Infection (2016) 44:107–110
DOI 10.1007/s15010-015-0805-7

CASE REPORT

Retrospective identification of a previously undetected clinical case of OXA-48-producing *K. pneumoniae* and *E. coli*: the importance of adequate detection guidelines

Ina Willemsen[1] · Joost van Esser[2] · Marjolein Kluytmans-van den Bergh[3,4] · Kai Zhou[5] · John W. Rossen[5] · Carlo Verhulst[1] · Kees Verduin[1] · Jan Kluytmans[1,4]

Received: 23 March 2015 / Accepted: 29 May 2015 / Published online: 11 June 2015

Abstract
Introduction The laboratory detection of OXA-48-carbapenemase-producing Enterobacteriaceae is difficult, as minimum inhibition concentrations for carbapenems are often below the clinical breakpoint. In 2011, the Dutch national guideline for the detection of highly resistant micro-organisms was issued, which includes recommendations on the use of carbapenem screening breakpoints for the detection of carbapenemase-producing Enterobacteriaceae.
Materials and Methods During a validation study of the Check-MDR CT103 microarray (Check-Points, Wageningen, The Netherlands) in 2013, an OXA-48-like carbapenemase gen was identified in two isolates that were previously obtained from a patient with non-Hodgkin lymphoma in 2007. Whole-genome sequencing (WGS) and subsequent BLAST Ringe Image Generator (BRIG) analysis were performed to establish the presence of OXA-48 carbapenemase encoding plasmids and their similarity.
Results This case report describes the first documented OXA-48-producing *Klebsiella pneumonia* (ST648) and *Escherichia coli* (ST866) in the Netherlands. A similar IncL/M plasmid was identified in both strains, suggesting within-patient horizontal transfer.
Conclusion This case illustrates that OXA-48-carbapenemase-producing Enterobacteriaceae can be unnoticed without adequate laboratory detection procedures. Our observation stresses the importance of uniform and adequate laboratory methods for the timely and accurate detection of important antimicrobial resistance.

Keywords OXA-48 · β-lactamase · Antimicrobial resistance · Plasmid transfer · Detection guidelines

Case report

In August 2006, a 63-year-old woman of Turkish origin was seen in the emergency room of our hospital because of abdominal pain, obstipation, and weight loss (8 kg in 2 months). She reported recent hospitalization in a Turkish hospital for less than 24 h during her holiday. She was diagnosed with a stage IVB diffuse B cell non-Hodgkin lymphoma with localisations in stomach and pleural cavity, for which eight R-CHOP$_{14}$ (rituximab, cyclophosphamide, doxorubicin, oncovin, and prednisone) courses with pegfilgrastim (to stimulate the level of white blood cells) were planned.

In December 2006, following her fourth R-CHOP course, she was admitted to the hospital because of abdominal pain and elevated infection parameters (high body temperature and increased C-reactive protein), and antimicrobial treatment with cefuroxime and metronidazole was started. A computed tomography (CT) scan showed fluid collections around liver and spleen, without signs of perforation or abscess. No residual lymphoma was seen.

✉ Ina Willemsen
ina.willemsen@gmail.com; iwillemsen@amphia.nl

[1] Laboratory for Microbiology and Infection Control, Amphia Hospital, PO Box 90158, 4800 RK Breda, The Netherlands

[2] Department for internal medicine, Amphia Hospital, Breda, The Netherlands

[3] Amphia Academy Infectious Disease Foundation, Amphia Hospital, Breda, The Netherlands

[4] Julius Center for Health Sciences and Primary Care, UMC Utrecht, Utrecht, The Netherlands

[5] Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

Table 1 Minimum inhibition concentrations (MIC), resistance genes, MLST, and plasmid(s)

	Isolate [1] *K. pneumoniae* abscess	Isolate [2] *K. pneumoniae* blood	Isolate [3] *E. coli* abscess	Isolate [4] *E. coli* rectal swab
Culture date	11-01-2007	9-3-2007	13-04-2007	21-04-2007
2007	Antimicrobial susceptibility, MIC mg/L)[a]			
Amoxicillin + clavulanic acid	>=32	>=32	>=32	>=32
Piperacillin + tazobactam	>=128	>=128	64	>=128
Cefuroxime	>=64	4	>=64	>=64
Ceftriaxone	<=1	<=1	>=64	>=64
Ceftazidime	<=1	<=1	>=64	>=64
Meropenem	1	1	<=0.25	8
Imipenem	4	8	<=1	8
Ciprofloxacin	<=0.25	<=0.25	>=4	>=4
Gentamicin	<=1	<=1	>=16	>=16
Tobramycin	<=1	<=1	>=16	>=16
Sulfamethoxazole	<=20	<=20	>=320	>=320
2013	Additional tests			
Etest meropenem	[b]	1	4	24
Etest imipenem	[b]	6	3	8
Etest ertapenem	[b]	1	>32	>32
Combined disk ESBL test				
Cefotaxime/cefotaxime + clav.acid	[b]	25/26 mm	10/12 mm	<10/13 mm
Ceftazidime/ceftazidime + clav.acid	[b]	28/28 mm	11/21 mm	12/19 mm
Microarray	[b]	OXA-48 like	OXA-48 like	OXA-48 like
			CTX-M-15 like	CTX-M-15 like
MLST	[b]	ST866	–	ST648
Plasmid(s)	[b]	*inc*L/M (pKPoxa-48N1)	–	*inc*L/M (pKPoxa-48N1)

