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Commentary

The difficulties of identifying and treating Enterobacterales with OXA-48-like carbapenemases

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Infections caused by Enterobacterales producing OXA-48-like carbapenemases have been a growing international problem for over a decade [1]. These increasingly prevalent enzymes are challenging to detect and there is no agreed reference standard treatment for infections due to bacteria that produce them. Few clinical outcome data have been published either for colistin combinations or for β-lactams combined with novel β-lactamase inhibitors, which vary in their ability to inhibit OXA-48-like enzymes. Further complicating the clinical picture, susceptibility breakpoints can indicate that isolates are susceptible to carbapenems despite the presence of OXA-48-like carbapenemases.

The family takes its name from OXA-48, the first member found, and includes several sequence variants, notably OXA-181 and OXA-232. All can be horizontally transmissible via plasmids and originated as gene escapes from the chromosomes of *Shewenella* spp. Classical OXA-48 was first reported in Turkey in 2001 and has since become the predominant carbapenemase among Enterobacterales infections in most of Europe (except Greece, Italy and Portugal), Northern Africa, South Africa and the Middle East (except Israel). OXA-181—four amino acids different from OXA-48—is widespread in India, though less prevalent than NDM metallo-carbapenemases. Isolates with OXA-48-like enzymes remain rare in the United States, Latin America and Australasia; there is a growing number of reports in East Asia [2].

The hydrolytic activity of OXA-48-like enzymes against carbapenems is modest, and imipenem and meropenem MICs, though raised, are lower than for isolates with *Klebsiella pneumoniae* carbapenemases (KPC) and metallo-β-lactamases (MBL) [3], often remaining within the 'susceptible' or 'intermediate' ranges defined by CLSI and EUCAST. Ertapenem resistance is usually unequivocal. A complicating factor is that OXA-48-like enzymes vary in hydrolytic activity; OXA-181 is a stronger carbapenemase than OXA-48, whereas OXA-163, an uncommon variant, is not a carbapenemase but (unlike OXA-48) does hydrolyse ceftazidime [4]. The resistance of producer strains reflects not only this hydrolytic efficiency, but also other factors, including the bacterial species, strength of enzyme expression, outer membrane permeability and any other resistance mechanisms present.

Most OXA-48-like enzymes except OXA-163 have little to no activity against third- and fourth-generation cephalosporins and their presence therefore may be suspected when oxyimino cephalosporin susceptibility is seen alongside elevated carbapenem MICs, especially if accompanied by high-level resistance to piperacillin/tazobactam. The complication is that many isolates with OXA-48-like enzymes co-produce extended-spectrum β-lactamases (ESBL), conferring resistance to third-generation cephalosporins, and making their phenotype hard to distinguish from that typical of isolates with combinations of ESBL production and impermeability. One approach, recommended by EUCAST, is to screen for high-level resistance to temocillin (typically MIC >64 mg/L) when meropenem MICs exceed a threshold of 0.12 mg/L. However, (1) temocillin disks are difficult to source in the countries where the drug is not licensed, (2) test panels for automated systems rarely incorporate the high temocillin concentrations needed, (3) temocillin MICs are anyway high for some genera, e.g. *Serratia* spp. or may be raised by other mechanisms, including MBLs and (4) occasional $bla_{\text{OXA-48}}$-positive isolates are susceptible to meropenem at ≤0.12 mg/L and/or temocillin at ≤32 mg/L [3].

Reliable detection of OXA-48-like enzymes requires immunochromography or PCR [2]. Biochemical assays, by matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) and Carba-NP, can be used but are less reliable than for MBLs or KPC types owing to the OXA enzymes' weak hydrolytic capacity. The need for such cost- and labour-intensive diagnostic methods is nonetheless

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underscored by discordance between *in vitro* susceptibility and *in vivo* outcomes with strains with OXA-48-like enzymes. In animal models of infection, carbapenems have poor bactericidal activity against Enterobacterales with OXA-48-like carbapenemases, even when MICs are low. In mice, seven OXA-48-harbouring Enterobacterales strains, including a *Klebsiella pneumoniae* isogenic pair (wild type ± OXA-48), were exposed to humanized antibiotic concentrations. The transconjugant with OXA-48 enzymes had a markedly reduced response to doripenem compared with its $bla_{\text{OXA-48}}$-free counterpart despite a doripenem MIC of only 0.38 mg/L and optimal pharmacodynamic driver attainment (100% fT > MIC). Likewise, doripenem's efficacy was marginal for the six additional isolates with $bla_{\text{OXA-48}}$ even with ≥70% fT > MIC [5]. By contrast, ceftazidime remained efficacious so long as ESBLs were absent.

