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Dynamics of Antimicrobial Resistance and Antimicrobial Consumption in a Secondary-Care Hospital in Serbia, 2019–2022: A Retrospective Observational Study

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Abstract

Antimicrobial resistance (AMR) is accelerating and driven by antimicrobial use. Hospital consumption and resistance data remain scarce in Serbia. This retrospective study assessed the prevalence of infectious agents among hospitalized patients (2019–2022), distribution and AMR rates of *Escherichia coli* and *Klebsiella pneumoniae*, antimicrobial consumption, and associations between AMR and antibiotic use (Spearman's correlation). All bacterial isolates were included for species-frequency analyses. For AMR and specimen distribution, repeat isolates of *E. coli* and *K. pneumoniae* per patient were excluded. Among 10,780 isolates, *E. coli* remained most frequent (24.7% in 2019; 24.3% in 2022), whereas *K. pneumoniae* increased from 7.7% to 16.9% ($p < 0.001$). *E. coli* resistance to cephalosporins, fluoroquinolones, and piperacillin/tazobactam increased significantly from 2019 to 2022 ($p < 0.05$). *K. pneumoniae* resistance increased to most agents ($p < 0.001$), with meropenem resistance rising from 11.7% to 52.0%. Multidrug resistance rose from 27.3% to 35.4% for *E. coli* (2020–2022; $p = 0.006$) and from 62.4% to 82.9% for *K. pneumoniae* (2019–2022; $p < 0.001$). Total hospital antibiotic consumption nearly doubled from 57 to 111.8 Defined Daily Doses per 100 bed days (2019–2021) and decreased to 52.0 (2022). Temporal concurrence of increased antimicrobial use during COVID-19 and escalating resistance underscores the need for strengthened surveillance and stewardship.

Keywords: antimicrobial resistance; antimicrobial consumption; *Escherichia coli*; *Klebsiella pneumoniae*; secondary-care hospital; COVID-19 pandemic

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1. Introduction

Antimicrobial resistance (AMR) is no longer a distant concern—it is already having a serious impact on global health [1]. According to the European Centre for Disease Prevention and Control (ECDC), in 2020 alone, bacterial AMR accounted for over 38,000 deaths within the European Union/European Economic Area (EU/EEA) [2]. In 2021, bacterial AMR was linked to 4.71 million deaths worldwide, with 1.14 million of those directly caused by resistant infections [3]. If current trends continue, projections suggest that by 2050, AMR could be directly responsible for 1.91 million deaths and associated

with a total of 8.22 million deaths globally, highlighting the urgent need for a unified global response [3]. The rapid rise of AMR has largely been fueled by the excessive and inappropriate use of antimicrobials in human medicine, veterinary sectors, agriculture, and the environment [4].

Resistance to last-resort antimicrobials is projected to rise substantially by 2035, with second-line antimicrobial resistance increasing by nearly 45% and third-line resistance, such as to carbapenems, potentially tripling compared to 2005 levels [5]. According to the Organization for Economic Co-operation and Development's (OECD) 2023 analysis, AMR in the EU/EEA countries imposes an estimated EUR 11.7 billion annual burden (approximately EUR 24 per capita) [5]. In the EU/EEA, AMR-related infections are estimated to result in approximately 9.5 million additional hospital bed–days annually, placing significant strain on hospital resources [5].

The World Health Organization (WHO) has classified carbapenem-resistant and third-generation cephalosporin-resistant *Enterobacterales* as critical priority pathogens [6]. An ECDC analysis of the AMR-attributable health burden in the EU/EEA during 2016–2020 identified third-generation cephalosporin-resistant *E. coli* and *K. pneumoniae* as two of the three leading pathogen–drug combinations [2]. Notably, *E. coli* and *K. pneumoniae* are among the most common causative agents of healthcare-associated infections overall across EU/EEA countries, underscoring their relevance in regional surveillance and infection-prevention strategies [7]. According to ECDC data for 2022, *K. pneumoniae* ranks third among the causes of bloodstream infections, while *E. coli* occupies the first position [8]. In addition to their recognized public health importance, *E. coli* and *K. pneumoniae* were selected because they are the most common clinically significant *Enterobacterales* isolated from hospitalized patients [7] and key targets for empirical therapy and antimicrobial stewardship.

The use of antimicrobials represents one of the most important modifiable drivers of AMR [9]. In 2019, antimicrobial consumption in Serbia was already among the highest in Europe, reaching 26.6 Defined Daily Doses (DDD) per 1000 inhabitants per day [10]. However, the Coronavirus Disease 2019 (COVID-19) pandemic hindered efforts to promote rational antimicrobial prescribing. Up to three-quarters of patients with COVID-19 received antimicrobials, even though coinfections occurred in only a small proportion of cases [11]. This global pattern reflects the broader challenge of antimicrobial overuse and underscores the importance of robust national surveillance, particularly as pandemic-related shifts in antimicrobial consumption may have influenced subsequent resistance trends.

At the national level in Serbia, systematic monitoring of antimicrobial consumption in the hospital sector remains unavailable, as does systematic and continuous surveillance of resistance among non-invasive isolates. Moreover, most published hospital AMR data in Serbia originate from tertiary-care centers [12,13], whereas secondary-care hospitals remain underrepresented despite their important role in inpatient care and antibiotic use.

The aims of this study were to evaluate the following: (i) the prevalence of causative bacterial agents of infections among hospitalized patients; (ii) the frequency and distribution of first isolates of *E. coli* and *K. pneumoniae* and their distribution by specimen type (invasive and non-invasive) and hospital ward; (iii) antimicrobial resistance rates and multidrug resistance trends in *E. coli* and *K. pneumoniae*; (iv) hospital antimicrobial consumption during 2019–2022; and (v) the association between antimicrobial consumption and resistance in *E. coli* and *K. pneumoniae*.

Hypothesis 1. *We hypothesized that antimicrobial resistance rates in E. coli and K. pneumoniae increased over the study period (2019–2022) and were correlated with annual hospital antimicrobial consumption.*

2. Materials and Methods

2.1. Study Design and Bacterial Isolates

This retrospective observational study covered the period from 1 January 2019 to 31 December 2022 and included all bacterial isolates from hospitalized patients recorded in the microbiology database of General Hospital Užice. No a priori sample size calculation was performed, as all available isolates were included. The study period (2019–2022) was selected to include a pre-pandemic baseline year (2019), the pandemic years (2020–2021), and the subsequent year (2022), enabling assessment of temporal changes in antimicrobial consumption and resistance trends as routine microbiology and stewardship monitoring indicated notable changes in resistance patterns and antimicrobial consumption during these years. The hospital is a secondary-level healthcare facility in western Serbia with 728 beds, including 38 intensive care unit (ICU) beds. During the COVID-19 pandemic, the hospital capacity was temporarily expanded by 152 beds in an auxiliary facility (COVID hospital) from July 2020. Hospital workflows were reorganized to accommodate COVID-19 care, including the establishment of dedicated COVID-19 wards and partial reallocation of ICU, surgical, and maternity capacities. These structural changes may have influenced patient case-mix and microbiological sampling patterns during the study period. Only isolates deemed clinically significant and likely causative agents of infection, according to routine clinical microbiological assessment, were included. Isolates representing expected physiological microbiota at the sampling site and/or recovered in non-significant quantities were excluded. For non-invasive specimens, the distinction between infection and colonization was determined using routine clinical microbiology criteria, taking into account relevant factors for each specimen type, including species identity, number of isolates, leukocyte presence, and dominance of the isolate in culture. Isolates interpreted as colonizers were excluded. Formal clinical diagnostic criteria were not applied. Bacterial species frequencies were determined for the full dataset, while antimicrobial susceptibility data and specimen-type distribution were analyzed specifically for *E. coli* and *K. pneumoniae*, for which only the first isolate per patient was considered. The isolate selection process is presented in Appendix A, Figure A1.

Species identification was carried out using a combination of conventional bacteriological methods—Gram staining, colony morphology assessment, and biochemical testing—and automated analysis with the VITEK 2 system (bioMérieux, Marcy-l'Étoile, France), according to the manufacturer's instructions. Antimicrobial susceptibility testing was conducted with disk diffusion and VITEK 2 (AST GN76, GN204) in accordance with European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [14]. Colistin susceptibility was determined by broth microdilution (Liofilchem, Roseto degli Abruzzi, Italy). Due to limited resources, colistin testing was not performed routinely for all isolates; it was conducted primarily for isolates resistant to all other tested agents and/or isolates recovered from critically ill patients where colistin was considered a potential therapeutic option. Test availability improved in 2021–2022, resulting in a higher number of isolates tested in these years. Therefore, colistin results were interpreted descriptively and were not used for temporal trend inference. Multidrug resistance (MDR) was defined according to Magiorakos et al. [15] as resistance to $\geq$1 agent in $\geq$3 antimicrobial categories.

In accordance with the methodology of the European Antimicrobial Resistance Surveillance Network (EARS-Net), invasive isolates were defined as those obtained normally sterile sites (e.g., blood, cerebrospinal fluid), while bacteria derived from colonized specimens were classified as non-invasive [16]. Invasive vs. non-invasive stratification was prespecified and applied to specimen-type distribution analyses. Antimicrobial resistance rates were analysed using the overall *E. coli* and *K. pneumoniae* first-isolate-per-patient

datasets (invasive and non-invasive isolates), because the number of invasive isolates per year was low and would limit the robustness of stratified analyses.

All patient data were de-identified and re-coded before data evaluation.

2.2. Antimicrobial Consumption

Hospital-level data on antimicrobials for systemic use (Anatomical Therapeutic Chemical (ATC) group J01) were obtained from the pharmacy's electronic database for 2019–2022. The number of hospital bed–days was obtained from the hospital's administrative records. Consumption was classified according to the WHO ATC classification and the DDD methodology, with consumption expressed as DDDs per 100 bed–days (DDD/100BD) [17]. Pharmacy consumption data were available only as aggregated annual hospital-level totals (without ward- or patient-level stratification).

