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ARTICLE

Faecal carriage of oxyiminocephalosporin-resistant Enterobacteriaceae among paediatric units in different hospitals in the south of France

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Abstract The aim of this study was to determine the presence of oxyiminocephalosporin-resistant (OCR) Gram-negative bacilli and extended-spectrum β-lactamase (ESBL)-producing isolates in stool specimens obtained from paediatric patients hospitalised for acute diarrhoea. We conducted a prospective, multicentre study over a period of 6 months in seven hospitals in the south of France. Samplings were carried out from infants admitted for acute diarrhoea with no previous antibiotic treatment in the last week. Bacteria in stool specimens were screened for the presence of OCR Gram-negative bacilli on Drigalski agar supplemented with ceftazidime and ESBL CHROMagar® media, and confirmed by the Rosco tablets test. Genetic detection was performed by the Check MDR® microarray and by polymerase chain reaction (PCR) and sequencing with bacterial DNA extracted from isolates. The presence of OCR enterobacteria was markedly high (177/1,118 patients, 15.2 %), with an important community origin (66.1 %). The majority of multidrug-resistant (MDR) bacteria were *Enterobacter cloacae* (106, 59.9 %) and *Escherichia coli* (61, 34.5 %). The prevalence of ESBL and CTX-M producers represented 5.2 and 4.3 % of the isolates, respectively. The main proportion of these ESBL carriers was

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found in children less than 1 year of age (53.4 %). One carbapenemase (IMP-1) was detected. The study revealed the wide dissemination of MDR bacteria in infants attending hospitals in the south of France during a non-outbreak situation, in particular, the spread of cefotaximase and the detection of a carbapenemase. This worrisome situation must reinforce the use of hygiene procedures and appropriate antibiotics to control the emergence and spread of OCR organisms.

Introduction

The potential of different antimicrobial agents to cause the emergence of multidrug resistance in the normal digestive microflora is of great importance. The diffusion of extended-spectrum β-lactamases (ESBLs) is worrisome [1]. More recently, the misuse of antibiotics has led to the emergence of other multidrug-resistant (MDR) bacteria, notably, carbapenem-resistant Enterobacteriaceae. Targeted surveillance of high-risk patients is essential to prevent outbreaks [2]. Indeed, this prevalence can be related to the increase in faecal carriers over time [3, 4]. To date, few studies have reported the prevalence of MDR bacteria among children [5–12]. The aim of this work was to evaluate the prevalence of oxyiminocephalosporin-resistant (OCR) enterobacteria in children hospitalised for acute diarrhoea.

Patients and methods

Study design and data collection

From November 2010 to April 2011, faecal samples from children hospitalised for acute diarrhoea in neonates and paediatrics departments in seven hospitals in the south of France (University Hospitals of Nîmes and Toulouse; General Hospitals of Aix-en-Provence, Aubagne, Cahors, Narbonne and Perpignan) were prospectively and consecutively collected. Sampling was carried out among children with acute diarrhoea and no previous antibiotic treatment in the last week during a non-outbreak period on the first day of hospitalization. The following clinical data were collected prospectively: demographic data, clinical ward, hospitalisation or surgical treatment in the last 12 months and transfer from another hospital or intensive care unit, and previous antibiotic treatment in the last 3 months. Children were deemed to be of community origin when they had never been hospitalised.

All the parents of the infants included in the study were informed of the protocol and accepted the research. The study was proposed to our local ethical committee (South Mediterranean III). The committee judged that no consent was needed in this study as the stool was not additional; this sample is systematic in diarrhoea.

Screening for OCR isolates

To screen for OCR Gram-negative bacilli, samples were placed in 1 mL sterile 0.9 % saline and then vortexed. From this suspension, 100 μl was inoculated on two culture media: two culture media were inoculated [Drigalski agar supplemented with ceftazidime (2 mg/L) and a chromogenic agar ESBL CHROMagar® (CHROMagar, France)]. Plates were incubated at 37 °C under aerobic conditions and assessed after 24 and 48 h of incubation. For the commercial media, the colour and intensity of the colonies were recorded according to the colour chart provided by the manufacturer.