Modestly raised carbapenem MICs, coupled with poor efficacy, are problematic because the CLSI and EUCAST susceptibility breakpoints for Enterobacterales are agnostic to genotype, potentially leading to inappropriate drug selection. In Europe, Enterobacterales with MIC ≤2 mg/L are considered meropenem susceptible (based on 1 g every 8 hr dosing) whereas an isolate with a meropenem MIC of 4 or 8 mg/L would be reported as 'susceptible—increased exposure' (based on 2 g, every 8 hr dosing, 3 hr infusion), but fully susceptible to meropenem/vaborbactam based on its single susceptibility breakpoint of ≤8 + 8 mg/L, (dosing of 2 + 2 g every 8 hr, 3 hr infusion). Similar problems arise with CLSI's Enterobacterales breakpoints for meropenem (≤1 mg/L susceptible, 2 mg/L intermediate, ≥4 mg/L resistant) and meropenem–vaborbactam (≤4 + 8 mg/L susceptible, 8 + 8 mg/L intermediate, 16 + 8 mg/L resistant). Both sets of criteria can assign an OXA-48-positive isolate as 'susceptible' to meropenem–vaborbactam but as 'intermediate'/'susceptible high-dose' to meropenem even though MICs are the same and vaborbactam does not inhibit OXA-48-like enzymes. To avoid confusion, laboratories should test meropenem as well as meropenem–vaborbactam and should consider the vaborbactam combination only when (1) it has substantially lower MICs than unprotected meropenem, with more than fourfold potentiation or (2) if they have confirmed the presence of a KPC (or other Class A) carbapenemase by PCR or other methods. They should not consider meropenem–vaborbactam when its MICs, or disk zones, are near identical to those of unprotected meropenem.

Clinical experience demonstrates how 'susceptible' *in vitro* carbapenem MICs may not predict clinical success for Enterobacterales producing OXA-48-like enzymes. Prior to the introduction of avibactam, treatment typically involved combinations of colistin, aminoglycosides, fluoroquinolones, and/or tigecycline with or without a carbapenem. Reported 28–30 day mortality rates with these regimens in severe infections were between 50% and 67% [6–8]. This was despite near universal activity of colistin and a reported meropenem MIC_{50} of 2 mg/L [7,8], indicating that at least half of these patients had 'meropenem-susceptible isolates' based upon the EUCAST breakpoint of 2 mg/L. As retrospective cohort series, these reports are limited by the lack of a control group, small numbers of patients and clinical heterogeneity; nevertheless both they and the animal studies just outlined all point to OXA-48-like enzymes being a surprisingly effective defence *in vivo*. Avibactam can inhibit OXA-48-like enzymes to a degree and, more particularly, is combined with ceftazidime and, prospectively, aztreonam, both of which are stable to these carbapenemases, though inactivated by the ESBLs that commonly accompany them. Published experience currently is limited to four retrospective cohort studies and one case series. De la Calle and colleagues report 23 patients (33% ICU) treated with ceftazidime-avibactam for infections caused by Enterobacterales with OXA-48-like enzymes (23 *K. pneumoniae* and 1 *E. coli*) [9]; 46% were 'susceptible' to meropenem and 62.5% to imipenem by EUCAST criteria, whereas all were susceptible to ceftazidime–avibactam, 8 + 4 mg/L. Clinical cure was achieved in 62.5% of patients. Sousa and colleagues report a cohort of 57 patients in the Canary Islands (Spain) treated with ceftazidime–avibactam for infections involving Enterobacterales with OXA-48-like enzymes [10]. Around half the patients were septic; the median Apache score was 24. Ceftazidime–avibactam was started due to empiric treatment failure in more than half of cases, a rate higher than the De la Calle cohort; nonetheless, a higher clinical cure rate (77%) was achieved. Castón and colleagues report a cohort of 31 carbapenem-resistant Enterobacterales (CRE) infections, including 19 involving organisms with OXA-48 enzymes; clinical cure achieved in 86% of ceftazidime–avibactam-treated patients but, unfortunately, no subgroup analysis was presented for the 'OXA-48 patients' [11]. Less encouragingly, Alraddadi and colleagues compared outcomes with or without ceftazidime–avibactam treatment for 38 patients with CRE, mostly (28/38) with OXA-48 enzymes, and reported a clinical cure rate of only 40% [12]. Finally, Temkin and colleagues reported 38 patients from Europe and Australia receiving compassionate use ceftazidime-avibactam as salvage therapy, including 13 with OXA-48-like enzymes [13]. Clinical cure was achieved for 8/13 (62%) patients with OXA-48 producers, but only 5 (38%) survived to discharge; survival was numerically higher (77%) for patients with KPC producers although this difference fell short of statistical significance (p 0.07). Although the generally high success rates with ceftazidime–avibactam, despite severe comorbidities and previous antibiotic failures, are encouraging, these case series are retrospective, subject to indication bias, and represent small populations. Prospective studies are needed to confirm the hypothesis that ceftazidime-avibactam is an effective treatment for infections due to Enterobacterales with OXA-48-like enzymes.

