



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

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Abstract

Urinary tract infections (UTIs) are some of the most common infections affecting humans worldwide. Occurrence of atypical, lactose non-fermenting, biochemically “inactive” strains of *E. coli* in clinical material has been described in the literature, which may cause a significant diagnostic challenge. The present retrospective microbiological study was carried out using isolates and data collected between January 1, 2013, and December 31, 2017, at the Institute of Clinical Microbiology. $n = 24,285$ positive urine samples were noted during the study period, out of which, samples positive for either *lac+* and *lac-* *E. coli* were included in the analysis. *E. coli* represented $n = 7075$ ($55.8\% \pm 4.6\%$) of outpatient and $n = 4916$ ($42.4\% \pm 3.6\%$) of inpatient isolates. $n = 401$ (3.3% ; $80.2 \pm 14.6/\text{year}$) *lac-* *E. coli* isolates were identified from urinary tract infections. The ratio of *lac-* *E. coli* isolates was significantly higher in outpatient samples (262 vs. 139). Resistance levels of *lac-* isolates for antibiotics commonly used for treating UTIs were significantly higher for both inpatient and outpatient isolates: norfloxacin, ciprofloxacin, fosfomycin and nitrofurantoin. It is essential to pay attention to the presence of *lac-* strains, and their omission from clinical material during diagnostic procedures may have significant consequences for epidemiological studies and therapy.

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

Introduction

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

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

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worrisome in drugs primarily used to treat UTIs, namely trimethoprim/sulfamethoxazole, fosfomycin, nitrofurantoin and the fluoroquinolones (Gajdács et al. 2019a; Jancel and Dudas 2002; Kaskatepe et al. 2017).

Members of the Enterobacterales order (previously: the *Enterobacteriaceae* family (Adelou et al. 2016)) are the most frequently associated with UTIs (including *E. coli* and *Klebsiella*, *Citrobacter*, *Enterobacter*, *Serratia*, *Proteus*, *Morganella* and *Providencia* species) (Park et al. 2017; Critchley et al. 2019); however, the pathogenic potential of Gram-positive cocci (*Enterococcus* spp., *Staphylococcus aureus*, *S. saprophyticus*), non-fermenting Gram-negative bacteria (e.g., *Pseudomonas aeruginosa*) (Gajdács et al. 2019b) and various yeasts (e.g., *Candida* species) should also be taken into consideration (Behzadi et al. 2015; Gajdács et al. 2019c). Nevertheless, the most common bacterial pathogen in UTIs is *E. coli* (namely uropathogenic *E. coli* or UPEC, recognized as a separate microbiological entity in the 1970s), corresponding to 70–95% of infections, based on various literature reports (Gajdács et al. 2019d; Behzadi 2019; Hozzari et al. 2020). *E. coli* is a commensal microorganism abundantly found in the gastrointestinal tract (producing Vitamin K for the host and having a protective role against other pathogens); however, if these bacteria breach into other anatomical regions, they act as opportunistic pathogens, owing to the plethora of virulence factors they possess (Gajdács et al. 2019d; Behzadi 2019; Hozzari et al. 2020; Jahandeh et al. 2015). *E. coli* is considered a biochemically active microorganism, while the hallmarks of biochemical identification include the ability to ferment lactose (*lac*+) and the decomposing of tryptophan into indole (Toledo and Trabulsi 1983). However, the occurrence of atypical, lactose non-fermenting (due to deficiency in the levels of lactose permease, encoded by *lacY* gene), often non-motile, biochemically “inactive” strains of *E. coli* in clinical material has been described in the literature, predominantly in the context of diarrheal (shigellosis-like) illnesses (Nicoletti et al. 1988; Rychert and Stephenson 1986; Bajpai et al. 2016). These non-fermenting atypical variants (*lac*-) may cause a significant diagnostic challenge; in addition, the few reports available on the prevalence of these isolates have highlighted the potential of these strains to harbor various virulence- and antibiotic-resistance determinants, clinically differentiating them from *lac*+ strains (Chang et al. 2014). Recently, an Australian study by Platell et al. highlighted that the *lac*- O75 clonal group of *E. coli* (a serotype that has been frequently associated with causing bacteremia and UTIs) had extensive levels of fluoroquinolone resistance (Platell et al. 2012).

