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Original Article

Prevalence of extended-spectrum and plasmid-mediated class C β-lactamases in clinical enteric bacteria

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Abstract

Objective: Extended-spectrum β-lactamases (ESBLs) and acquired AmpC β-lactamases complicate the detection and interpretation of antimicrobial resistance. This study provides a historical characterization of selected clinical Escherichia coli (E. coli) and Klebsiella pneumoniae (K. pneumoniae) isolates collected during 2004–2006.

Methods: From 4,478 E. coli and 1,302 Klebsiella spp. isolates, 107 isolates (59 E. coli and 48 K. pneumoniae) were selected on the basis of reduced susceptibility or resistance to cefoxitin and amoxicillin/clavulanate, together with reduced susceptibility to aztreonam or at least one third-generation cephalosporin. Phenotypic assays, including three-dimensional testing (TDT) with and without cloxacillin treatment, were compared with targeted PCR.

Results: At least one of the investigated TEM, SHV, or CTX-M gene families was detected in 105/107 isolates (98.1%); family-level TEM or SHV detection does not independently establish an ESBL variant. Acquired AmpC-family genes were detected in seven isolates (6.5%), including six E. coli (10.2%) and one K. pneumoniae (2.1%). Standard TDT showed 100% sensitivity and 70% specificity against the targeted PCR results. With cloxacillin treatment, the reported sensitivity was 42.9% and specificity was 93.3%.

Conclusion: These findings document resistance gene families and phenotypic–genotypic discordance in a resistance-enriched historical collection. They cannot estimate contemporary prevalence, establish temporal trends, or guide current empirical therapy. Contemporary isolate collections and validated molecular confirmation are needed to assess present-day epidemiology and diagnostic performance.

Keywords: Beta-lactamases, Escherichia coli, Klebsiella pneumoniae, microbial sensitivity tests.

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INTRODUCTION

Extended-spectrum β-lactamase (ESBL)-producing Enterobacterales and organisms carrying acquired AmpC β-lactamases remain important challenges in clinical microbiology. Their significance extends beyond resistance to individual β-lactams because multiple mechanisms can coexist and complicate susceptibility interpretation (1,2). Recent clinical evidence from Turkey confirms the continuing relevance of ESBL production and carbapenem resistance, although resistance estimates depend on the population, specimen type, and sampling strategy (3). Historical collections can contribute to this context only when their sampling period and limits of generalizability are explicit. Distinguishing acquired AmpC β-lactamase production from other causes of cephalosporin resistance remains difficult. In *Escherichia coli* (*E. coli*), increased expression of the intrinsic chromosomal ampC gene can resemble the phenotype of acquired AmpC enzymes. Recent studies combining phenotypic screening with PCR or whole-genome sequencing demonstrate that a positive AmpC screening result does not necessarily indicate an acquired AmpC gene (4,5). Consequently, molecular confirmation is valuable for resistance-mechanism characterization, while its interpretation depends on the targets included and the capacity to establish their genetic context. AmpC regulation varies across bacterial species and enzyme families. Some chromosomal AmpC enzymes are inducible, whereas intrinsic *E. coli* AmpC is generally non-inducible and may be overexpressed through regulatory-region mutations. Acquired AmpC enzymes should not all be described as constitutively expressed: AmpR-linked DHA-1 can be inducible, whereas CMY-2 typically exhibits constitutive expression. Experimental work in *Klebsiella pneumoniae* (*K. pneumoniae*) has demonstrated this distinction and the contribution of peptidoglycan recycling to DHA-1 induction (6). These differences are relevant when evaluating phenotypic detection strategies. The present study characterizes ESBL-associated and acquired AmpC gene families in resistance-selected *E. coli* and *K. pneumoniae* isolates obtained between January 2004 and December 2006 and examines their relationship with phenotypic assay results. Its purpose is to provide a historical microbiological description and an assessment of diagnostic discordance within that collection, rather than to estimate current resistance prevalence.

