Evaluation of the Antibacterial Activity of Piperacillin–tazobactam Against Burkholderia Pseudomallei in Vitro

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Research note

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Abstract

Objective: Melioidosis is a zoonotic disease caused by *Burkholderia pseudomallei* (*B. pseudomallei*). Because *B. pseudomallei* is naturally resistant to a variety of antibiotics, such as penicillin, ampicillin, first-and second-generation cephalosporins, macrolides, aminoglycosides, streptomycin, polymyxin, etc., the selection of drugs is limited. The present study aimed to evaluate the antibacterial activity of piperacillin/tazobactam against *B. pseudomallei* in vitro to provide a theoretical basis and a variety of therapeutic options for clinical application.The antimicrobial susceptibilities of all *B. pseudomallei* isolated from melioidosis patients were detected by Etest method.

Results: The MIC_{50} values of piperacillin/tazobactam, imipenem, meropenem, doxycycline, trimethoprim–sulfamethoxazole, ceftazidime, tetracycline, and amoxicillin/clavulanic acid were 0.25 μg/ml, 0.5 μg/ml, 0.5 μg/ml, 0.5 μg/ml, 1 μg/ml, 1 μg/ml, 2 μg/ml, and 2 μg/ml, respectively. Their MIC_{90} values were 0.5 μg/ml, 0.5 μg/ml, 1 μg/ml, 2 μg/ml, 2 μg/ml, 2 μg/ml, 4 μg/ml, and 4 μg/ml, respectively. All the isolates were uniformly sensitive (100%) to ceftazidime, imipenem and amoxicillin–clavulanic acid. The rate of antimicrobial sensitivity for doxycycline was 99.08%, for tetracycline-sulfamethoxazole 94.50%, and for tetracycline 94.49%.Both the MIC_{50} and MIC_{90} values of piperacillin/tazobactam were lower than those of the seven other antibiotics, indicating that it has strong antibacterial activity against *B. pseudomallei* in vitro.

Introduction

Melioidosis is a zoonotic disease caused by *Burkholderia pseudomallei* (*B. pseudomallei*). The disease is mainly epidemic in tropical and subtropical areas between latitudes of 20 degrees north and south, such as Southeast Asia and Northern Australia[1-3]. Hainan Island is the largest tropical island in southern China and is close to Thailand, Vietnam, Laos and other countries with a high incidence of melioidosis.The high temperature and humid tropical monsoon climate make Hainan Island become a major epidemic focus of *B. pseudomallei*. The clinical manifestations of melioidosis are complex and changeable. It can present acutely with fever and chills secondary to a localized infection or septicaemia. Chronic cases are seen when abscesses develop insidiously at various tissue sites or organs. Consistent with international guidelines[4-5], melioidosis is treated in Hainan with intravenous ceftazidime or meropenem for a minimum 2-week intensive phase, with longer intensive therapy for critically ill patients, followed by a minimum 3-month eradication phase with oral antibiotics (trimethoprim–sulfamethoxazole is the preferred agent, and amoxicillin/clavulanic acid or doxycycline is the second choice). However, recently, there have been reports of *B. pseudomallei* resistant to ceftazidime, trimethoprim–sulfamethoxazole (TMP–SMX), and amoxicillin–clavulanic acid from different melioidosis endemic countries of the world, including neighbouring India[1,6-10]. Piperacillin/tazobactam has a broad antibacterial spectrum, strong tissue permeability and high-concentration distribution in body fluid and blood after injection. However, it is rarely used in the clinic because of the lack of a recommendation of an MIC breakpoint from the American for Clinical and Laboratory Standards Institute (CLSI) for *B. pseudomallei*. In this study, we reviewed the treatment of 109 hospitalized patients with melioidosis and found that 2 patients were cured successfully with piperacillin/tazobactam. This observation aroused our interest in the activity of piperacillin/tazobactam against *B. pseudomallei* in vitro. With this information, the present study was undertaken to compare the MIC_{50} and MIC_{90} values of piperacillin/tazobactam, meropenem(with an EUCAST MIC breakpoint) and imipenem, doxycycline, amoxicillin/clavulanic acid, tetracycline, TMP–SMX, and ceftazidime (with CLSI-recommended MIC breakpoint)[11] for *B. pseudomallei* isolated from patients during the period of 2010–2018 in Hainan General Hospital by Etest to provide a theoretical basis for clinical application and provide additional choices for the treatment of melioidosis.

Materials And Methods

Bacterial isolates

A total of 109 *B. pseudomallei* isolated from melioidosis patients over a period of 8 years (June 2010 to May 2018) were collected in the Department of Microbiology of a 3000-bed tertiary care referral hospital in Haikou city (Hainan Province, China). *B. pseudomallei* isolates were presumptively identified by characteristic growth, Gram staining, oxidase test, and biochemical speciation (Vitek 2 Compact VT2.R 7.01; bioMérieux,France). Isolate identification was confirmed by Vitek MS (bioMérieux, France), and isolates were typed by multilocus sequence typing (MLST)[12]. The specimens included 70 blood cultures, 8 respiratory specimens, 5 urine samples and 26 purulent secretions.

