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Genetic Diversity of Chromosomal Metallo-β-Lactamase Genes in Clinical Isolates of *Elizabethkingia meningoseptica* from Korea

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This study was performed to characterize the chromosomal metallo-β-lactamases (MBLs) of *Elizabethkingia meningoseptica* isolated from Korea and to propose a clustering method of BlaB and GOB MBLs based on their amino acid similarities. Chromosomal MBL genes were amplified by PCR from 31 clinical isolates of *E. meningoseptica*. These PCR products were then cloned into a vector and electrotransformed into *E. coli* DH5α. Nucleotide sequencing was performed by the dideoxy chain termination method using PCR products or cloned DNA fragments. Antimicrobial susceptibilities were determined by the agar dilution method. PCR experiments showed that all 31 *E. meningoseptica* isolates contained both the *bla*B and the bla_{GOB} genes. DNA sequence analysis revealed that *E. meningoseptica* isolates possessed seven types of *bla*B gene, including five novel variants (*bla*B-9 to *bla*B-13) and 11 types of bla_{GOB} gene, including 10 novel variants (bla_{GOB-8} to bla_{GOB-17}). The most common combination of MBL was BlaB-12 plus GOB-17 (n=19). Minimum inhibitory concentrations of imipenem and meropenem for the electrotransformants harboring novel BlaB and GOB MBLs were two- or four-fold higher than those for the recipient *E. coli* DH5α. BlaB and GOB MBLs were grouped in three and six clusters including fifteen novel variants, respectively, based on amino acid similarities.

***Keywords*:** *E. meningoseptica*, metallo-β-lactamase, GOB, BlaB, cluster

Elizabethkingia meningoseptica, a ubiquitous bacterium in the natural and hospital environment, was renamed from *Chryseobacterium meningosepticum* in 2005 based on phylogenetic and phenotypic analysis (Kim *et al*., 2005). *E. meningoseptica* can cause significant diseases in human, though the members of *Chryseobacterium* spp. rarely have clinical significance (George *et al*., 1961). This microorganism causes infections most frequently in neonates and immunocompromised adults. Bloch *et al*. (1997) described meningitis (84%) as the predominant type of infection in neonates, followed by sepsis (13%) and pneumonia (3%), and the mortality rate reached up to 52%. In the older age-group, pneumonia (40%) was the most frequent type of infection, followed by sepsis (24%) and meningitis (18%). The mortality rates due to pneumonia and meningitis were more than 50%.

E. meningoseptica is intrinsically resistant to most β-lactams, including carbapenems, due to production of chromosomal metallo-β-lactamases (MBLs) (Steinberg and Rio, 2005). Bellais *et al*. (2000) reported that all *E. meningoseptica* isolates harbored two types of MBLs simultaneously: BlaB belonging to subclass B1 and GOB belonging to subclass B3 (Steinberg and Rio, 2005). However, a recent survey in China reported that only 55 of 170 *E. meningoseptica* isolates harbored both types of MBLs and 38 isolates harbored only one type of MBL (Chen *et al*., 2006). Furthermore, no MBL genes were detected in the remaining 77 isolates, even though many of these isolates were resistant to imipenem.

The present study was performed to characterize the chromosomal MBLs of *E. meningoseptica* isolated from Korea. In this study, 5 and 10 novel variants of BlaB and GOB, respectively, were detected by sequence analysis of the MBL genes found in *E. meningoseptica* isolates. We also propose a clustering method of BlaB and GOB MBLs based on their amino acid similarities.

Materials and Methods

Bacterial strains

Consecutive non-duplicate isolates of *E. meningoseptica* were collected from patients at a tertiary-care hospital in Korea from 1992 to 2003. The isolates were identified by conventional methods and by using the ATB 32GN system (bioMériux, France) or by 16S rDNA sequencing as described previously (Loffler *et al*., 2000). The isolates were stored at -70°C in skim milk until used for this study. *Escherichia coli* DH5α was used as a recipient strain for cloning (Lauretti *et al*.,

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Table 1. PCR and sequencing primers used in this study

