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ORIGINAL PAPER

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% ± 4.6%) of outpatient and $n = 4916$ (42.4% ± 3.6%) of inpatient isolates. $n = 401$ (3.3%; 80.2 ± 14.6/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)

	Lac- isolates		*lac* + isolates	
	Outpatient samples	Inpatient samples	Outpatient samples	Inpatient samples
Number of isolates	$n=262$	$n=139$	$n=200$	$n=200$
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% ($n=185$)	71.2% ($n=99$)	76.0% ($n=152$)	71.5% ($n=143$)

Antibiotic susceptibility results

The number and ratio of resistant *lac*- and *lac* + *E. coli* isolates (both from the inpatient and outpatient samples) are shown in Table 2. The highest levels of resistance were shown to norfloxacin, ampicillin, ciprofloxacin and trimethoprim/sulfamethoxazole in all sample groups, while lowest levels of resistance were shown against amikacin (< 5%), tigecycline (< 1%), imipenem and meropenem (0%). Overall, significant differences were observed between the resistance levels of the inpatient and outpatient sample groups for most of the β-lactam antibiotics (amoxicillin/clavulanic acid (5.6% vs. 10.9%; $p=0.039$), cefotaxime (9.6% vs. 29.2%; $p=0.011$), ceftriaxone (9.3% vs. 28.3%; $p=0.015$), ceftazidime (9.3% vs. 27.7%; $p=0.016$)) and gentamicin (6.5% vs. 15.1%; $p=0.02$). The prevalence of ESBL-positive isolates was also higher in the inpatient isolates (9.3% vs. 27.7%; $p=0.016$).

In contrast, such differences were not observed for β-lactams or any aminoglycosides if the groups were compared based on their *lac*- and *lac* + status. On the other hand, resistance levels of *lac*- isolates for antibiotics commonly used for treating UTIs were significantly higher for both inpatient and outpatient isolates: norfloxacin (outpatients: 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%; $p=0.037$, inpatients: 18.7% vs. 8.0%; $p=0.022$) and nitrofurantoin (outpatients: 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 ($p>0.05$).

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

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

Discussion

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).

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.

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 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 for the respective strains; therefore, it is not possible to ascertain which bacterial population is being referred to, e.g., in a sample of *E. coli* (Bajpai et al. 2016). To the best of our knowledge, this is the first study in Hungary, regarding the prevalence and resistance levels of lactose non-fermenting *E. coli* in urinary tract infections or otherwise. Among the main findings of our study, 3.3% (corresponding to $n = 401$ isolates) of *E. coli* was shown to be *lac*- over a 5-year surveillance period, which we compared to a stratified random sample of $n = 400$ *lac* + *E. coli*. Although the *lac*- strains represented a minor fraction of representative isolates, our study highlights that these bacteria may be misidentified or misrepresented in epidemiological studies, where only tube-based, presumptive biochemical tests are utilized (Barcaite et al. 2012). Resistance levels against β-lactams were significantly higher in isolates originating from inpatients; this finding has also been demonstrated in our previous studies (Gajdács et al. 2019a, d).

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

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

In our study results, the median age of the affected patients in the inpatient and outpatient groups varied considerably; however, this factor is probably unrelated to the lactose-fermentation status of these *E. coli* strains. More probably, this corresponds to the common phenomenon seen in the demographic characteristics of outpatient and inpatient UTIs; most of the outpatient samples usually originate from younger patients with a better general health status and less exposure to antibiotics; on the other hand, inpatient samples originate from older, hospitalized patients. The latter patient group is commonly affected by underlying conditions, and their lifetime antibiotic exposure is also considerably higher. This also corresponds to the higher resistance levels observed in inpatient samples. This phenomenon has been described in a plethora of studies, both in Hungary and elsewhere. Multidrug resistance in UTIs is a significant clinical problem (especially in the members of Enterobacterales, where the levels of ESBL- and carbapenemase-producing isolates are rapidly growing), which resulted in the "renaissance" in the utilization of older antibiotics, some of which have been specifically used for the treatment of UTIs. Fosfomycin, nitrofurantoin, mecillinam should all be considered as first-line antibiotics for uncomplicated urinary infections, while methenamine—a urinary antiseptic—has also been re-discovered in the twenty-first century (Ahmed et al. 2016; Doesschate et al. 2020). Fluoroquinolones have been extensively used for the therapy of UTIs; however, due to recent development regarding their side-effect profile (the Food and Drug Administration has issued a "black box" warning on their use) and the growing levels of drug resistance, their use as first-line agents in most clinical indications has been discouraged (Yarrington et al. 2019). Highlighting the significance of biochemical parameters, *lac*- isolates were significantly more prone to be resistant to fluoroquinolone antibiotics and drugs that should be used in the first line for uncomplicated UTIs.

Conclusions for future biology

Bacterial infections are still one of the most important factors of morbidity and mortality among communicable illnesses, and urinary tract infections are one of the most common infection types in human medicine. Gut bacteria, and among this group, *E. coli* is the most important urinary pathogen in both uncomplicated urinary tract infections of outpatient and in hospitalized patients; therefore, the precise knowledge of the epidemiological characteristics and susceptibility of these microorganisms is of utmost importance. The rapid emergence of antibiotic resistance in urinary pathogens is a global public health issue, affecting most Gram-negative bacteria. Most *E. coli* strains are biochemically active; however, it is essential to pay attention to the presence of atypical, *lac*- strains: Their omission from the clinical material during diagnostic procedures may have significant consequences for epidemiological studies and therapy. Our study has presented the relevance of *lac*- strains of *E. coli* over a long surveillance period, to encourage other diagnostic laboratories to pay close attention to this variant of *E. coli*. Based on the limited amount of available findings in the literature, differential workup of various clinical samples, the use of ancillary culture media, the interest and precision during selection of colonies during diagnostic processes and the availability of modern diagnostic modalities

were identified as possible explanations for the variable isolation frequency of *lac*- strains.

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Authors' Contributions M.G. conceived and designed the study, performed data collection and analysis, wrote and revised the full paper. M.Á. and A.L. performed the identification and antibiotic susceptibility testing of the respective isolates, wrote and revised the full paper. K.B. supervised the completion of the study, wrote and revised the full paper.

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Data Accessibility All data supporting the article's results and digital research materials are presented in the manuscript.

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