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Phenotypic susceptibility profiles of AmpC- and/or extended-spectrum beta-lactamase-(co)producing *Escherichia coli* strains

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AmpC β-lactamases hydrolyse broad-spectrum penicillins, cephamycins and third-generation cephalosporins have limited activity against cefepime and carbapenems and are only partially inhibited by tazobactam. In *Escherichia coli*, the chromosomal *ampC* gene (c*ampC*) is constitutively expressed at low levels. It is not inducible; however, mutations in its promoter/attenuator region can lead to its overexpression, resulting in varying resistance profiles against β-lactam antibiotics.[1] Additionally, *E. coli* can acquire plasmid-encoded *ampC* genes (p*ampC*), such as bla_{DHA}, bla_{CMY-2}, bla_{ACT}, $bla_{ACC,}$ bla_{MOX} and bla_{CFE}, which are constitutively expressed and can confer varying resistance profiles to β-lactam antibiotics.[2] Unlike c*ampC*, p*ampC* genes can spread within hospital settings through horizontal gene transfer, posing a significant challenge for infection control. For this reason, some hospital hygiene guidelines recommend measures to limit their dissemination.[3] Distinguishing between c*ampC* and p*ampC* genes is challenging based solely on antimicrobial susceptibility testing data and typically requires additional molecular tests, which may delay reporting and clinical decision-making.[4]

Cefoxitin is the most sensitive marker for detecting AmpC production in Enterobacterales, as this can be efficiently hydrolysed by most AmpC β-lactamases.[5] Based on the study of Polsfuss *et al.*,[6] cefoxitin resistance has been adopted as the sole screening marker for AmpC detection at the Institute of Medical Microbiology (IMM), University of Zurich. The current European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines for screening pAmpC production recommend using cefoxitin resistance combined with resistance to either ceftazidime and/or cefotaxime.[7] This schema was based on the work of Edquist *et al.*, which demonstrated that clinical resistance to third-generation cephalosporins significantly increases specificity by effectively excluding most cAmpC while maintaining sensitivity for pAmpC detection in *E. coli*.[8] Since third-generation cephalosporins are also used to screen for extended-spectrum beta-lactamase (ESBL) production, isolates suspected of AmpC production must also be examined for ESBL production. Notably, ESBL detection can be compromised by AmpC production, which can mask the synergy phenomena typically associated with ESBL activity.

To facilitate and expedite AmpC and ESBL detection in *E. coli*, we present disc diffusion susceptibility data profiles of 1792 cefoxitin-resistant *E. coli* strains isolated at the IMM between 2013 and 2024, primarily from patients at the University Hospital Zurich, for antibiotics whose efficacy can be compromised by AmpC and/or ESBL production, i.e. ceftazidime, cefotaxime, ceftriaxone, cefepime and piperacillin/tazobactam. Strains demonstrating a clear ESBL phenotype, characterized by resistance/reduced susceptibility to third-/fourth-generation cephalosporins, as well as the appearance of synergy phenomena between third-/fourth-generation cephalosporins and (amoxicillin/)clavulanic acid and/or (piperacillin/)tazobactam, were excluded from the analysis, since in this case further testing for ESBL detection is unnecessary, and additional AmpC detection holds no implication for hospital hygiene. Likewise, strains confirmed to produce carbapenemases were also excluded. The remaining isolates underwent phenotypic confirmatory tests for AmpC and ESBL production, which were considered as the standard method. AmpC production was confirmed using a double-disc synergy test, where a ≥4 mm increase in the cefoxitin inhibition zone in the presence of cloxacillin indicated AmpC positivity.[6] Similarly, ESBL production was confirmed by an increase in the inhibition zone of either ceftazidime and/or cefotaxime when clavulanic acid was added.[9] For isolates confirmed to produce AmpC, the ESBL confirmatory test was conducted on Mueller–Hinton agar supplemented with 250 mg/L cloxacillin (bioMérieux, Craponne, France). All antibiotic discs were obtained from Becton Dickinson (USA).

β-lactam resistance profiles of 1792 cefoxitin resistant *E. coli* clinical isolates

