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Case Report

Characterization of carbapenem-resistant *Morganella morganii* isolates from hospitals in Ningbo city, Zhejiang, China

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Abstract: Objective: Antimicrobial resistance mechanisms and transmission mode of carbapenem-resistant *Morganella morganii* from three hospitals in Ningbo, China from 2011 to 2015 were explored. Methods: The minimum inhibitory concentrations (MICs) of nine common antimicrobial drugs were measured by agar dilution method for carbapenem-resistant *Morganella morganii* isolates whose carbapenemase production was confirmed by modified Hodge test. PCR and subsequent sequencing analysis were conducted to analyze the β-lactamase-encoding genes in these isolates. Plasmid conjugation test and plasmid extraction were carried out to assess the transmission ability of the drug resistance and its possible mechanisms. Enterobacterial repetitive intergenic consensuse PCR (ERIC-PCR) was undertaken to evaluate the clonal relatedness among the isolates. Results: Nine *Morganella morganii* isolates that were isolated largely from sputum and urine samples were confirmed to be carbapenem-resistant by modified Hodge test. Nine isolates were all resistant to imipenem and meropenem (MIC 8-32 μg/mL), highly resistant to cefotaxime, cefepime, piperacillin/tazobactam, levofloxacin (MIC 8-256 μg/mL), and were contrarily susceptible to amikacin, fosfomycin, and tigecycline. Six isolates possessed the dominant bla_{KPC-2} and bla_{TEM} genes. Genes bla_{IMP-1}, bla_{VIM-1}, $bla_{CTX-M-14}$, bla_{DHA-3}, bla_{CMY-4}, and bla_{SHV} were detected in one to three isolates. Carbapenem resistance in eight isolates was successfully transferred to the recipient bacterium *Escherichia coli* J53 by plasmid conjugation test. Nine isolates were divided into four clonal types (6 isolates in Type A, and one isolate in Type B, C, and D each) by ERIC-PCR typing. Conclusions: The present study demonstrated that the hospital-isolated *Morganella morganii* strains were highly antimicrobial-resistant and harbored complex relevant drug resistance genes. Such resistance was prone to easy spread among strains. The monitoring of drug-resistant *Morganella morganii* strains should be strengthened.

Keywords: *Morganella morganii,* carbapenem-resistant, enterobacterial repetitive intergenic consensuse PCR, β-lactamase-encoding genes

Introduction

Morganella morganii (*M. morganii*) is the sole species of Enterobacteriaceae morganella. As a kind of opportunistic pathogen, it can cause nosocomial infections in the urinary tract and postoperative wounds as well as severe infections such as sepsis, pneumonia, myocarditis, encephalitis, pericarditis, chorioamnionitis, spontaneous bacterial peritonitis, etc [1-3]. However, *M. morganii* has not been well studied in aspects of antimicrobial resistance mechanisms and transmission modes in recent years. We thus aimed to characterize nine carbapenem-resistant *M. morganii* isolates from three hospitals regarding the antimicrobial resistance mechanisms and transmission mode.

Material and method

Bacterial isolates

Nine carbapenem-resistant *M. morganii* isolates were recovered from three hospitals from January 2011 to December 2015. The isolates were composed of seven isolates from the First

Table 1. Primers and amplification products of five extended-spectrum β-lactamase genes and two AmpC enzyme genes [5]

Genes	Primers	Sequence (5' to 3')	Size of products (bp)
bla_{CTX-M1}	Forward	ACGCTGTTGTTAGGAAGTGTG	759
	Revese	TTGAGGCTGGGTGAAGTAAG	
bla_{CTX-M2}	Forward	ACGCTACCCCTGCTATTTAG	830
	Revese	CAGAAACCGTGGGTTACG	
bla_{CTX-M9}	Forward	AGTGCAACGGATGATGTTCG	792
	Revese	GGCTGGGTAAAATAGGTCAC	
bla_{TEM}	Forward	CATTCAAATATGTATCCGCTC	953
	Revese	TTACCAATGCTTAATCAGTG	
bla_{SHV}	Forward	ACGCCGGGTTATTCTTATTTGTCGC	1031
	Revese	ATTACCGACCGGCATCTTTCCG	
bla_{DHA}	Forward	AACTTTCACAGGTGTGCTGGGT	450
	Revese	CCGTACGCATACTGGCTTTGC	
$bla_{ACT/MIR}$	Forward	TCGGTAAAGCCGATGTTGCGG	303
	Revese	CTTCCACTGCGGCTGCCAGTT	

Hospital of Ningbo City (one isolate in 2011, two isolates in 2012, 2013 and 2015, respectively), one isolate from Lihuili Medical Group of Ningbo City (in 2014), and one isolate from the Second Hospital of Ningbo City (in 2014). The isolates were identified using VITEK 2 Compact microbiological identification system (bioMe ´rieux, Hazelwood, MO, USA).

