OXA-48-like carbapenemases in the UK: an analysis of isolates and cases from 2007 - 2014

Jacqueline FINDLAY[1], Katie L. HOPKINS[1*], Richard LOY[1], Michel DOUMITH[1], Danièle MEUNIER[1], Robert HILL[1], Rachel PIKE[1], Nazim MUSTAFA[1], David M. LIVERMORE[1,2], and Neil WOODFORD[1]

[1]Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit, National Infection Service, Public Health England, London NW9 5EQ, UK; [2]Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK

Running heading: OXA-48-like carbapenemases in the UK

***Corresponding author**

Tel: +44 (0)208 327 6511; email: katie.hopkins@phe.gov.uk

Abstract

Objectives: OXA-48-like carbapenemases have spread worldwide since 2001. We analysed patient and microbiological data for UK isolates with these enzymes as confirmed by the national reference laboratory from November 2007 - December 2014.

Methods: MICs were determined using BSAC agar dilution. Isolates with reduced susceptibility or resistance to ≥1 carbapenem and high-level resistance to both piperacillin/tazobactam (MIC > 64 mg/L) and temocillin (MICs ≥ 128 mg/L) were screened by PCR for *bla*$_{\text{OXA-48-like}}$ genes. The genomes of around half of the isolates were sequenced, with MLST types, resistance genes and plasmid replicon types inferred. Patient data provided by sending laboratories were reviewed.

Results: Isolates (n=741) with OXA-48-like carbapenemases were submitted from 111 UK laboratories, representing 536 patients. Almost all (99%; 736/741) were Enterobacteriaceae, predominantly *Klebsiella pneumoniae* (55%; 408), and most (80%; 595) were from inpatients. WGS of 351 non-duplicate isolates identified *bla*$_{\text{OXA-48}}$ as the most common variant, found in two-thirds (235/351) of isolates, followed by *bla*$_{\text{OXA-181}}$ (68), *bla*$_{\text{OXA-232}}$ (32), *bla*$_{\text{OXA-244}}$ (10), *bla*$_{\text{OXA-484}}$ (5) and *bla*$_{\text{OXA-245}}$ (1). Among *K. pneumoniae* (163/351), *E.coli* (114/351), and *E. cloacae* (42/351), 119 STs were identified. Mapping analyses revealed that 63% (222/351) of isolates harboured plasmids that shared >99% identity to one of four known plasmids; pOXA-48a (44%; 154/351), pOXA-232 (10%; 34/351), pOXA181 (9%; 30/351), and pKP3-A (1%; 4/351); the remaining 37% of isolates harboured *bla*$_{\text{OXA-48-like}}$ in unknown environments.

Conclusions: OXA-48-like carbapenemases are an increasing problem in the UK. This study highlights both the role of successful plasmids and polyclonal nature of their dissemination.

Introduction

OXA-48-type carbapenemases were first identified in 2001 in a carbapenem-resistant *Klebsiella pneumoniae* isolate from the urine of a patient hospitalised in Istanbul, Turkey.[1] Since then, reports of infections caused by OXA-48-producing Enterobacteriaceae have escalated - particularly in Europe, Asia and Africa, and less so in the Americas.[2-5] There are six carbapenem-hydrolysing class D beta-lactamase (CHDL) subgroups that are clinically significant; OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, and OXA-143.[6] All except the OXA-48 group are predominantly found in *Acinetobacter* spp. isolates,[6] whereas OXA-48 enzymes are usually found in Enterobacteriaceae.[5] To date, 14 OXA-48-like variants have been described (OXA-48, -162, -163, -181, -199, -204, -232, -244, -245, -247, -370, -405, -436, and -484) of which 11 are CHDLs. These vary in sequence by one to five amino acids from the 'classical' OXA-48 variant and hydrolyse penicillins and carbapenems but not extended-spectrum cephalosporins (e.g. cefepime and ceftazidime).[1] In contrast to their CHDL counterparts, OXAs -163, -247 and -405 also differ from OXA-48 by one or two amino acids but additionally have a four-amino-acid deletion in the active site region: as a result they lack significant carbapenemase activity but do exhibit increased activity toward extended-spectrum cephalosporins.[7-9]

bla$_{\text{OXA-48}}$ has only been found in Enterobacteriaceae, and has been associated with outbreaks in Turkey, the Middle East and North Africa.[5, 10] Other variants have different geographical associations: in particular *bla*$_{\text{OXA-181}}$ and *bla*$_{\text{OXA-232}}$ have been epidemiologically linked to the Indian subcontinent and are often co-harboured with NDM enzymes.[11, 12]

The proliferation of OXA-48-like enzymes has been attributed both to successful clones and to plasmid spread. In 2011, OXA-48-positive *K. pneumoniae* ST395 was identified in patients in Morocco, the Netherlands and France, suggesting the country-to-country transfer of this clone,[13] with patient-linked transfer of OXA-48-producing *Enterobacter cloacae* from Morocco to France also documented.[14] On the other hand, pOXA-48a, a 61.9 kb self-

conjugative IncL/M plasmid, was shown to be the primary vehicle for dissemination of *bla*$_{OXA-48}$ in several outbreaks.[5, 15] This broad-host-range plasmid harbours *bla*$_{OXA-48}$ within Tn*1999* and occurs across several enterobacterial species.[5, 15]

This study describes the epidemiology of OXA-48-like carbapenemase producers submitted to PHE's Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit between 2007 and 2014.

Materials and Methods

Bacterial isolates, identification and susceptibility testing

Isolates were submitted to PHE's AMRHAI Reference Unit from clinical laboratories across the UK between November 2007 and December 2014 for investigation of unusual resistance, including to carbapenems.

Bacterial identification was carried out using chromogenic agars [CHROMagar™ Orientation (CHROMagar, Paris, France) and Brilliance UTI (Oxoid, Basingstoke, UK)] together with API-20E tests (bioMérieux SA, Marcy-l'Étoile, France) or, since August 2012, by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-ToF MS; Bruker Microflex LT, Bruker Daltonik GmbH, Bremen, Germany).