There are very few comparative studies available on the epidemiological features and resistance levels of *lac*+ and *lac*- strains of *E. coli* in clinical samples. Therefore, in the present study, our aim was to investigate the prevalence of

non-lactose (*lac*-) fermenting *E. coli* in the context of urine specimens over a long surveillance period, to see whether differential trends could be observed in the demographic characteristics of the affected patients and the antibiotic susceptibility of these isolates.

Materials and methods

Study design, data collection

The present retrospective microbiological study was carried out using data collected from the period between the January 1, 2013, and December 31, 2017 (a 5-year time frame) at the Institute of Clinical Microbiology (University of Szeged), which is the diagnostic microbiology laboratory of the Albert Szent-Györgyi Clinical Center, a primary- and tertiary-care teaching hospital in the Southern Great Plain of Hungary. Electronic search in the records of the MedBakter laboratory information system (LIS) for urine samples positive for *lac*+ and *lac*- *E. coli* (including identification methods, biochemical test results, susceptibility testing results) was conducted by the authors (M.G., Á.M. and A.L.) (Gajdács et al. 2019d).

Samples with clinically significant colony counts for *E. coli* ($> 10^5$ CFU/mL; however, this was subject to interpretation by the senior clinical microbiologists, based on the information provided on the clinical request forms for the microbiological analysis and international guidelines) that were positive for the nitrite and leukocyte-esterase tests were included in the data analysis (Gajdács et al. 2019a, d). Only the first isolate per patient was included in the study; however, isolates with different antibiotic-susceptibility patterns from the same patient were considered as different individual isolates. To evaluate the demographic characteristics of these infections, patient data were also collected, which was limited to sex, age at the sample submission and inpatient/outpatient status.

Identification of isolates

Ten microliters of each un-centrifuged urine sample was cultured on eosine methylene blue (EMB; Bio-Rad, Berkeley, CA, USA) and UriSelect chromogenic agar plates (Bio-Rad, Berkeley, CA, USA) with a calibrated loop, according to the manufacturer’s instructions and incubated at 37 °C for 24–48 h, aerobically. If relevant urinary pathogens (i.e., all isolates that were presumed to be Gram-negative bacteria) presented in significant colony count, the plates were passed on for further processing. Identification was primarily based on colony color and morphology, in addition to the biochemical reaction-based VITEK 2 Compact ID/AST (bioMérieux, Marcy-l’Étoile, France) automated system,

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

Antibiotic susceptibility testing

Antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion method (Liofilchem, Abruzzo, Italy) on Mueller–Hinton agar (MHA) plates, based on the methodological standards of EUCAST (EUCAST Clinical breakpoints-breakpoints and Accessed 18 Mar 2020). In addition, for the verification of discrepant results, the VITEK 2 Compact ID/AST (bioMérieux, Marcy-l'Étoile, France) automated system was also used (Gajdács et al. 2019a, d). The following antibiotics were tested (with disk potencies in brackets): ampicillin (10 µg), amoxicillin/clavulanic acid (10/10 µg), piperacillin (30 µg), cefotaxime (5 µg), ceftriaxone (30 µg), ceftazidime (10 µg), imipenem (10 µg), meropenem (10 µg), norfloxacin (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tobramycin (10 µg), amikacin (30 µg), tigecycline (15 µg), fosfomycin (200 µg with 50 µg glucose-6-phosphate), nitrofurantoin (100 µg), trimethoprim/sulfamethoxazole (1.25/23.75 µg).

