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Research Article

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More than just the gene: investigating expression using a non-native plasmid and host and its impact on resistance conferred by β-lactamase OXA-58 isolated from a hospital wastewater microbiome

J. Wu[1], T.P. Thompson[1], N.H. O'Connell[2,3], K. McCracken[4], J. Powell[2,3], B.F. Gilmore[1,3], C.P. Dunne[1,3], S.A. Kelly[1,3,*]

[1]School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom
[2]Microbiology Department, University Hospital Limerick, Limerick, V94 F858, Ireland
[3]School of Medicine and Centre for Interventions in Infection, Inflammation, and Immunity (4i), University of Limerick, Limerick, V94 T9PX, Ireland
[4]Keith McCracken Consulting Limited, The Manor House, Greencastle, Co. Donegal, F93 R9Y0, Ireland

*Corresponding author. School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom. E-mail: stephen.kelly@qub.ac.uk

Abstract

With the escalation of hospital-acquired infections by multidrug resistant bacteria, understanding antibiotic resistance is of paramount importance. This study focuses on the β-lactamase gene, bla_{OXA-58}, an important resistance determinant identified in a patient-facing hospital wastewater system. This study aimed to characterize the behaviour of the OXA-58 enzyme when expressed using a non-native plasmid and expression host. bla_{OXA-58} was cloned using a pET28a(+)/*Escherichia coli* BL21(DE3) expression system. Nitrocefin hydrolysis and antimicrobial susceptibility of OXA-58-producing cells were assessed against penicillin G, ampicillin, meropenem, and amoxicillin. bla_{OXA-58} conferred resistance to amoxicillin, penicillin G, and ampicillin, but not to meropenem. This was unexpected given OXA-58's annotation as a carbapenemase. The presence of meropenem also reduced nitrocefin hydrolysis, suggesting it acts as a competitive inhibitor of the OXA-58 enzyme. This study elucidates the phenotypic resistance conferred by an antimicrobial resistance gene (ARG) obtained from a clinically relevant setting and reveals that successful functional expression of ARGs is multifaceted. This study challenges the reliability of predicting antimicrobial resistance based solely on gene sequence alone, and serves as a reminder of the intricate interplay between genetics and structural factors in understanding resistance profiles across different host environments.

Impact Statement

A β-lactamase OXA-58 gene was cloned and expressed from a hospital wastewater microbiome using a non-native plasmid and expression host. Phenotypic resistance changed when expressed using this non-native system. OXA-58 annotated as a carbapenemase, but no resistance was conferred to meropenem. Meropenem may even act as a competitive inhibitor, reducing nitrocefin hydrolysis. This study illustrates how phenotypic antimicrobial resistance is multifaceted and depends on more than just the presence of a resistance gene.

Keywords: AMR; β-lactamase; carbapenemase; microbiome; OXA-58; wastewater

Introduction

Hospital-acquired infections (HAIs) caused by multidrug resistant (MDR) bacteria is an increasingly serious healthcare problem worldwide, spreading either by direct human transmission or through the environment (Chia et al. 2020, Sherry et al. 2022). β-lactamases constitute >200 kinds of hydrolytic enzymes responsible for high-level resistance in commonly prescribed antibiotics such as penicillins, cephalosporins, and carbapenems (Rawat and Nair 2010). The genes responsible are usually carried by plasmids, enabling horizontal transfer, further facilitating the emergence of antimicrobial resistance (AMR) and the spread of HAI (Onduru et al. 2021).

Microorganisms responsible for infection can be transferred within hospital environments, with resistant isolates and genes shown to be present in sinks, showers, or other surfaces (Souna et al. 2014, Muzslay et al. 2017, Zhou et al. 2021). Monitoring the transmission of β-lactamase-producing bacteria and antimicrobial resistance genes (ARGs) within the clinical setting is important, especially in moist niches, which can provide an ideal environment for the persistence of MDR strains.