Antibiotic susceptibilities (MICs) were determined by BSAC agar dilution[16] using AMRHAI's standard Gram-negative antibiotic panel, which includes ertapenem, meropenem and imipenem (the latter with/without 320 mg/L EDTA to detect metallo-carbapenemases), and interpreted using EUCAST breakpoints.[17]

Screening for carbapenemase genes

Isolates displaying high-level resistance to piperacillin/tazobactam (MICs ≥64 mg/L) and temocillin (MICs ≥128 mg/L), as well as reduced susceptibility or resistance to any carbapenem were tested for *bla*$_{\text{OXA-48-like}}$ genes by in-house PCR[1] and/or with a commercial microarray (Check-MDR CT102; Check-Points, Wageningen, The Netherlands).[18] In instances where imipenem potentiation by EDTA was observed, isolates were also tested by PCR for the presence of metallo-carbapenemases (*bla*$_{\text{NDM}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{IMP}}$, *bla*$_{\text{GIM}}$, *bla*$_{\text{SIM}}$ and *bla*$_{\text{SPM}}$) by in-house PCRs.[19]

Whole Genome Sequencing (WGS) and analyses

Three hundred and seventy isolates, temporally and geographically distributed throughout the study, were selected for WGS. Genomes were sequenced using the Nextera sample preparation method with the standard 2 × 100-base sequencing protocols on a HiSeq instrument (Illumina, San Diego, CA, USA). Data were analysed using an in-house bioinformatics pipeline as previously described.[20] Sequence types (STs) were inferred from WGS data where MLST schemes exist.

For plasmid analysis, sequencing reads were mapped against known OXA-48 plasmids, namely pOXA-48a (NC_019154), pOXA-232 (JX423831), pKP3-A (NC_019160), and pOXA181 (KP400525).

Analysis of patient demographic information

Patient data were obtained from the request forms sent with submissions from referring laboratories. A patient was categorized as ‘new’ if they were found to have OXA-48-like-positive isolates detected by AMRHAI for the first time and ‘known’ if any OXA-48-like-positive isolate, irrespective of species, had previously been identified from the patient by AMRHAI.

Results

Demographics of patients affected and distribution

During the study period, AMRHAI confirmed 741 OXA-48-like positive isolates from 111 laboratories throughout the UK and obtained from 536 patients. Figure 1 illustrates the temporal distribution of these isolates among 'new' and 'known' patients, and among submitting laboratories. The first OXA-48-like positive isolate was a *K. pneumoniae,* submitted to AMRHAI in November 2007 and obtained from the urine of a patient previously hospitalised in Turkey.[10]

Isolates with OXA-48-like carbapenemases were submitted from laboratories across all UK regions. The national distribution of affected patients was as follows: England (n=514), Scotland (n=13), Northern Ireland (n=6), and Wales (n=3). The greatest number of affected patients was in the London region (n=203), followed by the North West (n=143).

Most source patients were hospitalized (79%; 421/536) but a few were outpatients (7%; 38/536), in primary care (8%; 41/536) or in unknown settings (7%; 36/536). The mean patient age was 59.5 years and 54% (289/536) were male.

A travel history was reported for 130/536 (24%) patients. Of these, 55 patients had documented foreign travel to the following destinations; India (12), Turkey (9), Pakistan (5), Egypt (4), Libya (4), Spain (4), Kuwait (3), Malta (3), Sri Lanka (2), Tunisia (2) and single patients had travelled to Cyprus, Kenya, Morocco, Russia, Saudi Arabia, Singapore, and Syria. Twenty patients were known to have been hospitalised whilst abroad in Egypt (4), Libya (4), Turkey (4), India (3), Pakistan (2), Cyprus (1), Spain (1) and Sri Lanka (1).

Single OXA-48-like-positive isolates were referred from 408/536 (76%) patients and multiple isolates from the remaining 128 (24%). Amongst the patients with multiple OXA-48-like-positive isolates, 43/128 (34%) yielded isolates of different species or genera and 63/128 (49%) had isolates referred from different anatomical sites. The OXA-48-like-positive isolates

were referred over a period of <14 days in 65/128 (51%) instances, for 17/128 over a period of 14 to 28 days, 38/128 over a period of 1 to 6 months and over a period >6 months from 8 patients. Seventeen percent (127/741) of isolates were submitted from a single laboratory (laboratory 1), which serves 3 hospitals (to be subsequently known as hospitals A, B and C), over a 2-year period. Further details of the isolates from this laboratory are given below.

Microbiology

Most (99%; 736/741) submitted isolates were Enterobacteriaceae, comprising: *K. pneumoniae* (55%; 408/741), *E. coli* (29%; 218/741), *Enterobacter* spp. (9%; 68/741), *Klebsiella oxytoca* (3%; 24/741), *Citrobacter* spp. (2%; 14/741), *Serratia marcescens* (>1%; 3/741) and one *Raoutella ornithinolytica*. There were also five non-Enterobacteriaceae isolates comprising: *Pseudomonas aeruginosa* (n=3; all from one patient)[21] and *Shewanella* spp. (n=2; OXA-48-like enzymes are intrinsic in this genus).

If samples, rather than patients were considered as the denominator, most were taken in hospitals (89%; 656/741), but some were general practice urines (6%; 45/741) and a few from unknown settings (5%; 40/741). The most frequently reported specimen type was urine (29%; 215/741), followed by faeces/rectal swabs (27%; 202/741). Fifteen percent (110/741) of isolates were obtained from tissue and fluid samples, 11% (83/741) from blood cultures and line tips, 9% (68/741) from screening swabs, 5% (40/741) from respiratory samples, and 3% (23/741) were unknown specimen types (Table 1).

