Article

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Evolution of Antibiotic Resistance in *Escherichia coli* and *Klebsiella pneumoniae* from Urine Cultures

Inmaculada López-Sampedro[1], Itahisa Hernández-Chico[1], Esther Gómez-Vicente[2], Manuela Expósito-Ruiz[3], José María Navarro-Marí[2], José Gutiérrez-Fernández[1,2,*]

[1]Department of Microbiology, School of Medicine, University of Granada & Biosanitary Research Institute of Granada (ibs. GRANADA), 18016 Granada, Spain
[2]Laboratory of Microbiology, Virgen de las Nieves University Hospital & Biosanitary Research Institute of Granada (ibs. GRANADA), 18014 Granada, Spain
[3]Unit of Biostatistics, Department of Statistics, School of Medicine, University of Granada, 18016 Granada, Spain
*Correspondence: josegf@ugr.es (José Gutiérrez-Fernández)
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Objective: Determine the evolution of antibiotic resistance of symptomatic bacteriuria caused by *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) in Granada.
Material and Method: A descriptive retrospective study was carried out, including antibiograms of urine cultures in which microorganisms identified as *E. coli* and *K. pneumoniae*, were isolated in the Microbiology laboratory of the Hospital Universitario Virgen de las Nieves (Granada, Spain) between January 2016 and June 2021.
Results: *E. coli* was the most frequent isolate (10,048) and its resistance to ampicillin (59.45%) and ticarcillin (59.59%), and the increase to cefepime (15.07%) and amoxicillin-clavulanic acid (17.67%) is noteworthy. *K. pneumoniae* (2222) is notable for resistance to Fosfomycin (27.91%) and an increase to ciprofloxacin (37.79%) and amoxicillin-clavulanic acid (36.63%). Resistance is generally higher in hospitalized patients, males, and adults.
Conclusions: Antibiotic resistance to the studied *Enterobacteriaceae* is on the rise, requiring empirical treatment targeted to the population area.

Keywords: *Escherichia coli*; *Klebsiella pneumoniae*; symptomatic bacteriuria; antibiotic resistance

Introduction

Urinary tract infections (UTIs) are one of the most frequent pathologies in humans worldwide. Its clinic is wide and varies from uncomplicated cystitis to pyelonephritis or urosepsis [1]. It is because of its frequency and its possible complications, which makes it a common cause of hospitalizations, so they deserve special attention and early correct treatment. The latter can sometimes be complex at the outpatient level, since there is no defined treatment and, of first choice, no diagnostic test is performed before prescribing such treatment, which is contributing to the increase in resistance to antibiotics [2].

Because the etiology of this pathology is very broad, one option to try to alleviate the inappropriate use of antibiotics would be to know the local epidemiological data to determine the most appropriate empirical therapy [3]. The *family Enterobacteriaceae* comprises a large and heterogeneous group of gram-negative bacilli, including *Escherichia coli* (*E. coli*) and *Klebsiella pneumoniae* (*K. pneumoniae*) (components of the human gut microbiota and opportunistic pathogens). These microorganisms are the most frequently isolated in laboratories, being the most common cause of UTIs, both complicated and uncomplicated [4].

These bacteria also can generate resistance to antibiotics through different mechanisms, highlighting the production of extended-spectrum beta-lactamases (ESBL) and carbapenemases. Therefore, today, the Centers for Disease Control and Prevention affirms that carbapenem-resistant and ESBL-producing *Enterobacteriaceae* are urgent and serious threats to public health [5]. In addition, in the WHO (World Health Organization) list of priority resistant pathogenic bacteria, the *Enterobacteriaceae* family is listed as critical priority or level 1 [6].

The importance of this type of infection lies in its high frequency, partly due to the risk of recurrence since they are one of the most common both at the outpatient and hospital levels. This frequency translates into the prescription of numerous antibiotic treatments that, over time, lead to antibiotic resistance and motivate the need to find new treatments capable of fighting these infections [5]. In this regard, it is worth mentioning the SARS-CoV-2 pandemic, which has exponentially increased hospitalizations and the acquisition of nosocomial infections, favoring this cycle [7,8].

That is why, knowing the frequency of appearance of these pathogens in a community, analyzing different population groups according to sex, age, type of sample or distinguishing the origin of the infection (nosocomial or acquired in the community) and go further, interpreting the development of these pathogens in these groups, will show the evolution of wild and susceptible strains throughout the preselected study period and their possible modification of response to antibiotics. All these data have application both at the level of analysis and at the clinical level, since defining the groups in which bacteria with greater resistance are found would define at the clinical level the action that has to be carried out in terms of the administration of certain antibiotics. In this sense, it has been shown that knowledge of local epidemiological data is important to determine the empirical therapy to be used in UTIs [3]. Therefore, this study aims to analyze the evolution of antibiotic resistance of the main *Enterobacteriaceae* in our environment, *E. coli* and *K. pneumoniae.*

Material and Methods

A cross-sectional descriptive study was carried out, which included all the results of positive urine cultures (12,270 total isolates) were the microorganisms identified as *E. coli* and *K. pneumoniae* were selected in the Microbiology Laboratory of the Virgen de las Nieves University Hospital (Granada, Spain) between 1 January 2016, and 30 June 2021. The Hospital is a regional healthcare complex made up of three centers (General Hospital of Specialties, Maternal-Child Hospital and Hospital of Neuro-Traumatology and Rehabilitation) that has a third level care activity in the province. In our study, the assisted population consisted of patients exclusively from specialized care, as a sign of clinical importance. There was a lack of explicit information on urinary symptoms.

