Risk factors of fecal colonization with extended-spectrum β-lactamase-producing Enterobacteriaceae in special nursing homes in Japan

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Abstract

Objective- Japanese welfare facilities for the elderly are called as special nursing home (SNH), providing conventional-type with group care or unit-type with individual care. We investigated the risk factors of fecal colonization with extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) of elderly who required care at SNH in Japan.

Methods- The feces discharged on diaper were obtained from the total of one-hundred residents with fecal incontinence in 9 SNHs located in Tokyo, Japan. The samples were cultured on ESBL selection agar and ESBL-E were determined by the antimicrobial susceptibility test and genetic analysis. The status of the residents and the characteristics of facilities, especially about the incontinence care were obtained by questionnaire methods. Statistical analysis was performed to determine the factors related to carriage of ESBL-E.

Results- ESBL-E was isolated from 53 of 100 SNH residents. Risk factors of colonization among the individual residents were not found. The prevalence of ESBL-E carriage was significantly higher in the 6 conventional-type facility than in the 3 unit-type facility (p=.015). The cart for diaper exchange was used in 5 of 6 conventional-type facilities in 9 SNHs, and their residents tended to show high of ESBL-E colonization rate. The residents living in unit-type facilities which do not use gloves for changing diaper tended to show high ESBL-E colonization rate than other 2 facilities using gloves.

Conclusions- It is suggested that using the cart for changing diaper has relation to carry ESBL-E. In the facilities using cart, revision of their methods of excretion care will be needed.

Keywords

Extended-spectrum β-lactamase-producing *Enterobacteriaceae*; fecal carriage; risk factor, Japanese special nursing home; facility of conventional-type and unit-type

Introduction

Extended-spectrum β-lactamase-producing *Enterobacteriaceae* (ESBL-E) is an emerging infectious species raising global concerns [1-6]. Many *Enterobacteriaceae* species are part of the endogenous bacterial flora of the intestinal tract in humans, which poses a challenge in efforts to prevent spread of ESBL-E as part of healthcare-related infection control.

It was reported that the isolation rate of cefotaxime-resistant *Escherichia coli* from clinical samples at Japanese medical institutions had increased from 1% in 2000 to 12.9% in 2014 [7, 8]. Furthermore, the isolation rate of ESBL-E was higher in inpatients than in outpatients, although an increase over time was observed in both patient groups [9]. Japan Nosocomial Infections Surveillance under the jurisdiction of the Ministry of Health, Labor, and Welfare (JANIS) was launched in 2000, but the surveillance system remains nonexistent at care facilities for the elderly.

Publicly-run care facilities for the elderly are categorized into three types based on the medical dependency and admission purpose of residents: sanatorium medical facilities, long-term healthcare facilities for the elderly, and special nursing homes for the elderly (SNH, welfare facilities for the elderly in need of long-term care) [10]. Of these, SNHs admit people who have difficulties living at home, and become living places as an alternative to home. A study investigating the colonization risk of ESBL-E found that old age, need for nursing care, being bedridden, history of antimicrobial use, history of hospitalization, and diabetes were among the risk factors for colonization [2, 11-15]. Thus, residents of SNH are estimated to be particularly at high risk of ESBL-E colonization.

Japanese SNH has two different types based on its structure: conventional-type and unit-type facilities. Conventional-type facilities generally consist of multi-bed rooms with two to four residents in one room and a team of several caregivers assists their activity of daily living, such as eating and taking a bath. Excretion care at conventional-type facilities is also often performed on a set time schedule, and to efficiently provide care for multiple residents, conventional-type facilities use diaper carts loaded with unused clean instruments as well as used soiled instruments. In contrast, at unit-type facilities, approximately nine residents are housed in individual rooms in a single unit, to which one or two caregivers are assigned. The resident's living environment is similar to home, and the care is performed in accordance with each resident’s character and life rhythm. In addition, a single caregiver provides one-on-one care to each resident and can change diapers at each excretion by the residents without using diaper carts. The aims of this study are to reveal the prevalence and types of ESBL-E in feces of elderly residents in Japanese SNHs and the risk factors of ESBL-E colonization, including personal factors, facility factors, and excretion-care methods.

