Triple combination therapy with high-dose ampicillin/sulbactam, high-dose tigecycline and colistin in the treatment of ventilator-associated pneumonia caused by pan-drug resistant *Acinetobacter baumannii*: a case series study

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SUMMARY

Acinetobacter baumannii has evolved in recent decades as a major problem in carbapenem-resistant gram-negative nosocomial infections, associated with high mortality rates especially in intensive care units (ICUs). Recent reports highlight the increasing prevalence of resistance to colistin, a last resort therapeutic option for carbapenem-resistant *A. baumannii*. We retrospectively evaluated the potential efficacy, in terms of clinical and microbiological cure and mortality, of a combination of intravenous colistin and high-dose ampicillin/sulbactam and high-dose tigecycline, concurrently administered with inhaled colistin, in 10 ICU patients with ventilator-associated pneumonia (VAP) caused by carbapenem- and colistin-resistant *A. baumannii* strains, with high tigecycline MICs > 2μg/mL. Nine patients (90%) exhibited a successful clinical outcome, accompanied by microbiological eradication in seven of them. All clinically cured patients survived at 14 and 28 days. Acute kidney injury (AKI) was observed in one patient. In view of the increasing prevalence of pan-drug resistant *A. baumannii* infections in ICUs, its associated high rates of mortality and the lack of effective treatment options, we feel that there is an emerging need for our results to be further validated in larger prospective studies.

Keywords: *Acinetobacter baumannii*, ventilator-associated pneumonia, pan-drug resistant, colistin, tigecycline, sulbactam

INTRODUCTION

Acinetobacter baumannii has evolved over the last decades as a major problem in multidrug resistant gram-negative nosocomial infections, especially in the intensive care unit (ICU). Its intrinsic antimicrobial resistance together with its ability to easily adopt new resistance mechanisms has driven the evolution of extensively drug-resistant (XDR) and even pandrug-resistant (PDR) isolates [1]. The most frequent healthcare-associated infection caused by *A. baumanii* is ventilator-associated pneumonia (VAP). For carbapenemase-pro-

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ducing multi drug-resistant (MDR) *A. baumanii* strains, colistin represents the cornerstone of antimicrobial therapy, while tigecycline represents an additional option. However, the wide use of colistin for the treatment of MDR Gram negative nosocomial infections have forced the increasing rates of resistance to this last resort antibiotic. In a recently published Southern Europe multicentre study (Greece, Italy and Spain) on the molecular epidemiology, and antimicrobial susceptibility of *A. baumannii* isolates from respiratory tract samples of patients with VAP, carbapenem resistance was almost universal, while colistin resistance raised to 47.7% [1]. Over the course of this study, colistin resistance rates increased from 32% in 2012-13, to 62% in 2014-15. Of note, 34% of isolated strains were XDR and 31% PDR highlighting the difficulty in treating these patients [1].
We present herein, our tertiary centre experience with ten patients hospitalized in the intensive care unit (ICU), suffering from VAP caused by PDR *A. baumannii* strains, defined as carbapenem- and colistin-resistant with high tigecycline MICs >2μg/mL. The reported patients received a combination treatment consisting of intravenous colistin and high-dose ampicillin/sulbactam and high-dose tigecycline, concurrently with inhaled colistin.