Primer name	Sequence (5′→3′)[a]	Product size (bp)	Reference
BLAB-F	5′-GTG AAT GTA GCA GAG TGT TAA TG-3′	835	Bellais *et al*. (2000)
BLAB-R	5′-GTT GTC TGG TTA AGC GTT CG-3′		
BLABSEQ-F	5′-TAT GTA AAC GAC TGG ACG CAG TC-3′	Used for sequencing	This study
BLABSEQ-R	5′-CCC AGG TCC TTT GAA TCA G-3′		
BLABU-F	5′-GGA AAA GGD DAY ACR GCA GAT-3′	195	This study
BLABU-R	5′-RTG MCC TGC AAC RAC RTA CT-3′		
NBLAB-F	5′-TGT AAA CCG GGA GAA TTT AAT T-3′	847	This study
NBLAB2-R	5′-CTA CGA ATT CTT GGA AGA G-3′		
NBLABSEQ-R	5′-CCT GAA ACT CAT CCT TTC CTA-3′		
N1BD2BT-F	5′-TCA ACG GAA CCA AAT ACG C-3′	Used for sequencing	This study
N1BD2-F	5′-GGA AAG GAT GAG TTT CAG GTC TA-3′		
R1	5′-CAG TTC AAG CTT GTC CAG GAA TTC NNN NNN NGG CCT-3′	Used for sequencing	Sørensen *et al*. (1993)
R2	5′-CAG TTC AAG CTT GTC CAG GAA TTC NNN NNN NGC GCT-3′		
GOBS-F	5′-GGA GTG GTA AAA GAT GAA ATG TGC-3′	920	Bellais *et al*. (2000)
GOB-F	5′-GCT ATG AGA AAT TTT GCT ACA CTG-3′	882	
GOB-R	5′-TCA TAC TTA TTT ATC TTG GG-3′		
GOBSEQ-F	5′-ATA ACC CTG ACT TTG CTT CAT-3′	Used for sequencing	This study
GOBSEQ-R	5′-GCA CCT GTA TGG TCG TAG TG-3′		
GOBU2-F	5′-CST AGG AAC CTA TGA TTT GGC-3′	402	This study
GOBU2-R	5′-CAG GAA CCT TTT GTA TGT CC-3′		
GOBFULL2-F	5′-GGG ATT TCC GTA GAA TTA C-3′	1134	This study
GOBFULL2-R	5′-CTC CTG GAA ATA AAC ATC G-3′		
16SRNA-F	5′-AGA GTT TGA TCC TGG CTC AG-3′	1544	Loffler *et al*. (2000)
16SRNA-R	5′-AAG GAG GTG ATC CAG CCG CA-3′		

[a] D, A, G or T; Y, any pyrimidine (T or C); R, any purine (A or G); M, A or C; S, C or G; N; any nucleotide (A, G, C, or T).

1999). *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as minimum inhibitory concentration (MIC) reference strains.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities were determined by the agar dilution method using Mueller-Hinton agar (Difco Laboratories, USA) with an inoculum of 10^4 colony forming units according to the recommenddations of the CLSI (Clinical and Laboratory Standards Institute, 2006). The following antimicrobial agents were used: amoxicillin (Kun Wha Pharmaceuticals, Korea), piperacillin (Wyeth, USA), cephalothin (Sigma Chemical Co., USA), ceftazidime (GlaxoSmithKline, UK), cefotaxime (Handok, Korea), cefoxitin and imipenem (Merck Sharp & Dohme, USA), aztreonam and cefepime (Bristol-Myers Squibb, USA), moxalactam (Eli Lilly & Co., USA), and meropenem (Sumitomo, Japan).

Phenotypic detection of MBLs

Modified Hodge and imipenem-EDTA plus sodium mercaptopropionic acid (SMA) double disc synergy (DDS) tests were performed as described previously to distinguish MBL-producing isolates from MBL-non-producing ones (Lee *et al*., 2004).

Molecular analysis

Detection of genes encoding chromosome-borne BlaB and GOB MBLs was performed by PCR amplification with the primers listed in Table 1. PCR was carried out in a total volume of 100 μl, with 1 μl of heat-extracted template DNA, 20 pmol of each primer, and 2.5 U of *Ex Taq* DNA polymerase (TaKaRa, Japan). The reaction conditions were 94°C for 5 min; 35 cycles of 94°C for 30 sec, 50°C for 30 sec, and 72°C for 60 sec; and a final incubation at 72°C for 7 min. The PCR products were then subjected to direct sequencing. Both strands of all PCR products were sequenced twice with an automatic sequencer (ABI3700; Perkin-Elmer, USA). A simple two-step PCR was performed for sequencing of the 3′ and 5′ terminals of the open reading frame region of the *bla*B and bla_{GOB} genes as described previously (Sørensen *et al*., 1993). PCR products were ligated into pPCR-Script CamSK vector (Stratagene, USA) and electrotransformed into *E. coli* DH5α. Multiple deduced amino acid sequence alignments were carried out using the program CLUSTAL X version 1.8 (ftp://ftp-igbmc.u-strasbg.fr/pub/clustalx).

