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Letter to the Editor

International circulation of aztreonam/avibactam-resistant NDM-5-producing *Escherichia coli* isolates: successful epidemic clones

Editor Professor A Tsakris

Sir,

Considering that most metallo-β-lactamases (MBL)-producing Enterobacterales (including *Escherichia coli*), and particularly those producing NDM-type enzymes, are highly resistant both to β-lactams and most non-β-lactam antibiotics (aminoglycosides, trimethoprim/sulfamethoxazole, tetracycline, fluoroquinolones), the recently developed combination aztreonam/avibactam (ATM-AVI) offers a therapeutic alternative of choice [1,2]. However, elevated minimum inhibitory concentrations (MICs) for ATM-AVI have been observed among NDM-producing *E. coli* isolates [1,3,4].

Resistance to ATM-AVI has been reported, although the resistance mechanism remains largely unknown [5]. The aim of the present study was to evaluate whether clonally related ATM-AVI-resistant *E. coli* strains might be identified in distant geographical areas such as Pakistan and Switzerland and to characterise the isolates by whole-genome sequencing (WGS).

A series of clinical NDM-5-producing *E. coli* isolates ($n = 55$) obtained from two different countries (Switzerland and Pakistan) were analysed in this study. For Switzerland, NDM-5-producing *E. coli* isolates ($n = 24$) were obtained from various clinical sources (e.g. rectal swabs, blood cultures, urine, sputum). The isolates had been recovered from patients hospitalised in different parts of Switzerland (Aarau, Basel, Bellinzona, Bern, Coppet, Fribourg, Geneva, Lausanne, Luzern, Sion and Zurich) between 2018 and 2021. For Pakistan, the NDM-5-producing *E. coli* isolates ($n = 31$) were collected from rectal swabs from patients admitted to Benazir Bhutto Hospital in Rawalpindi from 2018–2019.

MICs of ATM and ATM-AVI were determined by standard broth microdilution in cation-adjusted Mueller–Hinton broth (Bio-Rad, Marnes-la-Coquette, France) and the results were interpreted according to the latest European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (www.eucast.org/clinical_breakpoints). AVI was tested at a fixed concentration of 4 μg/mL. Antimicrobial susceptibility testing was performed at least in duplicate. Since no breakpoint value for defining ATM-AVI resistance has yet been specified, that of ATM alone (>4 μg/mL; www.eucast.org/clinical_breakpoints) was arbitrarily chosen as a criterion of resistance. *Escherichia coli* ATCC 25922 wild-type strain was used as quality control strain.

The results of antimicrobial susceptibility testing revealed a significant proportion of Swiss and Pakistani NDM-5-producing *E. coli* isolates displaying MICs of ATM-AVI ranging from 2–16 μg/mL (29/55) (Table 1). Overall, a total of 13 NDM-5-producing *E. coli* isolates were considered as being resistant to ATM-AVI (MIC > 4 μg/mL). In addition, a total of 16 NDM-5-producing *E. coli* isolates had MICs of ATM-AVI ranging from 2–4 μg/mL, being further classified as 'intermediate resistant or less susceptible' to ATM-AVI considering the ATM susceptibility breakpoint of ≤1 μg/mL.

To further investigate the role of modified pencilling-binding protein 3 (PBP3) structure, against which ATM has potent selective and specific activity, and β-lactamase content as a source of reduced susceptibility to ATM-AVI, WGS of the 55 NDM-5-producing *E. coli* isolates was performed using Illumina sequencing technology. All 13 NDM-5-producing *E. coli* isolates exhibiting ATM-AVI MICs of >4 μg/mL had a 4-amino acid insertion in the PBP3 protein after residue 333 (YRIN or YRIK), which is consistent with previously published studies [1,3,4] (Table 1). It is likely that these 4-residue insertions in the PBP3 structure, located adjacent to the β-lactam binding pocket, have resulted from selective pressure from one or more of the β-lactam drugs rather than specifically from ATM [3]. Insertion of these additional four amino acids (YRIN or YRIK) has led to significant conformational change in the PBP3 structure, resulting in obstruction of the β-lactam binding pocket of PBP3 and thus hindering efficient binding of ATM, adversely impacting ATM-AVI activity against MBL-expressing Enterobacterales [3]. Moreover, Periasamy et al. reported that isolates carrying YRIK inserts demonstrated relatively higher MICs for ATM-AVI [4].

