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-

Received 2 February 2007; accepted 7 May 2007; electronically published 17 July 2007.

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Clinical Infectious Diseases 2007; 45:594–8

1058-4838/2007/4505-0011$15.00
DOI: 10.1086/520658

fazolin, cefoxitin, ceftazidime, ceftriaxone, cefepime, meropenem, 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 second panel of 17 antibiotics or combinations for gram-positive bacteria (benzylpenicillin, oxacillin, cefazolin, imipenem, gentamicin, ciprofloxacin, erythromycin, clindamycin, quinupristin-dalfopristin, linezolid, teicoplanin, vancomycin, tetracycline, nitrofurantoin, fusidic acid, rifampicin, and trimethoprim-sulfamethoxazole) were determined using Vitek card AST-P545. The susceptibility breakpoints for the antibiotics were those recommended by the Clinical and Laboratory Standards Institute, when applicable [14].

The combination of colistin and rifampicin was investigated against 8 paired colistin-susceptible and colistin-resistant strains by measurement of fractional inhibition concentration (FIC) indices [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_{90}, 8 μg/mL) (table 1) and colistin (MIC_{90}, 2 μg/mL) were the only 2 active antibiotics, as determined 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_{90} 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 aminoglycosides (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, erythromycin, 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 nitrofurantoin. Amikacin and tobramycin (but not gentamicin) had good antibacterial activity against most of these strains, regardless 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 example, 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-susceptible strains. No growth was observed for all the tested colistin-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 polymyxins (including colistin), as determined on the basis of MIC, is currently low [18], our group used an in vitro pharmacodynamic model to determine that resistance to colistin can be rapidly developed—even within 24 h—with colistin-heteroresistant *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 combinations with β-lactamase inhibitors, the MICs of most colistin-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; hydrophobicity, 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 resistance to colistin, thereby allowing rifampicin and the lipopolypeptides, macrolides, and streptogramins greater access to their target sites. Such antibiogram changes in colistin-resistant *A. baumannii* have great clinical potential to broaden the therapeutic options. Clinically achievable peak concentrations (ob-

Table 1. Susceptibilities of the paired colistin-susceptible and colistin-resistant strains to various antibiotics.

Antibiotic	Tested concentration range, μg/mL	Breakpoint for susceptible strains, μg/mL	MIC, μg/mL (no. of strains with MIC)	
			Parent strains	Colistin-resistant strains
Penicillins				
Ampicillin	2–32	≤8	16 (2), ≥32 (15)	2 (3), 4 (8), 16 (3), ≥32 (3)
Amoxicillin–clavulanic acid	2/1–32/16	NB	4 (3), 16 (2), ≥32 (12)	≤2 (13), 4 (2), 8 (1), ≥32 (1)
Ticarcillin–clavulanic acid	8/2–128/2	≤16	≤8 (4), 16 (1), ≥128 (12)	≤8 (16), ≥32 (1)
Piperacillin-tazobactam	4/4–128/4	≤16	≤4 (5), 64 (12)	≤4 (16), 64 (1)
Benzylpenicillin	0.03–0.5	NB	≥0.5 (17)	≥0.5 (17)
Oxacillin	0.25–4	NB	≥4 (17)	≥4 (17)
Cephalosporins				
Cefalotin	2–64	NB	≥64 (17)	≤2 (2), 8 (1), ≥64 (14)
Cefazolin	4–64	NB	≥64 (17)	≤4 (3), 16 (4), 32 (2), ≥64 (8)
Cefoxitin	4–64	NB	≥64 (17)	≤4 (9), 8 (2), 16 (1), 32 (2), ≥64 (3)
Ceftazidime	1–64	≤8	4 (1), 8 (3), 16 (1), ≥64 (12)	≤1 (12), 2 (1), 4 (2), 16 (1), ≥64 (1)
Ceftriaxone	1–64	≤8	8 (1), 16 (3), 32 (1), ≥64 (12)	≤1 (10), 2 (1), 4 (2), 8 (2), 16 (1), ≥64 (1)
Cefepime	1–64	≤8	2 (1), 4 (2), 8 (2), 16 (11), 32 (1)	≤1 (15), 2 (1), 16 (1)
Carbapenems				
Meropenem	0.25–16	≤4	≤0.25 (4), 0.5 (1), 8 (11), ≥16 (1)	≤0.25 (16), 4 (1)
Imipenem	1–16	≤4	≤1 (5), 8 (11), ≥16 (1)	≤1 (17)
Aminoglycosides				
Amikacin	2–64	≤16	≤2 (13), 4 (1), 8 (2), 16 (1)	≤2 (17)
Gentamicin	1–16	≤4	≤1 (3), 2 (1), 8 (1), ≥16 (12)	≤1 (5), 2 (1), 4 (5), 8 (1), ≥16 (5)
Tobramycin	1–16	≤4	≤1 (14), 2 (1), 4 (1), 8 (1)	≤1 (17)
Quinolones				
Nalidixic acid	2–32	NB	4 (5), ≥32 (12)	≤2 (3), 4 (2), 8 (2), 16 (8), ≥32 (2)
Ciprofloxacin	0.25–4	≤4	≤0.25 (2), 0.5 (2), 2 (1), ≥4 (12)	≤0.25 (4), 0.5 (2), 1 (8), 2 (1), ≥4 (2)
Norfloxacin	0.5–16	≤4	2 (4), 8 (1), ≥16 (12)	≤0.5 (2), 1 (2), 2 (8), 4 (3), ≥16 (2)
Sulphonamides and trimethoprim				
Trimethoprim	0.5–16	≤2	8 (2), ≥16 (15)	8 (1), ≥16 (16)
Trimethoprim-sulfamethoxazole	20 (1/19)–320 (16/304)	≤2/38	≤20 (4), ≥320 (13)	≤20 (4), 80 (1), 160 (1), ≥320 (11)
Macrolides and lincosamides				
Erythromycin	0.25–8	NB	≥8 (17)	≤0.25 (12), 0.5 (4), ≥8 (1)
Clindamycin	0.25–8	NB	≥8 (17)	0.5 (1), 4 (4), ≥8 (12)
Streptogramins: quinupristin-dalfopristin	0.25–16	NB	≥16 (17)	0.5 (1), 1 (8), 2(7), 8 (1)
Glycopeptides				
Teicoplanin	0.5–32	NB	≥32 (17)	≤0.5 (14), 1 (1), 2 (2)
Vancomycin	1–32	NB	≥32 (17)	≤1 (6), 8 (4), 16 (1), ≥32 (6)
Tetracycline	1–16	NB	4 (2), 8 (1), ≥16 (14)	≤1 (2), 2 (1), ≥16 (14)
Rifamycins: rifampicin	0.5–32	NB	2 (1), 16 (2), ≥32 (14)	≤0.5 (12), 1 (5)
Oxazolidinones: linezolid	0.5–8	NB	≥8 (17)	≥8 (17)
Other				
Fusidic acid	0.5–32	NB	≥32 (17)	≤0.5 (8), 1 (6), 2 (2), ≥32 (1)
Nitrofurantoin	16–512	NB	256 (1), ≥512 (16)	32 (1), 64 (1), 128 (1), 256 (6), ≥512 (8)

