Frequent isolation of extended-spectrum beta-lactamase-producing bacteria from fecal samples of individuals with severe motor and intellectual disabilities

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Frequent isolation of extended-spectrum beta-lactamase-producing bacteria from fecal samples of individuals with severe motor and intellectual disabilities☆

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ABSTRACT

Extended-spectrum beta-lactamase (ESBL) producing bacteria spread worldwide and became major concern for antibiotic treatment. Although surveillance reports in general hospitals and long-term care facilities are increasing, their frequencies in individuals with severe motor and intellectual disabilities (SMID) are so far unknown. In this study, we examined the frequency of ESBL in stool samples collected from 146 asymptomatic SMID subjects hospitalized in a single institution. With their clinical information, we evaluated possible risk factors for ESBL colonization. From 146 fecal samples, ESBL-producing bacteria were isolated in 45 cases (31%). Drug sensitivity testing showed that 82% of the isolates were resistant to levofloxacin but were sensitive to tazobactam/piperacillin and cefmetazole. The most frequent genotype was CTX-M-9 detected in 36/45 (80%). A high degree of disability, antibiotic use within three months before sampling and post-tracheostomy were statistically significant risk factors. Tube feeding was also strongly correlated with ESBL colonization ($p < 0.001$) and associated with lower micro-organismic diversities. Our findings are the first to reveal a high prevalence of ESBL in the fecal samples of SMID individuals and suggest possible relationships between high degree disability, tube feeding and latest histories of antibiotic use.

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1. Introduction

Recent advances in medical treatment and devices have enabled the survival of individuals with severe motor and intellectual disabilities (SMID) [1]. In Japan, severely handicapped people can be hospitalized for life at medical and/or social welfare institutions, and their fees are covered by the national insurance system. These institutions share common properties with long-term care facilities, although they also play roles in caring for post neonatal intensive care unit infants.

Young and old, crippled people are highly susceptible to infectious disorders due to their inability to manage their personal hygiene. Strong deformation of the body often causes difficulty in sputum discharge and defecation and subsequent infections. In addition, long-term group life care increases the risk of nosocomial infections. Although every patient with SMID is not immunocompromised, various antibiotics are frequently administered both for therapeutic and preventive purposes. However, such treatment induces intestinal microbial substitution and increases the risk of antibiotic resistance.

Among various forms of antibiotic resistance, enterobacteria acquiring antibiotic resistance may threaten a patient's life because they can easily translocate into the blood circulation [2]. Moreover, habitual constipation and intestinal motility disorders in SMID patients enlarge the intestinal cavity and make the mucosal barrier fragile.

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Bacteria that produce extended-spectrum beta-lactamase (ESBL) are resistant to several antibiotics, including third-generation cephalosporin. ESBL were first reported in Germany in 1983 [3] and showed worldwide distribution in the early 2000s, with numbers that continue to increase, especially in Southeast Asia and Eastern Mediterranean countries [4]. Recent reports also describe the prevalence of ESBL colonization in companion animals [5]. Although ESBL-producing bacteria asymptomatically colonize and are not pathogenic to healthy individuals, they occasionally induce fatal outcomes by sepsis due to their resistance to most antibiotics. As a result, surveillance of ESBL in general hospitals and long-term care facilities (LTCF) has increased [6–12]. Residents of LTCF share similar characteristics with SMID individuals, and the rate of ESBL colonization in residents of LTCF has been shown to be highly varied, ranging from close to zero up to 64.0% [12]. To the best of our knowledge, no studies have investigated ESBL colonization in individuals with SMID, and a high prevalence of ESBL is expected. Drug-resistant bacteria are a critical problem for SMID patients; therefore, it is important to survey and characterize their ESBL carrier status in order to select appropriate antibiotics.

In this study, we investigated the frequency of ESBL-producing enterobacteria among patients with SMID in a single institution and analyzed possible risk factors for their colonization.

2. Materials and methods

2.1. Study design and patients

This study was performed at Ashikaga-no-mori Ashikaga Hospital, a medical and social welfare institution located in the Northern Kanto region of Japan. One-hundred fifty-nine patients were admitted, with 12 rooms holding 2 persons and 35 rooms holding 4 persons. These patients had been hospitalized for life, as some had no family members who could take care of them and some needed daily medical care, such as tube feeding (40.1%), post-tracheostomy (25.2%), ventilator support (14.6%), etc. Fig. 1 shows the calculated Oshima's classification scores for intelligence quotient and physical abilities [13]. Ninety-three percent of the patients had scores of 1–4, indicating SMID. The study period was one year (March 2016 to February 2017). Informed consent and IRB approvals (Nihon University, No. 27-3-0; Ashikaga-no-mori Ashikaga Hospital, No. 27-2) were obtained before sampling. Written informed consent was obtained from the parents of the families because of the patients' severe mental retardation.