Wastewater (WW) disposal systems are considered a potential reservoir of ARGs, including β-lactamase genes, and infectious agents. Antibiotics and their metabolites are disposed of in the WW system following human consumption, favouring selection of ARG-carrying bacteria (Cirz et al. 2006, Peng et al. 2017). Pathogens carrying these genes proliferate and predominate in the environment. This can facilitate further horizontal transfer, including to those environmental strains which are not commonly considered pathogenic (Berendonk et al. 2015), also increasing the chance of ARG recombination, causing higher risk of MDR and resistance spread (Adekanmbi et al. 2020). The connection of hospital WW systems with

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urban water supplies and natural water courses also raise the concern of dissemination of AMR determinants and resistant strains outside the clinical setting (Lepuschitz et al. 2019). The increased detection of β-lactamase pollution highlights the importance of understanding the transmission of these genes from human sites to the natural environment (Aristizábal-Hoyos et al. 2019, Xin et al. 2019).

β-lactamase OXA-58 (OXA-58) is a class D β-lactamase first discovered in *Acinetobacter baumannii* in France in 2003 (Poirel et al. 2005). Class D β-lactamase genes are normally referred to as OXA genes, a group of genes responsible for resistance to carbapenems and other β-lactams in Gram-negative bacteria such as *Pseudomonas aeruginosa* and *Acinetobacter* species (Sahl et al. 2013). OXA genes can be transferred between bacterial species, and these resistant strains have been shown to cause infection outbreaks, both within and outside hospitals (Mentasti et al. 2020). OXA-58, along with OXA-23 and OXA-24, are the most common carbapenemases, and when present in their native plasmid and strain, have caused MDR *A. baumannii* nosocomial infections across the globe (Hamidian and Nigro 2019). Reports of OXA-58 producing Enterobacterales are rare, most commonly reported from *Proteus mirabilis* (Literacka et al. 2019, Bonnin et al. 2020). Other OXA-58 producing Gram-negative bacteria include *Pseudomonas*, *Rheinheimera*, *Stenotrophomonas*, *Shewanella*, *Raoultella*, *Vibrio*, *Pseudoalteromonas*, *Algoriphagus*, *Bowmanella*, and *Thalassospira* species (Xin et al. 2019). The mechanism of OXA-58 is similar to other OXA enzymes such as OXA-10. The nucleophilic hydroxyl group of the active site serine residue attacks the amide carbonyl carbon of the β-lactam ring and forms a covalent acyl-enzyme intermediate, eventually leading to complete opening of the β-lactam ring and liberation of the corresponding penicillinoate reaction product (Verma et al. 2011, Tooke et al. 2019). Bacteria that express OXA-58 genes typically but not universally demonstrate resistance to carbapenem antibiotics. For example, meropenem susceptibility has been demonstrated in KPC-producing *Escherichia coli* and *Klebsiella pneumoniae* locally (unpublished) and internationally (Chen et al. 2008, Yin et al. 2019).

In this study, a $bla_{\text{OXA-58}}$ gene was identified and amplified from environmental DNA extracted from a patient-facing WW pipe section which had undergone metagenomic sequencing (Kelly et al. 2023). This pipe section was recovered from a large teaching hospital in the Republic of Ireland that has experienced ongoing MDR infection outbreaks (O'Connor et al. 2016), and has experienced many MDR infection outbreaks, including linezolid resistant staphylococci, Extended spectrum beta lactamase (ESBL) producing *E. coli*, and ongoing issues with carbapenemase-producing Enterobacteriaceae (CPE) (Morris et al. 2012, O'Connell et al. 2014, 2022, O'Connor et al. 2015, 2016, 2017). The ward from which the pipes were removed had among the highest number of detected MDR bacteria in that hospital, including ESBL, Vancomycin Resistant Enterococci (VRE) and CPE. The aim of this study was to characterize the ability of $bla_{\text{OXA-58}}$ gene to confer resistance in a previously sensitive host, when cloned and heterologously expressed using a non-native plasmid and host system. As discovered, observations of phenotypic resistance were at odds with the enzyme's accepted annotation, highlighting the key role in expression vector and expression host in determining AMR characteristics, and not just the gene alone.