Carbapenemase alleles and typing of the isolates

WGS was undertaken for 351 non-duplicate isolates from single patients and their STs (where MLST schemes exist) and carbapenemase alleles were defined. These comprised: *K. pneumoniae* (n=163), *E. coli* (n=114), *E. cloacae* (n=42), *K. oxytoca* (n=13), *Citrobacter*

spp. (n=11), other *Enterobacter* spp. (n=5), *S. marcescens* (n=2), and *P. aeruginosa* (n=1). Their carbapenemase genes comprised: bla_{OXA-48} (66%; 230/351), $bla_{OXA-181}$ (18%; 62/351), $bla_{OXA-232}$ (7%; 24/351), $bla_{OXA-244}$ (3%; 10/351), $bla_{OXA-245}$ (<1%; 1/351), $bla_{OXA-484}$ (1%; 5/351), bla_{OXA-48} + bla_{NDM-1} (1%; 5/351), $bla_{OXA-181}$ + bla_{NDM-1} (2%; 6/351) and $bla_{OXA-232}$ + bla_{NDM-1} (2%; 8/351). The carbapenemase variants and allele combinations OXA-48, OXA-181, OXA-48 + NDM-1, and OXA-181 + NDM-1, were found in multiple species. OXA-232, OXA-245, OXA-484, and OXA-232 + NDM-1 were found only in *K. pneumoniae* isolates, whilst OXA-244 was found only in *E. coli* isolates. Numerous other genes encoding resistance to several other classes of antibiotics were also detected throughout all species (Table 2). Overall, 19/351 (5%) isolates had OXA-48-like enzymes together with another carbapenemase, 266/351 (76%) also harboured a predicted extended-spectrum beta-lactamase or plasmid-encoded AmpC, and 85/351 (24%) had OXA-48-like enzymes without any of these additional beta-lactamase types.

K. pneumoniae

One hundred and sixty-three non-duplicate *K. pneumoniae* isolates, from 56 laboratories across 10 UK regions, were sequenced. Individual laboratories submitted between 1 and 15 isolates. Forty-nine STs were identified, the most frequent being ST14 (27 isolates from 17 centres over 16-months), followed by ST231 (18 from 14 centres over 15-months), ST147 (17 from 13 centres over 7-years), ST101 (13 from 10 centres over 4-years), ST11 (10 from 7 centres over 16-months) and ST16 (6 from 5 centres over 3-months). The remaining 43 STs were each represented by five isolates or fewer. Five OXA-48-like variants were identified: bla_{OXA-48} (n=86), $bla_{OXA-181}$ (n=31), $bla_{OXA-232}$ (n=24), $bla_{OXA-484}$ (n=5) and $bla_{OXA-245}$ (n=1), and 16 isolates produced more than one carbapenemase: bla_{OXA-48} + bla_{NDM-1} (n=3), $bla_{OXA-181}$ + bla_{NDM-1} (n=5), and $bla_{OXA-232}$ + bla_{NDM-1} (n=8).

Multiple plasmid replicon types were identified including IncL/M, IncA/C, several IncF variants, IncHI2, IncX3 and ColE-like replicons. Plasmid mapping revealed that 70/86 (81%) of isolates with OXA-48-enzymes and one with OXA-245 had plasmids exhibiting >99% sequence identity to pOXA-48a, whereas among the 31 with OXA-181-enzymes 8 (26%) and 5 (16%) had plasmids with >99% sequence identity to pOXA181 and pKP3-A, respectively. All 32 isolates with OXA-232 enzymes had plasmids exhibiting >99% sequence identity to pOXA-232. pOXA-48a sequences were found in 37 STs, most frequently ST11 (n=8) and ST101 (n=9) whereas pOXA181 sequences were found in 5 STs (ST11, ST61, ST25, ST307, and ST709), each with 1-3 representatives; whilst pKP3-A sequences were found in 2 STs with one (ST395) and four representatives (ST147) respectively. pOXA-232 sequences were found in 7 STs (ST14, ST15, ST16, ST147, ST231, ST307 and ST395), most often ST14 (15 isolates from 13 centres), ST231 (11 from 8 centres), and ST147 (3 from 2 centres).

The earliest UK isolate with an OXA-48-like enzyme dated from 2007 and was from a patient who had previously been hospitalised in Turkey.[10] It was shown here to belong to ST147 and to harbour a pOXA-48a-like plasmid.

The most frequent submitter, laboratory 1, sent 15 isolates that were subjected to WGS over 22-months – 13 of which were obtained from hospital A. These fifteen represented 11 STs and all harboured plasmids with >99% sequence identity to pOXA-48a.

E. coli

One hundred and fourteen non-duplicate *E. coli* isolates, from 49 laboratories across 7 UK regions, were sequenced. Laboratories submitted between 1 and 16 isolates and 37 STs were identified. The most frequent of these were ST38 (53 isolates from 34 laboratories over 42-months) and ST410 (12 from nine laboratories over 20-months). The remaining 35 STs

were each represented by four isolates or fewer. Three OXA-48-like variants were identified; *bla*$_{OXA-48}$ (n=74), *bla*$_{OXA-181}$ (n=30), and *bla*$_{OXA-244}$ (n=10).

Multiple plasmid replicon types were identified including IncL/M, several IncF replicons, IncB/O, IncHI2, IncK and IncX3. Plasmid analyses revealed that 22/74 (30%) of *E. coli* with OXA-48-enzymes had plasmids exhibiting >99% sequence identity to pOXA-48a, and 22/30 (73%) of those with OXA-181 had plasmids that exhibited >99% sequence identity to pOXA181. pOXA-48a sequences were found in 17 STs, each with 1-4 representatives. pOXA181 sequences were found in 10 STs, predominantly ST410 (n=11) which were submitted from 8 laboratories across 5 regions. No plasmid could be identified in 70 isolates. Of these, 50 belonged to ST38, 41 of them harbouring *bla*$_{OXA-48}$ and nine harbouring *bla*$_{OXA-244}$. The most frequent submitter, laboratory 1, sent 16 isolates that were subject to WGS over a 21-month period - 14 of which were obtained from hospital A; these represented 14 STs, all had OXA-48 and 12/16 (75%) harboured pOXA-48a sequences.