[a] Determined by automated system VITEK2

[b] Isolate not stored at −80 °C and not available for further testing

Gastroscopy showed an ulcer not related to non-Hodgkin lymphoma.

A few weeks later, her clinical situation worsened and a CT-scan showed an abscess in the right upper abdomen. Subsequently, drainage was performed. Culture of fluid obtained from the abscess grew *K. pneumoniae*, with an MIC for meropenem of 1 mg/L and for imipenem of 4 mg/L, was measured using Vitek2 (isolate 1, Table 1). Treatment with trimethoprim–sulfamethoxazole was initiated (tested susceptible).

In February 2007, the patient was readmitted because of neutropenic fever and empiric treatment with piperacillin/tazobactam was started. When blood cultures grew a *K. pneumoniae* (isolate 2) that was resistant to piperacillin/tazobactam, therapy was switched to ceftriaxone.

Shortly afterwards, she was readmitted because of a relapse of her pre-hepatic abscess. Drainage showed an ESBL-producing, but carbapenem-susceptible, *E. coli* (isolate 3, Table 1). Following meropenem treatment, the patient recovered and the abscess resolved. Later that month, rectal swabs revealed an ESBL-positive *E. coli* with an Etest MIC for meropenem of 8 mg/L and for imipenem of 8 mg/L (isolate 4, Table 1). The patient, ultimately, died in August 2007 due to respiratory failure, secondary to rituximab lung injury.

In 2013, a study was performed in the microbiology laboratory to validate a commercial microarray (Check-MDR CT103, Check-Points, Wageningen, The Netherlands) for the detection of ESBL and carbapenemase genes. Isolate 3, ESBL positive, was included in the historical collection that was used for this validation. The microarray revealed the presence of an OXA-48-like carbapenemase gene [1]. Isolate 2 and 4, retrieved from the freezer, were also tested positive for OXA-48-like carbapenemase gene in the microarray. Whole-genome sequencing was performed for both the *E. coli* and *K. pneumoniae* isolates using the Nextera XT v2kit (Illumina, San Diego, CA, USA) and run on a Miseq (Illumina, San Diego, CA,USA) as described by Zhou et al. [2].

To identify the OXA-48 plasmid from the *E. coli* Sequence Type (ST) 866 and the *K. pneumoniae* ST648

