Journal of Microbiology, Immunology and Infection (2014) 47, 318–323

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ORIGINAL ARTICLE

Identification and epidemiological relatedness of clinical *Elizabethkingia meningoseptica* isolates from central Taiwan

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Received 17 July 2012; received in revised form 6 December 2012; accepted 27 March 2013
Available online 28 May 2013

KEYWORDS
Central Taiwan;
Elizabethkingia meningoseptica;
Epidemiological relatedness;
Identification

Background: Elizabethkingia meningoseptica is an opportunistic pathogen. Identification of *E. meningoseptica* based on conventional methods is rather labor- and time-consuming. The information on epidemiological relatedness and microbiological characteristics of *E. meningoseptica* isolates from central Taiwan was limited.
Methods: Forty *E. meningoseptica* isolates identified by conventional methods were collected by the Central Laboratory of Central Region Hospital Alliance between 2007 and 2011. The amplification of 16S ribosomalDNA gene by polymerase chain reaction with species-specific or universal primers following DNA sequencing was used as a standard identification method. The feasibility of Vitek 2 GN card was also evaluated. Some clinical information of the patients and the drug susceptibilities and epidemiological relatedness of the isolates were analyzed.
Results: For the 40 isolates, 39 *E. meningoseptica* and one *Chryseobacterium indologenes* were identified using 16S rDNA sequencing. Among the 39 isolates, all could be identified using species-specific primers, whereas only 84.6% could be identified by Vitek 2 GN card with excellent discrimination. All *E. meningoseptica* isolates were susceptible to minocycline but resistant to many drugs examined including ceftazidime, amikacin, colistin, and imipenem. The pulsed field gel electrophoresis (PFGE) patterns demonstrated that most isolates were quite genetic diversity. The patients had average age of 72.2 ± 14.5 years old (excluded one child patient of 1 year old) and 79.5% of patients were male. Twenty-three patients (59.0%) had underlying diseases.

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http://dx.doi.org/10.1016/j.jmii.2013.03.007

Conclusion: The designed species-specific primers could be used to identify *E. meningoseptica* with 100% of specificity and sensitivity, whereas the Vitek 2 GN card showed considerable ability in *E. meningoseptica* identification. The PFGE patterns showed that most isolates were genetic diversity enough to exclude the possibility of intrahospital spread.

Introduction

Elizabethkingia meningoseptica, previously known as *Chryseobacterium meningosepticum*,[1] is widely distributed in soil and water including municipal water supplies with chlorination.[2] Infections caused by *E. meningoseptica* via contaminated medical devices or surgically implanted devices have been documented.[3,4] This bacterium causes meningitis in premature and newborn infants, with high mortality.[5] Pneumonia, endocarditis, postoperative bacteremia, and meningitis generally associated with underlying diseases have also been reported in elderly adults.[6–9] An epidemiological study has proved that an outbreak caused by the possible clonal expansion of *E. meningoseptica* was acquired in a hospital environment[10]; infections caused by unrelated clones are also documented.[9]

E. meningoseptica is characterized as a nonglucose-fermentative, nonmotile, and oxidase-, catalase-, and indole-positive Gram-negative bacillus.[11,12] However, it is both labor- and time-consuming to identify *E. meningoseptica* by conventional methods based on biochemical parameters. The objective of the current study was to develop a species-specific primer pair for the identification of *E. meningoseptica*, evaluate the feasibility of using a Vitek 2 GN card, and review the medical records of 39 patients with *E. meningoseptica* infection in central Taiwan from 2007 to 2011.

Methods

Patients and bacterial isolates

E. meningoseptica isolates were collected from 40 patients by the Central Laboratory of Central Region Hospital Alliance (including the Taichung Hospital, Fongyuan Hospital, Changhwa Hospital, and Nantou Hospital) from August 2007 to January 2011. Identification was performed by conventional methods.[11,12] Briefly, oxidase- and indole-positive nonfermenters with the characteristics of no or pale yellow pigment, urease negative, gelatinase positive, and acidification of glucose, mannitol, and maltose, but not xylose and sucrose, was presumed to be *E. meningoseptica*. All the medical records of patients were studied retrospectively.