PATIENTS AND METHODS

We retrospectively evaluated the potential efficacy, in terms of clinical and microbiological cure and mortality, of a combination of intravenous colistin and high-dose ampicillin/sulbactam and high-dose tigecycline, concurrently administered with inhaled colistin, in 10 ICU patients with VAP caused by carbapenem- and colistin-resistant *A. baumannii* strains, with high tigecycline MIC >2 μg/mL. PDR phenotype was defined according to the international expert proposal for Interim standards guidelines [2]. Accordingly, PDR *A. baumannii* isolates were non-susceptible to all agents in all antimicrobial categories, except for tigecycline, where in the latest EUCAST clinical breakpoints (v. 8.1, May 15, 2018) no clinical breakpoint is set due to insufficient evidence [3]. However, previous clinical studies with XDR *A. baumannii* infections, have shown that when tigecycline MIC is >2 μg/ml, significantly higher therapeutic failures and mortality were observed either when tigecycline was used as monotherapy or as part of a combination therapy with colistin [4, 5]. From this point of view, the present study included patients with VAP caused by real PDR *A. baumannii* strains. Sulbactam alone has been found to have intrinsic activity against *Acinetobacter spp.*, but the only available preparation of sulbactam in Greece is within a fixed combination of ampicillin/sulbactam. However, it has been previously demonstrated that the activity of ampicillin/sulbactam against *Acinetobacter spp.* is exclusively due to sulbactam [6].
Patients were treated with intravenous colistin 9 million IU loading dose, followed after 12 h by maintenance dose of 4.5 million IU every 12 hours and high-dose intravenous tigecycline 200 mg loading dose followed after 12 h by 100 mg every 12 hours and high dose intravenous ampicillin/sulbactam 9 g (6+3) every 8 hours, while inhaled colistin 2 million IU every 8 hours was concurrently administered. In patients with a calculated creatinine clearance of less than 60 ml/min, according to the Cockcroft-Gault formula, the maintenance dose of colistin was reduced based on a modified formula from Garonzik et al. (daily maintenance colistin dose [IU] = $CL_{CR}/10 + 2$) [7, 8]. Ampicillin/sulbactam dose was also reduced according to the creatinine clearance value (<60 mL/min); for CL_{CR} 31-60 mL/min the dose was reduced by 25% without changes in interval dosing, while for CL_{CR} 7-30 mL/min the dose was reduced by 50% and administered twice daily [9].
The diagnostic criteria of VAP were in accordance with current clinical practice guidelines set by the Infectious Diseases Society of America and the American Thoracic Society: (a) mechanical ventilation >48 h; (b) satisfied two of the following: body temperature >38 °C or <36 °C, leukopenia or leukocytosis, or purulent secretions; (c) new or progressive chest infiltrates, for patients with underlying pulmonary or cardiac disease, two serial chest radiographs were required for assessment; (d) a quantitative culture of respiratory sample taken non-invasively (endotracheal aspiration) yielding ≥10^6 CFU/mL [10]. *A. baumannii* strains isolated from respiratory cultures deriving from ICU patients were identified using Vitek 2 Advanced Expert System (bioMérieux, Marcy l'Étoile, France). Antibiotic susceptibility was performed by the agar disk diffusion

method (Kirby-Bauer) for all antibiotics including carbapenems, while MIC to tigecycline was determined by Etest (AB Biodisk) and MIC to colistin by broth microdilution method. Results were interpreted according to EUCAST guidelines (EUCAST, 2018). Blood cultures (two sets from peripheral veins) were taken concomitant to tracheal aspirates at clinical suspicion of VAP and thereafter as clinically indicated (presence of fever ≥38.0 °C, clinical suspicion of bloodstream infection). Patients were considered immunocompromised if they had received a transplant, had a splenectomy, a gamma globulin deficiency, an HIV infection, were currently under anti-cancer chemotherapy, or received at least 20 mg of prednisone/day for ≥30 days. The Charlson comorbidity index determined the extent of comorbid illnesses [11]. A successful clinical outcome was defined as the resolution of symptoms and signs of infection and improvement of relevant laboratory data at the end of therapy. Microbiological success was defined as a follow up negative culture for *A. baumannii* at days 7 or 14. Mortality was evaluated at days 14 and 28. Patients with culture-proven polymicrobial infections were excluded. Patients receiving concurrent empiric coverage for MRSA were not excluded from our analysis. Therapeutic failure was considered if clinical findings worsened or persisted without improvement after 4-7 days of treatment. VAP-related death was considered when persistent symptoms and signs were present at the time of death without other definite causes. Acute kidney injury (AKI) was defined according to the RIFLE criteria; increase of serum creatinine of at least 50% from baseline (defined as Risk), doubled serum creatinine level from the baseline (defined as Injury), or three times increase in serum creatinine (defined as Failure) [12].

