i> , <i>Proteus</i> ,	
59	<i>Morganella</i> and <i>Providencia</i> species) (Park et al. 2017;	
60	Critchley et al. 2019); however, the pathogenic potential of	
61	Gram-positive cocci (<i>Enterococcus</i> spp., <i>Staphylococcus</i>	
62	<i>aureus</i> , <i>S. saprophyticus</i>), non-fermenting Gram-negative	
63	bacteria (e.g., <i>Pseudomonas aeruginosa</i>) (Gajdács et al.	
64	2019b) and various yeasts (e.g., <i>Candida</i> species) should	
65	also be taken into consideration (Behzadi et al. 2015;	
66	Gajdács et al. 2019c). Nevertheless, the most common bac-	
67	terial pathogen in UTIs is <i>E. coli</i> (namely uropathogenic	
68	<i>E. coli</i> or UPEC, recognized as a separate microbiological	
69	entity in the 1970s), corresponding to 70–95% of infections,	
70	based on various literature reports (Gajdács et al. 2019d;	
71	Behzadi 2019; Hozzari et al. 2020). <i>E. coli</i> is a commensal	
72	microorganism abundantly found in the gastrointestinal tract	
73	(producing Vitamin K for the host and having a protective	
74	role against other pathogens); however, if these bacteria	
75	breach into other anatomical regions, they act as opportu-	
76	nistic pathogens, owing to the plethora of virulence factors	
77	they possess (Gajdács et al. 2019d; Behzadi 2019; Hozzari	
78	et al. 2020; Jahandeh et al. 2015). <i>E. coli</i> is considered a	
79	biochemically active microorganism, while the hallmarks of	
80	biochemical identification include the ability to ferment lac-	
81	tose (<i>lac</i> +) and the decomposing of tryptophan into indole	
82	(Toledo and Trabulsi 1983). However, the occurrence of	
83	atypical, lactose non-fermenting (due to deficiency in the	
84	levels of lactose permease, encoded by <i>lacY</i> gene), often	
85	non-motile, biochemically “inactive” strains of <i>E. coli</i> in	
86	clinical material has been described in the literature, pre-	
87	dominantly in the context of diarrheal (shigellosis-like) ill-	
88	nesses (Nicoletti et al. 1988; Rychert and Stephenson 1986;	
89	Bajpai et al. 2016). These non-fermenting atypical variants	
90	(<i>lac</i> -) may cause a significant diagnostic challenge; in addi-	
91	tion, the few reports available on the prevalence of these	
92	isolates have highlighted the potential of these strains to	
93	harbor various virulence- and antibiotic-resistance deter-	
94	minants, clinically differentiating them from <i>lac</i> + strains	
95	(Chang et al. 2014). Recently, an Australian study by Platell	
96	et al. highlighted that the <i>lac</i> - O75 clonal group of <i>E. coli</i>	
97	(a serotype that has been frequently associated with caus-	
98	ing bacteremia and UTIs) had extensive levels of fluoroqui-	
99	nolone resistance (Platell et al. 2012).	
100	There are very few comparative studies available on the	
101	epidemiological features and resistance levels of <i>lac</i> + and	
102	<i>lac</i> - strains of <i>E. coli</i> in clinical samples. Therefore, in the	
103	present study, our aim was to investigate the prevalence of	
non-lactose (<i>lac</i> -) fermenting <i>E. coli</i> in the context of urine	104	
specimens over a long surveillance period, to see whether	105	
differential trends could be observed in the demographic	106	
characteristics of the affected patients and the antibiotic sus-	107	
ceptibility of these isolates.	108	
Materials and methods	109	
Study design, data collection	110	
The present retrospective microbiological study was carried	111	
out using data collected from the period between the January	112	
1, 2013, and December 31, 2017 (a 5-year time frame) at	113	
the Institute of Clinical Microbiology (University of Sze-	114	
ged), which is the diagnostic microbiology laboratory of	115	
the Albert Szent-Györgyi Clinical Center, a primary- and	116	
tertiary-care teaching hospital in the Southern Great Plain	117	
of Hungary. Electronic search in the records of the MedBak-	118	
ter laboratory information system (LIS) for urine samples	119	
positive for <i>lac</i> + and <i>lac</i> - <i>E. coli</i> (including identification	120	
methods, biochemical test results, susceptibility testing	121	
results) was conducted by the authors (M.G., Á.M. and A.L.)	122	
(Gajdács et al. 2019d).	123	
Samples with clinically significant colony counts for <i>E.</i>	124	
<i>coli</i> ($> 10^5$ CFU/mL; however, this was subject to interpre-	125	
ation by the senior clinical microbiologists, based on the	126	
information provided on the clinical request forms for the	127	
microbiological analysis and international guidelines) that	128	
were positive for the nitrite and leukocyte-esterase tests were	129	
included in the data analysis (Gajdács et al. 2019a, d). Only	130	
the first isolate per patient was included in the study; how-	131	
ever, isolates with different antibiotic-susceptibility patterns	132	
from the same patient were considered as different individ-	133	
ual isolates. To evaluate the demographic characteristics of	134	
these infections, patient data were also collected, which was	135	
limited to sex, age at the sample submission and inpatient/	136	
outpatient status.	137	
Identification of isolates	138	
Ten microliters of each un-centrifuged urine sample was	139	
cultured on eosine methylene blue (EMB; Bio-Rad, Berke-	140	
ley, CA, USA) and UriSelect chromogenic agar plates (Bio-	141	
Rad, Berkeley, CA, USA) with a calibrated loop, according	142	
to the manufacturer's instructions and incubated at 37 °C	143	
for 24–48 h, aerobically. If relevant urinary pathogens (i.e.,	144	
all isolates that were presumed to be Gram-negative bacte-	145	
ria) presented in significant colony count, the plates were	146	
passed on for further processing. Identification was primar-	147	
ily based on colony color and morphology, in addition to	148	
the biochemical reaction-based VITEK 2 Compact ID/AST	149	
(bioMérieux, Marcy-l'Étoile, France) automated system,	150	