16S rDNA identification

Genomic DNA extraction was performed using a genomic DNA purification kit (BioKit, Miaoli, Taiwan). Briefly, bacteria were suspended in normal saline to the turbidity of McFarland No. 6. After washing, lysis buffer and proteinase K stock solution were added and the mixture incubated at 56°C until complete lysis of bacteria was achieved. The sample was mixed with binding buffer and ethanol, and then transferred to a spin column. After centrifugation, DNA was eluted by water. A pair of species-specific primers (5′-GATTCGGCATCGGATTATATTG-3′ and 5′-CCACTTCAACCT TACTCAAGACTAAC-3′) was designed according to the conserved 16S rDNA region in *E. meningoseptica* with accession No. AY468482, AF207074, EU128743, and EU128722, then was applied to perform polymerase chain reaction (PCR). The PCR reaction mixture (50 μL) consisted of 25 pM primers (each), 1 μL genomic DNA, and 10 μL of 5× PCR Master Mix (GeneMark, Taichung, Taiwan). The PCR was performed as following: denaturation at 95°C for 5 minutes; 25 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 1 minute; and a final extension at 72°C for 5 minutes. The 475-bp product was analyzed by 1% agarose gel electrophoresis and visualized with ethidium bromide (EtBr) staining. In case no expected amplicon was obtained, a universal primer pair (5′-AGAGTTTGATCMTGGCTCAG-3′ and 5′-GGYTACCTTGTTACGACTT-3′)[13] was then used for PCR. The expected amplicon was 1488 bp long. All amplified DNA fragments were sequenced using ABI 3730 system with Big-Dye Terminator v3.1 Cycle Sequencing Kit (ABI Prism, Foster, CA, U.S.A.) and the DNA sequences data were searched using the Basic Local Alignment Search Tool (BLAST) in the National Center for Biotechnology Information database.

Biochemical discrimination and drug susceptibility assay

Vitek 2 GN card and antimicrobial susceptibility test (AST) card (bioMérieux, Marcy-l'Etoile, France) were used for biochemical discrimination and drug susceptibility assay of bacterial isolates, respectively. Identification (ID) message confidence levels for biochemical discrimination had six criteria: excellent: one choice of species with 96—99% probability; very good: one choice of species with 93—95% probability; good: one choice of species with 89—92% probability; acceptable: one choice of species with 85—88% probability; low discrimination: two to three choices of species with the sum of choices equal to 100% probability; unidentified organism: more than three or no choice of species. The minimal inhibitory concentrations (MICs) toward gentamicin, amikacin, ceftazidime, ceftriaxone, cefepime, chloramphenicol, ciprofloxacin, colistin, imipenem, piperacillin, piperacillin/tazobactam, minocycline, and trimethoprim/sulfamethoxazole were examined using AST card for Gram negative bacillus (GNB). The vancomycin MIC was determined by AST card for GPC. With the exception

of vancomycin which has not appropriate MIC breakpoint to be interpreted, the Clinical and Laboratory Standards Institute criteria for other non-*Enterobacteriaceae* was used to interpret the data from drug susceptibility assays.[14]

PFGE analysis

The bacteria were suspended in 3 mL cell suspension buffer (20 mM Tris, and 40 mM EDTA; pH 8.0) with an OD_{610} absorbance of 1.6—1.8. Agar plugs (200 μL bacterial aliquot, 100 μg proteinase K, and 200 μL 1% agarose) were prepared and treated with cell lysis buffer (50 mM Tris, 50 mM EDTA, 1% *N*-lauroylsarcosine, and 500 μg proteinase K; pH 8.0), then the DNA was digested with *Xho*I at 37°C for 4 hours. The *Xba*I-digested chromosomal DNA of *Salmonella enterica* serovar Braenderup H9812 was used as the molecular weight marker.[15] PFGE was performed using CHEF-DR III system (Bio-Rad, Hercules, CA, USA) and the data were analyzed using GelCompar II software (GelCompar II, Applied Maths NV, Belgium). The Dice similarity coefficients were calculated and clustering was done by unweighted pair group mean association (UPGMA).