PATIENTS AND RESULTS

The present study included ten patients with PDR *A. baumannii* VAP, while one patient additionally presented VAP-associated secondary bacteraemia. Patients had a mean age of 56.4±13.5 years, no one patient was immunocompromised, half of them had a Charlson comorbidity index ≥3 and were hospitalized in the ICU for a mean duration of 33.7±28.3 days. The mean duration of the

Table 1 - Clinical and microbiological outcome of PDR *A. baumannii* VAP treated with high-dose ampicillin/sulbactam and high-dose tigecycline and colistin combination.

Patient no	*Sex*	*Age*	*Immuno-compromised*	*Charlson comorbidity index*	*Days in ICU*	*Type of Infection*	*APACHE II score (admission)*	*Tigecycline MIC (μg/ml)*	*Co- administered antibiotics*	*Treatment duration*	*Microbiological success*	*Clinical success*	*14 d survival*	*28 d survival*	*AKI 48h*	*AKI 7d*	*AKI end of Tx*
1	M	38	No	0	14	VAP	27	4	ceftaroline	15	Yes	Yes	Yes	Yes	No	No	No
2	M	50	No	0	19	VAP	23	3	linezolid	11	Yes	Yes	Yes	Yes	No	No	No
3	M	62	No	4	34	VAP + BSI	25	3	vancomycin	14	No	Yes	Yes	Yes	Yes	Yes	Yes
4	F	49	No	0	14	VAP	20	4	linezolid	10	No	Yes	Yes	Yes	No	No	No
5	M	71	No	6	79	VAP	28	4	linezolid	12	Superinfection KPC Klebsiella	No	No	No	No	No	No
6	M	47	No	0	39	VAP	23	3	linezolid	7	Yes	Yes	Yes	Yes	No	No	No
7	M	51	No	0	90	VAP	20	12	linezolid	16	Yes	Yes	Yes	Yes	No	No	No
8	M	60	No	3	16	VAP	18	3	linezolid	14	Yes	Yes	Yes	Yes	No	No	No
9	F	51	No	5	11	VAP	22	3	linezolid	17	Yes	Yes	Yes	Yes	No	No	No
10	M	85	No	11	21	VAP	25	3	linezolid	14	Yes	Yes	Yes	Yes	No	No	No

M, male; F, female; PDR, pan-drug resistant; VAP, ventilator associated pneumonia; ICU, intensive care unit; BSI, blood stream infection; APACHE, Acute Physiology and Chronic Health Evaluation; MIC, minimum inhibitory concentration; KPC, *Klebsiella pneumoniae* carbapenemase; AKI, acute kidney injury; Tx, treatment

studied triple combination therapy for *A. baumannii* VAP was 13±3 days. The characteristics of the studied patients and their clinical and microbiological outcomes are presented in Table 1. Nine patients (90%) exhibited a successful clinical outcome accompanied by microbiological eradication in 7 of them. This discordance between clinical success and microbiological eradication has been previously described in non-sterile sites, especially when foreign bodies are involved, such as in patients with VAP, and constitutes colonization [13]. All clinically cured patients survived at 14 and 28d, while a patient died later from nosocomial sepsis owing to KPC-positive *Klebsiella pneumoniae* bacteraemia. The only patient who failed the combination treatment and died from VAP was the second older patient in this series (79 years old) with a high Charlson comorbidity index of 6, with the highest APACHE II score (28) on admission, who was complicated by KPC-positive *Klebsiella pneumoniae* superinfection. Colistin administration was proven safe in term of its potential nephrotoxicity and acute kidney injury (AKI) was developed in only one patient, the one with the accompanying bacteraemia from *A. baumannii,* who was receiving concomitant vancomycin for empiric MRSA coverage.

