First description of methyltransferases in extensively drug-resistant *Klebsiella pneumoniae* isolates from Saudi Arabia

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**Abstract**

*Purpose.* The resistance determinants for carbapenems, fluoroquinolones and aminoglycosides were characterized in 16 extensively drug-resistant *Klebsiella pneumoniae* (XDRKPN) strains collected from Saudi hospitals during 2014.

*Methodology.* PCR and sequencing were used to detect: *blaKPC, blaNDM, blaVIM, blaIMP-1, blaOXA-48, blaCTX-M, blaTEM, blaSHV* and ampC for β-lactam resistance; *qnrA, qnrB, qnrS, aac(6’)-Ib-cr, qepA* and mutations of gyrA and parC for fluoroquinolone resistance; and *aacA4, aacC2, aadA1, aphA6, armA* and *rmtB* for aminoglycoside resistance. Enterobacterial repetitive intergenic consensus sequence-based PCR was performed to detect the clonal relatedness.

*Results.* All isolates encoded *blaCTX-M, aacC2* and *aphA6*, together with mutations in gyrA and parC. *blaOXA-48, blaNDM-1, aadA1, aacA4, qnrB, aac(6’)-Ib-cr, armA* and/or *rmtB* were detected in different strains. At least 93.2% clonal relatedness was detected among these strains.

*Conclusions.* To our knowledge, this is the first report describing XDRKPN encoding at least seven resistance determinants and harbouring methyltransferases in Saudi Arabia.

*Klebsiella pneumoniae* is an important cause of hospital-acquired infections, such as blood-stream infections, wound infections, urinary-tract infections and respiratory-tract infections [1–3]. Among Gram-negative bacilli, it is second only to *Escherichia coli* as a cause of nosocomial infections [1]. The treatment of infections caused by *K. pneumoniae* has become more challenging due to the emergence of antibi-otic resistance to the major groups of antimicrobial agents, such as β-lactams, including carbapenems, fluoroquinolones and aminoglycosides [4–6].

The production of β-lactamases is the main resistance mechanism for β-lactams, in addition to permeability changes and target-binding modifications. Examples of β-lactamases include extended-spectrum β-lactamases (ESBLs) (*blaTEM, blaSHV* and *blaCTX-M*), plasmid-mediated *ampC*, carbapenemases (*blaKPC* and *blaOXA-48*), and metallo β-lactamases (*blaIMP, blaVIM* and *blaNDM*) [7]. Fluoroquinolone resistance is mainly mediated by mutations in the quinolone-resistance-determining regions (QRDRs) in the DNA gyrase (*gyrA* and *gyrB*) and topoisomerases (*parC* and *parE*) [8, 9]. Up-regulation of efflux pumps and plasmid-mediated quinolone resistance (PMQR) can also be involved. PMQR includes *qnrA, qnrB, qnrBC, qnrBD, qnrS, aac(6’)-Ib-cr* and *qepA* [8–10]. Aminoglycoside-modifying enzymes are the most common mechanism of aminoglycoside resistance, including acetyltansferases (AACs), adenyltransferases (AADs), phosphotransferases (APHs) and 16S rRNA methyltransferases, such as ArmA and RmtB [7, 11].

Acquiring resistance to different types of antibiotics leads to the development of multi-drug-resistant (MDR) organisms, which are defined as organisms that have acquired a non-susceptibility pattern to at least one antibiotic in three or more antimicrobial groups. An extensively drug-resistant (XDR) organism is any organism with non-susceptibility to at least one agent in all but two or fewer antibiotic categories [4, 12]. XDR *K. pneumoniae* (XDRKPN) strains are emerging worldwide [2, 3, 5, 11]. However, there are no reports characterizing XDRKPN strains from Saudi Arabia. The aim of this study was to detect XDRKPN isolates from hospitals in Saudi Arabia and to characterize the resistance.
determinants for the major groups of antibiotics, including fluoroquinolones and β-lactams such as carbapenems and aminoglycosides.