Broad-spectrum antimicrobial use (glycopeptides, 3rd–4th generation cephalosporins, monobactams, carbapenems, fluoroquinolones, polymyxins, piperacillin–tazobactam, linezolid) was analysed as a secondary indicator according to the European Surveillance of Antimicrobial Consumption Network (ESAC-Net) methodology [18], with a modification applied in calculating antimicrobial consumption. While ESAC reports consumption as DDDs per 1000 inhabitants per day, in this study, it was expressed as DDD/100BD. Given that both metrics represent relative measures of consumption, this modification was considered appropriate for the hospital setting.

The Drug Utilization 75% (DU75%) metric was applied to identify the antimicrobials accounting for 75% of total consumption and to describe prescribing concentration, thereby supporting stewardship prioritization [10]. DU75% was not intended as a direct measure of resistance drivers.

This study was approved by the Ethics Committee of the Healthcare Center Užice, General Hospital Užice (Number 1131/6, Date 10 February 2025).

2.3. Study Outcomes

The co-primary outcomes of this study were (i) antimicrobial resistance patterns and temporal trends among *E. coli* and *K. pneumoniae* isolates and (ii) hospital antimicrobial consumption, expressed as DDD/100BD and DU75%. Secondary outcomes included specimen-type distribution (invasive vs. non-invasive), ward-level distribution, prevalence of multidrug resistance, and associations between antimicrobial consumption and resistance.

2.4. Statistical Analysis

Descriptive statistics were used to summarize categorical variables as absolute and relative frequencies, *n* (%). Differences in proportions across study years were assessed using Pearson's chi-squared test (overall 2019–2022 and selected pairwise year-to-year comparisons where relevant). Associations between antimicrobial consumption and resistance rates were assessed using Spearman's rank correlation coefficient. Pairwise comparisons of column proportions for year-to-year resistance proportions were adjusted for multiple testing using the Bonferroni correction. Statistical analysis was performed using IBM SPSS Statistics for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). The significance level was set at $\alpha = 0.05$.

3. Results

The total number of hospitalized patients was 27,067 in 2019, 24,503 in 2020, 28,946 in 2021, and 27,760 in 2022. The number of COVID-19-positive hospitalized patients was 0 in 2019, 3263 in 2020, 7278 in 2021, and 1988 in 2022. Total hospital bed–days were 150,756, 133,332, 177,217, and 138,652, respectively. The lowest number of patients was recorded in

2020, representing a 9.5% decrease (2020) and a 6.9% increase (2021), respectively, compared with 2019.

The microbiology dataset did not allow reliable linkage of all isolates to unique patient identifiers across the full study period; therefore, patient counts are reported at the hospital level.

3.1. Comparative Distribution of Escherichia coli and Klebsiella pneumoniae Isolates

A total of 10,780 clinically significant isolates were identified during the study period, distributed as follows: 3331 in 2019, 2305 in 2020, 2440 in 2021, and 2704 in 2022.

Each pie chart represents the annual distribution of the most frequently isolated bacterial species as a proportion of the total number of isolates. Colours indicate different bacterial taxa: *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterococcus* spp., *Staphylococcus aureus*, *Acinetobacter* spp., *Proteus* spp., and other species. The "other species" category includes taxa representing <5% of all isolates. In the final chart, *Acinetobacter* spp. and *P. aeruginosa* were also <5% but were retained to illustrate their temporal trends relative to previous years.

E. coli remained the most frequently isolated species throughout all years. The proportion of *E. coli* among total isolates differed significantly across the study period (2019–2022) ($p = 0.019$). Its proportion declined from 24.7% ($n = 824$) in 2019 to 21.6% ($n = 526$) in 2021 ($p = 0.005$), while no significant differences were observed when comparing 2020 (24.8%, $n = 572$; $p = 0.972$) or 2022 (24.3%, $n = 656$; $p = 0.690$) with the baseline year.

In contrast, *K. pneumoniae* showed a steady and statistically significant increase, rising from 7.7% ($n = 258$) in 2019 to 16.9% ($n = 457$) in 2022 ($p < 0.001$), showing a marked increase in its annual proportion (Figure 1, Table A1).

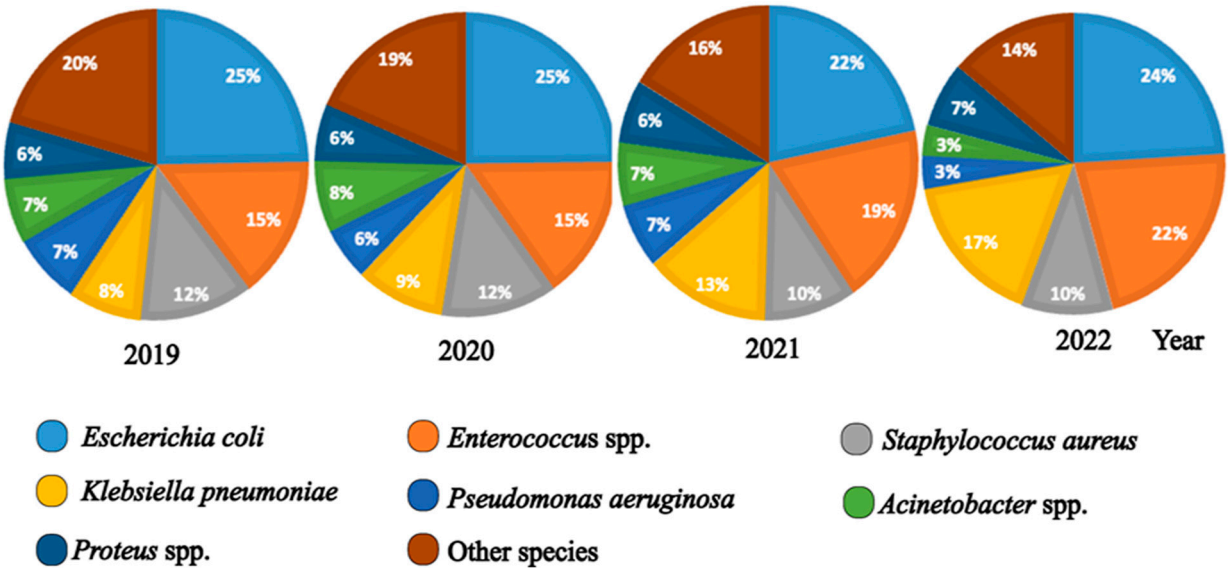


Figure 1. Distribution of the most frequently isolated bacterial species among all isolates, 2019–2022.

Among all isolates, 2578 *E. coli* and 1245 *K. pneumoniae* isolates were identified. After excluding repeated isolates from the same patient, 2217 *E. coli* and 935 *K. pneumoniae* first isolates were included in the susceptibility and specimen distribution analyses (Figure A1).

3.2. Specimen Distribution of Escherichia coli and Klebsiella pneumoniae

Subsequent resistance analyses were performed on the overall first-isolate-per-patient datasets for each species (combined invasive and non-invasive isolates).

For *E. coli*, invasive isolates accounted for 3.4% (n = 24) in 2019, 5.5% (n = 26) in 2020, 5.9% (n = 27) in 2021, and 5.4% (n = 31) in 2022, with no significant differences observed across study years (p = 0.155). Among non-invasive isolates, urine specimens predominated throughout the study period (68.2–69.2%), followed by skin and soft tissue samples (14.1–16.1%). The distribution of other specimen types is presented in Table 1. The proportions of urinary isolates (p = 0.170) and isolates from skin and soft tissue samples (p = 0.835) did not differ significantly between years.

Table 1. Distribution of clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* by specimen type, 2019–2022.

	2019	2019	2020	2020	2021	2021	2022	2022
Sample	***E. coli***	***K. pneumoniae***	***E. coli***	***K. pneumoniae***	***E. coli***	***K. pneumoniae***	***E. coli***	***K. pneumoniae***
	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**	***n* (%)**
Blood	24 (3.4)	12 (6.1)	26 (5.5)	9 (5.6)	27 (5.9)	20 (8.2)	31 (5.4)	30 (9.0)
CSF	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (0.3)
Urine	485 (68.2)	83 (42.1)	305 (63.9)	80 (50.0)	293 (64.4)	126 (51.6)	397 (69.2)	190 (56.9)
LRT	16 (2.3)	23 (11.7)	1 (0.2)	10 (6.3)	1 (0.2)	15 (6.1)	12 (2.1)	34 (10.2)
URT	5 (0.7)	9 (4.6)	3 (0.6)	10 (6.3)	13 (2.9)	10 (4.1)	6 (1.0)	5 (1.5)
SST	111 (15.6)	57 (28.9)	77 (16.1)	39 (24.4)	64 (14.1)	64 (26.2)	87 (15.2)	66 (19.8)
Genital	56 (7.9)	9 (4.6)	46 (9.6)	6 (3.8)	36 (7.9)	6 (2.5)	23 (4.0)	2 (0.6)
Punctate	14 (2)	4 (2.0)	19 (4)	6 (3.8)	21 (4.6)	3 (1.2)	18 (3.1)	6 (1.8)
Total	711 (100)	197 (100)	477 (100)	160 (100)	455 (100)	244 (100)	574 (100)	334 (100)

E. coli—*Escherichia* coli; *K. pneumoniae*—*Klebsiella pneumoniae*; CSF—cerebrospinal fluid; LRT—Lower respiratory tract; URT—Upper respiratory tract; SST—Skin and soft tissue; Punctate—specimens obtained from abdominal, biliary, and pleural punctates.

For *K. pneumoniae*, invasive isolates increased from 6.1% (n = 12) in 2019 to 9.3% (n = 31) in 2022; however, this trend was not statistically significant over the study period (p = 0.40). Among non-invasive isolates, urine specimens were the most frequent source, increasing from 42.1% (n = 83) in 2019 to 56.9% (n = 190) in 2022, with a statistically significant difference between years (p = 0.012). Skin and soft tissue samples represented the second most common source (19.8–28.9%), without significant variation over time (p = 0.088). Isolates from the lower respiratory tract accounted for 6.1–11.7% of cases, with no significant differences observed over the entire study period (p = 0.101). However, a significant increase was noted between 2021 and 2022, from 6.1% (n = 15) to 10.2% (n = 34) (p = 0.045) (Table 1).