Materials and methods

Environmental DNA extraction, sequencing, and read analysis

DNA was extracted from solid material inside a WW pipe section taken from a patient room, using well-established phenol: chloroform extraction methods (Boolchandani et al. 2017). DNA was purified for sequencing using a GeneJet PCR Purification Kit (Thermo Scientific, UK). Extracted DNA underwent QC on the AATI fragment analyser and library preparation was performed using the Illumina XT library prep kit, followed by library QC. These libraries were normalized and pooled and checked for index balance, before running on the Illumina NovaSeq 6000 SP 300 flow cell. This produced 150 bp paired-end reads at 50 M reads per sample. Sequencing reads were checked for quality using FASTQC v0.11.9 and showed high quality reads were produced across all samples. Fastq files were assembled into contigs using SPAdes v3.14.0 in—meta mode, using k-mer lengths 21, 33, and 55. ARGs were identified via the Resistance Gene Identifier (RGI), using the Comprehensive Antibiotic Resistance Database (CARD).

Amplification of $bla_{\text{OXA-58}}$ gene and preparation of *bla*OXA-58/pET28a(+) recombinant plasmid

The $bla_{\text{OXA-58}}$ gene was amplified from environmental DNA using Q5 High Fidelity DNA Polymerase (New England Biolabs, UK) and custom primers OXA-58-F (5′-TTTTTTGTCGACATGAAATTATTAAAAATA-3′) and OXA-58-R: (5′-TTTTTTGGATCCTTATAAATAATGAAAAC-3′). Agarose gel electrophoresis was used to verify amplification of the $bla_{\text{OXA-58}}$ gene and the PCR product was purified using a GenEluteTM PCR Clean-Up Kit (Sigma–Aldrich, UK), following the protocol provided by the manufacturer. Purified $bla_{\text{OXA-58}}$ DNA and the commercially available plasmid pET28a(+) (Novagen) were restricted with SalI-HF and BamHI-HF enzymes (New England Biolabs, UK) as per manufacturer's instructions. Plasmid DNA was also treated with Quick CIP (New England Biolabs, UK). Insert gene and plasmid DNA were ligated using T4 DNA Ligase (New England Biolabs, UK) at a molar ratio of 3:1 and incubated at 16°C overnight.

Transformation of competent cells and protein expression

NEB 5-alpha *E. coli* competent cells (New England Biolabs, UK) were transformed with recombinant *bla*OXA-58/pET28a(+) plasmid, following the manufacturer's protocol, and grown on LBA with appropriate antibiotic. Insert DNA was verified by agarose gel electrophoresis and sequencing. Recombinant plasmid was extracted from NEB 5-alpha cells using a GeneJET plasmid Miniprep Kit (ThermoFisher Scientific, UK) and used to transform *Escherichia coli* BL21 DE3 Chemically Competent cells (New England Biolabs, UK), following manufacturer's instructions. Transformants were cultured in 10 ml LBB containing appropriate antibiotic and induced in early log phase with 0.4 mmol l^{-1} IPTG. This culture was incubated at 37°C until it reached $OD_{550} = 1.2$. The culture was pelleted by centrifugation, cells were resuspended in 10 ml PBS, and sonicated on ice to lyse cells. The lysed mixture was centrifuged to remove cell debris and the soluble fraction was used as a cell-free extract for analysis and

