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EPIDEMIOLOGY, RISK FACTORS AND DURATION OF EXTENDED SPECTRUM BETA-LACTAMASE-PRODUCING ENTEROBACTERIACEAE CARRIAGE IN LONG-TERM-CARE FACILITIES

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Abstract: *Objectives:* To check the long-term care facilities reputation as high risk of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) cross-transmission. To evaluate the prevalence, incidence, risk factors and colonization duration of ESBL-E anal carriage in long term care institution for elderly patients. *Design:* A six-month prospective longitudinal study. *Setting:* 120 bed long-term care facilities at a teaching hospital. *Participants:* 115 patients. Intervention: rectal swabs or stools specimen patients sampled at inclusion and then monthly for ESBL-E detection. *Measurements:* Participants' characteristics (e. g. gender, age, Charlson index, functional activities), body mass index (BMI), nutritional parameters, feeding and meal management, C-Reactive Protein, serum albumin level, glycated haemoglobin. *Results:* Incidence density, prevalence and median duration of ESBL-E carriage were respectively 0.38 /1000 (95 % confidence interval 0.10-0.66) patient days, 4.4 % (95 % confidence interval 0.6-8.1) and 74 (interquartile range 26) days. The estimated probability of negativation was 59 % at 62 days. The main risk factors were male sex, stays in acute care unit during the survey and diet nature. *Conclusion:* Incidence density, prevalence, and rate of cross-transmission were low. Colonization duration could be linked to bacteria clonal characters.

Key words: Extended spectrum beta-lactamase, enterobacteriaceae, digestive carriage duration, long-term-care facilities, malnutrition.

Introduction

Incidence of extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-E) leads to an increase in carbapenem use, resulting in carbapenem resistance, especially due to carbapenemase.

As the index cases of some epidemics reported in teaching hospitals came from long-term care facilities (LTCF), these units were suggested as high risk of nosocomial cross-transmission of ESBL-E (1).

However, in LTCF, isolation precautions are controversies because of their potentially harmful consequences to the patient, including depression. So other procedures have to be found. To design preventive or curative trials, the evaluation of colonization duration is required.

This survey was performed to evaluate the prevalence, incidence, risk factors and colonization duration in long term care institution for elderly patients.

Patients and Methods

Setting and Study design

From 1st June 2010 to 30th November 2010, a prospective longitudinal study was conducted on 119 patients of the Reims teaching hospital LTCF, in France. This 2425-bed tertiary care hospital consists of acute care facilities (ACF) and a 120 bed LTCF. The patients' age was 83-years old on average and they required a large set of technical cares. They were included into the study if they were present in the unit on the 1st June 2010, and if they or their relatives consented. The study protocol was approved by the local ethics committee.

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Microbiological analysis

Screening consisted of a rectal swab or stools specimen patients sampled at inclusion and then monthly. Swabs were inoculated in pepton broth (10 mL) incubated overnight at 37°C. Fifty microliters were inoculated onto ChromID ESBL medium (bioMérieux, Marcy l'Etoile, France) and incubated for 18 h at 37°C. Enterobacteriaceae isolates were identified by Vitek 2 ID-GN (bioMérieux).

Antibiotic susceptibility was determined with the disk diffusion method on Mueller Hinton agar at 37°C for 18 h according to the European Committee on antimicrobial susceptibility testing (www.eucast.org/nc/antimicrobial-susceptibility-testing). ESBL production was detected by the double-disk synergy test using disks of 30 μg cefotaxime or ceftazidime alone and in combination with 10 μg clavulanate (Bio-Rad 92430 Marnes la Coquette France).