Results

Characteristics of patients and *E. meningoseptica* isolates

Among 39 patients with *E. meningoseptica* infection, the average age of patients was 72.2 ± 14.5 years (excluded one child patient), 31 (79.5%) were male, and 23 (59.0%) had underlying disease, including solid tumors (n = 7, 17.9%), chronic obstructive pulmonary disease (n = 6, 15.4%) and diabetes mellitus (n = 5, 12.8%) (Table 1). All isolates were collected from hospitalized patients. Among the *E. meningoseptica* isolates, 12 (30.8%) were from Fengyuan Hospital, 11 (28.2%) from Taichung Hospital, nine (23.1%) from Nantou Hospital, and seven (17.9%) from Changhua Hospital (Table 1). The most predominant sources were from blood (19 isolates), followed by sputum (16 isolates) (Table 1).

Table 1 Demographic information of 39 patients with *E. meningoseptica* infection

Hospital	Isolate no.	Age (y)	Sex	Source	Underlying diseases
FY	2	85	M	Sputum	None
FY	18	69	F	Sputum	Stroke, COPD
FY	19	56	M	Sputum	None
FY	24	44	M	Sputum	None
FY	26	58	M	Sputum	Esophageal cancer, DM
FY	33	61	M	Sputum	Stroke
FY	37	1	F	Blood	None
FY	41	77	M	Urine	None
FY	50	79	M	Blood	COPD
FY	81	79	M	Sputum	Renal failure
FY	82	81	M	Sputum	COPD
FY	95	29	M	Blood	None
T	3	82	M	Blood	Nasopharyngeal cancer
T	4	84	M	Blood	COPD
T	35	77	M	Blood	Lung cancer
T	55	79	M	Blood	DM, HHD
T	60	54	M	Sputum	None
T	68	92	M	Blood	None
T	79	84	M	Blood	Hypertension
T	83	69	F	Sputum	Colon cancer, COPD
T	84	86	M	Blood	DM, Hypertension
T	94	55	F	Sputum	Stroke
T	98	89	M	Blood	DM, Stroke
N	13	72	M	Sputum	None
N	28	88	M	Blood	Heart failure
N	42	69	F	Urine	Renal failure
N	43	83	M	Sputum	Respiratory failure
N	45	87	M	CVC	None
N	46	79	M	Blood	Lung cancer
N	52	70	M	Wound	None
N	66	86	M	Blood	None
N	86	80	M	Blood	COPD
C	7	57	M	Sputum	None
C	11	61	F	Blood	DM
C	15	52	F	Sputum	None
C	34	76	F	Blood	None
C	54	81	M	Sputum	Lung cancer
C	62	53	M	Blood	None
C	85	79	M	Blood	Heart failure, lung cancer

C = Changhua; COPD = chronic obstructive pulmonary disease; CVC = central venous catheter; DM = diabetes mellitus; F = female; FY = Fengyuan; HHD = hypertensive heart disease; M = male; N = Nantou; T = Taichung.