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

Statistical analyses

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

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

Results

Epidemiology, demographic characteristics

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

Among the positive samples, *E. coli* represented *n* = 7075 (55.8% ± 4.6%) of outpatient isolates and *n* = 4916 (42.4% ± 3.6%) of inpatient isolates, respectively; the highest percentages of *E. coli* among all urinary isolates were seen in 2015, while the lowest percentages were seen in 2017. Based on the phenotypic evaluation and the biochemical reactions by the VITEK 2 automated system, overall *n* = 401 (3.3%; 80.2 ± 14.6/year) *lac-* *E. coli* isolates were identified from urinary tract infections between 2013 and 2017. The ratio of *lac-* *E. coli* isolates was significantly higher in outpatient samples (*n* = 262; 3.7%), than in inpatient samples (*n* = 139; 2.8%) (*p* = 0.021).

Due to the pronounced differences (401 vs. 11,991) in the isolation rate of *lac+* and *lac-* *E. coli*, during statistical analyses (for demographic and susceptibility data), a random sample of *lac+* *E. coli* was created and used, with a similar sample size of *lac-* isolates. Randomization was performed *n* = 10 times (including *n* = 40 inpatient and *n* = 40 outpatient isolates randomly, per each study year for a total of *n* = 400 *lac+* *E. coli*) to assess whether these individual random samples presented with statistically significant differences. Based on the results of the preliminary statistical analysis, no relevant differences were found; thus, during the comparisons between *lac+* and *lac-* *E. coli* isolates, a random *lac+* sample (*n* = 400, 200–200 from inpatient and outpatient samples, respectively) was utilized.

The demographic characteristics associated with the *lac-* and *lac+* samples are presented in Table 1. Overall, 73.8% (*n* = 295) of *lac-* samples and 70.8% (*n* = 284) *lac+* originated from female patients (*p* > 0.05). The median age of patients of the *lac-* groups did not show relevant differences 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* iso-
 250 lates (both from the inpatient and outpatient samples) are
 251 shown in Table 2. The highest levels of resistance were
 252 shown to norfloxacin, ampicillin, ciprofloxacin and trimetho-
 253 prim/sulfamethoxazole in all sample groups, while lowest
 254 levels of resistance were shown against amikacin (< 5%),
 255 tigecycline (< 1%), imipenem and meropenem (0%). Over-
 256 all, significant differences were observed between the resist-
 257 ance levels of the inpatient and outpatient sample groups for
 258 most of the β -lactam antibiotics (amoxicillin/clavulanic acid
 259 (5.6% vs. 10.9%; *p* = 0.039), cefotaxime (9.6% vs. 29.2%;
 260 *p* = 0.011), ceftriaxone (9.3% vs. 28.3%; *p* = 0.015), ceftazi-
 261 dime (9.3% vs. 27.7%; *p* = 0.016) and gentamicin (6.5% vs.
 262 15.1%; *p* = 0.02). The prevalence of ESBL-positive isolates
 263 was also higher in the inpatient isolates (9.3% vs. 27.7%;
 264 *p* = 0.016).

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

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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 infec- 331
 282 tions in both community and healthcare settings; the epi- 332
 283 demiological significance of *E. coli* UTIs has also been 333
 284 highlighted in the context of our study. The pathogenic 334
 285 role of *E. coli* was noted by several reports from inter- 335
 286 national organizations: The World Health Organization 336
 287 has designated it to the priority-pathogen list (to facilitate 337
 288 the development of novel antimicrobial agents), while the 338
 289 Infectious Disease Society of America (IDSA) included 339
 290 it among the “ESKAPE” pathogens, pertaining to bacte- 340
 291 ria causing the highest levels of morbidity and mortal- 341
 292 ity worldwide (Rajendran et al. 2019; Gajdács 2019). *E.* 342
 293 *coli* is a microorganism that may cause life-threatening 343
 294 infections: The various subtypes of entero-virulent *E. coli* 344
 295 (EEC) strains are principal causes of diarrheal illnesses, 345
 296 both in the Western world and in developing countries 346
 297 (Ochoa and Contreras 2011). Among the extra-intestinal 347
 298 pathogenic *E. coli* (ExPEC) strains, UPEC isolates are 348
 299 the most common; nevertheless, sepsis-associated *E.* 349
 300 *coli* (SEPEC) and neonatal meningitis-associated *E. coli* 350
 301 (NMEC) strains have the potential to cause invasive, often 351
 302 lethal infections (Manges et al. 2019; Köhler and Dobrindt 352
 303 2011). Lactose non-fermenting *E. coli* strains have simi- 353
 304 larly been implicated in the pathogenesis of diarrhea, UTIs 354
 305 and invasive infections (Thompson et al. 1990; Barcaite 355
 306 et al. 2012).