2.2. Sample collection

Among the total of 159 patients, informed consent was obtained from 146 subjects before sampling. Fecal samples were collected throughout 9 months (March 2016 to December 2016). Fecal samples were taken once from each patient after informed consent was obtained. Each 200–400 mg fecal sample was obtained from a paper diaper of the patient. To avoid contamination, medical staff performed standard preventive measures when they collected samples and stored them in a −80 °C freezer.

2.3. ESBL identification

Bacterial isolates were analyzed with an automatic identification system, the MicroScan WalkAway 40 Plus System (Beckman Coulter, Pasadena, CA, USA). The Neg EN Combo1J panel was employed for bacterial identification and ESBL screening. ESBL expression and drug susceptibility were confirmed with the Neg MIC panel 3.31E for testing ceftazidime and cefotaxime with and without clavulanic acid according to the guideline of the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards) [14]. We defined patients with culture-positive fecal samples as ESBL-positive group.

2.4. Genotype identification of ESBL

Under the approval of the Biosafety Committee of Nihon University, these strains were handled as risk group 2 according to the laboratory biosafety manual of the World Health Organization, Geneva, 2004. DNA was directly extracted from 100 to 200 mg stool samples with a QIAamp DNA Stool Mini Kit (QIAGEN, Valencia, CA, USA). The genotypes of ESBL, including the CTX-M-1 group, CTX-M-2 group, CTX-M-9 group, TEM-type and SHV-type, were identified by PCR using previously reported primers [15,16]. The amounts and quality of the DNA samples were examined with a NanoDrop 1000 (Thermo Fisher Scientific Inc., Waltham, MA, USA). The PCR assays were performed with TaKaRa Ex Taq® (Takara Bio, Tokyo, Japan) in a 25 μL total amount of reaction mixture consisting of 19.9 μL distributed water, 2.0 μL 10× Taq polymerase buffer, 1.6 μL dNTP mixture, 0.2 μL of each primer (100 pmol/μL), 0.1 μL Taq DNA polymerase, and 1 μL of template DNA. PCR reactions were performed under the conditions recommended by Takara Bio using a Veriti™ thermal cycler (Applied Biosystems, Foster City, CA, USA). The PCR for each DNA was performed in duplicate to ensure the results. PCR products were electrophoresed with a microchip device, MultiNa (Shimazu corporation, Kyoto, Japan), followed by direct DNA sequencing with a 3730 xl DNA Analyzer and BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) performed at Eurofins Genomics Laboratory (Tokyo, Japan). The obtained sequences were searched in the Basic Local Alignment Search Tool using GENETYX® ver.13 software.

2.5. Data collection and statistical analysis

The bacterial profiles were compared with the patients' clinical information including age, sex, length of hospitalization, Oshima's classification and nutritional state, i.e., oral intake of a regular meal or tube feeding with an enteral nutrient. We also investigated the history of antibacterial drug use within the past three

Fig. 1
Oshima's classification, representing the degree of disablity. The patients with 1-4 scores are defined as SMID.

21	22	23	24	25	IQ 70-80
20	13	14	15	16	50-70
19	12	7	8	9	35-50
18	11	6	3	4	20-35
17	10	5	2	1	-20
Able to run	Able to walk	Have difficulty walking	Able to sit	Lay down	

months before sampling, tracheostomy, and probiotic use as possible risk factors of ESBL colonization. The patients were divided into two groups: an ESBL-positive group and ESBL-negative group. The data were not assumed to follow a specific distribution type; therefore, nonparametric tests were performed. The data were analyzed using SPSS software (ver. 17.0; AN IBM® COMPANY, Chicago, IL, USA). Because they were digitized, the patients' age and length of hospitalization were examined using the Mann-Whitney test. Because they were divided into two groups, the variables sex, disease history, Oshima's classification, history of antibacterial drug use, post-tracheostomy, nutrition and probiotic use were assessed using the χ^2 test. The significance of the p scores was set at $p < 0.05$.

2.6. Microbiome analysis

Eight samples were randomly selected from each of the patients fed via a feeding tube or fed orally. The microbiomes of the samples were analyzed by thermal restriction fragment length polymorphisms (T-RFLP) [17] at the TechnoSuruga Laboratory (Shizuoka, Japan). The samples were analyzed by the Nagashima method employing an original primer-enzyme combination that targeted the 16S rRNA gene. Fragments of approximately the same length are referred to as an operational taxonomic unit, which is represented as a relative ratio for each group of intestinal bacteria. *Bifidobacterium*, *Lactobacillales*, *Bacteroides*, *Prevotella*, *Clostridium* cluster IV, *Clostridium* subcluster XIVa, *Clostridium* cluster IX, *Clostridium* cluster XI, and *Clostridium* cluster XVIII were measured. The diversity of the flora was analyzed with the Simpson index and Shannon-Wiener index [18]. The data were compared between the tube fed patients and those fed orally as well as between the ESBL-positive and ESBL-negative groups using the Mann-Whitney test.