Identification using 16S rDNA sequencing and Vitek 2 methods

Forty *E. meningoseptica* isolates, identified by Central Laboratory of Central Region Hospital Alliance using conventional methods based on biochemical features, were reexamined using 16S rDNA sequencing as the gold standard. Thirty-nine isolates had expected amplicon amplified by species-specific primers and were identified as *E. meningoseptica* following DNA sequencing (with sequence similarity ranged from 99% to 100%, data not shown). The remaining one was identified as *Chryseobacterium indologenes* by the PCR using universal primer pair and DNA sequencing. The Vitek 2 GN card was also used to examine its feasibility in the identification of *E. meningoseptica* (Table 2). For the 39 *E. meningoseptica* isolates, 33 isolates were identified as *E. meningoseptica* with excellent discrimination (33 of 39, 84.6%), three isolates as *E. meningoseptica* with low discrimination (3 of 39, 7.7%), and three isolates as non-*E. meningoseptica* (2 as *C. indologenes* and 1 as *Stenotrophomonas maltophilia*). Thus, the sensitivity and specificity of Vitek 2 card were 84.6% and 100%, respectively, for the identification of *E. meningoseptica*. Interestingly, the 16S rDNA-sequencing-identified *C. indologenes* isolate was identified as *E. meningoseptica* with low discrimination by Vitek 2 GN card (Table 2).

Table 2 Comparison of Vitek 2 GN card with 16S rDNA sequencing in presumptive *E. meningoseptica* isolates identification

16S rDNA sequencing	Vitek 2 GN card			
	E. meningoseptica		*C. indologenes*	*S. maltophilia*
	Excellent discrimination	Low discrimination	Excellent discrimination	Excellent discrimination
E. meningoseptica ($n = 39$)	33 (84.6%)	3 (7.7%)	2 (5.1%)	1 (2.6%)
C. indologenes ($n = 1$)		1 (100%)		

Drug susceptibility

Drug susceptibilities of the 39 *E. meningoseptica* isolates were determined using the Vitek 2 AST card (Table 3). All isolates were resistant to amikacin, ceftazidime, colistin, and imipenem. The susceptibilities of the *E. meningoseptica* isolates to trimethoprim/sulfamethoxazole, ciprofloxacin, and cefepime were 74.4% (29 of 39), 35.9% (14 of 39), and 5.1% (2 of 39), respectively. The most potential drug was minocycline with 100% susceptibility.

PFGE analysis

The epidemiological relatedness of the 39 isolates from four hospitals in central Taiwan was determined by PFGE analysis (Fig. 1). There were seven isolates (no. 3, 13, 26, 35, 79, 85, and 98) found to be $\geq$77.8% similarity, whereas the remaining 32 isolates were of quite genetic diversity.

Discussion

Among the 39 *E. meningoseptica* clinical isolates collected from central Taiwan, the major sources were from blood (48.7%) and sputum (41.0%), respectively (Table 1). The specimen prevalence was quite consistent with that from a previous study.[16] The mean age of patients was 72.2 $\pm$ 14.5 years (excluding 1 child patient), and only three patients were younger than 50 years old (7.7%) (Table 1). Many reports concerning *E. meningoseptica* infection among newborn or young children are documented.[2,5,17] Infection with this bacterium among the elderly is also noted.[6–9,16] We found that all isolates except one were from adults, reflecting the low infectivity rate of this bacterium to children in central Taiwan. In addition, patients with various kinds of underlying disease(s) (59.0%) were observed as in previous reports,[9,18–20] but with less prevalence.

Table 3 Drug susceptibilities of 39 *E. meningoseptica* isolates

Antimicrobial agent	MIC_{50}	MIC_{90}	% Susceptibility
Gentamicin	$\geq$16	$\geq$16	0
Amikacin	64	64	0
Ceftazidime	64	64	0
Ceftriaxone	64	64	0
Cefepime	64	64	5.1
Chloramphenicol	64	64	0
Ciprofloxacin	$\geq$4	$\geq$4	35.9
Colistin	$\geq$16	$\geq$16	0
Imipenem	$\geq$16	$\geq$16	0
Piperacillin	$\geq$128	$\geq$128	0
Piperacillin/tazobactam	$\geq$128	$\geq$128	0
Minocycline	$\geq$1	$\geq$1	100
Trimethoprim/sulfamethoxazole	40	160	74.4
Vancomycin[a]	16	16	—

[a] The Clinical and Laboratory Standards Institute without MIC breakpoint for vancomycin.
MIC = minimum inhibitory concentration.