From 1 January to 31 December 2014, non-duplicate K. pneumoniae isolates were collected from patients in King Fahad Specialist Hospital-Dammam (KFSHD) and King Fahad University Hospital (KFUH) in the Eastern Province of Saudi Arabia. KFSHD is a 650-bed tertiary-care hospital and KFUH is a 450-bed general hospital. Organism-identification and drug-susceptibility testing were performed using the manufacturer’s instructions.

Minimum inhibitory concentrations (MICs) were interpreted according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The following drugs were tested: imipenem, meropenem, ertapenem, cefoxitin, cefotaxime, ceftazidime, ceftriaxone, cefepime, ciprofloxacin, gentamicin, amikacin, trimethoprim-sulfamethoxazole, piperacillin–tazobactam and colistin (Table 1). An Etest (epiSorter assay, bioMérieux) was used for tigecycline MIC. E. coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 strains were used as quality control strains for antimicrobial susceptibility testing.

A total of 989 K. pneumoniae isolates were collected from both hospitals during the period of this study. Based on the definitions of multi-drug-resistant K. pneumoniae (MDR PN) and XDRKPN mentioned above, 68 strains were MDRKPN, making the prevalence of MDRKPN strains 6.9% (68/989). Out of 68 MDRKPN, there were 16 XDRKPN isolates, which makes the prevalence of XDRKPN 1.6% (16/989). The XDRKPN strains were 100% resistant to the antibiotics tested, except colistin and tigecycline, where susceptibility was 100 and 87.5%, respectively. The XDRKPN strains were collected from urine (6), sputum (4), wounds (4) and blood (2).

For all 16 XDRKPN isolates, the PCR method was used to detect the resistance determinants for β-lactams, fluoroquinolones and aminoglycosides. All of the primers and PCR conditions were used as previously described in the literature [9–17]. Positive controls were included in each PCR. Molecular grade water was used to detect contamination. Amplicon sequencing was performed using an ABI 3730xl DNA Analyzer (Applied Biosystems).

The ARM-D for β-Lactamase 1D kit (Streck) and the Xpert Carba R kit (Cepheid) were used to examine the presence of carbapenemase genes, following the manufacturer’s instructions. Both kits can detect blaKPC, bladDM, blavIM, blamIP-1 and blaoXA-48. However, only the ARM-D kit detects blaCTX-M-15, bladTX-M-14, blarHA and blacMY-2. The ESBLs blatem and blasIV were also tested. Plasmid-mediated ampC genes were detected using the Philisa ampC ID kit (Streck), following the manufacturer’s instructions. blatem, blasIV, blatem, blasIV, blasIV, blasm and blasIV were detected in 13, 10, 6 and 3 strains, respectively. blatem, blasIV, blatem and blasm were not detected in any isolate.

Aminoglycoside-modifying enzyme genes were tested, including aacA4, aacC1, aacC2, aadA1, aadB, aphA6, armA and rmtB genes. Acetyltransferase gene aacC2 and phosphotransferase gene aphA6 were detected in all of the strains. In addition, aadA1 and armA were detected in 14 strains, while aacA4 and rmtB genes were detected in 9 and 4 strains, respectively (Table 1). Neither aadB nor aacC1 was detected.

PMQR and QRDRs were tested to detect their involvement in fluoroquinolone resistance. qnrB was detected in 12 strains (Table 1). When acquiring two point mutations Trp87Arg and Asp164Tyr, aacA4 [aac(6’)-Ib] can confer resistance not only to aminoglycosides but also to fluoroquinolones, and the gene is assigned as aac(6’)-Ib-cr [16]. A total of seven XDRKPN strains were found to harbour aac (6’)-Ib-cr upon sequence analysis. PCR did not detect qnrA, qnrC, qnrD and qepA. All isolates harboured double mutations in GyrA (Ser83Le/Phe/Tyr) and (Asp87Asn/Ala/Gly) and a single mutation in ParC (Ser80Ile or Glu84Iys).