3. Results

The patient profiles are shown in Table 1. ESBL-producing bacteria were isolated in 45/146 (31%) samples. There were 46 strains of ESBL-producing bacteria, with one sample containing ESBL-producing *Escherichia coli* (*E. coli*) and ESBL-producing *Proteus mirabilis*. In these strains, *E. coli* was the most frequently detected pathogen in 38/46 (83%) samples. The other pathogens were *Proteus mirabilis* (7%), *Klebsiella pneumoniae* (6%) and *Klebsiella oxytoca* (4%).

Drug susceptibility testing showed that 82% of the isolates were resistant to levofloxacin. These strains also tended to be resistant to minocycline but showed good sensitivity to tazobactam/piperacillin. These strains were also susceptible to cefmetazole. Carbapenem-resistant bacteria were not detected (Table 2).

Genotype analysis revealed that the CTX-M-9 group was present in 36/45 (80%) of the samples. The CTX-M-1 group was detected in 4 samples, and the CTX-M-2 group was not detected in any of the samples. Direct DNA sequencing confirmed the identification of these CTX-M groups. It also revealed that the SHV-type was found in 15 out of 16 samples; an additional sample showed a false

Table 1
Patient characteristics and statistical analysis.

	All subjects n = 146	ESBL-positive n = 45	ESBL-negative n = 101	P value
Age (year)				
	36.82 (4–77) 39.50[a] 17.130[b]	32.4 (4–68)	38.79 (4–77)	<0.05[c]
Sex				
Male	80	25	55	
Female	66	20	46	N.S.[d]
Disease history				
Cerebral palsy	64	15	49	N.S.[d]
Post-encephalitis/post-encephalopathy	24	12	12	<0.05[d]
Epilepsy/severe intellectual development disorder	14	5	9	N.S.[d]
Brain malformations	10	2	8	N.S.[d]
Chromosomal abnormalities	6	2	4	N.S.[d]
Abuse/drowning/traffic accident	6	5	1	<0.05[d]
Other[e]	22	4	18	N.S.[d]
Length of hospitalization (years)				
	23.3 (0–64) 14.67[a] 18.070[b]	17.6 (0–63)	25.8 (0–64)	<0.05[c]
Oshima's classification				
Score 1	89	38	51	<0.001[d]
Score ≥ 2	57	7	50	
Antibiotic use within 3 months				
Yes	57	26	31	
No	89	19	70	<0.01[d]
Post-tracheostomy				
Yes	31	23	8	
No	115	22	93	<0.001[d]
Nutrition				
Oral intake	84	8	76	
Tube feeding	62	37	25	<0.001[d]
Probiotic use				
Yes	12	2	10	
No	134	43	91	N.S.[d]

[a] Median value.
[b] Standard deviation.
[c] Evaluated using the Mann-Whitney analysis.
[d] Evaluated using the χ^2 analysis.
[e] Other: Muscular dystrophy, Multiple malformation syndrome, Rett syndrome, Tuberous sclerosis, Lesch-Nyhan syndrome, Sotos syndrome, or Cornelia de Lange syndrome.

Table 2
Results of the drug susceptibility testing of ESBL isolates.

Antibiotics	MIC[a] distribution (n = 46)									
	<0.5	1	2	4	8	16	32	64	MIC_{50}	MIC_{90}
Sulbactam/Ampicillin				2 strains[b] (<8[c])		4 (16), 40 (>16)			>16	>16
Tazobactam/Piperacillin					45 (<16)			1 (>64)	<16	<16
Sulbactam/Cefoperazone					18 (<16)		20 (32), 7 (>32)		32	>32
Cefmetazole	7	22	13	2	2				1	2
Cefepime			2	7	1	5	4 (32),27 (>32)		>32	>32
Meropenem	45			1					<0.5	<0.5
Amikacin			41 (<4)		5				<4	<4
Gentamicin		23 (<2),1 (<4)		1 (<8)		21 (>8)			<2	>8
Levofloxacin	5	3		38 (>4)					>4	>4
Minocycline		14 (<2)		16	11 (8), 5 (>8)				4	>8
Fosfomycin			31 (<4)		1 (<16), 3 (16), 11 (>16)				<4	>16
Sulfamethoxazole – Trimethoprim			21 (<2), 25 (>2)						>2	>2

[a] MIC: minimum inhibitory concentration.
[b] Number of strains.
[c] MIC distribution.

positive for the LEN-type beta-lactamase *Klebsiella pneumoniae*. Although the TEM-type was detected in 35 samples, direct DNA sequencing revealed that these strains were not ESBL and instead were of the TEM-1 type (Table 3).

The results of the statistical analysis are shown in Table 1. The Mann-Whitney analysis proved that an older age and long hospitalization period were not correlated with ESBL colonization. The χ^2 analysis showed significant differences in regards to a high degree of disability, antibiotic usage within 3 months before sampling, post-tracheostomy and nutrition between the ESBL-positive and ESBL-negative groups. Notably, tube feeding with an intestinal nutrient was strongly correlated with ESBL colonization ($p < 0.001$).

