Antibiograms of Multidrug-Resistant Clinical Acinetobacter baumannii: Promising Therapeutic Options for Treatment of Infection with Colistin-Resistant Strains

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Multidrug-resistant Acinetobacter baumannii infection has presented a global medical challenge. The antibiograms of paired colistin-susceptible and -resistant strains revealed increased susceptibility of colistin-resistant strains to most tested antibiotics, including those that are active against only gram-positive bacteria. Synergy between colistin and rifampicin was observed in the colistin-susceptible strains. The ability to form biofilm in the colistin-resistant strains was significantly lower (P < .001) than in the parent strains. Our study provides valuable information for potential expansion of our current therapeutic options against colistin-resistant A. baumannii infection.

Resistance to all major classes of antibiotics (except polymyxins) in Acinetobacter baumannii has substantially increased worldwide in the past decade [1–3]. The genetic potential of multidrug-resistant A. baumannii to carry and transfer diverse antibiotic resistance determinants [4] poses a major threat in hospitals [5]. A. baumannii is now regarded as one of the most difficult nosocomially acquired pathogens to treat and control [1, 2]. No novel antibiotics against multidrug-resistant A. baumannii will be commercially available within the next few years [1, 2]. The recently approved tigecycline is a therapeutic option; however, besides the potential for toxicity which is similar to that of tetracycline [2], a high percentage (78%) of resistance and intermediate susceptibility to tigecycline in multidrug-resistant A. baumannii has been reported recently in Israel, where tigecycline has never been used [6]. In many cases, colistin (polymyxin E) or polymyxin B is the only therapeutic option available for multidrug-resistant A. baumannii infection [2, 7, 8].

Unfortunately, a relationship between the increasing clinical use of colistin methanesulfonate, a nonactive prodrug of colistin [9], and resistance in A. baumannii has been reported [10]. Of potentially significant clinical concern is the recent observation of heteroresistance to colistin in clinical isolates of multidrug-resistant A. baumannii, against which colistin is believed to be very “active” on the basis of MICs [11]. It is inevitable that resistance to colistin will become more prevalent if it is used suboptimally [7, 12]. In addition, biofilm has been increasingly recognized as an antibiotic resistance mechanism in A. baumannii [13].

Therefore, it has become critical to determine what therapeutic options will be available to treat infections due to colistin-resistant A. baumannii. In this study, we investigated the differences between paired colistin-susceptible and colistin-resistant A. baumannii strains in antibiograms, responses to the combination of colistin and rifampicin, and biofilm-forming ability. Our study provides potentially valuable information on the treatment options for infections caused by colistin-resistant A. baumannii.

Methods. A. baumannii ATCC 19606 was purchased from the American Type Culture Collection. Also used in this study were 16 clinical strains recovered from 16 patients (collected during 2002–2004) at the Alfred Hospital (Melbourne, Australia). These strains belonged to 6 different groups fingerprinted by PFGE [11]. All 17 strains were susceptible to colistin, as determined on the basis of the MICs (0.25–2 µg/mL). From these parent colistin-susceptible strains, 17 paired colistin-resistant strains were obtained in a previous study [11] or by in vitro passaging. Strains were stored at −80°C before the experiments were performed.

MICs of colistin (sulfate) against colistin-resistant strains were measured by the microdilution broth method [14]. The antibiograms of these paired colistin-susceptible and colistin-resistant strains (n = 34) were determined using an automated system (Vitek; bioMérieux Vitek Systems), in the absence of colistin. The first panel of 20 antibiotics or combinations (ampicillin, amoxicillin–clavulanic acid, ticarcillin–clavulanic acid, piperacillin–tazobactam, cefalotin, ce-
fazolin, ceftoxitin, ceftazidime, ceftriaxone, cefepime, mero-
penem, amikacin, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, norfloxacin, nitrofurantoin, trimethoprim, and trimethoprim-sulfamethoxazole) were tested using Vitek card
AST-N044 for gram-negative bacteria. The MICs for the sec-
ond panel of 17 antibiotics or combinations for gram-positive
bacteria (benzylpenicillin, oxacillin, cefazolin, imipenem, gen-
tamicin, ciprofloxacin, erythromycin, clindamycin, quinupri-
stin-dalfopristin, linezolid, teicoplanin, vancomycin, tetracycline, nitrofurantoin, fusidic acid, rifampicin, and trimethoprim-sulfamethoxazole) were determined using Vitek card AST-P545. The susceptibility breakpoints for the an-
tibiotics were those recommended by the Clinical and Lab-
oratory Standards Institute, when applicable [14].