(a)
```
>OXA-58
MKLLKILSLVCLSISIGACAEHSMSRAKTSTIPQVNNSIIDQNVQALFNEISADAVF
VTYDGQNIKKYGTHLDRAKTAYIPASTFKIANALIGLENHKATSTEIFKWDGKPRFF
KAWDKDFTLGEAMQASTVPVYQELARRIGPSLMQSELQRIGYGNMQIGTEVDQFWLK
GPLTITPIQEVKFVYDLAQGQLPFKPEVQQQVKEMLYVERRGENRLYAKSGWGMAVD
PQVGWYVGFVEKADGQVVAFALNMQMKAGDDIALRKQLSLDVLDKLGVFHYL-
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(b)

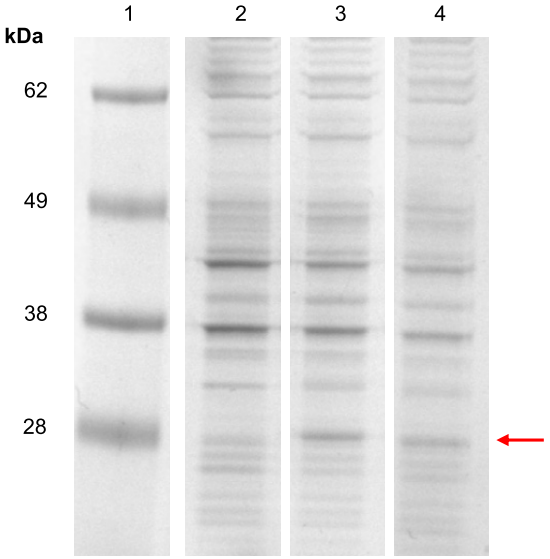

Figure 1. (a) Amino acid sequence of OXA-58 protein. (b) SDS-PAGE visualization showing SeeBlue Plus 2 reference ladder (lane 1); soluble fraction of *E. coli* BL21 DE3 containing empty pET28a(+) plasmid cell-free extract (lane 2); soluble fraction of *E. coli* BL21 DE3 containing *bla*OXA-58/pET28a(+) plasmid cell-free extract (lane 3); soluble fraction of *E. coli* BL21 DE3 containing *bla*OXA-58/pET28a(+) plasmid cell-free extract, with protein expressed induced by IPTG (lane 4). The overexpressed protein is indicated by the red arrow, close to the predicted size of approximately 31 kDa.

further assays. Protein expression was verified by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE).

Nitrocefin assay

The time-dependent hydrolysis of nitrocefin hydrolysis by *bla*OXA-58/pET28a(+)-containing transformants was examined using a previously described assay (O'Callaghan et al. 1972, Kahlmeter and Brown 2010). Nitrocefin in PBS solution was added to a 96-well plate to ensure final working concentrations of 0.0625 mmol l^{-1} and 0.125 mmol l^{-1}. Varying concentrations of meropenem and amoxicillin were also tested alongside nitrocefin, with nitrocefin stock solutions adjusted to ensure a final working concentration of 0.125 mmol l^{-1} for these assays. The reaction was started by the addition of 50 μl *bla*OXA-58/pET28a(+)/*E. coli* BL21 DE3 cell-free extract and absorbance was measured over 60 min at 490 nm in a FLUOstar Omega microplate reader (Ortenberg, Germany). *E. coli* BL21 DE3 cells containing empty pET28a(+) plasmid were used as a negative control.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was carried out to determine minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) using standard protocols (Matthew et al. 2006). MIC and MBC values for β-lactams penicillin G, meropenem, amoxicillin, and ampicillin, and the fluoroquinolone, ciprofloxacin (as a non-substrate control), were determined.

Statistical analysis

For Fig. 2a, Fig. 3a, and Fig. 3b, a one-way ANOVA, with Dunnett's test against the untreated control were used. *P*-values of $^{*}(P \leq 0.05)$, $^{**}(P \leq 0.01)$, $^{***}(P \leq 0.001)$, and $^{****}(P \leq 0.0001)$ indicate statistical significance. GraphPad Prism 10.2.3 was used for all analysis.

Results and discussion

Amino acid sequence and protein expression analysis of OXA-58 in *E. coli* BL21 DE3 cells

The $bla_{\text{OXA-58}}$ gene sequence identified from a patient-facing WW pipe section (GenBank accession number PP989441) and translated protein sequence were used as query terms against the National Center for Biotechnology (NCBI) database. The gene sequence resulted in a 100% identity with 100% query cover to a number of *Acinetobacter* sp. plasmids, with the amino acid sequence returning the same identity and query cover scores for 'Beta-lactamase class D', 'carbapenem-hydrolyzing oxacillinase', and 'carbapenem-hydrolyzing class D beta-lactamase OXA-58'. The amino acid sequence of OXA-58 is shown in Fig. 1a containing the the key residue ser83.

