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Antibiotic Susceptibilities of Escherichia coli and Klebsiella pneumoniae Isolates from Clinical Samples: 3-Year Analysis

ABSTRACT

Objectives

This study presents antibiotic susceptibility data for Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae) isolates recovered in our hospital between January 1, 2020, and December 31, 2022.

Methods

Over the 3-year period, we analyzed annual antibiograms and extended-spectrum β-lactamase (ESBL) positivity rates of E. coli and K. pneumoniae isolates recovered from urine and non-urine clinical specimens submitted to the clinical microbiology laboratory. Only isolates identified as causative agents of infection in adult patients were included. Data were stratified by specimen type into urinary and non-urinary groups; non-urinary specimens comprised blood, respiratory, and cerebrospinal fluid (CSF) samples. Antimicrobial susceptibility testing was performed using the disk diffusion method and the VITEK®2 Compact automated system (bioMérieux, France). ESBL production was assessed using the double-disk synergy test and the automated system. Antibiogram quality control was routinely performed monthly.

Results

A total of 4,129 E. coli and 1,385 K. pneumoniae isolates were included. Overall ESBL positivity was 21.0% for E. coli and 33.2% for Klebsiella spp. Over the study period, E. coli isolates showed susceptibility rates exceeding 80% for carbapenems, aminoglycosides, ceftriaxone, and fosfomycin.

Conclusion

Determining susceptibility profiles and ESBL positivity rates for commonly isolated pathogens such as E. coli and K. pneumoniae is critical. Healthcare institutions should perform these analyses regularly in accordance with national and international guidelines and share results with relevant stakeholders. Such efforts support local and national antimicrobial stewardship programs and guide empirical therapy strategies.

Keywords: Escherichia coli; ESBL; Klebsiella pneumoniae; antimicrobial susceptibility

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Introduction

Year-to-year and regional variation in antimicrobial resistance is clinically important, particularly for selecting empirical therapy in hospitalized patients and reducing morbidity and mortality (1). Antimicrobial resistance (AMR) is an escalating global health threat that requires urgent action through international collaboration (2). The World Health Organization (WHO) has projected that, without effective preventive measures, AMR could contribute to up to 10 million deaths annually by 2050 (3).

Institution-level surveillance of antimicrobial susceptibility patterns is essential for guiding empirical therapy (4–6). In this context, monitoring resistance trends among WHO-designated critical- and high-priority pathogens—often discussed in relation to ESKAPE organisms (7,8)—is particularly important.

Among Gram-negative bacteria, extended-spectrum β-lactamase (ESBL) production is a major mechanism of β-lactam resistance (9,10). Members of the Enterobacterales family, especially E. coli and K. pneumoniae, may hydrolyze penicillins and third-

generation cephalosporins via ESBL production, making detection and reporting of ESBLs crucial in both clinical care and microbiology laboratories (11). ESBL genes are frequently associated with co-resistance to other antimicrobial classes, further complicating therapy (11,12). Consequently, rising resistance contributes to increased morbidity and mortality (13).

Regular evaluation of institutional antibiogram data helps prevent the use of ineffective agents and reduces unnecessary use of broad-spectrum antibiotics when isolate-specific results are not yet available. Such analyses support appropriate empirical regimens and inform stewardship policies (4,14). Therefore, we aimed to analyze antibiotic susceptibility and ESBL positivity rates of E. coli and K. pneumoniae isolates recovered between January 2020 and December 2022.

Materials and Methods

Clinical isolates of E. coli and K. pneumoniae were obtained from cultures submitted to the clinical microbiology laboratory from adult inpatients and outpatients at Sincan Training and Research Hospital. Isolates were considered causative agents based on leukocyte presence, pure growth, and criteria defined by national and international guidelines. Antimicrobial susceptibility test results for isolates collected between January 1, 2020, and December 31, 2022, were retrospectively analyzed.

Clinical specimens were inoculated onto 5% sheep blood agar and eosin methylene blue (EMB) agar using sterile loops and incubated aerobically at 35–37°C. After overnight incubation, growth was evaluated. For normally sterile specimens (e.g., CSF and pleural fluid), if no growth was observed, incubation was extended for an additional 48 hours before final reporting.

Bacterial identification and antimicrobial susceptibility testing were performed using conventional methods and the VITEK®2 Compact automated system (bioMérieux, France). Susceptibility results were interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines. Data were stratified by specimen type as urine versus non-urine (blood, respiratory samples, and CSF), according to the number of isolates available for each category.

To minimize redundancy, duplicate isolates from the same patient were excluded and only the first isolate was included. Due to low isolate counts (<30), organisms recovered from pleural fluid, pericardial fluid, and other uncommon specimen types were excluded.

