Resistance Challenges Threatening the Treatment of *Pseudomonas aeruginosa* Infections with Levofloxacin: The Role of a Levofloxacin–Imipenem Combination for Prevention of Resistance

**Introduction**

*Pseudomonas aeruginosa* is an opportunistic pathogen that presents a serious infectious and therapeutic threat within the hospital environment (1–6). As a nosocomial pathogen, *P. aeruginosa* is a leading cause of hospital-acquired pneumonia, urinary tract infections, and bloodstream infections (2, 5–9), and treatment of serious *P. aeruginosa* infections can be problematic due to the ability of this pathogen to express multiple mechanisms of antibacterial resistance (5, 6, 10–12). Infections caused by multi-drug resistant *P. aeruginosa* have been associated with significant increases in patient morbidity and mortality, length of hospital stay, requirement for additional medical procedures and surgery, chronic care, and overall cost (13–15). Furthermore, if resistance emerges during the course of therapy, the length of hospital stay and overall costs of antibiotics are doubled in comparison with infections where only the baseline resistance profiles are expressed (16).

Among the fluoroquinolones, levofloxacin and ciprofloxacin remain the preferred anti-pseudomonal agents. Although the intrinsic potency of ciprofloxacin is greater than that of levofloxacin (based on MIC₅₀ data) (17–21), drug potency is only one factor affecting clinical success. Clinical efficacy is related to drug potency, drug pharmacokinetics, and the ability to achieve optimum pharmacodynamic exposure. Therefore, despite levofloxacin exhibiting a lower intrinsic potency, the superior pharmacokinetics of 750 mg levofloxacin compared with 400 mg ciprofloxacin equates to an almost identical pharmacodynamic

Historically, a combination of antibacterial agents has been the strategy to treat multi-drug resistant *P. aeruginosa* and to prevent the emergence of resistance during therapy. Unfortunately, the standard combination of an anti-pseudomonal β-lactam with an aminoglycoside is not always effective in achieving this goal. Therefore, the search for more effective mechanism-based combinations must be a priority. This review focuses on the resistance problems (global trends and molecular mechanisms) challenging the use of levofloxacin for treatment of *P. aeruginosa* and the potential role of a levofloxacin-imipenem combination for preventing emergence of resistance during therapy, even when the *P. aeruginosa* already lacks susceptibility to one or both drugs.

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target attainment for *P. aeruginosa* infections (22).

Mechanisms of resistance to some antibacterial classes (β-lactams and aminoglycosides) can be spread to *P. aeruginosa* on plasmids. However, resistance to levofloxacin and other fluoroquinolones is mediated through mutational changes within chromosomal genes. These mutational changes alter either the enzymatic targets of fluoroquinolones and/or the expression of multi-drug efflux pumps that extrude fluoroquinolones as a substrate (10, 23, 24). Whether these resistance mechanisms are expressed at the start of therapy or emerge during its course, they present serious therapeutic challenges for levofloxacin. This review will focus on the current global trends of levofloxacin resistance and the molecular mechanisms which continue to threaten levofloxacin efficacy against *P. aeruginosa*. In addition, the potential role of a levofloxacin-imipenem combination for preventing the emergence of resistance during therapy will be discussed.

**Global rates of levofloxacin resistance among *P. aeruginosa***

Selecting the appropriate antibacterial agent for treatment of serious *P. aeruginosa* infections is critical to optimizing clinical outcome. Antibacterial therapy of *P. aeruginosa* bloodstream infections is a perfect example. When an inappropriate drug is selected for therapy, mortality rates for *P. aeruginosa* bacteremia are significantly increased and can exceed 50% (25, 26). In contrast, when the infecting strain is susceptible to the antibiotic of choice, the mortality rates is less than 20% (25, 26).