The samples were initially processed following a strict work protocol established by the laboratory [7] to avoid the study of urine with contaminating microorganisms. For transport, tubes with boric acid were used as a preservative. No screening method was used prior to planting, so all urine received was cultured. The subsequent identification of the isolated microorganisms was carried out by MALDI-TOF mass spectrometry (Number 00556029, Biotyper, Brucker Daltonics, Billerica, MA, USA) and/or MicroScan WalkAway (Number 00695749, Beckman-Coulter, Brea, CA, USA). This system was used for the study of susceptibility to antibiotics. At the same time, susceptibility to beta-lactams was interpreted separately, registering susceptible phenotypes, ESBL phenotypes, IRT phenotypes (resistance to piperacillin-tazobactam and susceptibility to cephalosporins) and resistance profile phenotypes to both. The presence of carbapenemases such as oxacillinases (OXA), Verona integronencoded metallo-beta-lactamase (VIM), carbapenemase type IMP (IMPase), Klebsiella pneumoniae carbapenemases (KPC) and New Delli metallo-beta-lactamase (NDM) was also analyzed (Number NGB-CAR-S23-021, NG-Test CARBA 5, Guipry, France).

A descriptive statistical analysis was performed, calculating absolute and relative frequencies for qualitative variables, measures of central tendency and dispersion for quantitative variables. The normality of the data was contrasted with the Kolmogorov-Smirnov test. To compare the percentage of resistance to different antibiotics according to the year, Pearson's chi-square test was applied. In cases where the conditions of applicability of the test were not met (no more than 20% of the expected frequencies less than 5), Fisher's exact test was used. A $p < 0.05$ value was considered significant. Data were analyzed with R 4.4.1 software (IBM SPSS, Armonk, NY, USA).

Only the variables origin of the sample, microorganism, sex, and age of the patient were collected through the SIL of the laboratory (MODULAB®, Laboratorios Werfen, Barcelona, Spain, system used in the Public Health System of Andalusia as a support of the electronic medical record) for subsequent evaluation.

Results

The results obtained regarding the clinical distribution of all *E. coli* and *K. pneumoniae* isolates in our environment are shown in Table 1. Levofloxacin, amikacin, colistin, cefixime and ticarcillin have only been studied since 2019. The percentages of resistance by years are shown in Fig. 1 and Table 2. Overall, the results obtained from the statistical analysis of the *E. coli* and *K. pneumoniae* susceptibility study according to the years considered are shown in Table 3. For cefepime, trimethoprim-sulfamethoxazole, gentamicin, cefotaxime, ceftazidime, ertapenem, imipenem and tobramycin to *K. pneumoniae* the condition of application of the chi-square statistic is not met.

The statistical significance obtained according to the years and age groups ($\leq$14 and $>$14), the sex of the patients and the origin of the sample (community or hospital) is shown in Table 4. It shows that resistance is greater in hospitalized patients, men, and adults. However, there are no differences between children and adults for *K. pneumoniae* susceptibility. The results of the beta-lactam susceptibility study, where 8580 *E. coli* and 1930 *K. pneumoniae* isolates were analyzed, are shown in Table 5.

Discussion

This retrospective study describes the distribution and antibiotic resistance of bacterial species isolated from community-obtained, and hospitalized urinary tract samples from 2016 to 2021. The bacterial species studied, *E. coli* and *K. pneumoniae* are among the most important gram-negative etiological agents, both for their frequency and for their greater predisposition to present antibiotic

