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Major Article

Profiling the fecal carriage of β-lactamase genes in long-term care facility residents: A longitudinal study

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Background: The fecal carriage of β-lactamase (BL)–producing bacteria may play a major role in the spread of these organisms in long-term care facilities (LTCFs). The aims of this study were (1) to describe the gene profiles of fecal BL in 3 LCTFs in Taiwan and (2) to analyze the fecal carriage burden of BL genes between the residents (patient group) and staff (staff group) of LTCFs.

Methods: Thirty fecal samples were collected during June 2013 and July 2015: 20 were obtained from 10 residents both during hospitalization (T1) and 1 month after discharge (T2), and 10 were obtained from 10 staff members.

Results: In total, 80%, 70%, and 50% of the samples in the patient group at T1, staff group at T2, and patient group at T2, respectively, contained >2 BL genes. In the patient group, the predominant genes belonged to extended-spectrum BL genes (90%-100%) and AmpC BL genes (90%-100%). Furthermore, carbapenemase genes were approximately 20% during T1 and T2. The relative levels of SHV-type BLs were significantly higher ($P < .05$) in the patient group at T2 compared with the staff group.

Conclusions: In this study, we found a high carriage of fecal BLs among LTCF residents and staff. The monitoring of fecal BL carriage in LTCFs is needed for infection control measures and antibiotic choice for health care–associated infections.

An aging and increasingly disabled population has become a major concern in developed and developing countries,[1,2] with an increasing demand for long-term care services. The increasing use of antibiotics in long-term care facilities (LTCFs) has led to increased antimicrobial drug resistance.[3,4] Colonized LTCF residents may act as vectors for the transfer of resistant bacteria into acute care hospitals, where they may cause infections in the initial host or spread to other vulnerable patients.[5]

Currently, β-lactam antibiotics are extensively used for the treatment of several types of infections[6]; however, extended-spectrum β-lactamase (ESBL)–producing *Enterobacteriaceae* (ESBL-E) are a major health concern worldwide.[7] Although plasmid-mediated *AmpC* β-lactamases (BLs) are less common than ESBLs, *AmpC* BLs remain an emerging therapeutic problem.[8] Moreover, the spread of carbapenemase genes has recently emerged.[9] Although hospitalized patients may serve as BL reservoirs, the ubiquity of BLs in healthy individuals and their environments is another potential threat to weaker patients in hospitals, LTCFs, and the community, where they have been associated with increased morbidity, mortality, and cost.[10,11]

Several reports have emphasized the prevalence of ESBLs in health care–associated facilities. However, few studies have performed

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long-term follow-up to evaluate the duration of colonization among LTCF residents and the correlation between residents and staff.[12] Here, we designed a longitudinal study to investigate the abundance of fecal BLs in the residents and staff members of 3 LTCFs and a community hospital, and we describe the molecular epidemiologic phenomenon between LTCFs and hospitals. The aims of the study were (1) to describe the fecal BL gene profiles in LCTFs and (2) to analyze the fecal carriage burden of BL gene profiles between residents and staff in LTCFs.

MATERIALS AND METHODS

Study population and data collection

The study hospital was a 100-bed referral community hospital for the 3 study LTCFs in central Taiwan. This study was performed between June 2013 and July 2015. We performed a prevalence study concerning fecal BL genes in 3 LTCFs (the first was a 50-bed LTCF, the second was a 200-bed LTCF, and the third was a 100-bed LTCF attached to this community hospital) located in central Taiwan. The study was approved by the Ethics Committee of the Changhua Christian Hospital (CCH IRB no. 140318). Informed written consent was obtained from the residents or, if they were unable to consent, from their relatives. LTCF staff members were also screened. The following data were collected from the enrolled subjects: (1) clinical profiles, laboratory data, fecal samples, and questionnaire responses before discharge from the community hospital and return to LTCFs (timepoint 1 [T1]); and (2) clinical profiles, fecal samples, and questionnaire responses at the LTCFs 1 month after discharge from the community hospital (timepoint 2 [T2]). Risk factors for fecal carriage with BL genes and patient demographic data were recorded as follows: patient age, sex, diagnosis at discharge, comorbidities, antibiotic prescriptions, microbiologic data, and outcome. For the LTCF patients, the hospital ward of the previous acute admission, antibiotic treatments in the preceding 3 months, and other potential risk factors (urinary incontinence, decubitus ulcers, and immunosuppressive therapy) were also recorded. Two groups of subjects were defined: (1) a patient group was defined as LTCF residents; and (2) a staff group was defined as LTCF staff who had not been sick within 3 months of the study period. The hospitalized period was defined as 3 months before the discharge day, and the follow-up period was defined as 1 month since discharge. Demographic and risk factor data from all LTCF residents and staff were collected using a questionnaire when stool sampling was conducted. Resident data included the date of sampling, date of residence, age, sex, comorbidities, and a history of antibiotic therapies within the last 3 months. LTCF information included bed capacity, staffing care levels (staff per 10 beds), and monthly expenditure per resident (in U.S. dollars).