Strain identification and antimicrobial susceptibility testing

The VITEK 2 automated system (bioMérieux, Marcy l'Etoile, France) and the disk diffusion method were used for the biochemical identification and antibiotic susceptibility testing of all isolates that grew on the two media pathogens, respectively. The following antibiotics were tested: amoxicillin, amoxicillin + clavulanic acid, ticarcillin, ticarcillin + clavulanic acid, piperacillin, piperacillin + tazobactam, cefepime, cefazolin, cefotaxime, ceftazidime, cefoxitin, imipenem, ertapenem, meropenem and aztreonam. Strains were classified as antibiotic-sensitive, -intermediately resistant, or -resistant, according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (http://www.sfm-microbiologie.org).

To be retained in the study, enterobacteria should be resistant to oxyiminocephalosporins and *Pseudomonas aeruginosa* resistant to ceftazidime. ESBL and derepressed AmpC were detected by a combination of disk tests (NeoSensitabs tablets, ESBL + AmpC Screen Kit and ESBL Confirm ID Kit, Rosco Diagnostica) using the association between cefotaxime, ceftazidime, and clavulanic acid and cloxacillin. The results were interpreted following the manufacturer's recommendations.

Characterisation of β-lactamases resistance genes

Plasmid or chromosomal DNA was extracted from the isolates using the EZ1 DNA Tissue Kit on the BioRobot EZ1 extraction platform (Qiagen, Courtaboeuf, France). The genotypic characterisation of multidrug resistance mechanisms was determined by the Check-MDR CT102 microarray (Biocentrics, France) targeting ESBLs (bla_{TEM}, bla_{SHV} and bla_{CTX-M}) and carbapenemases (bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP} and bla_{NDM-1}) [13]. After detection, polymerase chain reaction (PCR) assays targeting the corresponding *bla* genes were performed and identified by sequencing the PCR products [14, 15]. We used a triplex PCR specific for the CTX-M-15-producing *Escherichia coli* O25b:H4-ST131 clone [16]. The detection of *ampC* promoter/attenuator

mutation in *E. coli* isolates and plasmid-mediated *ampC* genes in different suspected strains were performed by using PCR and sequencing [17, 18].

Results

Main characteristics of patients

A total of 1,118 stool specimens, one specimen for each hospitalised child, were examined. The characteristics of the study population are presented in Table 1. The repartition of the study population was: 55.4 % of male, with a median age of 1 year (range 0–16 years), 588 (52.6 %) infants were aged less than 1 year, 405 (36.2 %) were between 1 and 6 years of age, and 125 (11.2 %) more than 6 years old. Of the 1,118 children, 177 (15.2 %) harboured OCR enterobacteria. Moreover, 55 children harboured ceftazidime-resistant *Pseudomonas aeruginosa*. Single OCR isolates were identified in 151 patients, and two and three different MDR microorganisms were found in 23 and 3 patients, respectively.

Repartition of OCR strains

OCR isolates corresponded mostly to *E. cloacae* strains (106, 59.9 % of the OCR strains) and *E. coli* (61, 34.5 %). The prevalence of ESBL was 5.2 %, with 4.3 % CTX-M, 0.5 % SHV and 0.4 % TEM producers. The distribution of the different types of ESBL in the different centres is shown in Tables 1 and 2 and Fig. 1. Among the CTX-M producers, 36 (76.6 %) strains produced CTX-M-15 β-lactamases, mainly *E. coli* strains (31, 86.1 %). Only one strain among the 31 CTX-M-15-producing strains belonged to the *E. coli* O25b:H4-ST131 clone. TEM- and SHV-producing strains were equally prevalent in *E. cloacae* (45.5 % of the strains) and *E. coli* (54.5 % of the strains). No other species produced ESBL enzymes. One case (0.08 %) of carbapenemase carriage (IMP-1) was detected. Finally, we observed a very high amount of chromosomally AmpC derepressed carriage (10.2 %). No plasmid-mediated AmpC isolate was detected. In *E. coli* strains, *ampC* promoter/attenuator mutations were observed. These mutations concerned both promoter [at position −42: (C→T)] and attenuator [+32 (G→A) and +58 (C→T)], leading to increased expression of the chromosomal *ampC* gene.

