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In Vitro Activities of Novel Antimicrobial Combinations against Extensively Drug-Resistant *Acinetobacter baumannii*

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Extensively drug-resistant (XDR) *Acinetobacter* spp. have emerged as a cause of nosocomial infections, especially under conditions of intensive care. Unfortunately, resistance to colistin is increasing and there is a need for new therapeutic options. We aimed to study the effect of some novel combinations against XDR *Acinetobacter baumannii* in an *in vitro* pharmacokinetics-pharmacodynamics (PK/PD) model. Three nonrelated clinical strains of XDR *A. baumannii* were investigated. Antibiotic-simulated regimens were colistin at 3 MU every 8 h (q8h) (first dose, 6 MU), daptomycin at 10 mg/kg of body weight q24h, imipenem at 1 g q8h, and ertapenem at 1 g q24h. Combination regimens included colistin plus daptomycin, colistin plus imipenem, and imipenem plus ertapenem. Samples were obtained at 0, 1, 2, 4, 8, and 24 h. Among the single-agent regimens, only the colistin regimen resulted in significant reductions in $\log_{10}$ CFU per milliliter compared to the control for all the strains tested. Although colistin achieved bactericidal activity at 4 h, it was not able to reach the limit of detection (1 $\log_{10}$ CFU/ml). One strain had significant regrowth at 24 h without the emergence of resistance. Daptomycin-colistin combinations led to a significant reduction in levels of $\log_{10}$ CFU per milliliter that were better than those achieved with colistin as a single-agent regimen, reaching the limit of detection at 24 h against all the strains. The combination of imipenem plus ertapenem outperformed the colistin regimen, although the results did not reach the limit of detection, with significant regrowth at 24 h. Similarly, colistin-plus-imipenem combinations reduced the levels of $\log_{10}$ CFU per milliliter at 8 h, with significant regrowth at 24 h but with development of resistance to colistin. We have shown some potentially useful alternatives for the treatment of extensively drug-resistant *A. baumannii*. Among them, the daptomycin-colistin combination was the most effective and should be investigated in future studies.

Many Gram-negative rods display significant antimicrobial resistance. Among them, *Acinetobacter baumannii* deserves special consideration. Currently, carbapenem-resistant *A. baumannii* is included in the Infectious Diseases Society of America (IDSA) list of nosocomial pathogens of particular concern (1).

Therapeutic options for this pathogen are extremely limited, a situation made worse by the drying up of the pharmaceutical development pipeline for anti-infective agents. This has forced clinicians to return to older, previously discarded drugs, such as the polymyxins and tigecycline (despite controversies regarding its efficacy), or to drug combinations. However, their efficacy is limited because of resistance or heteroresistance to colistin, drug-drug interactions, or severe side effects such as renal failure (2).

Some authors have reported the efficacy of novel combinations, including daptomycin combinations, against multidrug-resistant (MDR) *A. baumannii* strains (3, 4).

The exact mechanisms involved in this synergistic combination are not fully understood. Daptomycin-colistin combinations have shown an absence of activity against other multidrug-resistant Gram-negative rods but have displayed significant activity against *A. baumannii* (4).

MATERIALS AND METHODS

Bacterial isolates. We used three nonrelated extensively drug-resistant (XDR) isolates of *Acinetobacter baumannii* (5). The strains were obtained from blood samples from two different hospitals.

All isolates were susceptible only to colistin and tigecycline.

Antibiotics and media. Colistin sulfate (COL), daptomycin (DAP), ertapenem (ERT), and imipenem (IMI) were purchased from Sigma-Aldrich Co., Madrid, Spain (colistin), Novartis Pharmaceuticals, Basel, Switzerland (daptomycin), and MSD, Madrid, Spain (ertapenem and imipenem).

Mueller-Hinton broth supplemented with 25 mg/liter calcium and 12.5 mg/liter magnesium (SMHB; Difco Laboratories, Spain) was used for all susceptibility testing and *in vitro* pharmacokinetics-pharmacodynamics (PK/PD) experiments. For experiments using daptomycin, SMHB was supplemented with 50 mg/liter calcium and 25 mg/liter magnesium as recommended by the CLSI guidelines (6).

Antibiotic concentrations were measured by high-performance liquid chromatography following previously published methods (7–9).