Complicating the selection of appropriate therapy is the reality that rates of antibacterial resistance among *P. aeruginosa* continue to increase globally and the isolation of multi-drug resistant strains from serious infections is occurring with increasing frequency (11, 27–29). Perhaps the greatest concern is the isolation of pan-resistant *P. aeruginosa* that are resistant to virtually all anti-psudomonal drugs (12). Outbreaks of pan-resistant *P. aeruginosa* infections have been reported in burns units, intensive care units (ICUs), cancer centers, and among cystic fibrosis patients (12, 30, 31).

The mechanisms of fluoroquinolone resistance characterized in clinical isolates of *P. aeruginosa* are summarized in the next section. Since these mechanisms of fluoroquinolone resistance affect the antibacterial activity of both levofloxacin and ciprofloxacin, it is not surprising that rates of resistance to both drugs are similar (18, 20, 21, 27, 32–35). A summary of global levofloxacin resistance rates among *P. aeruginosa* collected during the last decade are summarized in Table 1 (17–21, 27, 32–36). Although it is feasible to infer resistance rates for levofloxacin using ciprofloxacin data, only data specific for levofloxacin are presented. Furthermore, only data from studies defining the geographical region or country and dates of collection of isolates were included. Therefore, this summary is not meant to be a comprehensive analysis of global levofloxacin resistance but, rather, a snapshot of resistance trends from different geographical regions.

In the United States, national rates of levofloxacin resistance have ranged from approximately 20 to 30%, and similar rates have been reported in Canada, Germany and in larger studies focusing on the continents of Europe and North America. Some of the highest rates of resistance have been reported in Latin America (39%) and Italy (51%). While large scale studies such as these provide important data on resistance trends, it is important to remember that these studies can be misleading and serious regional or local resistance problems can be missed. Furthermore, these surveillance studies do not address the importance of epidemic outbreaks that can compromise therapy within individual communities or hospitals.

**Mechanisms of levofloxacin resistance among *P. aeruginosa***

Resistance to the fluoroquinolones is mediated by plasmid-encoded mechanisms and chromosomal mutations involving [1] target protection, [2] the production of inactivating enzymes, [3] alteration of drug targets, and [4] decreased drug accumulation. The DNA gyrase protecting protein, Qnr, and fluoroquinolone-modifying enzyme, AAC(6')Ib-cr, are both plasmid-encoded mechanisms primarily found among clinical isolates of the Enterobacteriaceae (37, 38). Qnr-mediated resistance has not been observed in *P. aeruginosa* despite attempts to locate the *qnr* gene in this organism (39, 40). Similarly, the *aac(6')Ib-cr* gene has not been identified in fluoroquinolone-resistant *P. aeruginosa*. Therefore, levofloxacin resistance among clinical isolates of *P. aeruginosa* is mediated through alteration of the intracellular targets (23, 24, 41) and decreased intracellular accumulation through the action of efflux pumps (10, 23, 42, 43). These two mechanisms are presented graphically in Figure 1 and summarized below.

**Target-mediated resistance.** Fluoroquinolones are bactericidal agents that inhibit the replication of DNA through interaction with the type II topoisomerases, DNA gyrase and topoisomerase IV (Figure 1A). The type II topoisomerases are critical enzymes involved in regulating DNA topology during replication and decatenating daughter chromosomes after replication (re-
Levofloxacin and *P. aeruginosa* viewed in reference 44). Both DNA gyrase and topoisomerase IV are multi-subunit enzymes composed of two subunits each of GyrA and GyrB (DNA gyrase) or ParC and ParE (topoisomerase IV) (44). Mutations within specific regions of each subunit, known as the quinolone resistance determining regions (QRDRs), alter the affinity of fluoroquinolones for the enzyme-DNA complex (Figure 1B). For Gram-negative bacteria, including *P. aeruginosa*, the primary target for fluoroquinolones is DNA gyrase (45). Therefore, it is not surprising that the most common and often first target-mediated mutations identified are within the QRDR of *gyrA* (23, 24, 46).