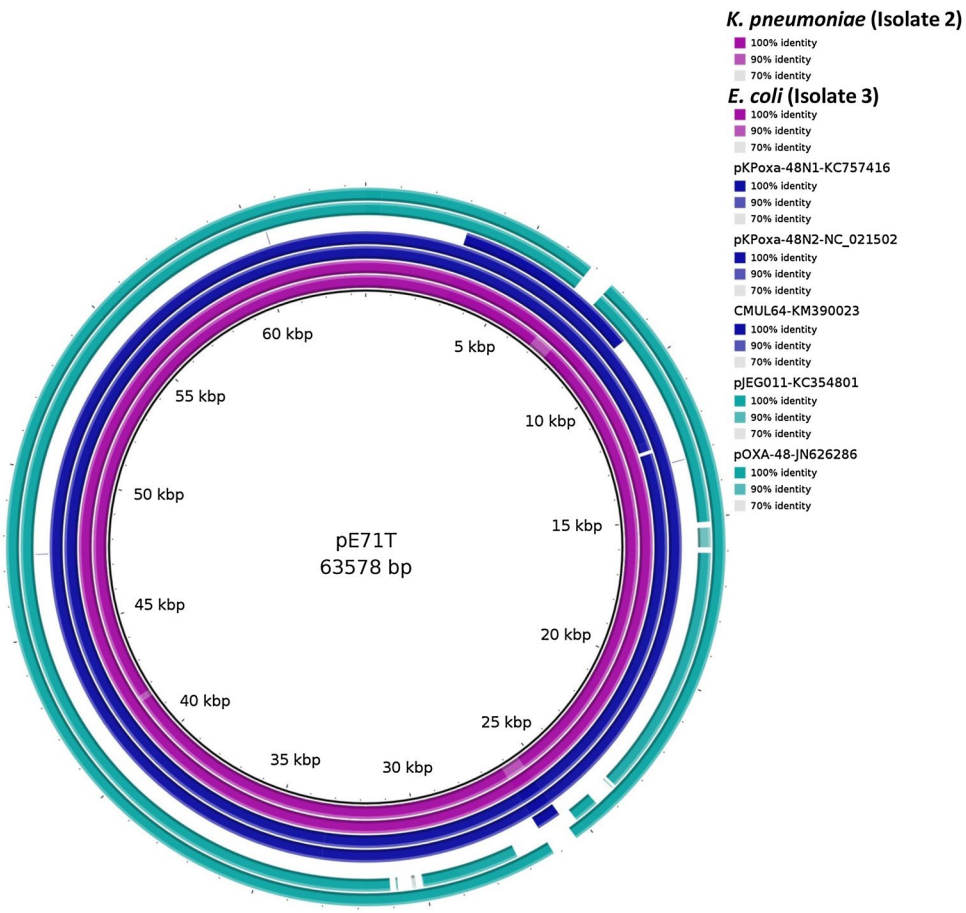

Fig. 1 Plasmid comparison generated by BRIG, with plasmid E71T used as a reference

strain (STs were determined by whole-genome sequencing), the fragments encoding OXA-48 and transposase were selected from both strains and were blasted in the GenBank. (http://blast.ncbi.nlm.nih.gov/Blast.cgi). In total, 7 matches with plasmids were detected with 100 % identity and 76–100 % coverage. A local BLAST was then performed using the genomes of the two strains to blast against the seven plasmids, using the BLAST Ring Image Generator (BRIG) [3]. Similar results were found for the fragments from both strains. The best hit was an IncL/M plasmid (accession number: KC335143) from *K. pneumoniae* E71T, sharing 99 % identity with the plasmid of our strains, with a coverage of 100 % (Fig. 1). In fact, another IncL/M plasmid pKPoxa-48N1 (accession number: KC575416.2) was also highly similar with our plasmid (99 % identity; coverage 98 %).

The class D β-lactamase OXA-48 conferring decreased susceptibility to carbapenems was first identified in a *K. pneumoniae* isolate in Turkey [4]. Since then, multiple other OXA-48-producing isolates of various Enterobacteriaceae species have been reported. At first, these strains were mainly found in Turkey [5, 6] but recently also in other parts of the world, i.e., northern Africa and Europe [7–9]. The first publication of an OXA-48-producing Enterobacteriaceae, in The Netherlands, until now was dated in 2010 [10]. This patient was transferred from a hospital in India. Our findings show that OXA-48-producing Enterobacteriaceae were present in the Netherlands several years earlier. Although the time between the patient's visit to Turkey and positive culture is quite long, we assume that this resistance trait originates from Turkey. Indeed, long-term carriage is a well-known phenomenon for resistant Gram-negative bacteria and OXA-48 is assumed to originate from Northern Africa and Turkey [5–7]. Unfortunately, isolate 1, the first isolate obtained from the patient, had not been stored. Therefore, we were not able to determine if this isolate also contained the OXA-48 gene.

ESBL-negative OXA-48 isolates are difficult to detect as the MICs for ceftazidime and/or ceftriaxone are usually in the susceptible range. After the acquisition of an additional CTX-M-15-bearing plasmid, the MICs (and zone differences in the combined disk ESBL test) increased considerable, as shown in Table 1. OXA-48 confers resistance to carbapenems but spares oxyiminocephalosporin.

We have to realise that the case, described in this case report, occurred at a time when only clinical breakpoints

were available. No screening breakpoints were used to identify strains suspected for carbapenemase production [11, 12]. This isolate would have been recognized as a possible carbapenemase producer using the current Dutch National guideline for the detection of resistant microorganisms; the MIC screening breakpoint for meropenem has been set at >0.25 mg/L for all Enterobacteriaceae, and the breakpoint for imipenem at >1 mg/L for *E. coli*, *Klebsiella* spp., *Salmonella* spp., *Enterobacter* spp., and *Citrobacter* spp. [13].

The presence of $bla_{\text{OXA-48}}$, in *K. pneumoniae* (first), as well as in *E. coli* (months later), suggests horizontal transfer of an OXA-48-encoding plasmid within one patient. Successful horizontal transfer of OXA-48-encoding plasmids within patients had been reported previously in the UK and France [14, 15]. In addition, Berger et al. showed that the mobilization of the OXA-48 gene is not restricted to the transfer of a single plasmid, but may also be involved in multiple rearrangements from different plasmids originating from different Enterobacteriaceae species [16].