MATERIALS AND METHODS

Isolate selection

Between January 2004 and December 2006, 4,478 *E. coli* and 1,302 *Klebsiella spp.* isolates were recovered from clinical specimens submitted to the Clinical Microbiology Laboratory of Marmara University Medical Faculty Hospital. Specimens included blood, abscess material, cyst fluid, wound samples, urine, bronchoalveolar lavage fluid, sputum, and catheter samples. From this population, 107 isolates (59 *E. coli* and 48 *K. pneumoniae*) were selected for further investigation based on the following combined criteria: (i) reduced susceptibility to aztreonam or at least one third-generation cephalosporin; (ii) intermediate susceptibility or resistance to cefoxitin; and (iii) intermediate susceptibility or resistance to amoxicillin/clavulanate. Antimicrobial susceptibility results were interpreted using the CLSI criteria applied during the study period (7). The selected collection included isolates associated with both hospital-acquired and community-acquired infections. Selection was based on resistance phenotypes rather than random sampling; therefore, the collection was enriched for suspected β-lactam resistance and was not representative of all clinical *E. coli* and *K. pneumoniae* isolates processed during the study period. The reported ESBL-associated and acquired AmpC gene-family positivity rates consequently describe only these 107 selected isolates and should not be interpreted as hospital-wide prevalence estimates.

Bacterial identification

Bacterial isolates were identified during routine laboratory processing using miniAPI or VITEK systems (bioMérieux, France) or the Phoenix system (Becton Dickinson, USA).

Phenotypic detection of ESBL and plasmid-mediated AmpC β-lactamases

Phenotypic detection of ESBL and plasmid-mediated AmpC β-lactamases *by modified double-disc assay*

The modified double-disc test was applied to isolates that met the selection criteria to detect ESBL and/or plasmid-mediated AmpC β-lactamase production (8). In the phenotypic evaluation in terms of ESBL production, a synergistic increase in the inhibition zone diameter of any of the ceftazidime, cefotaxime, cefepime and aztreonam discs placed around the amoxicillin-clavulanate disc, ESBL production was evaluated as positive.

Detection of ESBL with the E-test method

First, a bacterial suspension prepared from a fresh culture and adjusted to a 0.5 McFarland standard was spread over the surface of a Mueller–Hinton agar plate. E-test strips containing ceftazidime/ceftazidime-clavulanic acid (TZ/TZL) and cefotaxime/cefotaxime-clavulanic acid (CT/CTL) were then placed on the agar surface. The *K. pneumoniae* strain ATCC 700603 was used as a positive control for ESBL production. If the minimum inhibitory concentration (MIC) of the β-lactam antibiotic was at least eight times that of the corresponding β-lactam/β-lactamase inhibitor combination, the result was considered ESBL-positive (9, 10).

Phenotypic screening for AmpC β-lactamase activity

AmpC β-lactamase activity was assessed using the three-dimensional test (TDT), as described below. The test was repeated after treatment of the enzyme extract with cloxacillin to examine the effect of cloxacillin on the observed activity. These phenotypic procedures assessed AmpC-associated activity but did not establish whether the underlying resistance determinant was plasmid-borne. Isolates were therefore additionally investigated by multiplex PCR targeting the acquired AmpC gene families CIT, MOX, DHA, ACC, EBC, and FOX. Gene-family detection was interpreted separately from phenotypic activity and did not, by itself, establish plasmid localization. Three-dimensional testing (TDT) was applied to all strains included in the study that met the criteria described in section 1 (11-13). The TDT result was considered positive when growth of the ATCC reference strain on Mueller–Hinton agar (MHA) showed an indentation toward the disc at the intersection of the inhibition zone and the slit containing the bacterial enzyme extract (11,13,14).

Detection of ESBL and plasmid-mediated AmpC β-lactamases by genotypic methods

Genomic DNA was extracted from the bacterial isolates to identify plasmid-mediated AmpC β-lactamase and ESBL genes using the QIAamp DNA Mini Kit (QIAGEN), in accordance with the manufacturer's instructions. Polymerase chain reaction (PCR) assays were performed with primer sets detailed in Table 5 (15). Amplified products were separated by electrophoresis on 2% agarose gels prepared in 1× TBE buffer and stained with ethidium bromide. Electrophoresis was conducted at 90 V for 60 minutes, after which PCR bands were visualized under ultraviolet illumination, documented, and interpreted accordingly.