Bla$_{TEM}$, *bla*$_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{OXA}$ genes were detected by PCR and sequencing as previously described (2). The isolates were genotyped by Random Amplification of Polymorphic DNA (RAPD) (2)

Data collection

The following data were collected monthly: age, gender, number of ESBL-E carriers in the same unit, Charlson index, functional activities using the French public health insurance "Autonomie Gerontologie Groupe Iso-Ressources" (AG-GIR) score (http://www.ursaf.fr/images/ref-form-particulier-11510-01.pdf), digestive disorders, length of stay in LTCF and number of admissions in ACF. AGGIR score assess basic activities of daily living (BADL) and ranges from 0 (totally dependent for BADL) to 6 (totally independent for BADL). Caloric oral supplements were milk-, fruit juice- or cream-based. Blended diet included cream, compote, mashed potatoes, vegetable puree, reconstituted meat such as pâté. Mill put diet included minced meat, cooked fruits, compotes, hard boiled eggs, sardines, French bean, cottage pie. The antibiotic treatments within the year before the beginning and during the survey were collected separately. Values of C-Reactive Protein, serum albumin level, glycated haemoglobin were collected at inclusion, third and sixth months.

Statistical analysis

Incidence density was defined by all newly detected ESBL-E cases (colonized and infected) per 1000 patient days during the period following the first screening, and the colonization pressure as the ratio of ESBL-E colonized patient-days to the total number of patient-days during the study period. ESBL-E positive patients data were compared to ESBL-E negative ones using Chi-square, Student t-test, the two-tailed Fisher exact test and the Wilcoxon test. Relative risks confidence interval was determined using Taylor series. ESBL colonization rates were estimated with regard to survival curves using the Kaplan Meier method. Statistical significance was established at $p < 0.05$.

Results

Of 119 eligible patients, one died before the first sample and 3 declined the samples. Of the 115 definitively included, 10 refused further samples, 17 died and 2 were discharged during the survey. Ninety participated to the whole survey.

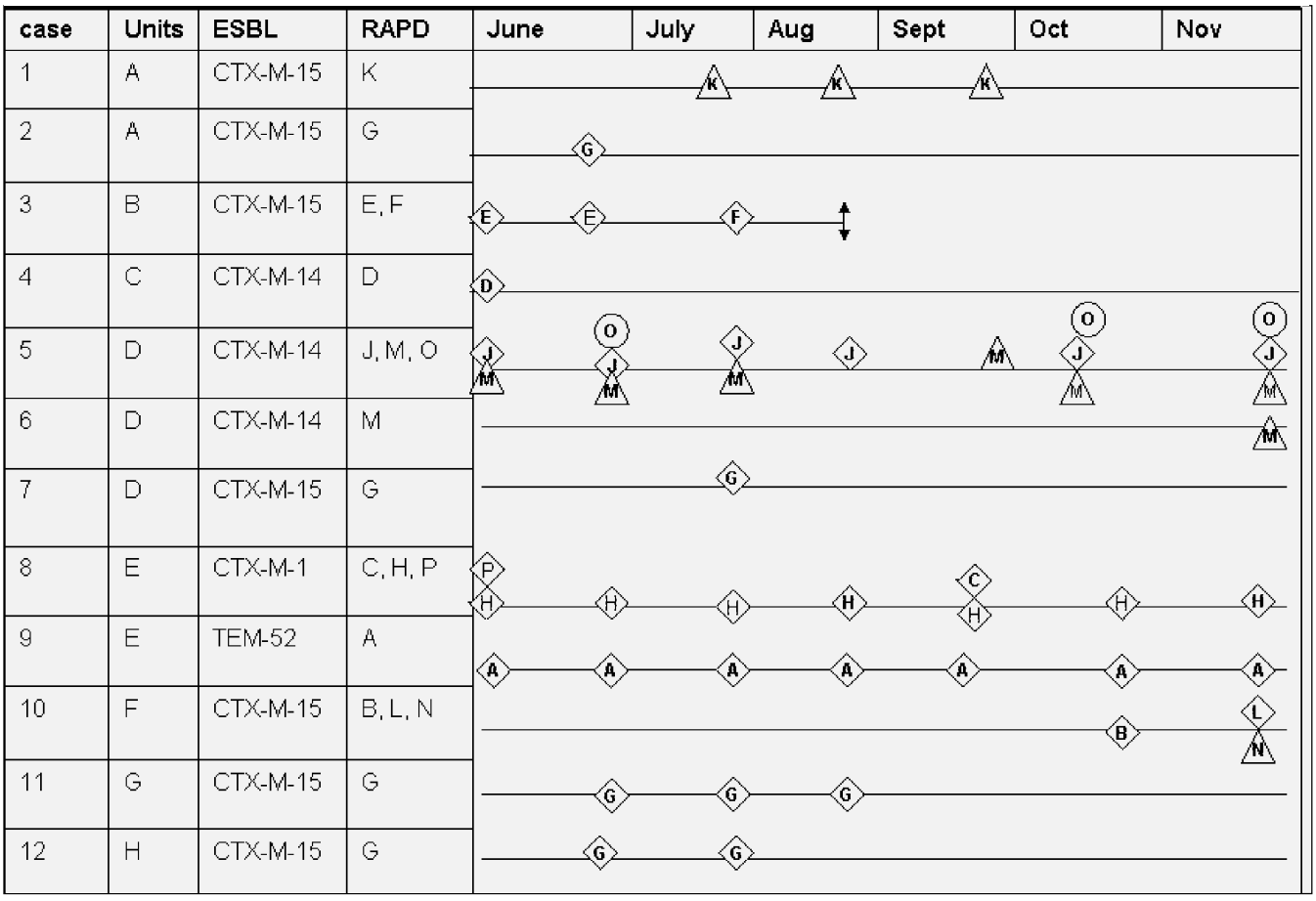
Table 1
Baseline characteristics and comorbid conditions[1]