Nucleotide sequence accession numbers

The nucleotide sequence data reported in this paper are available in the GenBank nucleotide database under accession numbers AY348324 (*bla*B-9), AY348325 (*bla*B-10), AY348326 (*bla*B-11), EF595958 (*bla*B-12), EF595959 (*bla*B-13), AY348327 (bla_{GOB-8}), AY647246 (bla_{GOB-9}), AY647247 (bla_{GOB-10}), AY647248 (bla_{GOB-11}), AY647249 (bla_{GOB-12}), AY647250 (bla_{GOB-13}), AY647251 ($bla_{GOB-13B}$), AY647256 (bla_{GOB-14}), AY775547 (bla_{GOB-15}), AY899331 (bla_{GOB-16}), and AY899332 (bla_{GOB-17}).

Results

Bacterial isolates

Clinical isolates of *E. meningoseptica* were recovered from 31 patients, including 6 infants less than 1 year of age, during the study period. The isolates were recovered from blood (n=8),

Table 2. Clinical characteristics of patients with infection caused by *E. meningoseptica*

Isolates			Patients		
No.	Specimen	MBLs	Age/sex	Underlying disease	Prognosis
YMC96/12/3780	Sputum	BlaB-2 + GOB-10	40/M	Liver cirrhosis	Expired
YMC95/08/2680	Bile juice	BlaB-2 + GOB-12	80/F	Cholangiocell cancer	Discharge
YMC94/04/2036	Eye discharge	BlaB-6 + GOB-11	21/F	Phthisis, bulbi	Improved
YMC94/03/3459	Blood	BlaB-9 + GOB-13	1 mo./F	Ventricular septal disease	Improved
YMC94/03/9135	Peritoneal fluid	BlaB-9 + GOB-13b	43/M	Liver cirrhosis	Improved
YMC95/08/2670	Bile juice	BlaB-9 + GOB-14	46/M	Gall bladder cancer	Transferred
YMC96/08/3211	Sputum	BlaB-9 + GOB-15	28/M	Acute myeloid leukemia	Improved
YMC95/12/447	Urine	BlaB-10 + GOB-1-like	69/F	Pulmonary tuberculosis	Improved
YMC95/05/3590	Sputum	BlaB-11 + GOB-8	46/F	Aneurysm	Improved
YMC00/12/R211	Sputum	BlaB-11 + GOB-9	58/M	Subarachnoid hemorrhage	Improved
YMC96/08/3109	Sputum	BlaB-11 + GOB-9	71/M	Multiple sore	Expired
YMC92/09/9131	Blood	BlaB-13 + GOB-16	1 mo./F	Hydrocephalus	Improved
YMC93/12/5003	Blood	BlaB-12 + GOB-17	59/M	Respiratory distress syndrome	Improved
YMC94/03/3719	Endotracheal tip	BlaB-12 + GOB-17	43/M	Pneumonia	Improved
YMC94/05/5402	Blood	BlaB-12 + GOB-17	4 mo./M	*Mitral Regurgitation*	Improved
YMC94/05/7796	Blood	BlaB-12 + GOB-17	10/M	Tetralogy of Fallot	Improved
YMC94/06/3876	Sputum	BlaB-12 + GOB-17	45/M	Acute lymphocytic leukemia	Expired
YMC94/06/5233	Blood	BlaB-12 + GOB-17	2/F	Patent ductus arteriosus	Improved
YMC94/06/5700	Blood	BlaB-12 + GOB-17	45/M	Rheumatic heart disease	Improved
YMC94/08/7718	Blood	BlaB-12 + GOB-17	43/M	Rheumatic heart disease	Expired
YMC94/09/3156	Sputum	BlaB-12 + GOB-17	66/F	Diabetes mellitus	Improved
YMC94/09/3158	Endotracheal tip	BlaB-12 + GOB-17	0 mo./F	Prematurity	Improved
YMC94/10/3013	Sputum	BlaB-12 + GOB-17	30/M	Viral encephalitis	Expired
YMC94/10/3481	Sputum	BlaB-12 + GOB-17	83/M	Intracerebral hemorrhage	Expired
YMC95/07/2262	Bile juice	BlaB-12 + GOB-17	54/M	Cholangiocell cancer	Improved
YMC95/08/2608	Bile juice	BlaB-12 + GOB-17	79/F	Cholangiocell cancer	Discharge
YMC95/08/3319	Sputum	BlaB-12 + GOB-17	37/F	Brain tumor	Improved
YMC95/08/3605	Sputum	BlaB-12 + GOB-17	3 mo./F	Atrial septal defect	Expired
YMC95/08/3794	Endotracheal tip	BlaB-12 + GOB-17	4 mo./M	Cystic hygroma	Expired
YMC96/09/3140	Endotracheal tip	BlaB-12 + GOB-17	42/F	Lymphoma	Improved
YMC95/12/2303	Wound	BlaB-12 + GOB-17	65/M	Respiratory distress syndrome	Expired

