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A combination of tigecycline, colistin, and meropenem against multidrug-resistant *Acinetobacter baumannii* bacteremia in a renal transplant recipient: pharmacodynamic and microbiological aspects

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ABSTRACT

Acinetobacter baumannii are emerging as the causal agents of healthcare-associated infections. We describe a renal transplant recipient who developed bacteremia caused by multiresistant *A. baumannii*, which received a combination of tigecycline, colistin, and meropenem in continuous infusion. The clinical outcome was favorable. In this article we made a molecular study of this multiresistant strain. Our analysis reveals the presence of a *bla*-$_{OXA-72}$ gene, a class D of oxacillinase belonging to *bla*-$_{OXA-40}$-like group, which constitutes the most disseminated familiy of carbapenemases in Spain. Thus, we found different susceptibility patterns of *A. baumannii* when we used different Mueller-Hinton agars with different manganese concentrations. Lastly, we explain the combination of these three antibiotics administered to increase microbiologic and pharmacodynamic yield.

Key words Multirresistant *Acinetobacter baumannii,* bacteremia, tigecycline, colistin, continuous infusion of meropenem, Manganese, Mueller-Hinton agar

Combinación de tigeciclina, colistina y meropenem en bacteriemia por *Acinetobacter baumannii* multirresistente en un receptor de trasplante renal: aspectos microbiológicos y farmacodinámicos

RESUMEN

Acinetobacter baumannii está emergiendo como uno de los agentes causales de las infecciones asociadas al sistema sanitario. Presentamos el caso del receptor del un trasplante renal que desarrollo una bacteriemia por *Acinetobacter baumannii* multirresistente, en el que se ensayó un tratamiento combinado de tigeciclina, colistina y meropenem en perfusión continua. La evolución fue favorable. En el presente artículo se establece un estudio molecular de esta cepa multirresistente. Nuestro análisis revela la presencia del gen bla-$_{OXA-72}$, una clase D de oxacilinasa perteneciente a un grupo bla-$_{OXA-40}$-like, que es la familia de carbapenemasas mas extendida en nuestro país. Además, se observaron diferentes patrones de sensibilidad de *A. baumannii* frente a tigeciclina en función del tipo de agar Mueller-Hinton, con diferentes concentraciones de manganeso. Por último se exponen los motivos microbiológicos y farmacodinámicos por los que se estableció la combinación de antibióticos

Palabras clave: *Acinetobacter baumannii* multirresistente, bacteriemia, tigeciclina, colistina, infusión continua de meropenem, manganeso, agar Mueller-Hinton

INTRODUCTION

Multiresistant pathogens are the consequence of an antimicrobial overuse, associated to the ease of spread drug resistant sequences among bacteria and a limited capacity of hospital infection control programs. *Acinetobacter baumannii* are emerging as the causal agents of healthcare-associated infections. The main determinants of the pathogenicity of *A. baumannii* are its scant nutritional requirements (allowing it to colonize and survive in the patient and on clinical material), its inherent multidrug resistance, and the ease with which it acquires resistance to new antimicrobial agents[1]. Predisposition to infection by this microorganism results from previous antimicrobial therapy, surgery, and hospitalization in critical care units. Nevertheless, this entity is increasingly found in more conventional units of the hospital, where inappropriate treatment could correlating with mortality, especially in infections such as pneumonia or bacteremia. Imipenem has been the most active antimicrobial agent against these microorganisms, but resistance to imipenem means that colistin and tigecycline may be the only remaining therapeutic options in infection by multiresistant *A.*

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Table 1 **Tigecycline, colistin and meropenem MICs with the E-test method, using 2 types of Mueller Hinton agar**

	(Mueller Hinton agar, bioMérieux®) with manganese supplementation			(Mueller Hinton agar, Becton Dickinson®) without manganese supplementation		
	Colistin	Tigecycline	Meropenem	Colistin	Tigecycline	Meropenem
AB 1st BC	0.094 mg/L	4 mg/L	32 mg/L	0.094 mg/L	1.5 mg/L	32 mg/L
AB 2nd BC	0.094 mg/L	6 mg/L	32 mg/L	0.094 mg/L	1.5 mg/L	32 mg/L
AB 3rd BC	0.38 mg/L	6 mg/L	32 mg/L	0.38 mg/L	1.5 mg/L	32 mg/L

AB 1st BC: ***A. baumannii*** isolated in the first blood culture (isolate HCSC33590) at 0 days of treatment; AB 2nd BC: ***A. baumannii*** isolated in the second blood culture (isolate HCSC34764) at 5 days of treatment; AB 3rd BC: ***A. baumannii*** isolated in the third blood culture (isolate HCSC36777) at 10 days of treatment.