Enterobacter spp.

Forty-seven non-duplicate *Enterobacter* spp. isolates, sent from 15 laboratories across 7 UK regions, were sequenced. These comprised; *E. cloacae* (n=42), *E. aerogenes* (n=4) and *E. hormaechei* (n=1). Individual laboratories submitted between 1 and 27 isolates. Forty-five isolates harboured *bla*$_{OXA-48}$ and the remaining 2 isolates harboured *bla*$_{OXA-48}$ + *bla*$_{NDM-1}$. Thirteen STs were identified among the *E. cloacae* isolates, each with between 1 and 17 representatives. The most frequently obtained ST was ST108 (17 isolates from 2 centres over 15-months), the remaining 12 STs were represented by 5 isolates or fewer. Multiple plasmid replicon types were identified including IncL/M, IncN, IncHI1 and several IncF replicons. Plasmid mapping revealed that 42/47 (89%) of isolates had DNA exhibiting >99% sequence identity to pOXA-48a. These comprised 40 *E. cloacae*, and single isolates of *E. aerogenes* and *E. hormaechei*. Thirteen STs were identified among the 40 *E. cloacae*

isolates but predominantly ST108 with 17 representatives. Most were submitted (16/17) from laboratory 1 and 14/16 were obtained from hospital A, over a 3-month period. Fifteen of these 16 (94%) harboured pOXA-48a sequences. In total, over half (27/48) of the *Enterobacter* spp. isolates with OXA-48-like enzymes were submitted by laboratory 1 over a 15-month period – 25 of which were obtained from hospital A, with these comprising 25 *E. cloacae* and one *E. hormaechei*. All 26 isolates produced OXA-48 and the 25 *E. cloacae* isolates represented 5 STs.

K. oxytoca

Thirteen non-duplicate *K. oxytoca* isolates, sent from 7 laboratories across 4 UK regions, were sequenced, with individual laboratories submitting between 1 and 4 isolates. All harboured the classical $bla_{\text{OXA-48}}$ variant. Five STs were identified, namely ST176 (6 isolates from 5 centres in four regions), ST27 (4 from one centre in one month), along with single representatives of ST36, ST95 and ST168. Multiple plasmid replicons were identified including IncL/M, IncHI2 and several IncF replicons. Plasmid mapping analyses revealed that all isolates shared >99% sequence identity with plasmid pOXA-48a.

Other species

The remaining 14 isolates that were sent for WGS comprised: *Citrobacter freundii* (n=9), *Citrobacter koseri* (n=2), *S. marcescens* (n=2) and *P. aeruginosa* (n=1). All harboured classical $bla_{\text{OXA-48}}$ except for one *C. freundii* isolate, which harboured $bla_{\text{OXA-181}}$ + $bla_{\text{NDM-1}}$, and the *P. aeruginosa* isolate that had $bla_{\text{OXA-181}}$. The *P. aeruginosa* isolate was previously found to belong to ST773 with $bla_{\text{OXA-181}}$ encoded within Tn*2013*.[21] Plasmid replicon types included IncL/M, several IncF replicons and IncA/C. Within the 9 *C. freundii* isolates 8 STs were identified. Five of these were obtained from inpatients in hospital A, 3 of which

harboured pOXA-48a sequences. In the remaining isolates 2 *C. koseri* isolates which produced OXA-48 shared >99% identity to plasmid pOXA-48a, and one OXA-181 producing *C. freundii* isolate shared >99% identity to pKP3-A.

Antibiotic susceptibility

MIC distributions for OXA-48-like-positive isolates are shown in Table 3. Ninety-nine percent (724/728) of isolates with available susceptibility data were resistant or non-susceptible to ertapenem; however 42% (312/735) and 54% (400/735) remained susceptible to imipenem and meropenem, respectively, based on EUCAST breakpoints. In all but 8 cases, MICs of meropenem were above the EUCAST screening cut-off concentration of 0.125 mg/L.[22] For the remaining 8 cases the imipenem MICs were also below the EUCAST screening cut-off of 1 mg/L but were above the ertapenem MIC cut-off of 0.125 mg/L. All 8 isolates were *E. coli* and analysis of 7/8 by WGS indicated that 6 different STs and 2 OXA-48-like variants ($bla_{\text{OXA-48}}$ (5) and bla_{OXA181} (2)) were represented. Piperacillin/tazobactam resistance (MIC >16 mg/L) was observed in 732/734 of tested isolates (data not shown). All the isolates that co-produced either NDM or VIM enzymes were non-susceptible to all three carbapenems. Non-susceptibility to ceftazidime and cefotaxime was observed in 69% and 89% of isolates. Non-susceptibility to the aminoglycosides amikacin, gentamicin and tobramycin was observed in 26%, 55% and 61% of isolates, respectively. Almost all (28/34) isolates that co-produced another carbapenemase were non-susceptible to all 3 aminoglycosides and all were resistant to ciprofloxacin. Most (91%; 659/722) isolates were susceptible to colistin.

Colistin resistance was observed in 63 isolates, submitted from 32 laboratories over 6 years, the majority (54/63) of which were *K. pneumoniae* and had MICs in the range to 4->32 mg/L. Sequencing of 21/54 colistin resistant *K. pneumoniae* isolates identified 9 STs, 6 of which were represented by a single isolate and the 4 remaining STs were as follows: ST14 (6), ST101 (4), ST147 (2), and ST231 (4). These 21 were submitted from 14 laboratories across

5 regions. For 10 isolates colistin MICs were >32mg/L; these comprised 7 *K. pneumoniae* and one *E. coli* along with two *S. marcescens* with inherent resistance. These were referred from 10 laboratories across 4 regions. Non-susceptibility to ciprofloxacin and tigecycline was observed in 63% and 32% of isolates, respectively.