3.3. Ward Distribution of Escherichia coli and Klebsiella pneumoniae

Escherichia coli was most frequently recovered from the Internal Medicine department across all years (22.0–26.3%; p = 0.411), followed by Neurology (15.0–18.7%; p = 0.278). Substantial proportions were also observed in Surgery (12.4–19.1%), where the distribution differed significantly over time (p = 0.019), increasing in 2019–2020 and slightly decreasing in 2021–2022. Obstetrics and Gynecology accounted for 8.4–12.3% of noted isolates (p = 0.128). In the ICU and Infectious Diseases, smaller yet consistent proportions were noted (approximately 3–6%), with isolates in ICU exhibiting significant variation throughout the study period (p = 0.013), declining from 4.9% in 2019 to 3.1% and 2.2% in 2020–2021, followed by an increase in 2022 (5.9%). In the Infectious Diseases Department, proportions remained consistent without significant variation (p = 0.913) (Table 2).

Table 2. Distribution of clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* by ward type, 2019–2022.

Ward	2019	2019	2020	2020	2021	2021	2022	2022
	E. coli	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*	*E. coli*	*K. pneumoniae*
	n (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)	*n* (%)
ICU	34 (4.9)	26 (13.2)	15 (3.1)	17 (10.6)	10 (2.2)	26 (10.7)	34 (5.9)	45 (13.5)
Surgery	87 (12.4)	32 (16.2)	91 (19.1)	29 (18.1)	73 (16.0)	45 (18.4)	97 (16.9)	47 (14.1)
Infectious Dis	36 (5.1)	12 (6.1)	25 (5.2)	6 (3.8)	23 (5.1)	40 (16.4)	34 (5.9)	54 (16.2)
Internal Med	164 (23.4)	26 (13.2)	105 (22.0)	25 (15.6)	111 (24.4)	33 (13.5)	151 (26.3)	34 (10.2)
Pulmo/Chest	19 (2.7)	6 (3.0)	4 (0.8)	3 (1.9)	4 (0.9)	16 (6.6)	11 (1.9)	10 (3.0)
Nephrology	21 (3.0)	7 (3.6)	14 (2.9)	17 (10.6)	19 (4.2)	10 (4.1)	19 (3.3)	12 (3.6)
Urology	29 (4.1)	26 (13.2)	22 (4.6)	17 (10.6)	24 (5.3)	14 (5.7)	25 (4.4)	34 (10.2)
Oncology	4 (0.6)	1 (0.5)	3 (0.6)	0 (0.0)	1 (0.2)	2 (0.8)	1 (0.2)	3 (0.9)
Neurology	105 (15.0)	15 (7.6)	89 (18.7)	18 (11.2)	85 (18.7)	30 (12.3)	98 (17.1)	55 (16.5)
Dermatology	10 (1.4)	18 (9.1)	2 (0.4)	5 (3.1)	3 (0.7)	3 (1.2)	0 (0.0)	2 (0.6)
Pediatrics	57(8.1)	7 (3.6)	36 (7.5)	3 (1.9)	20 (4.4)	8 (3.3)	19 (3.3)	6 (1.8)
Neonatology	9 (1.3)	19 (9.6)	7 (1.5)	21 (13.1)	16 (3.5)	13 (5.3)	7 (1.2)	16 (4.8)
Obs./Gyn.	86 (12.3)	6 (3.0)	57 (11.9)	3 (1.9)	50 (11.0)	5 (2.0)	48 (8.4)	3 (0.9)
Rehabilitation	33 (4.7)	2 (1.0)	3 (0.6)	3 (1.9)	4 (0.9)	1 (0.4)	20 (3.5)	10 (3.0)
Psychiatry	7 (1.0)	1 (0.5)	4 (0.8)	1 (0.6)	12 (2.6)	1 (0.4)	10 (1.7)	3 (0.9)
Total	701 (100.0)	197 (100.0)	477 (100.0)	160 (100.0)	455 (100.0)	244 (100.0)	574 (100.0)	334 (100.0)

ICU, Intensive care unit; Infectious Dis, Infective Diseases; Internal Med, Internal Medicine; Pulmo/Chest, Pulmonology/Chest medicine; Surgery, general surgery and surgical subspecialties (e.g., vascular surgery, plastic surgery, breast surgery, endocrine surgery, digestive surgery, neurosurgery, orthopedic and maxillofacial surgery; Obs./Gin.—Obstetrics/Gynecology)

K. pneumoniae isolates were most frequently recovered from Surgery (14.1–18.4%, $p = 0.490$) and the ICU (10.6–13.5%, $p = 0.659$) across the study period, with a marked increase in the contribution of Infectious Diseases in 2021–2022 (16.4%, $n = 40$ and 16.2%, $n = 54$, respectively) compared with 2019–2020 (6.1%, $n = 12$ and 3.8%, $n = 6$, respectively), which was statistically significant ($p < 0.001$). Neurology also increased over time (2019: 7.6%, $n = 15$ to 2022: 16.5%, $n = 55$), also marked as statistically significant ($p = 0.026$). However, Internal Medicine (2019: 13.2%, $n = 26$; 2020: 15.6%, $n = 25$; 2021: 13.5%, $n = 33$; 2022: 10.2%, $n = 34$, $p = 0.340$) and Urology (2019: 13.2%, $n = 26$; 2020: 10.6%, $n = 17$; 2021: 5.7%, $n = 14$; 2022: 10.2%, $n = 34$, $p = 0.062$) consistently accounted for a substantial proportion of isolates, consistently accounted for substantial proportions of isolates without statisticaly significant variation.

Across 2019–2022, *K. pneumoniae* was proportionally more frequent in the ICU than *E. coli* (12.2% vs. 4.2%), showing a borderline difference that did not meet the predefined threshold for statistical significance ($p = 0.051$).

3.4. Resistance of Escherichia coli Isolates to Antimicrobials

Overall, the lowest *E. coli* resistance rates to all tested antimicrobials, except trimethoprim/sulfamethoxazole (TRS), were recorded in 2020. Thereafter, resistance increased across most antimicrobial classes, as shown in (Figure 2; Table A2).

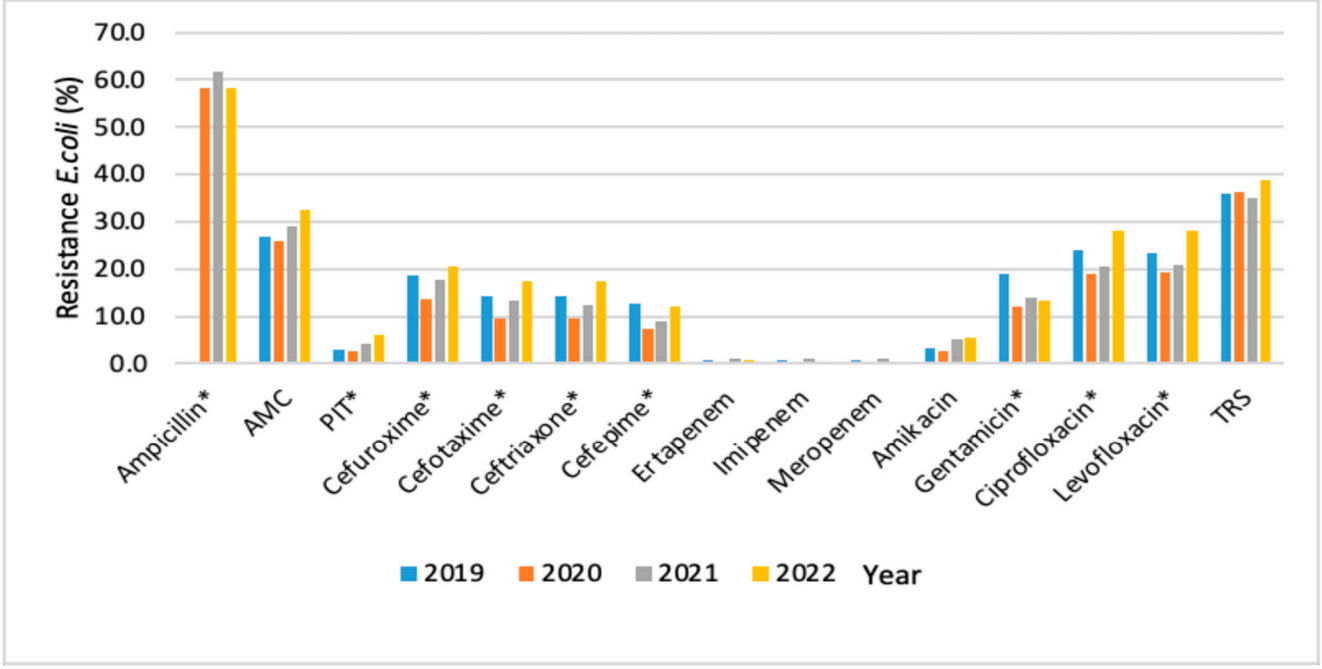


Figure 2. Resistance rates of *Escherichia coli* isolates to antimicrobials, 2019–2022. * Chi-square test for the overall comparison of resistance rates during 2019–2022, significant at $p < 0.05$; AMC—Amoxicillin/Clavulanic acid; PIT—Piperacillin/Tazobactam; Imipenem—imipenem/cilastatin; TRS—Trimethoprim/Sulfamethoxazole.

Resistance to cephalosporins differed significantly across the study period (2019–2022), including cefuroxime ($p = 0.028$), cefotaxime ($p = 0.004$), ceftriaxone ($p = 0.002$), and cefepime ($p = 0.009$). For descriptive purposes, resistance to cefuroxime increased from 18.6% in 2019 to 20.6% in 2022, cefotaxime from 14.4% to 17.4%, and ceftriaxone from 14.2% to 17.3%. Cefepime resistance showed a U-shaped pattern, decreasing from 12.7% in 2019 to 7.3% in 2020 and increasing again in 2021 (8.8%) and 2022 (12.2%). Resistance to piperacillin/tazobactam (PIT), ciprofloxacin, and levofloxacin also differed significantly across 2019–2022 ($p = 0.035$, $p = 0.003$, and $p = 0.004$, respectively), with higher resistance observed in 2022 than in 2019 for PIT (6.2% vs. 3.1%), ciprofloxacin (28.2% vs. 23.9%), and levofloxacin (28.1% vs. 23.9%) (Figure 2; Table A2).