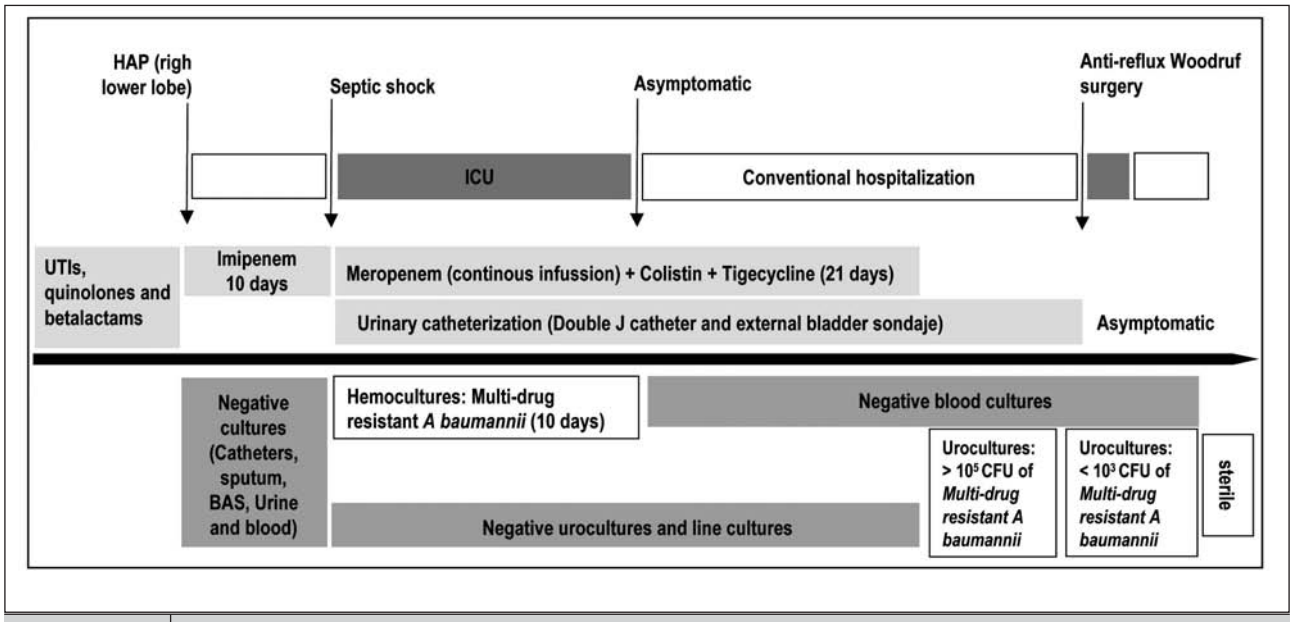

Figure 1 **Clinical and microbiological features during study period**

baumannii. Bacteremia caused by *A. baumannii* originates in the respiratory or urinary tract in 70% of cases. The predisposing factors are previous colonization by *A. baumannii*, previous exposure to antimicrobial drugs, and recent invasive procedures (eg, tracheostomy, central venous catheterization, urinary catheterization), and prognosis is worse in immunosuppressed patients. The overall mortality at 30 days is about 49%[2].

We describe a renal transplant recipient who developed bacteremia caused by multiresistant *A. baumannii* (penicillins, quinolones, rifampicin, cotrimoxazole, aminoglycosides, and carbapenems) and received a combination of tigecycline, colistin, and meropenem in continuous infusion. The clinical outcome was favorable.

CASE DESCRIPTION

The patient was a 71-year-old woman with chronic renal impairment secondary to AL amyloidosis. She had undergone a renal transplant 13 years before and suffered from suprarenal insufficiency that was being treated with fludrocortisone. The patient was also receiving tacrolimus (range 5-10 ng/ml), mycophenolate mofetil (range 2-3 ng/ml), and prednisone (5 mg/day) to prevent rejection, with good controls and corrected creatinine clearance of 56.35 ml/min. A recent urology workup showed grade II active vesicoureteral reflux in the transplanted kidney and a grade III cystocele, with residual urine and incontinence. She had been admitted in the last two months several times due to urinary tract infection and had received different antibiotic regimens, which included amoxicillin-clavulanate, ciprofloxacin, fosfomicin and third generation cephalosporins. She consulted with a 1-week history of fever, dyspnea, and nonproductive cough, with crackles in the right lung and an alveolar infiltrate in the right lower