Statistical Analysis

Data were summarized using descriptive statistics. Categorical variables were presented as frequencies and percentages, with the corresponding number of isolates in each species or subgroup used as the denominator. The diagnostic sensitivity and specificity of the three-dimensional test were assessed using targeted AmpC-family PCR results as the reference. Sensitivity was calculated as TP/(TP + FN) × 100, and specificity as TN/(TN + FP) × 100, where TP, FN, TN, and FP denote true-positive, false-negative, true-negative, and false-positive results, respectively. No inferential hypothesis tests were performed; therefore, no statistical significance threshold was applied. Standard TDT was evaluated in all 107 isolates, whereas the cloxacillin-treated procedure was evaluated only in the 37 initially TDT-positive isolates.

RESULTS

Phenotypic findings

Phenotypic test results are summarized in Table 1. Among 59 *E. coli* isolates, 49 (83.1%) were ESBL positive by the modified double-disk test (MDDT). E-test results were positive in 45 (76.3%) and indeterminate in 14 (23.7%). All 48 *K. pneumoniae* isolates were MDDT positive; E-test results were positive in 26 (54.2%) and indeterminate in 22 (45.8%). Of the 22 isolates with indeterminate results, 19 showed reduced susceptibility or resistance to imipenem, and 18 showed synergy only with aztreonam. No isolate exhibited an inducible AmpC phenotype by MDDT. Three-dimensional testing (TDT) was positive in 17 *E. coli* isolates (28.8%) and 20 *K. pneumoniae* (41.7%) isolates. Following cloxacillin treatment, four and one, respectively, remained positive. Among phenotypically ESBL-positive isolates, four of 49 *E. coli* isolates (8.2%) and one of 48 *K. pneumoniae* isolates (2.1%) had ceftazidime zone diameters exceeding the 22-mm CLSI screening threshold. One *K. pneumoniae* isolate also exceeded the 27-mm cefotaxime threshold. All 97 isolates met the aztreonam screening criterion (zone diameter ≤27 mm). Cefepime susceptibility was reported in 13 *E. coli* (26.5%) and eight *K. pneumoniae* isolates (16.7%).

Table 1. Phenotypic detection results by bacterial species

Assay	Result	*E. coli* (n = 59) n (%)	*K. pneumoniae* (n = 48) n (%)
ESBL: MDDT	Positive	49 (83.1)	48 (100.0)
ESBL: MDDT	Negative	10 (16.9)	0 (0.0)
ESBL: E-test	Positive	45 (76.3)	26 (54.2)
ESBL: E-test	Negative	0 (0.0)	0 (0.0)
ESBL: E-test	Indeterminate	14 (23.7)	22 (45.8)
Inducible AmpC: MDDT	Positive	0 (0.0)	0 (0.0)
Inducible AmpC: MDDT	Negative	59 (100.0)	48 (100.0)
AmpC activity: TDT	Positive	17 (28.8)	20 (41.7)
AmpC activity: TDT	Negative	42 (71.2)	28 (58.3)

Abbreviations: *E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae,* ESBL: Extended-spectrum β-lactamase, MDDT: Modified double-disk test, TDT: Three-dimensional test; AmpC: Class C β-lactamase. Phenotypic AmpC activity does not establish plasmid localization.

Genotypic findings

PCR detected at least one TEM, SHV, or CTX-M gene family in 105/107 isolates (98.1%): 58/59 *E. coli* isolates (98.3%) and 47/48 *K. pneumoniae* isolates (97.9%). Representative amplification products are shown in Figure 1. PCR testing using the acquired AmpC-family primer sets listed in Table 5 yielded positive results in seven isolates (6.5%): six *E. coli* isolates carried CIT-family genes, and one *K. pneumoniae* isolate carried both CIT- and EBC-family genes. Representative AmpC-family amplification products are shown in Figure 2. Individual and co-occurring gene-family profiles for both species are presented in Table 2, and overall and individual gene-family detection frequencies are summarized in Table 3. CTX-M predominated in *E. coli* (51/59, 86.4%). In *K. pneumoniae*, TEM was most frequent (36/48, 75.0%), followed by SHV (26/48, 54.2%) and CTX-M (18/48, 37.5%). Six of the seven acquired AmpC-positive isolates also carried at least one investigated ESBL-associated gene family. The distribution by infection source is shown in Table 4. Five AmpC-positive *E. coli* isolates were community-associated, including four with concurrent ESBL-associated gene-family detection. The remaining AmpC-positive *E. coli* isolate and the AmpC-positive *K. pneumoniae* isolate were hospital-associated.