Microbiome analyses are shown in Fig. 2. Although the population of "healthy bacteria" such as *Bifidobacterium* and *Lactobacillales* varied, patients with oral intake of a regular meal showed a significantly higher diversity of the microbiome than those with tube feeding. Among these samples, Nos. 1–8 and No. 16 were ESBL-positive samples. The ESBL-negative group showed higher diversity than the ESBL-positive group (Table 4).

4. Discussion

Recently, ESBL surveillance has increased in general hospitals and long-term care facilities. However, such investigation in individuals with SMID is rare. Therefore, we examined the frequency of ESBL-producing enterobacteria among SMID patients in a single institution and analyzed possible risk factors for ESBL colonization.

Our data revealed frequent isolation of ESBL-positive bacteria from patients with SMID. In Japan, the Ministry of Health, Labor and Welfare surveillance of multicenter institutions showed that the isolation rate of third-generation cephalosporin-resistant *E. coli* was 24.5% [19]. As for fecal specimens only, Nakamura, et al. reported that the carriage rate of ESBL-producing *E. coli* in the inpatient group was 12.5% [10]. Considering these data, the detection rate of 31% obtained in this study was higher than expected.

Drug susceptibility testing suggested that the carrier state was the result of routine antibiotic administration, as ESBL-producing bacteria have acquired resistance to commonly used antibiotics. In this study, frequently prescribed antibiotics were observed: such as levofloxacin (24%), cephalosporin (23%) and minocycline (16%). These oral antibiotics are often administered to individuals with SMID because of the difficulty in maintaining an intravenous route due to their limp position and rigidity. Thus, the selective pressure of these oral antibiotics likely led to the high rate of fluoroquinolone-resistant ESBL. Therefore, these antibiotics must be monitored for drug resistance.

Recent domestic [20] and foreign [21] reports recommend tazobactam/piperacillin and cefmetazole as first-line chemotherapy for ESBL infections. Our results are comparable to these findings because we did not encounter ESBL resistance against these antibiotics in this study. However, the susceptibility of ESBL to beta-lactams with/without a beta-lactamase inhibitor remains controversial. Fukuchi et al. reported cefmetazole-sensitive ESBL [22], while other strains, including CTX-M-15, were resistant [23]. In this sense, tazobactam/piperacillin and cefmetazole are also prescribed without strong evidence. For these reasons, carbapenem is used as the first choice for severe ESBL infections. However, considering the emergence of carbapenem-resistant bacteria, we propose reducing the use of carbapenem because most ESBL are susceptible to conventional antibiotics, including tazobactam/piperacillin and cefmetazole.

In this study, we employed total DNA extracted from stool directly for the ESBL genotyping. Because, we intended to detect every bacterium including some strains which were difficult to be separated by culture. This method of course has some limitations. First, we must avoid huge background of human genomic DNA and commensal microorganisms. Second, we cannot differentiate any bacteria which produce one or more different ESBLs. First problem was resolved by high yield DNA extraction system designed for prokaryotes. Second problem must be analyzed in future study. If the total DNA collected from fecal samples contains non-ESBL TEM-1 gene possessed by the majority of *E. coli*, the TEM ESBL gene possessed by minor population would be masked. In addition, two or more ESBL-producing genes were detected in 27% of the samples in this study. In this sense, we need to improve the sensitivities of our system to detect various ESBL genes possessed by minor populations of bacterial populations.

Table 3
Results of the ESBL genotype detection.

	Escherichia coli (83%)[a]	*Proteus mirabilis* (7%)	*Klebsiella pneumoniae* (6%)	*Klebsiella oxytoca* (4%)	Total
CTX-M-1 group	2	0	1	1	4
CTX-M-2 group	0	0	0	0	0
CTX-M-9 group	32 (+1)[b]	0 (+1)[b]	1	2	36 (+1)[b]
TEM	0	0	0	0	0
SHV	10	0	3	2	15
Other	4	2	0	0	6

[a] Frequency of each bacterial species.
[b] One sample had two ESBL-producing strains: *Escherichia coli* and *Proteus mirabilis*. Which bacteria or both carried CTX-*M*-9 was unidentifiable.