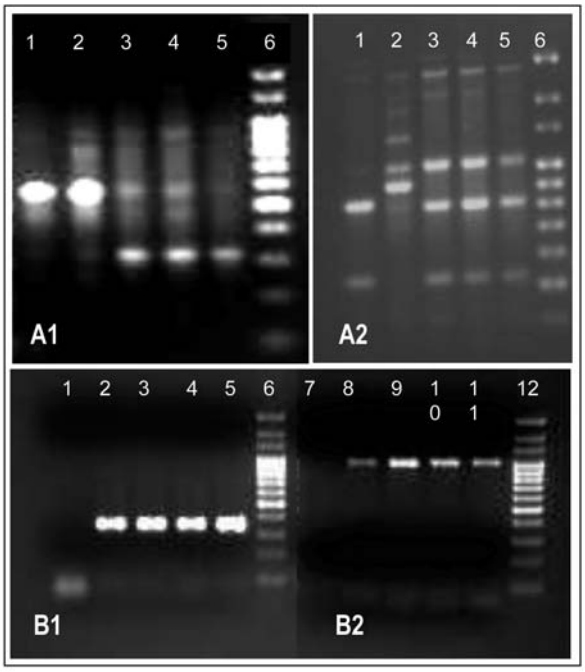

Figure 2 — **DNA fingerprinting obtained for *A. baumannii* isolates on agarose gels following RAPD-PCR with ERIC-2 primer (A1) and M13 primer (A2). Pannel B: PCRs for detection of β-lactamase genes. B1: oxa-51-like gene. B2 blaOXA-40-like gene. Lane 6, molecular-weight marker 2Kb; lanes 1 and 7 (negative) and 2 and 8 (positive), control strains; lane 3 and 9, isolate HCSC36777; lane 4 and 10, isolate HCSC34764; and lane 5 and 11, isolate HCSC33590.**

lobe in the chest radiograph. The suspicion of healthcare-associated pneumonia led physicians to start imipenem at 1 g every 8 hours. The clinical and radiographic outcome was good. All cultures were negative. Seven days after starting treatment, the patient presented dysuria, chills, a temperature of 39ºC, hypotension and a laboratory workup revealed 20,000 leukocytes per cubic milliliter. The suspicion of urinary sepsis led physicians to request blood cultures, the patient was transferred to the ICU, and hemodynamic support measures (hydration, double J catheter, urinary catheter, and linezolid to broaden the spectrum against resistant grampositive agents) were initiated. At 48 hours, the microbiology department reported that multiresistant *A. baumannii* had been isolated in blood cultures (isolate HCSC33590) only susceptible to colistin (MIC to sulbactam < 32 mg/L, to meropenem 32 mg/L, to tigecycline of 4 mg/L and to amikacin > 64 mg/L). A 21-day triple regimen was started. This was composed of tigecycline at a 100-mg loading dose and 50 mg/12h, colistin (MIC 0,094 mg/L) at 1 million IU every 12 hours, and meropenem at dose of 1 gram three times a day in continuous infusion during 8 hours, to warrantee the drug molecular stability (doses of colistin and meropenem were adjusted with renal clearance). The patient remained bacteremic for 10 days. Blood cultures obtained on days 5 and 10 were positives, but did not turn positives afterwards. Neither of the 2 echocardiograms recorded during the bacteremic episode showed signs of endocarditis. Ten days after finished treatment, the patient underwent surgery for reimplantation of the transplanted ureter using the Woodruff antireflux technique. The double J catheter and the urinary catheter were maintained for a further 15 days. During this period, and after the foreign devices were withdrawn, the patient had 2 positive urine cultures with isolation of *A. baumannii* (20,000 and 10,000 CFU) with the same susceptibility pattern that those obtained in blood cultures. She progressed favorably until symptoms disappeared, and was discharged 60 days after admission. The urine cultures remained negative at subsequent checkups (figure 1).