151 the results of which were recorded (Gajdács et al. 2019a,
 152 *d*). For the verification of discrepant identification results,
 153 matrix-assisted laser desorption/ionization time-of-flight
 154 mass spectrometry (MALDI-TOF MS by the Microflex
 155 MALDI Biotyper; Bruker Daltonics, Bremen, Germany)
 156 was utilized. The sample preparation methodology and the
 157 technical details for mass spectrometry measurements were
 158 described elsewhere (Takach et al. 1997). The MALDI Bio-
 159 typer RTC 3.1 software (Bruker Daltonics) and the MALDI
 160 Biotyper Library 3.1 were used for spectrum analysis. Dif-
 161 ferentiation of *lac* + and *lac*- *E. coli* strains was carried out
 162 based on the abovementioned tests.

163 Antibiotic susceptibility testing

164 Antimicrobial susceptibility testing was performed using the
 165 Kirby–Bauer disk diffusion method (Liofilchem, Abruzzo,
 166 Italy) on Mueller–Hinton agar (MHA) plates, based on the
 167 methodological standards of EUCAST (EUCAST Clinical
 168 breakpoints-breakpoints and Accessed 18 Mar 2020). In
 169 addition, for the verification of discrepant results, the
 170 VITEK 2 Compact ID/AST (bioMérieux, Marcy-l’Étoile,
 171 France) automated system was also used (Gajdács et al.
 172 2019a, *d*). The following antibiotics were tested (with disk
 173 potencies in brackets): ampicillin (10 µg), amoxicillin/clavu-
 174 lanic acid (10/10 µg), piperacillin (30 µg), cefotaxime
 175 (5 µg), ceftriaxone (30 µg), ceftazidime (10 µg), imipenem
 176 (10 µg), meropenem (10 µg), norfloxacin (10 µg), ciproflox-
 177 acin (5 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin
 178 (30 µg), tigecycline (15 µg), fosfomycin (200 µg with 50 µg
 179 glucose-6-phosphate), nitrofurantoin (100 µg), trimetho-
 180 prim/sulfamethoxazole (1.25/23.75 µg).

