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Molecular profiling of antimicrobial resistance determinants among ESBL- producing Enterobacteriaceae isolated from community-acquired urinary tract infections in Tétouan, Morocco

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Abstract

Purpose

Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae increasingly cause community-acquired urinary tract infections and may harbor additional resistance determinants. This study characterized ESBL-producing urinary Enterobacteriaceae previously identified in Tétouan, Morocco.

Methods

All 20 phenotypically confirmed ESBL-positive isolates (16 Escherichia coli and four Klebsiella pneumoniae) identified among 422 community-acquired urinary Enterobacteriaceae isolates collected in 2022–2023 underwent molecular analysis. Conventional PCR targeted three β-lactamase genes (blaCTX-M, blaTEM, blaSHV), three plasmid-mediated quinolone resistance (PMQR) genes (qnrA, qnrB, qnrS), and three aminoglycoside resistance genes (aac(6')-Ib, aac(3)-IIa, ant(2'')-Ia). Phenotypic susceptibility profiles were compared descriptively with molecular findings.

Results

blaCTX-M was detected in 19/20 isolates (95.0%), followed by blaTEM (9/20, 45.0%) and blaSHV (3/20, 15.0%). At least one PMQR determinant was detected in 19 isolates (95.0%), with qnrB predominating (80.0%). Among aminoglycoside resistance genes, aac(6')-Ib was most frequent (90.0%), followed by aac(3)-IIa (60.0%) and ant(2'')-Ia (30.0%). Co-occurrence of blaCTX-M and aac(6')-Ib was observed in 18 isolates (90.0%), while 15 (75.0%) simultaneously carried blaCTX-M, qnrB, and aac(6')-Ib. Overall, 96 resistance-gene detections were recorded, corresponding to a mean of 4.8 genes per isolate. All isolates remained susceptible to imipenem and ertapenem, whereas 11/20 (55.0%) were resistant to amikacin.

Conclusions

CTX-M-type ESBLs predominated among community-acquired urinary isolates in Tétouan and frequently co-occurred with PMQR and aminoglycoside resistance determinants. This co-carriage indicates a broad resistance determinant repertoire among these community-associated isolates. Continued regional molecular surveillance, including plasmid and genomic analyses, is needed to clarify the genetic context and potential transmission pathways of these determinants and inform antimicrobial stewardship in northern Morocco.

Introduction

Antimicrobial resistance among Enterobacteriaceae is a major challenge in the management of urinary tract infections (UTIs) and increasingly limits empirical antimicrobial treatment options worldwide [1, 2].

Escherichia coli and *Klebsiella pneumoniae* are among the predominant uropathogens in both community and healthcare settings and have acquired resistance to multiple antimicrobial classes, including β-lactams, fluoroquinolones, aminoglycosides, and folate-pathway inhibitors [3, 4]. The emergence of resistant Enterobacteriaceae in community settings is of particular concern because resistant strains can circulate outside healthcare facilities and contribute to the broader dissemination of antimicrobial resistance [4, 5].

Extended-spectrum β-lactamases (ESBLs) are important mediators of resistance to β-lactam antibiotics. They hydrolyze penicillins, extended-spectrum cephalosporins, and aztreonam, thereby reducing the effectiveness of these agents [6]. ESBL determinants are frequently associated with mobile genetic elements, particularly plasmids, which can facilitate the acquisition and dissemination of antimicrobial resistance genes among Enterobacteriaceae [7]. Over the past two decades, the global emergence of CTX-M enzymes has reshaped the molecular epidemiology of ESBL-producing Enterobacteriaceae, making blaCTX-M, blaTEM, and blaSHV among the most frequently investigated β-lactamase genes [6, 8].

The clinical and epidemiological significance of ESBL-producing isolates extends beyond β-lactam resistance because they may carry additional resistance determinants. Plasmid-mediated quinolone resistance (PMQR) genes, including qnrA, qnrB, and qnrS, can reduce susceptibility to fluoroquinolones and facilitate the selection of additional chromosomal resistance mechanisms [9, 10]. Likewise, aminoglycoside resistance genes such as aac(6')-Ib, aac(3)-IIa, and ant(2")-Ia encode aminoglycoside-modifying enzymes that enzymatically inactivate aminoglycosides and are frequently located on the same mobile genetic elements as ESBL genes [11, 12]. Consequently, the co-occurrence of these determinants within ESBL-producing isolates may therefore result in broader multidrug-resistance profiles and further restrict therapeutic options. Molecular detection of resistance genes, including PCR and sequencing-based assays, complements phenotypic susceptibility testing by providing information on specific resistance determinants that may not be apparent from the phenotype alone [13].

In Morocco, molecular studies have identified CTX-M enzymes as predominant ESBL determinants among clinical Enterobacteriaceae isolates, with PMQR and aminoglycoside resistance determinants also reported among ESBL-producing strains [13–16]. However, available molecular data remain geographically heterogeneous and are concentrated in selected regions of the country. Molecular profiling of community-associated ESBL-producing Enterobacteriaceae from northern Morocco, particularly Tétouan, remains limited.

Building on our previously published cross-sectional surveillance of community-acquired urinary tract infections in Tétouan, comprising 422 nonduplicate Enterobacteriaceae isolates [17], the present study provides a molecular follow-up of the phenotypically confirmed ESBL-producing isolates identified within that cohort. Specifically, we aimed to characterize nine selected antimicrobial resistance-associated genes, including β-lactamase genes (blaCTX-M, blaTEM, and blaSHV), plasmid-mediated quinolone resistance (PMQR) genes (qnrA, qnrB, and qnrS), and aminoglycoside resistance genes (aac(6')-Ib,

aac(3)-IIa, and ant(2")-Ia). We also sought to compare the detected molecular determinants descriptively with the corresponding phenotypic antimicrobial susceptibility profiles.