MATERIAL AND METHODS

Blood cultures were processed by the clinical microbiology laboratory using the BACTEC 9240 system (Becton Dickinson and Company, Franklin Lakes, New Jersey, USA). *A. baumannii* complex was identified by both standard microbiological techniques and the Vitek system (bioMérieux, Marcy l'Étoile, France). Antimicrobial

susceptibility testing of *A. baumannii* isolates was determined by the Vitek commercial microdilution technique and Clinical Laboratory Standards Institute (CLSI) breakpoints were applied to define susceptibility and resistance. Susceptibility testing of the 3 isolates to tigecycline, colistin, and meropenem was performed using the Etest method (AB Biodisk, Solna, Sweden). Interpretation of tigecycline susceptibility against *A. baumannii* requires Mueller Hinton agar with no cationic supplements (manganese). MIC readings of the tigecycline E-test strips in Mueller Hinton agar with manganese were higher, and close to or reaching the susceptibility breakpoint (table 1)[3]. When we used Mueller Hinton agar poorly supplemented in manganese, all strains were susceptible to tigecycline with low MIC ranges. Because there are no standardized CLSI breakpoints for tigecycline susceptibility, provisional MIC breakpoints were used. Some authors consider an MIC <2 mg/L or below as susceptible, MIC 2-8 mg/L as intermediately resistant, and MIC > 8 mg/L as resistant, as suggested by pharmacokinetic and pharmacodynamic data in various in vitro studies[1,4-5]. We and other authors considered MIC ≤ 2 mg/L as susceptible, MIC 4 mg/L as intermediately resistant, and MIC ≥ 8 mg/L as resistant[6].

In order to verify whether the 3 *A. baumannii* isolates obtained from the patient during admission were the same strain, we analyzed the clonal relationship between them by RAPD-PCR typing with both M13 and ERIC-2 primers. The analysis showed that the 3 isolates were of the same strain and different from 2 control strains of our collection (figure 2A). The isolates were also analyzed to determine the presence of betalactamases associated with resistance to carbapenems. PCR for carbapenemase genes was performed for the following genes: *bla*-$_{IMP}$, *bla*-$_{VIM}$, *bla*$_{OXA23}$-like, *bla*$_{OXA40}$-like, *bla*$_{OXA58}$-like and *bla*$_{OXA51}$-like. PCR screenings were carried out in a final volume of 50 µL. Primers and conditions were those described elsewhere[7]. The sequence analyses were accomplished after PCR products were purified using the UltraClean PCR Clean-up kit (Mobio Laboratories). Sequencing was performed on both strands with the ABI Prism BigDye Terminator kit (Applied Biosystems, Foster City, California, USA). The assay was carried out according to a standard protocol. Similarity searches and alignments for the nucleotide sequences and predicted protein sequences were performed with the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST). As expected, *bla*$_{OXA51}$-like genes were found in all isolates (figure 2B). PCR with OXA-23 and OXA-24 primers amplified fragments of expected size. Nevertheless, the sequence of these fragments revealed that amplicon obtained with OXA-23 primers shows 97% homology with a putative TonB-dependent receptor protein instead with an oxacillinase gene. Partial sequencing of the OXA-40 fragment indicated the presence of *bla*$_{OXA72}$ (figure 2B). No other positive results were detected by PCR. An inhibitor-potentiated disk diffusion methods (IPD) using imipenem-EDTA were carried out for screening of metallo-betalactamase production, and no metallo-betalactamases were detected, using both (IPD and PCR) techniques.

DISCUSSION

Infection by *A. baumannii* is probably one of the most typical examples of multiresistant nosocomial infection. It is related to previous consumption of antimicrobials (especially carbapenems and third-generation cephalosporins), stay in the ICU, and invasive procedures (eg, tracheostomy, vascular and urinary catheterization). This agent is frequently responsible of pneumonia, bacteremia, surgical wound infections, and urinary tract infections. It is a predictor of extended mean stay, the need for intubation, and mortality, especially in pneumonia and bacteremia, with an overall 30-day mortality about 49%[2]. Multiresistance among *A. baumannii* is high, with the result that carbapenems are often the treatment of choice. However, resistance to this class of betalactams has been increasingly reported throughout the world during the last decade. The most common carbapenemases in *A. baumannii* are those belonging to class D. A multiresistant epidemic clone of *A. baumannii* carrying the *bla*-$_{OXA-40}$-like gene has been reported to be disseminated in Portugal and Spain[8].

Treatment of an infection caused by a multiresistant organism requires a combination of microbiologically active antibiotics administered in such a way as to increase pharmacodynamic yield by acting on the focus and its reservoirs, according to the model of infection. Tigecycline is active against *A. baumannii* with a high volume of distribution (approximately 6.8 L/kg at 100 mg and 13 L/kg at 200 mg), a long half-life, and a postantibiotic effect[9]. The serum peak of the drug at the marketed dose (50 mg tid after a loading dose of 100 mg) is approximately 0.63 ± 0.28 mg/L, which is below the MIC of most *A. baumannii* isolates in vitro (50% of isolates published have an MIC ≥ 1 mg/L and 32% an MIC ≥ 2 mg/L). However, it has an AUC/MIC ratio that predicts efficacy. There have recently been 2 reports of cases of isolation of *A. baumannii* in the blood of patients receiving tigecycline at standard doses[9]. Although the drug has not been approved in bloodstream infections, microbiological and pharmacodynamic yield (AUC/MIC) would probably be greater in this infection model by doubling the marketed dose (100 mg tid), even for a bacteriostatic drug.

