Failure to detect carbapenem-resistant *Escherichia coli* producing OXA-48-like using the Xpert Carba-R assay®

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Sir,

The emergence and spread of carbapenem-resistant *Enterobacteriaceae* (CRE) is one of the most challenging public health issues today. Therefore, rapid and accurate detection of infected patients or carriers is mandatory to prevent outbreak development. In their recent review, P. Savard and T.M. Perl underlined the putative contribution of molecular techniques to screen CRE carriers directly from clinical samples [1]. The GenExpert® system (Cepheid, Sunnyvale, CA, USA) has developed a real-time PCR technique with ready-to use cartridges. This molecular-based technique can be implemented as a point-of-care test in clinical settings. Genetic tools that do not require technical skill or expanded time consumption should be preferred, in particular when they can be implemented within 24 h, like the GenExpert® technology.

Recently, using this platform, a test has been developed for early detection of the clinically relevant bla_{KPC}, bla_{IMP}, bla_{VIM} and bla_{NDM} carbapenemase genes and, if needed, directly from rectal swabs (Xpert MDRO Assay) [2]. The panel of those carbapenemase genes was recently expanded to include the bla_{OxA-48} gene (Xpert Carb-R) (Carretto et al., 14th European Congress of Clinical Microbiology and Infectious Diseases, P1184). In Europe there has been rapid spread of CRE, with OXA-48-producing bacteria becoming the most prevalent [3].

The OXA-48-like group comprises 11 variants (as of August 2014), and the corresponding producers exhibit variable resistance patterns. Indeed some may be extended spectrum β-lactamase producers and therefore resistant to expanded-spectrum cephalosporins, while others may be of reduced susceptibility or resistant to carbapenems [4]. In addition several OXA-48-like β-lactamases possess specific hydrolytic properties [4], such as OXA-163 (differing from OXA-48 by a single amino acid substitution and a four amino acid deletion) conferring resistance to broad-spectrum cephalosporins but hydrolysing carbapenems only marginally. That makes the suspicion and detection of OXA-48-like producers sometimes really difficult. Recently EUCAST has lowered the carbapenem screening cut-off to detect carbapenemase-producing bacteria with moderately elevated MICs (http://www.eucast.org/resistance_mechanisms).

Among the OXA-48 variants, OXA-181 differs from OXA-48 by four amino acids but possesses very similar hydrolytic activity toward β-lactams [5]. The $bla_{OxA-181}$ gene was originally identified from a *Klebsiella pneumoniae* isolate recovered in the Sultanate of Oman and then in other enterobacterial species in different geographical area, including UK, New Zealand, Singapore, Australia, Canada, France, Norway, Sri Lanka and Romania; the source of contamination being of Indian origin in most cases [4,5]. In South Africa, OXA-181 is the most prevalent carbapenemase and in Singapore, OXA-181 is now considered to be significantly established. Recent data from the French National Reference Center for Antibiotic Resistance in 2013 identified 11 OXA-181 producers out of 636 carbapenemase producers (P. Nordmann, personal communication).

Here we report failures in detecting a bla_{OxA-48}-like gene in *Escherichia coli* using a molecular technique. On 11 May 2014, a 69-year-old patient was admitted to the medical intensive care unit (ICU) of a tertiary-care university hospital in a Paris suburb (Henri Mondor Hospital) for acute pulmonary oedema. During the previous month the patient had been hospitalized for pneumonia in an ICU in the Comoro Islands (Africa). Rectal screening was performed at hospital admission to screen for multidrug-resistant organisms. It yielded (i) a negative signal using the Xpert Carb-R® cartridge and (ii) two different morphotypes of *E. coli* (Ecol1 and Ecol2) from a concomitant culture performed on selective screening medium (ChromID® OXA-48; bioMérieux, Craponne, France). On 26 May 2014, another patient (65 years old) consulted at the emergency ward of our hospital for the management of a chronic end-stage renal failure.

