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Emergence of Resistance to Novel β-Lactam–β-Lactamase Inhibitor Combinations Due to Horizontally Acquired AmpC (FOX-4) in *Pseudomonas aeruginosa* Sequence Type 308

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The growing prevalence of nosocomial infections produced by multidrug-resistant (MDR) and extensively multidrug-resistant (XDR) *Pseudomonas aeruginosa* strains is associated with significantly increased morbidity and mortality (1). This increasing threat results from the extraordinary capacity of *P. aeruginosa* for developing resistance to nearly all available antibiotics by the selection of mutations in chromosomal genes, the growing prevalence of transferable resistance determinants (such as carbapenemases or extended-spectrum β-lactamases [ESBLs]), and the global dissemination of MDR/XDR strains, the so-called high-risk clones (2, 3).

The recent introduction of novel β-lactam–β-lactamase inhibitor combinations, such as ceftolozane-tazobactam or ceftazidime-avibactam, has substantially improved our arsenal for combating infections by MDR/XDR *P. aeruginosa* (1, 4, 5). However, ceftolozane-tazobactam or ceftazidime-avibactam are not effective against horizontally acquired class B carbapenemases (metallo-β-lactamases), which are a growing threat in hospitals worldwide (2). Moreover, while ceftolozane-tazobactam or ceftazidime-avibactam are stable against hydrolysis by the intrinsic chromosomal cephalosporinase AmpC from *P. aeruginosa*, resistance may emerge during treatment due to the selection of mutations in the Ω-loop of the β-lactamase (6, 7). Here, we describe a case of ceftolozane-tazobactam and ceftazidime-avibactam resistance caused by the production of a horizontally acquired AmpC β-lactamase, adding further complexity and concern to the current scenario of *P. aeruginosa* resistance mechanisms.

In 2017, an XDR *P. aeruginosa* strain (HUIGC-PA1) was isolated from a urine sample obtained from a urinary catheter of a female spine trauma patient transferred for rehabilitation to Hospital Universitario Insular de Gran Canaria (Gran Canaria, Spain) 1 week previously. During the prior 2 months, the patient had received several broad-spectrum antibiotics, including cefotaxime and cefepime, for hospital-acquired pneumonia (*Escherichia coli*) and wound infections (*Staphylococcus epidermidis*). The isolation of XDR *P. aeruginosa* from the urine was considered colonization, and the patient was discharged 2 months later without antibiotic treatment.

The susceptibility profile of HUIGC-PA1 is shown in Table 1. The strain was resistant or showed borderline susceptibility to all β-lactams tested (including ceftolozane-tazobactam and ceftazidime-avibactam), tobramycin, and ciprofloxacin, remaining only susceptible to amikacin and colistin. Phenotypic and molecular assays for horizontally acquired carbapenemases and ESBLs (8) yielded negative results. Therefore, the strain was subjected to whole-genome sequencing (WGS) using previously described approaches (9) to determine the potential mechanisms involved. The presence of hori-

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TABLE 1 MICs for the FOX-4-producing clinical strain HUIGC-PA1 and for PAO1 producing a cloned FOX-4

Antibiotic (EUCAST 2019 breakpoints [μg/ml])[a]	MIC (μg/ml)[b]			
	HUIGC-PA1	PAO1	PAO1(pUCPAC)	PAO1(pUCPFOX-4)
Piperacillin-tazobactam (S ≤ 16; R > 16)	32	4	128	32
Ceftazidime (S ≤ 8; R > 8)	>64	1	32	>64
Cefepime (S ≤ 8; R > 8)	16	1	4	16
Ceftolozane-tazobactam (S ≤ 4; R > 4)	16	0.5	1	16
Ceftazidime-avibactam (S ≤ 8; R > 8)	32	1	2	32
Aztreonam (S ≤ 16; R > 16)	16	4	16	16
Imipenem (S ≤ 4; R > 4)	32	4	4	4
Meropenem (S ≤ 2; R > 8)	8	1	2	2
Ciprofloxacin (S ≤ 0.5; R > 0.5)	>16	≤0.25	≤0.25	≤0.25
Tobramycin (S ≤ 4; R > 4)	>16	≤1	≤1	≤1
Amikacin (S ≤ 8; R > 16)	8	≤4	≤4	≤4
Colistin (S ≤ 2; R > 2)	2	2	2	2