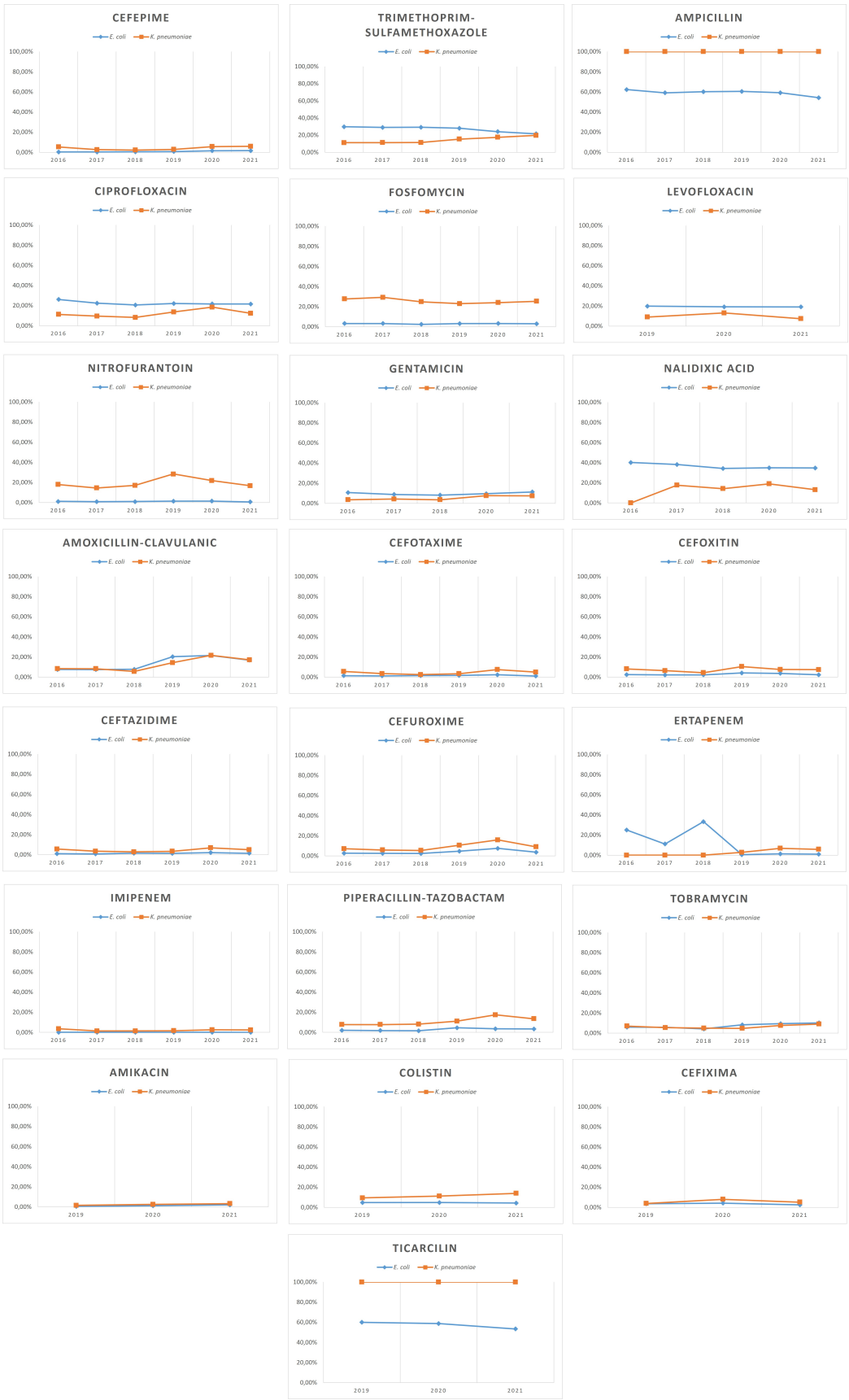

Fig. 1. Percentages of resistance to antibiotics by years.

Table 1. Clinical distribution in number and percentage of all *E. coli* and *K. pneumoniae* isolates in our environment.

	Isolates	Age*		Sex		Origin		Type of sample				
		Child	Adult	Man	Woman	Hospitalizations	Community					
E. coli	10,048							CB	NC	PUC	UMM	PRUC
2016	1784	303 (17.08)	1481 (82.92)	599 (33.58)	1185 (66.42)	452 (25.34)	1332 (74.66)	62 (3.47)	12 (0.67)	117 (6.56)	1280 (71.75)	313 (17.54)
2017	2091	425 (20.33)	1666 (79.67)	676 (32.33)	1415 (67.67)	897 (42.89)	1194 (57.1)	68 (3.25)	10 (0.48)	117 (5.6)	1551 (74.18)	345 (16.49)
2018	1856	319 (17.18)	1537 (82.82)	538 (28.9)	1318 (71.1)	911 (49.09)	945 (50.91)	36 (1.93)	5 (0.27)	111 (5.98)	1461 (78.71)	243 (13.1)
2019	2000	253 (12.65)	1747 (87.35)	571 (28.55)	1429 (71.45)	951 (47.55)	1049 (52.45)	20 (0.99)	7 (0.35)	109 (5.45)	1482 (74.1)	382 (19.1)
2020	1587	182 (11.46)	1405 (88.53)	521 (32.82)	1066 (67.17)	741 (46.69)	846 (53.31)	8 (0.5)	5 (0.31)	96 (6.05)	1188 (74.85)	290 (18.27)
2021	730**	83 (11.37)	647 (88.63)	208 (28.49)	522 (71.5)	349 (47.8)	381 (52.2)	4 (0.55)	6 (0.82)	41 (5.61)	558 (76.44)	121 (16.57)
K. pneumoniae	2222											
2016	367	35 (9.54)	332 (90.46)	161 (43.87)	206 (56.13)	154 (41.96)	213 (58.03)	13 (3.54)	7 (1.9)	53 (14.44)	202 (55.04)	92 (25.06)
2017	453	34 (7.51)	419 (92.49)	193 (42.61)	260 (57.39)	215 (47.79)	238 (52.2)	4 (0.88)	11 (2.42)	51 (11.26)	297 (65.56)	90 (19.87)
2018	351	11 (3.14)	340 (96.86)	129 (36.75)	222 (63.24)	172 (49)	179 (51)	5 (1.42)	5 (1.42)	57 (16.23)	231 (65.81)	53 (15.09)
2019	444	17 (3.89)	427 (96.17)	183 (41.21)	261 (58.78)	257 (57.88)	187 (42.11)	5 (1.12)	3 (0.67)	53 (11.94)	278 (62.61)	105 (23.65)
2020	435	16 (3.68)	419 (96.32)	196 (44.95)	239 (54.81)	265 (60.91)	170 (39.08)	1 (0.23)	5 (1.15)	76 (17.47)	249 (57.24)	104 (23.9)
2021	172**	0 (0)	172 (100)	80 (46.51)	92 (53.49)	101 (58.72)	71 (41.28)	0 (0)	3 (1.75)	14 (8.14)	112 (65.11)	43 (25)

*Age: Child: ≤14 years; Adult: >14 years. **Until 30 June. CB, Children's collecting bag; NC, nephrostomy catheter; PUC, permanent urinary catheter; UMM, urine of medium urination; PRUC, provisional urinary catheter.