In *E. coli* isolates, notable year-to-year variations in resistance proportions (adjusted with Bonferroni correction, $p < 0.05$) were observed for gentamicin between 2019–2020 and 2019–2022; for ampicillin between 2019–2020, 2019–2022, and 2020–2022; for ciprofloxacin between 2020–2022 and 2021–2022; for levofloxacin, cefuroxime, ceftriaxone, and cefotaxime only between 2020 and 2022; and for cefepime between 2019 and 2020.

Although resistance to amoxicillin/clavulanic acid (AMC) and amikacin increased from 2020 (25.8% and 2.7%, respectively) to 2022 (32.3% and 5.4%, respectively), the differences did not reach statistical significance over the 2019–2022 period ($p = 0.077$ and $p = 0.064$, respectively). No significant changes in carbapenem resistance were observed over the study period. The lowest resistance rates to ertapenem, imipenem, and meropenem were recorded in 2019 (0.5%, 0.2%, and 0.2%, respectively), while the highest rates were observed in 2021 (1.0%, 1.0%, and 0.9%, respectively) (Figure 2; Table A2).

3.5. Resistance of Klebsiella pneumoniae Isolates to Antimicrobials

During 2019–2022, resistance rates among *K. pneumoniae* isolates showed a significant increase across years for most antimicrobials tested ($p < 0.001$), while the increase for AMC was smaller but still statistically significant ($p < 0.05$), likely due to high baseline resistance.

The most pronounced increase was observed for carbapenems, with resistance rates increasing from 19.7% (ertapenem; $n = 23$), 10.7% (imipenem; $n = 19$), and 11.7% (meropenem; $n = 22$) in 2019 to 69.4% (ertapenem; $n = 170$), 53.4% (imipenem; $n = 172$), and 52.0% (meropenem; $n = 173$) in 2022 ($p < 0.001$ for all).

In *K. pneumoniae* isolates, notable year-to-year variations in resistance proportions (adjusted using Bonferroni correction, $p < 0.05$) were seen for the years 2019–2021, 2019–2022, 2020–2021, and 2021–2022 concerning amikacin, ciprofloxacin, levofloxacin, cefepime, imipenem, meropenem, ertapenem, and PIT. The proportions for gentamicin significantly varied between 2019–2022 and 2020–2022, whereas for AMC, the variation occurred only between 2020 and 2022. For TRS and cefuroxime, the resistance proportion varied for 2019–2021 and 2019–2022, whereas for ceftriaxone and cefotaxime, the data varied for 2019–2021, 2019–2022, and 2020–2022, as well.

Colistin susceptibility was assessed in a targeted subset of isolates; among tested isolates, the highest resistance rate (39.3%) was observed in 2020 (Figure 3; Table A3).

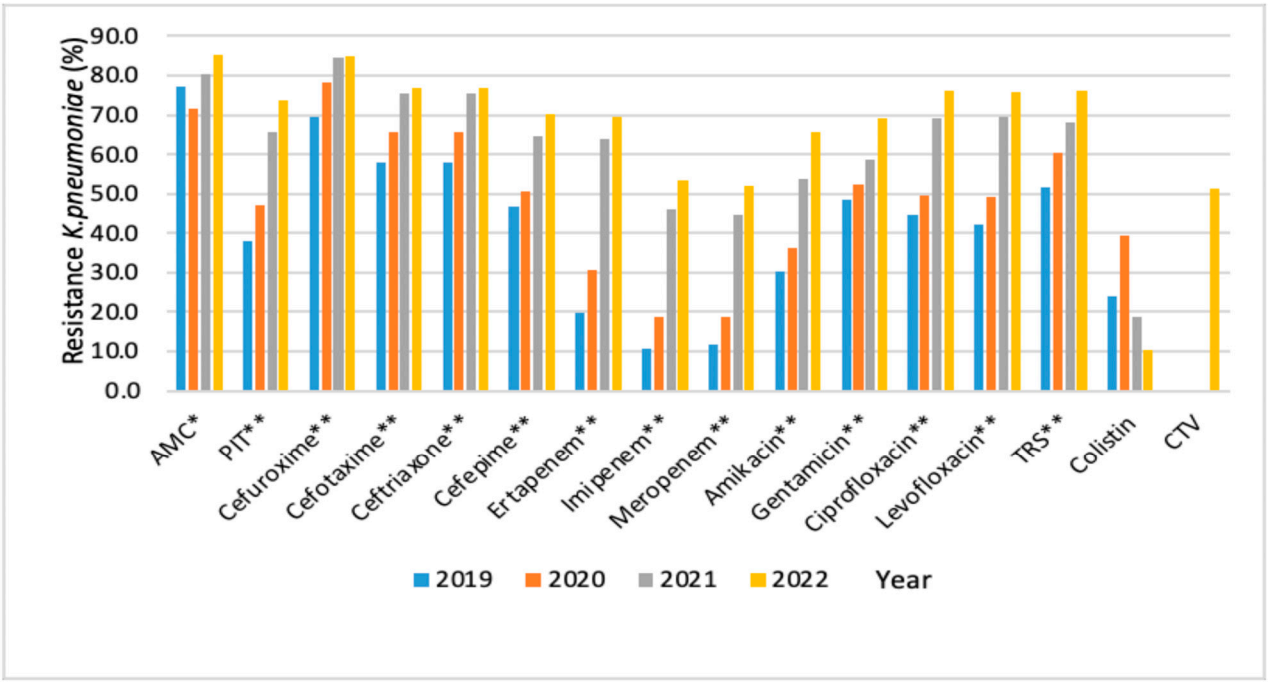


Figure 3. Resistance of *Klebsiella pneumoniae* isolates to antimicrobials 2019–2022. * Chi-square test for the overall comparison of resistance rates during 2019–2022, significant at $p < 0.05$; ** Chi-square test for the overall comparison of resistance rates during 2019–2022, significant at $p < 0.001$; AMC—Amoxicillin/Clavulanic acid; PIT—Piperacillin/Tazobactam; Imipenem—imipenem/cilastatin; TRS—Trimethoprim/Sulfamethoxazole; CTV—ceftazidime/avibactam.

Unlike *E. coli*, during 2019–2022, *K. pneumoniae* resistance rates increased significantly across years ($p < 0.001$), and the number of resistant isolates for most antimicrobials showed an upward trend, peaking in 2022 (Figure 4).

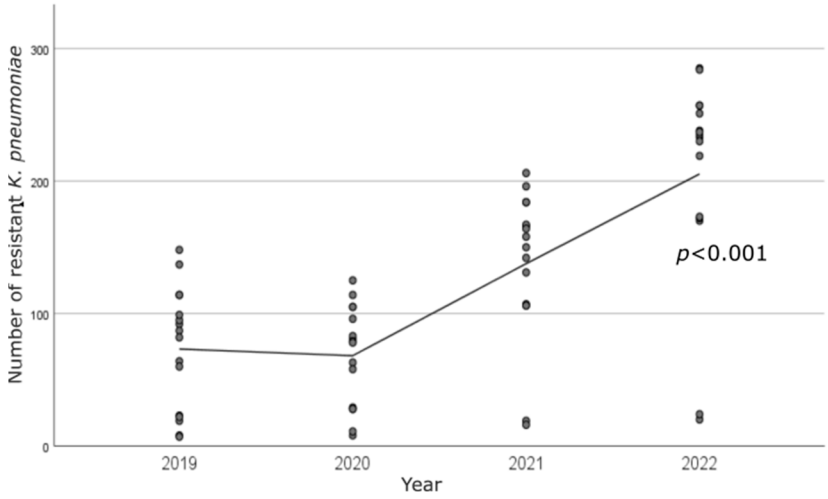


Figure 4. Correlation analysis of resistance in *Klebsiella pneumoniae* isolates to individual antimicrobials, 2019–2022.

3.6. Trend in the Proportion of MDR Escherichia coli and MDR Klebsiella pneumoniae

The proportion of MDR *E. coli* isolates differed significantly across the study period (2019–2022) (p = 0.041), rising from 32.1% (n = 228) in 2019 to 35.4% (n = 203) in 2022 (Figure 5). A detailed ward- and year-specific distribution of MDR *E. coli* isolates is provided in the Supplementary Material (Table S1).

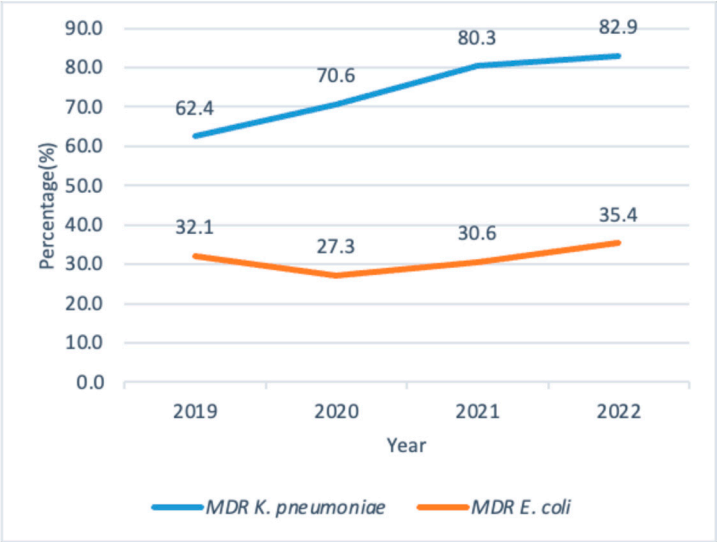


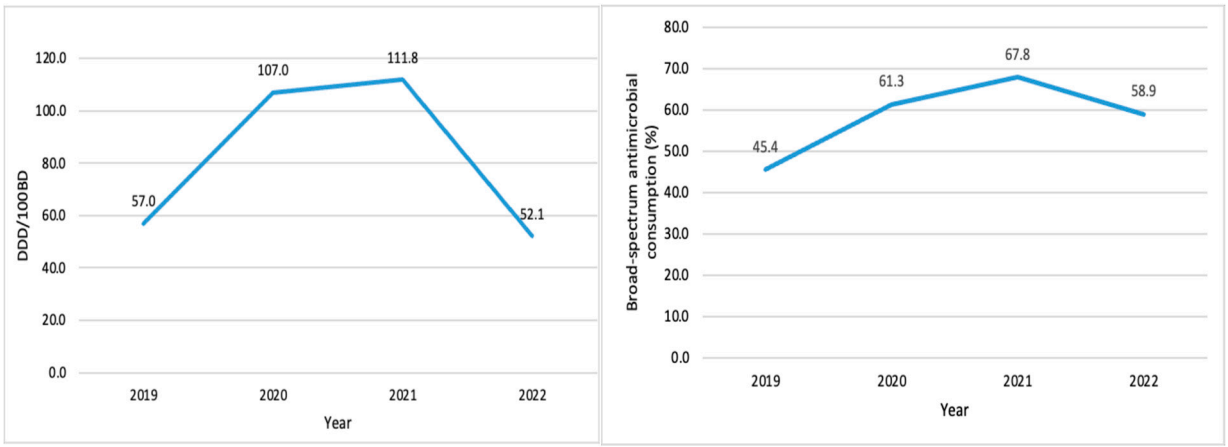
Figure 5. Trend in the proportion of multidrug-resistant (MDR) *Klebsiella pneumoniae* and MDR *Escherichia coli* isolates, 2019–2022. (%)—The proportion of MDR isolates relative to the total number of isolates of each species.