The combination of colistin and rifampicin was investi-
gated against 8 paired colistin-susceptible and colistin-resis-
tant strains by measurement of fractional concentration
indices (FIC) [15]. The tested concentrations of
(colistin (sulfate) and rifampicin (sodium)) were 0.0625–4 µg/
ml and 0.125–128 µg/ml, respectively. An FIC of ≤0.5 was
defined as synergy [16].

The biofilm-forming ability of 5 paired strains was measured
using crystal violet [17]. Results for each strain were expressed
as the mean ± standard deviation of independent samples in
40 wells and were compared using Student’s t test (Microsoft
Excel).

Results. Amikacin (MIC<sub>90</sub> 8 µg/ml) (table 1) and colistin
(MIC<sub>90</sub> 2 µg/ml) were the only 2 active antibiotics, as deter-
mined on the basis of MICs, against all of the tested colistin-
susceptible strains in this study. MICs of colistin for colistin-
resistant strains were in the range of 16 to 1024 µg/ml, with
an MIC<sub>90</sub> of 512 µg/ml. Compared with their parent colistin-
susceptible strains, the majority of colistin-resistant strains showed increased susceptibility (at least 2 dilutions in MICs), in
the absence of colistin, to β-lactam/β-lactamase inhibitors,
cephalosporins, carbapenems, fluoroquinolones, and aminog-
lycosides (table 1). Surprisingly, for the antibiotics that are
usually inactive against gram-negative bacteria, considerable
decreases in MICs, in the absence of colistin, were observed in
the colistin-resistant strains for rifampicin, fusidic acid, eryth-
romycin, teicoplanin, and quinupristin-dalfopristin (table 1).
There was practically no significant difference between the
paired strains with regard to susceptibility to benzylpenicillin,
oxacillin, trimethoprim-sulfamethoxazole, linezolid, and nitro-
furantoin. Amikacin and tobramycin (but not gentamicin) had
good antibacterial activity against most of these strains, regard-
less of whether they were susceptible or resistant to colistin
(table 1). No clear trend was observed in the antibiogram
changes among the strains in the same PFGE group. For ex-
ample, strain 16 was from the same PFGE group as strains 1,
4, 5, and 9–15; however, its colistin-resistant derivative strain
was still resistant to most of the tested antibiotics, whereas the
other strains in this PFGE group were susceptible.

FIC results (FIC range, 0.14–0.53) (table 2) demonstrated
synergy between colistin and rifampicin against colistin-sus-
ceptible strains. No growth was observed for all the tested co-
listin-resistant strains, even at a rifampicin concentration of
0.125 µg/ml; therefore, the FICs were not able to be calculated.
The biofilm-forming ability decreased significantly (P<.001)
figure 1) after the strains became resistant to colistin.

Discussion. Because colistin methanesulfonate is a prodrug
of colistin [9], colistin (sulfate) was used in the current study.
Although the incidence of resistance of A. baumannii to poly-
myxins (including colistin), as determined on the basis of MIC,
is currently low [18], our group used an in vitro pharmacody-
namic model to determine that resistance to colistin can be
rapidly developed—even within 24 h—with colistin-heterores-
istant A. baumannii [19]. Therefore, the question arises: what
antibiotic is available to treat colistin-resistant A. baumannii
infection?

