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

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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.
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 years.
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