Research letters

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References

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Spread of Klebsiella pneumoniae isolates producing OXA-48 β-lactamase in a Tunisian university hospital

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Sir,

The emergence and dissemination of Klebsiella pneumoniae isolates harbouring carbapenemases is a serious problem. Since the initial report of the OXA-48 enzyme in a K. pneumoniae isolate from Turkey in 2001,1 OXA-48 producers have been reported in many countries of the world.2-4 Current reports indicate that OXA-48 producers are widespread, mostly from Mediterranean countries as well as other countries in Europe.2-4 In North Africa, OXA-48 producers have been identified in Morocco and Tunisia.3,4 The outbreaks of OXA-48-producing K. pneumoniae isolates have been described in several cities in Turkey, once in the UK and recently in France.6 In the present report, we describe the spread of OXA-48 associated with CMY-4- and CTX-M-14-producing K. pneumoniae clinical isolates in Sfax University Hospital.

During a 6 month period (October 2009–March 2010), 153 clinical isolates of K. pneumoniae with reduced susceptibility to extended-spectrum cephalosporins and/or imipenem were recovered in Sfax University Hospital. Among these isolates, 21 (13.7%) produced the blaOXA-48 gene. These isolates were recovered from patients in eight different wards.

The antibiogram determined by the disc diffusion method and MICs determined by agar dilution and interpreted according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) revealed that all isolates were resistant to ticarcillin (MICs > 2048 mg/L) (Table 1). All but one isolate were resistant to extended-spectrum cephalosporins, including cefotaxime (MICs 4–1024 mg/L), ceftazidime (MICs 0.5–256 mg/L) and cefepime (MICs 2–64 mg/L). All isolates but one (Kp11) were susceptible to imipenem (MIC<sub>90</sub> = 2 mg/L; MIC range = 0.5–8 mg/L). This isolate, Kp11, was intermediate to imipenem but susceptible to extended-spectrum cephalosporins. However, all isolates were resistant to ertapenem (MIC<sub>90</sub> = 8 mg/L; MIC range = 2–32 mg/L).

The β-lactamase genes detected by PCR as described previously and screening the 21 OXA-48-positive K. pneumoniae isolates were as follows: bla<sub>OXA-48</sub> (1 isolate); bla<sub>OXA-48</sub> + bla<sub>CMY-4</sub> + bla<sub>TX-M-14</sub> (14 isolates); bla<sub>OXA-48</sub> + bla<sub>CMY-4</sub> + bla<sub>CTX-M-14</sub> + bla<sub>OXA-1</sub> (3 isolates); bla<sub>OXA-48</sub> + bla<sub>CMY-4</sub> + bla<sub>TX-M-14</sub> + bla<sub>TEM-1</sub> (1 isolate); bla<sub>OXA-48</sub> + bla<sub>CTX-M-14</sub> + bla<sub>OXA-1</sub> (1 isolate); and bla<sub>OXA-48</sub> + bla<sub>CMY-4</sub> + bla<sub>CTX-M-15</sub> (1 isolate).

The bla<sub>OXA-48</sub> gene to Escherichia coli J53 was observed in 20 OXA-48-producing isolates. However, conjugation and electroporation experiments failed for Kp4, suggesting that in this isolate the bla<sub>OXA-48</sub> gene might be chromosomally located. However, the bla<sub>OXA-48</sub> gene was shown to be mostly plasmid-borne and associated with insertion sequence IS1999 but not integrons.2 Using a series of PCR primers, two IS1999 insertion sequences were found surrounding the bla<sub>OXA-48</sub> gene in all our isolates, as found in the prototype OXA-48-positive K. pneumoniae 11978 isolate from Turkey.1,2

Molecular analysis of E. coli transconjugants showed that the bla<sub>OXA-48</sub> and bla<sub>CMY-4</sub> genes were detected on the same plasmid, explaining the resistance to β-lactams of the K. pneumoniae isolates and their transconjugants. Plasmid analysis showed...
Table 1. Characteristics of the K. pneumoniae strains and their respective transconjugants

| Incompatibility groups | Types of β-lactamase detected | MICS (mg/L) | TIC, TIM, CAZ, CTX, FEP, IPM, ETP, eritopenem, PMCs were determined by susceptibility testing. |
|------------------------|--------------------------------|-------------|-------------------------------------------------------------------------------------------------
|                        |                                |             | that 19 isolates harboured a plasmid of ~70–80 kb belonging to the same incompatibility group, IncA/C. This is the first description of the localization of blaOXA-48 in an A/C plasmid. In fact, the majority of studies reported that the blaOXA-48 gene was carried by a similar 70 kb plasmid for which the incompatibility group was indeterminate by the PCR-based replicon typing method. However, it was demonstrated recently that this plasmid would be of incompatibility group P<sup>+</sup>.<sup>7</sup> CMY-4 was reported to be located only in the A/C replicon.<sup>5</sup> In our hospital CMY-4 was described, associated with VIM-4 metalloenzyme, in an epidemic K. pneumoniae clone. In our study, it may be hypothesized that the CMY-4-encoding plasmid had acquired OXA-48 in a single replicon IncA/C, as it was described in IncA/C plasmid pCC416 encoding VIM-4 and CMY-4 β-lactamases.<sup>5</sup>

PFGE revealed four clones (Table 1). The majority of isolates (18 K. pneumoniae isolates) were genetically related and belonged to clone A. Among these isolates, 13 produced OXA-48 + CMY-4 + CTX-M-14, 3 produced OXA-48 + CMY-4 + CTX-M-15 + OXA-1, 1 produced OXA-48 + CMY-4 + CTX-M-14 + TEM-1 and 1 produced OXA-48 + CTX-M-14 + OXA-1. The three remaining isolates were sporadic and they produced OXA-48, OXA-48 + CMY-4 + CTX-M-14 and OXA-48 + CMY-4 + CTX-M-15.