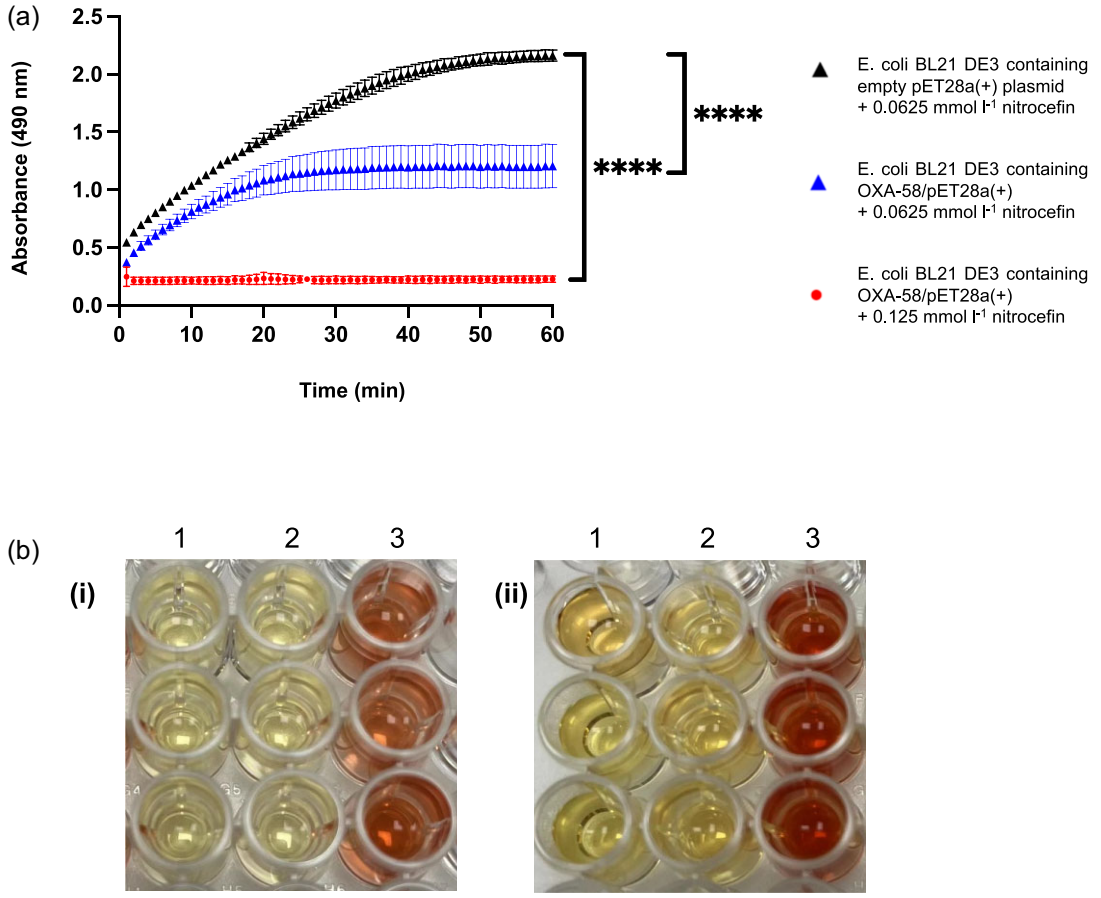

Figure 2 A. Nitrocefin hydrolysis assay comparing turnover of varying concentrations of *bla*OXA-58/pET28a(+)-containing *E. coli* BL21 DE3 cell-free extract and compared to empty vector controls. The reaction was started with the addition of cell-free extract and followed over 60 min. Asterisks denote significant differences between OXA-58 inserted plasmid compared to the empty vector control ($t = 60$). (B) Visual endpoint measurements of the nitrocefin hydrolysis assay showing OXA-58-catalysed hydrolysis of the substrate at 60 min with (i) 0.0625 mmol l^{-1} nitrocefin and (ii) 0.125 mmol l^{-1} nitrocefin. Column 1: Water + nitrocefin; Column 2: Empty vector + nitrocefin; Column 3: OXA-58 + nitrocefin.

Following PCR amplification and cloning into a pET28a(+) plasmid, recombinant plasmids were used to transform *E. coli* BL21 DE3 cells and protein expression was induced using IPTG. The resultant protein was around 31 kDa, commensurate with the protein sequence and predicted molecular weight. Cells containing the recombinant plasmid which were not induced by IPTG also exhibited overexpression of OXA-58 protein, indicating 'leaky expression' often associated with this system. No expression of OXA-58 was observed in the empty vector negative control (**Fig. 1B**).

Nitrocefin conversion and antibiotic resistance profile

Time-dependent hydrolysis of nitrocefin was clearly observed with increases in absorbance with both 0.125 mmol l^{-1} and 0.0625 mmol l^{-1} nitrocefin, with increases in absorbance at 490 nm with both concentrations (Fig. 2). This demonstrated the expressed enzyme was active and able to turn over the nitrocefin substrate. The antimicrobial susceptibility profile of *bla*OXA-58/pET28a(+)-containing *E. coli* BL21 (DE3) cells was assessed against the β-lactams penicillin G, meropenem, amoxicillin and ampicillin, as well as the fluoroquinolone ciprofloxacin as a non-β-lactam control. An empty vector transformant was used as a negative control. Newly-acquired resistance was observed to a number of β-lactam antibiotics in *bla*OXA-58/pET28a(+)-containing transformants versus empty vector control (Table 1). These were penicillin G (256 vs. 4 mg l^{-1}), amoxicillin (128 vs. <1 mg l^{-1}), and ampicillin (128 vs. <1 mg l^{-1}), but not to ciprofloxacin or meropenem. The lack of resistance to meropenem was somewhat unexpected, as OXA-58 enzymes are annotated as carbapenemases and most strains producing this enzyme can hydrolyse meropenem (Coelho et al. 2006). However, such a phenomenon has been described before with the transfer of a $bla_{\text{OXA-58}}$ gene to *E. coli*, resulting in higher MIC values for amoxicillin and ticarcillin, but not for meropenem (Chen et al. 2008). Slow hydrolysis rates may account for the lack of resistance conferred to meropenem. Other factors have been shown to affect the activity of OXA-type β-lactamases, such as the addition of bicarbonate (Studentova et al. 2015).