In summary, this case report shows that OXA-48 carbapenemase can easily be missed without adequate detection procedures. Therefore, this resistance trait was not recognized at the time that the patient was hospitalized. Our observation stresses the importance of uniform and adequate detection methods for early recognition and optimal management of patients with resistant pathogens.

Conflict of interest All authors report no conflicts of interest relevant to this article.

References

1. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Nordmann P. Evaluation of a DNA microarray for the rapid detection of extended-spectrum beta-lactamases (TEM, SHV and CTX-M), plasmid-mediated cephalosporinases (CMY-2-like, DNA, FOZ, ACC-1, ACT/MIR and CMY-1-like/MOX) and carbapenemases (KPC, OXA-48, VIM, IMP and NDM). J Antimicrob Chemother. 2012;67:1865–9.
2. Zhou K, Ferdous M, de Boer RF, Kooistra-Smid AMD, Grundman AW, Friedrich AW, Rossen JWA. The mosaic genome structure and phylogeny of Shiga toxin-producing *Escherichia coli* O104:H4 is driven by short-term adaptation. Clin Microbiol Infect. 2014;. doi:10.1016/j.cmi.2014.12.009.
3. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST ring image generator (BRIG): simple prokaryote genome comparisons. BMC Genom. 2011;12:402.
4. Poirel L, Heritier C, Tolun V, Nordmann P. Emergence of oxacillinase-mediated resistance to imipenem in Klebsiella pneumoniae. Antimicrob Agents Chemother. 2004;48:15–22.
5. Aktaş Z, Kayacan CB, Schneider I, Can B, Midilli K, Bauernfeind A. Carbapenem-hydrolyzing oxacillinase, OXA-48, persists in Klebsiella pneumoniae in Istanbul. Turkey. Chemotherapy. 2008;54:101–6.
6. Carrër A, Poirel L, Eraksoy H, Cagatay AA, Badur S, Nordmann P. Spread of OXA-48-positive carbapenem-resistant Klebsiella pneumoniae isolates in Istanbul. Turkey. Antimicrob Agents Chemother. 2008;52:2950–4.
7. Benouda A, Touzani O, Khairallah MT, Araj GF, Matar GM. First detection of oxacillinase-mediated resistance to carbapenems in Klebsiella pneumoniae from Morocco. Trop Med Parasitol. 2010;104:327–30.
8. Decre D, Birgand G, Geneste D, Maury E, Petit E, Petit JC, Barbut F, Arlet G Possible importation and subsequent cross-transmission of OXA-48-producing Klebsiella pneumoniae, France, 2010. Euro Surveill. 2000; 15(46). **(pii: 19718)**.
9. Cuzon G, Naas T, Bogaerts P, Glupczynski Y, Huang TD, Nordmann P. Plasmid-encoded carbapenem-hydrolyzing beta-lactamase OXA-48 in an imipenem-susceptible Klebsiella pneumoniae strain from Belgium. Antimicrob Agents Chemother. 2008;52:3463–4.
10. Kalpoe JS, Al Naiemi N, Poirel L, Nordmann P. Detection of an Ambler class D OXA-48-type β-lactamase in a Klebsiella pneumoniae strain in The Netherlands. J Med Microbiol. 2011;60:677–8.
11. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing: 15th information supplement. Wayne PA: CLIS. 2005; M100–S15.
12. European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint tables for interpretation of MICs and zone diameters. Version 3.1, valid from 2013-02-11. http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/Breakpoint_table_v_3.1.pdf.
13. NVMM guideline for the detection of November 15th 2012. http://www.nvmm.nl/system/files/2012.11.15%20richtlijn%20BRMO%20%28version%202.0%29%20-%20RICHTLIJN.pdf. Accessed 22 Okt 2014.
14. Dimou V, Dhanji H, Pike F, Livermore DM, Woodford N. Characterization of Enterobacteriaceae producing OXA-48-like carbapenemases in the UK. J Antimicrob Chemother. 2012;67:1660–5.
15. Poirel L, Bonnin R, Nordmann P. Genetic features of widespread plasmid coding for the carbapenemase OXA-48. Antimicrob Agents Chemother. 2012;56:559–62.
16. Berger S, Alauzet C, Aissa N, Henard S, Rabaud C, Bonnet R, Lozniewski A. Characterization of a new blaOXA-48 carrying plasmid in Enterobacteriaceae. Antimicrob Agents Chemother. 2013;57:4064–7.