Specifically, the mutational substitution of threonine at position 83 of GyrA with an isoleucine (Thr83→Ile) appears to be the most common and usually the first mutational step involved in target-mediated levofloxacin resistance (23, 24, 29, 47). Data from our own study of seven levofloxacin-resistant *P. aeruginosa* clinical isolates demonstrated that six strains exhibited the Thr83→Ile mutation, with five of these strains exhibiting this mutation as the only QRDR change (23). The highest level of levofloxacin resistance (MIC = 64 µg/ml) was observed in one strain that exhibited QRDR mutations within both *gyrA* and *parC*. Amino acid substitutions within ParC, primarily the serine residue at position 87, is the second most common mutation and appears to represent the next mutational step towards high-level fluoroquinolone resistance (24, 29, 41, 46).

Although less common, mutations within the QRDRs of *gyrB* and *parE* have also been described in fluoroquinolone resistant isolates of *P. aeruginosa* (24).

**Efflux-mediated resistance**

The outer membrane of *P. aeruginosa* is an effective molecular barrier for antibiotics, and data suggest it is 100-fold less permeable than the outer membranes of other Gram-negative bacteria (48). To augment this antibacterial mechanism, *P. aeruginosa* encodes on its chromosome the genes for dozens of efflux pumps belonging to five distinct superfamilies (49). Efflux pumps of the resistance-nodu-

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**Table 1. Global rates of levofloxacin resistance among *Pseudomonas aeruginosa***

<table>
<thead>
<tr>
<th>Country or region</th>
<th>Source of isolates</th>
<th>Dates of study</th>
<th>Number of isolates</th>
<th>Percentage resistance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>United States</td>
<td>Non-ICU</td>
<td>1998</td>
<td>4,776</td>
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</tr>
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<td></td>
<td>2001</td>
<td>9,345</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>ICU</td>
<td>1998</td>
<td>339</td>
<td>20.4</td>
<td>36</td>
</tr>
<tr>
<td></td>
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<td></td>
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<td>1,652</td>
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<td>2,146</td>
<td>24.5</td>
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<tr>
<td>United States</td>
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<td>464</td>
<td>21.6</td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>All sources</td>
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<td>404</td>
<td>27.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>32</td>
</tr>
<tr>
<td>United States</td>
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<td>689</td>
<td>23.2</td>
<td>18</td>
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<tr>
<td></td>
<td></td>
<td>2005</td>
<td>589</td>
<td>22.4</td>
<td></td>
</tr>
<tr>
<td>United States</td>
<td>Hospitalized patients</td>
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<td>606</td>
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<tr>
<td>United States</td>
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<td>21,059</td>
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<tr>
<td>Canada</td>
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<tr>
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<td>33</td>
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<tr>
<td>North America</td>
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<td>144</td>
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<td>Europe</td>
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<td>6,616</td>
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<td>Asia-Pacific</td>
<td>All sources</td>
<td>1999-2001</td>
<td>757</td>
<td>17.0</td>
<td>19</td>
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<sup>a</sup> All *Pseudomonas aeruginosa* isolates were collected from hospital laboratories.

<sup>b</sup> Percentage nonsusceptible based upon reported percentage susceptible data.

<sup>c</sup> Percentage nonsusceptible is an average of rates for each year 1997 to 1999.

Abbreviation: ICU = intensive care unit.
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cell division (RND) superfamily have attracted the most attention because of their established role in intrinsic and acquired resistance to antimicrobials. RND efflux pumps typically exist in a tripartite complex structure consisting of an outer membrane factor, cytoplasmic membrane transporter, and the periplasmic membrane fusion protein which serves to connect the inner and outer membrane components (Figure 1B). This complex forms a channel spanning the entire membrane allowing the transport of compounds from the periplasmic space and cytoplasm to the extracellular environment (Figure 1B) (50).

