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 InHI2 plasmids pEC-IMP and pEC-IMPQ, 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 I 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 IncHI2-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 IncHI2 plasmid related to pEC-IMPQ, that associates the qnrB2 gene with SHV-12 and TEM-1.

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Transparency declarations

None to declare.

Supplementary data

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

References


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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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Keywords: MICs, MBCs, fluoroquinolones

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Sir,

RD-3 is a new investigational quinolone derivative with the formula bis(4,9,9a,10-tetrahydro-9-phenyl-3H-pyrrolizino-[1, 2-b]quinolin-7-)methane 12, synthesized by the Department of Organic Chemistry, University of Madras, Chennai, India (Figure 1). This drug has shown significant activity against Gram-positive and Gram-negative organisms.1

Mycoplasma pneumoniae is a significant cause of upper and lower respiratory tract infections in persons of all age groups.2 In some cases, these organisms can cause severe, systemic disease, especially in the setting of a debilitated or immunocompromised host. Current treatment alternatives are limited primarily to drugs in the macrolide, lincosamide, tetracycline and fluoroquinolone classes, and agents in these classes exhibit differential in vitro activities against these organisms. Though macrolides are the treatment of choice for M. pneumoniae respiratory infections, the emergence of macrolide-resistant strains of M. pneumoniae has been reported in Japan.3 Mycoplasma hominis most frequently causes diseases of the genitourinary tract. In this study, we examined the activity of RD-3 against 61 clinical isolates of M. pneumoniae collected from patients with pneumonia and 40 genital tract isolates of M. hominis. Ten of the isolates were resistant to tetracycline (MIC50, ≥8 mg/L).

The comparator agents moxifloxacin, ciprofloxacin, levofloxacin, gatifloxacin, gemifloxacin, azithromycin, doxycycline, tetracycline and clindamycin were purchased from Sigma-Aldrich (St Louis, MO, USA). Azithromycin was tested only against M. pneumoniae since M. hominis is always resistant to macrolides,2 and clindamycin was tested only against M. hominis since it is not recommended for the treatment of M. pneumoniae.4

Antimicrobial powders were used according to the manufacturer’s protocol. Working dilutions of the drugs were prepared fresh on the day of the assay.

Mycoplasmas were tested by the agar dilution method using a Steers replicator as described previously.2 M. pneumoniae ATCC 29342 and M. hominis ATCC 43521 were used as reference strains. The MICs of the compounds tested against the reference strains for quality control were reproducible throughout the study. The inoculum was derived from actively growing cultures in broth medium supplemented with 20% serum and 10% fresh yeast extract.5 The MIC was the least amount of antimicrobial agent that completely prevented colony formation by a Steers replicator as described previously.2 The MBC was defined as the concentration of the antimicrobial at which no growth was apparent, as shown by lack of colour change in the broth after prolonged incubation.

MICs of RD-3 and other antimicrobial agents are shown in Table 1. RD-3 and azithromycin were the drugs most active against M. pneumoniae (MIC50, 0.001 mg/L; MIC90, 0.016 mg/L). Gemifloxacin (MIC50, 0.03 mg/L; MIC90, 0.125 mg/L) was more active than moxifloxacin (MIC50, 0.06 mg/L; MIC90, 0.125 mg/L), gatifloxacin (MIC50, 0.125 mg/L; MIC90, 0.125 mg/L), doxycycline (MIC50, 0.25 mg/L; MIC90, 0.25 mg/L), levofloxacin (MIC50, 1 mg/L; MIC90, 1 mg/L), tetracycline (MIC50, 1 mg/L; MIC90, 1 mg/L) and ciprofloxacin (MIC50, 4 mg/L; MIC90, 4 mg/L). The MBCs of RD-3 (MBC50, 0.001 mg/L; MBC90, 0.016 mg/L) and moxifloxacin (MBC50, 0.06 mg/L; MBC90, 0.125 mg/L) showed that these molecules were bactericidal against each of the 10 isolates of M. pneumoniae tested.

M. hominis was highly susceptible to RD-3 (MIC50, 0.03 mg/L; MIC90, 0.06 mg/L), (Table 1). RD-3 was as active as clindamycin (MIC50, 0.03 mg/L), and was more active than moxifloxacin (MIC50, 0.06 mg/L; MIC90, 0.06 mg/L), gatifloxacin (MIC50, 0.125 mg/L; MIC90, 0.125 mg/L), levofloxacin (MIC50, 0.25 mg/L; MIC90, 0.5 mg/L), ciprofloxacin (MIC50, 1 mg/L; MIC90, 1 mg/L), doxycycline (MIC50, 2 mg/L; MIC90, 2 mg/L) and tetracycline (MIC50, 8 mg/L; MIC90, 8 mg/L).

Our study indicates that RD-3 is active in vitro against Mycoplasma species that are clinically important in humans with activity that was comparable to azithromycin and superior to the fluoroquinolones tested. Earlier work demonstrated that RD-3 inhibited gyrase supercoiling with potency similar to ciprofloxacin and down-regulated gyrase A gene expression in

Figure 1. Chemical structure of RD-3.