Materials and Methods

Study design and setting

This laboratory-based molecular investigation was conducted as a follow-up study to a previously published cross-sectional surveillance of community-acquired urinary tract infections in Tétouan, Morocco [17] (Fig. 1). The source surveillance cohort comprised 422 nonduplicate Enterobacteriaceae isolates recovered from urine cultures of outpatients between April 1, 2022, and December 31, 2023, in three private clinical microbiology laboratories. Bacterial identification, antimicrobial susceptibility testing, and phenotypic ESBL screening were performed as part of the original surveillance study. The original study design, patient selection, specimen collection, laboratory procedures, and epidemiological analyses have been reported previously [17].

Bacterial isolates

The present investigation included all 20 phenotypically confirmed ESBL-producing Enterobacteriaceae isolates identified among the 422 isolates comprising the original surveillance cohort [17]. These isolates constituted the complete ESBL-positive subset identified in the parent study, and all were included in the molecular characterization. No additional isolate sampling or selection was performed.

Phenotypic antimicrobial susceptibility testing

Bacterial identification and antimicrobial susceptibility testing of the source cohort were performed in the original surveillance study using the automated VITEK 2 system (bioMérieux, France) and the VITEK® 2 AST-N233 card, respectively [17]. Susceptibility was interpreted according to the EUCAST recommendations applied in that study, with *Escherichia coli ATCC 25922* used for quality control. ESBL production was confirmed using the Modified Double Disc Synergy Test (MDDST) as presented in **Online Resource 1**. For the present molecular investigation, the corresponding phenotypic susceptibility results of the 20 ESBL-positive isolates were retrieved from the original records and used for descriptive genotype–phenotype comparisons. Further details of the phenotypic procedures are available in the parent publication [17].

Molecular characterization of antimicrobial resistance genes

All molecular characterization procedures, including genomic DNA extraction, PCR amplification, and agarose gel electrophoresis, were performed at the National Center for Scientific and Technical Research (CNRST), Rabat, Morocco.

DNA preparation and PCR targets

Genomic DNA was extracted from the ESBL-producing isolates using the MagPurix Bacterial DNA Extraction Kit according to the manufacturer's instructions. Detailed information on the DNA extraction procedure is provided in **Online Resource 2**. Conventional PCR assays were performed to detect nine antimicrobial resistance genes, including the β-lactamase-associated genes *blaCTX-M*, *blaTEM*, and *blaSHV*, the plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, *qnrB*, and *qnrS*, and the aminoglycoside resistance genes *aac(6')-Ib, aac(3)-IIa*, and *ant(2")-Ia*. Forward and reverse primers were prepared at a concentration of 10 µM. Primer sequences and expected amplicon sizes are provided in **Online Resource 3**.

PCR amplification: PCR amplification was performed using NZYPROOF 2× Green Master Mix (NZYTech, Lisbon, Portugal). Each 25-µL reaction mixture contained 12.5 µL of 2× master mix, 1 µL each of the forward and reverse primers, approximately 100 ng of template DNA, and nuclease-free water to a final volume of 25 µL. Amplification was carried out in a Veriti ABI thermal cycler under the following conditions: an initial denaturation at 95°C for 3 min, followed by 35 cycles of denaturation at 95°C for 30 s, primer-specific annealing for 30 s under the conditions specified in the laboratory protocol, extension at 72°C for 60 s, and a final extension at 72°C for 5 min.

Agarose gel electrophoresis: PCR products were separated by electrophoresis on 1% agarose gels using a 1-kb DNA ladder as the molecular size marker. Amplicons were visualized and documented using a G:BOX gel documentation system. A gene was considered present when a PCR product of the expected size was detected. The expected sizes of all PCR amplicons are provided in **Online Resource 3**. PCR products were not sequenced; therefore, gene detection was based on amplification of products of the expected size. Plasmid characterization was not performed.

PCR Result Interpretation

The agarose gel electrophoresis images were independently evaluated by two researchers (A.Z. and L.F.). The presence or absence of each target gene was determined based on the presence or absence of a band at the expected amplicon size. Any discrepancies between the two evaluations were resolved by joint review and consensus before the final results were recorded for analysis.

Statistical analysis

The analysis was strictly descriptive. Categorical variables were summarized as frequencies and percentages using the 20 ESBL-producing isolates as the denominator. Continuous variables are presented as the mean ± standard deviation and range, and the median number of detected resistance genes per isolate was also calculated. Phenotypic antimicrobial susceptibility data and molecular findings were compared descriptively to assess genotype–phenotype concordance. No inferential statistical tests, regression analyses, or hypothesis testing were performed.

Results

3.1. Characteristics of the ESBL-producing Enterobacteriaceae subset

The original epidemiological surveillance included 422 nonduplicate Enterobacteriaceae isolates recovered from outpatient urine cultures in Tétouan, Morocco, between April 2022 and December 2023 [17]. Among these, 20 isolates (4.7%) were phenotypically confirmed as ESBL producers and constituted the complete ESBL-positive subset investigated in the present molecular study. The ESBL-producing isolates comprised 16 (80.0%) *Escherichia coli* and four (20.0%) *Klebsiella pneumoniae*.

All 20 ESBL-positive isolates were included in the molecular characterization, with no additional isolate sampling. Thus, the 422 isolates represent the source surveillance cohort, whereas the molecular findings reported below are based exclusively on the 20 ESBL-positive isolates. The overall study workflow and isolate-selection process are illustrated in Fig. 1.

