might have occurred in Enterobacteriaceae originating from an animal host. The presence of the qnrB2 gene in animal isolates and zoonotic pathogens opens the possibility that genetic exchange and plasmid acquisition of the qnrB2 gene could occur in the faecal flora of the animals. Interestingly, p137.25 belongs to the IncN plasmid family that is able to replicate in different enterobacterial strains, but also seems prevalent in faecal flora from animals. In fact, a study performed on a large collection of E. coli from the USA demonstrated that the prevalence of IncN plasmids is high in avian E. coli (10%–16%) but negative in E. coli from faeces of healthy humans.15 This evidence supports the hypothesis that the Salmonella 137.25 strain acquired the qnrB2 gene on an IncN plasmid circulating in avian bacterial flora. This strain could cause infections in humans through the food chain and the resistance plasmid contributes to the dissemination of the qnrB2 gene in other Enterobacteriaceae.

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References


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Novel genetic environment of qnrB2 associated with TEM-1 and SHV-12 on pB1004, an IncHI2 plasmid, in Salmonella Bredeney BB1047 from Spain

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Sir,

Resistance to fluoroquinolones through plasmid-mediated qnr genes, especially when associated with resistance to cephalosporins by extended-spectrum b-lactamases (ESBLs), is an emerging phenomenon in enterobacteria. This is worrisome in the case of Salmonella, as these are two of the major antimicrobials used in invasive salmonellosis.1 We report here the presence of a qnrB2 gene in a Salmonella Bredeney clinical isolate, BB1047, from Spain. The gene was borne by a self-transferable IncHI2 plasmid, pB1004, associated with blaTEM-1 and blasmHV-12. The genetic environment of qnrB2 revealed a common evolutionary origin with plasmid pEC-IMPQ recently described in Enterobacter cloacae clinical isolates from Taiwan.
In 2005, a 76-year-old woman in an intensive care unit in South Spain suffered from insidious wound infection. Microbial analysis showed that Salmonella Bredeney, BB1047, was the causative agent. Complete antimicrobial profile revealed that the bacterium possessed an unusual multidrug resistance profile [Table S1, available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/)]. In order to characterize the genetic basis of the resistance profile, conjugation experiments were performed. Resistance to β-lactams, aminoglycosides, chloramphenicol, tetracycline and fluoroquinolones could be transferred en bloc into Escherichia coli K802N (res−, gyrA, NalR) at a frequency of $1 \times 10^{-5}$ per donor cfu, giving rise to transconjugant BB1048 (Table S1). Using the S1-PFGE method, the size of the plasmid in BB1047 and BB1048 was estimated to be $\sim 315$ kbp, and was named pB1004 (data not shown). Hybridization with β-lactamase probes (OXA, CMY, CTX, TEM and SHV) and sequencing revealed that the genes encoding TEM-1 and SHV-12 were located in pB1004. In order to characterize pB1004, the plasmid was extracted, digested with HindIII, ligated into pUC19, transformed into E. coli and plated on brain heart infusion agar plates containing ampicillin (50 mg/L). Two different clones were obtained, bearing a 7613 bp and a 4489 bp fragment that were completely sequenced in both strands by genome walking (GenBank accession numbers EU643617 and FJ973574). The 4489 bp fragment encoded the trhA and trhL genes, as well as most of the putative replication origin of the plasmid. It was 100% identical to the IncHI2 plasmids pK29 from Klebsiella pneumoniae (GenBank accession number EF382672), R478 from Serratia marcescens (GenBank accession number BX664015) and the recently described plasmids pEC-IMP and pCE-IMPQ in E. cloacae from Taiwan. When compared with the rest of the sequences present in the databases, the fragment differed by 26 nucleotides from other IncHI2 plasmids, like plasmid pAPEC-O1-R from E. coli (GenBank accession number DQ517526). Thus, pB1004 can be assigned to the R478 subfamily of the IncHI2 plasmids described to date.

Sequence analysis of the 7613 bp revealed the presence of the qnrB2 gene, the structural gene of the QnrB2 protein. To assess whether qnrB2 was responsible for quinolone resistance in this strain (Table S1), the gene was amplified together with the putative promoter and terminator regions with primers qnrB2F (5′-CGACCGGGAAAATTGACATG-3′) and qnrB2R (5′-CAGGGATGGATTTCAGAACC-3′), and cloned into pCR2.1. The resulting transformant, BB1049, showed reduced susceptibility to quinolones and fluoroquinolones (Table S1) demonstrating that qnrB2 contributes to the phenotype of BB1047 against these antimicrobials.