During the period 2019–2022, MDR *K. pneumoniae* isolates exhibited a clear and statistically significant increasing trend (p < 0.001), rising from 62.4% (n = 123) in 2019 to 82.9% (n = 277) in 2022 (Figure 5). A detailed ward- and year-specific distribution of MDR *K. pneumoniae* isolates is provided in the Supplementary Material (Table S2). In addition, the ward- and year-specific distribution of the three most common MDR phenotypes of *K. pneumoniae* is shown in Table S3 and Figure S1.

3.7. Antimicrobial Consumption

Annual antimicrobial consumption increased from 57.0 DDD/100BD in 2019 to 111.8 DDD/100BD in 2021, representing an increase of 96.1%. In 2022, annual consumption

decreased to 52.0 DDD/100BD, corresponding to an 8.8% reduction compared with 2019 (Figure 6a).



(**a**) (**b**)

Figure 6. (**a**) Trend in annual total antimicrobial consumption expressed as defined daily doses per 100 bed–days (DDD/100 BD), 2019–2022; (**b**) The overall proportion of broad-spectrum antimicrobial consumption (glycopeptides, third- and fourth-generation cephalosporins, monobactams, carbapenems, fluoroquinolones, polymyxins, piperacillin with tazobactam, linezolid) among the total antimicrobial consumption expressed as percentages, 2019–2022.

The secondary indicator of hospital antimicrobial consumption, the proportion of 'broad-spectrum antimicrobials' consumption, showed the highest increase in 2020 (+16.7%), followed by a slightly slower increase in 2021 (+6.5%) and a slower decline in 2022 (−8.9%) (Figure 6b).

Antimicrobials Accounting for 75% (DU75) of Annual Hospital Consumption

Detailed annual hospital consumption of antimicrobials is shown in Table 3. Ceftriaxone was the most frequently used antimicrobial across all years. During 2020–2021, particularly in 2021, antimicrobial consumption was largely dominated by a limited number of agents—ceftriaxone, meropenem, amikacin, vancomycin, and metronidazole—which together accounted for approximately 75% of total use (Table 3, Figure A2).

Table 3. Antimicrobials accounting for 75% (DU75) of annual hospital consumption, expressed as defined daily doses per 100 bed–days (DDD/100 BD) and relative proportions, 2019–2022.

	2019	2020	2021	2022
Antimicrobial	**DDD/100BD (%)**	**DDD/100BD (%)**	**DDD/100BD (%)**	**DDD/100BD (%)**
Ceftriaxone	14.52 (25.49)	38.75 (36.22)	46.6 (41.66)	21.5 (41.30)
Metronidazole	6.44 (11.30)	7.76 (7.25)	6.87 (6.14)	5.36 (10.30)
Cefazolin	3.76 (6.61)	/	/	1.57 (3.01)
Cefuroxime	3.59 (6.30)	3.95 (3.69)	/	2.23 (4.28)
Amikacin	3.26 (5.73)	5.26 (4.91)	9.11 (8.14)	3.19 (6.14)
Vancomycin	3.04 (5.34)	10.19 (9.53)	8.7 (7.78)	2.13 (4.08)
Gentamicin	2.97 (5.21)	/	/	/
Meropenem	2.22 (3.90)	8.99 (8.40)	13.47 (12.04)	2.12 (4.07)
Azithromycin	2.22 (3.90)	7.7 (7.20)	/	/
TRS	1.96 (3.44)	/	/	1.83 (3.51)
Total	43.98 (77.20)	82.6 (77.20)	84.75 (75.77)	39.93 (76.70)

Table 3. *Cont.*

	2019	2020	2021	2022
Antimicrobial	**DDD/100BD (%)**	**DDD/100BD (%)**	**DDD/100BD (%)**	**DDD/100BD (%)**
Others	12.99 (22.80)	24.38 (22.80)	27.09 (24.23)	12.13 (23.30)
Total annual consumption	56.97 (100)	106.98 (100)	111.84 (100)	52.06 (100)

TRS—Trimethoprim/Sulfamethoxazole.

3.8. Correlation Between Antimicrobial Consumption and Bacterial Resistance

Despite the increase in antimicrobial consumption and the corresponding rise in *K. pneumoniae* resistance to antimicrobials over the study period, no statistically significant correlation was observed between antimicrobial use and resistance for both *E. coli* and *K. pneumoniae* (Figure 7).

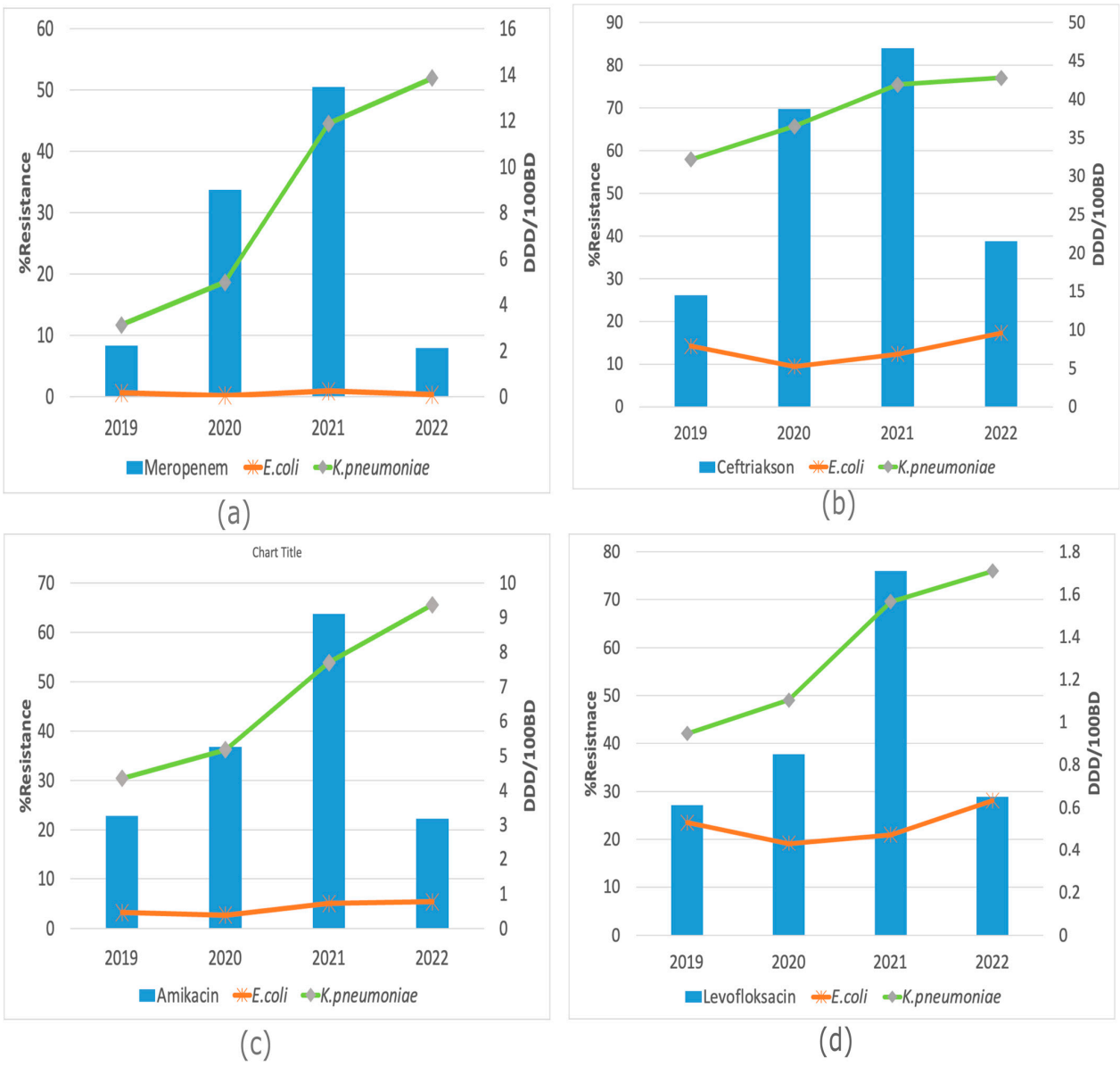


Figure 7. Relationship between antimicrobial consumption and resistance rates of *E. coli* and *K. pneumoniae* during the period 2019–2022 for each antimicrobial. (**a**) Meropenem; (**b**) Ceftriaxone; (**c**) Amikacin; (**d**) Levofloxacin.

Bars depict antimicrobial consumption for the respective antimicrobial expressed as defined daily doses per 100 bed–days (DDD/100 BD), while lines indicate bacterial resistance rates to the same antimicrobial, expressed as the percentage of resistant *E. coli* and *K. pneumoniae* isolates relative to the total number of isolates within each species.

The most notable changes were observed in the annual consumption of meropenem between 2019 and 2021 (2.2 and 13.5 DDD/100 BD, respectively), alongside an increase in *K. pneumoniae* resistance to meropenem (11.7% and 44.5%, respectively) (Figure 7).