Antimicrobial susceptibility testing and modified Hodge confirmatory test

The minimum inhibitory concentrations (MICs) of levofloxacin (LEV), ceftriaxone (CRO), cefepime (FEP) were determined by the E-test strips (OXOID, UK). MICs of amikacin (AK), fosfomycin (FOS), piperacillin/tazobactam (TZP), tigecycline (TIG), imipenem (IPM), and meropenem (MEM) were determined by the agar dilution method. The postulated carbapenemase-producing isolates were confirmed by the modified Hodge confirmatory test according to Clinical and Laboratory Standards Institute recommendations [4]. *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC700603 were used for quality control.

Polymerase chain reaction (PCR) amplification and DNA sequence analysis of common carbapenemase genes

Genomic DNA of *M. morganii* isolates was obtained with bacterial genomic DNA miniprep kit (Axygen Scientific, Union City, CA, USA) and used as the template for PCR assays. The primers used to amplify bla_{KPC}, bla_{NDM-1}, bla_{GES}, bla_{SME}, $bla_{IMI-1/NmcA}$ and bla_{SHV-38} have been previously described [5, 6]. PCR products were sequenced using an ABI 3730 sequencer (Applied Biosystems, Foster City, CA, USA), and the sequences were then compared with the reported sequences on GenBank (**Table 1**).

ERIC-PCR typing

Genomic DNA was used as template in enterobacterial repetitive intergenic consensuse PCR (ERIC PCR) analysis. The sequences of the primers and PCR conditions were used as described by Versalovic [7].

Conjugation experiment

Conjugation experiments were conducted with carbapenem-resistant *M. morganii* isolates as donor bacteria and sodium azide-resistant *Escherichia coli* J53 as the recipient bacteria. A single colony of each donor and recipient bacterium was used to prepare a saline suspension of 0.5 McFarland turbidity unit. Then, 500 uL suspension of each donor and recipient bacterium was concurrently inoculated into four mL of bouillon culture medium The donor and recipient bacteria inoculated culcture was incubated for 16-18 h. Subsequently, 0.2 mL of the culture was coated on MacConkey plate containing meropenem (0.5 μg/mL) and sodium azide (125 μg/mL). The plate was incubated for 16-18 h before presumptive zygosporic colonies were picked on the plate. The species identification and drug susceptibility test were subsequently conducted, and the PCR was used to detect the relevant resistance genes. The PCR products were sequenced (Sangon Biotech [Shanghai] Co., Ltd.) through the dideoxy chain termination to identify the zygote.

Results

Distribution of the isolates

Of nine carbapenem-resistant *M. morganii* isolates collected from three hospitals in this study, eight were from sputum and one from urine (**Table 2**).

Table 2. Clinical distribution of nine carbapenem-resistant *M. morganii* isolates

Isolate	Isolation date	Hospital	Department	Specimen	Patient's disease	Age
CRM01	2011-1-23	NBDY	Geriatric department	Sputum	Pulmonary infection	76
CRM02	2012-4-11	NBDY	ICU	Sputum	Chronic obstructive pulmonary disease	67
CRM03	2012-4-28	NBDY	ICU	Sputum	Pneumonia	68
CRM04	2013-6-12	NBDY	ICU	Sputum	Urine	55
CRM05	2013-9-25	NBDY	Urologic department	Urine	Chronic nephrosis	51
CRM06	2014-6-11	LHL	ICU	Sputum	Spontaneous subarachnoid hemorrhage	35
CRM07	2014-8-17	NBDE	ICU	Sputum	Cerebral infection	87
CRM08	2015-9-14	NBDY	Urologic department	Urine	Urinary tract infection	45
CRM09	2015-12-5	NBDY	ICU	Sputum	Bronchitis	72

Abbreviation: CRM, carbapenem-resistant *M. morganii*; NBDY, the First Hospital of Ningbo; LHL, Lihuili Medical Group of Ningbo; NBDE, the Second Hospital of Ningbo.