Determination of MIC

Antimicrobial susceptibility testing was performed by Etest (bioMérieux) according to the manufacturer's instructions. This method of testing has previously been shown to be comparable with the gold standard of microbroth dilution[13-14]. Minimum inhibitory concentrations (MICs) were read after 18 h and 24 h of incubation at 37 °C under aerobic conditions. For TMP/SMX, the MIC was read at 80% inhibition as per the manufacturer's instructions. The MIC (μg/ml) interpretations for susceptible (S),

intermediate (I), and resistant (R) to imipenem, doxycycline, amoxicillin/clavulanic acid, tetracycline, TMP/SMX, and ceftazidime were carried out following the CLSI published guideline M45-A2 [11]. The MIC interpretation for meropenem was carried out following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guideline. The MIC_{50} and MIC_{90} of all antibiotics were calculated. The quality control strains were *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC27853). All the quality control results were in accordance with the CLSI.

Results

The results show that the mainly MIC values of imipenem, meropenem, ceftazidime, amoxicillin/clavulanic acid and tetracycline were 0.25-0.5 μg/ml, 0.5-1.0 μg/ml, 1.0-2.0 μg/ml, 2.0-4.0 μg/ml and 1.0-4.0 μg/ml, respectively. The mainly MIC values of piperacillin/tazobactam, doxycycline and TMP/SMX were 0.25-0.5 μg/ml, 0.25-1.0 μg/ml, and 0.5-2.0 μg/ml, respectively. The detailed MIC interpretive values used in the study are shown in Table 1.

Table 1

The concentration distribution and accumulation rate of eight antimicrobial agents against 109 *Burkholderia pseudomallei* isolates from Haikou,Hainan Province,China.

Drug concentration (μg/ml)	Meropenem		Amox–Clav		Pip–Tazo		TMP–SMX		Imipenem		Doxycycline		Ceftazidime	
	N	Cumulative rate (%)	N	Cumulative rate (%)	N	Cumulative rate (%)	N	Cumulative rate (%)	N	Cumulative rate (%)	N	Cumulative rate (%)	N	Cumu rate (%
0.008	0	0.0	—	—	—	—	0	0.0	0	0.0	—	—	—	—
0.016	0	0.0	—	—	—	—	0	0.0	0	0.0	—	—	—	—
0.032	0	0.0	—	—	—	—	0	0.0	0	0.0	—	—	—	—
0.064	0	0.0	0	0.0	0	0.0	1	1.0	0	0.0	0	0.0	0	0.0
0.125	0	0.0	0	0.0	1	1.0	4	4.6	0	0.0	0	0.0	0	0.0
0.25	1	1.0	0	0.0	89	82.6	12	15.6	20	18.3	18	16.5	0	0.0
0.5	71	66.1	1	1.0	17	98.2	29	42.2	86	97.2	44	56.9	2	1.8
1	33	96.3	1	1.8	2	100.0	42	80.7	2	100.0	35	89.0	71	67.0
2	1	97.2	77	72.5	0	100.0	16	95.4	0	100.0	7	95.4	35	99.1
4	0	97.2	29	99.9	0	100.0	5	100.0	0	100.0	4	99.1	1	100.0
8	3	100.0	1	100.0	0	100.0	0	100.0	0	100.0	0	99.1	0	100.0
16	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	1	100.0	0	100.0
32	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0	0	100.0
64	—	—	0	100.0	0	100.0	—	—	—	—	0	100.0	0	100.0
128	—	—	0	100.0	0	100.0	—	—	—	—	0	100.0	0	100.0
256	—	—	0	100.0	0	100.0	—	—	—	—	0	100.0	0	100.0

N: Number of strains, *Amox*–Clav: amoxicillin/clavulanic acid, *Pip*–*Tazo*: piperacillin–tazobactam, *TMP*–*SMX*: trimethoprim/sulfamethoxazole