Discussion

This report reviews all isolates producing an OXA-48-like carbapenemase and referred to PHE's AMRHAI Reference Unit from laboratories across the UK between November 2007 and December 2014. Over this study period 741 OXA-48-like-positive isolates were obtained from 536 patients across all UK regions.

The majority of isolates were from clinical specimens, predominantly urines. All isolates were resistant to ≥2 classes of antibiotics and most were non-susceptible at EUCAST breakpoints to at least one of the three carbapenems tested. A high rate of susceptibility was maintained only to colistin (91%), with amikacin (74% susceptible) and tigecycline (68%) next in rank order. High levels of resistance to the third-generation cephalosporins, ceftazidime and cefotaxime, could be attributed to the co-carriage of ESBL/AmpC enzymes in 76% of sequenced isolates. There was huge variation in susceptibility to other antibiotics tested in this study, attributable to the presence of a plethora of other resistance genes, as identified in the WGS analyses (Table 2), sometimes including other carbapenemase genes - 5% (34/741) of isolates with OXA-48-like enzymes also harboured either a bla_{NDM} (33/741) or bla_{VIM} (1/741) allele. It follows that there can be no 'standard' antibiotic regimen for the treatment of infections caused by OXA-48-like producers without additional susceptibility testing and/or resistance gene data, although ceftazidime-avibactam shows promise based on *in vitro* data.[23] Although colistin, tigecycline and amikacin retained the highest levels of susceptibility their individual indications may make them unsuitable for the treatment of some

infections. For example tigecycline cannot be used for the treatment of urinary tract infections[24] and colistin use has been associated with both neuro- and nephrotoxicity.[25]

At the time of writing there are 14 known OXA-48-like variants, 11 of them CHDLs, and WGS analysis of 351 non-duplicate UK isolates identified five of the CHDL types. OXA-48 was by far the most common variant, found in two-thirds (235/351) of isolates. The earliest OXA-48-positive isolate identified in the UK was obtained in 2007 from a patient who had recently been hospitalised in Turkey; this isolate was shown here to be an ST147 *K. pneumoniae* carrying a plasmid with >99% identity to pOXA-48a. pOXA-48a has been implicated in early OXA-48 dissemination in Turkey.[15, 26]

A travel history was available for only 24% of patients, of whom 10% had documented travel to 18 different countries/islands, several of which have previously reported outbreaks involving bacteria with OXA-48-like enzymes.[2, 4, 5, 27] All five patients with reported travel to Turkey and 6/7 with travel to other Middle Eastern or North African countries whose isolates were analysed by WGS were found to carry organisms with $bla_{\text{OXA-48,}}$ as repeatedly found in Turkey.[26, 27] By contrast, both OXA-181 and OXA-232 have been associated with the Indian subcontinent,[12, 28] and were found in 12/13 sequenced isolates from patients reporting travel to India, Pakistan or Sri Lanka. These data further underscore the role that international travel may play in carbapenemase dissemination. Notably four *K. pneumoniae* with OXA-48-like enzymes were from patients transferred to the UK for intensive care treatment of injuries received during the Libyan 'Emergency' of 2011.

Forty-four percent (154/351) of sequenced isolates, and 153/235 of those with classical $bla_{\text{OXA-48}}$ possessed DNA with >99% sequence identity to pOXA-48a, an approx. 62 kb IncL/M plasmid first associated with $bla_{\text{OXA-48}}$, in Turkey and North Africa and now with multiple polyclonal and cross species outbreaks.[15] pOXA-48a-like sequences were found here in multiple species and sequence types. Except for one isolate with $bla_{\text{OXA-245}}$ these all carried $bla_{\text{OXA-48}}$. The demonstration of both the broad range and success of this plasmid

supports an earlier and much smaller analysis where we found IncL/M OXA-48 plasmids amongst several enterobacterial species and STs.[10] Of the remaining 197 isolates sequenced, 68 carried DNA with >99% identity to one of three plasmids: pOXA-232 (32/68), pOXA181 (30/68) or pKP3-A (6/68). pOXA-232 and pKP3-A are ColE-like plasmids, of approx. 6 kb and 7.6 kb respectively, originally discovered in *E. coli* and *K. pneumoniae* isolates obtained from patients following hospitalisation in India.[28, 29] All of the 32 sequenced isolates harbouring $bla_{\text{OXA-232}}$ and six of 69 isolates with $bla_{\text{OXA-181}}$ contained sequences with >99% sequence identity to pOXA-232 and pKP3-A respectively. Thirty further isolates with OXA-181 enzymes were shown to carry DNA with >99% sequence identity to pOXA181, an IncX3 plasmid of approx. 51.5 kb that was first found in an *E. coli* ST410 isolate obtained from the blood sample of a patient in China who had no history of travel to the Indian subcontinent.[30]

We found $bla_{\text{OXA-48-like}}$ genes in multiple species and STs and also that there were some clones that were particularly successful as a vehicle for $bla_{\text{OXA-48-like}}$ dissemination. In *K. pneumoniae* some STs (such as ST14 and ST147) were associated with multiple OXA-48-like plasmids, indicating the success of the ST but not indicating expansion of a specific clone/plasmid pairing. Although *K. pneumoniae* ST395 has been associated with outbreaks in Europe and Morocco,[13] we found only two representatives among those sequenced and these harboured different OXA-48-like variants, $bla_{\text{OXA-48}}$ and $bla_{\text{OXA-181}}$, in different genetic environments; one in a pKP3-A sequence and the other in an unidentified environment.

Rather alarmingly, 16% of isolates were submitted from a single hospital, hospital A, representing at least six species and 33 STs. Within the sixty-one isolates from hospital A that were sequenced most (54/61) harboured pOXA-48a sequences. This suggests the local spread of pOXA-48a amongst different genera, species and STs within this hospital and is indicative of the success of pOXA-48a in dissemination.

