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Simultaneous quantification of antimicrobial agents for multidrug-resistant bacterial infections in human plasma by ultra-high-pressure liquid chromatography–tandem mass spectrometry

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ABSTRACT

Antibiotic-resistant bacterial infection is one of the most serious clinical problems worldwide. Vancomycin, teicoplanin, daptomycin, and colistin are glycopeptide and lipopeptide antibiotics that are frequently used to treat multidrug-resistant bacterial infections. Therapeutic drug monitoring is recommended to ensure both safety and efficacy and to improve clinical outcomes. This study developed a fast, simple, and sensitive ultra-high-pressure liquid chromatography–tandem mass spectrometry (UHPLC–MS/MS) method for the simultaneous determination of the concentrations of these four drugs in human plasma. The sample preparation process includes a simple protein denaturation step using acetonitrile, followed by an 11-fold dilution with 0.1% formic acid. Eight target peaks for the four drugs can be analyzed within 3 min using a Kinetex™ 2.6 μm C18 column. The mass spectrometry parameters were optimized, and two transitions for each target peak were used for multiple reaction monitoring, which provided high sensitivity and specificity. The UHPLC–MS/MS method was validated over clinical concentration ranges. The intra-day and inter-day precisions for the ratio of the peak area of each analyte to the peak area of the internal standard were all below 12.7 and 14.7% relative standard deviations, respectively. The accuracy at low, medium, and high concentrations of the eight target peaks was between 89.3 and 110.7%. The standard curves for the analytes were linear and had coefficients of determination higher than 0.997. The limits of detection were all below 70 ng mL^{-1}. The use of this method to analyze patient plasma samples confirmed that it is effective for the therapeutic drug monitoring of these four drugs and can be used to improve the therapeutic efficacy and safety of treatment with antibiotics.

1. Introduction

Drug-resistant bacterial infection is one of the most serious clinical problems worldwide. Several multidrug-resistant (MDR) microorganisms, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.*, have recently been referred to by the term "ESKAPE" because of their escape from antibiotics [1]. Of these MDR microorganisms, the prevalent Gram-positive bacterium methicillin-resistant *S. aureus* (MRSA) is associated with complicated skin-structure infections, hospital-acquired infections, and ventilator-associated pneumonia. In 2005, an estimate of 18,650 in-hospital deaths were due to invasive MRSA infections in the United States [2]. The emergence of MRSA is one of the most important aspects of nosocomial infections worldwide in the last two decades. The prevalence of hospital-acquired MRSA (HA-MRSA) is greater than 50% in North America, South America, Asia and Malta [3]. In additional to Gram-positive MDR bacterial infections, outbreaks of Gram-negative MDR bacterial infections including *A. baumannii* (MDRAB) and MDR *P. aeruginosa* (MDRPA) have been reported in Europe, North America, Argentina, Brazil, China, Taiwan, Hong Kong, Japan, and Korea in recent years [4,5]. It has been reported that MDRAB and MDRPA infections significantly increase treatment complexity and the duration of hospital stays for patients. Studies have demonstrated that ensuring the effectiveness of antibiotic treatment is of prime importance for the control of MDR infections. Glycopeptide antibiotics such as vancomycin and

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teicoplanin (six sub-components), lipopeptide antibiotic daptomycin, and oxazolidinone antibiotic linzolid are now widely used to treat MRSA. In contrast to the many antibiotics available to treat Gram-positive bacterial infections, there are very few effective antibiotics for treating Gram-negative MDR strains. Colistin, an old lipopeptide antibiotic with known nephrotoxicity and neurotoxicity, was withdrawn in the 1970s but is now being used to treat Gram-negative MDR strains. [6].

Various factors such as drug absorption, liver and kidney deficiency, obesity, and critical illness affect pharmacokinetic (PK) performance and result in different drug concentrations in patients' plasma. Among the antimicrobial agents for multidrug-resistant bacterial infections, vancomycin, teicoplanin, daptomycin and colistin are renally excreted, and therapeutic drug monitoring (TDM) is recommended for patients being treated with the aforementioned four drugs to ensure pharmaceutical efficacy and prevent toxicity, especially for patients with renal and critical illnesses. In contrast to these four antibiotics, no dose adjustment is recommended for patients with renal insufficiency when receiving linezolid due to MDR bacterial infections [7]. It has been reported that the trough concentrations of vancomycin and teicoplanin should be greater than 10–20 mg L^{-1} to ensure their efficacy [8,9]. A daptomycin concentration above 0.5 μg mL^{-1} should be maintained to inhibit 90% of several staphylococcal strains (MIC_{90}) [10], and effective peak plasma concentration of daptomycin was suggested to be higher than 60 times of the MIC [11]. Steady state plasma concentration of colistin was suggested to be between 1 and 5 μg mL^{-1} [12]. Because of the limited pharmacokinetic/pharmacodynamic data available for colistin and daptomycin, both of the therapeutic index and treatment regimen are still being seriously discussed. Severe adverse effects, including nephrotoxicity, ototoxicity, and hypotension, have been reported for vancomycin. Studies indicate that higher incidences of adverse effects occur when the plasma vancomycin concentration is above 80–100 mg L^{-1} [13,14]. Teicoplanin is less toxic than vancomycin. However, patients have reported experiencing adverse reactions such as ototoxicity and nephrotoxicity when using teicoplanin [15]. Both myopathy and eosinophilic pneumonia have been observed in