[a]R, resistance; S, sensitivity.
[b]pUCP24 plasmids produced a cloned PDC-1 (wild-type AmpC) from PAO1 (pUCPAC) or FOX-4 (pUCPFOX-4).

zontally acquired resistance determinants and the sequence type (ST) was determined using online databases (https://cge.cbs.dtu.dk//services/).

The HUIGC-PA1 isolate was documented to belong to the ST308 high-risk clone (3) and OprD (nt220Δ1) and quinolone resistance-determining region mutations (GyrA T83I and ParC S87L) could mostly explain carbapenem and fluoroquinolone resistance, respectively. However, the most interesting findings resulted from the analysis of the horizontally acquired mechanisms, since, in addition to enzymes explaining aminoglycoside resistance (AadB and AacA4), an unexpected transferable AmpC (FOX-4) was detected. Thus, we then aimed to determine whether the production of FOX-4 could explain the resistance profile of HUIGC-PA1, including ceftolozane-tazobactam and ceftazidime/avibactam. For this purpose, bla_{FOX-4} was PCR amplified using the primers FOX-4F (5′-TCGAATTCCATTCACCACGAGAATAACCAT-3′) and FOX4R (5′-TCAAGCTTGA TATTTAGCGGGCGGACTC-3′), cloned into pUCP24, and electroporated into PAO1 as previously described (10). As shown in Table 1, the cloned FOX-4, in contrast to wild-type PAO1 *P. aeruginosa* AmpC (PDC-1), conferred resistance to ceftolozane-tazobactam and ceftazidime-avibactam, in addition to piperacillin-tazobactam, aztreonam, ceftazidime, and cefepime. Thus, our results were consistent with previous findings showing that FOX-4 is a highly proficient broad-spectrum cephalosporinase and that it is weekly inhibited by avibactam (11, 12).

From the epidemiological perspective, it was highly interesting to document that HUIGC-PA1 was isolated in the same hospital from where the first (and to our knowledge, the only, thus far) description of FOX-4 from an *Escherichia coli* isolate was made nearly 20 years before (13). Indeed, the analysis of the genetic context of bla_{FOX-4} revealed that it was identical to that of the 3.3-kb fragment reported for the original FOX-4-producing *E. coli* isolate. Moreover, bla_{FOX-4} was flanked by two copies of IS*91*-like transposases, and thus it was linked to an insertion sequence common region (ISCR) element. However, all attempts to transfer FOX-4 through conjugation and electroporation, according to previously described protocols (14), yielded consistently negative results. Moreover, an analysis of WGS data using plasmidSPAdes software (15) failed to identify the integron containing the bla_{FOX-4} gene in a plasmid contig, suggesting the chromosomal location of the β-lactamase.

In any case, ongoing analysis of stored and prospective *Enterobacteriaceae* and *P. aeruginosa* MDR isolates will provide key information for understanding the epidemiology and persistence of this unusual broad-spectrum transferable AmpC β-lactamase in a single hospital for nearly 20 years.

In summary, our work, together with the recent report by Bour et al. (16), reports the emergence of horizontally acquired AmpC β-lactamases as a mechanism of resistance to novel β-lactam combinations, such as ceftolozane-tazobactam and ceftazidime-avibactam, adding further complexity and concern to the current scenario of *P. aeruginosa* resistance.

Data availability. The complete sequence of HUIGC-PA1 has been deposited in the European Nucleotide Archive under accession number ERS3900446.

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