For the penicillin class and carbapenems, including the com-
binations with β-lactamase inhibitors, the MICs of most co-
istin-resistant strains were substantially lower than those for
colistin-susceptible strains—in some cases, >16 times lower
(table 1). Different generations of cephalosporins had slightly
different susceptibilities for colistin-resistant strains, generally
with the activity in the ascending order of first generation,
second generation, third generation, and fourth generation. It
is very likely that the outer membrane of the colistin-resistant
strains became much more permeable and that, therefore, the
susceptibility to the cell wall–targeted antibiotics increased.

Generally, colistin-resistant strains were more susceptible to
quinolones than their colistin-susceptible parent strains (table
1). Differences in the susceptibility to the 3 aminoglycosides
(amikacin, gentamicin, and tobramycin) were not always the
same for the paired strains. The susceptibility to tetracycline
did not increase for most of the colistin-resistant strains, relative
to the parent strains (table 1). This suggests that the outer-
membrane impermeability is only one of the mechanisms of
tetracycline resistance in A. baumannii [20].

Interestingly, the colistin-resistant strains had substantially
increased susceptibility (table 1) to most of the antibiotics that
are usually inactive against gram-negative bacteria; hydropho-
bicity, negative charge, or the large molecular size of these
antibiotics may decrease the potential to permeate the outer
membrane [21]. It is very likely that substantial changes in the
outer membrane of A. baumannii occurred as a result of resis-
tance to colistin, thereby allowing rifampicin and the lipo-
polypeptides, macrolides, and streptogramins greater access to
their target sites. Such antibiogram changes in colistin-resistant
A. baumannii have great clinical potential to broaden the ther-
apapeutic options. Clinically achievable peak concentrations (ob-
Table 1. Susceptibilities of the paired colistin-susceptible and colistin-resistant strains to various antibiotics.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Tested concentration range, µg/mL</th>
<th>Breakpoint for susceptible strains, µg/mL</th>
<th>MIC, µg/mL (no. of strains with MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Parent strains</td>
<td>Colistin-resistant strains</td>
</tr>
<tr>
<td>Penicillins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>2–32</td>
<td>≤8</td>
<td>16 (2), &gt;32 (15)</td>
</tr>
<tr>
<td>Amoxicillin–clavulanic acid</td>
<td>2/1–32/16</td>
<td>NB</td>
<td>4 (3), 16 (2), &gt;32 (12)</td>
</tr>
<tr>
<td>Ticarcillin–clavulanic acid</td>
<td>8/2–128/2</td>
<td>&lt;16</td>
<td>≤8 (4), 16 (1), &gt;128 (12)</td>
</tr>
<tr>
<td>Piperacillin–tazobactam</td>
<td>4/4–128/4</td>
<td>≤16</td>
<td>≤4 (5), 64 (12)</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>0.03–0.5</td>
<td>NB</td>
<td>&gt;0.5 (17)</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>0.25–4</td>
<td>NB</td>
<td>&gt;4 (17)</td>
</tr>
<tr>
<td>Cephalosporins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefalotin</td>
<td>2–64</td>
<td>NB</td>
<td>&gt;64 (17)</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>4–64</td>
<td>NB</td>
<td>&gt;64 (17)</td>
</tr>
<tr>
<td>Cefoxitin</td>
<td>4–64</td>
<td>NB</td>
<td>&gt;64 (17)</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>1–64</td>
<td>≤8</td>
<td>4 (1), 8 (3), 16 (1), &gt;64 (12)</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>1–64</td>
<td>≤8</td>
<td>8 (1), 16 (3), 32 (1), &gt;64 (12)</td>
</tr>
<tr>
<td>Cefepime</td>
<td>1–64</td>
<td>≤8</td>
<td>2 (1), 4 (2), 8 (2), 16 (11), 32 (1)</td>
</tr>
<tr>
<td>Carbapenems</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Meropenem</td>
<td>0.25–16</td>
<td>≤4</td>
<td>&lt;0.25 (4), 0.5 (1), 8 (11), &gt;16 (1)</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1–16</td>
<td>≤4</td>
<td>≤1 (5), 8 (11), &gt;16 (1)</td>
</tr>
<tr>
<td>Aminoglycosides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>2–64</td>
<td>≤16</td>
<td>≤2 (13), 4 (1), 8 (2), 16 (1)</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>1–16</td>
<td>≤4</td>
<td>≤1 (3), 2 (1), 8 (1), &gt;16 (12)</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>1–16</td>
<td>≤4</td>
<td>≤1 (14), 2 (1), 4 (1), 8 (1)</td>
</tr>
<tr>
<td>Quinolones</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>2–32</td>
<td>NB</td>
<td>4 (5), &gt;32 (12)</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>0.25–4</td>
<td>≤4</td>
<td>&lt;0.25 (2), 0.5 (2), 2 (1), &gt;4 (12)</td>
</tr>
<tr>
<td>Norfloxacin</td>
<td>0.5–16</td>
<td>≤4</td>
<td>2 (4), 8 (1), &gt;16 (12)</td>
</tr>
<tr>
<td>Sulphonamides and trimethoprim</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>0.5–16</td>
<td>≤2</td>
<td>8 (2), &gt;16 (15)</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>20 (1/19)–320 (16/304)</td>
<td>≤2/38</td>
<td>≤20 (4), &gt;320 (13)</td>
</tr>
<tr>
<td>Macrolides and lincosamides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>0.25–8</td>
<td>NB</td>
<td>&gt;8 (17)</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.25–8</td>
<td>NB</td>
<td>≥8 (17)</td>
</tr>
<tr>
<td>Streptogramins: quinupristin-dalfopristin</td>
<td>0.25–16</td>
<td>NB</td>
<td>&gt;16 (17)</td>
</tr>
<tr>
<td>Glycopeptides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.5–32</td>
<td>NB</td>
<td>≥32 (17)</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1–32</td>
<td>NB</td>
<td>≥32 (17)</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1–16</td>
<td>NB</td>
<td>4 (2), 8 (1), &gt;16 (14)</td>
</tr>
<tr>
<td>Rifamycins: rifampicin</td>
<td>0.5–32</td>
<td>NB</td>
<td>2 (1), 16 (2), &gt;32 (14)</td>
</tr>
<tr>
<td>Oxazolidinones: linezolid</td>
<td>0.5–8</td>
<td>NB</td>
<td>≥8 (17)</td>
</tr>
<tr>
<td>Other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fusidic acid</td>
<td>0.5–32</td>
<td>NB</td>
<td>≥32 (17)</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>16–512</td>
<td>NB</td>
<td>256 (1), &gt;512 (16)</td>
</tr>
</tbody>
</table>