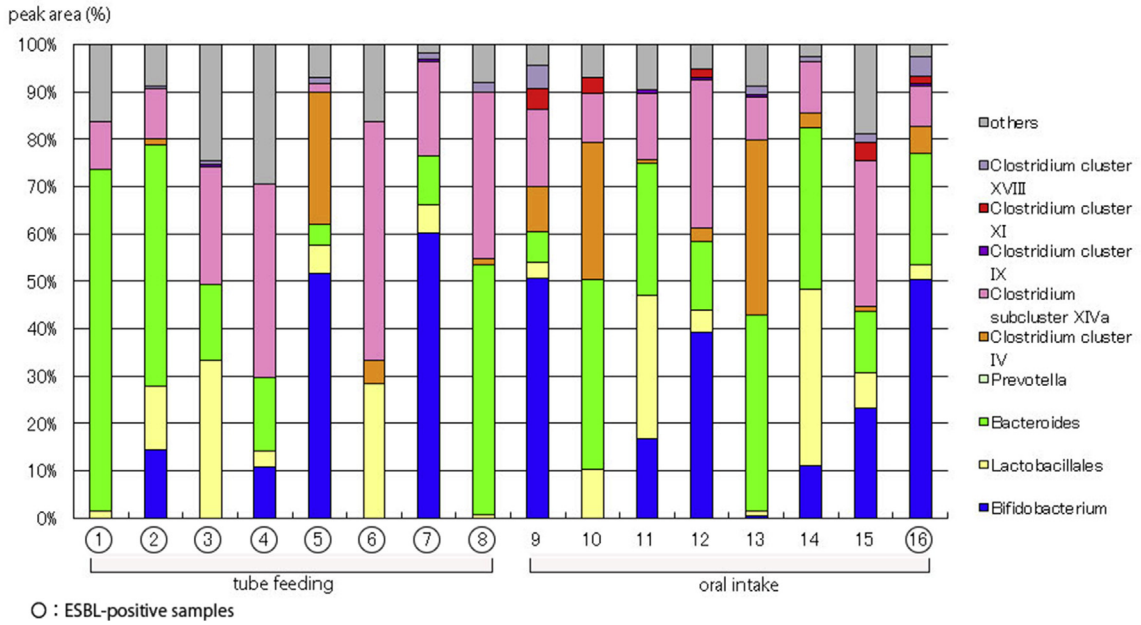

Fig. 2. Results of the intestinal flora measurement, measured by T-RFLP analysis. The samples were randomly selected from patients fed via a feeding tube (Nos. 1–8) and patients fed orally (Nos. 9–16); the latter group showed a higher diversity of the microbiome. This result was obtained by calculating the Simpson index (Mann-Whitney analysis, $p < 0.05$) and was confirmed using the Shannon-Wiener index (Mann-Whitney analysis, $p < 0.01$). Among these samples, Nos. 1–8 and No. 16 were ESBL-positive samples.

As shown in Table 4, the most frequent genotype observed in this study was CTX-M-9 group. This finding was consistent with other reports employing separated bacteria indicating CTX-M-9 as the most frequent and increasing genotype among Asian countries [4,8]. However, we cannot exclude the possibility of hospital-acquired infection of CTX-M-9-positive ESBL without other genetic information. In order to identify molecular epidemiology of these genes, we need to analyze their genetic backgrounds by isolation of bacterial strains and subsequent pulsed-field gel electrophoresis.

Clinical risk factors have been reported for the colonization of ESBL [12,24], such as older age, male sex, physical disability, invasive medical devices, previous administration of antibiotics within the preceding year, surgical procedures within 30 days, and urinary tract infections. However, previous surveillance efforts have targeted patients who were hospitalized in general hospitals or LTCF and were different from SMID patients who have a wide range of medical and social conditions. For example, we observed no correlation between the presence of ESBL and older age or a longer period of hospitalization. We speculate that some hospitalized patients might be colonized with ESBL prior to hospitalization.

Table 4
Microbiome diversity analysis.

(a) Nutrition			
	Tube feeding	Oral intake	P value[b]
Shannon-Wiener index	1.760 ± 0.104[a]	2.199 ± 0.057	<0.01
Simpson index	0.629 ± 0.033	0.723 ± 0.015	<0.05
(b) ESBL			
	Positive	Negative	P value[b]
Shannon-Wiener index	1.803 ± 0.101[a]	2.207 ± 0.066	<0.01
Simpson index	0.635 ± 0.029	0.730 ± 0.015	<0.05

[a] Mean ± standard deviation.
[b] Evaluated using the Mann-Whitney analysis.

As expected, there was a significant correlation between disease severity based on Oshima's classification and the prevalence of intestinal ESBL. In addition, we observed a high prevalence of ESBL-positive bacteria among post-tracheostomy patients. However, each patient with a tracheostomy was fed through a tube, and we therefore cannot determine tracheostomy to be an independent factor. The preventive potential of probiotics has also rarely been investigated due to the small population size and sample availability.

Interestingly, we observed a strong correlation between ESBL colonization and tube feeding with an intestinal nutrient ($p < 0.001$). This finding can be explained as follows. First, these patients are likely treated more frequently with oral antibiotics for their compromised states. Second, prolonged infusion of nutritional supplements may lead to drastic changes in intestinal flora, increasing resistant bacteria.

We next assessed the possible floral changes in an environment with ESBL-producing bacteria using a microbiome analysis. Although no reports in the English literature suggest if there are dynamic changes in the microbiome or the presence/absence of antibiotic resistance, frequent and high dose use of single or multiple antibiotics might affect bacterial populations in the gastrointestinal tract. In addition, we also investigated the possible microbiome differences between tube fed patients and those fed orally. Our results revealed that all of the patients with a feeding tube had ESBL colonization. Their microbiome also had lower diversity than the patients fed orally, most of which did not have ESBL colonization. However, it is difficult to clarify whether this relationship is caused by or the result of antibiotic use and tube feeding. We are trying to eradicate ESBL through more strict sterilization, restricted use of antibiotics and the prescription of probiotics. If these procedures effectively eradicate ESBL, then we can obtain better hygiene conditions for SMID patients.