3.2. Sociodemographic characteristics of patients with ESBL-producing isolates

Of the 20 isolates, 9 (45.0%) were recovered from female patients and 11 (55.0%) from male patients. The mean patient age was 27.3 ± 30.1 years (range, < 1 to 78 years).

The sociodemographic characteristics of patients from whom ESBL-producing isolates were recovered are summarized in Table 1.

3.3. Phenotypic antimicrobial susceptibility profile

All 20 isolates were resistant to ampicillin and ticarcillin, while resistance to amoxicillin/clavulanic acid was observed in 16 (80.0%) isolates. Resistance to cefoxitin occurred in 5 (25.0%) isolates. All 20 isolates were resistant to ceftriaxone and ceftazidime, whereas 19 (95.0%) were resistant to cefotaxime. All isolates remained susceptible to imipenem and ertapenem. Resistance to amikacin, gentamicin, and tobramycin was observed in 11 (55.0%), 14 (70.0%), and 18 (90.0%) isolates, respectively. Ciprofloxacin resistance occurred in 9 (45.0%) isolates, while 11 (55.0%) were resistant to trimethoprim-sulfamethoxazole. Detailed isolate-level phenotypic results are provided in **Online Resource 4** and Fig. 2.

3.4. Distribution of antimicrobial resistance genes

Among the β-lactamase genes investigated, *blaCTX-M* was detected in 19 isolates (95.0%), followed by *blaTEM* in 9 (45.0%) and *blaSHV* in 3 (15.0%).

PMQR genes were widely distributed. *qnrB* was detected in 16 isolates (80.0%), followed by *qnrA* in 11 (55.0%) and *qnrS* in 2 (10.0%). Overall, at least one PMQR determinant was detected in 19 of the 20 isolates (95.0%).

Among aminoglycoside resistance determinants, *aac*(6')-*Ib* was the most frequently detected gene (18/20; 90.0%), followed by *aac*(3)-*IIa* (12/20; 60.0%) and *ant*(2")-*Ia* (6/20; 30.0%). The distribution of

resistance genes is summarized in Table 2, while the complete molecular profile of each isolate is presented in **Online Resource 5** and Fig. 2.

3.5. Co-occurrence of antimicrobial resistance determinants

The combination of *blaCTX-M* and *aac(6')-Ib* was the most frequently observed two-gene combination, occurring in 18 isolates (90.0%), followed by *blaCTX-M* and *qnrB* in 15 isolates (75.0%) and *aac(6')-Ib* and *qnrB* in 15 isolates (75.0%). *blaCTX-M* also co-occurred with *aac(3)-IIa* in 12 isolates (60.0%), *qnrA* in 11 (55.0%), and *blaTEM* in 9 (45.0%). Among three- and four-gene combinations, *blaCTX-M–qnrB–aac(6')-Ib* was detected in 15 isolates (75.0%), while *blaCTX-M–qnrA–aac(6')-Ib* and *blaCTX-M–aac(6')-Ib–aac(3)-IIa* were each detected in 11 (55.0%). The combination *blaCTX-M–qnrA–qnrB–aac(6')-Ib* occurred in 9 isolates (45.0%). The most frequent combinations of antimicrobial resistance genes are presented in Table 3, and the complete gene combinations for individual isolates are provided in **Online Resource 6.**

3.6. Distribution of the molecular resistance burden

Overall, 96 resistance-gene detections were recorded among the 20 isolates, corresponding to a mean of 4.8 genes per isolate (median, 5; range, 2–8). Twelve isolates (60.0%) carried four or five resistance genes, whereas three isolates (15.0%; isolates 17, 18, and 20) harbored seven or more. Isolate 19 exhibited the lowest molecular resistance burden, carrying two genes, *qnrB and ant(2'')-Ia*, and none of the three investigated β-lactamase genes. The overall distribution of antimicrobial resistance gene burden among the ESBL-producing isolates is presented in Table 4.

3.7. Genotype–phenotype concordance

Resistance to ceftriaxone and ceftazidime was observed in all 20 isolates (100.0%), while 19 (95.0%) were resistant to cefotaxime. *blaCTX-M* was detected in 19 isolates (95.0%). One *K. pneumoniae* isolate (isolate 19) was negative for *blaCTX-M*, *blaTEM*, and *blaSHV* despite exhibiting an ESBL-confirmed phenotype.

PMQR determinants were more frequently detected than phenotypic ciprofloxacin resistance. Nineteen isolates (95.0%) carried at least one PMQR gene, whereas 9 (45.0%) were ciprofloxacin-resistant.

Similarly, *aac(6')-Ib, aac(3)-IIa*, and *ant(2'')-Ia* were detected in 18 (90.0%), 12 (60.0%), and 6 (30.0%) isolates, respectively, whereas resistance to amikacin, gentamicin, and tobramycin occurred in 11 (55.0%), 14 (70.0%), and 18 (90.0%) isolates, respectively.

The relationship between phenotypic antimicrobial resistance and the corresponding molecular determinants is summarized in Table 5 and illustrated in Fig. 2.

Discussion

This study extends the previously published phenotypic surveillance of 422 community-acquired urinary *Enterobacteriaceae* isolates in Tétouan [17], by providing a targeted molecular characterization of the 20 ESBL-positive isolates identified within that cohort. All ESBL-positive isolates were included in the molecular analysis. The isolates showed a broad resistance profile, with frequent co-occurrence of β-lactamase, quinolone, and aminoglycoside resistance determinants. Notably, *blaCTX-M* and *aac(6')-Ib* co-occurred in 90.0% of isolates, while *blaCTX-M–qnrB–aac(6')-Ib* was detected in 75.0%. This co-carriage indicates that ESBL-producing isolates in the community may harbor resistance determinants affecting several antimicrobial classes, potentially limiting empirical treatment options [18, 19].

