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Short Communication

Performance of the Xpert® Carba-R v2 in the daily workflow of a hygiene unit in a country with a low prevalence of carbapenemase-producing Enterobacteriaceae

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ABSTRACT

Early detection of patients colonised with carbapenemase-producing Enterobacteriaceae (CPE) is crucial for implementing proper infection control measures. Here we evaluated the biological performance of the Xpert® Carba-R v2 (Cepheid) in the daily workflow of a hygiene unit in a country with a low CPE prevalence. Patients repatriated from countries known for high CPE prevalence or contact patients of a known CPE carrier were targeted as being 'high-risk patients' for CPE carriage. Between September 2015 and March 2016, 241 'high-risk patients' for CPE carriage were screened using the Xpert® Carba-R v2 and by plating on chromID® CARBA Smart medium (bioMérieux) with and without ertapenem-containing enrichment culture for 24 h. Of these patients, 81.7% were previously hospitalised abroad and 18.3% were contact patients of known CPE carriers. The Xpert® Carba-R v2 was able to detect 12 OXA-48-like, 1 KPC and 1 OXA-48-like/NDM carriers. For 2 of the 14 Xpert® Carba-R v2-positive samples, cultures remained negative even on two additional screenings (performed at Days 4 and 7). The Xpert® Carba-R v2 presents 100% sensitivity, 99.13% specificity, 85.71% positive predictive value and 100% negative predictive value. This study demonstrated that the Xpert® Carba-R v2 kit is well adapted for rapid screening of high-risk patients even in low prevalence regions (in <1 h versus 24/48 h for culture). This assay may guide infection control programmes to limit the spread of CPE.

1. Introduction

The fight against bacteria is turning again into one of the greatest challenges faced by our society, especially with the spread of carbapenem-resistant Enterobacteriaceae, leaving only a few, if any, effective therapeutic options to treat severe infections caused by these bacteria [1,2]. Carbapenem resistance is often the result of combined mechanisms such as outer membrane permeability defects and production of non-carbapenemase β-lactamases [e.g. acquired or overexpressed chromosomally encoded cephalosporinases and extended-spectrum β-lactamases (ESBLs)]. Nowadays, carbapenem resistance in Enterobacteriaceae is increasingly related to the production of carbapenemases belonging either to Ambler class A (KPC-type), Ambler class B or metallo-β-lactamases (MBLs) (such as NDM-, VIM- and IMP-types), or Ambler class D (OXA-48-like) [2,3]. Hence, dissemination of carbapenemase-producing Enterobacteriaceae (CPE) is a matter of great clinical concern [1,2].

The epidemiology of CPE varies from country to country in terms of prevalence of carbapenem resistance as well as the carbapenemases involved [2]. Indeed, several countries such as Greece, Italy, Israel and Puerto Rico may be considered as endemic for KPC; India, Bangladesh and Pakistan for NDM; and North African countries and Turkey for OXA-48 [2]. In other countries such as the UK, Sweden and France, CPE are still rare and are frequently associated with patient transfers from countries with a high CPE prevalence [4,5]. Early detection of colonised patients is crucial for fast implementation of strict hygiene measure, reinforced contact precautions or even cohorting of these patients [6,7]. Detection of CPE is primarily based on chromogenic culture plates containing antibiotics, but rapid molecular diagnostic assays have been in-

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creasingly used directly on rectal swabs, allowing a reduced time to results reporting in order to rapidly implement proper infection control measures [8,9]. The performance of such tests depends largely on the local prevalence. According to experience in Israeli, 15% of patients with CPE identified by PCR had negatives cultures with conventional culture-based techniques [9]. However, in low prevalence regions, false-positive results could be higher. There are still no clear recommendations on when to use molecular methods for identification of CPE gastrointestinal carriage, especially in low prevalence regions.

The aim of the present study was to determine the biological performance of a novel version of the Xpert® Carba-R v2 assay, which has been improved for detection of $bla_{OXA-181}$ and $bla_{OXA-232}$ genes [10], for direct screening of CPE carriage from rectal swabs in the daily workflow of a hygiene unit of a 2000-bed French university hospital in a low CPE prevalence setting. As the French national prevalence for CPE is still low (<1%), active surveillance testing by molecular methods would not be reasonable for patients admitted to high-risk wards (e.g. intensive care units) [4,5]. Therefore, in this study only patients considered as 'high-risk patients' for CPE colonisation according to the French national recommendations, e.g. patients directly admitted from abroad or previously hospitalised in areas with a high prevalence of CPE as well as contact patients of known carriers, were included.

2. Materials and methods

2.1. Prospective study design

This study was conducted between September 2015 and March 2016 at Hôpital Bicêtre (Le Kremlin-Bicêtre, France), which is a 2000-bed teaching hospital. A total of 241 patients were included in the study as they were considered as 'high-risk patients' for CPE carriage following the French national recommendations, e.g. patients directly admitted from abroad or previously hospitalised in areas with a high prevalence of CPE (require one screening upon admission) as well as contact patients of known carriers (require three screenings normally at 1-week intervals, but in this study the time interval was reduced to 1, 4 and 7 days). Patients were kept under reinforced contact precautions until screening results were available. Positive CPE carriers were then cohorted in a dedicated ward with dedicated staff. Rectal swabs were performed using double CultureSwab™ EZ II (BD/BBL™, Le Pont-de-Claix, France).