181 The interpretation of the results was based on the official
 182 EUCAST breakpoints at the time of isolation (v.8.0-v.9.0).
 183 Phenotypic detection and confirmation of extended-spectrum
 184 β -lactamase (ESBL) production were carried out using the
 185 ESBL Disk Test Set (Liofilchem, Abruzzo, Italy) (Gajdács
 186 et al. 2019a, *d*). *S. aureus* ATCC 29213, *E. faecalis* ATCC
 187 29212, *P. mirabilis* ATCC 35659, *E. coli* ATCC 25922, *K.*
 188 *pneumoniae* ATCC 700603 and *P. aeruginosa* ATCC 27853
 189 were used as quality control strains. During data analysis,
 190 intermediately susceptible results were grouped with and
 191 reported as resistant.

192 Statistical analyses

193 Descriptive statistical analysis (including means or medians
 194 with ranges and percentages to characterize data) was per-
 195 formed using Microsoft Excel 2013 (Redmond, WA, USA,
 196 Microsoft Corp.). Statistical analyses were performed with
 197 SPSS software version 24 (IBM SPSS Statistics for Win-
 198 dows 24.0, Armonk, NY, USA, IBM Corp.), using the Chi-
 199 square test or Student's *t* test. The normality of variables was

200 tested using Shapiro–Wilk tests. *p* values <0.05 were con-
 201 sidered statistically significant. Randomization of *lac* + *E.*
 202 *coli* sample was carried out using the RANDOM function
 203 in Microsoft Excel 2013 (Suresh 2011).

204 Results

205 Epidemiology, demographic characteristics

206 During the respective 5-year study period, $n=24,285$ uri-
 207 nary samples were received in the Institute of Clinical
 208 Microbiology that turned out to be positive for a significant
 209 urinary pathogen; out of these samples, $n=12,690$ (52.3%)
 210 originated from outpatient clinics, while $n=11,595$ (47.7%)
 211 was sent by inpatient departments ($p>0.05$). The majority
 212 of samples were midstream urine ($n=18,107$; 74.6%), fol-
 213 lowed by catheter-specimen urine ($n=5299$; 21.8%), while
 214 first-stream urine ($n=859$; 3.5%) and bladder tap ($n=20$;
 215 0.1%) represented a minor fraction of urine samples.

216 Among the positive samples, *E. coli* represented
 217 $n=7075$ ($55.8\% \pm 4.6\%$) of outpatient isolates and $n=4916$
 218 ($42.4\% \pm 3.6\%$) of inpatient isolates, respectively; the highest
 219 percentages of *E. coli* among all urinary isolates were seen
 220 in 2015, while the lowest percentages were seen in 2017.
 221 Based on the phenotypic evaluation and the biochemical
 222 reactions by the VITEK 2 automated system, overall $n=401$
 223 ($3.3\% ; 80.2 \pm 14.6/\text{year}$) *lac*- *E. coli* isolates were identified
 224 from urinary tract infections between 2013 and 2017. The
 225 ratio of *lac*- *E. coli* isolates was significantly higher in out-
 226 patient samples ($n=262$; 3.7%), than in inpatient samples
 227 ($n=139$; 2.8%) ($p=0.021$).