Table 2. Resistance (in %) of various antibiotics from total isolates, from urine cultures, of *E. coli* and *K. pneumoniae* between 2016–2021.

	Cefep	Trim-sulf	Amp	Cipro	Fosfo	Levof	Nitrof	Gent	Nalidic acid	Amox-clav	Cefot	Cefoxi	Ceftaz	Cefur	Ertap	Imip	Piper-tazo	Tobra	Amik	Colis	Cefix	Ticarc
E. coli (10,048)																						
2016	7.00%	32.62%	65.81%	30.16%	3.81%	-	1.35%	11.60%	44.23%	8.07%	10.76%	2.75%	5.94%	11.04%	0.28%	0.05%	2.41%	7.40%	-	-	-	-
2017	8.03%	32.09%	63.18%	28.17%	3.83%	-	1.20%	10.90%	42.95%	8.13%	11.05%	2.58%	6.84%	11.86%	0.24%	0.10%	2.10%	8.56%	-	-	-	-
2018	7.68%	31.74%	63.59%	25.99%	3.22%	-	1.24%	9.34%	39.15%	8.32%	10.47%	2.58%	6.98%	10.58%	0.43%	0.11%	1.99%	6.44%	-	-	-	-
2019	10.15%	31.15%	64.35%	27.70%	4.15%	21.70%	1.50%	-	-	22.10%	11.35%	2.75%	10.85%	13.65%	0.60%	0.05%	5.05%	11.60%	0.75%	4.35%	11.30%	53.80%
2020	12.41%	27.47%	63.89%	28.73%	4.28%	26.15%	1.51%	12.73%	41.46%	24.89%	13.80%	4.16%	13.67%	17.96%	1.70%	0.19%	4.35%	14.18%	1.58%	5.17%	15.12%	63.14%
2021	15.07%	25.48%	59.45%	29.04%	3.42%	25.34%	1.10%	13.29%	41.78%	17.67%	14.66%	2.60%	14.93%	16.30%	1.37%	0.00%	3.29%	14.52%	2.33%	5.20%	15.75%	59.59%
K. pneumoniae (2222)																						
2016	24.80%	29.70%	100.00%	26.70%	28.34%	-	19.62%	18.53%	0.00%	13.08%	25.61%	9.81%	22.89%	26.16%	4.90%	3.81%	10.63%	20.71%	-	-	-	-
2017	23.40%	30.68%	100.00%	28.70%	31.13%	-	17.00%	23.62%	4.86%	13.91%	25.61%	7.06%	22.08%	26.49%	3.97%	1.32%	9.71%	21.63%	-	-	-	-
2018	19.37%	26.78%	100.00%	23.36%	27.64%	-	20.80%	15.95%	27.92%	11.11%	21.08%	5.13%	17.38%	23.65%	3.13%	1.99%	10.26%	15.95%	-	-	-	-
2019	20.72%	28.15%	100.00%	27.25%	25.90%	17.12%	24.55%	-	-	23.65%	21.40%	6.53%	21.62%	26.80%	4.50%	1.80%	15.77%	16.44%	1.80%	9.91%	18.69%	100.00%
2020	31.26%	36.32%	100.00%	37.47%	25.06%	28.05%	22.53%	22.53%	31.72%	35.17%	32.87%	8.51%	32.41%	38.39%	11.72%	2.07%	22.99%	25.06%	3.22%	10.80%	32.64%	100.00%
2021	33.14%	40.12%	100.00%	37.79%	27.91%	27.91%	22.67%	22.67%	31.40%	36.63%	32.56%	8.14%	32.56%	35.47%	14.53%	2.33%	22.67%	30.81%	5.23%	11.63%	31.98%	100.00%

Cefep, cefepime; Trim-sulf, trimethoprim-sulfamethoxazole; Amp, ampicillin; Cipro, ciprofloxacin; Fosfo, fosfomycin; Levof, levofloxacin; Nitrof, nitrofurantoin; Gent, gentamicin; Nalidic acid, nalidixic acid; Amox-clav, amoxicillin-clavulanic acid; Cefot, cefotaxime; Cefoxi, cefoxitin; Ceftaz, ceftazidime; Cefur, cefuroxime; Ertap, ertapenem; Imip, imipenem; Piper-tazo, piperacillin-tazobactam; Tobra, tobramycin; Amik, amikacin; Colis, colistin; Cefix, cefixime; Ticarc, ticarcilin; Gentamicin and nalidixic acid were not studied in 2019.

Table 3. Statistical significance of the susceptibility study of *E. coli* and *K. pneumoniae* according to the years considered.