The predominance of *blaCTX-M* was consistent with the extended-spectrum cephalosporin phenotype: *blaCTX-M* was detected in 19/20 isolates, while resistance to ceftriaxone and ceftazidime was universal and resistance to cefotaxime occurred in 95.0%. This pattern is consistent with reports from Morocco, where *CTX-M-15* predominates among community ESBL-producing isolates from Meknes, Settat, and Rabat (range, 78.79–100%) [13, 16, 20], as well as studies from North Africa and Tanzania reporting *CTX-M* frequencies of 81.6–98.96% [21–27]. Although *CTX-M-15* predominates among ESBL-producing Enterobacteriaceae in Morocco and neighboring countries [13, 16, 20, 26], the family-level PCR used in this study did not allow allele-level discrimination.

blaTEM and *blaSHV* were detected in 45.0% and 15.0% of isolates, respectively, and frequently occurred with *blaCTX-M*. In Moroccan community isolates, reported prevalences of *blaTEM* and *blaSHV* range from 73.21–88.9% and 26.78–55.6%, respectively [13, 20], while Egyptian studies reported *TEM* in 60.7% and *SHV* in 42.8% [24]. Such variation may reflect differences in bacterial species, geographical setting, study population, and molecular methods [28, 29]. The frequent co-occurrence of these genes with *blaCTX-M* suggests accumulation of multiple β-lactamase determinants within individual isolates [6].

However, β-lactamase genes represented only one component of the resistance landscape. The high prevalence of *qnr* genes and aminoglycoside-modifying enzyme (AME) genes indicates that the Tétouan isolates carried a broader multiclass resistance determinant repertoire. At least one PMQR determinant was detected in 95.0% of isolates, with *qnrB* predominating (80.0%). Nationally, this was higher than prevalences reported in Meknes (22–24%) and Casablanca (23.1%) [30, 31], and exceeded the 17.6–46.0% reported in several international studies [26, 32–34]. Although comparisons are limited by differences in study populations and molecular methods, the high prevalence of *qnrB* indicates that this determinant was common among the ESBL-producing isolates investigated in Tétouan.

Aminoglycoside resistance genes were also frequent, with *aac*(6')-*Ib*, *aac*(3)-*IIa*,

and *ant*(2")-*Ia* detected in 90.0%, 60.0%, and 30.0% of isolates, respectively. Similar variation has been reported according to bacterial species and geographical setting, including higher AME frequencies among ESBL-producing *K. pneumoniae* than *E. coli* in Spain [35], and detection of these determinants in 60.82% of ESBL-producing isolates in North India [34]. More importantly, their repeated co-occurrence with β-lactamase and PMQR genes indicates that aminoglycoside resistance frequently formed part of broader resistance profiles [36]. Although the genetic location of these determinants was not

investigated, their recurrent co-occurrence is compatible with the association of resistance determinants on mobile genetic elements described in other studies [6, 37, 38].

The genotype–phenotype comparison further demonstrated that molecular detection did not consistently correspond to phenotypic resistance [39]. Although 95.0% of isolates carried at least one *qnr* gene, only 45.0% were ciprofloxacin-resistant. This is compatible with the low-level quinolone protection conferred by Qnr proteins, which may facilitate selection of chromosomal mutations associated with higher-level fluoroquinolone resistance [40]. Similarly, resistance to amikacin was observed in 11/20 isolates (55.0%), despite frequent detection of aminoglycoside resistance genes and resistance to gentamicin and tobramycin. This finding likely reflects the distinct substrate specificities of AMEs and the contribution of additional resistance mechanisms not investigated in the present study, including the aac(6')-Ib-cr variant and 16S rRNA methylases [41, 42]. These findings reinforce the complementary value of phenotypic and molecular approaches for resistance characterization.

One of the major molecular findings of this study was the extensive co-carriage of antimicrobial resistance determinants.

Ninety-six resistance genes were detected across 20 isolates (median, 5 genes/isolate), with 60.0% carrying four or five genes and three carrying seven or more. *blaCTX-M* frequently co-occurred with *aac(6')-Ib, qnrB*, and *aac(3)-IIa*. Similar multidrug resistance architectures have been reported in Morocco, where *bla*CTX-M-15 co-occurs with additional β-lactamase, quinolone, and aminoglycoside resistance genes on transferable plasmids [13, 16], as well as in neighboring countries [23, 24]. Comparable patterns have also been described in India, reflecting the widespread accumulation of resistance genes within mobile genetic elements [34]. The observed co-carriage demonstrates that multiple resistance determinants occurred within the same isolates, but the present PCR-based approach cannot distinguish whether these patterns resulted from shared mobile genetic elements, chromosomal acquisition, or clonal relatedness.

Isolate 19 (*K. pneumoniae*) represented an important genotype–phenotype discordance, exhibiting an ESBL phenotype despite lacking *bla*CTX-M, *bla*TEM, and *bla*SHV. Other ESBL determinants (e.g., blaPER, blaVEB, or blaGES), AmpC β-lactamases, or permeability defects such as porin loss, alone or in combination may explain this phenotype; however, these possibilities were not investigated in the present study [28, 39, 43]. The contribution of untested or uncommon β-lactamase determinants cannot be excluded. The isolate nevertheless carried *qnrB* and *ant*(2")-*Ia*, demonstrating that additional resistance determinants were present despite the absence of the three investigated ESBL genes. Comparable genotype–phenotype discordances have been reported in *K. pneumoniae* [23, 24]. This finding highlights the limitations of targeted PCR panels and supports broader molecular characterization when phenotypic and targeted genotypic results disagree [35].