Ceftazidime	Cefotaxime	Ceftriaxone	Piperacillin-Tazobactam	Cefepime	AmpC pos		AmpC neg		Total
					ESBL neg	ESBL pos	ESBL neg	ESBL pos	
Cefoxitin-resistant isolates resistant to ceftazidime and susceptible to cefotaxime									
					80 (94.1 %)	0 (0 %)	5 (5.9 %)	0 (0 %)	85
					26 (78.8 %)	0 (0 %)	7 (21.2 %)	0 (0 %)	33
					3 (30 %)	0 (0 %)	7 (70 %)	0 (0 %)	10
					10 (76.9 %)	1 (7.7 %)	2 (15.4 %)	0 (0 %)	13
					1 (33.3 %)	0 (0 %)	2 (66.7 %)	0 (0 %)	3
					6 (46.2 %)	0 (0 %)	7 (53.8 %)	0 (0 %)	13
					6 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	6
					132 (81%)	1 (0.6%)	30 (18%)	0 (0 %)	163
Cefoxitin-resistant isolates susceptible to ceftazidime and resistant to cefotaxime									
					10 (90.9 %)	1 (9.1 %)	0 (0 %)	0 (0 %)	11
					10 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	10
					1 (20 %)	0 (0 %)	4 (80 %)	0 (0 %)	5
					0 (0 %)	4 (33.3 %)	1 (8.3 %)	7 (58.3 %)	12
					0 (0 %)	0 (0 %)	2 (40 %)	3 (60 %)	5
					1 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	1
					22 (50%)	5 (11%)	7 (16%)	10 (23%)	44
Cefoxitin-resistant isolates resistant to both ceftazidime and cefotaxime									
					106 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	106
					52 (96.3 %)	0 (0 %)	2 (3.7 %)	0 (0 %)	54
					1 (100 %)	0 (0 %)	0 (0 %)	0 (0 %)	1
					0 (0 %)	0 (0 %)	1 (100 %)	0 (0 %)	1
					132 (91 %)	3 (2.1 %)	6 (4.1 %)	4 (2.8 %)	145
					104 (94.5 %)	4 (3.6 %)	2 (1.8 %)	0 (0 %)	110
					24 (27.6 %)	18 (20.7 %)	0 (0 %)	45 (51.7 %)	87
					39 (19.8 %)	31 (15.7 %)	22 (11.2 %)	105 (53.3 %)	197
					338 (48%)	56 (8%)	33 (5%)	154 (22%)	701
Cefoxitin-resistant isolates total number									
					974 (54%)	68 (4%)	584 (33%)	166 (9%)	1792
Cefoxitin-resistant isolates also resistant to either ceftazidime and/or cefotaxime (EUCAST guidelines)									
					614 (67%)	62 (7%)	72 (8%)	165 (18%)	913
					108 (57.8%)	3 (1.6%)	76 (40.7%)	0	187
Cefoxitin-resistant isolates susceptible (without and/or with increased exposure) to both ceftazidime and cefotaxime									
					252 (36.4%)	3 (0.4%)	436 (63%)	1 (0.1%)	692
					360 (41 %)	6 (0.7 %)	512 (58.2 %)	1 (0.1 %)	879
Cefoxitin-resistant isolates susceptible to ALL other tested antibiotics									
					244 (44.7 %)	1 (0.2 %)	296 (54.2 %)	3 (0.5 %)	546

Figure 1. β-Lactam resistance profiles of 1792 cefoxitin-resistant *E. coli* clinical isolates. Red indicates clinical resistance, green susceptibility and yellow susceptibility at increased exposure.

The inclusion of resistance to ceftazidime and/or cefotaxime as suggested in the EUCAST AmpC screening schema expectedly allowed for a significant reduction in AmpC confirmatory testing, decreasing the number from 1792 to 913 (50.1%, see Figure 1). This likely resulted in equal sensitivity and increased specificity for pAmpC detection. Among the remaining isolates susceptible (with or without increased exposure) to ceftazidime and/or cefotaxime, AmpC production (most likely resulting from c*ampC* overexpression) was confirmed in 366/879 isolates (41%). Notably, AmpC production was identified in 245/546 cefoxitin-resistant isolates (44.9%) susceptible to all other tested antibiotics, further underscoring the high sensitivity but low specificity of cefoxitin alone as a screening agent for pAmpC.

Among the isolates screened positive for AmpC production based on the EUCAST guidelines, AmpC production was identified in 133/163 isolates (81.6%) resistant only to ceftazidime, 27/44 isolates (61.4%) resistant only to cefotaxime and 394/701 isolates (56.2%) resistant to both cephalosporins. Additionally, among the 85 isolates resistant to ceftazidime but susceptible to all other tested antibiotics, AmpC production was confirmed in 80 isolates (94%), highlighting ceftazidime as the most sensitive third-generation cephalosporin marker for AmpC detection. Notably, AmpC production was detected in all 106 isolates (100%) resistant to both ceftazidime and cefotaxime but susceptible to other tested antibiotics.

Overall, among isolates screened positive for AmpC production based on the EUCAST guidelines, the most prevalent group included isolates resistant to ceftazidime, cefotaxime and ceftriaxone, with AmpC production confirmed in 135/145 isolates (93.1%). Moreover, among isolates additionally resistant to piperacillin/tazobactam, AmpC production was detected in 109/110 cases (99%). As expected, additional resistance to cefepime was often associated with ESBL production, which was detected in 199/284 isolates (70%), while AmpC (co-) production was confirmed in only 112/284 cases (39.4%).

A limitation of our study is the lack of molecular confirmation of pAmpC. However, the primary aim was to establish a phenotypic framework for the early identification of AmpC and ESBL producers based on disc diffusion data, which may help refine local diagnostic strategies and support infection control efforts.

In summary, resistance to ceftazidime ± cefotaxime serves as an excellent predictor of AmpC production in cefoxitin-resistant *E. coli* isolates. Additional resistance to ceftriaxone and/or piperacillin/tazobactam enhances both the sensitivity and specificity of AmpC detection, while resistance to cefepime is a strong indicator of ESBL (co-)production. These findings can be leveraged to refine and expedite diagnostic algorithms for AmpC and ESBL detection in *E. coli*.

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Transparency declarations

None to declare.

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