Effect of other antibiotics on nitrocefin hydrolysis

Our study elucidates the AMR profile of β-lactamase OXA-58 when expressed in *E. coli* BL21 DE3 cells using a pET28(a)+ vector. The gene sequence was obtained from a pipe removed from a medical ward with a longstanding and notable history of MDR bacteria including CPE. In this source hospital, four patients to date have been detected with organisms ex-

Table 1. MIC and MBC values for a range of antibiotics against *E. coli* BL21 DE3 cells harbouring the *bla*OXA-58/pET28a(+) plasmid and negative control *E. coli* BL21 DE3 cells containing empty pET28a(+) plasmid.

	pET28a(+) control		*bla*OXA-58/pET28a(+)	
	MIC (mg l^{-1})	MBC (mg l^{-1})	MIC (mg l^{-1})	MBC (mg l^{-1})
Penicillin G	4	16	256	>256
Meropenem	<0.0625	<0.0625	<0.0625	<0.0625
Amoxicillin	<1	2	128	128
Ampicillin	<1	2	128	128
Ciprofloxacin	<0.125	<0.5	<0.125	<0.5

pressing OXA-58 enzymes, all *Acinetobacter* species, and two of these patients attended this ward (not simultaneously) two years prior to the collection of the samples. Three of these isolates showed reduced susceptibility to meropenem (MIC range 2–4 mg l^{-1}) and one isolate was fully resistant (MIC > 32 mg l^{-1}). Three isolates were detected from screening specimens and one from a clinical respiratory sample. All were detected prior to the introduction of molecular screening (EntericBio CPE, Serosep, Limerick, Ireland) in October 2020, which detects the NDM, KPC, OXA-48-like, VIM, IMP, and GES carbapenemase genes (Vanstone et al. 2018), but not OXA-58 or other less common genes.

The gene sequence and its translated amino acid sequence aligned closely with known *Acinetobacter* sp. β-lactamase class D enzymes in the NCBI database. The β-lactamase activity of the expressed OXA-58 was confirmed through nitrocefin hydrolysis assays. Furthermore, antimicrobial susceptibility tests revealed that *E. coli* cells harbouring the $bla_{\text{OXA-58}}$ gene showed increased resistance to specific β-lactams, including penicillin G, amoxicillin, and ampicillin. Notably, these transformants did not display resistance to meropenem, a result that deviates from conventional understanding of this enzymatic class, given that OXA-58 enzymes are typically annotated as carbapenemases. This observation is in accordance, however, with previous reports which show a differential resistance profile when $bla_{\text{OXA-58}}$ is transferred to *E. coli* (Héritier et al. 2005, Chen et al. 2008). In this study we have expressed a periplasmic protein using a non-native plasmid and expression host, without removing the leader sequence. A functional enzyme was still produced and capable of operating effectively in the periplasmic space, as evidenced by the phenotype resistance conferred on the transformed *E. coli* against penicillin G, amoxicillin, and ampicillin. However, expression using this non-intrinsic system resulted in no resistance being conferred against meropenem, which would be expected when this gene is expressed in its native host. This shows that phenotypic resistance is determined by more than just the presence of a genetic resistance determinant, and other factors, such as plasmid vector, expression host, and cloning strategy also play a role. In the case of the expression host, and given that the expressed enzyme operates as a periplasmic protein, factors such as different membranes from one species to another may also play a role.