**NOTE.** NB, no breakpoint data were available for these antibiotics against *Acinetobacter baumannii.*
Table 2. Fractional inhibition concentration (FICs) of the combination of colistin and rifampicin against colistin-susceptible strains of *Acinetobacter baumannii*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>FIC of colistin-susceptible strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.31</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
</tr>
<tr>
<td>6</td>
<td>0.19</td>
</tr>
<tr>
<td>7</td>
<td>0.14</td>
</tr>
<tr>
<td>8</td>
<td>0.27</td>
</tr>
<tr>
<td>10</td>
<td>0.50</td>
</tr>
<tr>
<td>14</td>
<td>0.19</td>
</tr>
<tr>
<td>16</td>
<td>0.38</td>
</tr>
</tbody>
</table>

NOTE. FICs of the colistin-resistant strains could not be calculated, because there was no growth even at the lowest concentration of rifampicin (0.125 μg/mL) used in the FIC measurement.

The biofilm-forming ability of the paired colistin-susceptible (black bars) and colistin-resistant (gray bars) strains. The biofilm formation was determined from the optical density at 520 nm (OD520nm) of crystal violet–stained biofilm.

In summary, our study provides valuable information for potential expansion of our current therapeutic options against colistin-resistant *A. baumannii* infection with use of antibiotics that are only active against gram-positive bacteria. Given that there are no novel antibiotics in the drug development pipeline, and given that colistin resistance is increasingly reported, novel combinations of antibiotics have to be investigated for treatment of infection due to colistin-resistant *A. baumannii*. Additional pharmacokinetic and pharmacodynamic evaluations of such combinations are warranted before the agents are used clinically.

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References