The molecular findings have implications for community-acquired UTI management. As previously reported [17], ESBL-producing isolates exhibited near-universal resistance to third-generation cephalosporins and frequent resistance to ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin,

and tobramycin. The additional molecular evidence of multiclass resistance suggests that empirical therapy may be unreliable when based solely on the community origin of infection. Culture and susceptibility testing should therefore guide treatment whenever feasible, particularly in patients with recurrent UTIs, recent antimicrobial exposure, or treatment failure. Although imipenem and ertapenem remained active against all 20 isolates, amikacin resistance was observed in 11 (55.0%). These findings should not be interpreted as evidence for general empirical use of any specific agent. Continued antimicrobial stewardship and surveillance for emerging carbapenemase and high-level aminoglycoside resistance determinants remain important [44].

The public health relevance extends beyond individual infections. The detection of multiple resistance determinants among community-associated isolates indicates a substantial resistance burden outside hospital settings, although this study cannot establish transmission through plasmids, horizontal gene transfer, or clonal expansion. Molecular surveillance should therefore extend beyond hospitals and include community laboratories within regional and national antimicrobial resistance networks. Future studies combining allele-level sequencing, plasmid analysis, and whole-genome sequencing could determine the genetic context of these determinants and distinguish local clonal expansion from acquisition of resistance through mobile elements. The genomic characterization of ESBL-producing E. coli from Rabat provides an important reference point [16], but multicenter studies are required to determine whether the resistance profiles observed in Tétouan extend to other regions of Morocco.

This study has several limitations. First, molecular characterization was restricted to 20 ESBL-producing isolates identified within a source cohort of 422 urinary Enterobacteriaceae isolates. Although all ESBL-positive isolates identified in the original surveillance were included, the molecular sample size limits the precision and generalizability of the observed gene frequencies. Furthermore, the source cohort originated from three private clinical microbiology laboratories in a single city and may not represent the wider Moroccan population. Second, the molecular investigation relied on a targeted conventional PCR panel covering nine resistance-associated genes. PCR products were not sequenced, preventing allele-level confirmation, while the absence of plasmid analysis and whole-genome sequencing precluded the assessment of genetic linkage, plasmid localization, and clonal relationships. Finally, genotype–phenotype comparisons were descriptive, and additional resistance mechanisms and gene-expression patterns were not investigated.

Conclusion

This study provides the first molecular profiling of ESBL-producing Enterobacteriaceae causing community-acquired urinary tract infections in Tétouan, northern Morocco. The predominance of *blaCTX-M* and the frequent co-occurrence of PMQR and aminoglycoside resistance determinants indicate that these isolates carried resistance determinants spanning multiple antimicrobial classes. Extending the epidemiological and phenotypic findings [17], the molecular profiles demonstrate a substantial resistance burden among community-associated ESBL-producing Enterobacteriaceae in Tétouan. Further studies incorporating resistance-gene sequencing, whole-genome sequencing, and plasmid

characterization are needed to define the genetic context and transmission of these determinants. Continued integration of molecular and phenotypic surveillance may support antimicrobial stewardship and early detection of emerging resistance in the community.

Table 1

Sociodemographic and bacteriological characteristics of ESBL-producing Enterobacteriaceae isolates included in the molecular analysis (n = 20)| Sociodemographic characteristics of patients and bacterial species distribution among the 20 phenotypically confirmed ESBL-producing *Enterobacteriaceae* isolates included in the molecular characterization.

Characteristic	**n**	**%**
Sex		
Male	11	55.0
Female	9	45.0
Age group, years		
≤ 17	10	50.0
18–44	4	20.0
45–64	2	10.0
≥ 65	4	20.0
Mean age ± SD, years	27.3 ± 30.1	—
Age range, years	0.05–78	—
Bacterial species		
Escherichia coli	16	80.0
Klebsiella pneumoniae	4	20.0

Percentages were calculated using the total number of ESBL-producing *Enterobacteriaceae* isolates included in the molecular analysis (n = 20). SD, standard deviation.

Table 2

Distribution of antimicrobial resistance genes among ESBL-producing Enterobacteriaceae isolates (n = 20)| Distribution of β-lactamase, plasmid-mediated quinolone resistance (PMQR), and aminoglycoside resistance genes detected by conventional PCR among the 20 ESBL-producing *Enterobacteriaceae* isolates included in the molecular analysis.

Resistance category	Target gene	Positive isolates, n	%
ESBL (β-lactamase)	*bla*CTX-M	**19**	**95.0**
	*bla*TEM	**9**	**45.0**
	*bla*SHV	3	15.0
PMQR	*qnrA*	**11**	**55.0**
	qnrB	**16**	**80.0**
	qnrS	2	10.0
Aminoglycoside resistance	*aac(6')-Ib*	**18**	**90.0**
	aac(3)-IIa	12	60.0
	ant(2'')-Ia	6	30.0

Percentages were calculated using the total number of molecularly characterized isolates (n = 20).

Table 3

Most frequent co-occurrence of antimicrobial resistance genes among ESBL-producing Enterobacteriaceae isolates (n = 20)| Frequency of the most common combinations of antimicrobial resistance genes detected by conventional PCR among the 20 ESBL-producing *Enterobacteriaceae* isolates.

Gene combination	n	%
blaCTX-M + *aac(6')-Ib*	18	90.0
blaCTX-M + qnrB	15	75.0
qnrB + *aac(6')-Ib*	15	75.0
blaCTX-M + *aac(3)-IIa*	12	60.0
blaCTX-M + qnrA	11	55.0
qnrA + *aac(6')-Ib*	11	55.0
aac(6')-Ib + *aac(3)-IIa*	11	55.0
blaCTX-M + *blaTEM*	9	45.0
qnrA + qnrB	9	45.0
blaCTX-M + qnrB + *aac(6')-Ib*	15	75.0
blaCTX-M + qnrA + *aac(6')-Ib*	11	55.0
blaCTX-M + *aac(6')-Ib* + *aac(3)-IIa*	11	55.0
blaCTX-M + qnrA + qnrB + *aac(6')-Ib*	9	45.0

Selected frequent gene combinations detected by conventional PCR are presented. Percentages were calculated using the total number of molecularly characterized isolates (n = 20). Gene combinations were not mutually exclusive.