There are some limitations of this study. First, this was a single-institution study, and the sample size was small. For SMID patients, medical and nursing care protocols for antibiotic usage are different at every institution. Thus, we must differentiate possible causes of

ESBL colonization in different situations. The varied backgrounds of SMID patients also make it difficult to align the baseline when performing statistical analyses. Second, this was a retrospective study. The clinical state of each individual with SMID is influenced by many factors, including nutritional status and antibiotic administration. Finally, with single point sampling, we could not discriminate between transient and long-lasting changes of intestinal flora. In this sense, repeated sampling and precise analysis of clinical information might improve our understanding of the intestinal flora in severely handicapped persons.

In conclusion, this study is the first to reveal the high prevalence of ESBL-producing bacteria in SMID patients. To prevent the spread of infection, an emphasis on standard and contact precautions is recommended. ESBL strains tend to acquire resistance to frequently used antibiotics; therefore, we suggest the importance of regular surveillance of antibiotic use and ESBL colonization. Among the risk factors analyzed, tube feeding with intestinal nutrients was strongly correlated with ESBL colonization. However, a multicenter study is required in future to obtain a more detailed isolation rate of ESBL in SMID patients. The relationship between the diversity of the microbiome, tube feeding and resistant bacteria should also be investigated with larger sample sizes.

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Conflicts of interest

None.

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和文の要約

重症心身障害児者の糞便では ESBL 産生菌の頻度が高い

【背景】
新生児医療や救命救急医療の進歩に伴い、多様な基礎疾患を持つ重症心身障害児者（重症児者）の長期生存が可能となった。重症児者は易感染性から抗菌薬を投与される機会が多く、また重症化を危惧し広域抗菌薬が選択されることが多いため、多剤耐性菌の出現が問題となる。
多剤耐性菌のひとつである基質特異性拡張型 β ラクタマーゼ（extended-spectrum beta-lactamase: ESBL）産生菌の検出数は年々増加傾向にあり、一般病棟や長期療養施設におけるサーベイランスは多数報告されている。しかし重症児者における多剤耐性菌の研究は調べうる限りない。日本には国立の重症児者施設が存在するが他国においては稀であり、重症児者を対象とした研究自体が少ないからである。
ESBL 産生菌の保菌のリスク因子は一般的に、高齢、長期入院、デバイスの留置、セフェム系抗菌薬使用などが挙げられるが、重症児者は近年高齢化が進み、入院は長期に及んでいる。抗菌薬を投与される機会も多いため、これまで報告されているリスク因子を多く持つ集団であり、耐性菌の検出率が高いことが予測された。
また、重症児者は腸管運動の機能障害に伴い腸閉塞の発症頻度が高く、腸管内圧が亢進した際は、腸管内の細菌が容易に腸管粘膜を通過して血液中に入り、敗血症や敗血性ショックを引き起こす。多剤耐性菌による敗血症は致命的であり、事前に腸管内に ESBL 産生菌を保菌しているかを把握することは重要である。
本研究では、重症児者の糞便における ESBL 産生菌の検出状況や薬剤感受性、遺伝子型、保菌のリスク等について解析した。

【方法】
対象は、単一の重症心身障害者施設に入所する患者 146 名である。同施設と日本大学医学部の倫理委員会承認のもと、同意が得られた患者から無症候時の糞便を採取した。ESBL 産生菌は CLSI の基準に基づき、スクリーニング試験および確認試験で同定した。薬剤感受性検査には MicroScan WalkAway 40 Plus System の MIC panel を用いた。遺伝子型は TEM 型、SHV 型、CTX-M-1 group、CTX-M-2 group、CTX-M-9 group について、既報のプライマーを用いた PCR 法と電気泳動により同定し、配列は Direct DNA sequence で確認した。
臨床的背景は年齢、性別、入院期間、大島分類のスコア、経管栄養の有無、3 か月以内の抗菌薬使用歴、気管切開の有無、整腸剤使用歴について後方視的に調査し、統計解析を行った。また、経口摂取群、経管栄養群からそれぞれ無作為に患者を選び、腸内常在細菌叢を解析した。