Phenotypic resistance findings suggest meropenem was not efficiently hydrolysed by the OXA-58 enzyme when expressed using the *E. coli* BL21(DE3)/pET28a(+) system. The findings do not prove whether this was due to inefficient binding, or in the hydrolysis step itself. If meropenem binded efficiently with OXA-58 but was not well-hydrolysed, it may in fact act as a competitive inhibitor for the OXA-58 active site. In order to test this hypothesis, the nitrocefin hydrolysis assay was set up in the presence of varying concentrtaions of meropenem (Fig. 3a). These data show that in the presence of meropenem, nitrocefin hydrolysis is reduced, possibly due to the fact that meropenem is occupying the active site of OXA-58 and preventing nitrocefin breakdown. This effect was also observed with amoxicillin, but to a lesser, dose-dependent degree (Fig. 3b). In this case, it appears that amoxicillin is acting as a competing substrate to nitrocefin for the OXA-58 active site, but amoxillin is efficiently hydrolysed by OXA-58 and the hydrolysis product is released, effectively freeing OXA-58 to catalyse further hydrolysis of nitrocefin. In this way, the inhibitory effect of amoxicillin is less pronounced compared to meropenem, and a dose dependent trend can be observed.

The complex interaction between meropenem and various classes of β-lactamases has been extensively studied. Class A, C, and D β-lactamases generally fail to confer *in vitro* resistance to carbapenems (Franceschini et al. 2002). Surprisingly, meropenem has been shown to be extremely slowly hydrolysed substrate of blaC, a specific β-lactamase gene (Kong et al. 2009). This slow hydrolysis might be due to the enzyme's inefficient attempts to orientate the substrate within its active site, and by the time this occurs, the antibiotic has reached lethal concentrations. It is conceivable that as the substrate (meropenem) flexes in the active site, the active site nucleophile and substrate electrophilic centre may come into sufficient proximity randomly, allowing intermittent hydrolysis, giving rise to a slow hydrolysis rate.

Previous work on the OXA-58 Class D β-Lactamase of *Acinetobacter baumannii* revealed that active-site plasticity is essential to carbapenem hydrolysis (Pratap et al. 2016). This complex concept refers to the ability of the enzyme's active site to change shape or orientation to facilitate the reaction. The structural requirements for the catalysis of carbapenems by these enzymes were explored, confirming the mechanistic relevance of a hydrophobic bridge formed over the active site. This structural feature stabilizes the hydrolysis-productive acyl-enzyme species formed from the carbapenem substrates. The plasticity of the active site, therefore, drives the mechanism of carbapenem hydrolysis towards efficiency, providing a nuanced understanding of how these enzymes function.