By analysing the PBP3 sequence of 16 NDM-producing isolates identified as intermediate resistant to ATM-AVI (MICs of 2–4 μg/mL), the same 4-amino acid insertions (YRIN or YRIK) were identified. However, identical insertions were found within the PBP3 sequence among NDM-5-producing *E. coli* with lower MICs of ATM-AVI (0.5–1 μg/mL), therefore indicating that such modified PBP3, even if likely enhancing the occurrence of elevated ATM-AVI MICs, is not enough to confer ATM-AVI resistance and that other mechanism(s) were likely involved. Previous studies described insertions of four specific amino acids in the PBP3 sequence (YRIN, YRIK or YRIP) in NDM-producing *E. coli* isolates in India, Turkey, China, Thailand, Kuwait and Lebanon, showing reduced susceptibility to ATM-AVI (MICs of 4–16 μg/mL) [1,3,4,6].

By analysing the WGS data, all of the NDM-5-producing *E. coli* isolates categorised as 'resistant' and most of those classified as 'less susceptible' carried plasmid-borne bla_{CMY} β-lactamase genes, among which the bla_{CMY-42} allele was predominant (Table 1). To sum up, most of the NDM-5-producing *E. coli* isolates exhibiting ATM-AVI MICs of ≥4 μg/mL produced CMY-42 and possessed an insertion in the PBP3 sequence, and all isolates with low but not wild-type MICs of ATM-AVI (0.5–2 μg/mL) lacked a CMY-encoding gene but possessed an insertion in PBP3. Altogether, these data confirm that amino acid insertions in PBP3 constitute a source of decreased susceptibility to ATM-AVI, whereas association of PBP3 insertion with production of CMY-42 is responsible for resistance to ATM-AVI [1,3,5–7]. It has been shown that the presence of this modified PBP3 is not sufficient to confer resistance to ATM-AVI, however its presence with CMY-42 production likely played a significant role in decreased susceptibility to ATM-AVI [1,5]. Alm et al.

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Table 1
Minimum inhibitory concentrations (MICs) and insertions in the penicillin-binding protein (PBP3) sequence of NDM-5-producing *Escherichia coli* isolates from Switzerland and Pakistan