In our laboratory, susceptibility testing for urinary E. coli isolates is routinely performed using disk diffusion in accordance with EUCAST guidelines (version 13.0, 2023). Disks (Bioanalyse®, Türkiye) included amikacin (AN, 30 µg), gentamicin (GN, 10 µg), ampicillin (AM, 10 µg), amoxicillin/clavulanic acid (AMC, 20/10 µg), ceftriaxone (CRO, 30 µg), cefepime (FEP, 30 µg), trimethoprim/sulfamethoxazole (SXT, 1.25/23.75 µg), piperacillin/tazobactam (TZP, 30/6 µg), fosfomycin (FOS, 200 µg; only for uncomplicated E. coli urinary tract infection), meropenem (MEM, 10 µg), and ciprofloxacin (CIP, 5 µg).

For isolates from non-ICU patients, disk diffusion was used, whereas isolates from ICU patients were tested using the VITEK®2 system.

ESBL production was assessed using the double-disk synergy test and the VITEK®2 system, as previously described by Akpaka et al. (10). Quality control of susceptibility testing was performed monthly.

Ethical approval was obtained from the Ankara Bilkent City Hospital Clinical Research Ethics Committee (Decision No: E1-23-4360).

Statistical Analysis

ESBL positivity and antibiotic susceptibility rates were calculated descriptively.

Results

During the 3-year period, 4,129 E. coli and 1,385 K. pneumoniae isolates were included. Among E. coli isolates, 2,448 (59.3%) were obtained from outpatients and 1,681 (40.7%) from hospitalized patients. For K. pneumoniae, 432 (31.2%) isolates were from outpatients and 953 (68.8%) from hospitalized patients.

In 2020, 1,192 E. coli and 424 K. pneumoniae isolates were recovered; 914 (76.7%) and 313 (73.8%) of these, respectively, originated from urine specimens. (If “A total of 914 urine isolates were analysed over the three-year period” is correct, this sentence should be clarified because 914 is already the 2020 urine count.)

E. coli accounted for 57% of isolates in 2020, 58% in 2021, and 61% in 2022. For K. pneumoniae, the corresponding proportions were 19% (n = 313), 20% (n = 299), and 17% (n = 419), respectively. The overall median age was 45 years. Of 5,514 patients, 3,606 (65.4%) were female and 1,908 (34.6%) were male. Median age was 39 years (range: 18–99) in females and 56 years (range: 18–90) in males.

ESBL positivity rates for E. coli and K. pneumoniae were 20.3% (242/1,192) and 35.2% (150/425) in 2020, 21.1% (228/1,076) and 34.9% (148/424) in 2021, and 21.4% (401/1,866) and 30.4% (163/536) in 2022, respectively.

For E. coli, susceptibility rates exceeding 90% were observed for meropenem, piperacillin/tazobactam, ceftriaxone, and fosfomycin. For K. pneumoniae, the highest susceptibility was observed for meropenem (>90%). Annual isolate counts and susceptibility distributions are presented in Tables 1–3.

Discussion

Analysis and reporting of institutional susceptibility data are central to antimicrobial stewardship. Because identification and susceptibility testing may take time, institution-specific antibiogram data can support appropriate empirical therapy selection (14–16). Regional resistance patterns vary, and institutional data complement clinical guidelines in guiding

empirical choices (17).

Previous studies have reported that susceptibility rates for K. pneumoniae are often lower in non-urine specimens than in urine specimens, consistent with our findings. In our dataset, E. coli showed the highest susceptibility (>90%) to amikacin, ceftriaxone, piperacillin/tazobactam, and meropenem, while meropenem showed the highest susceptibility among K. pneumoniae isolates (>90%).

According to the 2023 CAESAR report (based on 2021 data), resistance rates for E. coli and K. pneumoniae vary substantially across regions, with high resistance to third-generation cephalosporins and fluoroquinolones reported in many settings (18). Urinary tract infections remain among the most common adult infections, with E. coli the leading pathogen and K. pneumoniae also frequently isolated (19). Multiple studies have similarly highlighted carbapenems and amikacin among the most active agents, while resistance to ciprofloxacin, trimethoprim/sulfamethoxazole, and ceftriaxone may be substantial depending on region and setting (20).

Comparing our findings with earlier institutional data suggests a modest increase in ESBL positivity over time. In the present study, ESBL positivity was 21.0% (871/4,129) for E. coli and 33.2% (461/1,385) for Klebsiella spp., indicating a continuing upward trend and reinforcing the need for coordinated stewardship efforts between clinical and microbiology teams.

This study included only adult patients and demonstrated a slight increase in resistance rates over time. The larger number of processed specimens in 2022 may reflect increased routine hospital attendance following the containment of the COVID-19 pandemic. Continued emphasis on infection prevention measures is warranted.

Conclusion

The increasing prevalence of antimicrobial resistance is concerning. Carbapenems remain among the most reliable treatment options in settings with high ESBL rates. Routine surveillance of susceptibility and ESBL positivity for common pathogens such as E. coli and K. pneumoniae is essential for guiding empirical therapy and supporting antimicrobial stewardship. Institutions should conduct these analyses regularly in accordance with national and international guidelines and disseminate findings to relevant stakeholders to inform local and national stewardship initiatives and empirical treatment strategies.