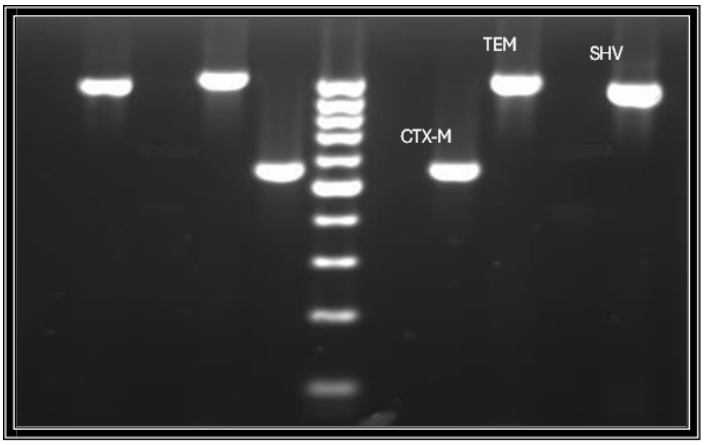

Figure 1. Agarose gel electrophoresis of PCR products targeting the SHV, TEM, and CTX-M β-lactamase gene families in control and clinical isolates.
Electrophoresis was performed on a 2% agarose gel at 90 V for 60 min. Lanes 1, 3, and 4: positive controls for SHV, TEM, and CTX-M, respectively; lane 2: negative control (*Escherichia coli* ATCC 25922); lane 6: 100-bp DNA ladder; lane 7: *E. coli* isolate 1423-04, positive for CTX-M; lane 8: *E. coli* isolate 496-05, positive for TEM; lane 9: *E. coli* isolate 1348-04, negative for all three investigated gene families; lane 10: *Klebsiella pneumoniae* isolate 360-04, positive for SHV.

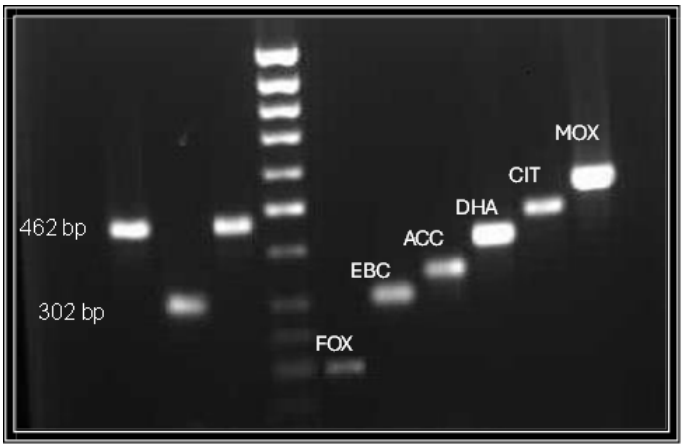

Figure 2. Agarose gel electrophoresis of PCR products targeting acquired AmpC β-lactamase gene families in control and clinical isolates.
Electrophoresis was performed on a 2% agarose gel at 90 V for 60 min. Lane 1: negative control (*Escherichia coli* ATCC 25922); lanes 2–7: positive controls for MOX (520 bp), CIT (462 bp), DHA (405 bp), ACC (346 bp), EBC (302 bp), and FOX (190 bp), respectively; lane 8: 50-bp DNA ladder; lane 9: *E. coli* isolate 240-05, positive for CIT; lanes 10 and 11: *Klebsiella pneumoniae* isolate 360-04, positive for EBC and CIT, respectively; lane 12: *E. coli* isolate 1492-04, negative for the investigated acquired AmpC gene families.

Table 2. Individual and co-occurring resistance gene-family profiles

Detected gene-family profile	*E. coli* (n = 59) n (%)	*K. pneumoniae* (n = 48) n (%)
SHV only	0 (0.0)	4 (8.3)
TEM only	4 (6.8)	17 (35.4)
CTX-M only	18 (30.5)	0 (0.0)
CIT only	1 (1.7)	0 (0.0)
SHV + TEM	0 (0.0)	7 (14.6)
SHV + CTX-M	2 (3.4)	7 (14.6)
TEM + CTX-M	27 (45.8)	4 (8.3)
TEM + CIT	2 (3.4)	0 (0.0)
CTX-M + CIT	1 (1.7)	0 (0.0)
SHV + TEM + CTX-M	2 (3.4)	7 (14.6)
SHV + TEM + CIT	1 (1.7)	0 (0.0)
TEM + CTX-M + CIT	1 (1.7)	0 (0.0)
SHV + TEM + CIT + EBC	0 (0.0)	1 (2.1)
None of the investigated families	0 (0.0)	1 (2.1)
Total	59 (100.0)	48 (100.0)