The qnrB2 gene was described for the first time in Citrobacter koseri (GenBank accession number DQ351242), and was detected thereafter in Proteus mirabilis (GenBank accession number EF488762), E. cloacae (GenBank accession number EU131184), Citrobacter freundii (GenBank accession numbers AB281054 and FJ232918), K. pneumoniae (GenBank accession numbers AJ609296 and FJ943244) and Salmonella enterica serovars Enteritidis, Keurmassar and Mbandaka. The

Figure 1. Schematic representation of the genetic environments of the qnrB2 gene described to date. Grey shading denotes $\geq 99\%$ nucleotide identity. Black lines beneath Salmonella Bredeney indicate the regions that have been amplified by overlapping PCR and sequenced using the nucleotide sequence of pEC-IMPQ for the design of appropriate primers. This figure appears in colour in the online version of JAC and in black and white in the print version of JAC.
genetic environment of qnrB2 has been described in the Salmonella Enteritidis and Keurmassar serovars, as well as in the Klebsiella strains and partially in C. koseri, being highly similar in all bacteria described to date (Figure 1). In pB1004, immediately upstream of qnrB2, an IS26 is followed by two genes homologous to genes located in the chromosome of the marine bacterium Marinobacter aquaeolei VT8 (GenBank accession number CP000514). The intM gene encodes IntM, a 513 amino acid protein with 68.3% identity with Maqu_0026, encoding the catalytic domain of the IS21 transposase. Downstream of intM, istB codes for IstB, an ATP-binding protein with 83.4% identity with Maqu_0025 of M. aquaeolei involved in the transposition of IS21-like elements. The two genes are located in the same order in the chromosome of M. aquaeolei VT8. Interestingly, both genes are present in the recently described Taiwanese InH12 plasmids pEC-IMP and pEC-IMPO, the latter also bearing the qnrB2 gene. Further analysis by PCR and sequencing of the genetic environment of qnrB2 revealed the existence of an 8939 bp deletion including an ISCR1 element and a class 1 integron with the blaIMP-8 metallo-β-lactamase gene.

Here we report the identification of a qnr gene in Salmonella in Spain. Previously, qnrB2 has been identified in Enterobacter spp. and qnrA has been detected in E. coli, E. cloacae and K. pneumoniae, whereas the qnrS gene has been shown to be present in a K. pneumoniae clinical isolate in this country. In other countries like France, the UK, Germany, Israel, Australia, the USA, Taiwan, the Netherlands and Senegal, the qnrB gene is largely present. In the latter four, Salmonella spp. was the host bacterium of the qnrB2 gene. In Taiwan, the gene was present in the serovar Enteritidis, and possessed a similar genetic environment to that described previously for Salmonella Keurmassar. Interestingly, the single qnrB2-bearing Salmonella originating from a Dutch broiler chicken also belongs to serovar Bredeney (isolate 137.25) and was suspected to be potentially linked to the Bredeney isolate from Spain. However, the genetic environment flanking the qnrB2 gene and the plasmid incompatibility groups are completely different for the two Bredeney isolates from the Netherlands and Spain (IncN-p137.25 and IncH12-PB1004, respectively), suggesting independent events of acquisition of qnrB2-carrying plasmids in these isolates.

In contrast, the genetic environment of this qnrB2, as well as the partial sequence of the plasmid backbone, reveals a striking common evolutionary origin of pB1004 with pEC-IMPQ. This is further supported by the plasmid size of a Taiwanese plasmid that has been shown to be 324 kbp, as compared with the 315 kbp from pB1004. It is tempting to speculate that the ISCR1 element has been responsible for the deletion of the 8939 bp in pB1004 through rolling-circle replication. However, detailed analysis of the junctions reveals that the deletion occurred upstream of the replication origin of the ISCR1 element. Further, a perfect IR1 (‘left inverted repeat’) structure in the IS26 element downstream of qnrB2 is evident in pB1004, indicating that insertion of IS26 occurred after deletion of the 8939 bp fragment. These results confirm emergence in Spain of pB1004, an IncH12 plasmid related to pEC-IMPO, that associates the qnrB2 gene with SHV-12 and TEM-1.

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Transparency declarations

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Supplementary data

Table S1 is available as Supplementary data at JAC Online (http://jac.oxfordjournals.org/).

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In vitro activity of the new quinolone derivative RD-3 against clinical isolates of Mycoplasma pneumoniae and Mycoplasma hominis

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