4. Discussion

By examining four years of data, this study highlights the evolving relationship between antimicrobial resistance and antimicrobial consumption in two common bacterial pathogens. The impact of the COVID-19 pandemic on antimicrobial resistance varied across countries, with most studies reporting increased antimicrobial resistance [13,19–22]. Reorganization of hospital services, including suspension of elective procedures and limiting admissions to emergency and COVID-19 cases, was commonly reported in the literature [23,24] and was also implemented in our hospital, resulting in a temporary reduction in patient numbers and bed–days in 2020. Hospital activity subsequently peaked in 2021 before returning number of hospitalisations toward pre-pandemic levels in 2022.

4.1. Prevalence and Specimen Distribution of Escherichia coli and Klebsiella pneumoniae Isolates

As expected, *E. coli* remained the most frequently isolated species throughout the study period. The overall decrease in the total number of both bacterial isolates in 2020 is likely attributable to the reduced number of hospitalized patients during that period, coupled with the implementation of stricter hospital infection prevention measures in response to the epidemic. In contrast, the proportion of *K. pneumoniae* increased over time, which may be related to changes in case-mix and the higher burden of severe hospital-acquired infections during the COVID-19 period. This finding is supported by a national study from Serbia, which reported *K. pneumoniae* as one of the leading causative agents of bacterial infections among hospitalized patients with COVID-19 [12].

An expectedly low proportion of invasive isolates was observed for both bacterial species. Among non-invasive isolates, urine was the most frequent specimen type for *E. coli* as well as for *K. pneumoniae*, confirming their predominant role as uropathogens. This distribution is consistent with the findings of Drakul et al. [25], who reported *E. coli* as the most frequently isolated pathogen from urine samples, followed by *K. pneumoniae*. Notably, the proportion of urinary *K. pneumoniae* isolates increased significantly over the study period. In addition, the proportion of lower respiratory tract isolates increased between 2021 and 2022, which may be partly related to the high burden of severe COVID-19 cases during the later pandemic period and the increased use of invasive mechanical ventilation in hospitalized patients.

4.2. Prevalence and Ward Distribution of Escherichia coli and Klebsiella pneumoniae Isolates

Ward-level distribution provided additional clinical context for interpreting resistance dynamics during 2019–2022. *E. coli* was most frequently recovered from Internal Medicine and Neurology wards across all years, with relatively stable proportions, consistent with its predominant role as a uropathogen and a common cause of infections managed outside critical care settings. ICU contribution showed a U-shaped pattern, likely reflecting pandemic-related changes in hospital organization and sampling intensity rather than a true epidemiological shift.

In contrast, *K. pneumoniae* showed a ward distribution more typical of healthcare-associated pathogens, with persistently high proportions in Surgery and the ICU. A marked increase in isolates from the Infectious Diseases and Neurology ward in 2021–2022 may reflect changes in case-mix during the later pandemic period, including a higher burden of severely ill patients, longer hospital stays, and increased exposure to invasive procedures and broad-spectrum antimicrobials. However, ward-level transmission and outbreaks, or clonal spread of carbapenemase-producing lineages, cannot be excluded due to the lack of molecular typing data. Overall, these findings highlight that temporal resistance trends should be interpreted in light of shifting ward structure and patient populations, warranting cautious interpretation of consumption–resistance associations in the absence of patient-level covariates.

4.3. Resistance of Escherichia coli and Klebsiella pneumoniae to Antimicrobials

The decrease in overall *E. coli* resistance observed in 2020 may reflect changes in patient case-mix and hospital activity during the pandemic, including fewer elective procedures and strengthened infection prevention and control measures. In contrast, resistance of *E. coli* to most antimicrobial agents increased again during 2021–2022, a trend also reported in Mexico, where resistance rose across nearly all tested agents, including carbapenems [26]. Although the proportion of carbapenem-resistant *E. coli* increased fourfold in 2021 compared with 2020, the absolute number of such isolates remained very low, and no statistically significant difference in carbapenem resistance was detected across the entire study period. At the national level in Serbia, carbapenem-resistant invasive *E. coli* also remained rare, increasing from 0.4% in 2019 to 3.2% in 2021 and declining to 1.5% in 2022, a trend consistent with our findings [27].

Resistance of *E. coli* and *K. pneumoniae* to cephalosporins, aminoglycosides, and fluoroquinolones, as well as resistance of *K. pneumoniae* to carbapenems, was approximately 20% lower than the national data for Serbia for invasive isolates in 2022 [27]. This difference may be partly related to the secondary-care setting of our hospital, whereas national surveillance also includes tertiary-care centres, which typically report higher resistance burdens, and possibly also to the implementation of more effective infection control measures compared with other secondary-level facilities. Compared with *K. pneumoniae*, *E. coli* showed a more stable and less consistent resistance profile over time.

Resistance of *K. pneumoniae* to most tested antimicrobials steadily increased during the pandemic, with particularly high levels observed toward its end. A similar trend was reported in hospitals in Turkey [24]. In our study, the most pronounced rise—over fourfold—was observed for carbapenems. According to a report by the Centers for Disease Control and Prevention (CDC), hospital-onset carbapenem-resistant *Enterobacterales* in the United States increased by 35% during the COVID-19 pandemic [28]. Rises in carbapenem resistance have also been documented in studies from Serbia, Slovenia, Italy, Mexico, and India, highlighting that similar increases were reported internationally during the pandemic period, in parallel with our findings. [13,19,22,26,29,30].

However, by 2022, our isolates showed resistance rates comparable to those reported for general hospitals in Serbia for third-generation cephalosporins (76.9% vs. 75.5%) and carbapenems (53.4% vs. 55.9%) according to data from the Annual Report on Infectious Diseases in the Republic of Serbia for 2022 from the Institute of Public Health of Serbia "Dr Milan Jovanović Batut" [31].

Cross-country comparisons should be interpreted cautiously, as Serbia differs from many EU/EEA settings in terms of antimicrobial prescribing practices, availability of hospital-sector consumption surveillance, limited antimicrobial stewardship capacity, inconsistent infection prevention and control practices, and patient referral patterns. There-

fore, although our findings are broadly consistent with international reports of increased broad-spectrum antibiotic use and rising resistance during the COVID-19 period, the magnitude and dynamics of these changes may not be directly comparable across countries.

The marked increase in *K. pneumoniae* resistance, particularly to carbapenems, should be interpreted cautiously. Besides changes in antimicrobial consumption, alternative explanations may include clonal spread of a successful hospital-adapted lineage, introduction and dissemination of carbapenemase-producing strains (e.g., OXA-48-like or NDM-producing isolates), or multiple parallel transmission events occurring across wards. In addition, the COVID-19 period was associated with major changes in hospital organization and case-mix, including increased ICU burden and intensive use of invasive devices, which may have contributed to a higher diagnostic intensity and altered microbiological sampling patterns. Because molecular typing and carbapenemase characterization were not available in this study, we cannot determine whether the observed rise reflects expansion of a single clone/outbreak or broader endemic transmission, which limits the epidemiological interpretation and the ability to propose targeted containment measures.

At the surveillance level, the prevalence of the MDR *E. coli* phenotype simultaneously resistant to third-generation cephalosporins, fluoroquinolones, and aminoglycosides is routinely monitored [8]. This was the most prevalent MDR *E. coli* phenotype identified in the present study, showing an almost threefold increase from 2020 to a peak in 2022 (5.9%), while remaining substantially lower than the prevalence reported among invasive *E. coli* isolates in Serbia (17%) in the same year [27]. No clear ward-level predominance of this MDR phenotype was observed, as its distribution across hospital wards remained heterogeneous without a consistent concentration in a specific department.

In 2019, the MDR phenotype of *K. pneumoniae* most commonly involved simultaneous resistance to third-generation cephalosporins, fluoroquinolones, and aminoglycosides (23.4%). Its frequency subsequently declined as resistance to carbapenems increased, giving rise to a broader MDR phenotype. Overall, the prevalence of MDR *K. pneumoniae* isolates increased by approximately one third, rising from 62.4% in 2019 to 82.9% in 2022. Although these values are high, they remained lower than those reported in the ICU of a tertiary-level institution in Serbia, where MDR *K. pneumoniae* accounted for 91.2% even before the pandemic [32].

Beyond overall MDR prevalence, ward-specific distribution of MDR phenotypes was also observed. In the Neonatology ward, MDR phenotype I—defined by resistance to amoxicillin/clavulanic acid, cephalosporins, aminoglycosides, and trimethoprim/sulfamethoxazole—was consistently detected in high numbers throughout the study period. This phenotype was uncommon in other wards and remained susceptible to fluoroquinolones and carbapenems. Given the ward-specific persistence of this resistance profile, it may reflect circulation of a hospital-adapted strain within Neonatology. This pattern could also be influenced by local prescribing practices, as fluoroquinolones are not used in neonatal care and carbapenems are rarely administered.

In 2019, the most frequent MDR phenotype overall was phenotype II, characterized by resistance to amoxicillin/clavulanic acid, narrow- and broad-spectrum cephalosporins, aminoglycosides, trimethoprim/sulfamethoxazole, fluoroquinolones, and piperacillin/tazobactam, while remaining susceptible to carbapenems and colistin. This phenotype was most frequently observed in Urology. Over the subsequent three years, the total number of MDR isolates increased, particularly those additionally resistant to carbapenems. Such isolates (phenotype III) were most frequently recovered from Infectious Diseases, Neurology, the ICU, Surgery, and Internal Medicine. Notably, the proportion of *K. pneumoniae* isolates recovered from the Infectious Diseases ward increased significantly in 2021–2022 compared with 2019–2020 ($p < 0.001$), coinciding with the higher frequency of MDR phenotype III

in these years. Phenotype III was also consistently detected in Nephrology and Urology, which is expected given the role of *K. pneumoniae* as a frequent cause of catheter-associated urinary tract infections.

The increasing contribution of Surgery and, particularly, the marked rise in Infectious Diseases and Internal Medicine during 2021–2022 may reflect changes in patient case-mix and healthcare exposure during the later pandemic period. In 2022, a higher number of isolates resistant to all tested antimicrobial groups was observed, including isolates with additional resistance to colistin and ceftazidime/avibactam.

Overall, these ward-specific patterns should be interpreted cautiously, as they may reflect localized transmission dynamics and changes in patient case-mix during the COVID-19 period. The lack of molecular typing and carbapenemase characterization limits the epidemiological interpretation of these findings.