Strain	ST	Origin	Other β-lactamases	MIC (μg/mL) ATM	ATM-AVI	Insertion in PBP3
148C	167	Pakistan	**CMY-42**, TEM-1B	32	**8**	YRIN
272A	167	Pakistan	**CMY-42**, TEM-1B	64	**8**	YRIN
278A	167	Pakistan	**CMY-42**, TEM-1B	32	**8**	YRIN
279D	167	Pakistan	**CMY-2**	16	4	YRIK
177B	167	Pakistan	CTX-M-15, OXA-1	>128	1	YRIN
278C	167	Pakistan	CTX-M-15, OXA-1	>128	1	YRIN
279B	167	Pakistan	CTX-M-15, OXA-1	>128	1	YRIN
52B	167	Pakistan	CTX-M-15, OXA-1, TEM-1B	>128	0.5	YRIN
53C	167	Pakistan	CTX-M-15, OXA-1, TEM-1B	>128	0.5	YRIN
60E	167	Pakistan	CTX-M-15, OXA-1, TEM-1B	>128	0.5	YRIN
N590	167	Switzerland	**CMY-42**	64	**8**	YRIN
N640	167	Switzerland	**CMY-42**-like, TEM-1 B	64	4	YRIN
N665	167	Switzerland	CTX-M-15, OXA-1	>128	2	YRIN
N783	167	Switzerland	–	1	1	YRIN
N461	167	Switzerland	CTX-M-15, OXA-1	>256	0.5	YRIN
N898	167	Switzerland	CTX-M-15, OXA-1	>256	0.5	YRIN
N568	167	Switzerland	CTX-M-15, OXA-1	>256	0.5	YRIN
N1153	167	Switzerland	**CMY-2**, CTX-M-15, OXA-1, TEM-1B	>256	**8**	YRIK
N1146	167	Switzerland	**CMY-42**, TEM-1B	32	**8**	YRIN
240F	205	Pakistan	**CMY-42**, TEM-1B	32	**16**	YRIK
290A	205	Pakistan	**CMY-42**, TEM-1B	32	4	YRIK
N901	354	Switzerland	CTX-M-24, TEM-1B	>256	2	YRIP
N897	354	Switzerland	CTX-M-24, TEM-1 B	>256	1	YRIP
N442	354	Switzerland	CTX-M-24, TEM-1	>256	0.5	YRIP
52A	361	Pakistan	**CMY-42**	64	4	YRIN
N689	361	Switzerland	OXA-244	4	2	YRIN
N1013	361	Switzerland	**CMY-42**-like	128	**8**	YRIN
280A	405	Pakistan	CTX-M-15, OXA-1	>128	2	YRIK
128A	405	Pakistan	CTX-M-15, OXA-1	>128	2	YRIK
78B	405	Pakistan	CTX-M-15, OXA-1	>128	1	YRIK
N775	405	Switzerland	TEM-1B	16	2	YRIK
N489	405	Switzerland	CTX-M-15, TEM-1B	>256	1	YRIK
N525	405	Switzerland	CTX-M-15	>256	1	YRIK
N1416	405	Switzerland	**CMY-42**, CTX-M-15, OXA-1, TEM-1	>256	**16**	YRIK
142A	617	Pakistan	**CMY-42**, TEM-1B	64	**8**	YRIN
119A	617	Pakistan	CTX-M-15	>128	2	YRIN
N1470	617	Switzerland	**CMY-42**	64	**16**	YRIN
228D	648	Pakistan	–	8	1	YRIK
266D	648	Pakistan	–	8	1	YRIK
N679	648	Switzerland	–	4	2	YRIK
N935	648	Switzerland	TEM-1B	256	2	YRIK
131C	940	Pakistan	OXA-1, TEM-1B	2	2	YRIN
120A	940	Pakistan	OXA-1, TEM-1B	2	1	YRIN
132B	940	Pakistan	OXA-1, TEM-1B	4	1	YRIN
129A	940	Pakistan	OXA-1, TEM-1B	4	1	YRIN
80A	940	Pakistan	OXA-1, TEM-1B	2	0.5	YRIN
125A	940	Pakistan	OXA-1, TEM-1B	2	0.5	YRIN
129B	940	Pakistan	OXA-1, TEM-1B	2	0.5	YRIN
N1076	940	Switzerland	**CMY-42**, TEM-1B	64	**8**	YRIN
296C	1284	Pakistan	**CMY-42,** TEM-1B	32	4	YRIN
N653	1284	Switzerland	CTX-M-15, TEM-1b, OXA-1	>256	0.5	YRIN
N1014	1588	Switzerland	CTX-M-15, SHV-1	>256	1	YRIN
278B	2450	Pakistan	CTX-M-15, OXA-1	>128	1	YRIN
246A	2659	Pakistan	**CMY-131**, TEM-1B	64	**8**	YRIN
78A	2851	Pakistan	**CMY-42**, CTX-M-15, TEM-1B	>128	4	YRIN
E. coli ATCC 25922	–	–	–	≤0.125	≤0.125	–

ST, sequence type; ATM, aztreonam; ATM-AVI, aztreonam/avibactam.