In contrast to the ST diversity among *K. pneumoniae* isolates with OXA-48-like enzymes ST38 accounted for almost half of all the sequenced *E. coli* isolates with OXA-48-llike enzymes (53/114). ST38 has previously been associated with bla_{CTX-M} carriage in multiple countries.[31] Plasmid mapping could not establish a location for bla_{OXA-48} in most (50/53) of these isolates. In a previous study[10] the authors failed to obtain any plasmid transformants from ST38 isolates and, more recently, it was reported that bla_{OXA-48} and $bla_{OXA-244}$ can be chromosomally-encoded in ST38 isolates.[32] This may apply here, but establishing this would require utilisation of longer read sequencing techniques (e.g. PacBio and MinION).

In summary, this study has shown an increase in OXA-48-like enzymes in the UK over a seven-year period. We suggest that the accumulation of OXA-48-like carbapenemases within the UK is due to repeated import, coupled with both the dissemination of successful plasmids, notably pOXA-48a and the spread of successful clones (e.g. *E. coli* ST38); the linkage to plasmid spread was especially strong.

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Sharpe & Dohme Corp and Nordic Pharma. Shareholdings in: Dechra, GSK, Merck Sharpe & Dohme Corp, Perkin Elmer, Pfizer amounting to <10% of portfolio value.

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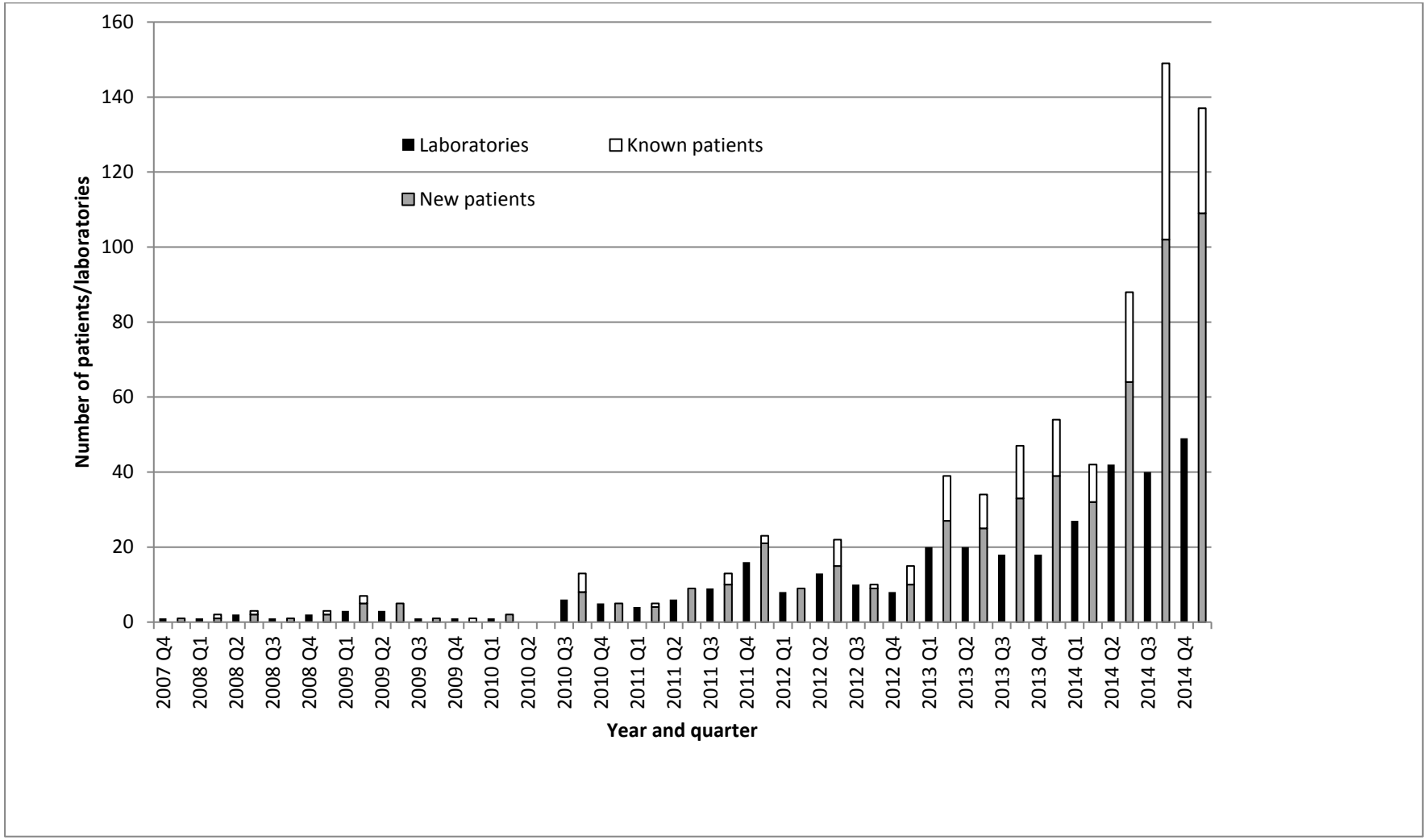
519 Figure 1. Numbers of new and known affected patients and laboratories sending OXA-48-positive isolates per quarter during the study period.



520

521 Table 1. Sources of OXA-48-like-positive isolates.

	Hospital setting								
Species	Urines	Screening swabs	Blood cultures and line tips	Respiratory	Tissue and fluid	Faeces/Rectal swabs	Not known	GP urines	Total
K. pneumoniae	91	27	48	28	58	110	8	15	385
E. coli	47	22	18	7	28	58	2	25	207
Enterobacter spp.	6	15	9	1	9	16	10	1	67
K. oxytoca	2	2	7	0	2	6	0	4	23
*Citrobacte*r spp.	5	2	1	0	0	4	0	0	12
S. marcescens	0	0	0	0	1	0	0	0	1
R. ornithinolytica	0	0	0	0	0	1	0	0	1
other spp.[a]	0	0	0	2	2	1	0	0	5
Total	151	68	83	38	100	196	20	45	701

522

	Unknown setting							
Species	Urines	Screening swabs	Blood cultures and line tips	Respiratory	Tissue and fluid	Faeces/Rectal swabs	Not known	Total
K. pneumoniae	9	0	0	1	7	4	2	23
E. coli	8	0	0	0	2	1	0	11
Enterobacter spp.	1	0	0	0	0	0	0	1
K. oxytoca	0	0	0	1	0	0	0	1
Citrobacter spp.	1	0	0	0	0	1	0	2
S. marcescens	0	0	0	0	1	0	1	2
Total	19	0	0	2	10	6	3	40

523 [a]- other species comprise *P. aeruginosa* (n=3) and *Shewanella* spp. (n=2).