228 Due to the pronounced differences (401 vs. 11,991) in
 229 the isolation rate of *lac* + and *lac*- *E. coli*, during statistical
 230 analyses (for demographic and susceptibility data), a ran-
 231 dom sample of *lac* + *E. coli* was created and used, with a
 232 similar sample size of *lac*- isolates. Randomization was per-
 233 formed $n=10$ times (including $n=40$ inpatient and $n=40$
 234 outpatient isolates randomly, per each study year for a total
 235 of $n=400$ *lac* + *E. coli*) to assess whether these individual
 236 random samples presented with statistically significant dif-
 237 ferences. Based on the results of the preliminary statistical
 238 analysis, no relevant differences were found; thus, during
 239 the comparisons between *lac* + and *lac*- *E. coli* isolates, a
 240 random *lac* + sample ($n=400$, 200–200 from inpatient and
 241 outpatient samples, respectively) was utilized.

242 The demographic characteristics associated with the *lac*-
 243 and *lac* + samples are presented in Table 1. Overall, 73.8%
 244 ($n=295$) of *lac*- samples and 70.8% ($n=284$) *lac* + origi-
 245 nated from female patients ($p>0.05$). The median age of
 246 patients of the *lac*- groups did not show relevant differences
 247 to those of the *lac* + group ($p>0.05$).

Table 1 Demographic characteristics associated with *lac-* and *lac+E. coli* isolates (2013–2017)

	<i>Lac-</i> isolates		<i>lac+</i> isolates	
	Outpatient samples	Inpatient samples	Outpatient samples	Inpatient samples
Number of isolates	<i>n</i> =262	<i>n</i> =139	<i>n</i> =200	<i>n</i> =200
Median age (years)	54	73	52	72
Age range (years)	0.5–97	0.3–91	0.3–94	0.4–96
% of female patients affected	70.6% (<i>n</i> =185)	71.2% (<i>n</i> =99)	76.0% (<i>n</i> =152)	71.5% (<i>n</i> =143)

248 **Antibiotic susceptibility results**

249 The number and ratio of resistant *lac-* and *lac+E. coli* isolates (both from the inpatient and outpatient samples) are
250 shown in Table 2. The highest levels of resistance were
251 shown to norfloxacin, ampicillin, ciprofloxacin and trimetho-
252 prim/sulfamethoxazole in all sample groups, while lowest
253 levels of resistance were shown against amikacin (<5%),
254 tigecycline (<1%), imipenem and meropenem (0%). Over-
255 all, significant differences were observed between the resis-
256 tance levels of the inpatient and outpatient sample groups for
257 most of the β -lactam antibiotics (amoxicillin/clavulanic acid
258 (5.6% vs. 10.9%; *p*=0.039), cefotaxime (9.6% vs. 29.2%;
259 *p*=0.011), ceftriaxone (9.3% vs. 28.3%; *p*=0.015), ceftazi-
260 dime (9.3% vs. 27.7%; *p*=0.016)) and gentamicin (6.5% vs.
261 15.1%; *p*=0.02). The prevalence of ESBL-positive isolates
262 was also higher in the inpatient isolates (9.3% vs. 27.7%;
263 *p*=0.016).

265 In contrast, such differences were not observed for
266 β -lactams or any aminoglycosides if the groups were com-
267 pared based on their *lac-* and *lac+* status. On the other
268 hand, resistance levels of *lac-* isolates for antibiotics com-
269 monly used for treating UTIs were significantly higher for
270 both inpatient and outpatient isolates: norfloxacin (outpa-
271 tients: 58.0% vs. 44.0%; *p*=0.033, inpatients: 69.2% vs.
272 51.0%; *p*=0.024), ciprofloxacin (outpatients: 29.0% vs.
273 19.5%; *p*=0.046, inpatients: 37.4% vs. 25.5%; *p*=0.037),
274 fosfomycin (outpatients: 10.3% vs. 6.0%; *p*=0.037, inpa-
275 tients: 18.7% vs. 8.0%; *p*=0.022) and nitrofurantoin (out-
276 patients: 4.6% vs. 2.0%; *p*=0.049, inpatients: 6.5% vs.
277 2.5%; *p*=0.046) (Table 2). No significant correlation was
278 found between lactose positivity and ESBL prevalence
279 (*p*>0.05). AQ3 9