respiratory specimens (n=15), and others (n=8) (Table 2). All the isolates were recovered from in-patients at least 3 days after admission, except 2 from out-patients. *E. meningoseptica* infections exhibited frequent association to cardiovascular diseases (n=5) in infants and children, and to chronic liver diseases, including cholangiocell cancer (n=3), gall bladder cancer (n=1), and liver cirrhosis (n=2), in adults. Nineteen patients improved with antimicrobial treatment, while 9 patients, including two infants, died. Six patients died as a direct result of infection, while remaining 3 patients died for other reasons (data not shown). A high mortality rate (47%, 7/15) was observed when *E. meningoseptica* was recovered from respiratory specimens (Table 2).

Genotypes of metallo-β-lactamases

All the isolates exhibited positive results in the modified Hodge and imipenem-EDTA plus SMA DDS tests, thus indicating the production of MBLs (data not shown). PCR experiments detected both genes encoding BlaB and GOB MBLs in all *E. meningoseptica* isolates. DNA sequence analysis revealed that *E. meningoseptica* isolates possessed 7 types of *bla*B gene, including 5 novel variants (*bla*B-9 to *bla*B-13), and 11 types of bla_{GOB} gene, including 10 novel variants (bla_{GOB-8} to bla_{GOB-17}). The most common combination of MBLs was BlaB-12 plus GOB-17 (n=19) (Table 2). The MIC range of imipenem for the isolates was 16-64 mg/L (Table 3). MICs of imipenem and meropenem for the electrotransformants harboring novel BlaB and GOB MBLs were two- or four-fold higher than those for the recipient *E. coli* DH5α (Table 3).

Genetic relations of metallo-β-lactamases

Alignment of the BlaB and GOB sequences of *E. meningoseptica* isolates revealed heterogeneity, with 87 to 100% and 80 to 100% deduced amino acid identity (Figs. 1 and 2). All the residues suspected to be the Zn1 (His^{116}, His^{118}, and His^{196}, according to the standard numbering scheme for class B β-lactamases) and Zn2 (Asp^{120}, Cys^{221}, and His^{263}) ligands were conserved in all five novel BlaB enzymes (Fig. 1). The Zn (Asp^{120}, His^{121}, and His^{263}) ligands of GOB enzymes were also conserved in 10 novel GOB enzymes (Fig. 2). GOB-11, GOB-13, GOB-14, and GOB-15 enzymes had insertion of two amino acid residues upstream of the cleavage site.

Table 3. MIC ranges of β-lactams for clinical isolates of *E. meningoseptica*, their transformants, and *E. coli* DH5α

Bacteria (No. of isolates)	MIC range (μg/ml)									
	IPM	MEM	AZT	CEP	CTX	CAZ	FEP	MOX	FOX	PIP
Cinical isolates of *E. meningoseptica* (31)	16-64	16-64	32-64	>128	32->128	>128	32->128	32->128	16->128	8-64
Transformants with recombinant plasmids carrying novel *bla*B genes (5)	0.25	0.03	0.12	4	0.12-0.25	0.25-1	0.03-0.06	0.12-0.25	4-8	1-4
Transformants with recombinant plasmids carrying novel bla_{GOB} genes (11)	0.25-0.5	0.03	0.06-0.12	4-8	0.06-0.12	0.25-8	≤0.03-0.03	0.06-0.5	4-16	1-2
E. coli DH5α	0.12	0.015	0.03	4	0.03	0.12	≤0.03	0.12	4	0.5

Abbreviations: IPM, imipenem; MEM, meropenem; AZT, aztreonam; CEP, cephalothin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; MOX, moxalactam; FOX, cefoxitin; PIP, piperacillin.

The 13 BlaB enzymes were grouped in three clusters based on their amino acid similarities (Fig. 3). (i) The BlaB-1 cluster includes four enzymes (BlaB-1, BlaB-2, BlaB-3, and BlaB-11). (ii) The BlaB-4 cluster includes three enzymes (BlaB-4, BlaB-12, and BlaB-13). (iii) The BlaB-5 cluster includes the remaining six enzymes (BlaB-5 to BlaB-10). The evolutionary distance between the BlaB-1 and BlaB-5 clusters was short.