Table 4

Distribution of antimicrobial resistance gene burden among ESBL-producing Enterobacteriaceae isolates (n = 20)| Distribution of the total number of antimicrobial resistance genes detected by conventional PCR in each ESBL-producing *Enterobacteriaceae* isolate.

Number of resistance genes detected	Number of isolates	Percentage (%)	Isolate IDs
2	1	5.0	19
3	2	10.0	1, 10
4	6	30.0	2, 4, 7, 9, 11, 12
5	6	30.0	3, 5, 6, 8, 14, 16
6	2	10.0	13, 15
7	2	10.0	18, 20
8	1	5.0	17

The number of resistance genes corresponds to the total number of PCR-positive targets detected among the nine investigated antimicrobial resistance genes. Percentages were calculated using the total number of molecularly characterized isolates (n = 20).

Table 5

Genotype–phenotype concordance between antimicrobial resistance determinants and phenotypic resistance among ESBL-producing Enterobacteriaceae isolates (n = 20)| Comparison of phenotypic antimicrobial resistance profiles with the corresponding antimicrobial resistance genes detected by conventional PCR among the 20 ESBL-producing *Enterobacteriaceae* isolates.

Antimicrobial class	Phenotypic resistance marker(s)	Resistant isolates, n (%)	Gene(s) investigated	Gene-positive isolates, n (%)	Interpretation
Extended-spectrum cephalosporins	CTX, CRO, CAZ	CTX: **19 (95.0)**; CRO: **20 (100)**; CAZ: **20 (100)**	*blaCTX-M*	**19 (95.0)**	*blaCTX-M* was detected in **19 (95.0%) isolates**, closely matching the high frequency of resistance to the tested third-generation cephalosporins.
	CTX, CRO, CAZ	CTX: **19 (95.0)**; CRO: **20 (100)**; CAZ: **20 (100)**	*blaTEM*	**9 (45.0)**	*blaTEM* was detected in **9 (45.0%) isolates** and frequently co-occurred with other resistance determinants, including *blaCTX-M*.
	CTX, CRO, CAZ	CTX: **19 (95.0)**; CRO: **20 (100)**; CAZ: **20 (100)**	*blaSHV*	**3 (15.0)**	*blaSHV* was detected in **3 (15.0%) isolates**, all of which were *K. pneumoniae*, and occurred together with other β-lactamase determinants.
Fluoroquinolones	CIP	**9 (45.0)**	*qnrA*, *qnrB*, *qnrS*	**19 (95.0)**	At least one PMQR determinant was detected in **19 (95.0%) isolates**, whereas ciprofloxacin resistance was observed in **9 (45.0%)**, indicating that PMQR gene detection was more frequent than phenotypic ciprofloxacin resistance.

Antimicrobial class	Phenotypic resistance marker(s)	Resistant isolates, n (%)	Gene(s) investigated	Gene-positive isolates, n (%)	Interpretation
Aminoglycosides	GN, TOB, AK	GN: **14 (70.0)**; TOB: **18 (90.0)**; AK: **11 (55.0)**	*aac(6')-Ib*	**18 (90.0)**	*aac(6')-Ib* was the most frequently detected aminoglycoside resistance determinant (**18/20, 90.0%**), occurring alongside substantial phenotypic resistance to gentamicin and tobramycin.
	GN	**14 (70.0)**	*aac(3)-IIa*	**12 (60.0)**	*aac(3)-IIa* was detected in **12 (60.0%)** isolates, while gentamicin resistance occurred in **14 (70.0%)**; the difference indicates that phenotypic resistance was not fully accounted for by detection of this gene alone.
	GN, TOB, AK	GN: **14 (70.0)**; TOB: **18 (90.0)**; AK: **11 (55.0)**	*ant(2")-Ia*	**6 (30.0)**	*ant(2")-Ia* was detected in **6 (30.0%)** isolates and was observed mainly in isolates carrying multiple resistance determinants.

Phenotypic susceptibility was determined using the VITEK® 2 AST-N233 card according to the applicable EUCAST recommendations. Genotypic resistance was assessed by conventional PCR targeting β-lactamase, PMQR, and aminoglycoside resistance genes. Percentages were calculated using the total number of ESBL-producing *Enterobacteriaceae* isolates included in the molecular analysis (**n = 20**). **Abbreviations**: AK, amikacin; CAZ, ceftazidime; CIP, ciprofloxacin; CRO, ceftriaxone; CTX, cefotaxime; ERT, ertapenem; ESBL, extended-spectrum β-lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing; GN, gentamicin; IMP, imipenem; PMQR, plasmid-mediated quinolone resistance; PCR, polymerase chain reaction; TOB, tobramycin.

Declarations

Competing interests:

The authors declare that they have no relevant financial or non-financial interests to disclose.

Ethics approval:

The present molecular investigation was conducted as a follow-up analysis of the previously described surveillance study [17]. All patient data and bacterial isolates were anonymized and used solely for research purposes. The study protocol was approved by the Institutional Review Board of the Hospital–University Ethics Committee of Tangier (CEHUT) (IRB No. AC111JV/2–025). The study was conducted in accordance with the ethical standards of the Declaration of Helsinki and its later amendments or comparable ethical standards.