The sensitivity of the 109 strains of *B. pseudomallei* to the eight antibiotics was as follows. Piperacillin/tazobactam had no MIC breakpoint in the CLSI guidelines. All the isolates were uniformly sensitive (100%) to ceftazidime, imipenem, and amoxicillin–clavulanic acid. Of the 109 isolates, 103 (94.49%) were susceptible to tetracycline at the MIC breakpoint of ≤4.0 μg/ml; 5 isolates had intermediate resistance to tetracycline (MIC=8 μg/ml); and 1 isolate was resistant (MIC ≥ 16.0 μg/ml). Moreover, 108 isolates (99.08%) were susceptible to doxycycline at the MIC breakpoint of ≤4 μg/ml, and 1 isolate was resistant (MIC≥16.0 μg/ml), whereas none had intermediate breakpoint. Finally, 104 (94.5%) were susceptible to TMP/SMX at the MIC breakpoint of ≤2/38 μg/ml, and 6 isolates was resistant (MIC≥4.0/76 μg/ml). There was no intermediate breakpoint according to the CLSI guidelines for TMP/SMX. In addition,isolates with an MIC of >2 μg/ml for meropenem were considered resistant, and isolates with an MIC≤2 μg/ml were susceptible based on the EUCAST guidelines. Therefore, 106 isolates (97.24%) were susceptible, and 3 isolates (2.76%) were resistant to meropenem. The MICs of antibiotics that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of the 109 isolates are shown in Table 2.

Table 2

Minimum inhibitory concentrations (MICs) of eight antimicrobial agents against 109 *Burkholderia pseudomallei* isolates from Haikou, Hainan Province, China.

Antimicrobial agent	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Imipenem	0.5	0.5
Meropenem	0.5	1
Ceftazidime	1	2
Amoxicillin/clavulanic acid	2	4
Tetracycline	2	4
Doxycycline	0.5	2
TMP/SMX	1	2
Piperacillin–tazobactam	0.25	0.5

All the results were analysed, and the order of the MIC_{50} for the eight antimicrobial agents was piperacillin/tazobactam MIC_{50} (0.25 μg/ml) < imipenem MIC_{50} (0.5 μg/ml), meropenem MIC_{50} (0.5 μg/ml), doxycycline MIC_{50} (0.5 μg/ml) <TMP/SMX MIC_{50} (1 μg/ml), ceftazidime MIC_{50} (1 μg/ml) < tetracycline MIC_{50} (2 μg/ml), amoxicillin/clavulanic acid MIC_{50} (2 μg/ml). In addition, the MIC_{90} from low to high was piperacillin/tazobactam (0.5 μg/ml), imipenem (0.5 μg/ml), meropenem (1 μg/ml), ceftazidime (2 μg/ml), doxycycline (2 μg/ml), TMP/SMX (2 μg/ml), amoxicillin/clavulanic acid (4 μg/ml), tetracycline (4 μg/ml) and so on.

In addition, based on the peak blood concentration (Cmax) of China and Italy dosage forms of piperacillin/tazobactam commonly used in the clinic, the ratios of C_{max} to MIC_{50} and MIC_{90} are shown in Table 3.

Table 3

Piperacillin/tazobactam dosage forms and the ratios of peak blood concentration (Cmax) to MIC_{50} and MIC_{90} are listed

Dosage form (China)	1.25 g	2.5 g	5.0 g	Dosage form(Italy)	2.25 g	3.375 g	4.5 g
C_{max} (μg/ml)	42.7	80.3	192.5	C_{max} (μg/ml)	134	242	298
C_{max}/MIC_{50}	170.8	321.2	770	C_{max}/MIC_{50}	536	968	1192
C_{max}/MIC_{90}	85.4	160.5	385	C_{max}/MIC_{90}	268	484	596
Dosage form, C_{max} from piperacillin/tazobactam instructions							

Discussion

Melioidosis is an important public health disease in Southeast Asia and Australia, but it is still considered a potential emerging infectious disease in tropical developing countries[2,13-18]. A 2016 modelling study estimated that there are ~165,000 cases of melioidosis in humans per year worldwide, of which 89,000 (54%) are estimated to be fatal[19]. Although quinolones have slight antibacterial activity in vitro, their treatment failure rate is relatively high[8,20]. The mortality of melioidosis ranges from 14 to 40% and may be as high as 80% if effective antibiotics are not given[21]. With the early use of antibiotics, the mortality rate can also be reduced to under 10%[22]. However, *B. pseudomallei* is intrinsically resistant to many antibiotics (such as penicillin, ampicillin, first- and second-generation cephalosporins, macrolides, aminoglycosides, streptomycin, polymyxin, etc.)[23-24]. At present, only TMP/SMX, ceftazidime, tetracycline, doxycycline, amoxicillin/clavulanic acid and imipenem have MIC breakpoints for *B. pseudomallei* in the CLSI guidelines[11]. Therefore, the selection of antimicrobial agents is limited for the treatment of melioidosis.