Compared to other carbapenemases such as OXA-10 and OXA-13, OXA-58 is believed to have less affinity to meropenem due to a difference in the hydrophobic pocket formed by side chains Leu-170 and Val-132 (Verma et al. 2011). Significant increases or decreases in carbapenem resistance mediated by the same OXA-58-containing plasmid can also occur when incorporated into different host cells (Héritier et al. 2005, Chen et al. 2008).

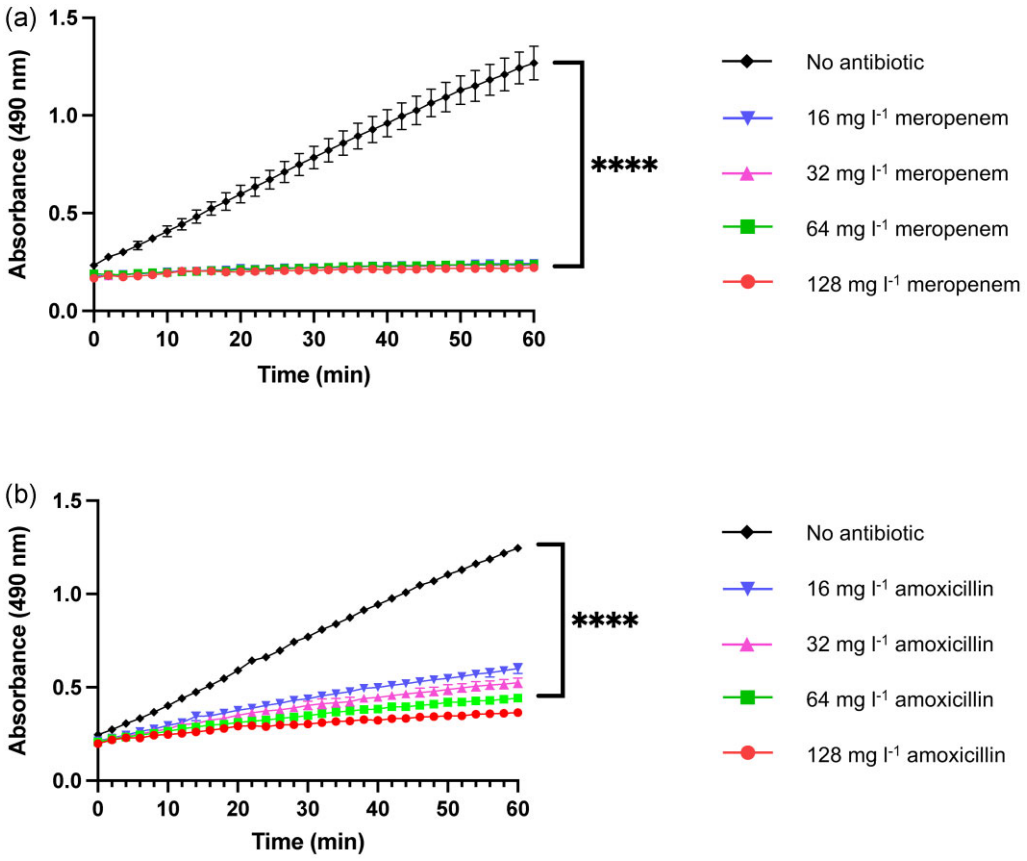

Figure 3. Nitrocefin hydrolysis assay comparing turnover of nitrocefin by OXA-58-pET28a(+)-containing *E. coli* BL21 DE3 cell-free extract in the presence of varying concentrations of (A) Meropenem, and (B) Amoxicillin. Significance levels were the same for each meropenem concentration compared to no antibiotic control and therefore only one set is displayed. Asterisks denote significant differences between the presence and absence of meropenem ($t = 60$). This also applies for amoxicillin concentrations shown in **B**.

Our study has provided further important data showing that an identical resistance profile is not necessarily conferred simply by transferring an ARG from its native strain into a new host. The unique characteristics of OXA-58, especially when compared to other carbapenemases, underscore the multifaceted nature of AMR. This study serves as a reminder of the intricate interplay between genetics and structural factors in understanding resistance profiles across different host environments.

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Author contributions

J. Wu (Conceptualization [supporting], Data curation [lead], Formal analysis [equal], Methodology [lead], Writing – original draft [lead]), T.P. Thompson (Data curation [supporting], Formal analysis [supporting], Methodology [supporting], Writing – original draft [supporting], Writing – review & editing [supporting]), N.H. O'Connell (Resources [supporting], Writing – review & editing [supporting]), K. McCracken (Project administration [supporting], Resources [supporting]), J. Powell (Resources [supporting], Writing – review & editing [supporting]), B.F. Gilmore (Conceptualization [supporting], Methodology [supporting], Supervision [supporting], Writing – review & editing [supporting]), C.P. Dunne (Resources [supporting], Supervision [supporting], Writing – review & editing [supporting]), and S.A. Kelly (Conceptualization [lead], Methodology [supporting], Supervision [lead], Writing – original draft [supporting], Writing – review & editing [lead])

Conflict of interest: Thomas Thompson is on the editorial board of *Letters in Applied Microbiology*. He was not involved in the review or editorial process for this paper, on which he is listed as author.

Data availability

The data underlying this article are available in the GenBank Nucleotide Database at https://www.ncbi.nlm.nih.gov/genbank/and can be accessed with unique identifier, PP989441. All remaining data are incorporated into the article.

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