The present work indicates that the spread of the blaOXA-48 gene is not driven by the dissemination of a single K. pneumoniae clone that harboured the same A/C replicon (isolates belonging to clone A), but by the diffusion of this same replicon in different clones (two genetically unrelated isolates producing OXA-48 + CMY-4 (Kp10) and OXA-48 + CMY-4 + CTX-M-15 (Kp12)) or by the localization of blaOXA-48 in a different replicon [one isolate harbouring a plasmid with an indeterminate incompatibility group (Kp11)] and probably in the chromosome for another isolate, Kp4.

OXA-48-producing isolates co-expressed several β-lactamases, including the class A extended-spectrum β-lactamase SHV-2a and especially CTX-M-15 and the narrow-spectrum β-lactamases TEM-1, OXA-1 and OXA-47.<sup>2</sup><sup>–</sup><sup>4</sup> The Tunisian OXA-48-producing K. pneumoniae isolates in France co-produced TEM-1 and CTX-M-15.<sup>3</sup> All our OXA-48-producing isolates, except one, co-produced at least two β-lactamases, such as CMY-4, CTX-M-14, OXA-1, TEM-1 and CTX-M-15.

This study demonstrates the alarming diffusion of the blaOXA-48 gene among expanded-spectrum cephalosporin-resistant K. pneumoniae (13.7%), mainly associated with the blaCMY-4 and blaCTX-M-16 genes. The emergence of carbapenem resistance among Enterobacteriaceae is worrying since carbapenems are often the last resort for treating infections due to third-generation cephalosporin-resistant isolates. Thus, there is a clear need for a structured nationwide prevalence study of OXA-48-producing Enterobacteriaceae in Tunisia.

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Dissemination of the New Delhi metallo-β-lactamase-1 (NDM-1) among Enterobacteriaceae in a tertiary referral hospital in north India

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Sir,
Of the different classes of carbapenemases, class B enzymes are clinically the most significant; they possess the widest substrate hydrolysis range, including penicillins, cephalosporins and carbapenemases, but not monobactams. In recent years, metallo-β-lactamase (MBL) genes have spread from Pseudomonas aeruginosa to members of the Enterobacteriaceae. An alarming report by the HPA UK in 2009 warned of a rapidly proliferating novel carbapenem-hydrolysing β-lactamase (carbapenemase) designated New Delhi MBL-1 (NDM-1) among the Enterobacteriaceae family, identified in UK hospital patients. Moreover, the common presence of these β-lactamase genes in transferable mobile elements means that these genes could reach virtually any Gram-negative bacterium and this provides an added risk of dissemination in the community.

Our present work was undertaken with the objective of detecting the blNDM-1 gene among clinical isolates of the Enterobacteriaceae family in a tertiary referral hospital in north India.

A total of 780 consecutive, non-duplicate isolates of Escherichia coli (n = 528), Klebsiella pneumoniae (n = 126), Citrobacter species (n = 84), Enterobacter aerogenes (n = 22), Proteus mirabilis (n = 11) and Morganella morganii (n = 9) were recovered from different clinical specimens from patients who were admitted to different wards, as well as from those who attended the outpatient departments of S.S. Hospital, BHU, Varanasi, India. The study was conducted from February 2010 to July 2010. The work was approved by the Ethics Committee.

An imipenem/EDTA disc potentiation test was performed for phenotypic detection of MBLs. Antimicrobial susceptibility tests were performed using the Kirby–Bauer disc diffusion method and results were interpreted according to the CLSI recommendations. For partial gene PCR amplification, primers specific for the blNDM-1 gene were used for reaction with bacterial DNA as template. PCR was performed as described previously.

Random amplified polymorphic DNA (RAPD) was performed using primer 7 (5’-GTTGATCGGA-3’) and isolates were typed according to their band patterns.

Sixty-four isolates were phenotypically found to be MBL producers. On performing PCR for all of the MBL-producing isolates the presence of the gene encoding NDM-1 was confirmed among 54 isolates, consisting of E. coli (n = 30), Citrobacter species (n = 12) and K. pneumoniae (n = 12), with an overall occurrence of 6.9% (54/780). Similar to this study, Deshpande et al. reported 22 NDM-1-producing Enterobacteriaceae in a short span of 3 months, while in a previous multicentre study, the occurrence was reported to be 2% from this centre. Most often, NDM producers were recovered from intensive care unit patients (35.1%). The age of the patients ranged from 1 day to 85 years, with 32 male patients and 22 female patients (Table S1, available as Supplementary data at JAC Online).

The presence of class 1 integrons was demonstrated in all of the NDM-1-harbouring isolates. On typing the NDM-1-harbouring isolates, 18 patterns of E. coli, 8 patterns of K. pneumoniae and 5 patterns of Citrobacter species were found by RAPD. These results suggest horizontal transmission of the gene at both the intraspecies and interspecies level.

Disc diffusion susceptibility testing showed that 46 (85.1%) of the NDM-1-producing isolates were susceptible to polymyxin B and 25 (46.2%) were susceptible to tigecycline. For other antimicrobials, 51.8% showed susceptibility to piperacillin/tazobactam and 22.2%, 5.5% and 1.8% showed susceptibility to amikacin, gentamicin and tobramycin, respectively. Among 23 urinary tract isolates, 8 showed susceptibility to nitrofurantoin. As many as 27 (50%), 26 (48.1%) and 16 (29.6%) isolates were found to be susceptible to ertapenem, imipenem and meropenem, respectively. A previous study has demonstrated 32.4% similarity of NDM-1 to VIM-1/VIM-2-type MBL and it