Materials and Methods

Samples collection and ESBL-E screening culture

The samples of feces discharged on diaper were obtained from the total of one-hundred residents who were bedridden and using diaper for fecal incontinence in 9 SNHs located in Tokyo, Japan, between August to December in 2015. The feces discharged on diaper were sampled in the sterile tube and were cultured on ChromID ESBL agar (SYSMEX bioMerieux, France) for screening of ESBL-E within 6 hours after sampling. The colonies on the agar were isolated on Drigalski agar. All strains with different morphotypes were identified by MALDI-Biotyper, with methodology of the matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (Bruker Daltonics, Germany). This study had the approval of the ethics committee of Faculty of Health Care and Nursing, Juntendo University with approval number 27-15. The written consents from the residents and/or substitute family for them and the president of SNH were obtained.

Antimicrobial susceptibility test

For ESBL-E, *E. coli*, *Klebsiella* spp., and *Proteus mirabilis* isolated by screening culture, antimicrobial susceptibility tests for screening were performed using Dry-plate DP31 (Eiken Chemical, Japan) included 18 antimicrobials; piperacillin, cefazolin, cefotiam, cefotaxime, ceftazidime, cefepime, flomoxef, cefpodoxime, sulbactam/ampicillin, aztreonam, imipenem, meropenem, gentamicin, amikacin, sulfamethoxazole-trimethoprim, fosfomycin, minocycline, and levofloxacin. For confirming ESBL-E, Dry-plate DPD1 (Eiken Chemical, Japan) including cefotaxime, ceftazidime and cefpodoxime (with and without clavulanate for each of them), and ciprofloxacin, was used following the instruction manual. ESBL-E was determined by the criteria of Clinical and Laboratory Standards Institute [16].

Genetic analysis

For extracting DNA from ESBL-E, Cica Geneus DNA Extraction Reagent (Kanto Kagaku Co. Ltd., Japan) was used according to the manufacturer's protocol. β-lactamase genes, such as CTX-M, TEM, SHV, OXA, IMP, KPC, and VIM, were investigated using PCR methods as previously described [17, 18]. The CTX-M genes were identified by sequencing and Basic Local Alignment Search Tool search. Complete sequence match with known CTX-M could be accepted as identification of CTX-M.

Questionnaire methods

The status of the residents and the characteristics of SNHs were obtained by questionnaire methods. The questionnaire was composed of following contents: age, sex, required care level, urinary catheter placement, previous history of urinary tract infection, previous use of antimicrobials in the previous 3 months and previous hospitalization within 1 year before sampling of stool. For SNHs, information of capacity, type of facility (conventional-type or unit-type), procedure of excretion care such as wearing personal protective equipment,

frequency and timing of diaper change, using cart and role assignment for changing diaper, was obtained from the caregivers or the nurses in charge.

Statistical analysis

Characteristics of the residents and SNHs of the ESBL-E carriers were compared with of the non-carriers. The statistical analysis was performed using IBM SPSS ver.19. For univariate analysis, Chi-square test or Fisher's exact test were used for categorical variables, and a *p*-value of less than <.05 was considered as significant. Multivariate analysis using logistic regression analysis with stepwise method was performed to evaluate the risk factors of ESBL-E carriage. Odds ratios (OR) and 95% confidence interval (CI) were calculated.

Results

Detection of ESBL-E

A total of 57 ESBL-E strains were detected in the fecal samples 53 of 100 residents (53.0%) living in nine SNHs in the Tokyo metropolitan area. The following bacterial species were isolated from the residents; *E. coli* (n = 46), *K. pneumoniae* (n = 1), *K. oxytoca* (n = 2), and *P. mirabilis* (n = 8).

ESBL-E exhibited resistance to a high percentage of fluoroquinolones, with 97% of ciprofloxacin and 88% of levofloxacin, 95% of fourth-generation cephalosporins, and 75% of monobactams. Meanwhile, all detected ESBL-E were susceptible to carbapenems.

Of the 57 strains identified as ESBL-E, 54 strains (94.7%) possessed CTX-M. Specifically, CTX-M (n=44), CTX-M + OXA (n=1), CTX-M + SHV (n=1), CTX-M + TEM (n=8) were found by PCR, respectively. Among the 54 strains with CTX-M, 36, nine, and eight strains belonged to the CTX-M-9, CTX-M-1, and CTX-M-2 groups, respectively, whereas the group was not identified in three CTX-M strains. One strain was assigned to both of CTX-M-9 group and CTX-M-1 group (Figure 1). One strain possessed TEM alone, and no β-lactamase gene could be found in two strains.