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*.

Strain	FIC of colistin-susceptible strain
3	0.31
5	0.53
6	0.19
7	0.14
8	0.27
10	0.50
14	0.19
16	0.38

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.

tained from the respective product information) of erythromycin (2.01 μg/mL; Erythrocin I.V., Abott Australasia), fusidic acid (11.6 μg/mL; Fucidin, CSL Ltd.), quinupristin-dalfopristin (3.20 and 7.96 μg/mL, respectively; Synercid I.V., Aspen Pharmacare, Australia), rifampicin (6–32 μg/mL; Rifadin, Aventis), teicoplanin (20–50 μg/mL; Targocid, Aventis), and vancomycin (63 μg/mL; Vancocin CP, Eli Lilly Australia) in plasma or serum after administration of the recommended dose are substantially higher than their MICs against the colistin-resistant *A. baumannii* (table 1), even with protein binding considered. Currently, these antibiotics are not clinically used for *A. baumannii* infection, with the exception of occasional use of rifampicin.

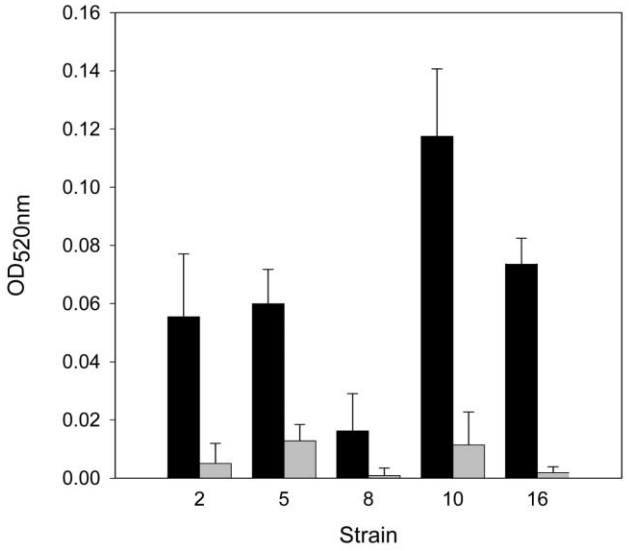

Figure 1. Biofilm-forming ability of the paired colistin-susceptible *(black bars)* and colistin-resistant *(gray bars)* strains. The biofilm formation was determined from the optical density at 520 nm (OD_{520nm}) of crystal violet–stained biofilm.

A substantial increase of multidrug resistance in *A. baumannii* and a dry antibiotic development pipeline have forced clinicians to use combinations of colistin with other antibiotics [22]. Several combinations (with rifampicin, doxycycline, or azithromycin) have been investigated for activity against *A. baumannii* [23, 24], but studies have mainly focused on colistin plus rifampicin. In this study, FICs against colistin-susceptible strains (table 2) also support clinical use of the combination of colistin and rifampicin. The colistin-resistant strains were substantially more susceptible to rifampicin (MIC, ≤0.125 μg/mL). Such increased susceptibility to rifampicin in colistin-resistant *A. baumannii* strains strongly supports recent clinical use of the combination of colistin with rifampicin for treatment of *A. baumannii* infection [23, 25]. In addition, the significantly decreased biofilm formation in colistin-resistant strains will benefit clinical situations, considering that biofilm of *A. baumannii* is related to decreased susceptibility to antibiotics [26].

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.

Acknowledgments

Financial support. Australian National Health and Medical Research Council. J.L. is an Australian National Health and Medical Research Council R. Douglas Wright Research Fellow.

Potential conflicts of interest. All authors: no conflicts.

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