Table 1. Susceptibilities of M. pneumoniae and M. hominis to RD-3 and other antimicrobial agents

<table>
<thead>
<tr>
<th>Organism/drug</th>
<th>MIC (mg/L)</th>
<th>range</th>
<th>50%a</th>
<th>90%b</th>
</tr>
</thead>
<tbody>
<tr>
<td>M. pneumoniae (61)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RD-3</td>
<td>0.001–0.016</td>
<td>0.001</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>azithromycin</td>
<td>0.001–0.016</td>
<td>0.001</td>
<td>0.016</td>
<td></td>
</tr>
<tr>
<td>gemifloxacin</td>
<td>0.004–0.125</td>
<td>0.03</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>moxifloxacin</td>
<td>0.06–0.25</td>
<td>0.06</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>gatifloxacin</td>
<td>0.016–0.25</td>
<td>0.125</td>
<td>0.125</td>
<td></td>
</tr>
<tr>
<td>doxycycline</td>
<td>0.016–1</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>levofloxacin</td>
<td>0.0125–2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>tetracycline</td>
<td>0.25–1</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ciprofloxacin</td>
<td>0.5–8</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

M. hominis (40)  
| clindamycin    | 0.008–0.5  | 0.03  | 0.03  |
| RD-3           | 0.016–0.25 | 0.03  | 0.06  |
| moxifloxacin   | 0.06–0.25  | 0.06  | 0.06  |
| gatifloxacin   | 0.016–0.25 | 0.125 | 0.125 |
| levofloxacin   | 0.125–1    | 0.25  | 0.5   |
| ciprofloxacin  | 0.25–2     | 1     | 1     |
| doxycycline    | 0.008–2    | 2     | 2     |
| tetracycline   | 0.5–16     | 8     | 8     |

*MICs at which 50% of the isolates tested were inhibited (MIC50).
*MICs at which 90% of the isolates tested were inhibited (MIC90).
Research letters

Escherichia coli. These in vitro data indicate that RD-3 may show considerable promise for both genital and respiratory infections with mycoplasmas, but its clinical utility will depend upon its toxicity and pharmacokinetics.6

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References


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Susceptibility of pneumococci causing meningitis in Spain and prevalence among such isolates of serotypes contained in the 7-valent pneumococcal conjugate vaccine

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Sir,

Streptococcus pneumoniae remains the most common cause of bacterial meningitis in children in the USA,1 although since the introduction of the 7-valent pneumococcal conjugate vaccine (PCV-7) rates have decreased despite the increase in meningitis caused by non-PCV-7 serotypes.2 In Spain the decrease in the prevalence of PCV-7 serotypes among the global population of invasive pneumococci after the introduction of PCV-7 was not as marked as in the USA, probably due to irregular and lower coverage. After vaccine introduction in 2001, distribution was via the private market because of the selective introduction into childhood vaccination calendars (it was only introduced in the Madrid region from November 2006).3,4 A recently published ecological analysis of invasive isolates over time in Spain suggested that PCV-7 vaccination in children had produced a herd effect (with respect to prevalence of PCV-7 isolates and antibiotic susceptibility) in adults.3 It has also been reported that the incidence of pneumococcal meningitis among children <5 years old significantly decreased in Spain from 2001 to 2006, without evidence of changes in the incidence of meningitis caused by non-vaccine serotypes.5 Although a small proportion of invasive pneumococcal infections present as meningitis, it has a high case-fatality rate. Whether the empirical use of cefotaxime for meningitis needs to be continued in countries with increasing PCV-7 uptake needs to be monitored. We considered isolates from CSF received in the Spanish Reference Laboratory for Pneumococci (SRLP) in the current decade to analyse their susceptibility and prevalence of PCV-7 serotypes.

All CSF isolates of S. pneumoniae sent voluntarily from all over the country to the SRLP (passive, laboratory-based surveillance system) from January 2000 to December 2008 were analysed. Isolates were serotyped by Quellung reaction and/or dot blot assay, and susceptibility was determined by agar dilution.3 Current CLSI meningitis susceptibility breakpoints for penicillin (MIC ≤ 0.06 mg/L) and cefotaxime (MIC ≤ 0.5 mg/L) and susceptibility breakpoints of MIC ≤ 1 mg/L for vancomycin and MIC ≤ 2 mg/L for levofloxacin were used.6 Trends over time were explored by linear regression analysis. P ≤ 0.05 was considered significant.

Data are shown in Table 1. Of the 1397 CSF isolates received between January 2000 and December 2008, 923 (66.1%) were from adults and 474 (33.9%) from children ≤14 years of age. No significant trends in the percentage of CSF isolates among invasive isolates were found in the three populations: total population (R² = 0.008, P = 0.823), adults (R² = 0.240, P = 0.180) and children (R² = 0.395, P = 0.070), although in children there was a continuous decrease from 15.5% in 2003 to 9.8% in 2008. The prevalence of PCV-7 serotypes among CSF isolates showed significant decreasing linear trends in the total population (R² = 0.914, β = −0.956, P < 0.001), adults (R² = 0.819, β = −0.905, P < 0.001) and children (R² = 0.870, β = −0.933, P < 0.001), with a significantly higher decreasing slope in children than in adults (B coefficient = −6.307, 95% CI = −8.485 to −4.128 in children versus B coefficient = −3.495, 95% CI = −4.963 to −2.027 in adults).