Seven RND efflux pumps of *P. aeruginosa* have been characterized to date, but only four of these, MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY-OprM, have been associated with fluoroquinolone resistance in clinical isolates (reviewed in references 51, 52). Another RND efflux pump, MexVW-OprM, was implicated in fluoroquinolone resistance for a laboratory mutant (53), but this pump has not yet been linked to resistance in clinical isolates. Only the MexAB-OprM efflux pump is produced at sufficient levels in “wild-type” strains of *P. aeruginosa* to play a role in the relatively low baseline susceptibility to multiple classes of antibiotics, including levofloxacin (51, 54, 55). In contrast to MexAB-OprM, the other three pumps are more quiescent in wild-type *P. aeruginosa* and do not appear to affect the baseline susceptibility to levofloxacin (reviewed in reference 51). Although overexpression of all four efflux pump systems decreases susceptibility to fluoroquinolone (23, 43, 51, 56–58), efflux
Pump overexpression alone does not always result in clinical resistance to levofloxacin or ciprofloxacin. Two recent studies from our laboratory have evaluated strains of *P. aeruginosa* (clinical isolates and laboratory-selected mutants) that overexpress different RND efflux pumps but remain susceptible to ciprofloxacin (MIC = 1 µg/ml) and/or exhibit intermediate susceptibility to levofloxacin (MIC = 4 µg/ml) (23, 59). The highest levels of fluoroquinolone resistance are often observed in isolates that [1] overexpress an efflux pump in combination with a target mutation (23, 60, 61), [2] express a combination of target mutations within both DNA gyrase and topoisomerase IV (41, 46) and/or [3] simultaneously overexpress multiple efflux pumps (62, 63).

**Levofloxacin-imipenem to prevent emergence of resistance**

As discussed previously, infections caused by multidrug-resistant *P. aeruginosa* can present serious therapeutic challenges, especially when resistance emerges during the course of therapy. Clinicians must always be aware of the resistance threat when initiating therapy against *P. aeruginosa*, and appropriate therapy of serious infections should include a combination of drugs. In contrast to the strategy of combining penicillins and aminoglycosides to provide synergistic killing for serious enterococcal infections, the goal of combination therapy for *P. aeruginosa* is focused more on preventing the emergence of resistance during therapy.

Historically, the combination of choice for *P. aeruginosa* has been an anti-pseudomonal β-lactam and an aminoglycoside. Some may describe this combination as “mechanism-based” since the drugs have unrelated mechanisms of action and target different cellular components. In theory, the aminoglycoside should kill any mutant subpopulations expressing resistance to the β-lactam (mediated through derepression of AmpC cephalosporinase) and the β-lactam in the combination should kill any cells expressing resistance to the aminoglycoside. Unfortunately, the addition of an aminoglycoside does not always prevent clinical failures due to the emergence of AmpC-mediated resistance (64–68), and the search for more effective combinations must be a priority with this pathogen.

**Rationale for levofloxacin-imipenem combination therapy.** Similar to the combination of an aminoglycoside with a β-lactam, levofloxacin-imipenem is a mechanism-based combination. The rationale for this combination centers around the drug-specific mechanisms of resistance expressed by *P. aeruginosa* to each of these drugs. As shown in Figure 1 and discussed above, the general mechanisms responsible for levofloxacin resistance among *P. aeruginosa* are alterations in the target enzymes and increased expression of RND efflux
30

pumps. Although target modifications significantly decrease susceptibility to levofloxacin, this mechanism is specific for fluoroquinolones and does not affect the susceptibility to imipenem. Furthermore, overexpression of the known RND efflux pumps does not directly affect the susceptibility to imipenem since this carbapenem is not a pump substrate (69, 70). When P. aeruginosa develops resistance to imipenem and other carbapenems, the mechanism can involve either [1] the acquisition of a carbapenem-hydrolyzing enzyme or [2] a mutational change affecting the expression and/or function of the OprD outer membrane porin (Figure 1B) (69, 71). Neither of these resistance mechanisms affect the susceptibility of P. aeruginosa to levofloxacin.