Discussion

This prospective multisite study highlights the high faecal carriage of MDR bacteria in young children hospitalised for acute diarrhoea in seven French hospitals in the south of France, notably, the great importance of CTX-M β-lactamases and the emergence of a carbapenemase. This high level of faecal carriage of OCR is a surprising finding, considering the proportion of infants (53 % of all the included children), the studied population (children with acute diarrhoea at admission to hospital) and the high proportion (73 %) of community origin encountered. We observed that 38 % and 53.4 % of the OCR and ESBL carriers were less than 1 year old and 74.3 % of the ESBL carriers have never been hospitalised. Moreover, all the ESBL carriers were less than 1 year old and 96 % of all these carriers have never had

Table 1 Demographic characteristics of the study population and distribution of oxyiminocephalosporin-resistant (OCR) enterobacteria

Characteristics	ESBL carriers *n*=58	AmpC carriers *n*=118	Carbapenemase carrier *n*=1	Total *n*=1118
Age (range)	0 (0–10)	1.5 (0–16)	7	1 (0–16)
<1 year	31 (53.4)	36 (30.5)	–	588 (52.6)
Between 1 and 6 years	25 (43.1)	73 (61.9)	–	405 (36.2)
>6 years	2 (3.4)	9 (7.6)	1	125 (11.2)
Male/female, *n* (%)	32 (55.2)/26 (44.8)	68 (57.6)/50 (42.4)	1/0	619 (55.4)/499 (44.6)
Previous hospitalisation or lived in an institution in the last year	15 (25.9)	45 (38.1)	1	299 (26.7)
Distribution of OCR isolates				
Toulouse (*n*=405)	23	49	–	72 (17.8)
Perpignan (*n*=158)	10	25	–	35 (22.2)
Aix en Provence (*n*=217)	5	27	–	32 (14.7)
Nîmes (*n*=152)	10	10	–	20 (13.2)
Aubagne (*n*=134)	4	4	1	9 (6.7)
Cahors (*n*=30)	6	1	–	7 (23.3)
Narbonne (*n*=22)	–	2	–	2 (9.1)

Table 2 Oxyiminocephalosporin-resistant (OCR) Gram-negative bacilli recovered from stool specimens

Strains	Number	ESBL[a]						Carbapenemases	Hyperproduction of AmpC
		CTX-M-15	CTX-M-14	TEM-24	TEM-19	SHV-4	SHV-5	IMP-1	
E. cloacae	106 (45.7)	5	3	3	–	1	2	1	91
E. coli	61 (26.3)	31	8	1	1	–	3	–	17
P. aeruginosa	55 (23.7)	–	–	–	–	–	–	–	55
C. koseri	6 (2.6)	–	–	–	–	–	–	–	6
E. aerogenes	4 (1.7)	–	–	–	–	–	–	–	4
Total enterobacteria	177 (76.3)	36	11	4	1	1	5	1	118
Total	232 (100)	36	11	4	1	1	5	1	173

[a] *ESBL* Extended spectrum β-lactamase

antibiotic exposure, respectively. In this young population, the worldwide *E. coli* O25b:H4-ST131 clone was detected in one case (a community acquisition), showing a weak spread of this strain in the community (no antibiotic exposure was recorded in the last 3 months).