Rows represent mutually exclusive profiles; percentages use the species total. Sixteen of the 17 *K. pneumoniae* isolates with TEM-only detection showed reduced susceptibility or resistance to imipenem. Gene-family detection does not establish enzyme expression, an ESBL-conferring TEM/SHV allele, or plasmid localization.

Abbreviations: *E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae,* SHV: Sulfhydryl variable, TEM: Temoniera, CTX-M: Cefotaximase-Munich, CIT: *Citrobacter freundii*-derived AmpC family, EBC: *Enterobacter cloacae*-derived AmpC family, ESBL: Extended-spectrum β-lactamase.

Table 3. Overall and individual resistance gene-family detection.

PCR findings	*E. coli* (n = 59) n (%)	*K. pneumoniae* (n = 48) n (%)
Any TEM, SHV, or CTX-M	58 (98.3)	47 (97.9)
Any acquired AmpC family	6 (10.2)	1 (2.1)
SHV	5 (8.5)	26 (54.2)
TEM	37 (62.7)	36 (75.0)
CTX-M	51 (86.4)	18 (37.5)
CIT	6 (10.2)	1 (2.1)
EBC	0 (0.0)	1 (2.1)
MOX, DHA, or ACC	0 (0.0)	0 (0.0)

Overall positivity was 105/107 (98.1%) for TEM, SHV, or CTX-M and 7/107 (6.5%) for acquired AmpC families. Categories overlap because isolates may carry several families. The single AmpC-positive *K. pneumoniae* isolate carried both CIT and EBC.

Abbreviations: PCR: Polymerase chain reaction, *E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae*, TEM: Temoniera, SHV: Sulfhydryl variable, CTX-M: Cefotaximase-Munich, AmpC: Class C β-lactamase, CIT: *Citrobacter freundii*-derived AmpC family, EBC: *Enterobacter cloacae*-derived AmpC family, MOX: MOX-type AmpC family, DHA: Dhahran-type AmpC family, ACC: Ambler class C family.

Table 4. Gene-family detection according to infection source

Species and source	n	AmpC only n (%)	ESBL-associated only n (%)	Both n (%)	Neither n (%)
E. coli **Hospital**	15	0 (0.0)	14 (93.3)	1 (6.7)	0 (0.0)
E. coli **Community**	44	1 (2.3)	39 (88.6)	4 (9.1)	0 (0.0)
K. pneumoniae **Hospital**	31	0 (0.0)	29 (93.5)	1 (3.2)	1 (3.2)
K. pneumoniae **Community**	17	0 (0.0)	17 (100.0)	0 (0.0)	0 (0.0)

Percentages use the number of isolates in each source-specific row. ESBL-associated denotes TEM, SHV, or CTX-M family detection; AmpC denotes an acquired AmpC family. These are gene-family categories and do not establish an ESBL-conferring allele or plasmid localization.
Abbreviations: *E. coli: Escherichia coli, K. pneumoniae: Klebsiella pneumoniae,* AmpC: Class C β-lactamase, ESBL: Extended-spectrum β-lactamase, TEM: Temoniera, SHV: Sulfhydryl variable, CTX-M: Cefotaximase-Munich.

Table 5. Primer sets used for acquired AmpC-family PCR

Target	Primer	Primer Sequence*	Size (bp)
MOX-1,2, CMY-1, CMY-8 - CMY-11	MOX-MF MOX-MR	GCT GCT CAA GGA GCA CAG GAT CAG ATT GAC ATA GGT GTG GTG C	520
LAT-1,-4,BIL-1, CMY 2-CMY-7	CIT-MF CIT-MR	TGG CCA GAA CTG ACA GGC AAA TTT CTC CTG AAC GTG GCT GGC	462
DHA-1, DHA-2	DHA-MF DHA-MR	AAC TTT CAC AGG TGT GCT GGG T CCG TAC GCA TAC TGG CTT TGC	405
ACC	ACC-MF ACC-MR	AAC AGC CTC AGC AGC CGG TTA TTC GCC GCA ATC ATC CCT AGC	346
MIR-1, ACT-1	EBC-MF EBC-MR	TCG GTA AAG CCG ATG TTG CGG CTT CCA CTG CGG CTG CCA GTT	302
FOX-1, FOX-5b	FOX-MF FOX-MR	AAC ATG GGG TAT CAG GGA GAT G CAA AGC GCG TAA CCG GAT TGG	190