***In vitro* PK/PD model.** A previously described *in vitro* PK/PD model consisting of a 250-ml one-compartment glass chamber with multiple ports for delivery and removal of medium, delivery of antibiotics, and collection of bacterial and antimicrobial samples was used (10, 11). The model was set with a targeted initial bacterial inoculum of ~10^8 CFU/ml.

Antibiotics were administered as boluses into the central compartment via an injection port. Peristaltic pumps supplying fresh media were set to simulate antibiotic half-lives ($t_{1/2}$).

Samples (1 ml of media) were obtained at 0, 1, 2, 4, 8, and 24 h and applied to tryptic soy agar (TSA) plates using drop plating. Plates were read after 24 h of incubation at 35°C. For all samples, antimicrobial carryover was accounted for by serial dilution of the plated samples or by vacuum filtration if the drug level of the anticipated dilution was near the

Received 1 March 2015 Returned for modification 12 April 2015
Accepted 7 September 2015
Accepted manuscript posted online 14 September 2015
Citation Córdoba J, Coronado-Álvarez NM, Parra D, Parra-Ruiz J. 2015. *In vitro* activities of novel antimicrobial combinations against extensively drug-resistant *Acinetobacter baumannii*. Antimicrob Agents Chemother 59:7316–7319. doi:10.1128/AAC.00493-15.

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TABLE 1 MIC of antimicrobial agents

Antimicrobial agent	MIC (mg/liter) for strain:		
	AJ001	AJ002	AJ003
Colistin	2	2	4
Daptomycin	256	256	256
Imipenem	32	64	32
Ertapenem	64	128	64
Tigecycline	1	1	1

MIC value for the organism. The limit of detection of these methods of colony count determination was 2 $\log_{10}$ CFU/ml (extended to 1 $\log_{10}$ CFU/ml by vacuum filtration).

Bactericidal activity was defined as >3 $\log_{10}$ CFU/ml kill from the initial inoculum.

Emergence of resistance was evaluated by performing susceptibility testing of colonies recovered at 24 h according to CLSI guidelines (6).

Experiments were performed in duplicate to account for biological variability.

Simulated antibiotic regimens. For colistin, the regimen consisted of a 6-MU loading dose (maximum concentration of free, unbound drug in serum [fC_{max}], 4.5 mg/liter; $t_{1/2}$, 4 h; protein binding, 50%) followed by 3 MU administered every 8 h (q8h) (fC_{max}, 3 mg/liter) (12); for daptomycin, the regimen consisted of 10 mg/kg of body weight q24h (fC_{max}, 11.3 mg/liter; $t_{1/2}$, 8 h; protein binding, 92%) (13); for ertapenem, the regimen consisted of 1 g q24h (fC_{max}, 7.75 mg/liter; $t_{1/2}$, 4 h; protein binding, 95%) (14); and for imipenem, the regimen consisted of 1 g q8h (fC_{max}, 80 mg/liter; $t_{1/2}$, 1 h; protein binding, 20%) (15).

Statistical analysis. Changes in bacterial CFU counts per milliliter were compared by analysis of variance (ANOVA) and Turkey's *post hoc* test. A P value of <0.05 was considered significant.

RESULTS

Susceptibilities of the isolates are displayed in Table 1. Observed PK parameters were within 15% of the targeted values.

The daptomycin-colistin combination demonstrated bactericidal activity against all three clinical isolates of XDR *A. baumannii* evaluated (Fig. 1), reaching the limit of detection at 24 h. No other combination therapy reached the limit of detection.

Colistin was the only single-agent regimen that was able to produce a significant reduction in the bacterial count, reaching a bactericidal effect against all isolates at 4 h; however, there was regrowth in all three isolates at 24 h, without emergence of resistance. Despite regrowth at 24 h, colistin produced a significant reduction of the bacterial count compared to controls against all three strains ($P = 0.012$).

The imipenem regimen led to a small reduction of the bacterial count at 2 h, followed by bacterial regrowth. At 24 h, there were no statistically significant differences between the results seen with imipenem, ertapenem, and daptomycin used as single-agent regimens and control results.

Imipenem-colistin and imipenem-ertapenem combinations outperformed colistin as a single-agent regimen and demonstrated a bactericidal effect at 4 h, although there was significant regrowth at 24 h. The imipenem-colistin combination led to the emergence of resistance to colistin.

DISCUSSION

Using this *in vitro* pharmacokinetic/pharmacodynamic model of bacteremia, we have shown the efficacy of the daptomycin-colistin combination against three nonrelated strains of extensively drug-resistant *Acinetobacter baumannii*. Daptomycin-colistin combinations outperformed colistin as a single-agent regimen and colistin-imipenem combinations.