Table 3. MIC values of eight commonly used antimicrobial agents in nine carbapenem-resistant *M. morganii* isolates

Isolate	MIC (μg/mL)								
	IPM	MEM	CTX	FEP	TZP	LEV	AK	FOS	TIG
CRM01	>32	16	>256	64	>256	16	1	32	2
CRM02	>32	16	>256	64	>256	32	1	32	4
CRM03	>32	16	>256	64	>256	32	1	32	4
CRM04	>32	32	>256	64	>256	8	1	32	8
CRM05	>32	32	>256	64	>256	16	2	32	8
CRM06	16	8	>256	128	>256	16	8	16	16
CRM07	8	16	>256	128	>256	32	4	16	8
CRM08	>32	32	>256	64	>256	16	4	32	4
CRM09	16	8	>256	128	>256	16	4	8	4

Abbreviation: IMP, imipenem; MEM, Meropenem; CTX, cefotaxime; PEP, cefepime; TZP, piperacillin/tazobactam; LEV, levofloxacin; AK, amikacin; FOS, fosfomycin; TIG, Tigecycline.

MIC values of eight common antimicrobial agents

Nine isolates were all resistant to imipenem and meropenem (MIC 8-32 μg/mL), highly resistant to cefotaxime, cefepime, Tazocin, levofloxacin (MIC 8-256 μg/mL), and were contrarily susceptible to amikacin, fosfomycin, and tigecycline (**Table 3**).

Modified Hodge test

Carbapenemase-production was proved for eight isolates (No. 1-6 and 8-9) by Hodge test. Isolate No. 7 was negative for Hodge test.

Drug resistance genes

Genes encoding carbapenemases were detected in six of nine carbapenem-resistant *M. morganii* isolates. DNA sequencing and blasting with NCBI sequences proved that genes bla_{KPC-2} and bla_{TEM-1} (6/9 isolates, No. 1-5 and No. 8) were the top two most found. The numbers of isolates with bla_{VIM-1}, bla_{IMP-1}, bla_{SHV-6}, bla_{CMY-4}, bla_{DHA} and $bla_{CTX-M-14}$ varied between one and three (**Table 4**).

Plasmid conjugation test

Eight *M. morganii* isolates (No. 1-8) showed the transfer of drug resistance. Substantial increase in drug resistance was observed in the transconjugants after the conjugation (**Table 5**).

ERIC-PCR typing

ERIC-PCR results were interpreted according to Tenover standards. Nine *M. morganii* isolates were subtyped into four subtypes (designated as A through D). Type A included six isolates (No. 1 through 5 and No. 8), as well as Type B, C, and D each having one isolate (**Figure 1**).

Discussion

M. morganii as well as carbapenem-resistant strains is not commonly found clinically, but this pathogen is still one of the important bacteria causing hospital-acquired infections [8]. In the present study, seven isolates were isolated from sputum and urine of patients in ICU (**Table 2**). ERIC-PCR indicated that six isolates belonged to the same clonal lineage, which showed a clustering trend in this pathogen (**Figure 1**). This clustering trend suggested whether this pathogen was of the same transmission source or lacked clonal diversity, a phenomenon that required further investigation.

Table 4. Presence of drug resistance genes in nine carbapenem-resistant *Morganella morganii* isolates

Isolate	Drug resistance genes							
	bla_{KPC-2}	bla_{IMP-1}	bla_{VIM-1}	$bla_{CTX-M-14}$	bla_{TEM-1}	bla_{DHA-3}	bla_{CMY-4}	bla_{SHV-6}
CRM01	+	-	-	-	+	-	+	-
CRM02	+	-	-	-	+	-	-	-
CRM03	+	-	-	-	+	-	-	-
CRM04	+	-	-	-	+	-	-	-
CRM05	+	-	-	+	+	-	-	-
CRM06	-	+	-	-	-	-	-	+
CRM07	-	-	-	-	-	+	-	+
CRM08	+	-	-	-	+	-	+	-
CRM09	-	-	+	+	-	-	-	+

Table 5. MIC values of eight commonly used antimicrobial agents after plasmid transfer conjugation of nine *M. morganii* isolates

Transconjugants	MIC (μg/mL)								
	IPM	MEM	CTX	FEP	TZP	LEV	AK	FOS	TIG
CRM01-c	>32	16	>256	64	>256	16	1	32	2
CRM02-c	>32	16	>256	64	>256	32	1	32	4
CRM03-c	>32	16	>256	64	>256	32	1	32	4
CRM04-c	>32	32	>256	64	>256	8	1	32	8
CRM05-c	>32	32	>256	64	>256	16	2	32	8
CRM06-c	16	8	>256	128	>256	16	8	16	16
CRM07-c	8	16	>256	128	>256	32	4	16	8
CRM08-c	>32	32	>256	64	>256	16	4	32	4
CRM09-c	16	8	>256	128	>256	16	4	8	4

Abbreviation: IMP, imipenem; MEM, Meropenem; CTX, cefotaxime; PEP, cefepime; TZP, piperacillin/tazobactam; LEV, levofloxacin; AK, amikacin; FOS, fosfomycin; TIG, Tigecycline.