Table 2 Antibiotic susceptibilities associated with *lac-* and *lac+E. coli* isolates (2013–2017)

	<i>lac-</i> isolates		<i>lac+</i> isolates	
	Outpatient samples	Inpatient samples	Outpatient samples	Inpatient samples
Number of isolates	<i>n</i> =262	<i>n</i> =139	<i>n</i> =200	<i>n</i> =200
Ampicillin	45.0% (<i>n</i> =118)	51.1% (<i>n</i> =71)	48.0% (<i>n</i> =96)	50.5% (<i>n</i> =101)
Amoxicillin/clavulanic acid	5.3% (<i>n</i> =14)	10.8% (<i>n</i> =15)	6.0% (<i>n</i> =12)	11.0% (<i>n</i> =22)
Piperacillin	6.9% (<i>n</i> =18)	12.2% (<i>n</i> =17)	8.5% (<i>n</i> =17)	11.0% (<i>n</i> =22)
Cefotaxime	8.8% (<i>n</i> =23)	28.1% (<i>n</i> =39)	10.5% (<i>n</i> =21)	30.0% (<i>n</i> =60)
Ceftriaxone	8.4% (<i>n</i> =22)	26.6% (<i>n</i> =37)	10.5% (<i>n</i> =21)	29.5% (<i>n</i> =59)
Ceftazidime	7.6% (<i>n</i> =20)	26.6% (<i>n</i> =37)	10.5% (<i>n</i> =21)	28.5% (<i>n</i> =57)
Imipenem	0% (<i>n</i> =0)	0% (<i>n</i> =0)	0% (<i>n</i> =0)	0% (<i>n</i> =0)
Meropenem	0% (<i>n</i> =0)	0% (<i>n</i> =0)	0% (<i>n</i> =0)	0% (<i>n</i> =0)
Norfloxacin	58.0% (<i>n</i> =152)	69.2% (<i>n</i> =96)	44.0% (<i>n</i> =88)	51.0% (<i>n</i> =102)
Ciprofloxacin	29.0% (<i>n</i> =76)	37.4% (<i>n</i> =52)	19.5% (<i>n</i> =39)	25.5% (<i>n</i> =51)
Gentamicin	6.1% (<i>n</i> =16)	15.1% (<i>n</i> =21)	7.0% (<i>n</i> =14)	15.0% (<i>n</i> =30)
Tobramycin	4.6% (<i>n</i> =12)	8.6% (<i>n</i> =12)	5.5% (<i>n</i> =11)	8.5% (<i>n</i> =17)
Amikacin	2.7% (<i>n</i> =7)	3.5% (<i>n</i> =5)	3.5% (<i>n</i> =7)	4.5% (<i>n</i> =9)
Tigecycline	0% (<i>n</i> =0)	0.7% (<i>n</i> =1)	0% (<i>n</i> =0)	0.5% (<i>n</i> =1)
Fosfomycin	10.3% (<i>n</i> =27)	18.7% (<i>n</i> =26)	6.0% (<i>n</i> =12)	8.0% (<i>n</i> =14)
Nitrofurantoin	4.6% (<i>n</i> =12)	6.5% (<i>n</i> =9)	2.0% (<i>n</i> =4)	2.5% (<i>n</i> =5)
Trimethoprim/sulfamethoxazole	25.9% (<i>n</i> =68)	33.1% (<i>n</i> =46)	27.0% (<i>n</i> =54)	31.0% (<i>n</i> =62)