The main prevalence of OCR faecal carriage is between 1 and 6 years (54.2 %); this was due to the carriage of AmpC derepressed strains. Different studies have previously reported the prevalence of faecal carriage of ESBL-producing isolates and highlighted that the community could be a reservoir of these organisms [7–12]. Marked regional variations were observed in the incidence and genotype of these strains. The prevalence was low in European countries (1.1 to 3 %) [7–9] compared to other parts of the

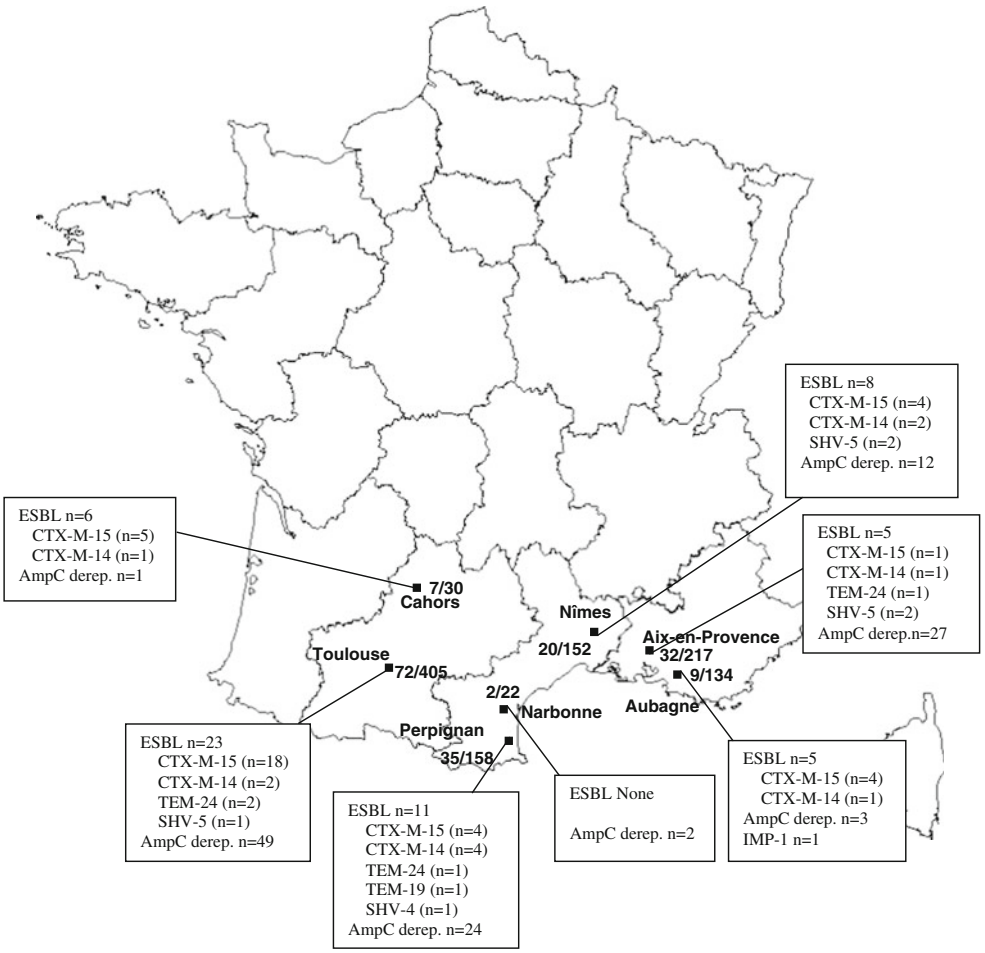

Fig. 1 Distribution of multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae strains described in the seven hospitals in the south of France

world (21 to 58 %) [5–12]. The only previous French study in a paediatric unit showed that the prevalence of the MDR bacteria was 4.2 % in 2009 with 2.1 % of ESBL carriers [8]. Our study demonstrated an expansion of the resistant bacteria that could be explained by the differences in settings. In neonates, infections/colonisations due to MDR strains are classically associated with preterm low birth weight, prolonged mechanical ventilation, prior use and duration of antibiotic treatment (notably third-generation cephalosporins and aminoglycosides), and invasive devices [19]. The outcome of the infants was not associated with mortality [20]. This worrisome situation was increased by the detection of one case (0.08 %) of carbapenemase carriage (IMP-1) in a 7-year-old child who had multiple hospitalisations and received different courses of antibiotic treatment during these indwellings. This metallo-β-lactamase IMP was detected in our previous study, confirming its low but real circulation in France [21].