Author Contributions

All authors contributed to conception and study design, data acquisition/analysis, and manuscript drafting and revision.

Declarations

Ethics Committee Approval: Approved by the Ankara Bilkent City Hospital Clinical Research Ethics Committee (Decision No: E1-23-4360).

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This can be attributed to the increase in routine hospital visits following the containment of the COVD-19 pandemic. In addition, it is imperative that infection prevention measures are taken.

Table 1. Antibiotic Susceptibility Rates of *E. coli* and *K. pneumoniae* Isolates in 2020 (%)

Antibiotic Disk	*E. coli* Urine (%)	*E. coli* Non-urine (%)	Antibiotic Disk	*K. pneumoniae* Urine (%)	*K. pneumoniae* Non-urine (%)
AN	98	94	AN	64	53
GN	81	43	GN	75	58
AM	24	13	AM	4	3
AMC	55	35	AMC	36	13
CRO	96	91	CRO	74	65
FEP	83	54	FEP	69	62
SXT	59	26	SXT	63	37
TZP	90	90	TZP	82	72
FOS*	95	NT	FOS	NT	NT
MEM	99	96	MEM	92	86
CIP	63	52	CIP	75	70
Total	n:914	n:278	Total	n:313	n:112

***Non-urine**: CSF (Cerebrospinal Fluid), Blood, DTA (Deep Tracheal Aspirate), Sputum, Wound Culture Samples *: Only for E. coli NT: Not tested*

AN (Amikacin), GN (Gentamicin), AM (Ampicillin), AMC (Amoxicillin/Clavulanic Acid), CRO (Ceftriaxone), FEP (Cefepime), SXT (Trimethoprim/Sulfamethoxazole), TZP (Piperacillin/Tazobactam), FOS (Fosfomycin), MEM (Meropenem), CIP (Ciprofloxacin)

Table 2. Antibiotic Susceptibility Rates of *E. coli* and *K. pneumoniae* Isolates in 2021 (%)

Antibiotic Disk	*E. coli* Urine (%)	*E. coli* Non-urine (%)	Antibiotic Disk	*K. pneumoniae* Urine (%)	*K. pneumoniae* Non-urine (%)
AN	97	93	AN	65	55
GN	85	53	GN	77	63
AM	30	16	AM	4	3
AMC	57	34	AMC	33	12
CRO	92	88	CRO	62	50
FEP	81	54	FEP	70	61
SXT	52	26	SXT	66	46
TZP	90	90	TZP	82	72
FOS*	96	NT	FOS	NT	NT
MEM	97	95	MEM	91	88
CIP	65	55	CIP	75	68
Total	n:873	n:198	Total	n:299	n:125

***Non-urine**: CSF (Cerebrospinal Fluid), Blood, DTA (Deep Tracheal Aspirate), Sputum, Wound Culture Samples *: Only for E. coli NT: Not tested*

AN (Amikacin), GN (Gentamicin), AM (Ampicillin), AMC (Amoxicillin/Clavulanic Acid), CRO (Ceftriaxone), FEP (Cefepime), SXT (Trimethoprim/Sulfamethoxazole), TZP (Piperacillin/Tazobactam), FOS (Fosfomycin), MEM (Meropenem), CIP (Ciprofloxacin)

Table 3. Antibiotic Susceptibility Rates of *E. coli* and *K. pneumoniae* Isolates in 2022 (%)

Antibiotic Disk	*E. coli* Urine (%)	*E. coli* Non-urine (%)	Antibiotic Disk	*K. pneumoniae* Urine (%)	*K. pneumoniae* Non-urine (%)
AN	97	93	AN	61	52
GN	86	52	GN	74	62
AM	31	20	AM	4	4
AMC	55	31	AMC	31	13
CRO	92	90	CRO	57	50
FEP	75	51	FEP	65	57
SXT	53	30	SXT	65	44
TZP	90	90	TZP	82	72
FOS*	95	NT	FOS	NT	NT
MEM	97	93	MEM	88	86
CIP	63	54	CIP	72	65
Total	n:1468	n:398	Total	n:419	n:117

***Non-urine**: CSF (Cerebrospinal Fluid), Blood, DTA (Deep Tracheal Aspirate), Sputum, Wound Culture Samples *: Only for E. coli NT: Not tested*

AN (Amikacin), GN (Gentamicin), AM (Ampicillin), AMC (Amoxicillin/Clavulanic Acid), CRO (Ceftriaxone), FEP (Cefepime), SXT (Trimethoprim/Sulfamethoxazole), TZP (Piperacillin/Tazobactam), FOS (Fosfomycin), MEM (Meropenem), CIP (Ciprofloxacin)

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