Consent to participate:

The Institutional Review Board waived the requirement for informed consent because the clinical specimens were collected as part of routine diagnostic procedures.

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No funding was received for this study.

Author Contribution

L. F.: Conceptualization, methodology, investigation, data curation, formal analysis, visualization, and writing—original draft. A. E.: Methodology, investigation, data curation, formal analysis, visualization, and writing—original draft. Z. E. : Investigation, supervision, and validation. Z. M.: Supervision, methodology, and interpretation of results. N. E.: Supervision and critical review of the manuscript. All authors contributed to the interpretation of the findings, critically revised the manuscript for important intellectual content, approved the final version, and agreed to be accountable for all aspects of the work.

Data Availability

All data underlying the findings are provided within the article and its supplementary files.

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Figures

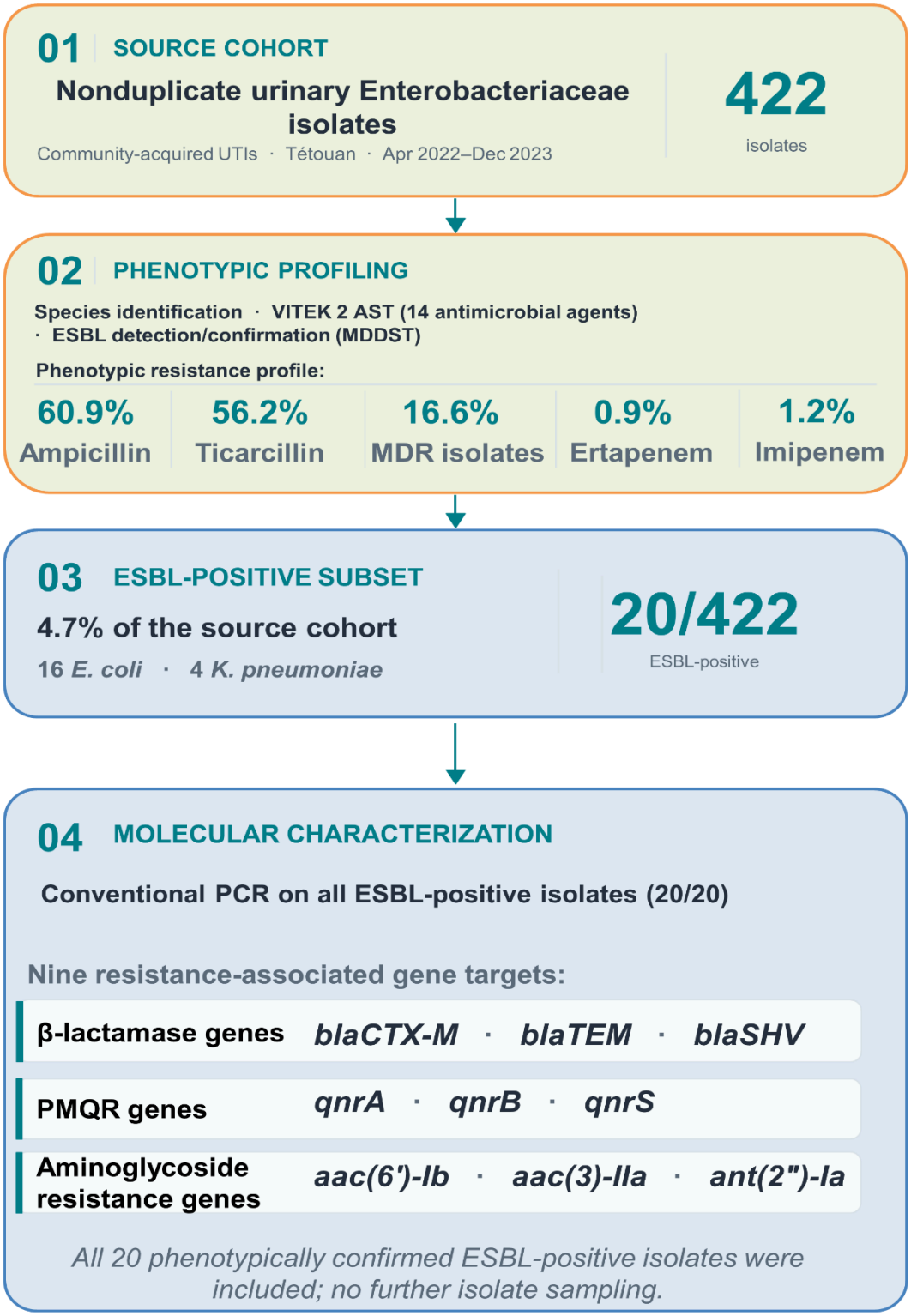


Figure 1

Fig. 1 Study design and workflow of phenotypic and molecular characterization of community-acquired urinary Enterobacteriaceae in Tétouan, Morocco. The figure illustrates the overall study design and workflow. The first three stages represent the source cohort, phenotypic antimicrobial resistance profiling, and identification of ESBL-positive isolates, as reported in the previously published surveillance study [17]. Building on this work, the present study focused on the molecular

characterization of all 20 phenotypically confirmed ESBL-positive isolates. Conventional PCR was used to investigate nine resistance-associated gene targets belonging to three categories: β-lactamase genes, plasmid-mediated quinolone resistance (PMQR) genes, and aminoglycoside resistance genes. No additional isolate sampling was performed. Abbreviations: AST, antimicrobial susceptibility testing; ESBL, extended-spectrum β-lactamase; MDR, multidrug resistance; MDDST, Modified Double Disc Synergy Test; PMQR, plasmid-mediated quinolone resistance.