TABLE 1. Results of antimicrobial susceptibilities and carbapenemase genes carriage of the three carbapenem-resistant *Escherichia coli*

Strain	Antimicrobial susceptibility[a]	Antimicrobial resistance[a]	Carbapenem MICs and susceptibility[a]	Carba NP test	Carbapenemase genes PCR screening[b]	Sequencing
Ecol1	Fox, Amk, Tob, Gm, Sxt,	Amp, CoAmox-clav, Tic, Tic-clav, Pip, Pip-taz, Ctx, Caz, Fep, Ofx	Ertapenem: 0.75 mg/L (I) Imipenem: 0.5 mg/L (S) Meropenem: 0.25 mg/L (S)	Positive	bla_{OxA-48}	OXA-181
Ecol2	Fox, Amk, Tob, Ofx	Amp, CoAmox-clav, Tic, Tic-clav, Pip, Pip-taz, Ctx, Caz, Fep, Gm, Sxt,	Ertapenem: 0.5 mg/L (S) Imipenem: 0.5 mg/L (S) Meropenem: 0.5 mg/L (S)	Positive	bla_{OxA-48}	OXA-181
Ecol3	Fox, Amk, Tob, Gm, Ofx	Amp, CoAmox-clav, Tic, Tic-clav, Pip, Pip-taz, Fox, Ctx, Caz, Fep, Tob, Gm, Sxt	Ertapenem: 0.75 mg/L (I) Imipenem: 0.5 mg/L(S) Meropenem: 0.38 mg/L (S)	Positive	bla_{OxA-48}	OXA-181

[a]Abbreviations: Amp, ampicillin; CoAmox-clav, amoxicillin-clavulanate; Tic, Ticarcillin; Tic-clav, Ticarcillin-clavulanate acid; Pip, piperacillin; Pip-taz, Piperacillin-tazobactam; Fox, cefoxitin; Ctx, cefotaxime; Caz, ceftazidime; Fep, Cefepime; Amik, Amikacin; Tob, tobramycin; Gm, gentamicin; Ofx, ofloxacin; Sxt, Trimethoprim-sulfamethoxazole; according to EUCAST interpretation guidelines for clinical isolates [http://www.eucast.org].
[b]Including bla_{VIM}, bla_{NDM}, bla_{KPC} and bla_{OxA-48}.

He was returning from Cambodia where he had been dialysed several times. Although leading to a negative signal from the Xpert Carb-R® cartridge, his rectal swabbing yielded an *E. coli* isolate (Ecol3) also after culture onto selective media for CRE.

The three *E. coli* isolates were subsequently investigated. According to the manufacturer's recommendations, the negative results of the molecular screening using rectal swabs were confirmed by performing the test directly on bacterial cultures (Table 1). Antimicrobial susceptibility testing was performed using a disk diffusion method according to EUCAST guidelines (http://www.eucast.org) and MICs of carbapenem were determined by the Etest® method (bioMérieux). Finally, the Carba NP® test was performed as previously described on cultured bacteria. The three isolates exhibited different antimicrobial susceptibility patterns including a low-level ertapenem resistance and a positive Carba NP® test (Table 1). The three *E. coli* isolates were genetically unrelated according to the results of rapid amplified polymorphic DNA protocol (data not shown). In-house PCR to detect carbapenemase genes followed by sequencing was performed as described [5]. It showed that they were all positive for bla_{OxA-48}-like genes and sequencing identified the $bla_{OxA-181}$ gene in all three isolates.

The lack of detection of OXA-181-producing isolates constitutes here a pitfall for the implementation of a molecular genetic approach as a first screening test for detecting CRE. Although OXA-181 differs from OXA-48 by only four amino acid substitutions [6], the corresponding genes differ by 43 out of 798 bp. This might explain the lack of amplification and/or detection by the Xpert® technique. Although OXA-181 producers were initially mainly identified from India, here the two cases originated from other geographical areas (Africa and South-East Asia). This suggests that the prevalence of OXA-181 producers is certainly underestimated. The exact prevalence of the $bla_{OxA-181}$ gene remains unknown because molecular identification of the bla_{OxA-48} gene is not always followed by sequencing of the corresponding gene. In addition, false-negative results may occur when using techniques including primers targeting the bla_{OxA-48}-like gene in a variable region.

This report underlines that molecular diagnostic techniques in the current phase of their development should not be considered as reference standards for the detection of CRE carriers. Those molecular-based techniques may detect only a limited number of known genes. They should be reserved for rapid identification of carriers during management of outbreaks when the index case has been identified.

Transparency declaration

The authors declare that they have no conflicts of interest.

Acknowledgements

We thank Jeanne-Marie Le Glaunec, Nathalie Launay and Florence Cizeau for technical support.

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