Other new combinations offer less. Ceftolozane/tazobactam has MICs ≤4 mg/L for most ceftazidime-susceptible/intermediate (i.e. ESBL-negative) Enterobacterales with OXA-48-like enzymes but ceftazidime-resistant counterparts are consistently resistant. This is surprising, since tazobactam should inhibit the ESBLs responsible for cephalosporin resistance. This anomaly could be due to inactivation of tazobactam by OXA-48 enzymes, but this hypothesis has not been tested experimentally. Neither vaborbactam nor relebactam inhibits OXA-48-like enzymes, meaning that their combinations with carbapenems have no advantage against producers.

In summary, many complications surround OXA-48-like enzymes. They are hard to identify when molecular diagnostics are rationed or unavailable; there is conflict between frequent *in vitro* susceptibility to carbapenems and poor *in vivo* efficacy and there are no prospective clinical data to inform antibiotic selection. When using the EUCAST screening cut-off approach, suspected producers are not flagged until several days into empirical therapy, which may then transpire to be inadequate. PCR or immunochromotographic methods allow swift reliable detection of producers and should be considered for routine deployment where OXA-48-like enzymes are prevalent. Future clinical evaluations of antibiotics to treat infections due to strains with OXA-48-like enzymes should be informed by translational studies using *in vivo* models of infection, which can measure the bactericidal activity achievable in tissue with clinical drug exposure, which may help rule out ineffective therapies. Comparative study designs using these models may distinguish among ceftazidime/avibactam—already licensed—and the several β-lactam and β-lactamase inhibitor drugs and combinations in development and active *in vitro* (e.g. cefepime/zidebactam, cefepime/VNRX-5133, cefiderocol).

Unless these challenges are faced, OXA-48 carbapenemases, seemingly feeble *in vitro*, have considerable capacity to undermine patients' therapy and, by spreading 'beneath the radar', healthcare systems too.

Transparency declaration

D.M.L.: Advisory Boards or ad-hoc consultancy Accelerate, Allecra, Antabio, Centauri, Entasis, Integra-Holdings, Meiji, Melinta, Menarini, Mutabilis, Nordic, ParaPharm, Pfizer, QPEX, Roche, Shionogi, T.A.Z., Tetraphase, VenatoRx, Wockhardt, Zambon. Paid lectures: Astellas, bioMerieux, Beckman Coulter, Cardiome, Cepheid, Merck/MSD, Menarini, Nordic, Pfizer and Shionogi. Relevant shareholdings or options: Dechra, GSK, Merck, PerkinElmer, Pfizer, T.A.Z, amounting to <10% of portfolio value. D.P.N. is a consultant, speakers bureau member or has received research funding from Allergan, Bayer, Cepheid, Merck, Melinta, Pfizer, Wockhardt, Shionogi, Tetraphase.

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