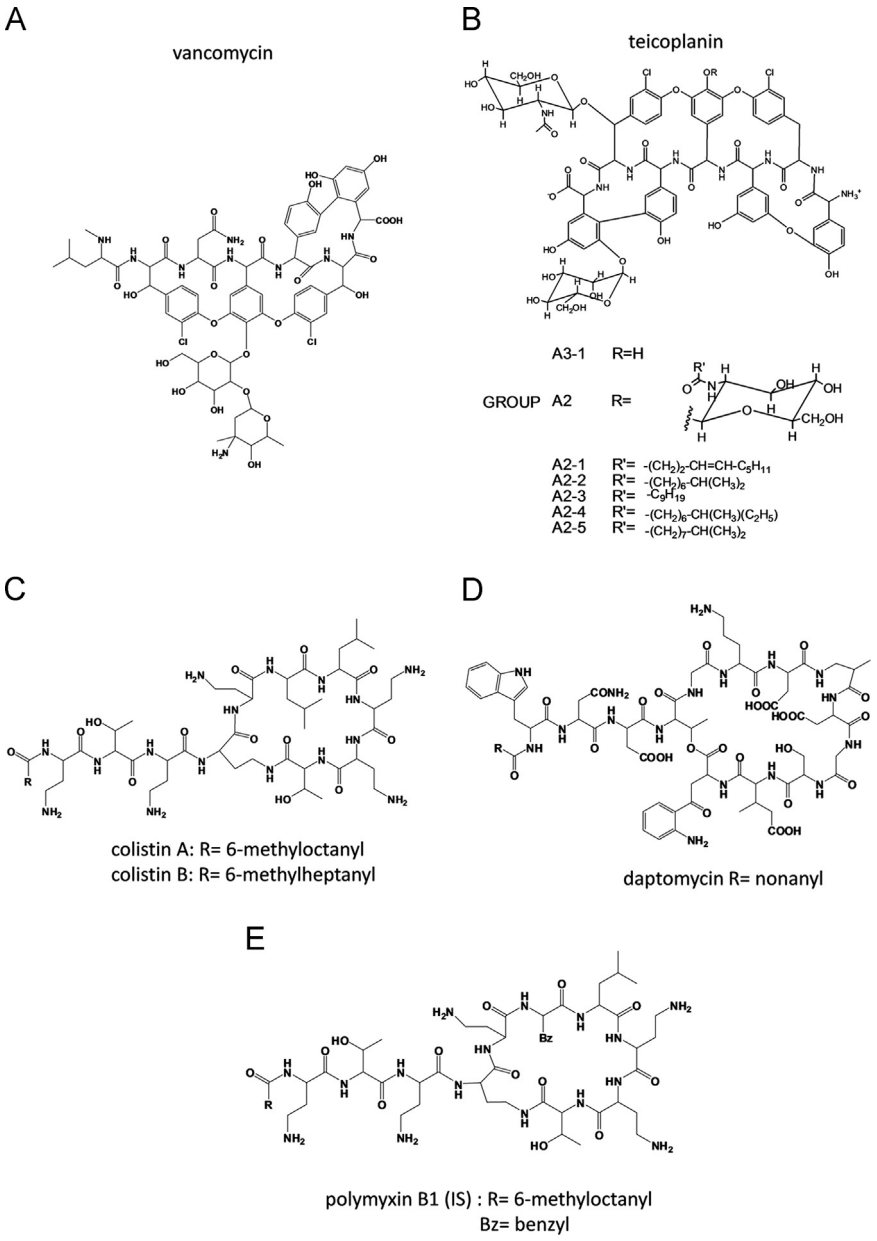

Fig. 1. Structures of (A) vancomycin, (B) the six sub-components of teicoplanin, (C) colistin A and colistin B, (D) daptomycin, and (E) IS: polymyxin B1.