DISCUSSION

VAP and bacteraemia caused by XDR *A. baumannii* strains, defined as resistant to all antibiotics except colistin and tigecycline, result in >50% mortality rates [5, 14]. In infections by XDR *A. baumannii* strains, a number of studies attempted to answer the question whether there is any benefit of colistin-based combination therapy over monotherapy with colistin, but the results were contradictory [14]. However, a recent randomised controlled trial including 406 patients with severe infections caused by carbapenem-resistant *A. baumannii* demonstrated that the addition of meropenem to colistin did not improve clinical outcome [15]. Regarding the use of tigecycline in VAP, as previously stated significantly higher mortality was observed with this treatment in MICs >2 μg/mL [4, 5]. However, in the above-mentioned studies the standard dose of tigecycline, 100 mg loading followed by 50 mg every 12 hours, was used. Several concerns have been raised about tigecycline distribution in the lung, although its levels in patients with pneumonia are expected to be higher than healthy volunteers. Standard doses are probably inadequate to reach maximally efficacy, especially against resistant pathogens on the upper end of the MIC distribution (1 to 2 μg/mL) [16]. High dose tigecycline therapy, defined as 200 mg loading dose followed by 100 mg every 12 hours, attains a better PK-PD profile and is associated with significantly improved outcomes [16]. With regard to the comparison of antimicrobial options usually administered to treat patients with nosocomial pneumonia caused by MDR/XDR *A. baumannii*, in a recent metanalysis of twenty-three studies including 15 antimicrobial treatments, sulbactam (normal and high-dose) was the best treatment in terms of survival benefit, followed by fosfomycin and colistin, combined inhaled and intravenous colistin, high-dose tigecycline and colistin monotherapy [17]. With the emergence of colistin resistance in *A. baumannii*, our therapeutic armamentarium to treat these infections becomes very limited. New antibiotics against Gram negative bacilli do not offer a solution to this problem, because ceftolozane-tazobactam has no reliable activity against *A. baumannii* and ceftazidime-avibactam has activity similar to ceftazidime alone against *A. baumannii* [18]. Therefore, in carbapenem- and colistin-resistant (PDR) *A. baumannii* severe infections, it is reasonable to use combination regimens to take advantage of antibiotic synergism. Diverse combinations of antibiotics have demonstrated a synergistic action against XDR *A. baumannii*, such as colistin combined with rifampicin or tigecycline or sulbactam or vancomycin and carbapenems combined with colistin, or sulbactam or aminoglycosides or rifampicin [14]. The combination of high dose ampicillin/sulbactam with colistin has demonstrated promising results in *in-vitro*, experimental and clinical studies, but clinical data is scarce, especially in patients with PDR *A. baumannii* severe infections [19-21].

Patients analysed in the present study suffered from VAP by PDR *A. baumannii*, resistant to carbapenems and colistin, with high tigecycline MIC >2 μg/mL. This difficult to treat population received a salvage combination treatment regimen, consisted of intravenous colistin and high dose ampicillin/sulbactam and high-dose tigecycline. Also, inhaled colistin as adjunctive to its intrave-

nous administration was concurrently administered. The rationale of this combined regimen was to take advantage of the best available antimicrobial weapons to fight a very serious infectious enemy. The very high rates of clinical response and 28d survival (90%) observed in the present case series are promising and have not been previously attained with dual combination treatments. Moreover, it is noteworthy that this positive clinical response and survival was attained in VAP cases caused by PDR *A. baumannii* strains, as compared to most previously published studies which refer to XDR *A. baumannii* infections. The mean duration of the triple combination therapy in our study was 13±3 days, which is longer than the currently recommended 7-day course of antimicrobial therapy in VAP [10]. However, in VAP caused by non-glucose-fermenting Gram negative bacilli including Pseudomonas and Acinetobacter, there is a concern that short courses of antibiotics are associated with recurrent infection, as demonstrated in a systematic review including 6 randomised controlled trials [22].