280 **Discussion**

281 *E. coli* is the most common cause of urinary tract infections in both community and healthcare settings; the epidemiological significance of *E. coli* UTIs has also been highlighted in the context of our study. The pathogenic role of *E. coli* was noted by several reports from international organizations: The World Health Organization has designated it to the priority-pathogen list (to facilitate the development of novel antimicrobial agents), while the Infectious Disease Society of America (IDSA) included it among the “ESKAPE” pathogens, pertaining to bacteria causing the highest levels of morbidity and mortality worldwide (Rajendran et al. 2019; Gajdács 2019). *E. coli* is a microorganism that may cause life-threatening infections: The various subtypes of entero-virulent *E. coli* (EEC) strains are principal causes of diarrheal illnesses, both in the Western world and in developing countries (Ochoa and Contreras 2011). Among the extra-intestinal pathogenic *E. coli* (ExPEC) strains, UPEC isolates are the most common; nevertheless, sepsis-associated *E. coli* (SEPEC) and neonatal meningitis-associated *E. coli* (NMEC) strains have the potential to cause invasive, often lethal infections (Manges et al. 2019; Köhler and Dobrindt 2011). Lactose non-fermenting *E. coli* strains have similarly been implicated in the pathogenesis of diarrhea, UTIs and invasive infections (Thompson et al. 1990; Barcaite et al. 2012).

307 In our study, the primary isolation of the bacteria from urine samples was carried out on eosine methylene blue and UriSelect chromogenic agar plates; although these culture media may have a role in the phenotypic misidentification of *lac* + and *lac* - strains in our local context, there are no data (from the literature or from our personal experiences) suggesting that the isolation frequency differs during the use of these culture media. Thus, all *E. coli* isolates (in fact, all Gram-negative bacteria isolated from urine samples) were included in the identification process for the VITEK automated system which has been extensively characterized as a reliable method for identification and susceptibility testing of Gram-negative bacteria. Any discrepancies were clarified during the use of the MALDI-TOF MS system; as this method employs a protein-based identification system (irrespective of the *lac* + or *lac* - status of the strains) (Takach et al. 1997), there was very limited chance of misidentification or misrepresentation in our results.

326 From a clinical perspective, it is important to attain the knowledge about the most frequent etiological agents of UTIs and their susceptibility-levels to predict the clinical course of an infection and to select for adequate empiric antibiotic therapy (Abbo and Hooton 2014). However, it

331 may be difficult to interpret the results of several authors as in most cases, biochemical characteristics (as differentiating factors, e.g., *lac*- and *lac* + status) are not reported 332 for the respective strains; therefore, it is not possible to 333 ascertain which bacterial population is being referred to, 334 e.g., in a sample of *E. coli* (Bajpai et al. 2016). To the best 335 of our knowledge, this is the first study in Hungary, regarding 336 the prevalence and resistance levels of lactose non- 337 fermenting *E. coli* in urinary tract infections or otherwise. 338 Among the main findings of our study, 3.3% (corresponding 339 to $n=401$ isolates) of *E. coli* was shown to be *lac*- over 340 a 5-year surveillance period, which we compared to a stratified 341 random sample of $n=400$ *lac* + *E. coli*. Although the 342 *lac*- strains represented a minor fraction of representative 343 isolates, our study highlights that these bacteria may be 344 misidentified or misrepresented in epidemiological studies, 345 where only tube-based, presumptive biochemical tests 346 are utilized (Barcaite et al. 2012). Resistance levels against 347 β -lactams were significantly higher in isolates originating 348 from inpatients; this finding has also been demonstrated in 349 our previous studies (Gajdács et al. 2019a, d). 350