Characteristics	Patients (n=115)
Mean (SD) age (years)	81.1 (8.9)
Male sex %	34.8
Patients in acute care units	
within the year prior to inclusion %	37.4
during the survey %	20.0
Underlying diseases	
Mean (SD) Charlson score	3.8 (2.2)
Mean (SD) AG-GIR score	2.2 (1.2)
Mean (SD) BMI	25.4 (5.4)
Diabetes %	26.1
Dementia syndrome %	53.0
Disorientated %	60.0
Self -moving %	35.7
Incontinent %	73.0
Indwelling urinary catheter %	11.3
Intermittent urinary catheterization %	2.6
Urinary tract infection %	4.3
Diet	
- usual %	27.8
- blended %	34.8
- mill put %	35.7
- enteral %	6.1
Food supplementation %	51.3
Meal in dining-room %	31.3
Biological data at inclusion :	
Mean (SD) Albuminemia (g/l)	32.2 (4.7)
Mean (SD) C Reactive protein (mg/l)	11.8 (15.1)
Mean (SD) HbA1c (%)	5.9 (1.0)
Antibiotics received within the year prior to inclusion	
At least one antibiotic %	72.2
Penicillins %	49.6
Cephalosporins %	29.6
Carbapenem %	0.0
Fluoroquinolones %	24.3
Glycopeptides %	0.9
Aminoglycosides %	3.5

1. % for qualitative variables; AG-GIR score: Autonomy Gerontology Groups Iso-Resources; BMI: Body mass index

The median length of stay in LTCF was 3.2 (interquartile range IR 6.1) years. Eighty-four patients (73.0 %) had urinary incontinence and 69 (60.0 %) were disorientated (Table 1). Patients' functional activities were

Figure 1
Carriage duration according to units, patients, ESBL and genotypes



◊ *E. coli* ; Δ *K. pneumoniae* ; O *P. mirabilis* ; ↕ Death ; ____ Period of inclusion into the survey; RAPD : Random Amplification of Polymorphic DNA ; ESBL : Extended Spectrum Beta-lactamase

Table 2
Risk factors for ESBL-E carriage[1]