identified a total of 14 NDM-producing *E. coli* isolates with decreased susceptibility to ATM-AVI with amino acid insertions in the PBP3 sequence and also carrying a plasmid-borne bla_{CMY} gene, particularly bla_{CMY-42} [3]. Also, a previous study showed that a series of *E. coli* isolates exhibiting elevated MICs of ATM-AVI (8 μg/mL) carried three serine β-lactamase genes (bla_{CMY-42}, $bla_{OXA-1/-30}$ and bla_{TEM-1}), membrane porin alterations, and a 4-amino acid insertion in PBP3 [6]. In another study from India, CMY-type enzymes, particularly CMY-42, were identified in *E. coli* isolates with ATM-AVI MICs of ≥2 μg/mL [7]. Very recently, a single ATM-AVI-resistant NDM-5-producing *E. coli* isolate was identified in food products. By further analysis of the WGS data of that strain, a 4-amino acid insertion (YRIN) in PBP3 after residue 333 and a plasmid-borne bla_{CMY-42} β-lactamase were identified [8]. Nordmann et al. reported the recent emergence of ATM-AVI resistance not only with MBL-producing strains but also with carbapenem-hydrolysing class D β-lactamases (OXA-48 and OXA-181), even though this combination is not yet in clinical use [9].

Genome analyses of the isolates revealed a variety of *E. coli* clonal backgrounds exhibiting decreased susceptibility to ATM-AVI. Sequence types (STs) identified both among Swiss and Pakistani isolates were ST167 (n = 19), ST940 (n = 8), ST405 (n = 7), ST648 (n = 4), ST361 (n = 3), ST617 (n = 3) and ST1284 (n = 2), of which five (ST167, ST405, ST361, ST940 and ST1284) had been previously reported from India (most commonly), Kuwait, Thailand, Turkey, Colombia, Venezuela and the USA [3,4]. The remain-

ing six sequence types (ST205, ST354, ST1588, ST2450, ST2659 and ST2851) included sporadic isolates, identified either in Switzerland or in Pakistan. Of note, *E. coli* ST167 was recently described as a high-risk clone that has disseminated globally and has been identified both in humans and animals, often showing a multidrug-resistant phenotype [1,10]. Very recently in India, Estabrooka et al. identified a high prevalence of *E. coli* isolates with elevated MICs for ATM-AVI. In their study, most isolates were clonally unrelated but had amino acid insertions within their PBP3 and belonged to ST167, ST405, ST648, ST361, ST1284, ST617 and ST205 [7], which have also been identified in the present study either from Switzerland or Pakistan. In most isolates the $bla_{\text{NDM-5}}$ gene was located exclusively on an F-type plasmid, and a minority were located on a narrow-host-range IncX3 plasmid. The F-type plasmids were multi-replicon plasmids and belonged to various plasmid multilocus sequence types clustering into five type groups (FII, FIB, FII-FIA, FII-FIB and FII-FIA-FIB), of which four (IncF[F36:A4:B-], IncF[F36:A1:B1], IncF [F2:A4:B-] and IncF[F36:A-:B32]) were identified both among Swiss and Pakistani isolates. The $bla_{\text{CMY-42}}$ alleles are located on either IncIγ or IncI1 plasmids. The occurrence of some given clonally-related NDM-5-producing *E. coli* isolates with reduced susceptibility/resistance to ATM-AVI in Switzerland, India and Pakistan may further indicate the international spread of such multidrug-resistant superbugs.

In conclusion, our study revealed a variety of NDM-5-producing *E. coli* isolates with diverse clonal backgrounds exhibiting decreased susceptibility or resistance to ATM-AVI that have already disseminated worldwide (Switzerland, Pakistan, India, Kuwait, Thailand, Turkey, Colombia, Venezuela and the USA). As it has been shown since 2010 that the main reservoir of NDM producers is Southeast Asia, it is likely that these Swiss *E. coli* isolates originate directly or indirectly from Southeast Asian countries and from community spread.

This quite novel ATM-AVI resistance trait represents one of the ultimate evolutions of resistance towards pandrug resistance that may compromise the effectiveness of ATM-AVI, which represents one of the latest and rescue therapies for treating MBL infections.

Here we present evidence of the international spread of multidrug-resistant clones that may remain undetected [11]. In our opinion, *E. coli* is the most important pathogen for humans since (i) it is a cause of the most prevalent human infection, i.e. urinary tract infection, (ii) it is a commensal in humans and animals and (iii) it can be transmitted from animals to humans directly or via the food chain or/and the environment.

GenBank accession numbers

The raw sequence data have been deposited in GenBank under BioProject numbers **PRJNA630933** and **PRJNA645311**.

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Competing interests

None declared.

Ethical approval

Not required.

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