351 In the following, a brief summary is presented regarding 352 the available literature on the differential aspects of *lac*- and 353 *lac* + *E. coli* clinical isolates. Among the first reports on the 354 subject was the publication of Thompson et al., reporting 355 a prevalence of 4.0% for *lac*- *E. coli*; in this study, the isolates 356 were originating from stool samples and most of the *lac*- *E. coli* isolates were Verotoxin producers (Thompson 357 et al. 1990). Versalovic et al. estimated that around 5.0% of 358 all *E. coli* clinical isolates (irrespective of the sample type) 359 should be a lactose non-fermenter (Versalovic et al. 2011). 360 This ratio has been proven to be correct by the study of 361 Barcaite et al. from Lithuania, during which the study group 362 screened pregnant women and neonates for Group B *Streptococcus* and *E. coli* colonization (Barcaite et al. 2012). In 363 consecutive studies from India (starting in 1995), Bhat et al. 364 showed that 12.4% of urinary *E. coli* isolates are lactose 365 non-fermenters (Bhat and Bhat 1995), while in studies with 366 similar settings, Raksha et al. (in 2003) (Raksha et al. 2003), 367 Radha et al. (in 2010) (Radha and Jeya 2010) and Bajpai 368 et al. (in 2016) (Bajpai et al. 2016) detected *lac*- *E. coli* in 369 9.0%, 6.3% and 3.6% of urine samples, respectively. Kaczmarek 370 et al. characterized $n=58$ *lac*- and *lac* + *E. coli* bacteria 371 isolated from pregnant women and neonates in Poland, 372 using phenotypic and genotypic methods; in their report, 373 *lac*- isolates showed higher levels of resistance to ticarcillin 374 and ticarcillin/clavulanic acid, while no difference was seen 375 in the number of genes carried for virulence factors (Kaczmarek 376 et al. 2017; Kaczmarek et al. 2011). Yaratha et al. 377 compared the epidemiological and clinical characteristics 378 of $n=150$ *lac*- and *lac* + *E. coli* clinical isolates from urine 379 samples in a New York tertiary-care hospital: In this report, 380 no differences were observed in the clinical outcomes of the 381

384 respective infections. However, they have noted that lac- isolates showed significantly higher levels of resistance to third
 385 generation cephalosporins and cefepime, while no such difference was seen for other urinary antibiotics (Yaratha et al.
 386 2017). Hossain et al. characterized lac- isolates isolated from
 387 stool samples in Bangladesh: In this study, 16.0% of *E. coli*
 388 were lac-, and non-fermenters showed significantly higher
 389 levels of resistance to fluoroquinolones and trimethoprim/
 390 sulfamethoxazole (Hossain 2012). The highest prevalence
 391 of non-fermenters was seen in a report from the Republic
 392 of Korea by Chang et al.: 19.7% were lac- and the 075 sero-
 393 type was the most prominent among tested strains; however,
 394 they have found higher resistance in lac+ *E. coli* against
 395 ciprofloxacin (Chang et al. 2014). The pronounced differences
 396 among the reported isolation frequencies (~3–20%)
 397 may be attributable to several factors: (i) As most of these
 398 studies discussed mainly used common culture media for the
 399 primary isolation of these species from the clinical samples,
 400 differential levels of isolation are presumably not due to the
 401 “loss at culture,” which is a common phenomenon, when
 402 considering fastidious microorganisms; (ii) the workup of
 403 different sample types (i.e., urine, stool, high vaginal swabs
 404 and so on) entails the use of different ancillary culture media
 405 and different incubation times (24–72 h), which may affect
 406 the expression of different enzymes, the sensitivity/speci-
 407 ficity of the media and therefore, the detection rate of lac-
 408 isolates; (iii) depending of the financial situation of clinical
 409 microbiology laboratories, different identification schemes
 410 may put into place: Some laboratories are only capable of
 411 using tube-based presumptive tests, others may use semi-
 412 automated (e.g., API) or automated biochemical identifica-
 413 tion (e.g., VITEK), and the most up-to-date institutions may
 414 utilize MALDI-TOF MS and PCR; all of these methods have
 415 different sensitivities and relevance in detecting lac- isolates;
 416 (iv) the interest and precision at the selection of colonies
 417 during diagnostic processes and the attitude toward the exact
 418 identification and characterization of these UTI pathogens
 419 may also play a role; as in most cases, laboratories do not
 420 bother with the detailed characterization of the causative
 421 agents to this extent, because they do not consider this as
 422 a potential diagnostic inaccuracy; in addition, most clin-
 423 icians are only concerned with susceptibility results to guide
 424 therapy.