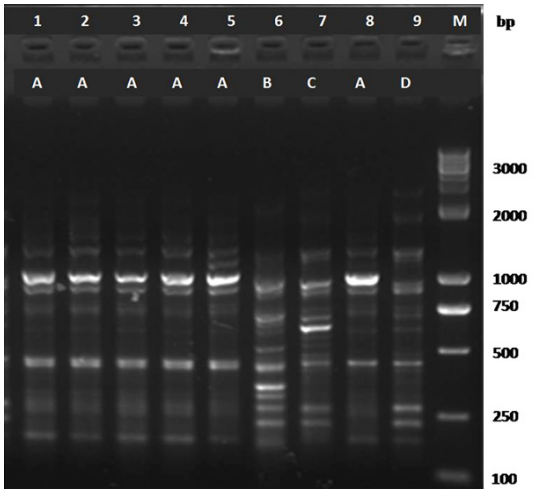

Figure 1. ERIC-PCR typing of nine carbapenem-resistant *Morganella morganii* isolates. Four clonal types were found with Type A of six isolates and Type A, B, C and D of one isolate.

Previous studies demonstrated that carbapenem resistance in *M. morganii* was mostly caused by metal β-lactamase production which was detected in 88.9% of strains investigated by modified Hodge test [9, 10]. We also found similar high level of antimicrobial resistance. The present study detected three categories of resistance genes by PCR (**Table 4**): genes encoding carbapenemases (bla_{KPC-2}, bla_{VIM-1}, and bla_{IMP-1}); genes encoding extended-spectrum β-lactamase ($bla_{CTX-M-4}$, bla_{SHV-1}, and bla_{TEM-6}); and those encoding AmpC enzyme (bla_{DHA-3}, bla_{CYM-4}). These findings were consistent with previous studies [11, 12]. bla_{KPC-2} was found in this study to be the dominant carbapenemase gene (6/9). This gene encodes the most common type A carbapenemases and has been detected first in *Klebsiella pneumoniae*, and subsequently, in various species of Enterobacteriaceae bacteria, including *Escherichia coli, Serratia marcescens,* and *Proteus mirabilis* [13, 14]. Our study proved that bla_{KPC-2} was highly associated with the different levels of carbapenem resistance in *M. morganii.* Based on the PCR and drug resistance phenotypes (**Table 3**), we concluded that the high presence of β-blactam-related genes such as $bla_{CTX-M-4}$, bla_{TEM-1}, bla_{DHA-3}, bla_{SHV-6}, bla_{CYM-4} and other AmpC enzyme genes was the main reason for the high-level resistance to cephalosporin and enzyme inhibitor compound preparation piperacillin/tazobactam in *M. morganii*. When AmpC enzyme and ESBLs enzyme existing in the same bacteria, this situation is referred to as super-extended-spectrum β-lactamase (SSBL). The bacteria with SSBL production were resistant to both cephalosporins and antibacterial agents containing enzyme inhibitors. This type of bacteria often contains plasmids conferring resistance to quinolone and sulfa [15], which often causes pan-resistance. Quinolone resis-

tance is proved to be related to plasmid-mediated quinolone resistance gene *qnrD* [13, 14]. Nonetheless, this quinolone resistance-relevant gene was not found in the present study, which needs further study.

The present study proved that after conjugation *Escherichia coli* J53 developed resistance to carbapenem, and acquired drug-resistant plasmids and most of drug resistance genes (except bla_{VIM}) that were similar to the donor bacteria. The obtained transconjugants were resistant to cephalosporins and levofloxacin. PCR amplification and sequencing results showed that nine *M. morganii* isolates and their transconjugants carried the same profile of resistance genes except for bla_{VIM}. These findings indicated that the antimicrobial resistance genes were positioned on the plasmid and could be transmitted horizontally.

Drug-resistance plasmids in *M. morganii* strains can facilitate the spread of drug resistance among different bacteria. Therefore, the monitoring of drug-resistant *M. morganii* strains should be strengthened and effective isolation measures should be implemented [16]. The present study also showed that the carbapenem-resistant isolates were susceptible to amikacin, fosfomycin, and tigecycline. These results suggest that the combination of effective antimicrobials can be taken to achieve the beneficial treatment outcome by rational drug choices according to antimicrobial susceptibility testing results.

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Disclosure of conflict of interest

None.

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