Piperacillin, as with all other β-lactam antibiotics, interferes with the final stage of peptidoglycan synthesis by inhibiting penicillin-binding proteins (PBPs), which crosslink peptidoglycan polymers[25]. Tazobactam extends piperacillin's spectrum of activity to include bacteria producing many Ambler class A β-lactamases,

narrow- and extended-spectrum (TEM-, SHV-, and CTX-M-type) β-lactamases and some class C (AmpC-type) β-lactamases[26-27]. Piperacillin-tazobactam has the broadest spectrum of activity among the penicillin class of β-lactam antibiotics and is generally active against most of the typical human pathogens, including aerobic and anaerobic gram-positive and gram-negative bacteria[25]. Piperacillin/tazobactam is widely distributed in various tissues and body fluids after injection, and its clinical indications are also relatively broad, such as respiratory tract infection, bacterial septicaemia, and subcutaneous abscess[25]. The clinical infection of the target bacteria we studied is also complex and diverse and is considered "like a hundred diseases". Piperacillin/tazobactam has a sensitive CLSI breakpoint for Enterobacteriaceae bacteria but no MIC breakpoints for non-fermentative bacteria except *Pseudomonas aeruginosa*, *Acinetobacter baumannii* and *Haemophilus influenzae*.

Therefore, we compared the MICs of piperacillin/tazobactam with those of six antibiotics(with a CLSI MIC breakpoints) and meropenem (with an EUCAST MIC breakpoint) to provide additional choices for the treatment of melioidosis in this study. The results showed that the MIC_{50} and MIC_{90} of piperacillin/tazobactam were 0.25 μg/ml and 0.5 μg/ml, respectively. The MIC_{50} of piperacillin/tazobactam was lower than that of the other seven antibiotics. In addition, the MIC_{90} was also lower than that of the other six antibiotics, except for imipenem (the MIC_{90} of piperacillin/tazobactam was equal to that of imipenem). This result indicates that the antibacterial activity of piperacillin/tazobactam is stronger than that of the other tested antibiotics in vitro. Regarding the pharmacokinetic characteristics in vivo (see Table 3), it was found that both the peak value of the plasma concentration of China and Italy formulations is far higher than the MIC_{50} and MIC_{90} values of piperacillin/tazobactam. It is generally believed that antibiotics have bacteriostatic or bactericidal effects in vivo when the blood concentration of antibiotics is 4-6 times higher than the MIC value of target bacteria. In the published literature, the criteria used in these studies to analyse the susceptibility to piperacillin/tazobactam and meropenem to *B. pseudomallei* were mostly based on the MIC breakpoint of *P. aeruginosa* in the CLSI guidelines[28]. In studies published in Bangladesh, Dutta et al. found that 20 isolates of *B. pseudomallei* were uniformly sensitive (100%) to piperacillin–tazobactam (refer to the MIC breakpoint of *P. aeruginosa* in the CLSI guidelines), and both the MIC_{50} and MIC_{90} values were 2 μg/ml[28]. These values are obviously higher than the results of our study. However, the number of samples in Dutta's study may be too few, and both the MIC_{50} and MIC_{90} values of all isolates to three common antibiotics (imipenem, ceftazidime, and amoxicillin/clavulanic acid) were relatively higher (great than or equal to 2 μg/ml, agar dilution method). This result indicated that the MIC values of the 20 isolates of *B. pseudomallei* in Dutta's study were relatively high. In view of the above information, this study still has certain reference significance. It can be used as the theoretical basis for the clinical treatment of *B. pseudomallei* infection with piperacillin–tazobactam.

In this study, we conducted a preliminary study on the MIC of piperacillin–

tazobactam for *B. pseudomallei* in vitro. We hope that this study can play a valuable role, arousing more attention to antibiotic selection for *B. pseudomallei* infection to improve the cure rate and reduce mortality. Therefore, the laboratory should work closely with the clinic to obtain a more clinical pharmacodynamic summary of piperacillin/tazobactam in the treatment of *B. pseudomallei* infection and provide more powerful evidence for its treatment.

Limitations

In this paper, we have not summarized the MIC breakpoint of piperacillin-tazobactam to *B. pseudomallei*, but still need a lot of data analysis and summary.

Abbreviations

MIC: minimum inhibitoryconcentration;TMP–SMX:trimethoprim–sulfamethoxazole; *Amox*–Clav: amoxicillin/clavulanic acid;*Pip*–*Tazo*: piperacillin–tazobactam; CLSI:Clinical and Laboratory Standards Institute;EUCAST:the European Committee on Antimicrobial Susceptibility Testing;MLST: multilocus sequence typing; N: Number of strains;PBPs:penicillin-binding proteins.

Declarations

Ethics approval and consent to participate

The Medical Ethics Committee of Hainan general hospital approved this study protocol.

Consent for publication

Not applicable.

Availability of data and materials

All relevant data of this study are within the paper.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Meihui Huang performed the culture, identifcation, literature search and manuscript writing. Hua Wu performed the antimicrobial susceptibility tests. Xiaojun Zhou was involved in storage and identifcation of organisms and primary data analysis. Qinfang Cao was involved in analyzed the clinical data.Xuming

Wang was involved in study design, analysis of the data and manuscript writing. All authors read and approved the fnal manuscript.

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