2.2. Detection of carbapenemase-producing Enterobacteriaceae

CPE detection was performed using the Xpert® Carba-R v2 (Cepheid, Toulouse, France) as recommended by the manufacturer. In addition, culture on CPE screening medium was used as the gold standard, which consisted of (i) direct plating on ChromID® CARBA SMART medium (bioMérieux, Marcy-l'Étoile, France) and (ii) plating on an enrichment culture in 10 mL of trypticase soy broth supplemented with ertapenem (0.5 μg/mL) for 24 h and subsequent plating on ChromID® CARBA SMART medium for an additional 24 h. Antibiotic susceptibility testing according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [11] and the Carba NP test [12] were performed on each type of colony grown on ChromID® CARBA SMART medium (directly or after enrichment). Finally, all isolates that gave a positive Carba NP test and/or were suspected of being CPE according to the algorithm for carbapenemase detection of the Antibiogram Committee of the French Society of Microbiology (CA-SFM) [based on the inhibition zone diameters of imipenem (10 μg), ticarcillin/clavulanate (75/10 μg) and temocillin (30 μg) for the screening of non-carbapenemase-producers among enterobacterial isolates with decreased susceptibility to carbapenems] [13], were confirmed by in-house PCR and sequencing [2,14]. The workflow is detailed in Fig. 1.

2.3. Statistical analysis

The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for all tested isolates. Statistical analysis was performed using 95% confidence intervals (CIs) calculated using Student's *t*-test.

3. Results

Of the 241 patients considered as 'high-risk patients' for CPE carriage between September 2015 and March 2016, 81.7% (197/241) were previously hospitalised abroad (61% in Africa, 19% in Europe, 12% in the Middle East, 4% in Asia, 3% in South America and 1% in North America), whilst 18.3% (44/241) were contact patients of known carriers.

The Xpert® Carba-R v2 was able to detect 12 OXA-48-like, 1 KPC and 1 OXA-48-like/NDM carriers (Table 1). Of these 14 Xpert® Carba-R v2-positive PCR tests, 2 could not be cultured (Table 1, Patients 13 and 14), even though Patient 13 was known as an OXA-48 carrier in another hospital. In these two cases, cultures remained negative even on two additional screenings (performed at Days 4 and 7). Accordingly, the Xpert® Carba-R v2 results were considered as false positive and these patients were considered negative for CPE.

Of note, for one patient the Xpert® Carba-R v2 test was positive for two carbapenemases (OXA-48 and VIM). Culture results confirmed the presence of an OXA-48-producing *Klebsiella pneumoniae* and an *Enterobacter cloacae* co-producing OXA-48 and NDM-1. This patient was known to also be colonised with a VIM-1-producing *Proteus mirabilis* 1 week earlier. This latter CPE was not cultured on ChromID® CARBA SMART medium. Thus, in case of multiple CPE colonisation [15], one carbapenemase-encoding gene might have been missed by the Xpert® Carba-R v2 (here bla_{NDM-1}).

The overall performance of the Xpert Carba-R v2 for CPE carriage detection was as follows: sensitivity, 100% (95% CI 73.54–100%); specificity, 99.13% (95% CI 96.88–99.89%); PPV, 85.71% (95% CI 57.19–98.22%); and NPV, 100% (95% CI 98.39–100%).

4. Discussion

The observed performance of the Xpert Carba-R v2 for CPE carriage detection was higher than that recently published by Tato et al. who reported a sensitivity, specificity, PPV and NPV of 96.6% (95% CI 92.2–98.9%), 98.6% (95% CI 97.1–99.4), 95.3% and 99.0%, respectively [16]. Tato et al. evaluated the first version of the Xpert Carba-R assay, which was not able to detect some OXA-48 variants (OXA-181 and OXA-232), which resulted in lower performance. The Xpert® Carba-R v2 assay, with improvement in detection of $bla_{OXA-181}$ and $bla_{OXA-232}$ genes, is now well adapted for the French epidemiology of CPE, which reflects that of many countries especially in northern Europe, thus allowing improved biological performance [10].

The question of false-positive PCR results compared with culture remains an important issue. Should these patients be considered as positive and thus be cohorted with culture-positive patients, with the risk of acquiring of another CPE, or should these patients be considered negative for CPE carriage? In our hospital, PCR-positive and culture-negative patients were considered negative for CPE. No CPE transmission could be evidenced around these two patients during their hospital stay. Whether these patients were decolonised or whether they were simply not excreting CPE anymore is not known. Ruppé at al. recently showed that 90% of healthy travellers who acquired ESBLs became decolonised after 3 months, although CPE colonisation over several years has also been described [17,18]. Stan-