CTX-M of the 54 strains were differentiated in detail by sequencing. CTX-M-2 (n=5), CTX-M-15 (n=5), CTX-M-18 (n=11), CTX-M-27 (n=27) and CTX-M-15 and -18 (n=1) were identified, respectively. CTX-M group was identified in CTX-M type could not be identified in 5 strains.

Personal factors for colonization with ESBL-E

Table 1 shows the relationship between personal factors collected from questionnaires and detection of ESBL-E. ESBL-E were detected at a significantly higher rate among the residents of conventional-type facilities than among the residents of unit-type facilities in detail, 45 of 75 residents (60%) at conventional-type facilities and eight of 25 residents (32%) at unit-type

facilities carried ESBL-E (p=.015). In other factors, no significant differences were observed between the ESBL-E carriers and non-carriers.

Characteristic of facilities participated in this study

Table 2 describes the characteristics of the facilities included in this study. No differences in basic characteristics of study participants, such as mean age, mean level of care needed, or presence of physical illness or dementia, were observed between the residents of the two facility types.

The percentage of ESBL-E carriers was significantly higher among the residents of convention-type facilities, as mentioned above (60% at conventional-type facilities and 32% at unit-type facilities, respectively, p=.015), but the percentage of the carriers varied from 25% to 100% among convention-type facilities, and from 22% to 66% among unit-type facilities.

The procedures of changing diapers also varied among the facilities. All 6 conventional-type facilities carried out scheduled rounds for changing diapers several times a day, and the 5 facilities (83.3%) used diaper carts, loading clear unused materials and trash boxes to discard used diapers as well. All the conventional facilities used disposable gloves when they changed diapers, but in contrast, all 3 unit-type facilities did not use diaper carts, and tended not to use personal protective equipment for changing diapers, even though a facility which did not use any protective equipment seemed to show relatively higher percentage of ESBL-E carriers.

Discussion

This study found that 53% of residents among Japanese SNH residents in need of nursing care carried ESBL-E, with CTX-M as the major ESBL type. The prevalence of ESBL-E carriers was significantly higher among residents living in convention-type facilities.

The prevalence of ESBL-E among SNH residents in this study was reasonable, compared with the previous studies investigating facility residents, ranging widely from 3.4 % to 71.6 % [2, 11, 13, 19]. However, compared with the 12.9 % isolation rate of cefotaxime-resistant *E. coli* in clinical samples reported in the 2014 surveillance of medical institutions in Japan, the prevalence of ESBL-E carriers was higher among the participants of our study [7]. Fecal incontinence and need of nursing care were recognized as related factors for colonization with ESBL-E, so the participants of this study reflected high-risk population of ESBL-E carriage [2, 13, 20].

The 2007 Guideline for Isolation Precautions issued by the US Centers for Disease Control and Prevention states that ESBL-E is the subject to implement contact precautions [21]. Among the subjects investigated in this study, none of the carriers of ESBL-E exhibited infection symptoms; thus, these carriers usually do not have chance of bacterial testing at the time of hospitalization with causes unrelated to infection. However, medical institutions should

anticipate the possibility of colonization with resistant bacteria in hospitalized residents of SNH, based on the high prevalence of ESBL-E carriers among the residents of long-term care facilities, for successful prevention of their nosocomial spread [22].

The major ESBL-coding gene in Japan is CTX-M [4, 8, 9, 17, 23-27], and in this study, CTX-M was found in 94.7 % of ESBL-E. The most prevalent CTX-M type found in each facility was different from each other. Furthermore, detection rates and CTX-M types of ESBL-E differed between facilities, raising concerns of the possibility of horizontal transmission in each facility. Future studies using molecular epidemiological analysis are needed to verify horizontal transmission within facilities.

Among the SNH residents investigated in this study, few had used antimicrobials within a year and no relationship between antimicrobial use and ESBL-E carriage was found. However, third-generation cephalosporins and fluoroquinolones accounted for the majority of the antimicrobials used in the present study (date not shown). It is known that prescription of fluoroquinolones for treatment of SNH applies selective pressure and may increase resistance to fluoroquinolones [6, 19, 22]. The ESBL-E detected in this study showed resistance to several antimicrobials, including β-lactams and fluoroquinolones, suggesting that fluoroquinolone resistance is becoming the norm for ESBL-E. Unnecessary use of third-generation cephalosporins and fluoroquinolones should be avoided not to increase ESBL-E among residents in SNH [28, 29].