Finally, we observed a very high amount of chromosomally AmpC derepressed carriage (10.2 %). A great number of infants (69.5 %) carrying these strains have previously received previously courses of antibiotics treatment during the last 3 months. This level confirms a problem in antibiotic use explaining this resistance. Interestingly, in *E. coli* strains, we observed some known mutations necessary to convert the weak promoter of *ampC* to the strongest promoter consensus sequence. In the same way, 55 children harboured ceftazidime-resistant *Pseudomonas aeruginosa*. Very few studies have reported prevalence in this population: this prevalence varied between 6 and 57 % [22, 23]. Antibiotics ineffective against *P. aeruginosa* significantly increased the risk of colonisation [24]. We observed this trend in our population, with a majority of children (54.5 %) having previous hospitalisations and antibiotic courses. Even if the study described colonisation, we could not exclude the consequence of this emergence in the infection situation, inducing very restricted antimicrobial treatment options.

Conclusion

The results of this multicentre study are worrying. Basic hygiene (strict compliance to hand washing to prevent cross-transmission in hospitals, but also in the community, nurseries and schools) must be reinforced, risk factors for the acquisition of these strains must be detected and the elective pressure, on account of antibiotics misuse, should be discouraged.

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Conflict of interest The authors report no conflicting interests.

References

1. Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Caniça MM, Park YJ, Lavigne JP, Pitout J, Johnson JR (2008) Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother 61:273–281
2. Lucet JC, Decré D, Fichelle A, Joly-Guillou ML, Pernet M, Deblangy C, Kosmann MJ, Régnier B (1999) Control of a prolonged outbreak of extended-spectrum β-lactamase-producing Enterobacteriaceae in a university hospital. Clin Infect Dis 29:1411–1418
3. Cantón R, Novais A, Valverde A, Machado E, Peixe L, Baquero F, Coque TM (2008) Prevalence and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae in Europe. Clin Microbiol Infect 14:144–153
4. Sasaki T, Hirai I, Niki M, Nakamura T, Komalamisra C, Maipanich W, Kusolsuk T, Sa-Nguankiat S, Pubampen S, Yamamoto Y (2010) High prevalence of CTX-M β-lactamase-producing Enterobacteriaceae in stool specimens obtained from healthy individuals in Thailand. J Antimicrob Chemother 65:666–668
5. Lo WU, Ho PL, Chow KH, Lai EL, Yeung F, Chiu SS (2010) Fecal carriage of CTXM type extended-spectrum β-lactamase-producing organisms by children and their household contacts. J Infect 60:286–292
6. Andriatahina T, Randrianirina F, Hariniana ER, Talarmin A, Raobijaona H, Buisson Y, Richard V (2010) High prevalence of fecal carriage of extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in a pediatric unit in Madagascar. BMC Infect Dis 10:204
7. Strömdahl H, Tham J, Melander E, Walder M, Edquist PJ, Odenholt I (2011) Prevalence of faecal ESBL carriage in the community and in a hospital setting in a county of Southern Sweden. Eur J Clin Microbiol Infect Dis 30:1159–1162
8. Janvier F, Mérens A, Delaune D, Soler C, Cavallo JD (2011) Fecal carriage of third-generation cephalosporins-resistant *Enterobacteriaceae* in asymptomatic young adults: evolution between 1999 and 2009. Pathol Biol (Paris) 59:97–101
9. Millar MR, Walsh TR, Linton CJ, Zhang S, Leeming JP, Bennett PM; ALSPAC Study Team. Avon Longitudinal Study of Pregnancy and Childhood (2001) Carriage of antibiotic-resistant bacteria by healthy children. J Antimicrob Chemother 47:605–610
10. Souza TB, Morais MB, Tahan S, Melli LC, Rodrigues MS, Scaletsky IC (2009) High prevalence of antimicrobial drug-resistant diarrheagenic *Escherichia coli* in asymptomatic children living in an urban slum. J Infect 59:247–251
11. Woerther PL, Angebault C, Jacquier H, Hugede HC, Janssens AC, Sayadi S, El Mniai A, Armand-Lefèvre L, Ruppé E, Barbier F, Raskine L, Page AL, de Rekeneire N, Andremont A (2011) Massive increase, spread, and exchange of extended spectrum β-lactamase-encoding genes among intestinal Enterobacteriaceae in hospitalized children with severe acute malnutrition in Niger. Clin Infect Dis 53:677–685
12. Ho PL, Wong RC, Chow KH, Yip K, Wong SS, Que TL (2008) CTX-M type β-lactamases among fecal *Escherichia coli* and *Klebsiella pneumoniae* isolates in non-hospitalized children and adults. J Microbiol Immunol Infect 41:428–432
13. Naas T, Cuzon G, Bogaerts P, Glupczynski Y, Nordmann P (2011) Evaluation of a DNA microarray (Check-MDR CT102) for rapid detection of TEM, SHV, and CTX-M extended-spectrum β-lactamases and of KPC, OXA-48, VIM, IMP, and NDM-1 carbapenemases. J Clin Microbiol 49:1608–1613
14. Pitout JD, Hanson ND, Church DL, Laupland KB (2004) Population-based laboratory surveillance for *Escherichia coli*-producing extended-spectrum beta-lactamases: importance of community isolates with bla_{CTX-M} genes. Clin Infect Dis 38:1736–1741