Extensively drug-resistant *A. baumannii* has become a serious problem worldwide. Limited antibiotic options and high severity of disease in patients suffering from XDR *A. baumannii* infections account for the high rates of mortality described.

Although other authors have reported the efficacy of colistin-daptomycin combinations against MDR *A. baumannii* strains, they used different methodologies, mainly time-kill curves and checkerboard assays (3) or the Etest agar dilution method (4). We used an *in vitro* PK/PD model that resembles human pharmacokinetics and mimics *A. baumannii* bacteremia.

Other combinations of anti-Gram-positive agents with colistin against *A. baumannii* have been studied. Significantly enhanced *in vitro* killing activity has been described with vancomycin (16), teicoplanin (17), and telavancin (18). Similarly, glycopeptide-colistin combinations have been studied *in vivo* with promising results (19). Very recently, Petrosillo et al. reported the efficacy of glycopeptide-colistin combinations in intensive care unit (ICU) patients infected with multidrug-resistant Gram-negative microorganisms (60% *A. baumannii*). Patients who received glycopeptide-colistin combinations had an increased chance of survival (20). However, renal failure was more frequent among patients who received combined therapy with glycopeptides, limiting its utility.

Daptomycin has no renal toxicity (21), and it can attenuate the nephrotoxicity of other antimicrobials (22), so the daptomycin-colistin combination is not expected to increase renal toxicity as described with glycopeptide-colistin combinations, making this combination an attractive option in cases of XDR *A. baumannii* infection.

Colistin is thought to increase the permeability of the Gram-negative outer membrane, rendering Gram-negative bacteria susceptible to antimicrobials which otherwise would have no effect against them. Although this mechanism of action could explain the enhanced activity of vancomycin, teicoplanin, or rifampin combinations (16, 23), it does not fully explain the effect of the daptomycin-colistin combination against *A. baumannii*.

Phee et al. (4) reported that the daptomycin-colistin combination had no effect against colistin-susceptible strains of *Escherichia coli*, *Klebsiella pneumonia*, *Enterobacter cloacae*, or *Pseudomonas aeruginosa* but demonstrated high efficacy against all colistin-susceptible strains of *A. baumannii*. These results are consistent with data published previously where a mutant strain of *E. coli* that lacked the outer membrane (*E. coli imp*) was fully susceptible to vancomycin (24) whereas the strain remained fully resistant to daptomycin and its derivatives (25). It has been suggested that daptomycin, after binding into cell membrane, induces alterations in the operon encoding YycFG, a two-component system present only in Gram-positive organisms and involved in controlling the cytoplasmic membrane integrity, leading to rapid cell death without lysis (26, 27). This proposed mechanism of action might explain daptomycin's absence of activity against Gram-negative bacteria. However, the high activity displayed against *A. baumannii* but not against many other Gram-negative bacterial species suggests the presence of striking differences between *A. baumannii* and other Gram-negative bacteria that should be further studied.