Beyond the efficacy of the triple combination antibiotic therapy used in the present study, the increased survival rate observed could be additionally attributed to host and pathogen factors. Regarding host factors, studied patients were relatively young (only two patients were >65 years old), without underlying immunosuppression (100%) or major comorbidities (Charlson comorbidity index was 0 in 5 patients). Also, although not analysed in the present study, differences in pathogenicity among carbapenem-resistant *A. baumannii* clones might explain the positive clinical outcomes [23]. Mutations that lead to antimicrobial resistance might impair bacterial fitness and virulence potential. Previous studies have shown that emergence of colistin resistance in *A. baumannii* strains, especially in mutants that lose lipopolysaccharide, cost a fitness and virulence deficit [24, 25].

Regarding the safety of the triple antibiotic combination, three patients developed diarrhoea, but none of these cases were associated with *Clostridium difficile* infection. Acute kidney injury (AKI) developed in only one patient, the one with the accompanying bacteraemia from *A. baumannii,* who was receiving concomitant vancomycin for empiric MRSA coverage. Previous studies have demonstrated that the presence of bloodstream infection and the concomitant administration of colistin with glycopeptides are independently associated with high risk of AKI development in critically ill patients [12]. Therefore, AKI in this patient was attributed to the aforementioned co-factors and not solely to the combination treatment regimen.

A major limitation of our findings is that this is only a small case series study, retrospectively looking at the results of our experience and clinical practice, when dealing with such difficult to treat infections. In view of the increasing prevalence of PDR *A. baumannii* infections in ICUs, its associated high rates of mortality and the lack of effective treatment options, we feel that there is an emerging need our results to be further validated in larger prospective studies.

Conflicts of interest

The authors declare no competing financial interests.

REFERENCES

[1] Nowak J., Zander E., Stefanik D., et al. High incidence of pandrug-resistant *Acinetobacter baumannii* isolates collected from patients with ventilator-associated pneumonia in Greece, Italy and Spain as part of the MagicBullet clinical trial. *J. Antimicrob. Chemother.* 72, 3277-3282, 2017.

[2] Magiorakos A.P., Srinivasan A., Carey R.B., et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin. Microbiol. Infect.* 18, 268-281, 2012.

[3] The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1, 2018. http://www.eucast.org.

[4] Chuang Y.C., Cheng C.Y., Sheng W.H., et al. Effectiveness of tigecycline-based versus colistin- based therapy for treatment of pneumonia caused by multidrug-resistant *Acinetobacter baumannii* in a critical setting: a matched cohort analysis. *BMC Infect. Dis.* 14, 102, 2014.

[5] Cheng A., Chuang Y.C., Sun H.Y., et al. Excess mortality associated with colistin-tigecycline compared with colistin-carbapenem combination therapy for extensively drug-resistant *Acinetobacter baumannii* bacteremia: a multicenter prospective observational study. *Crit. Care Med.* 43, 1194-1204, 2015.

[6] Corbella X., Ariza J., Ardanuy C., et al. Efficacy of sulbactam alone and in combination with ampicillin in no-