524

525

526 Table 2. Characteristics of 351 non-duplicate isolates that were subjected to WGS.

		Carbapenemases				Other resistance genes		
Species	No. of isolates	OXA-48-like alleles **(no.)**	OXA + NDM alleles (n=13)	STs **(no. if >1)**	Replicons	Beta-lactamases (variants)	Aminoglycoside resistance genes	Others
K. pneumoniae	163	OXA-48**(86)**, OXA-181**(31)**, OXA-232**(24)**, OXA-245**(1)**, OXA-484**(5)**	OXA-48+NDM-1**(3)**, OXA-181+NDM-1**(5)**, OXA-232+NDM-1**(8)**	11**(10)**, 14**(27)**, 15**(4)**, 16**(6)**, 17, 25**(2)**, 35, 36, 39, 43, 45**(4)**, 48**(2)**, 101**(13)**, 104, 105, 111, 133**(2)**, 147**(17)**, 152, 187**(2)**, 231**(18)**, 253, 294, 299, 307**(7)**, 323, 327, 336**(3)**, 359, 392**(3)**, 395**(2)**, 405**(2)**, 461**(2)**, 659, 685, 709, 831**(2)**, 922, 985, 1141, 1164, 1473**(5)**, 1680**(2)**, 1819, 1821, 1825, 1827, 1834, 2205	A/C, ColKP3, FIA, FIB, FII, HI2, L/M, X3	*bla*$_{TEM-1}$, *bla*$_{SHV}$ (1,11,28,33,39,75,76,100,103,159), *bla*$_{CTX-M}$ (14,15,16), *bla*$_{OXA}$ (1,9), *bla*$_{DHA-1}$, *bla*$_{CMY-4}$	*strA*, *strB*, *aadA1*, *aadA2*, *aadA3*, *aadA5*, *armA*, *aac(3)-lla*, *aac(3)-lld*, *aac(6')Ib-cr*, *rmtF*	*oqxA*, *oqxB*, *qnrB1*, *qnrS1*, *qnrB66*, *arr-2*, *arr-3*, *sul1*, *sul2*, *fosA*, *mphA*, *msrE*, *ereA*, *ereB*, *ereC*, *ermB*, *mdfA*, *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA14*, *catA1*, *catB3*, *cmlA1*, *sat2*, *tetA*, *tetD*
E. coli	114	OXA-48**(74)**, OXA-181**(30)**, OXA-244**(10)**		10**(4)**, 28, 38**(53)**, 58, 59, 69, 73, 83, 95, 127, 131, 167, 205**(2)**, 224, 227**(2)**, 354**(2)**, 361, 399**(3)**, 401, 405**(4)**, 410**(12)**, 428, 648, 681, 940**(3)**, 1170, 1284**(2)**, 1431, 1722, 2139, 2164, 2179, 3221, 3541, 6328, 6329, 6330	B/O, FIA, FIB, FII, HI2, K, L/M, X3	*bla*$_{TEM}$ (1,33,169,190), *bla*$_{CTX-M}$ (14,15,24,27,82), *bla*$_{OXA}$ (1,10), *bla*$_{CMY}$ (2,42,44,54,59,61)	*strA*, *strB*, *aadA1*, *aadA2*, *aadA3*, *aadA5*, *aadA23*, *aac(3)-lld*, *aac(3)-lla*, *aph(6)-ld*, *aac(6')Ib-cr*, *rmtB*	*qnrB1*, *qnrS1*, *qepA*, *mdfA*, *mphA*, *ermB*, *msrE*, *dfrA1*, *dfrA5*, *dfrA7*, *dfrA12*, *dfrA14*, *dfrA17*, *fosA*, *sul1*, *sul2*, *catA1*, *cmlA1*, *floR*, *sat2*, *tetA*, *tetD*
E. cloacae complex	42	OXA-48**(40)**	OXA-48+NDM-1**(2)**		FIB, FII, HI1, L/M	*bla*$_{TEM-1}$, *bla*$_{OXA-1}$, *bla*$_{SHV}$ (5,12), *bla*$_{CTX-M}$ (9,15,82), *bla*$_{ACT}$ (7,14,15,16)	*strA*, *strB*, *aadA2*, *aadA3*, *aadA12*, *aac(3)-lla*, *ant(2")-la*, *aac(6')-llc*, *aac(6')Ib-cr*	*qnrA1*, *qnrB1*, *qnrS1*, *ereA*, *mphA*, *dfrA12*, *dfrA14*, *dfrA16*, *dfrA18*, *fosA*, *sul1*, *sul2*, *catA1*, *catA2*, *tetA*, *tetD*
K. oxytoca	13	OXA-48**(13)**		27**(4)**, 36, 95, 168, 176**(6)**	FII, HI2, L/M	*bla*$_{TEM-1}$, *bla*$_{OXY}$ (1,2,5,6)	*strA*, *strB*, *aac(3)-lla*	*qnrA1*, *mphA*, *dfrA18*, *sul1*, *tetD*, *tetK*
C. freundii	11	OXA-48**(10)**	OXA-181+NDM-	ND	A/C, FIB,	*bla*$_{TEM-1}$, *bla*$_{CMY-48}$,		*qnrB12*, *dfrA7*, *sul1*,