Our results did not identify personal risk factors of SNH residents for colonization with ESBL-E. In addition to old age and need for nursing care, personal risk factors of colonization with ESBL-E include use of antimicrobials in the last 3 months, previous history of hospitalization in the last year, indwelling urinary catheter, previous history of urinary tract infection, and diabetes [2, 11-15]. Dementia is also a risk factor of carrying multidrug-resistant organisms [22, 30]. The small number of study participants with such complicated risk factors in this study was likely to underlie our findings.

We found that colonization with ESBL-E was significantly more frequent among the residents of conventional-type facilities (60%) than those resided at the unit-type facilities (32%). Diaper carts are used in the most of conventional-type facilities in this study and good for the efficiency of excretion care for groups, but are likely linked to the spread of ESBL-E. A study on colonization with ESBL-E in nursing homes in Sweden found that 11% of nursing home residents living in individual rooms carried ESBL-E [31]. Our results support the efficacy of unit-type facilities to prevent ESBL-E colonization in Japan, as well.

Wearing personal protective equipment is recognized as a standard precaution to handle excreted materials. Actually, only staff at conventional-type facilities used diaper carts, carried hand sanitizers, and wore aprons during diaper exchange, whereas these practices were not used at unit-type facilities, even though conventional-type facilities showed higher percentage of ESBL-E carriers than unit-type facilities. The higher carriage rate in conventional-type facilities

suggests that the use of personal protective equipment and hand sanitizers do not have sufficient effects to prevent transmission of ESBL-E in SNH. Considering these results, the structure of the multi-bed conventional-type facility and use of carts for changing diaper seem to have large influence on the transmission ESBL-E because of the excretion care for the residents is performed continuously. Further evaluation of the effectiveness of strict space-isolation of each resident in conventional-type facilities is necessary in the future. As the essential basis, staff must have appropriate knowledge and skills of standard precaution that are paramount for infection control under limited number of staff and structures; referring nursing care methods, elimination of shared instruments, proper implementation of hand hygiene and personal protective equipment because one unit-type facility without any precautions showed relatively higher carriage rate than other unit-type facilities. [15, 21, 30, 32].

Some limitations exist in this study. First, this study focused on elderly people with fecal incontinence using diaper in SNHs. Therefore, the prevalence of ESBL-E found in this study cannot be extrapolated to all SNH residents without using diaper. Second, the number of subjects at each facility who participated in this study varied, and detailed genetic analysis of ESBL-E detected was not performed; thus, the evaluation of horizontal transmission at each facility and whether carrying ESBL-E occurred via humans or environmental factors could not be performed.

In conclusion, we detected ESBL-E in 53 of a total of 100 residents of SNH in Japan. While we found no personal risk factors for colonization with ESBL-E. However, it is suggested that living in conventional-type facilities and scheduled diaper change using the cart for changing diaper has relation with carriage of ESBL-E. In multi-bed, conventional-type facilities using cart, revision of their methods of excretion care is necessary.

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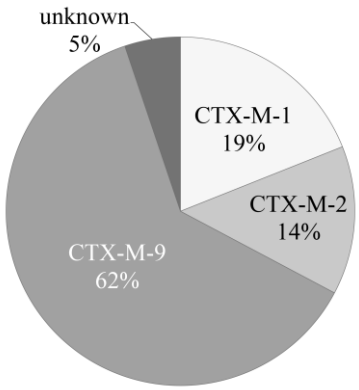

Figure 1. Percentage of CTX-M group typing of ESBL-E isolated from SNH's residents
ESBL-E, extended-spectrum β-lactamase-producing *Enterobacteriaceae*; SNH, special nursing home.