The 18 GOB enzymes grouped in six clusters based on their amino acid similarities (Fig. 4). (i) The GOB-1 cluster includes three enzymes (GOB-1, GOB-2, and GOB-18). (ii) The GOB-3 cluster comprised seven enzymes (GOB-3 to GOB-6 and GOB-8 to GOB-10). (iii) The GOB-7 cluster includes one member. (iv) The GOB-11 cluster includes four enzymes (GOB-11, GOB-13, GOB-14, and GOB-15). (v) The GOB-12 cluster includes one member. (vi) Finally, the GOB-16 cluster includes the remaining two enzymes (GOB-16 and GOB-17).

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          37                                                                             * * +                                158
BlaB-1    QENPDVKIE KLKDNLYVYTTYNTFNGT KYAANAVYLVTDKGVVVIDCPWGEDKFKSFTDEIY KKHGKK VIMNIATHSHDDRAGGLEYFGKI GAKTYSTKMTDSILAKEN
BlaB-2    --------D ------------------ ----------------------------------- ------ ----------------------- ------------------
BlaB-3    --------- ------------------ ----------------------------------- ------ --I-------------------- ------------------
BlaB-4    --T-E---- ------------------ -----------S-------S----E---------- -R---- --------------------KSL ----------------D-
BlaB-5    -Q---I--- ---------------K-- --------M----------S--------------- ------ ----------------------L ------------------
BlaB-6    -Q---I--- ---------------K-- --------M----------S--------------- ------ ----------------------L ------------------
BlaB-7    -Q---I--- ---------------K-- --------M-----I----S--------------- ------ ----------------------L ------------------
BlaB-8    -Q---I--- ---------------K-- --------M----------S--------------- ------ ----------------------L ------------------
BlaB-9    -Q---I--- ---------------K-- --------M----------S--------------- ------ ----------------------L ------------------
BlaB-10   -Q---I--- ---------------K-- --------M----------S--------------- ------ ----------------------L ------------------
BlaB-11   --------- ------------------ ----------------------------------- ------ ----------------------- ------------------
BlaB-12   --T-E---- ------------------ -----------S-------S----E---N------ -R---- --------------------KSL -V--------------D-
BlaB-13   --T-E---- ------------------ -----------S-------S----E---N------ -R---- --------------------KSL -V--------------D-

        159                                   *                       +                                         +            280
BlaB-1           KPRAQYTFDNNKSFKVGKSEFQVYYPGKGHTADNVVVWF   PKE  KVLVGGCIIKSADSKDLGYIGEAYVNDWTQSVHNIQQKFSGAQYVVAGHDD          WKDQ
BlaB-2           ---------------------------------------   ---  ---------------------------------------------------          ----
BlaB-3           ---------------------------------------   ---  ---------------------------------------------------          ----
BlaB-4           ------------------D--------------H-----   --D  -----------G------F-----------------K-PNV----------          ----
BlaB-5           ----K------------NT--------------------   --D  --------V--G-------------------I------PDV----------          ----
BlaB-6           ----K------------NT--------------------   --D  --------V--G------F------------I------PDV----------          ----
BlaB-7           ----K-------------T--------------------   --D  --------V--G------F------------I------PDV----------          ----
BlaB-8           ----K-------------T--------------------   --D  --------V--G------F------------I------PDV----------          ----
BlaB-9           ----K------------NT--------------------   --D  --------V--G------F------------I------PDV----------          ----
BlaB-10          ----K-------------T--------------------   --D  --------V--G------F------------I------PYV----------          ----
BlaB-11          ---------------------------------------   ---  ---------------------------------------------------          ----
BlaB-12          ------------------D--------------------   --D  -----------G------FL----------------K--PNV---------          ----
BlaB-13          ------------------D--------------------   --D  -----------G---N--FL----------------K--PNV---------          ----