Characteristics	ESBL-E+ (n=12)	ESBL-E- (n=103)	Relative Risk (95% CI)	p-value
Male sex %	66.7	31.1	3.7 (1.2-11.7)	0.02[2]
Patients in acute care units				
- within the year prior to inclusion %	58.3	35.0	2.3 (0.6-11.)	0.1[4]
- during the survey %	50.0	16.5	4.0 (1.4-11.2)	0.02[2]
Diabetes %	50.0	23.3	2.8 (1.0-8.1)	0.08[4]
Dementia syndrome %	33.3	57.6	0.4 (0.1-1.3)	0.1[2]
Mill put diet %	66.7	32.3	3.6 (1.2-11.3)	0.04[3]
Penicillins within the year prior to inclusion %	75.0	46.6	3.0 (0.8-10.7)	0.06

1. % for qualitative variables 2. two-tailed Chi-square; 3. with Yates correction; 4. Fisher exact test; ESBL-E: extended-spectrum beta-lactamase *Enterobacteriaceae*; ESBL-E+: ESBL-E carrier; ESBL-E-: patient negative for ESBL-E detection

very reduced (mean GIR score 2.2) at inclusion. Body mass index was normal for all the patients. Five patients were treated for an urinary tract infection. Eighty-three patients (72.2 %) received antibiotic treatment during the year before inclusion, and 57 (49.6 %) penicillins. (Table 1).

Twelve patients were found ESBL-E carriers, 5 at inclusion and 7 during the survey (Figure 1). The ESBL-E prevalence was 5/115 (4.4%, 95 % confidence interval CI 0.6-8.1)) at the beginning of the survey and 5/90 (5. 6 %, 95% CI 0.8-10.3) at the end. The incidence density was 0.38 (95 % CI 0.10-0.66) per 1000 patients days.

Colonization pressure was 5.2 (95 % CI 4.9 -5.6) per 100 patients days. The median duration of ESBL-E carriage was 74 (IR 26) days. The estimated probability of negativation was 59 % at 62 days and 65 % at 90 days of stay. Three patients were colonized for 6 months (Figure 1). After negativation no positive results have been observed in 6 patients for a 2- to 6-months period.

Risk factors

In comparison to non-colonized patients, colonized patients were more often men (66.7% vs 31.1%; p = 0.02) and stays in ACF during the survey were more numerous (50.0% vs 16.5%; p = 0.02) (Table 2). They had more diabetes mellitus (50.0 % vs 23.3 %, p = 0.08), and less dementia syndrome (33.3 % vs 57.6%; p = 0.1). Food supplements were less often prescribed in colonized patients (33.3% vs 53.4%; p = 0.2) and mill put diet more often (66.7% vs 32.0% p = 0.04) (Table 2). During the year before the survey, more colonized patients were hospitalized in acute care units (58.3% vs 35.0%, p=0.1) and received penicillins (75.0 vs 46.6 %, p=0.06) than non-colonized ones. No differences were observed for other variables (Table 2).

In comparison to other ESBL-E carriers, the three patients carrying ESBL-E for the whole period had more dementia syndrome (100% vs 11% p = 0.02), were less constipated (0.0% vs 77.8% p=0.05) and were hospitalized in units with more ESBL-E carriers (mean 3.7 ± 0.6 vs 1.4 ± 1.4; p=0.02). Patients carrying ESBL-E for less than 62 days had higher GIR (mean 2.3 ± 0.8 vs 1.5 ± 0.5; p=0.04) and higher serum albumin level (mean 35.6 ± 1.8 vs 31.6 ± 2.8 mg/l; p = 0.01)

Microbiological results

Among the 704 samples performed, 38 were ESBL-E positive. The species identified were *Escherichia coli* (n= 33), *Klebsiella pneumoniae* (n=11), *Proteus mirabilis* (n=3) and 78.5 % of them were susceptible to both aminoglycosides and fluoroquinolones (Figure 1) .

