Brief report

Presence of quinolone resistance to qnrB1 genes and blaOXA-48 carbapenemase in clinical isolates of Klebsiella pneumoniae in Spain

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A study is presented on the presence of quinolone resistance qnrB1 genes in clinical isolates belonging to the largest series of infections caused by OXA-48-producing Klebsiella pneumoniae in a single-centre outbreak in Spain. Evidence is also provided, according to in vitro results, that there is a possibility of co-transfer of plasmid harbouring blaOXA-48 with another plasmid harbouring qnrB1 in presence of low antibiotic concentrations of fluoroquinolones, showing the risk of multi-resistance screening.

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Introduction

Klebsiella pneumoniae is a gram-negative rod of the family Enterobacteriaceae and a common cause of community, nosocomial and opportunistic infections. An emerging association between carbapenems and fluoroquinolone (FQ) resistance is a significant problem in managing such infections. The main mechanisms for transferable carbapenem resistance in this microorganism are due to the emergence of carbapenemases (MBL, KPC and OXA-48-like groups). Several low-level plasmid-mediated FQ resistance (PMQR) mechanisms have been described to date in K. pneumoniae: Qnr proteins, the Aac(6′)-Ib-cr enzyme, the plasmid-mediated efflux pumps, QepA and OqxAB. Since the increase in MIC values produced by these plasmid determinants is less than the concentrations commonly used in commercial panels, it is difficult to detect this sort of mechanism in routine practice using commercial microdilution panels.

OXA-48 is a carbapenem-hydrolysing oxacillinase that was first described in a clinical isolate of K. pneumoniae and then, OXA-48-producing Enterobacteriaceae have been isolated in several countries in Northern Africa, the Middle East and Europe. It is not easy to ascertain the real prevalence of OXA-48-producing
enterobacteria since it is difficult sometimes to detect it routinely in the clinical microbiology laboratory due to low carbapenems MIC values. Bacteria expressing \( \textit{bla}_{OXA-48} \) also commonly express \( \textit{bla}_{CTX-M-15} \) and have permeability defects; only then they are resistant to carbapenems, particularly ertapenem (ERT). The largest series of infections caused by \( \textit{OXA-48} \)-producing \( \textit{K. pneumoniae} \) in a single-centre outbreak was recently reported in Spain.\(^3\) The predominant clone was assigned sequence type (ST) 405 and harboured \( \textit{bla}_{TEM-1} \), \( \textit{bla}_{SHV-7} \), \( \textit{bla}_{CTX-M-15} \), \( \textit{bla}_{OXA-1} \) and \( \textit{bla}_{OXA-48} \) genes. In addition to the multiresistant genetic background, the 78.5% of the isolates of this clone showed a pattern compatible (nalidixic acid susceptible and reduced susceptibility to FQ) with \( \textit{qnr} \) genes. Herein we characterize the additional determinants detected on two strains obtained at the beginning of this outbreak (May 2011).

### Methods

\( \textit{K. pneumoniae} \) 471 and 971 were selected and belonged to two different clones (ST405 and a sporadic clone) of this outbreak. Both isolates were recovered from clinical samples: one from urine and the other one from a wound sample.

Whole-cell DNA of these isolates was used as a template for PCR amplification. Screening for PMQR genes (\( \textit{qnrA} \), \( \textit{qnrB} \), \( \textit{qnrS} \), \( \textit{qnrC} \), \( \textit{qnrD} \), \( \textit{qnrVC} \), \( \textit{qepA} \), \( \textit{oqxA} \) and \( \textit{oqxB} \)) was performed.\(^4\) DNA bands compatible with \( \textit{qnrB} \), \( \textit{aac(6')-Ib-cr} \) and \( \textit{oqxA} \) were identified and confirmed by sequencing (with \( \textit{qnrB} \) identified as \( \textit{qnrB1} \)). Furthermore, the QDR sequences of the \( \textit{qnrA} \) and \( \textit{parC} \) genes did not identify mutations associated with FQ resistance in either of the two isolates.

Localisation of the \( \textit{qnrB} \), \( \textit{aac(6')-Ib-cr} \), \( \textit{oqxA} \), \( \textit{oqxB} \), \( \textit{bla}_{OXA-48} \) and \( \textit{bla}_{CTX-M-15} \) genes (plasmidic and/or chromosomal) was determined by (i) Southern-blot using electroporated plasmid extracts obtained with the Kieser method; and by (ii) conjugation (performed 3 times) using \( \textit{Escherichia coli} \) J53 AzR and selection on plates containing 100 mg/L sodium azide combined with 0.03, 0.06, 0.125, 0.25 or 0.5 mg/L of ciprofloxacin (CIP) and/or 0.25 mg/L of ERT.\(^5\)

### Results and discussion

\( \textit{K. pneumoniae} \) 471 and 971 isolates showed reduced susceptibility to FQ (Table 1) but remained susceptible to nalidixic acid.

Transconjugants were obtained in the three selection conditions and three patterns were observed: (1) transconjugants with only reduced susceptibility to FQ and cephalosporins that hybridized with \( \textit{qnrB1} \), \( \textit{aac(6')-Ib-cr} \) and \( \textit{bla}_{CTX-M-15} \) all of which were located on a single 180-kb plasmid (selected with FQ); (2) transconjugants with only reduced susceptibility to carbapenems that hybridized with \( \textit{bla}_{OXA-48} \) located into a 70-kb plasmid (selected with ERT); and (3) transconjugants with reduced susceptibility to carbapenems, cephalosporins and FQ carrying the two 180- and 70-kb plasmids (selected with CIP plus ERT or only CIP) (Table 1).

### Table 1

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>( \textit{K. pneumoniae} ) 971</th>
<th>( \textit{K. pneumoniae} ) 471</th>
<th>( \textit{K. pneumoniae} ) 971</th>
<th>( \textit{K. pneumoniae} ) 471</th>
<th>( \textit{K. pneumoniae} ) 971</th>
<th>( \textit{K. pneumoniae} ) 471</th>
<th>( \textit{E. coli} ) J53AzR</th>
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<td>1</td>
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### References