        281                 301
BlaB-1    RSIQHTLDLINEYQQKQKASN
BlaB-2    ----R----------------
BlaB-3    T---------S----------
BlaB-4    TA-H------S----------
BlaB-5    T---------SD---------
BlaB-6    T---------S----------
BlaB-7    T---------S----------
BlaB-8    T---------S----------
BlaB-9    T---------S----------
BlaB-10   T---------S----------
BlaB-11   ----R----------------
BlaB-12   TA--------S----------
BlaB-13   TA--------S----------
```

Fig. 1. Comparison of amino acid sequences of the BlaB β-lactamases from *E. meningoseptica* isolates. The hyphen (-) indicates identical amino acid sequences. The asterisk (*) and cross (+) indicate the residues known to be involved either directly or indirectly with Zn1 and Zn2 cofactors, respectively.

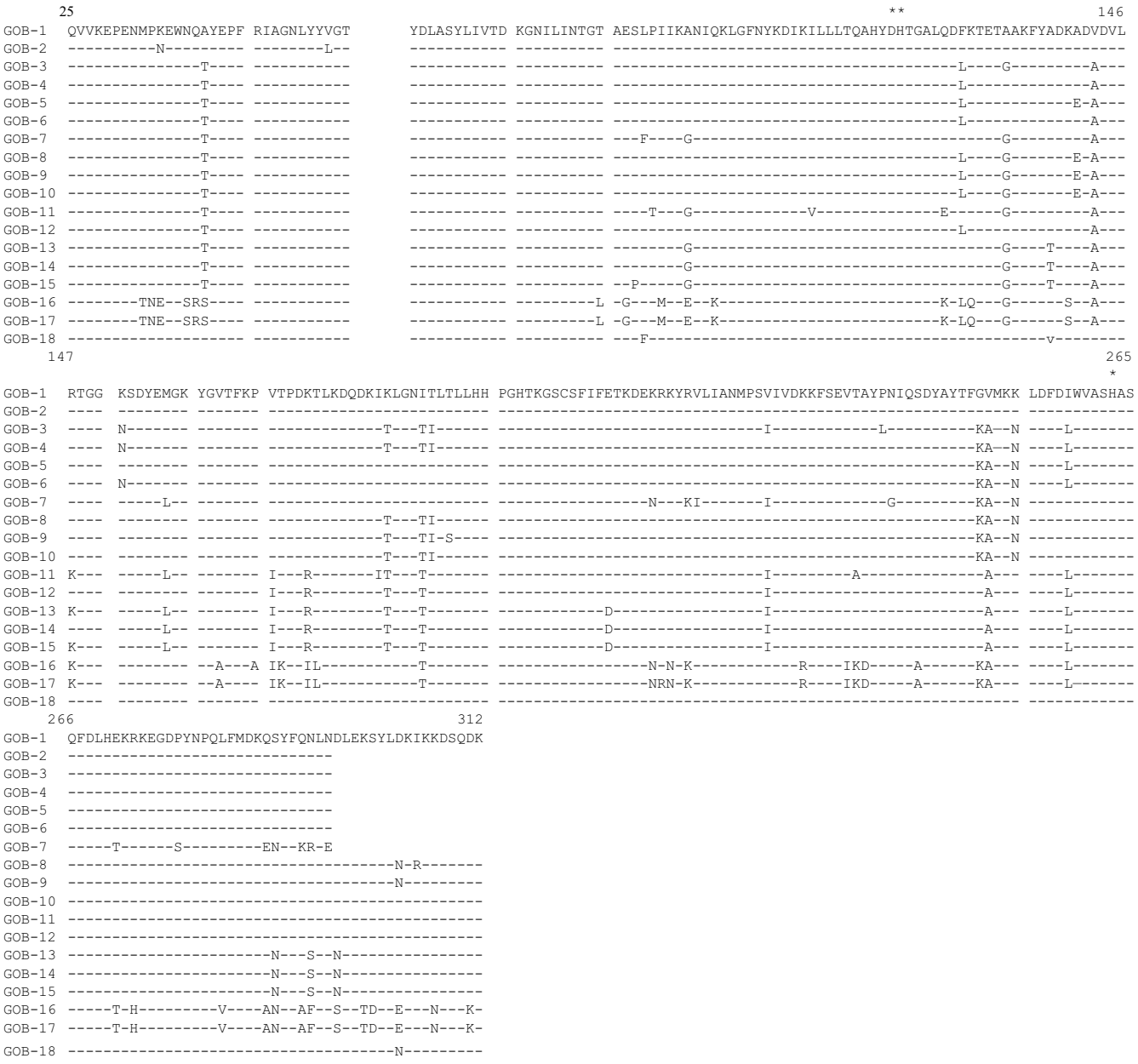

Fig. 2. Comparison of amino acid sequences of the GOB β-lactamases from *E. meningoseptica* isolates. The hyphen (-) indicates identical amino acid sequences. The asterisk (*) indicates the residues known to interact with Zn^{2+} cofactors.

Discussion

Our results showed that all *E. meningoseptica* isolates harbored both BlaB and GOB MBLs. Eight variants of Bla-B (BlaB-1 to BlaB-8) and 8 variants of GOB (GOB-1 to GOB-7 and GOB-18) have been described since the first detection of BlaB1 and GOB-1 in 1998 and 2000, respectively (Rossolini *et al*., 1998; Bellais *et al*., 2000; Woodford *et al*., 2000; Kirby *et al*., 2004; Morán-Barrio *et al*., 2007). We identified 5 more BlaB variants and 10 more GOB variants in this study. Transformants carrying these new variants genes exhibited two- to four-fold higher level of MICs for imipenem and meropenem than the recipient, suggesting that BlaB and GOB enzymes might play a role in carbapenem resistance of *E. meningoseptica*.

E. meningoseptica has known to cause fatal infections in neonates and immunocompromised adults with severe underlying diseases. Our results also showed a high mortality rate (29%, n=9). However, 7 of 9 patients were died for aggravation of underlying diseases. Only 2 patients were died as a direct result of pulmonary infection. It was noteworthy that the patients were coinfected by other major pathogens, such as *A. baumannii* and *P. aeruginosa*. The results suggest that *E. meningoseptica* might not cause fatal results by itself against the previous reports (Bloch *et al*., 1997).

A variety of therapeutic regimens for the treatment of *E. meningoseptica* infection has been expressed. A previous study

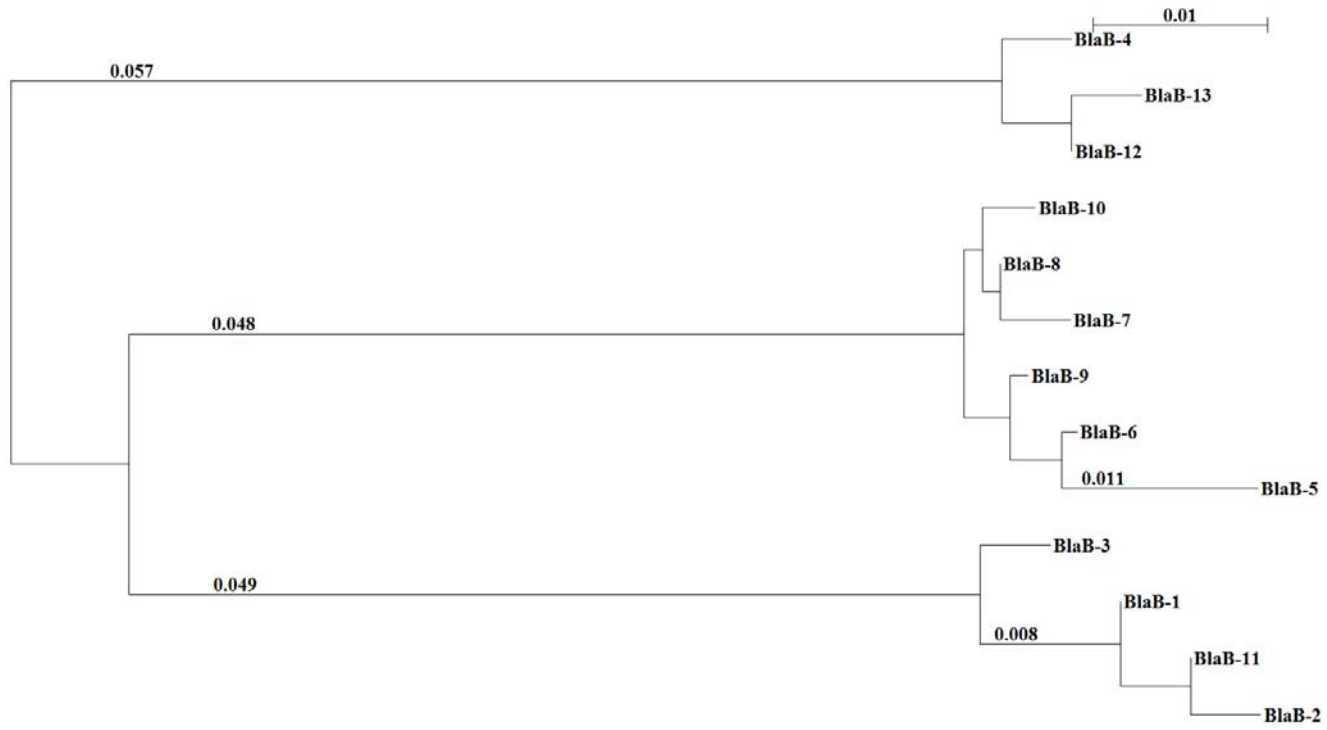

Fig. 3. Dendrogram of 13 BlaB enzymes constructed by CLUSTAL X. The phylogenetic relations between the enzymes reported in the BlaB family show three types of BlaB enzymes.

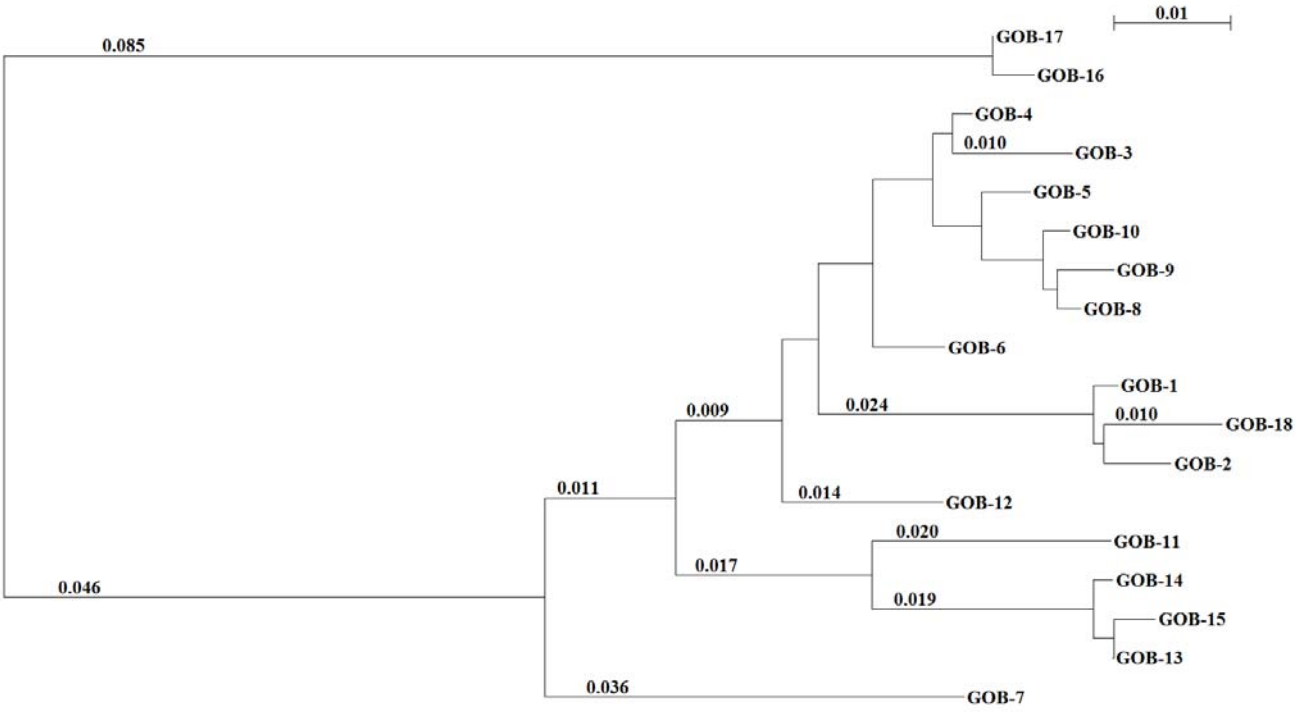

Fig. 4. Dendrogram of 18 GOB enzymes constructed by CLUSTAL X. The phylogenetic relations between the enzymes reported in the GOB family show six types of GOB enzymes.

recommended vancomycin alone or in combination with other antimicrobial agents as treatment of choice for *E. meningoseptica* infections (Ratner, 1984). However, Kirby *et al.* (2004) described that the drug had poor *in vitro* activity. They described that fluoroquinolones, including gatifloxacin, garenoxacin, and levofloxacin, exhibited the highest potency against this microorganism. Recently, Lin *et al*. (2009) described that tigecycline exhibited a potent *in vitro* activity against clinical *E. meningoseptica* isolates, while colistin did not. In our study, most patients were treated with combination regimens comprised of expanded-spectrum β-lactams, such as aztronam, cefoperazone, and ceftriaxone, and fluoroquinolones and/or aminoglycosides. Both 2 patients, who died as a direct result of infection, were treated with cefoperazone and pefloxacin. Addition of fluoroquinolones to the treatment regimen did not improve clinical course. It was interesting that 3 patients improved without antimicrobial treatment. Further studies are needed to establish a guideline for the treatment of *E. meningoseptica* infections.

In summary, all *E. meningoseptica* isolates harbored both BlaB and GOB enzymes. BlaB and GOB MBLs were grouped in three and six clusters including fifteen novel variants, respectively, based on amino acid similarities.

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