Increased endurance	Decreased endurance	No significant changes
E. coli (10,048)		
Cefepime ($p < 0.0001$)	Trimethoprim-sulfamethoxazole ($p < 0.0001$)	Fosfomycin ($p = 0.6997$)
Amoxicillin-clavulanic acid ($p < 0.0001$)	Ampicillin ($p = 0.01527$)	Levofloxacin ($p = 0.5732$)
Ceftazidime ($p < 0.0001$)	Ciprofloxacin ($p < 0.0001$)	Cefotaxime ($p = 0.2419$)
Piperacillin-tazobactam ($p < 0.0001$)	Nitrofurantoin ($p < 0.0001$)	Imipenem ($p = 0.7996$)
Cefuroxime ($p < 0.0001$)	Gentamicin ($p < 0.0001$)	Colistin ($p = 0.8981$)
Tobramycin ($p < 0.0001$)	Nalidixic acid ($p = 0.00172$)	Cefixime ($p = 0.1784$)
Amikacin ($p < 0.0001$)	Cefoxitin ($p = 0.00036$)	Ticarcilin ($p = 0.07893$)
	Ertapenem ($p < 0.0001$)	
K. pneumoniae (2222)		
Ciprofloxacin ($p < 0.01$)	Nitrofurantoin ($p < 0.01$)	Fosfomycin ($p = 0.4454$)
Amoxicillin-clavulanic acid ($p < 0.01$)	Cefoxitin ($p < 0.01$)	Levofloxacin ($p = 0.3343$)
Cefuroxime ($p < 0.01$)		Nalidixic acid ($p = 0.4219$)
Piperacillin-tazobactam ($p < 0.01$)		Amikacin (N.S.)
		Cefixime ($p = 0.08311$)
		Colistin ($p = 0.6394$)

Ampicillin and ticarcillin are 100% resistant in *K. pneumoniae.*

resistance [4,9]. In general, the existence of publications that show the evolution of resistance in the Spanish territory in a generic way is scarce since most reference is made to a specific population or to a single antibiotic. In addition, this study represents the first contribution in this regard in Granada in the last ten years.

Of the isolates studied (12,270), more than 70% were identified as E. *coli*, being the most frequent bacterium among the uropathogens analyzed. *E. coli* resistance to some antibiotics was higher in the current study compared to previous studies conducted in the same community [10]. However, if we compare it with studies carried out in the community of Madrid (2012–2015) [11] and Navarra (2014–2016) [12] from urinary tract samples, the resistance rates were like those obtained on this occasion.

This study showed a considerable increase in resistance to amoxicillin-clavulanic acid, both for the species *E. coli* ($p < 0.0001$) and for *K. pneumoniae* ($p < 0.01$), which coincides with previous studies carried out in our area [13], presumably due to the significant consumption of this antibiotic. In Spain, an increase in resistance of almost 15% has been observed in UTI isolates in just 5 years against this antibiotic [14]; In the study by Medina *et al.* [11] In patients with urological disorders, a percentage of resistance to this antibiotic of 34.90% and 38.70% in the case of trimethoprim-sulfamethoxazole in *E. coli*, percentages higher in both cases than those obtained in our environment (Table 2). In contrast, other national research showed percentages of resistance in the same species according to our results. Grados *et al*., in 2019 [15], obtained in community patients a 16.60% resistance against amoxicillin-clavulanic acid, 23.90% for ciprofloxacin, 6.60% for cefotaxime, 2.20% for Fosfomycin and 29.30% for trimethoprim-sulfamethoxazole. On the other hand, Cantón *et al*., in 2019 [16], whose study was carried out based on data obtained from 10 hospitals, obtained 22.30% in the case of amoxicillin-clavulanic acid, 37% for ciprofloxacin and 9.90% for cefotaxime, all of them superior when dealing with patients of hospital origin, which was obtained in our analysis (Table 4).

For *K. pneumoniae*, a 10% increase in resistance has been observed in piperacillin-tazobactam ($p < 0.01$), in addition to, being a versatile antibiotic that is used for all types of infections in the daily routine, after the SARS-CoV-2 pandemic, there has been an increase in resistance to the new combinations of beta-lactam inhibitors-beta-lactamases [17].

However, the decrease in resistance to other antibiotics is striking, such as nitrofurantoin ($p < 0.0001$ for *E. coli* and $p < 0.01$ for *K. pneumoniae*), currently in disuse, and hence its reduction, and gentamicin ($p < 0.0001$ for *E. coli*), although its resistance increases again in 2021, going from 9.34% to 13.29% (Table 2). This last fact could be related again to the SARS-CoV-2 pandemic. The rest of the antibiotics are intended for complicated UTIs, which may justify their lower use and, therefore, the reduction of resistance.

According to the results obtained from *E. coli* isolates in pediatric age, they have a lower antibiotic resistance than in adulthood. In addition, in the hospital environment resistance increases. These results are consistent with a previous study conducted only in children <2 years in the same hospital between the years 2011–2014 [18] and with what was obtained by Moya *et al.* [19], where the percentages of resistance in pediatric patients were 14% for amoxicillin-clavulanic acid, 2% for cefuroxime, and 3% for gentamicin.

Table 4. Statistical analysis of *E. coli* and *K. pneumoniae* according to age, sex, and sample origin.