Table 1. Risk factors of ESBL-E colonization for residents living SNH

	ESBL-E strains				
	Positive (n=53)	Negative (n=47)	OR	(95% CI)	*p* value
Sex (men), n (%)	10 (19%)	6 (13%)	1.22	(0.79-1.88)	.41
Age (less than 80-years)	6 (11%)	9 (19%)	0.72	(0.38-1.38)	.27
Indwelling urinary catheter	2 (4%)	1 (2%)	1.27	(0.56-2.89)	1.00
Previous history of urinary tract infection	7 (13%)	6 (13%)	1.02	(0.59-1.75)	.95
Previous use of antimicrobials within 3 months	7 (13%)	11 (23%)	0.50	(0.18-1.41)	.19
Previous hospitalization within 1 year	13 (25%)	9 (19%)	1.15	(0.77-1.76)	.52
Length of SNH stay (less than 24 months)	18 (34%)	21 (45%)	0.80	(0.54-1.20)	.27
Comorbidity					
Diabetes mellitus	7 (13%)	5 (11%)	1.12	(0.67-1.87)	.69
Cardiac diseases	14 (26%)	8 (17%)	1.27	(0.87-1.87)	.26
Cerebrovascular disease	14 (26%)	14 (30%)	0.92	(0.60-1.42)	.71
Musculoskeletal disorder	16 (30%)	14 (30%)	1.01	(0.68-1.51)	.97
Urogenital disease	4 (8%)	3(6%)	1.09	(0.56-2.12)	1.00
Pressure ulcer	3 (6%)	0 (0%)	1.94	(1.60-2.35)	.25
Gastrogavage	6 (11%)	3 (6%)	1.29	(0.78-2.13)	.50
Type of facility for living resident					
Conventional-type (6 facilities; n=75)	45 (60%)	30 (40%)	1.88	(1.03-3.42)	.015

ESBL-E, extended-spectrum β-lactamase-producing *Enterobacteriaceae*; SNH, special nursing home; OR, odds ratio; CI, confidence interval.

Table 2. Characteristic of facilities participated in this study

Type of facility	**Conventional-type**							**Unit-type**			
Facility ID	A	C	D	E	G	H	Total	B	F	I	Total
Capacity of facility (beds)	70	50	90	100	54	80	444	120	52	93	265
Number of participants of this study (%)	31 (44)	2 (4.0)	8 (8.9)	13 (13)	9 (17)	12 (15)	75 (17)	10 (8.3)	9 (17)	6 (6.5)	25 (9.4)
Number of participants of ESBL-E colonization (%)	24 (77)	2 (100)	2 (25)	8 (62)	4 (44)	5 (42)	45 (60)	6 (66)	2 (22)	0 (0.0)	8 (32)
Average age of participants (±SD)	88.4 ±6.8	83.0 ±7.1	86.5 ±7.9	90.5 ±7.4	89.1 ±4.5	85.6 ±8.9	88.1 ± 7.2*	85.5 ±9.2	86.3 ±11.8	88.5 ±10.5	86.5 ±10.1*
Average of required care level of participants	4.9	5	5	5	4.7	4.3	4.81	4.9	4.8	4.5	4.76
Average length of SNH stay (months, ±SD)	31.9 ±35.5	51.5 ±29.0	26.0 ±11.3	46.5 ±34.8	56.0 ±23.5	41.3 ±38.4	38.7 ±33.2*	53.0 ±47.1	69.3 ±43.2	31.2 ±30.5	53.6 ±43.2*
Number of methods of changing diaper implemented (%)	3 (100)	1 (33)	3 (100)	3 (100)	2 (67)	3 (100)	median 3 (83)	0 (0)	1 (33)	2 (67)	median 1 (33)
Timing on schedule	Y	Y	Y	Y	Y	Y	6 (100)			Y	1 (33)
Assignment to particular staff	Y		Y	Y		Y	4 (67)		Y	Y	2 (67)
Using of diaper cart	Y		Y	Y	Y	Y	5 (83)				0 (0)
Number of infection prevention practices implemented (%)	4 (100)	2 (50)	1 (25)	2 (50)	3 (75)	3 (75)	median 3 (63)	0 (0)	1 (25)	2 (50)	median 1 (25)
Wearing gloves	Y	Y	Y	Y	Y	Y	6 (100)		Y	Y	2 (67)
Wearing apron	Y			Y	Y	Y	4 (67)				0 (0)
Wearing mask	Y	Y					2 (33)			Y	1 (33)
Carrying hand antiseptic	Y				Y	Y	3 (50)				0 (0)

SNH, special nursing home; SD, standard deviation; Y, yes.

* p value>0.05

NOTE: In long-term care insurance in Japan, eight daily living care categories are used to certificate the needs of care level for the elderly: “long-term care level” 1-5, “support level” 1 and 2, and “not certified”. The elderly in bedridden status, with dementia, etc., are certified as “long-term care level”, and require long-term care services. Especially, the elderly in “long-term care level 5” need care at all of the activity of daily living.