The most frequent enzyme was CTX-M-15, produced by E. coli in seven patients and *K. pneumoniae* in two (Figure 1). CTX-M-14 was produced by *E. coli* in three patients, *K. pneumoniae* in two and *P. mirabilis* in one, and TEM-52 was produced by *E. coli* in one patient. Each patient carrying the strains of different species produced one single enzyme (Figure 1).

RAPD genotyping classified *E. coli* isolates in 11 groups and *K. pneumoniae* in 3 groups (Figure 1). The type G pattern was observed for CTX-M-15-producing *E. coli* in four cases, hospitalized in different units. None of them were persistent carriers (Figure 1). One episode of cross-transmission was identified for two patients (5 and 6) hospitalized in the same unit. Three patients had different RAPD types *E. coli*. The persistent clones A, J and H did not prove more resistant to the different antibiotic classes than the others.

Discussion

Long-term care facilities have been regarded as high-risk places for cross transmission of multiresistant bacteria. In our study the prevalence and the cross-transmission of ESBL-E were not as high as expected. The autonomy score was weak and urinary incontinence was more frequent than in other reports (73.0 % vs 43-65%) (3,4). That should have increase the prevalence of ESBL-E in our study. But ESBL-producing *Enterobacteriaceae* prevalences showed a wide variation (0-75 %) in nursing homes or LTCF in different countries, perhaps owing to the proportion in dementia syndrome as well as the approach of assistance in toilets use, depending on the facilities (4, 5). They were mostly higher than in ours, especially for *E. coli*, despite a similar proportion of antibiotic treatment (6). Further studies are thus required to evaluate the evolution of the ESBL-E prevalence in LTCFs. Some carriers could not have been detected but the repetitive sampling increased sensitivity. There was no consensus about ESBL-E carriage detection with rectal swab. Swab incubation into broth, as performed in our study, could have increased the detection but was rarely reported.(7) The high proportion of strains susceptible to both aminoglycosides and fluoroquinolones in our study was unusual (6, 8, 9).

Few reports on ESBL-E incidence density are available for LTCF. The French ESBL-E national incidence density showed an increase from 0.13 to 0.32 per 1000 patients days between 2002 and 2009 in LTCFs in France. (http://www.invs.sante.fr/publications/2011/surveillance_bacteries_ multiresistantes/ rapport_raisin_resultats_2009.pdf).

Our study suggested that LTCF patients acquired the ESBL-E in ACF, in part because the duration of stay in acute care unit, during the month prior to the sampling, was longer for the colonized patients; yet comorbidities frequency was similar between the two groups. In addition the rate of cross-transmission was low, perhaps because *E. coli* was the predominant species and was known to be less involved in patient-to-patient transmission than *K. pneumoniae* (Figure 1) (10).

The length of colonization duration in our study was similar to that reported in one six-month study (11). Therefore frequent screenings during the first two months after a positive sample are not necessary. However, after two or three months they have to be repeated because the patient could have become negative or the species could have changed. Two categories of patients could be characterized according to their colonization: some patients were colonized for the six months and the others lost their ESBL-E. That raises the question of whether permanent or long-term carriers

could be distinguished as observed for methicillin resistant *Staphylococcus aureus*. Another possibility is that some genotypes have more factors that promote colonization of the host. But these patients were more dement and in units where ESBL-E were more prevalent.

There are few reports about nutritional effect on ESBL-E carriage in LTCF (12). It should be noted that patients receiving food supplements were less colonized than the others, despite a lower body mass index at inclusion (23.7 vs 27.3 p = 0.001) and patients colonized for less than two months had a higher serum albumin level. Therefore food supplementation could be an element of the prevention of ESBL-E in LTCF. The relation between mill put diet and ESBL-E carriage is more difficult to explain because food control did not evidence anormal bacterial load.

In summary LTCF did not appear to have a major role into ESBL-E transmission. However according to the patients lengths of stay, food supplements could be tried to shorten carriage duration.

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