		1**(1)**		FII, L/M	*bla*$_{MAL-1}$		*sul2*, *tetD*
Other *Enterobacter* spp.	5	OXA-48**(5)**	45**(3)**, 51, 66**(5)**, 90, 93**(3)**, 104**(4)**, 106, 108**(17)**,135, 145, 268, 269, 279**(3)**	H, L/M, N	*bla*$_{TEM-1}$, *bla*$_{CTX-M-14}$, *bla*$_{ACT-37}$, *bla*$_{SHV-12}$	*strA*, *strB*, *aadA1*, *aadA2*, *aph(6)-Id*, *aac(6')-Ilc*, *aph(3')-Vib*	*qnrA1*, *ereA*, *dfrA18*, *floR*, *tetA*, *sul1*, *sul2*, *sul3*
S. marcescens	2	OXA-48**(2)**	ND	L/M	*bla*$_{CTX-M}$ (14,82)	*strA*, *strB*, *aph(3')-Vib*	
P. aeruginosa	1	OXA-181**(1)**	773		*bla*$_{PAO}$, *bla*$_{OXA-50}$	*aac(3)-Ie, aph(3')-IIb, aadA1*	*catB7*, *dfrB5*, *sul1*

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528 Table 3. MIC distributions for OXA-48-like-positive isolates (n=741).

				Number of isolates with MIC (mg/L)												
Carbepenemase gene(s)	Isolates	Antibiotic (range tested, mg/L)	EUCAST breakpoints ≤S/>R	≤0.125	0.25	0.5	1	2	4	8	16	32	64	≥128	NA	%S
OXA-48-like	Enterobacteriaceae	Ertapenem (0.125-16)	≤0.5/>1			4	15	53	124	147	351[a]				7	<1
	other spp.[c]														6	NA
NDM + OXA-48-like	Enterobacteriaceae										33[a]					0
VIM + OXA-48-like	Enterobacteriaceae										1[a]					0
OXA-48-like	Enterobacteriaceae	Imipenem (0.06-128)	≤2/>8		5	28	92	186	180	98	47	22	20	17[a]	6	45
	other spp.[c]					1			1			1	1	2[a]		17
NDM + OXA-48-like	Enterobacteriaceae								1	4	6	3	7	12[a]		0
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0
OXA-48-like	Enterobacteriaceae	Meropenem (0.06-32)	≤2/>8	7[b]	38	105	138	110	69	35	81	112[a]			6	57
	other spp.[c]			1[b]			1				1	3[a]				33
NDM + OXA-48-like	Enterobacteriaceae									1	4	28[a]				0
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0
OXA-48-like	Enterobacteriaceae	Ceftazidime (0.125-256)	≤1/>4	14[b]	57	69	86	31	28	26	23	47	83	231[a]	6	33
	other spp.[c]			2[b]				2	2							33
NDM + OXA-48-like	Enterobacteriaceae													33[a]		0
VIM + OXA-48-like	Enterobacteriaceae													1[a]		0
OXA-48-like	Enterobacteriaceae	Cefotaxime (0.125-256)	≤1/>2	1[b]	3	28	48	40	45	42	23	22	33	405[a]	11	12
	other spp.[c]			1[b]											5	100
NDM + OXA-48-like	Enterobacteriaceae													33[a]		0
VIM + OXA-48-like	Enterobacteriaceae													1[a]		0
OXA-48-like	Enterobacteriaceae	Amikacin (0.5-64)	≤8/>16			26[b]	138	186	124	58	47	30	86[a]		6	77
	other spp.[c]						2		1			1	2[a]			50
NDM + OXA-48-like	Enterobacteriaceae							2	2	2			27[a]			18
VIM + OXA-48-like	Enterobacteriaceae												1[a]			0
OXA-48-like	Enterobacteriaceae	Gentamicin (0.125-32)	≤2/>4	3[b]	55	175	85	10	11	10	22	324[a]			6	47
	other spp.[c]				1			1	1	2	1					33
NDM + OXA-48-like	Enterobacteriaceae					1						32[a]				3
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0
OXA-48-like	Enterobacteriaceae	Tobramycin (0.125-32)	≤2/>4	1[b]	17	119	114	29	36	59	87	230[a]			9	40
	other spp.[c]						1	1		1		3[a]				33
NDM + OXA-48-like	Enterobacteriaceae										5	28[a]				0
VIM + OXA-48-like	Enterobacteriaceae											1[a]				0

OXA-48-like	Enterobactericaeae	Ciprofloxacin	≤0.5/>1	191[b]	45	33	24	11	37	343[a]			17	39
	other spp.[c]	(0.125-8)		1[b]			1			4[a]				17
NDM + OXA-48-like	Enterobactericaeae									31[a]			2	0
VIM + OXA-48-like	Enterobacteriaceae									1[a]				0
OXA-48-like	Enterobactericaeae	Colistin	≤2/>2			397[b]	204	20	11	16	13	21[a]	19	91
	other spp.[c]	(0.5-32)				2[b]	2	1	1					83
NDM + OXA-48-like	Enterobactericaeae					19[b]	12	1	1					97
VIM + OXA-48-like	Enterobacteriaceae						1							100
OXA-48-like	Enterobactericaeae	Tigecycline	≤1/>2		185[b]	140	144	118	65	24	6[a]		19	69
	other spp.[c]	(0.25-16)			1[b]			1					4	50
NDM + OXA-48-like	Enterobactericaeae				8[b]	3	6	12	3		1[a]			52
VIM + OXA-48-like	Enterobacteriaceae									1				0

529 S, susceptible; R, resistant; NA, not available.

530 The dotted vertical lines indicate intermediate breakpoints and the solid vertical lines indicate resistant breakpoints.

531 [a]MIC greater than or equal to indicated value.

532 [b]MIC less than or equal to indicated value.

533 [c]other spp. comprise *Pseudomonas aeruginosa* and *Shewanella* spp..

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