Like in any other organ, tigecycline has good tisular diffusion to kidney, due to it high distribution volume. However, the urinary tract concentration of this drug is low. The major route of elimination, after hepatic glucuronidation, was feces (60%), but secondary elimination pathway of this drug is renal excretion of unchanged N-acetyl-9-aminominocycline glucuronided conjugates (about 30%). This circumstance reduces the use of tigecycline in urosepsis, although the urinary concentrations of this drug should be enough for treating and uncomplicated urinary tract infection. In our patient, tigecycline was chosen for

reasons of sensitivity; although it could be useful against the hypothetic reservoir generated by *A. baumannii* in the double J catheter (this had to remain in place for more than 20 days and may have been responsible for the positive *A. baumannii* counts in urine). Furthermore, it proved to be non-nephrotoxic in a patient who had undergone renal transplant.

Polymyxins are polypeptides with a low volume of distribution (favorable against bacteremia) and known activity against *A. baumannii*. Out of necessity, colistin has recently been reinstated. It has proven to be effective even in critical situations, but shows nephrotoxicity, especially with previous renal disease. However, heteroresistance phenomena[10] have been observed, and these could perhaps be minimized by modifying the posology. In pharmacodynamic models against *A. baumannii* in vitro, dose-lethality curves showed that colistin at 3, 6, 12, or 24 mg/L reduced bacterial load by about 3 log CFU/mL after 2-3 hours; however, growth began to increase again after the third hour approximately. The addition of a second dose of colistin at 12 hours was unable to reduce the bacterial load[11]. In fact, Tan et al[12] observed greater logarithmic decreases in vitro in regimens that spaced the dose of colistin, and reported the vulnerability of this exposure during the 12-24 hours before the emergence of resistance among the heteroresistant *A. baumannii* isolates. They also recommended avoiding monotherapy, especially in immunodepressed patients. However, they administered colistin with ceftazidime (intrinsically resistant to *A. baumannii*, MIC ≥ 64 mg/L) in continuous infusion, and again there was a logarithmic decrease of 3 log CFU/mL in 3 hours, although regrowth was delayed or did not occur[13]. A similar effect has recently been reported[14]; dose-lethality curves showed that regrowth was prevented by combining tigecycline with colistin in a multiresistant strain of *K. pneumoniae* VIM-1, SHV-12. Our isolate was sensitive to colistin (MIC 0.094 mg/L) and was administered at 1 million units every 12 hours looking for minimize toxicity in a renal transplant recipient and to avoid this period of vulnerability. Nevertheless, a slight increase in the MIC to colistin was observed in the isolate from the third blood culture after 10 days of treatment (table 1).

Our patient's regimen was extended by the addition of meropenem at a loading dose of 500 mg followed by a continuous infusion of 125 mg/h (3 g/d), in order to maintain serum levels at a minimum of 10 mg/L for 24 hours, despite the fact that our isolate of *A. baumannii* was resistant (MIC > 16 mg/L). The usefulness of imipenem in experimental models of infection caused by *A. baumannii*, even against strains resistant to carbapenems[15] was the first reason for including meropenem. We also intended to reproduce the pharmacodynamic conditions favorable to colistin reported by Kroeger et al.[11].

In conclusion, infection by carbapenem-resistant *A. baumannii* is increasingly common in our setting. The dissemination of multiresistant clones carrying class D betalactamases has been detected throughout the world. In Spain, Ruiz et al.[8] have studied the prevalence of *A. baumannii* that produces the OXA-40-like gene (similar to that found in our study). This makes treatment difficult and obliges us to search for alternative solutions, especially when treating immunocompromised patients. The combination of tigecycline and colistin could be microbiologically and pharmacodynamically useful in the treatment of bacteremic infections caused by *A. baumannii*. The sensitivity of *A. baumannii* to tigecycline should be confirmed in agars without manganese suplementation.

LIMITATIONS

The impossibility of carrying out in our institution the chromatographic determination of drugs serum levels and the fact that most of the sources used were animal models or in vitro studies mean that some aspects of the present study remain hypothetical.

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