425 In our study results, the median age of the affected
 426 patients in the inpatient and outpatient groups varied consider-
 427 ably; however, this factor is probably unrelated to the lac-
 428 tose-fermentation status of these *E. coli* strains. More prob-
 429 ably, this corresponds to the common phenomenon seen in
 430 the demographic characteristics of outpatient and inpatient
 431 UTIs; most of the outpatient samples usually originate from
 432 younger patients with a better general health status and less
 433 exposure to antibiotics; on the other hand, inpatient sam-
 434 ples originate from older, hospitalized patients. The latter

435 patient group is commonly affected by underlying condi-
 436 tions, and their lifetime antibiotic exposure is also consider-
 437 ably higher. This also corresponds to the higher resistance
 438 levels observed in inpatient samples. This phenomenon has
 439 been described in a plethora of studies, both in Hungary and
 440 elsewhere. Multidrug resistance in UTIs is a significant clin-
 441 ical problem (especially in the members of Enterobacterales,
 442 where the levels of ESBL- and carbapenemase-producing
 443 isolates are rapidly growing), which resulted in the “rena-
 444 issance” in the utilization of older antibiotics, some of which
 445 have been specifically used for the treatment of UTIs. Fos-
 446 fomycin, nitrofurantoin, mecillinam should all be considered
 447 as first-line antibiotics for uncomplicated urinary infections,
 448 while methenamine—a urinary antiseptic—has also been
 449 re-discovered in the twenty-first century (Ahmed et al. 2016;
 450 Doesschate et al. 2020). Fluoroquinolones have been exten-
 451 sively used for the therapy of UTIs; however, due to recent
 452 development regarding their side-effect profile (the Food
 453 and Drug Administration has issued a “black box” warn-
 454 ing on their use) and the growing levels of drug resistance,
 455 their use as first-line agents in most clinical indications has
 456 been discouraged (Yarrington et al. 2019). Highlighting the
 457 significance of biochemical parameters, lac- isolates were
 458 significantly more prone to be resistant to fluoroquinolone
 459 antibiotics and drugs that should be used in the first line for
 460 uncomplicated UTIs.

Conclusions for future biology

461 Bacterial infections are still one of the most important
 462 factors of morbidity and mortality among communicable
 463 illnesses, and urinary tract infections are one of the most
 464 common infection types in human medicine. Gut bacteria,
 465 and among this group, *E. coli* is the most important uri-
 466 nary pathogen in both uncomplicated urinary tract infec-
 467 tions of outpatient and in hospitalized patients; therefore,
 468 the precise knowledge of the epidemiological characteris-
 469 tics and susceptibility of these microorganisms is of utmost
 470 importance. The rapid emergence of antibiotic resistance in
 471 urinary pathogens is a global public health issue, affecting
 472 most Gram-negative bacteria. Most *E. coli* strains are bio-
 473 chemically active; however, it is essential to pay attention to
 474 the presence of atypical, lac- strains: Their omission from
 475 the clinical material during diagnostic procedures may have
 476 significant consequences for epidemiological studies and
 477 therapy. Our study has presented the relevance of lac- strains
 478 of *E. coli* over a long surveillance period, to encourage other
 479 diagnostic laboratories to pay close attention to this variant
 480 of *E. coli*. Based on the limited amount of available find-
 481 ings in the literature, differential workup of various clinical
 482 samples, the use of ancillary culture media, the interest and
 483 precision during selection of colonies during diagnostic pro-
 484 cesses and the availability of modern diagnostic modalities

488 were identified as possible explanations for the variable iso-
489 lation frequency of *lac-* strains.

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496 formed data collection and analysis, wrote and revised the full paper.
497 M.Á. and A.L. performed the identification and antibiotic susceptibility
498 testing of the respective isolates, wrote and revised the full paper.
499 K.B. supervised the completion of the study, wrote and revised the
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