In order to detect the clonal relatedness of XDRKPN isolates, the enterobacterial repetitive intergenic consensus sequence-based PCR (ERIC-PCR) methodology was used, as described by Rivera et al. [18]. The DNA fingerprint patterns generated by ERIC-PCR were analysed using Gel Compar II software, version 6 (Applied Maths). ERIC-PCR analysis differentiated the 16 strains into 2 unique clusters and a single strain (number 16) (Table 1). A total of six strains from KFSHD were identical and combined together with 97% clonally related similarity (cluster A). Cluster B was composed of nine strains (three isolates from KFSH and six isolates from KFUH) with more than 97% clonal relatedness (Table 1). Clusters A and B have 95.3% clonal relatedness between them and 93.2% relatedness with strain 16 (Table 1). Despite this clonal relatedness, the patient data revealed no patient transport between the two hospitals. All of the routine environmental samples and studies during the collection period for these XDRKPN isolates were negative. In addition, there were no reports of outbreaks caused by XDRKPN from either hospital. These results suggest that the K. pneumoniae strains are highly homogeneous, with there being great potential for the spread and dissemination of these strains in the community. Further epidemiological and clinical follow-up studies are required in the future to trace the source of the spread within the community and in hospitals in the same region. Several factors could have contributed to the emergence of MDR organisms in the region, including the use of antibiotics over the counter and excessive prescription of these agents. The implementation of antimicrobial stewardship programmes to make strict policies for antibiotic use and more infection control surveillance programmes are necessary to minimize the spread of these MDR organisms.

The prevalence of MDRKPN strains in this study is alarming, but the MDRKPN rate is still lower than those observed in several global surveillance studies conducted in the decade after 2000, where 20–80% of K. pneumoniae were
MDRKPN [1]. Studies in China reported MDRKPN rates of as high as 61.4\% [5]. XDRKPN strains have been reported in several countries, such as China, Italy, Switzerland, Taiwan and Brazil [2–4, 6, 19, 20]. Despite being the first report on XDRKPN from Saudi Arabia, this study was only performed in two hospitals. Nationwide surveillance studies are needed to detect the prevalence of MDRKPN and XDRKPN in Saudi Arabia more accurately.

The data revealed that bla\textsubscript{OXA-48} followed by bla\textsubscript{NDM-1} was the main resistance mechanism to carbapenems, which correlates with other studies from the Gulf States, the Middle East, Africa, China, the USA, Europe and India [1, 5, 21–27]. For strains 5 and 14 (Table 1), it is possible that carbapenem resistance could be due to the expression of bla\textsubscript{CTX-M-15} coupled with the changes of the permeability in the outer membrane, which is a well-described mechanism in the literature [4, 12, 21, 27]. Interestingly, this is, to our knowledge, the first report of strains co-encoding bla\textsubscript{CTX-M-15}, bla\textsubscript{OXA-48} and bla\textsubscript{NDM-1} in the Gulf Cooperation Council (GCC) region.

However, several studies worldwide reported \textit{K. pneumoniae} isolates harbouring multiple genes responsible for carbapenem resistance [4, 12, 21, 27].

<table>
<thead>
<tr>
<th>Isolate no</th>
<th>Source</th>
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<th>Resistance-associated genes</th>
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methyltransferases in the GCC region. Methyltransferases have been reported worldwide, in Asia, Europe and North and South America [2, 4, 6, 17, 29]. Interestingly, aacA4, aacC2 and aphA6 were also detected, indicating that the major groups of aminoglycoside-modifying enzymes contributed to the aminoglycoside resistance. An interesting feature of these isolates is the coexistence of a large number of antibiotic-resistance-determinant genes in these strains. At least seven antimicrobial-resistant determinants were detected in each strain. To the best of our knowledge, this is the first study to report such a large number of resistance elements in bacterial isolates from Saudi Arabia and other GCC states.

The major resistance determinants for carbapenems, aminoglycosides, and fluoroquinolones are co-encoded in the same isolates. It is well known that these resistance genes can be encoded on mobile DNA, such as plasmids and integrons. Therefore, they can be transferred horizontally among organisms and patients in hospital settings, which results in the global dissemination of MDR organisms [2, 6, 11, 20].

In conclusion, XDRKPN strains from Saudi Arabia are presented for the first time. As far as we know, several resistance determinants are presented for the first time from the GCC, such as methyltransferases. This study sheds light on the importance of these isolates to minimize their spread in health-care centres and the environment.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References