307 In our study, the primary isolation of the bacteria from 352
 308 urine samples was carried out on eosine methylene blue 353
 309 and UriSelect chromogenic agar plates; although these 354
 310 culture media may have a role in the phenotypic mis- 355
 311 identification of *lac* + and *lac* - strains in our local context, 356
 312 there are no data (from the literature or from our personal 357
 313 experiences) suggesting that the isolation frequency dif- 358
 314 fers during the use of these culture media. Thus, all *E. coli* 359
 315 isolates (in fact, all Gram-negative bacteria isolated from 360
 316 urine samples) were included in the identification process 361
 317 for the VITEK automated system which has been exten- 362
 318 sively characterized as a reliable method for identification 363
 319 and susceptibility testing of Gram-negative bacteria. Any 364
 320 discrepancies were clarified during the use of the MALDI- 365
 321 TOF MS system; as this method employs a protein-based 366
 322 identification system (irrespective of the *lac* + or *lac* - 367
 323 status of the strains) (Takach et al. 1997), there was very 368
 324 limited chance of misidentification or misrepresentation 369
 325 in our results.

326 From a clinical perspective, it is important to attain the 370
 327 knowledge about the most frequent etiological agents of 371
 328 UTIs and their susceptibility-levels to predict the clinical 372
 329 course of an infection and to select for adequate empiric 373
 330 antibiotic therapy (Abbo and Hooton 2014). However, it

331 may be difficult to interpret the results of several authors 332
 333 as in most cases, biochemical characteristics (as differen- 334
 335 tiating factors, e.g., *lac* - and *lac* + status) are not reported 336
 337 for the respective strains; therefore, it is not possible to 338
 339 ascertain which bacterial population is being referred to, 340
 341 e.g., in a sample of *E. coli* (Bajpai et al. 2016). To the best 342
 343 of our knowledge, this is the first study in Hungary, regard- 344
 345 ing the prevalence and resistance levels of lactose non- 345
 346 fermenting *E. coli* in urinary tract infections or otherwise. 346
 347 Among the main findings of our study, 3.3% (correspond- 347
 348 ing to $n = 401$ isolates) of *E. coli* was shown to be *lac* - over 348
 349 a 5-year surveillance period, which we compared to a strat- 349
 350 ified random sample of $n = 400$ *lac* + *E. coli*. Although the 350
 351 *lac* - strains represented a minor fraction of representative 351
 352 isolates, our study highlights that these bacteria may be 352
 353 misidentified or misrepresented in epidemiological stud- 353
 354 ies, where only tube-based, presumptive biochemical tests 354
 355 are utilized (Barcaite et al. 2012). Resistance levels against 355
 356 β -lactams were significantly higher in isolates originating 356
 357 from inpatients; this finding has also been demonstrated in 357
 358 our previous studies (Gajdács et al. 2019a, d).