***:** Primer sequences are shown in the 5′ to 3′ direction. Product sizes are given in base pairs.
Abbreviations: PCR: Polymerase chain reaction, AmpC: Class C β-lactamase, MOX: MOX-type AmpC family, CMY: Cephamycinase, LAT: LAT-type AmpC enzyme, BIL: BIL-type AmpC enzyme, CIT: *Citrobacter freundii*-derived AmpC family, DHA: Dhahran-type AmpC family, ACC: ACC-type AmpC family, MIR: MIR-type AmpC enzyme, ACT: ACT-type AmpC enzyme, EBC: *Enterobacter cloacae*-derived AmpC family, FOX: FOX-type AmpC family, MF: Forward primer, MR: Reverse primer, bp: base pairs.

DISCUSSION

This study describes a resistance-enriched collection from 2004–2006. Targeted PCR detected at least one TEM, SHV, or CTX-M gene family in 105/107 isolates (98.1%) and acquired AmpC-family genes in 7/107 isolates (6.5%). These are proportions within the selected collection, not prevalence estimates for all clinical isolates. The hospital surveillance figures reported for 2004, 2005, and 2006 were 20.6%, 21.7%, and 22.6% for ESBL-producing *Klebsiella* spp. and 10.4%, 14.5%, and 18.1% for *E. coli*, respectively. The difference between the hospital surveillance figures and the proportions observed in the selected study population illustrates the influence of resistance-based inclusion criteria. Neither set of historical figures establishes current local epidemiology. The CTX-M family was detected in 86.4% of the selected *E. coli* isolates and 37.5% of *K. pneumoniae* isolates. Among the selected *K. pneumoniae* isolates, TEM was the most frequently detected gene family (36/48, 75.0%), followed by SHV (26/48, 54.2%) and CTX-M (18/48, 37.5%). These findings document the presence of these gene families in this historical collection but cannot identify the variants currently predominating. A 2025 systematic review documented multiple CTX-M genes across human, animal, and environmental sources in the United States (16). Recent multicenter clinical data from Turkey likewise demonstrate the continuing burden of ESBL production and carbapenem resistance (3). However, that study involved older adults hospitalized with urinary tract infections, so its estimates are not directly comparable with those derived from our mixed-specimen, resistance-selected collection. The publication dates of recent studies should be distinguished from their isolate collection dates. A key interpretive distinction concerns the resolution of the molecular assays. Detection of TEM or SHV at the gene-family level does not establish that an ESBL-conferring variant is present, because these families also include genes encoding non-ESBL enzymes (1). Accordingly, the 98.1% molecular positivity rate should not be treated as an independently sequence-confirmed ESBL prevalence. Similarly, CIT- or EBC-family amplification identifies the targeted family but does not establish a specific allele or demonstrate plasmid localization. Detection of several gene families in the same isolate is evidence of co-carriage, not a quantitative measurement of enzyme expression. The absence of sequence-level and plasmid-context characterization limits more detailed mechanistic conclusions. Recent diagnostic studies help explain why phenotypic and molecular results may diverge. Teng et al. found acquired AmpC genes in 72 of 250 *E. coli* isolates with positive AmpC screens, and alternative phenotypic criteria showed different sensitivity–specificity trade-offs (4). Hinić et al. evaluated a rapid molecular assay against whole-genome sequencing and showed that many phenotypically AmpC-positive *E. coli* isolates instead carried changes associated with intrinsic chromosomal AmpC overexpression (5). These findings support confirmatory molecular testing when the objective is to identify acquired AmpC mechanisms. They do not directly validate our TDT procedure because the assays, isolate selection, and reference methods differ. Within our historical collection, standard TDT showed a sensitivity of 100% and a specificity of 70% using targeted PCR results as the reference; after cloxacillin treatment, sensitivity was 42.9% and specificity was 93.3%. The sensitivity estimates rest on only seven PCR-positive isolates and are therefore imprecise. Furthermore, performance against a limited PCR panel cannot establish accuracy for all AmpC variants or all causes of an AmpC-like phenotype. The cloxacillin-treated procedure should not be equated with contemporary inhibitor-based combination-disk algorithms without a direct comparative evaluation. In a study published in 2023, Rao et al. detected acquired AmpC genes in 30/348 neonatal bloodstream isolates and reported reduced phenotypic detection in isolates co-harboring NDM (17). These