It is hypothesized that a levofloxacin-imipenem combination will prevent the emergence of resistance during therapy of P. aeruginosa since levofloxacin would remain active against any subpopulations exhibiting imipenem-specific resistance mechanisms, and imipenem in the combination would eliminate any subpopulations exhibiting resistance to levofloxacin through target modifications and/or overexpression of the MexAB-OprM, MexCD-OprJ, or MexXY-OprM efflux pumps. One potential challenge for this combination involves P. aeruginosa that overexpress the MexEF-OprN efflux pump. Although imipenem is not a substrate for this pump, these strains have been shown to exhibit a concurrent decrease in the expression of oprD and dual resistance to fluoroquinolones and imipenem (55, 58, 72). Theoretically, this dual resistance could overcome the antibacterial activity of the combination.

Levofloxacin-imipenem prevents emergence of resistance to susceptible clinical isolates of P. aeruginosa. The ability of levofloxacin-imipenem to prevent the emergence of resistance during therapy of P. aeruginosa was evaluated with three susceptible clinical isolates in a two-compartment in vitro pharmacokinetic model (IVPM) (73). Logarithmic-phase cultures (1 x 10^8 CFU/ml) were introduced into the peripheral compartment of the IVPM, and were treated with simulated human doses of 750 mg of levofloxacin alone (q24h), 250 mg of imipenem alone (q8h), and a combination of levofloxacin-imipenem. The dose of 250 mg imipenem was chosen for these studies to promote the emergence of resistance and to provide a greater therapeutic challenge for the combination. When resistance emerged during the course of therapy, mutant populations were confirmed for changes in susceptibility by agar dilution methodology and the potential involvement of efflux pumps and OprD as mechanisms of resistance was evaluated.

The pharmacodynamics of levofloxacin, imipenem, and the combination of levofloxacin-imipenem were similar against all three P. aeruginosa, with one representative strain shown in Figure 2. Resistance emerged rapidly during treatment of all strains with either levofloxacin or imipenem alone (Figure 2A). As predicted, imipenem-selected resistance was associated with the decreased expression of oprD (not shown), whereas levofloxacin was found to select for a variety of mutant phenotypes, some of which were associated with the overexpression of multi-drug efflux pumps (Figure 2B). Interestingly, in studies with all three strains, mutant subpopulations overexpressing mexEF-oprN and exhibiting dual resistance to both levofloxacin and imipenem were associated with the observed emergence of resistance. In contrast to the failures of each drug alone, the levofloxacin-imipenem combination rapidly eradicated all three P. aeruginosa strains from the IVPM and prevented the emergence of resistance (Figure 2A). Eradication in these experiments was defined as the viable counts decreasing to less than the 10 CFU/ml limit of detection. In summary, the combination of levofloxacin-imipenem was effective in preventing the emergence of resistance, even when resistance was associated with overexpression of the mexEF-oprN efflux pump and dual resistance to both drugs in the combination.

Levofloxacin-imipenem against P. aeruginosa already lacking susceptibility to one or both drugs in the combination. Data from the first study suggested that levofloxacin-imipenem effectively prevents emergence of resistance during therapy of susceptible P. aeruginosa. However, with antibacterial resistance already established within the environment, it was important to evaluate this combination against strains of P. aeruginosa already lacking susceptibility to one or both drugs through characterized mechanisms of resistance (59). In this second study, a two-compartment IVPM was used to evaluate the pharmacodynamics of levofloxacin alone (750 mg q24h), imipenem alone (250 mg or 1g q12h), and a combination of levofloxacin-imipenem against a panel of five characterized P. aeruginosa mutants summarized in Table 2. Pharmacodynamic interactions are shown in Figure 3.