359 In the following, a brief summary is presented regarding 352
 360 the available literature on the differential aspects of *lac* - and 353
 361 *lac* + *E. coli* clinical isolates. Among the first reports on the 354
 362 subject was the publication of Thompson et al., reporting 355
 363 a prevalence of 4.0% for *lac* - *E. coli*; in this study, the iso- 356
 364 lates were originating from stool samples and most of the 357
 365 *lac* - *E. coli* isolates were Verotoxin producers (Thompson 358
 366 et al. 1990). Versalovic et al. estimated that around 5.0% of 359
 367 all *E. coli* clinical isolates (irrespective of the sample type) 360
 368 should be a lactose non-fermenter (Versalovic et al. 2011). 361
 369 This ratio has been proven to be correct by the study of 362
 370 Barcaite et al. from Lithuania, during which the study group 363
 371 screened pregnant women and neonates for Group B *Strep-* 364
 372 *tococcus* and *E. coli* colonization (Barcaite et al. 2012). In 365
 373 consecutive studies from India (starting in 1995), Bhat et al. 366
 374 showed that 12.4% of urinary *E. coli* isolates are lactose 367
 375 non-fermenters (Bhat and Bhat 1995), while in studies with 368
 376 similar settings, Raksha et al. (in 2003) (Raksha et al. 2003), 369
 377 Radha et al. (in 2010) (Radha and Jeya 2010) and Bajpai 370
 378 et al. (in 2016) (Bajpai et al. 2016) detected *lac* - *E. coli* in 371
 379 9.0%, 6.3% and 3.6% of urine samples, respectively. Kacz- 372
 380 marek et al. characterized $n = 58$ *lac* - and *lac* + *E. coli* bac- 373
 381 teria isolated from pregnant women and neonates in Poland, 374
 382 using phenotypic and genotypic methods; in their report, 375
 383 *lac* - isolates showed higher levels of resistance to ticarcillin 376
 384 and ticarcillin/clavulanic acid, while no difference was seen 377
 385 in the number of genes carried for virulence factors (Kacz- 378
 386 marek et al. 2017; Kaczmarek et al. 2011). Yaratha et al. 379
 387 compared the epidemiological and clinical characteristics 380
 388 of $n = 150$ *lac* - and *lac* + *E. coli* clinical isolates from urine 381
 389 samples in a New York tertiary-care hospital: In this report, 382
 390 no differences were observed in the clinical outcomes of the 383

384 respective infections. However, they have noted that *lac*- iso- 437
 385 lates showed significantly higher levels of resistance to third 438
 386 generation cephalosporins and cefepime, while no such dif- 439
 387 ference was seen for other urinary antibiotics (Yaratha et al. 440
 388 2017). Hossain et al. characterized *lac*- isolates isolated from 441
 389 stool samples in Bangladesh: In this study, 16.0% of *E. coli* 442
 390 were *lac*-, and non-fermenters showed significantly higher 443
 391 levels of resistance to fluoroquinolones and trimethoprim/ 444
 392 sulfamethoxazole (Hossain 2012). The highest prevalence 445
 393 of non-fermenters was seen in a report from the Republic 446
 394 of Korea by Chang et al.: 19.7% were *lac*- and the O75 sero- 447
 395 type was the most prominent among tested strains; however, 448
 396 they have found higher resistance in *lac* + *E. coli* against 449
 397 ciprofloxacin (Chang et al. 2014). The pronounced differ- 450
 398 ences among the reported isolation frequencies (~3–20%) 451
 399 may be attributable to several factors: (i) As most of these 452
 400 studies discussed mainly used common culture media for the 453
 401 primary isolation of these species from the clinical samples, 454
 402 differential levels of isolation are presumably not due to the 455
 403 “loss at culture,” which is a common phenomenon, when 456
 404 considering fastidious microorganisms; (ii) the workup of 457
 405 different sample types (i.e., urine, stool, high vaginal swabs 458
 406 and so on) entails the use of different ancillary culture media 459
 407 and different incubation times (24–72 h), which may affect 460
 408 the expression of different enzymes, the sensitivity/speci- 461
 409 ficity of the media and therefore, the detection rate of *lac*- 462
 410 isolates; (iii) depending of the financial situation of clinical 463
 411 microbiology laboratories, different identification schemes 464
 412 may put into place: Some laboratories are only capable of 465
 413 using tube-based presumptive tests, others may use semi- 466
 414 automated (e.g., API) or automated biochemical identifica- 467
 415 tion (e.g., VITEK), and the most up-to-date institutions may 468
 416 utilize MALDI-TOF MS and PCR; all of these methods have 469
 417 different sensitivities and relevance in detecting *lac*- isolates; 470
 418 (iv) the interest and precision at the selection of colonies 471
 419 during diagnostic processes and the attitude toward the exact 472
 420 identification and characterization of these UTI pathogens 473
 421 may also play a role; as in most cases, laboratories do not 474
 422 bother with the detailed characterization of the causative 475
 423 agents to this extent, because they do not consider this as 476
 424 a potential diagnostic inaccuracy; in addition, most clini- 477
 425 cians are only concerned with susceptibility results to guide 478
 426 therapy. 479