findings highlight the continuing importance of coexisting resistance mechanisms but do not provide a directly comparable prevalence estimate. The detection of CIT-family genes in six *E. coli* isolates and the co-detection of CIT- and EBC-family genes in one *K. pneumoniae* isolate describe the gene families identified in this collection. They do not establish that CMY-2 or any other allele was present. Nor does a negative induction test exclude acquired AmpC: CMY-2 and AmpR-linked DHA-1 have different regulatory behavior, as demonstrated experimentally in *K. pneumoniae* (6). The reduced imipenem susceptibility observed in some *K. pneumoniae* isolates also cannot be attributed to a specific carbapenemase or used to infer clonal relatedness from the available gene-family and susceptibility profiles. Genomic typing and additional resistance-mechanism testing would be required. Clinical interpretation must use contemporary evidence rather than historical susceptibility categories alone. Recent therapeutic literature emphasizes that the activity of cefepime against ESBL-producing Enterobacterales may be compromised by β-lactamase-mediated hydrolysis, while combining cefepime with the β-lactamase inhibitor enmetazobactam can restore activity against certain ESBL-producing isolates (2). These contemporary treatment developments should not be extrapolated to our historical isolates, for which neither therapeutic outcomes nor the efficacy of inhibitor combinations was evaluated.

Our study did not evaluate treatment outcomes and therefore cannot determine therapeutic efficacy or justify present-day treatment recommendations. The observation of acquired AmpC-family genes among community-associated isolates is also relevant from a One Health perspective. A recent systematic review identified shared β-lactamase genes, including CMY-2 and several CTX-M variants, across human, animal, and environmental sources (16). A prospective household study provided genomic evidence that resistant Enterobacterales were shared between companion animals and cohabiting humans (18). These studies support investigation of interconnected reservoirs, but they do not establish an animal source, transmission route, or direction of transmission for the isolates in our collection.

Limitations

The age of the dataset is a major limitation. All isolates were collected in 2004–2006, and no contemporary comparator collection was included. Subsequent changes in circulating lineages, resistance determinants, antimicrobial exposure, infection-control practice, diagnostic methods, and susceptibility breakpoints restrict the present-day applicability of these findings. Adding recent references provides context but cannot remedy this temporal limitation or demonstrate a trend. Additional limitations include the single-center setting, resistance-based isolate selection, the small number of acquired AmpC-family-positive isolates (n=7), and the absence of reported sequence-level characterization, plasmid localization, genomic relatedness, or clinical outcome analyses. Susceptibility categories reflect the historical methods and interpretive framework; a modern reclassification cannot be assumed. Future studies should use contemporaneous, consecutively sampled isolates, current susceptibility criteria, and molecular methods capable of resolving alleles and genetic context. Resistance-based isolate selection introduced selection bias by enriching the collection for organisms with suspected ESBL and/or AmpC-associated resistance. Consequently, the observed gene-family positivity rates may overestimate the corresponding proportions in an unselected clinical isolate population and should not be interpreted as hospital-wide prevalence estimates. This selection may also influence the observed performance of the phenotypic assays, limiting the generalizability of these findings to routine, unselected isolates.

CONCLUSION

This study provides a historical description of ESBL-associated and acquired AmpC gene families in selected E. coli and K. pneumoniae isolates collected between 2004 and 2006. The observed phenotypic–genotypic discordance illustrates the limitations of inferring resistance mechanisms from screening assays alone. The reported proportions and test-performance estimates apply to this selected collection and cannot be extrapolated to contemporary prevalence, temporal trends, or treatment efficacy. Updated local surveillance with molecular confirmation is required to determine the current distribution and clinical relevance of these resistance mechanisms.

Conflict of interests

The authors declare no conflicts of interest.

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