4.4. Antimicrobial Consumption

In our hospital, annual antimicrobial consumption increased markedly at the onset of the COVID-19 pandemic, reaching peak values in 2020–2021 that were almost twofold higher than pre-pandemic levels. This was followed by a decline in 2022, when consumption fell below 2019 values. This reduction occurred in 2022, in parallel with strengthened antimicrobial stewardship activities and infection control measures in our hospital.

Comparable trends have been reported in other settings. In Turkey, antimicrobial consumption increased from 72.58 DDD/100BD in the pre-pandemic period to 81.14 DDD/100BD during the early pandemic [24]; although baseline consumption in that hospital was higher than in our setting, the relative increase observed during the pandemic was greater in our hospital, with 2020 values exceeding those reported in Turkey.

Even more pronounced increases were documented elsewhere. In Spain, a 77% rise in hospital antimicrobial consumption during the first pandemic wave was reported, increasing from 79.94 to 141.10 DDD/100 BD [33]. Similar increases were observed in studies from the United States, Portugal, Spain, and Pakistan [34–38].

However, trends differed in some settings. Spanish hospitals reported a decline in antimicrobial consumption as early as the second quarter of 2020 [39], whereas in our study, a reduction was not observed until 2022.

Although direct comparison is limited by differences in measurement approaches, the observed increase in broad-spectrum antimicrobial consumption in our study, representing an approximately 50% relative increase (from 45.4% in 2019 to 67.8% in 2021), is consistent with the EU/EEA-reported shift toward broader-spectrum and last-line agents, particularly carbapenems, during the same period [40].

To complement DDD/100 bed–days, we used the Drug Utilization 75% (DU75%) metric to identify the antimicrobials accounting for 75% of total consumption, as an indicator of prescribing concentration and stewardship priorities, rather than a direct driver of resistance. During the study period, marked changes in antimicrobial consumption patterns were observed. Broad-spectrum agents, particularly ceftriaxone, meropenem, and vancomycin, exhibited marked increases during 2020–2021, whereas narrow-spectrum antimicrobials, including first- and second-generation cephalosporins, gentamicin, and trimethoprim/sulfamethoxazole, demonstrated a decline. The increased use of carbapenems may have been influenced by rising resistance to third-generation cephalosporins among Gram-negative pathogens and a shift toward broader-spectrum empirical therapy during the pandemic period. At the same time, cefazolin use declined, as it is primarily employed for surgical prophylaxis, and the number of surgical procedures decreased during the pandemic, a trend observed both in our hospital and worldwide [24].

In our study, ceftriaxone was the most frequently prescribed antimicrobial. This finding is consistent with reports from a Serbian tertiary-care hospital treating COVID-19 patients, where ceftriaxone also predominated, followed by fluoroquinolones and carbapenems [41]. In contrast, in our hospital, carbapenem use exceeded that of fluoroquinolones, which were restricted due to the risk of *Clostridioides difficile* infection. According to Gajić et al. [12], metronidazole, glycopeptides, and cephalosporins were most frequently prescribed in other Serbian tertiary-care centers treating COVID-19 patients, which contrasts with our findings, where ceftriaxone, vancomycin, and meropenem predominated. Increases in broad-spectrum antimicrobial use during the first pandemic waves have also been reported in hospitals in Switzerland and Turkey [23,24]. In contrast, studies from Israel highlight that strict adherence to antimicrobial stewardship guidelines during successive COVID-19 waves resulted in reduced consumption [42]. A decline following the same pattern was achieved in our hospital, although only in 2022, reflecting both accumulated knowledge about COVID-19 and renewed stewardship and infection control efforts.

4.5. Correlation Between Antimicrobial Resistance of Escherichia coli and Klebsiella pneumoniae and Antimicrobial Consumption

Changes in *E. coli* resistance did not mirror trends in the consumption of the corresponding antimicrobials, with resistance remaining relatively stable despite marked fluctuations in consumption. Notably, levofloxacin consumption increased in 2020–2021, whereas *E. coli* resistance decreased in 2020 before rising again in 2022, indicating a non-parallel ("U-shaped") pattern. For *K. pneumoniae*, resistance increased during 2020–2021 in parallel with increased antimicrobial use; however, resistance did not decline in 2022 despite reduced consumption, showing only a slower rate of increase. Overall, no statistically significant correlations between antimicrobial consumption and resistance were observed. These findings should be interpreted cautiously, as consumption–resistance relationships may require longer observation periods to be reliably detected, as highlighted by Peñalva et al. [43]. In addition, resistance to one antimicrobial may be influenced by the use of another through co-selection mechanisms [43]; for example, broad-spectrum cephalosporins can co-select fluoroquinolone resistance when resistance determinants are carried on the same plasmid [44]. Moreover, changes in infection prevention and control practices can substantially shape resistance trends, particularly for *K. pneumoniae*, which is predominantly associated with healthcare-related infections [43]. Given the retrospective observational design and the lack of significant correlations, causal relationships cannot be inferred.

By contrast, Medic et al. [45] (2023) reported significant positive associations in Serbia, particularly between meropenem, ceftriaxone, and levofloxacin consumption and resistance of invasive *K. pneumoniae*, as well as between ertapenem use and resistance of invasive *K. pneumoniae* and *E. coli*. These discrepancies may reflect differences in study design, the inclusion of invasive versus non-invasive isolates, and the higher resistance burden typically observed in tertiary-care settings compared with secondary hospitals such as ours.

Beyond hospital practices, resistance levels in human isolates are also influenced by antimicrobial use in food-producing animals, highlighting the need for an integrated One Health approach to antimicrobial resistance.

Several limitations should be noted. First, this was a single-center study, which may limit the generalizability of the findings to other hospitals in Serbia, particularly tertiary-care institutions. Second, due to the retrospective design and limitations of the microbiology database, we were unable to assess molecular epidemiology and reliably link all isolates to patient-level clinical data (e.g., demographics, comorbidities, COVID-19 status, prior antibiotic exposure, or clinical outcomes). Therefore, multivariable analyses to control

for confounding could not be performed. Consequently, temporal changes in resistance and antimicrobial consumption should be interpreted as ecological trends, with possible residual confounding, as the consumption–resistance correlation was based on aggregated annual hospital-level data and therefore has limited sensitivity to ecological bias and time-lagged effects. Third, prescribing appropriateness was not assessed; therefore, changes in antimicrobial consumption cannot be attributed to guideline-concordant empirical therapy, overtreatment, or shifts in disease severity. In addition, COVID-19-related changes in infection prevention and control practices and hospital reorganization may have influenced both sampling intensity and transmission dynamics, independently of antimicrobial use. Fourth, due to restricted material resources and national drug registration constraints, susceptibility testing was not available for all antimicrobials throughout the study period, precluding assessment of extensively drug-resistant (XDR) and pandrug-resistant (PDR) phenotypes. Colistin testing was performed selectively and not systematically across study years, which may introduce selection bias and limits interpretation of temporal changes. Susceptibility for newer β-lactam/β-lactamase inhibitor combinations was introduced after their clinical availability. Testing for ceftazidime/avibactam was introduced in 2022; therefore, resistance to these agents may therefore be underestimated.

Despite these limitations, the inclusion of both invasive and non-invasive isolates of *E. coli* and *K. pneumoniae* over a four-year period, combined with hospital antimicrobial consumption data, provides valuable insight into local resistance dynamics in a secondary-care hospital setting.

5. Conclusions

This study provides insight into antimicrobial resistance and antimicrobial consumption patterns in a secondary-care hospital in Serbia during 2019–2022. Resistance in *K. pneumoniae*, particularly to carbapenems, increased substantially over the study period, while hospital antimicrobial consumption peaked in 2020–2021 and declined in 2022. The findings support the need for sustained local surveillance and targeted stewardship efforts focusing on high-use agents (e.g., ceftriaxone and meropenem) and high-risk settings such as the ICU.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/microbiolres17020040/s1. Figure S1: Dynamics of the number of MDR *Klebsiella pneumoniae* isolates resistant to eight antibiotic classes by ward; Table S1: Distribution of MDR *Escherichia coli* isolates by hospital ward, 2019–2022; Table S2: Distribution of MDR *Klebsiella pneumoniae* isolates by hospital ward, 2019–2022; Table S3: Distribution of a the three most common MDR phenotypes of *K. pneumoniae* by ward.

Author Contributions: Conceptualization, N.O. and I.G.; writing—original draft preparation, M.B., M.J.P. and A.T.; writing—review and editing, N.O. and I.G.; results visualization, M.J.P. and A.T.; supervision, I.G. and N.O. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of the Healthcare Center Užice, General Hospital Užice (protocol code 1131/6, 10 February 2025).

Informed Consent Statement: The bacterial isolates were obtained during routine microbiological diagnostics performed as part of standard clinical care. The study retrospectively evaluated relevant laboratory data and antimicrobial consumption data obtained from the hospital pharmacy, all of which were fully anonymised before analysis. No identifying patient or participant information (including personal details, images, or videos relating to any individual) was evaluated or used in

the study. In accordance with local legislation, informed consent for participation is not required for non-interventional, retrospective studies based on anonymised data.