socomial infections caused by multiresistant *Acinetobacter baumannii. J. Antimicrob. Chemother.* 42, 793-802, 1998.
[7] Garonzik S.M., Li J., Thamlikitkul V., et al. Population pharmacokinetics of colistin methanesulfonate and formed colistin in critically ill patients from a multicenter study provide dosing suggestions for various categories of patients. *Antimicrob. Agents Chemother.* 55, 3284-3294, 2011.
[8] Karaiskos I., Giamarellou H. Multidrug-resistant and extensively drug-resistant Gram-negative pathogens: current and emerging therapeutic approaches. *Expert Opin. Pharmacother.* 15, 1351-1370, 2014.
[9] Betrosian A.P., Frantzeskaki .F, Xanthaki A., Douzinas E.E. Efficacy and safety of high-dose ampicillin/sulbactam vs. colistin as monotherapy for the treatment of multidrug resistant *Acinetobacter baumannii* ventilator-associated pneumonia. *J. Infect.* 56, 432-436, 2008.
[10] Kalil A.C., Metersky M.L., Klompas M., et al. Management of adults with hospital-acquired and ventilator-associated pneumonia: 2016 Clinical Practice Guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin. Infect. Dis.* 63, e61-e111, 2016.
[11] Charlson M., Szatrowski T.P., Peterson J., Gold J. Validation of a combined comorbidity index. *J. Clin. Epidemiol.* 47, 1245-1251, 1994.
[12] Rocco M., Montini L., Alessandri E., et al. Risk factors for acute kidney injury in critically ill patients receiving high intravenous doses of colistin methanesulfonate and/or other nephrotoxic antibiotics: a retrospective cohort study. *Crit. Care* 17, R174, 2013.
[13] Kiem S., Schentag J.J. Correlations between microbiological outcomes and clinical responses in patients with severe pneumonia. *Infect. Chemother.* 45, 283-291, 2013.
[14] Poulikakos P., Tansarli G.S., Falagas M.E. Combination antibiotic treatment versus monotherapy for multidrug-resistant, extensively drug-resistant, and pandrug-resistant Acinetobacter infections: a systematic review. *Eur. J. Clin. Microbiol. Infect. Dis.* 33, 1675-1685, 2014.
[15] Paul M., Daikos G.L., Durante-Mangoni E., et al. Colistin alone versus colistin plus meropenem for treatment of severe infections caused by carbapenem-resistant Gram-negative bacteria: an open-label, randomised controlled trial. *Lancet Infect. Dis.* 18, 391-400, 2018.
[16] De Pascale G., Montini L., Pennisi M., et al. High dose tigecycline in critically ill patients with severe infections due to multidrug-resistant bacteria. *Crit. Care* 18, R90, 2014.
[17] Jung S.Y., Lee S.H., Lee S.Y., et al. Antimicrobials for the treatment of drug-resistant *Acinetobacter baumannii* pneumonia in critically ill patients: a systemic review and Bayesian network meta-analysis. *Crit. Care* 21, 319, 2017.
[18] van Duin D., Bonomo R.A. Ceftazidime/avibactam and ceftolozane/tazobactam: second-generation beta-lactam/beta-lactamase inhibitor combinations. *Clin. Infect. Dis.* 63, 234-241, 2016.
[19] Lenhard J.R., Smith N.M., Bulman Z.P., et al. High-dose ampicillin-sulbactam combinations combat polymyxin-resistant *Acinetobacter baumannii* in a hollow-fiber infection model. *Antimicrob. Agents Chemother.* 61, e01268-e01216, 2017.
[20] Leelasupasri S., Santimaleeworagun W., Jitwasinkul T. Antimicrobial susceptibility among colistin, sulbactam, and fosfomycin and a synergism study of colistin in combination with sulbactam or fosfomycin against clinical isolates of carbapenem-resistant *Acinetobacter baumannii. J. Pathog.* 2018, 3893492, 2018.
[21] Betrosian A.P., Frantzeskaki F., Xanthaki A., Georgiadis G. High-dose ampicillin-sulbactam as an alternative treatment of late-onset VAP from multidrug-resistant Acinetobacter baumannii. *Scand. J. Infect. Dis.* 39, 38-43, 2007.
[22] Pugh R., Grant C., Cooke R.P., Dempsey G. Short-course versus prolonged-course antibiotic therapy for hospital-acquired pneumonia in critically ill adults. *Cochrane Database Syst Rev.* CD007577, 2015.
[23] Nishida S., Ono Y. Comparative analysis of the pathogenicity between multidrug-resistant *Acinetobacter baumannii* clinical isolates: isolation of highly pathogenic multidrug-resistant *A. baumannii* and experimental therapeutics with fourth-generation cephalosporin cefozopran. *Infect. Drug Resist.* 11, 1715-1722, 2018.
[24] Da Silva G.J., Domingues S. Interplay between colistin resistance, virulence and fitness in *Acinetobacter baumannii*. *Antibiotics (Basel).* 6, 2017.
[25] Carretero-Ledesma M., Garcia-Quintanilla M., Martin-Pena R., Pulido M.R., Pachon J., McConnell M.J. Phenotypic changes associated with colistin resistance due to lipopolysaccharide loss in *Acinetobacter baumannii. Virulence* 9, 930-942, 2018.