For molecular identification of *E. meningoseptica*, the designed species-specific primer pair could correctly identify all isolates. To evaluate the specificity of the species-specific primer pair, 35 *C. indologenes* and 13 *C. gleum* clinical isolates collected from the central Taiwan were also examined and none of them had 16S rDNA fragment being amplified (data not shown). Generally, bacterium could be identified by amplification of 16S rDNA gene using universal primer pair, followed by DNA sequencing.[13] However, DNA sequencing of PCR product was unnecessary when species-specific primer pair was used to amplify 16S rDNA fragment from presumptive *E. meningoseptica*. It will be beneficial in both cost and time saving. Using the Vitek 2 GN card, 84.6% isolates (33 of 39) could be identified with excellent discrimination (Table 2), whereas six isolates were of an unacceptable level: 7.7% (3 of 39) with low discrimination as *E. meningoseptica*, 5.1% (2 of 39) as *C. indologenes*, and 2.6% (1 of 39) as *S. maltophilia*. The Vitek 2 has been used to identify *E. meningoseptica* without confirmation by molecular identification.[9,21] In this study, the Vitek 2 GN card was also used for culture identification to evaluate its feasibility. Our results indicated the Vitek 2 GN card with considerable ability in *E. meningoseptica* identification. Interestingly, all of the three misidentified isolates (two as *C. indologenes* and one as *S. maltophilia*) showed hemolytic activity on sheep blood agar plate within 72 hours, whereas only two of the other 36 isolates (5.6%) possessed the hemolytic ability (data not shown), suggesting the atypical biochemical characterizations of these isolates.

For the drug susceptibilities of *E. meningoseptica*, the results revealed that this bacterium was highly resistant to many drugs examined, implying the difficulty for proper therapy.[21,22] According to a previous report,[6,21] all isolates were sensitive to minocycline. During comparison with the study conducted in Taichung Veterans General Hospital,[21] a medical center in central Taiwan, the MIC_{50} of ciprofloxacin and piperacillin/tazobactam significantly promoted from 0.5 to $\geq$4 and 8 to $\geq$128, respectively. However, a study performed in northern Taiwan showed similar MIC_{50} of these drugs as our study.[22] It is worthy to continuously monitor the tendencies of drug resistances in this area in the future.