Sample collection and identification of BL genes

Fecal samples were collected from patients hospitalized in Nantou Christian hospital (T1). After hospital discharge, the patients were transferred to LTCFs. Fecal samples were collected from each subject in the LTCF 1 month after discharge (T2). Fecal samples were also collected from LTCF staff. After sampling, each sample was stored at –20°C until further processing. DNA was extracted using the DNA stool Mini Kit (Qiagen, Valencia, CA) following the manufacturer's protocol and immediately stored at –20°C.

The fecal carriage of BL genes was tested, and all polymerase chain reaction (PCR) reactions followed the directions of Dallenne et al.[13] A total of 19 BL genes were classified into 3 groups: (1) group A: ESBL genes, including SHV, OXA-1 like, CTXM Gp1, CTXM Gp2, CTXM Gp9, CTXM MG8/25, GES, PER, and VEB; (2) group B: AmpC BL genes, including ACC, FOX, MOX, DHA, CIT, and EBC; and (3) group C: carbapenemase genes, including IMP, VIM, OXA-48, and KPC. To completely identify the PCR amplicons in the fecal samples, the amplicons were subjected to DNA sequencing using an ABI 3730 XL DNA Analyzer (Applied Biosystems, Foster City, CA). DNA sequences were compared with those registered in the National Center for Biotechnology Information database. The quantity of the *shv* β-lactamase gene was normalized against the 16S rRNA gene. Universal primers were used to determine the 16S rRNA genes for all bacteria by PCR.

Statistical analysis

Differences between the relative amount of *shv* genes in the fecal samples of the staff and patient groups were analyzed using Student *t* test, as appropriate. Differences between 2 groups of isolates were considered to be significant at the 0.05 level. Data entry and analyses were performed using SPSS software version 15.0 (SPSS, Chicago, IL).

RESULTS

Description of enrolled subjects

Initially, 20 LTCF residents were evaluated during the study period, but 10 residents withdrew from the study. Therefore, a total of 10 LTCF residents (patient group) completed the stool specimen collection, and their samples were analyzed according to the protocol. A total of 10 subjects (staff group) completed the stool specimen collection according to the protocol during the study period. In the patient group, 3 out of 10 (30%) patients were men, and the mean age ± SD was 77.2 ± 7.7 years. Table 1 shows the major diagnosis of each patient at discharge. The top 3 major diagnoses at discharge were pneumonia (n = 9), urinary tract infection (n = 4), and bacteremia (n = 2). The mean number ± SD of comorbidities was 6.5 ± 1.4 diseases, and most of the patients showed chronic obstructive pulmonary disease, dementia, type 2 diabetes mellitus, anemia, atherosclerosis heart disease, bacteremia, and an old cerebrovascular accident (Table 1).

The mean number ± SD of antibiotics used during the hospitalization period was 3.8 ± 1.9, and most were β-lactams (Table 1). Three out of 10 patients received oral β-lactams after discharge (follow-up period), and the remaining patients received oral ciprofloxacin. The top 3 clinical isolates from 6 out of 10 patients during the hospitalization period were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus* spp, and none of the patients was recorded during the follow-up period (Table 1). All patients were treated with β-lactam drugs during the hospitalization period, and the appearance of fecal BL genes was distributed in all patients at T1 and T2 sampling times.

Distribution of BL genes

A total of 80% and 50% of samples contained >2 BL genes in the T1 and T2 patient groups, respectively (Fig 1). Within the 3 LTCFs, the predominant genes belonged to groups A (90%-100%) and B (90%-100%). The average monthly expenditure per resident ranged from $540-$1,100, and the number of staff per 10 beds ranged from 1.92-4.22 (Table 2). Ten samples carried group C carbapenemase genes, including 2 (20%) in the patient group at T1, 2 (20%) in the patient group at T2, and 6 (60%) in the staff group. Importantly, *Klebsiella pneumoniae* carbapenemase (KPC) carriage in the residents (2/10) and staff (3/10) were disclosed, and Verona integron-encoded metallo-BL carriage in the residents (3/10) and staff (2/10) in LTCFs were also disclosed.