	E. coli			*K. pneumoniae*		
	Age*	Sex**	Origin***	Age*	Sex**	Origin***
Cefepime	$p = 0.0062$	$p = 0.05529$	$p = 0.3467$	$p = 0.2854$	$p = 0.1481$	$p = 0.01782$
Trimethoprim sulfamethoxazole	$p = 0.0003$	$p = 0.001$	$p = 0.0016$	$p = 0.3704$	$p < 0.001$	$p = 0.004744$
Ampicillin	$p = 0.2095$	$p < 0.0001$	$p = 0.0046$	$p = 1$	$p = 1$	$p = 1$
Ciprofloxacin	$p < 0.0001$	$p < 0.0001$	$p = 0.04075$	$p = 0.003597$	$p < 0.001$	$p = 0.1604$
Fosfomycin	$p < 0.0001$	$p = 0.6379$	$p = 0.239$	$p = 0.01092$	$p = 0.6329$	$p = 0.7885$
Levofloxacin	$p < 0.0001$	$p < 0.0001$	$p = 0.027$	$p = 0.111$	$p < 0.001$	$p = 0.06989$
Nitrofurantoin	$p = 0.0049$	$p = 0.578$	$p = 0.08547$	$p < 0.001$	$p = 0.0058$	$p = 0.8416$
Gentamicin	$p < 0.0001$	$p < 0.0001$	$p = 0.7738$	$p = 0.1699$	$p = 0.002557$	$p = 0.1675$
Nalidixic Acid	$p < 0.0001$	$p < 0.0001$	$p = 0.3039$	$p = 0.01499$	$p < 0.001$	$p = 0.4711$
Amoxicillin Clavulanic Acid	$p < 0.0001$	$p < 0.001$	$p < 0.001$	$p = 0.7162$	$p < 0.001$	$p < 0.001$
Cefotaxime	$p < 0.0001$	$p = 0.9863$	$p = 0.00325$	$p = 0.1765$	$p = 0.01018$	$p < 0.001$
Cefoxitin	$p < 0.0001$	$p = 0.04491$	$p = 0.0016$	$p = 0.146$	$p = 0.0021$	$p = 0.1753$
Ceftazidime	$p < 0.0001$	$p = 0.7543$	$p < 0.0001$	$p = 0.1945$	$p = 0.0428$	$p = 0.001681$
Cefuroxime	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$	$p = 0.02669$	$p < 0.001$	$p < 0.001$
Ertapenem	$p = 0.06135$	$p = 0.4311$	$p = 0.4073$	$p = 0.5958$	$p = 0.002911$	$p = 0.226$
Imipenem	$p = 0.6774$	$p = 0.4563$	$p = 0.0071$	$p = 0.3947$	$p = 0.4071$	$p = 0.02028$
Piperacillin-Tazobactam	$p = 0.2221$	$p < 0.0001$	$p = 0.1781$	$p = 0.4873$	$p < 0.001$	$p = 0.03106$
Tobramycin	$p < 0.0001$	$p < 0.0001$	$p = 0.7711$	$p = 0.2381$	$p = 0.0718$	$p = 0.01198$
Amikacin	$p = 0.5759$	$p = 0.9678$	$p = 0.7958$	$p = 1$	$p = 0.1038$	$p = 0.7788$
Colistin	$p = 0.1606$	$p = 0.7417$	$p = 0.5784$	$p = 0.76$	$p = 0.7633$	$p = 0.4652$
Cefixime	$p = 0.02997$	$p = 0.3022$	$p = 0.0078$	$p = 0.3981$	$p = 0.007522$	$p < 0.001$
Ticarcilin	$p = 0.2736$	$p < 0.0001$	$p = 0.002176$	$p = 1$	$p = 1$	$p = 1$

Age*: % of higher resistance in >14 years. Sex**: % of higher resistance in men. Origin***: % of higher resistance in hospitalized patients. Statistical significance: $p < 0.05$.

Table 5. Study of beta-lactam resistance in *E. coli* (8580) and *K. pneumoniae* (1930), isolated from urine cultures, between 2016–2021.

	2016	2017	2018	2019	2020	2021
Susceptible *E. coli*	1254 (89.51%)	1416 (85.40%)	1249 (86.62%)	1529 (82.25%)	1215 (80.09%)	568 (80.80%)
E. coli phenotype ESBL	117 (8.35%)	216 (13.03%)	174 (12.07%)	232 (12.58%)	235 (15.47%)	111 (15.79%)
E. coli phenotype IRT	16 (1.14%)	16 (0.96%)	9 (0.62%)	69 (3.71%)	35 (2.31%)	16 (2.28%)
E. coli phenotype AmpC/Carba ± ESBL	14 (0.99%)	10 (0.60%)	10 (0.69%)	29 (8.56%)	32 (2.12%)	8 (1.14%)
Carbapenemase-producing *E. coli*	1 (7.14%)	1 (10%)	0 (0%)	0 (0%)	2 (6.25%)	2 (25%)
OXA-48 like		1			2	1
KPC						1
VIM		1				
NDM	1					
Susceptible *K. pneumoniae*	216 (69.45%)	258 (69.73%)	208 (54.03%)	277 (68.23%)	219 (55.58%)	98 (59.76%)
K. pneumoniae phenotype ESBL	59 (18.97%)	76 (20.54%)	50 (17.54%)	60 (14.79%)	77 (19.54%)	26 (15.85%)
K. pneumoniae phenotype IRT	11 (3.54%)	11 (2.97%)	7 (2.46%)	26 (6.40%)	28 (7.11%)	9 (5.49%)
K. pneumoniae phenotype AmpC/Carba ± ESBL	25 (8.04%)	25 (6.76%)	20 (7.02%)	43 (10.59%)	70 (17.77%)	31 (18.90%)
Carbapenemase-producing *K. pneumoniae*	12 (48%)	14 (56%)	7 (35%)	13 (30.23%)	33 (47.14%)	19 (61.29%)
OXA-48 like	2	8	2	8	29	18
KPC	9	4	5			1
VIM	1	2		1		
NDM				4	4	