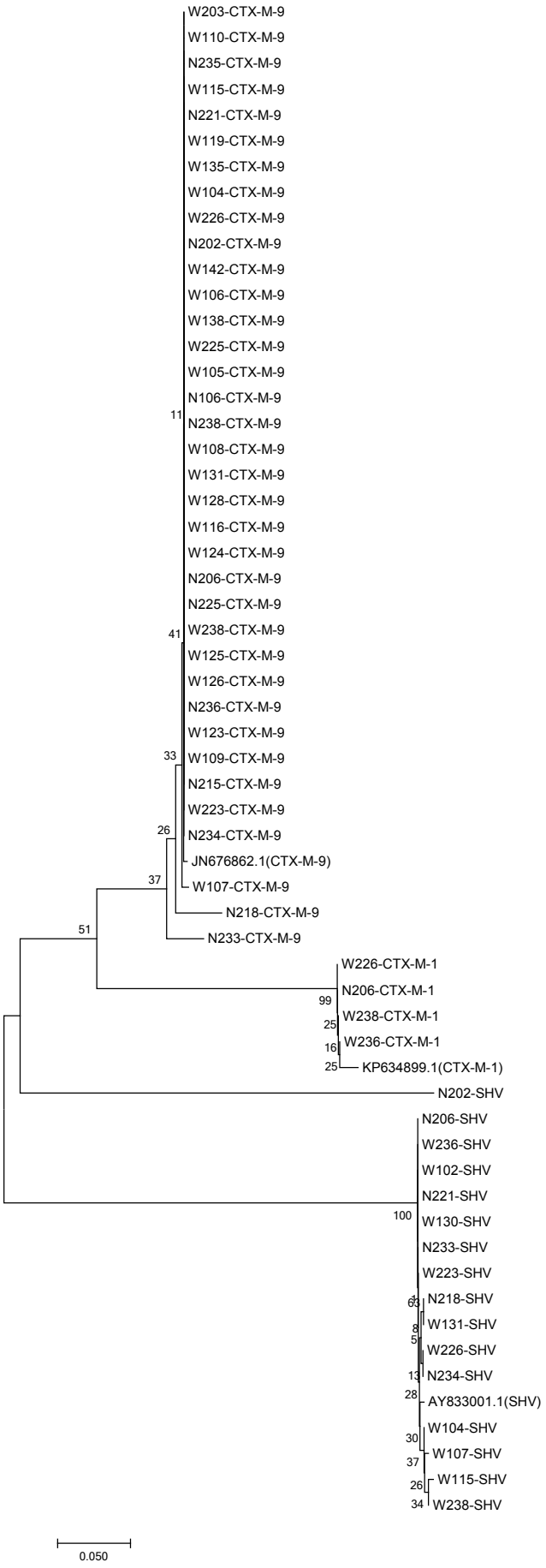
【結果】
ESBL 産生菌は全糞便検体の 31%から検出された。83%の菌種は *Escherichia coli* であった。ほとんどの菌株はピペラシリン/タゾバクタムおよびセフメタゾールに感性があり、カルバペネム耐性株は検出されなかった。しかしレボフロキサシンに耐性を示す株は 82%に及び、ミノマイシンにも耐性傾向を認めた。遺伝子型は CTX-M-9 group が 80%を占めた。
対象者の年齢は平均 36.8 歳（4-77 歳、中央値 39.50、標準偏差 17.130）、入院期間は平均 23.3 年（0-64 年、中央値 14.67、標準偏差 18.070）、自然食を経口摂取しているのは 84 名、経管栄養を行っているのは 62 名であった。
ESBL 産生菌陽性者の方が、低年齢、入院期間は短く、重症度は高いことが示された（Mann-Whitney 検定）。
ESBL 産生菌の保菌は 3 か月以内の抗菌薬使用歴、気管切開のある患者に多く、特に経管栄養とは強い関連を認めた（$p<0.001$、χ^2検定）。
整腸剤は全例ビフィズス菌（ラックビー微粒）を投与していたが、ESBL 保菌との相関は認めなかった。
抗菌薬の予防的投与は、クラリスロマイシンが 12 名（ESBL 陽性 9 名、陰性 3 名）、エリスロマイシンが 4 名（ESBL 陽性 3 名、陰性 1 名）に、いずれも慢性気管支炎や副鼻腔炎に対して処方されていた。ST 合剤は 2 名（ESBL 陽性 2 名、陰性 0 名）に、尿路感染症予防のために使用されていた。その他、臨時の抗菌薬投

与では第三世代セフェム系、レボフロキサシン、ミノマイシンの使用が多く、呼吸器感染症、尿路感染症、皮膚感染症に対する使用であった。
腸内常在細菌叢解析では、経管栄養群および ESBL 産生菌陽性群において、有意に菌叢の多様性が低いことが分かった。