427 In our study results, the median age of the affected 480
 428 patients in the inpatient and outpatient groups varied consid- 481
 429 erably; however, this factor is probably unrelated to the lac- 482
 430 tose-fermentation status of these *E. coli* strains. More prob- 483
 431 ably, this corresponds to the common phenomenon seen in 484
 432 the demographic characteristics of outpatient and inpatient 485
 433 UTIs; most of the outpatient samples usually originate from 486
 434 younger patients with a better general health status and less 487
 435 exposure to antibiotics; on the other hand, inpatient sam-
 436 ples originate from older, hospitalized patients. The latter

437 patient group is commonly affected by underlying condi- 438
 439 tions, and their lifetime antibiotic exposure is also consider- 439
 440 ably higher. This also corresponds to the higher resistance 440
 441 levels observed in inpatient samples. This phenomenon has 441
 442 been described in a plethora of studies, both in Hungary and 442
 443 elsewhere. Multidrug resistance in UTIs is a significant clin- 443
 444 ical problem (especially in the members of Enterobacterales, 444
 445 where the levels of ESBL- and carbapenemase-producing 445
 446 isolates are rapidly growing), which resulted in the “renais- 446
 447 sance” in the utilization of older antibiotics, some of which 447
 448 have been specifically used for the treatment of UTIs. Fos- 448
 449 fomicin, nitrofurantoin, mecillinam should all be considered 449
 450 as first-line antibiotics for uncomplicated urinary infections, 450
 451 while methenamine—a urinary antiseptic—has also been 451
 452 re-discovered in the twenty-first century (Ahmed et al. 2016; 452
 453 Doesschate et al. 2020). Fluoroquinolones have been exten- 453
 454 sively used for the therapy of UTIs; however, due to recent 454
 455 development regarding their side-effect profile (the Food 455
 456 and Drug Administration has issued a “black box” warn- 456
 457 ing on their use) and the growing levels of drug resistance, 457
 458 their use as first-line agents in most clinical indications has 458
 459 been discouraged (Yarrington et al. 2019). Highlighting the 459
 460 significance of biochemical parameters, *lac*- isolates were 460
 461 significantly more prone to be resistant to fluoroquinolone 461
 462 antibiotics and drugs that should be used in the first line for 462
 463 uncomplicated UTIs.

463 Conclusions for future biology 463

464 Bacterial infections are still one of the most important 464
 465 factors of morbidity and mortality among communicable 465
 466 illnesses, and urinary tract infections are one of the most 466
 467 common infection types in human medicine. Gut bacteria, 467
 468 and among this group, *E. coli* is the most important uri- 468
 469 nary pathogen in both uncomplicated urinary tract infec- 469
 470 tions of outpatient and in hospitalized patients; therefore, 470
 471 the precise knowledge of the epidemiological characteris- 471
 472 tics and susceptibility of these microorganisms is of utmost 472
 473 importance. The rapid emergence of antibiotic resistance in 473
 474 urinary pathogens is a global public health issue, affecting 474
 475 most Gram-negative bacteria. Most *E. coli* strains are bio- 475
 476 chemically active; however, it is essential to pay attention to 476
 477 the presence of atypical, *lac*- strains: Their omission from 477
 478 the clinical material during diagnostic procedures may have 478
 479 significant consequences for epidemiological studies and 479
 480 therapy. Our study has presented the relevance of *lac*- strains 480
 481 of *E. coli* over a long surveillance period, to encourage other 481
 482 diagnostic laboratories to pay close attention to this variant 482
 483 of *E. coli*. Based on the limited amount of available find- 483
 484 ings in the literature, differential workup of various clinical 484
 485 samples, the use of ancillary culture media, the interest and 485
 486 precision during selection of colonies during diagnostic pro- 486
 487 cesses and the availability of modern diagnostic modalities 487

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

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497 M.Á. and A.L. performed the identification and antibiotic susceptibil-
498 ity 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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504 Compliance with ethical standards

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