In UTIs caused by ESBL microorganisms in children, the suggested oral antibiotic treatment is ciprofloxacin or cotrimoxazole [20], obtaining in our study a significant difference with higher resistance in adults ($p < 0.0001$; $p < 0.001$). In addition, in 2018, Rodríguez *et al.* [21] obtained, for infections caused by ESBL-producing *E. coli*, higher percentages of resistance in the case of patients over 15 years of age, than in pediatric patients.

In the sex subgroup, the highest percentage of resistance is found in men, even though symptomatic bacteriuria is widely more frequent in women (in 2021 71.5% of *E. coli* isolates were in women), which coincides with the results obtained in the study carried out in Navarra [12]. This can be justified, according to the data collected, by a higher proportion of temporary probes in women and a greater number of permanent probes in men, which are samples of poorer quality, tend to be infected more easily and are more susceptible to such infection being caused by resistant bacteria.

As for *K. pneumoniae,* about the sex of the patients, there are no significant differences except for some exceptions such as gentamicin ($p = 0.002557$) and amoxicillin-clavulanic acid ($p < 0.001$), the latter being especially relevant since in men the resistance is 45%, while in women it is 58.3%. This can be justified by the increased number of symptomatic bacteriuria suffered by women and requiring first-line and sometimes second-line treatment.

On the other hand, the results obtained for *E. coli* show a relevant increase in the percentage of resistance in cefepime (from 7% in 2016 to 15.07% in 2021), this fact may be motivated by the change in the cut-off points by international organizations over the years. This increase in resistance also occurs in tobramycin (from 7.40% in 2016 to 14.52% in 2021) (Table 2).

For *K. pneumoniae*, a very substantial increase in resistance in amoxicillin-clavulanic acid ($p < 0.01$) (from 13.08% in 2016 to 36.63% in 2021) stands out, together with ciprofloxacin ($p < 0.01$) and piperacillin-tazobactam ($p < 0.01$), mostly caused by ESBL-producing microorganisms. Similarly, García *et al.* [22] showed 95.50% resistance to amoxicillin-clavulanic acid in ESBL producers, 86.40% to ciprofloxacin and 31.80% to piperacillin-tazobactam.

These results are quite alarming, but the justification for most lies in their beta-lactam origin, in addition this last analysis only included ICU patients. Ampicillin and ticarcillin are 100% resistant, so they are not suitable for fighting infections by this microorganism (Table 2).

A significant increase in isolates of *E. coli* and *Klebsiella* spp. producers of ESBL, has been previously demonstrated in our environment [23,24], being especially worrying the close relationship between the production of ESBL and resistance to fluoroquinolones [25], this increase justifies the high percentage of resistance of ticarcillin (59.59% in 2021) so it is not suitable for treating infections by this microorganism. By this same mechanism of resistance, very frequent in *E. coli* isolates [9], the percentages of resistance obtained for ceftazidime ($p < 0.0001$) and cefuroxime ($p < 0.0001$) can be justified, since, by definition, no susceptibility should appear in ESBL organisms and a significant increase in resistance has been observed throughout the study. Whereas, for ampicillin (59.45% in 2021) and cefotaxime (14.66% in 2021), these resistance percentages were high from the beginning.

With all this analysis of the data obtained, for all our *E. coli*, in this study, and according to the Spanish Association of Urology [26] and the European Association of Urology [27], the recommended first-line empirical treatment would be Fosfomycin in single doses of 3 grams, since it meets the requirements imposed by the first association: That has a low prevalence of bacterial resistance (<20%) and that it is easy to comply with. Trimethoprim-sulfamethoxazole (provided resistance is less than 20%), norfloxacin, ciprofloxacin, amoxicillin-clavulanic acid and cefepime are proposed as second-line treatment. Trimethoprim-sulfamethoxazole, in the population of Granada, could not be used since the resistance of *E. coli* is 25.48%, higher than the established limit. Norfloxacin, despite being recommended in the guidelines, is not studied in this laboratory so we cannot provide information about it, assuming a limitation of the study.