【考察】
JANIS の報告や一次施設のサーベイランスと比較すると、重症児者における ESBL 産生菌の検出頻度は高いことがわかった。重症児者は過去に集中治療を行った背景を持つ患者が多く、その際多くの抗菌薬投与やデバイス留置を行い、ESBL 産生菌を保菌した可能性がある。また急性期病棟でのケアによる院内感染曝露も考えられる。重症児者は急性期を過ぎても、易感染性から抗菌薬を投与される機会が多く、重症化を危惧し第三世代セフェムやより広域スペクトラムの抗菌薬が選択されることがある。以上が ESBL 産生菌の検出頻度が高かった理由と考えられるが、医療者の手を介した院内伝播の可能性も否定できない。
ESBL 産生菌の保菌のみでは抗菌薬投与は不要であり、有症時の適切な抗菌薬投与と、感染拡大の予防が重要である。
ESBL 保菌者の感染発熱時は、カルバペネム系抗菌薬が第一選択となるが、当施設の ESBL 産生菌はピペラシリン/タゾバクタムやセフメタゾールでも治療可能であるため、重症度に応じてこれらの薬剤を検討することが必要である。
また、重症児者は点滴ルートの確保が困難であるため、治療には内服製剤の第三世代セフェム系やレボフロキサシン、ミノマイシンが頻用され、これら薬剤の選択圧を強く受けていると考えられた。特に当該施設では、レボフロキサシン耐性 ESBL 産生菌が増加していた。使用頻度の高い抗菌薬を把握し、事前に ESBL 産生菌の薬剤耐性状況を把握することは、適切な抗菌薬選択に繋がると考える。
一方で耐性菌のさらなる増加を防ぐためには、抗菌薬の適正使用、つまり重症度に応じた抗菌薬の de-escalation や、感染巣に応じた治療期間を心掛けること、定期的な監視培養を行い、保菌状況を把握することが必要である。
遺伝子型は CTX-M-9 group が最も多く、本邦のサーベイランスと一致した。遺伝子型の偏りがあり、分子系統樹解析でも単独 ESBL のアウトブレイクを否定することは困難であったが、より詳細な検討には検体採取方法を変更し、MLST 解析や PFGE 解析が必要である。
ESBL 産生菌の一般的な保菌リスクとして、高齢、長期入院が挙げられるが、本研究では ESBL 産生菌陽性者の方が低年齢で、入院期間が短く、必ずしも一般的なリスクが当てはまらないことが示された。
重症度の高い患者や抗菌薬使用歴のある患者、特に経管栄養を行っている患者は ESBL 産生菌を保菌している可能性が高いため、急性期の治療における抗菌薬選択に配慮が必要である。
ESBL 産生菌が経管栄養と強い相関を示した理由として、経管栄養群は経口摂取群よりもより重症度が高く、抗菌薬投与や院内感染暴露が多かった可能性が挙げられる。当施設における経管栄養の注入方法を確認したところ、チューブやバッグの共用はなく、院内感染の原因となる手技は指摘できなかった。引き続き標準予防策を徹底することが重要である。
腸内細菌叢の検討では、ESBL 産生菌の保菌および経管栄養が腸内常在細菌叢の多様性の低下に関わることが示唆された。長期に及ぶ経管栄養剤の使用により、腸内細菌叢が変化し、耐性菌が除菌されにくい腸内環境が形成された可能性も考えられるが、この仮説の証明にはさらなる検討が必要である。
ESBL 産生菌は菌種を越えて伝播するため、接触感染予防として手袋やガウンの着用を徹底すべきである。排泄物の処置をした後には必ず手袋を変えてから、その他の部位の処置をすること、またドアノブや手すりを介した院内伝播の可能性も考慮し、処置前後の手指消毒を改めて徹底することが必要である。

【結論】
本研究は、重症児者において ESBL 産生菌の検出頻度が高いことを示した初めての報告である。サーベイランス結果に基づく抗菌薬の適正使用と、感染予防対策が重要である。より詳細な検討には多施設研究が望まれる。また、耐性菌と経管栄養、腸内細菌叢の多様性低下の関係性については、より多くの検体を用いて層別化を図り解析する必要がある。

系統樹解析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W110-CTX-M-9
N235-CTX-M-9
W115-CTX-M-9
N221-CTX-M-9
W119-CTX-M-9
W135-CTX-M-9
W104-CTX-M-9
W226-CTX-M-9
N202-CTX-M-9
W142-CTX-M-9
W106-CTX-M-9
W138-CTX-M-9
W225-CTX-M-9
W105-CTX-M-9
N106-CTX-M-9
11
N238-CTX-M-9
W108-CTX-M-9
W131-CTX-M-9
W128-CTX-M-9
W116-CTX-M-9
W124-CTX-M-9
N206-CTX-M-9
N225-CTX-M-9
41
W238-CTX-M-9
W125-CTX-M-9
W126-CTX-M-9
N236-CTX-M-9
W123-CTX-M-9
33
W109-CTX-M-9
N215-CTX-M-9
W223-CTX-M-9
26
N234-CTX-M-9
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W107-CTX-M-9
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51
N233-CTX-M-9
W226-CTX-M-1
N206-CTX-M-1
99
W238-CTX-M-1
25
W236-CTX-M-1
16
25
KP634899.1(CTX-M-1)
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N206-SHV
W236-SHV
W102-SHV
N221-SHV
100
W130-SHV
N233-SHV
W223-SHV
N218-SHV
W131-SHV
W226-SHV
N234-SHV
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26
W115-SHV
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