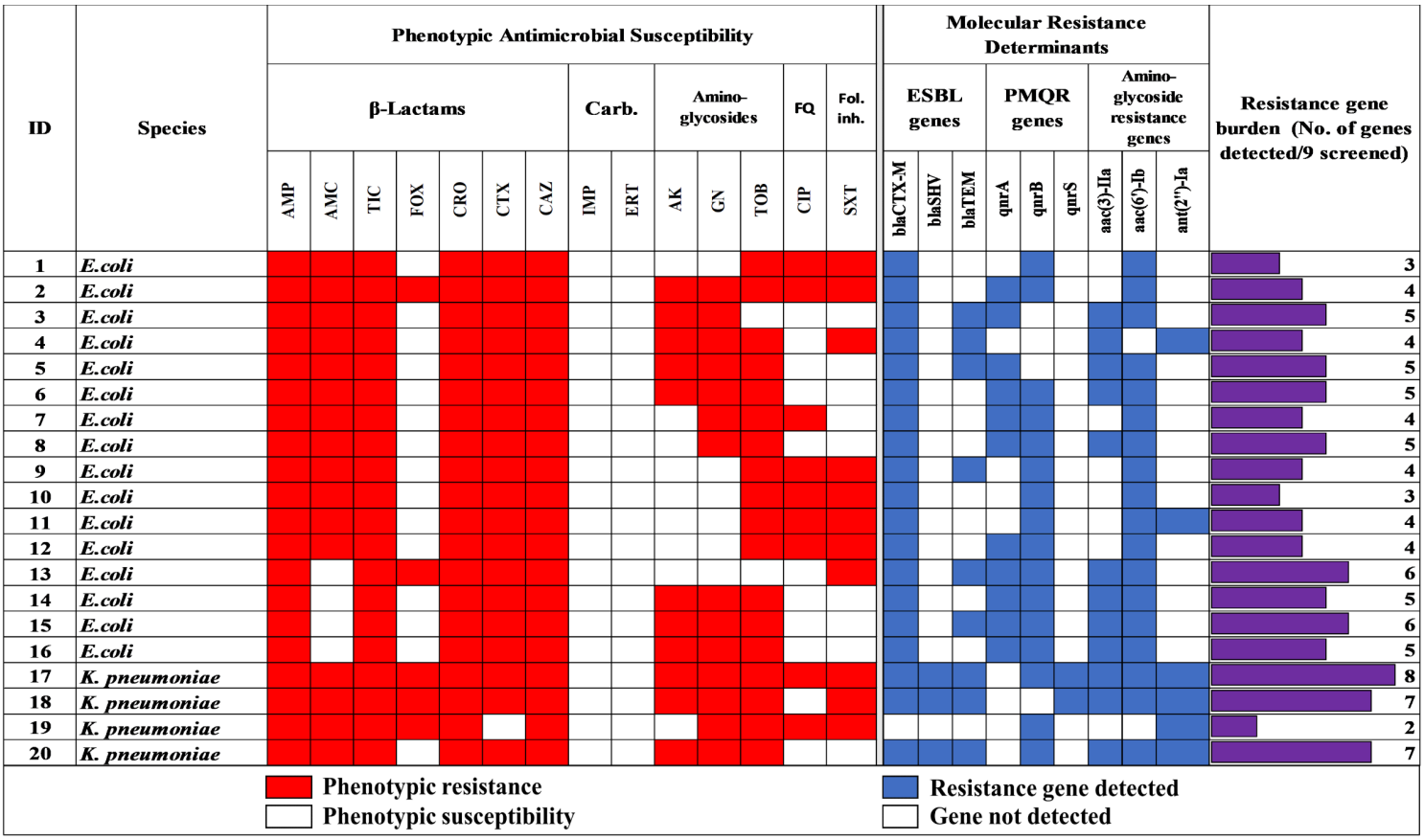


Figure 2

Fig. 2 Phenotypic antimicrobial susceptibility, molecular resistance determinants, and resistance gene burden among ESBL-producing Enterobacteriaceae isolates (n = 20)| Heatmap showing the phenotypic antimicrobial susceptibility profiles and molecular resistance determinants of the 20 ESBL-producing Enterobacteriaceae isolates recovered from community-acquired urinary tract infections. Red squares indicate phenotypic resistance to the tested antimicrobial agents, whereas blue squares indicate the presence of resistance genes detected by PCR. Purple bars represent the resistance gene burden, calculated as the total number of resistance genes detected per isolate. Abbreviations: Species: *E. coli*, *Escherichia coli*; *K. pneumoniae*, *Klebsiella pneumoniae*. Antimicrobial classes: Pen., penicillins; Cep., cephalosporins; Carb., carbapenems; Amin., aminoglycosides; FQ, fluoroquinolones; Fol. inh., folate inhibitors. Antimicrobial agents: AMP, ampicillin; AMC, amoxicillin/clavulanic acid; TIC, ticarcillin; FOX, cefoxitin; CRO, ceftriaxone; CTX, cefotaxime; CAZ, ceftazidime; IMP, imipenem; ERT, ertapenem; AK, amikacin; GN, gentamicin; TOB, tobramycin; CIP, ciprofloxacin; SXT,

trimethoprim/sulfamethoxazole. Resistance genes: *blaCTX-M*, CTX-M β-lactamase gene; *blaSHV*, SHV β-lactamase gene; *blaTEM*, TEM β-lactamase gene; *qnrA*, *qnrB*, and *qnrS*, plasmid-mediated quinolone resistance genes; *aac(6')-Ib*, aminoglycoside 6'-N-acetyltransferase gene; *aac(3)-IIa*, aminoglycoside 3-N-acetyltransferase IIa gene; *ant(2")-Ia*, aminoglycoside 2"-O-nucleotidyltransferase Ia gene.

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