Data Availability Statement: The original contributions presented in this study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AMR	Antimicrobial resistance
ECDC	European Centre for Disease Prevention and Control
EU/EEA	European Union/European Economic Area
OECD	Organisation for Economic Co-operation and Development
WHO	World Health Organization
COVID-19	Coronavirus Disease 2019
DDD	Defined Daily Dose
ICU	Intensive care unit
EUCAST	European Committee on Antimicrobial Susceptibility Testing
MDR	Multidrug resistance
EARS-Net	European Antimicrobial Resistance Surveillance Network
ATC	Anatomical Therapeutic Chemical
DDD/100BD	DDDs per 100 bed–days
ESAC-Net	European Surveillance of Antimicrobial Consumption Network
DU75%	Drug Utilization 75%
CDC	Centers for Disease Control and Prevention
AMC	Amoxicillin/Clavulanic acid
PIT	Piperacillin/tazobactam
TRS	Trimethoprim/Sulfamethoxazole
PDR	Pandrug-resistant
XDR	Extensively drug-resistant

Appendix A

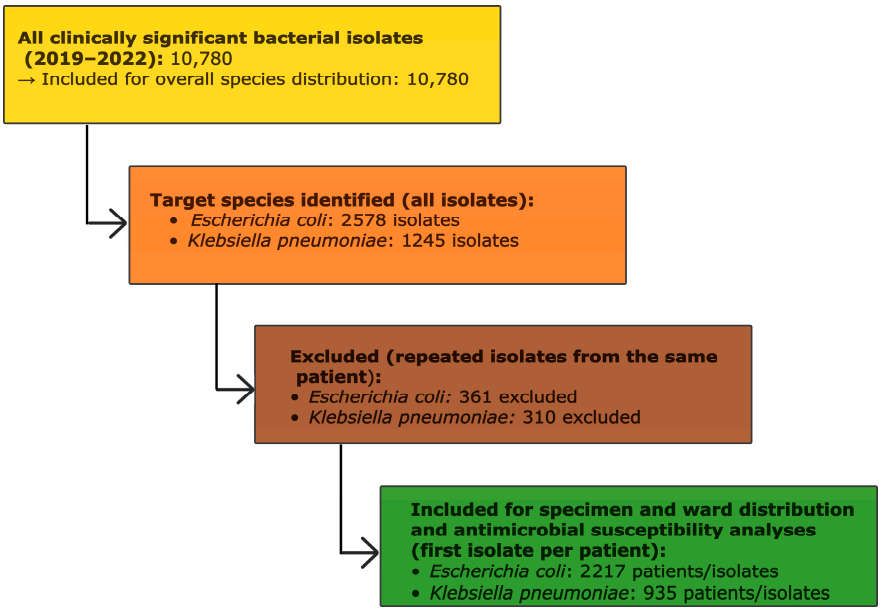


Figure A1. Flow chart of isolate selection and inclusion.

Table A1. Distribution of the most frequently isolated bacterial species among all isolates, 2019–2022.

	2019	2020	2021	2022
Bacteria	**% (*n*)**	**% (*n*)**	**% (*n*)**	**% (*n*)**
Escherichia coli	824 (24.7)	572 (24.8)	526 (21.6)	656 (24.2)
Enterococcus spp.	495 (14.9)	351 (15.3)	463 (19.0)	569 (20.9)
Staphylococcus aureus	403 (12.1)	287 (12.5)	247 (10.1)	267 (9.9)
Klebsiella pneumoniae	258 (7.8)	219 (9.5)	311 (13.6)	457 (16.9)
Pseudomonas aeruginosa	246 (7.4)	130 (5.6)	166 (6.8)	96 (3.6)
Acinetobacter spp.	225 (6.8)	178 (7.7)	162 (6.6)	90 (3.3)
Proteus spp.	196 (5.9)	139 (6.0)	160 (6.6)	190 (7.0)
Other species	684 (20.5)	427 (18.5)	405 (16.6)	379 (14.0)
Total	3331 (100)	2305 (100)	2440 (100)	2704 (100)

Table A2. Resistance rates of *Escherichia coli* isolates to antimicrobials, 2019–2022.

	2019	2020	2021	2022	
Antimicrobial	**% (*n*)**	**% (*n*)**	**% (*n*)**	**% (*n*)**	***p* Value**
Ampicillin	77.3 (467/604)	58.2 (242/416)	61.6 (244/396)	67.1 (306/456)	0.001 *
AMC	26,8 (186/694)	25.8 (120/466)	29.1 (129/443)	32.3 (184/569)	0.077
PIT	3.1 (18/581)	2.6 (10/384)	4.1 (15/366)	6.2 (29/466)	0.035 *
Cefuroxime	18.6 (131/706)	13.6 (65/477)	17.6 (80/455)	20.6 (118/572)	0.028 *
Cefotaxime	14.4 (102/710)	9.6 (46/477)	13.4 (61/454)	17.4 (100/574)	0.004 *
Ceftriaxone	14.2 (101/711)	9.4 (45/477)	12.3 (56/455)	17.3 (99/574)	0.002 *
Cefepime	12.7 (90/711)	7.3 (35/477)	8.8 (40/455)	12.2 (70/574)	0.009 *
Ertapenem	0.7 (4/616)	0.5 (2/438)	1.0 (4/395)	0.8 (4/492)	0.801
Imipenem	0.6 (4/644)	0.2 (1/452)	1.0 (4/417)	0.4 (2/532)	0.462
Meropenem	0.6 (4/650)	0.2 (1/453)	0.9 (4/424)	0.4 (2/535)	0.472
Amikacin	3.2 (23/710)	2.7 (13/476)	5.1 (23/454)	5.4 (31/572)	0.064
Gentamicin	18.8 (133/706)	12.2 (58/476)	13.9 (63/454)	13.4 (77/574)	0.005 *
Ciprofloxacin	23.9 (167/699)	18.9 (87/460)	20.7 (93/450)	28.2 (158/561)	0.003 *
Levofloxacin	23.5 (164/698)	19.1 (88/460)	20.9 (94/449)	28.1 (158/562)	0.004 *
TRS	35.8 (253/707)	36.2 (172/475)	35.1 (158/450)	38.8 (221/569)	0.595

* Chi-square test for the overall comparison of resistance rates during 2019–2022, significant at $p < 0.05$; AMC—Amoxicillin/Clavulanic acid; PIT—Piperacillin/Tazobactam; TRS—Trimethoprim/Sulfamethoxazole.

Table A3. Resistance of *Klebsiella pneumoniae* isolates to antimicrobials 2019–2022.

	2019	2020	2021	2022	
Antimicrobial	**% (*n*)**	**% (*n*)**	**% (*n*)**	**% (*n*)**	***p* Value**
AMC	77.1 (148/192)	71.7 (114/159)	80.3 (196/244)	85.3 (285/334)	0.003 *
PIT	38.1 (64/168)	47.0 (63/134)	65.8 (150/228)	73.7 (233/316)	<0.001 **
Cefuroxime	69.54 (137/197)	78.1 (125/160)	84.4 (206/244)	85.0 (284/334)	<0.001 **
Cefotaxime	57.9 (114/197)	65.6 (105/160)	75.4 (184/244)	77.0 (257/334)	<0.001 **
Ceftriaxone	57.9 (114/197)	65.6 (105/160)	75.4 (184/244)	77.0 (257/334)	<0.001 **
Cefepime	46.7 (92/197)	50.6 (81/160)	64.8 (158/244)	70.4 (235/334)	<0.001 **
Ertapenem	19.7 (23/117)	30.5 (29/95)	64.1 (107/167)	69.4 (170/245)	<0.001 **
Imipenem	10.7 (19/188)	18.9 (28/148)	46.1 (106/230)	53.4 (172/322)	<0.001 **
Meropenem	11.7 (22/188)	18.7 (28/150)	44.5 (106/238)	52.0 (173/333)	<0.001 **
Amikacin	30.5 (60/197)	36.3 (58/160)	53.9 (131/243)	65.6 (219/334)	<0.001 **
Gentamicin	48.5 (95/196)	52.2 (83/159)	58.7 (142/242)	69.3 (230/332)	<0.001 **

Table A3. *Cont.*

	2019	2020	2021	2022	
Antimicrobial	**% (*n*)**	**% (*n*)**	**% (*n*)**	**% (*n*)**	***p* Value**
Ciprofloxacin	44.6 (87/195)	49.7 (79/159)	69.2 (166/240)	76.3 (238/312)	<0.001 **
Levofloxacin	42.1 (82/195)	49.1 (78/159)	69.6 (167/240)	76.0 (237/312)	<0.001 **
TRS	51.6 (99/192)	60.4 (96/159)	68.1 (164/241)	76.3 (251/329)	<0.001 **
Colistin	24.1 (7/29)	39.3 (11/28)	18.8 (16/85)	10.5 (24/229)	NA
CTV	0/0	0/0	0/0	51.4 (36/70)	NA

* Chi-square test for the overall comparison of resistance rates during 2019–2022, significant at $p < 0.05$; ** Chi-square test for the overall comparison of resistance rates during 2019–2022, significant at $p < 0.001$; AMC—Amoxicillin/Clavulanic acid; PIT—Piperacillin/Tazobactam; TRS—Trimethoprim/Sulfamethoxazole; CTV—ceftazidime/avibactam; NA, not applicable, CTV tested only in 2022.

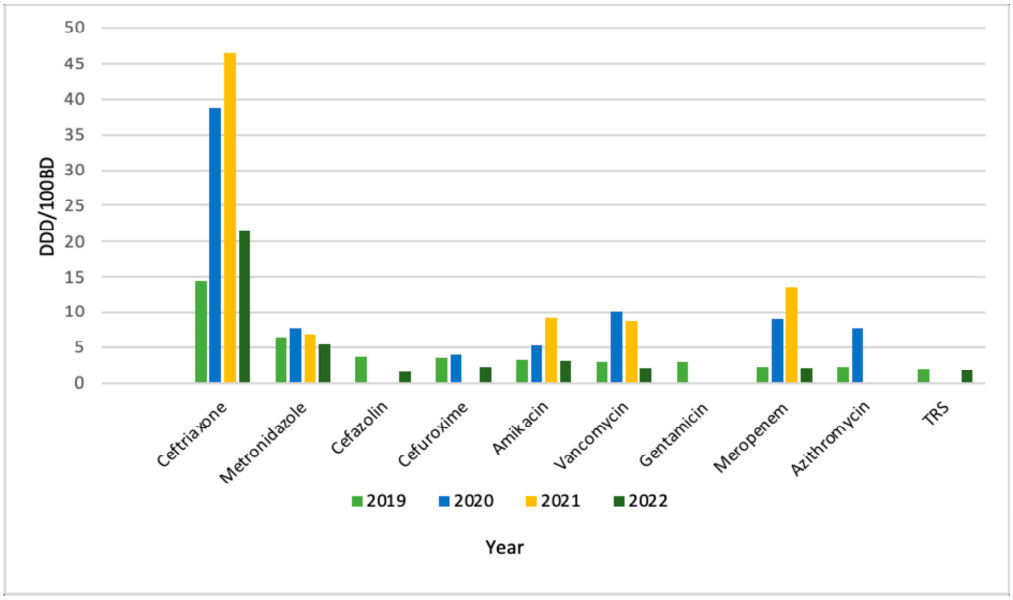


Figure A2. Antimicrobials accounting for 75% (DU75) of annual hospital consumption, expressed as defined daily doses per 100 bed–days (DDD/100 BD) and relative proportions, 2019–2022.

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