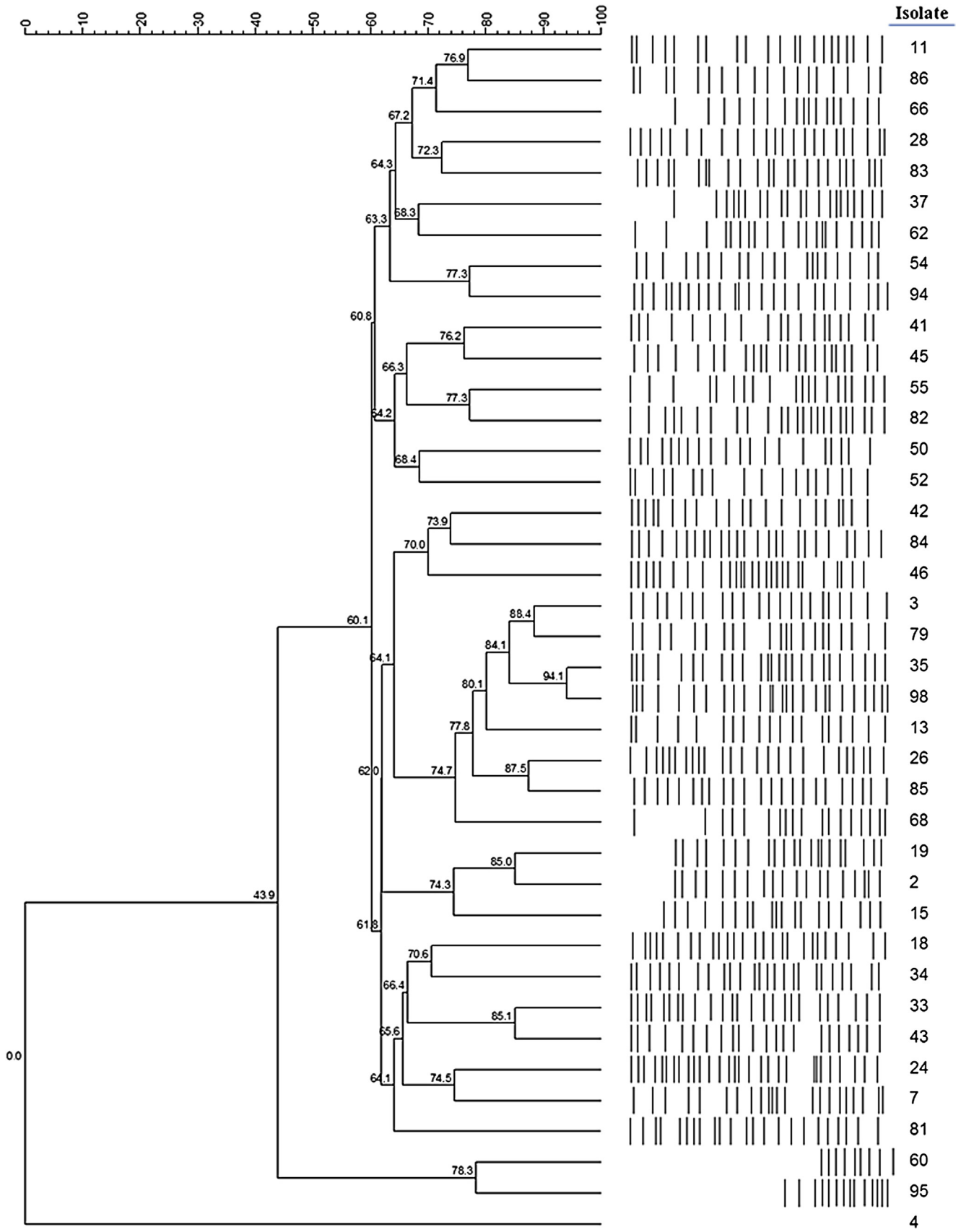

Figure 1. Pulsed field gel electrophoresis dendrogram of 39 *E. meningoseptica* isolates. Isolates were grouped according to *Xho*I restriction patterns by GelCompar II software.

PFGE analysis revealed that seven isolates were found to be ≥77.8% similarity, whereas the other isolates in central Taiwan were genetically diverse. *E. meningoseptica* that caused outbreaks in the neonatal intensive care unit by a single strain have been reported.[4,23] However, isolates from bloodstream infections in northern Taiwan were demonstrated to be clonally unrelated.[9,24] In this study, most isolates proved to be nonclonally related even though all of them were collected from hospitalized patients. However, it was notified that four isolates with ≥84.1% similarity (Fig. 1), including isolate 3, 35, 79, and 98, were collected from elderly patients hospitalized in the same hospital (Table 1). It raised the concern for the possible occurrence of an outbreak in that hospital during the collection period.

In conclusion, the PCR method using our species-specific primers could be used to identify *E. meningoseptica* clinical isolates with 100% of sensitivity and specificity. Microbiological characterization of the identified *E. meningoseptica* revealed valuable information about the drug resistance pattern and genetic diversities of clinical isolates from central Taiwan. Continuous monitoring of infections caused by this bacterium in this area is needed for the public health.

Conflicts of interest

All contributing authors declare no conflicts of interest.

Acknowledgments

This study was supported by a grant from the Fongyuan Hospital, Taichung, Taiwan (100-005). We are also very thankful to members of the Central Laboratory of the Central Region Hospital Alliance, who contributed to the collection of clinical isolates.

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