We have also explored other combinations, such as imipenem-

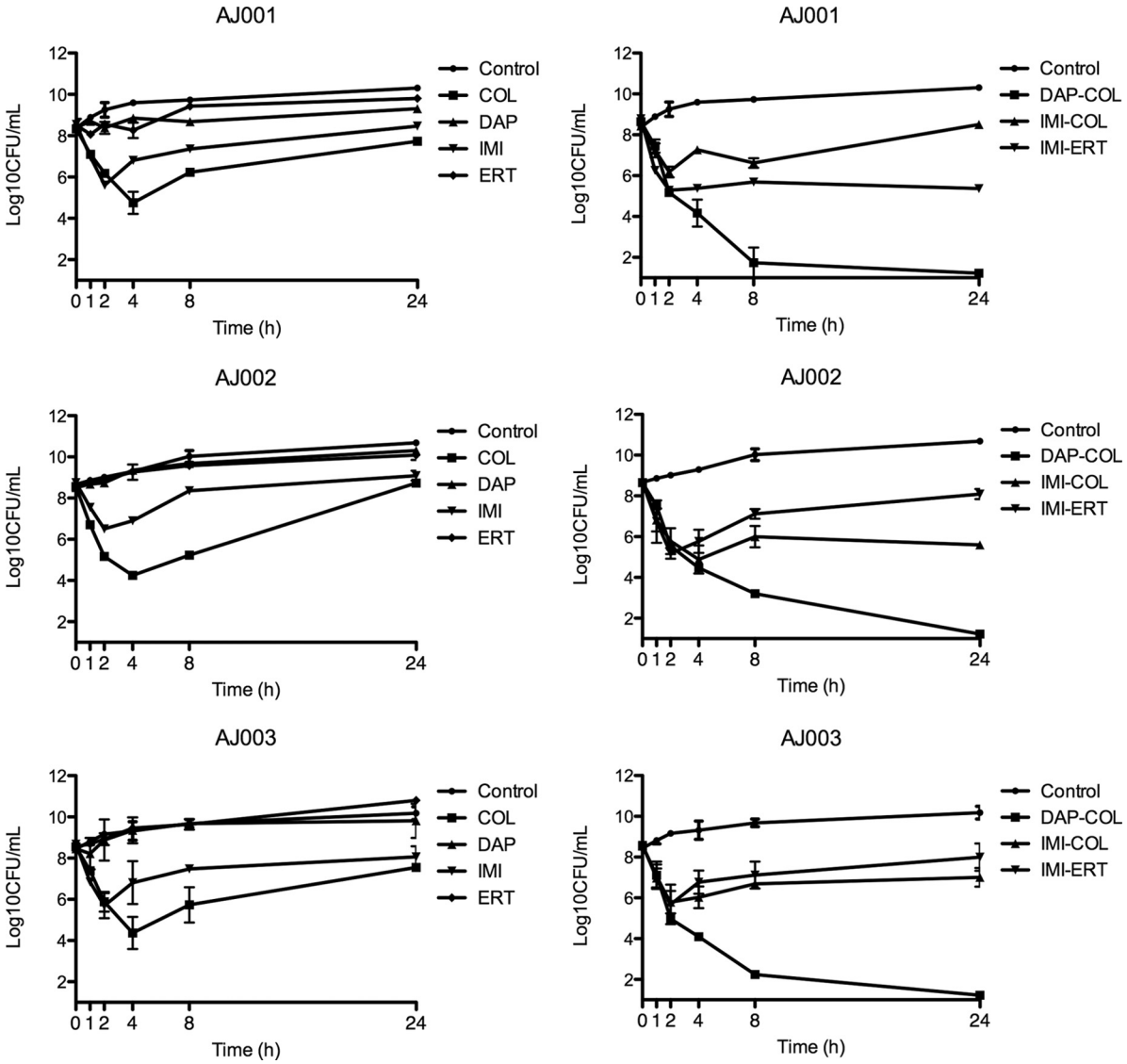

FIG 1 Activity of different single-agent regimens and combination regimens against three nonrelated strains of extensively drug-resistant *A. baumannii*. Symbols represent the mean numbers of viable colonies at each time interval. COL, colistin; DAP, daptomycin; IMI, imipenem, ERT, ertapenem.

colistin or double-carbapenem combinations, that have proven to be synergistic against other MDR Gram-negative organisms (28, 29). However, we did not find any activity with the ertapenem-imipenem combination and found limited efficacy of imipenem-colistin combinations. Interestingly, the imipenem-colistin combination led to the emergence of colistin resistance, but administration of colistin as a single-agent regimen did not result in colistin resistance. We do not have an explanation for this, but our results suggest that there might be some kind of interaction with imipenem and colistin.

Our work has some limitations. First, we tested only three strains of *A. baumannii*, so our results may not be generalizable to all strains; however, the consistency of the results against all three strains, and the fact that we used three unrelated strains, makes us believe that our results can be generalized. Second, our model ran for only 24 h and thus would not detect antimicrobial activity that might appear after 24 h or mechanisms of resistance. We also have to admit that the high inoculum we used might have impaired the activity of imipenem. However, recent data from studies using the colistin-carbapenem combination did not show differences at low ($<\sim10^8$ CFU/ml) or high ($\sim10^{11}$ CFU/ml) bacterial density (30).

Finally, we acknowledge that *in vitro* studies do not always translate into similar results in clinical practice, so caution has to be advised prior to use. However, similar results have been reported with three other methodologies (3, 4), giving robustness to our results.

In the present situation of almost no therapeutic option against some strains of XDR *A. baumannii*, we believe that our results represent a novel strategy to add to the limited options against XDR *A. baumannii*.

ACKNOWLEDGMENT

We declare that we have no conflicts of interest.

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