The proposed third-line treatment would be nitrofurantoin, which is widely active in our medium, however, it has a drawback at the time of administration since it requires greater adherence to treatment. The latter is conventionally considered an excellent therapeutic option against uncomplicated cystitis in the United States [28], presenting in our study only 1.10% resistance in 2021 for *E. coli* isolates. On the contrary, for the species *K. pneumoniae* it reaches 22.67% in 2021. However, nitrofurantoin should be used exclusively in the treatment of acute cystitis for up to seven days, because serious adverse reactions (pulmonary and hepatic) have been reported advising against its use in prolonged or intermittent prophylactic treatments of months duration [29]. For all *K. pneumoniae*, with all the data obtained analyzed, for symptomatic bacteriuria, the recommended treatment in our area would be imipenem, amikacin and colistin. There is no guidance to justify this proposed treatment.

Resistance to beta-lactam antibiotics, quinolones, and co-trimoxazole are generally higher in Spain than in other European countries [30,31]. Regarding the study of beta-lactam antibiotics, among E. *coli* isolates an increase in the ESBL phenotype was observed in the last two years of the study, while the IRT or piperacillin-tazobactam-resistant phenotypes and cephalosporins did not increase (Table 5). This fact could be related to the SARS-CoV-2 pandemic that began in 2020, which led to an increase in prolonged hospitalizations, hospital infections and greater use of this antibiotic empirically [17]. The same occurs in the case of the species *K. pneumoniae*, where it was observed, after the

beginning of the pandemic, an increase in phenotypes resistant to both piperacillin-tazobactam, and cephalosporins, with an increase in OXA type carbapenemases. The latter is of great importance, since carbapenems are considered antibiotics of last resort, whose use is limited in the hospital setting. However, the pandemic may have contributed to the rise of resistance to these, as prevention of the unknown led to increased empirical use of broad-spectrum antibiotics in hospitals. This fact has led to an increase in infections due to multidrug-resistant bacteria, especially in intensive care units, with most of these infections being related to high-risk clones producing carbapenemases [17], as reflected in our results. For the *E.coli* species, it goes from 7.14% resistance caused by carbapenemases in 2016, to 25% in 2021. Meanwhile, in *K. pneumoniae* it goes from 48% in 2016, to 61.29% in 2021, worrying percentages that put at risk the available therapeutic options and highlight the need for strategies that use alternative agents [32]. Previous studies highlight this increase in the dissemination, since 2013 of carbapanemase-producing *Enterobacteriaceae* in our environment, more specifically OXA-48-producing *K. pneumoniae*, with infections associated with high mortality and acquired mainly in hospital [33].

In the hospital setting, despite the low prevalence of coinfections and secondary infections in patients with COVID-19, a high percentage of them have received antimicrobial treatment, with the most commonly used antimicrobial families being fluoroquinolones, macrolides, cephalosporins and combinations of beta-lactams with lactamase inhibitors [17], which is related to antibiotics with a high percentage of resistance from our study, highlighting combinations of beta-lactams with beta-lactamase inhibitors and fluoroquinolones.

Informing the medical community about the evolution of these resistances over the years, the objective of this study, is crucial to maintain proper management of antibiotics, as well as the health of patients who present infections. In addition, this study shows the increase of more resistant phenotypes in *E. coli* and *K. pneumoniae* uropathogenic since the beginning of the SARS-CoV-2 pandemic, so it is necessary to investigate other phenotypes of resistance and study their clinical involvement when making decisions with empirical treatment. In any case, the COVID-19 pandemic and its consequences on antibiotic resistance have demonstrated the need to maintain antimicrobial stewardship and infection control programs. As well as knowing the mistakes made during this period and their consequences to avoid them in future similar situations.

Conclusions

Antibiotic resistance to the studied Enterobacteriaceae is on the rise, requiring empirical treatment targeted to the population area.

Abbreviations

UTIs, urinary tract infections; ESBL, extended-spectrum beta-lactamases.

Availability of Data and Materials

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding autor.

Author Contributions

JGF—conceptualization; ILS, IHC, EGV, MER, JGF—methodology; ILS, IHC, MER—formal analysis; JMNM—resources; ILS, IHC, JGF—writing—original draft preparation; ILS, IHC, EGV, JGF—writing—review and editing. All authors have done supervision, project administration. All authors have read and agreed to the published version of the manuscript.

Ethics Approval and Consent to Participate

The study protocol was conducted in accordance with the Declaration of Helsinki and the ethical considerations of epidemiological research. This was a non-interventional study, with no further investigation to routine procedures. The biological material was used only for the standard diagnosis of urinary tract infections, following the prescriptions of doctors. No additional sampling or modification of the routine diagnostic protocol was performed. Data analyses were performed using a completely anonymous database, where subjects were replaced by different infectious episodes, occurring at least 6 weeks apart from the previous one, if any. The entity that granted permission to access and use the data was the Clinical Management Unit of Clinical Microbiology of the Virgen de las Nieves University Hospital (Granada, Spain). For these reasons, ethics committee approval was considered unnecessary according to national guidelines.

The study protocol was carried out in accordance with the Helsinki Declaration. Data analyses were performed using an anonymous database. Therefore, approval was considered unnecessary according to the guidelines of our country (Law on Data Protection-Organic Law 15/1999 of 13 December on the protection of data of a personal nature, https://www.boe.es/eli/es/lo/1999/12/13/15).

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Conflict of Interest

Given his role as Editorial Board member, José Gutiérrez-Fernández had no involvement in the peer-review of this article and has no access to information regarding its peer-review.

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