Against two strains overexpressing the MexAB-OprM or MexCD-OprJ efflux pumps, levofloxacin-imipenem prevented the emergence of high-level resistance observed with single drug therapy and achieved eradication of each strain (Figures 3A and 3B). Although eradication of the MexEF-OprN overexpressing, OprD down-regulated strain was not achieved (Figure 3C), the combination decreased viable counts by a total of
Levofloxacin and *P. aeruginosa* 5 logs and prevented the emergence of high-level resistance observed with each drug alone (Figure 3C). Levofloxacin-imipenem also eradicated the imipenem-resistant strain lacking OprD, thus preventing the emergence of resistance observed with levofloxacin monotherapy (Figure 3D). Levofloxacin-imipenem could not prevent emergence of higher resistance from a dual-resistant strain lacking OprD and overexpressing MexXY when the 250 mg dose of imipenem was simulated in the combination (Figure 3E). However, when the dose of imipenem was increased to 1g, viable counts at 30 hr remained 5 logs below the starting inoculum and emergence of resistance was suppressed.

**Future considerations for fluoroquinolone-carbapenem combination therapy**

The data obtained from these studies provide support for the use of levofloxacin-imipenem to prevent the emergence of resistance during therapy of *P. aeruginosa* infections. Even when the target strain is already lacking susceptibility to one or both drugs, the combination effectively prevents the emergence of higher levels of resistance while achieving eradication of most strains. Although a non-traditional dose of 250 mg imipen-

### Table 2. Characteristics of the *Pseudomonas aeruginosa* panel

<table>
<thead>
<tr>
<th>Strain number</th>
<th>Characterized resistance mechanisms</th>
<th>Levofloxacin MIC (µg/ml)</th>
<th>Imipenem MIC (µg/ml)</th>
</tr>
</thead>
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<tr>
<td>K-155</td>
<td>MexAB-OprM overexpression</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>164CD-921C</td>
<td>MexCD-OprJ overexpression</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>PAO1-Tokai#1</td>
<td>MexEF-OprN overexpression and OprD down-regulation</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>244</td>
<td>OprD deficient</td>
<td>0.5</td>
<td>16</td>
</tr>
<tr>
<td>289</td>
<td>MexXY overexpression and OprD deficient</td>
<td>4</td>
<td>32</td>
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</table>

*This table has been adapted from Table 1 of *J Antimicrob Chemother* 2006; 57 (5): 999–1003 as published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. Abbreviation: MIC = minimum inhibitory concentration.*
Figure 3. Pharmacodynamics of levofloxacin, imipenem, and a levofloxacin-imipenem combination against *Pseudomonas aeruginosa* K-1455 (Panel A), *P. aeruginosa* 164CD-921C (Panel B), *P. aeruginosa* PAO1-Tokai#1 (Panel C), *P. aeruginosa* 244 (Panel D), and *P. aeruginosa* 289 (Panel E). Each data point represents the mean number of viable bacterial counts for duplicate experiments. Error bars represent the standard deviation. This figure previously appeared in *J Antimicrob Chemother* 2006; 57 (5): 999–1003 as published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy.
Imipenem (q8h or q12h) was evaluated in these studies, the rationale for this low dose was to challenge the combination and promote the emergence of imipenem-resistance. The data presented should not be used to support the use of 250 mg imipenem for the treatment of *P. aeruginosa*, even in the context of combination therapy with levofloxacin. Maximum recommended doses of imipenem should be used when treating infections with this challenging pathogen.

The potential role of other fluoroquinolones and carbapenems should also be considered with this mechanism-based approach to combination therapy. As mentioned previously, levofloxacin and ciprofloxacin are generally comparable with regards to rates of resistance among *P. aeruginosa* and the similarity of their pharmacodynamics against *P. aeruginosa*. Therefore, the potential role of ciprofloxacin in combination with imipenem is of clinical interest and should be evaluated. Furthermore, the potential role of other carbapenems in this combination is also of interest. Imipenem was selected as the carbapenem of choice for these studies because it is the only FDA-approved carbapenem that is not recognized as a substrate by the characterized RND efflux pumps. However, the ability of levofloxacin-imipenem to suppress the emergence of resistance by MexEF-OprN overexpressing/OprD downregulated populations provides a more optimistic view for the potential use of other carbapenems in the combination. Further studies with meropenem and the recently approved doripenem would be of clinical interest.

### References

14. Aboush V, Navon-Venezia S,
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Levofloxacin and P. aeruginosa