15. Moland ES, Hanson ND, Black JA, Hossain A, Song W, Thomson KS (2006) Prevalence of newer beta-lactamases in Gram-negative clinical isolates collected in the United States from 2001 to 2002. J Clin Microbiol 44:3318–3324
16. Blanco M, Alonso MP, Nicolas-Chanoine MH, Dahbi G, Mora A, Blanco JE, López C, Cortés P, Llagostera M, Leflon-Guibout V, Puentes B, Mamani R, Herrera A, Coira MA, García-Garrote F, Pita JM, Blanco J (2009) Molecular epidemiology of *Escherichia coli* producing extended-spectrum β-lactamases in Lugo (Spain): dissemination of clone O25b:H4-ST131 producing CTX-M-15. J Antimicrob Chemother 63:1135–1141
17. Pérez-Pérez FJ, Hanson ND (2002) Detection of plasmid-mediated AmpC β-lactamase genes in clinical isolates by using multiplex PCR. J Clin Microbiol 40:2153–2162
18. Corvec S, Prodhomme A, Giraudeau C, Dauvergne S, Reynaud A, Caroff N (2007) Most *Escherichia coli* strains overproducing chromosomal AmpC β-lactamase belong to phylogenetic group A. J Antimicrob Chemother 60:872–876
19. Singh N, Patel KM, Léger MM, Short B, Sprague BM, Kalu N, Campos JM (2002) Risk of resistant infections with Enterobacteriaceae in hospitalized neonates. Pediatr Infect Dis J 21:1029–1033
20. Chiu S, Huang YC, Lien RI, Chou YH, Lin TY (2005) Clinical features of nosocomial infections by extended-spectrum β-lactamase-producing *Enterobacteriaceae* in neonatal intensive care units. Acta Paediatr 94:1644–1649
21. Vidal-Navarro L, Pfeiffer C, Bouziges N, Sotto A, Lavigne JP (2010) Faecal carriage of multidrug-resistant Gram-negative bacilli during a non-outbreak situation in a French university hospital. J Antimicrob Chemother 65:2455–2458
22. Yoshioka H, Fujita K, Maruyama S (1983) Faecal carriage of *Pseudomonas aeruginosa* in newborn infants. J Hosp Infect 4:41–44
23. Cooke EM, Shooter RA, O'Farrell SM, Martin DR (1970) Faecal carriage of *Pseudomonas aeruginosa* by newborn babies. Lancet 2:1045–1046
24. Thuong M, Arvaniti K, Ruimy R, de la Salmonière P, Scanvic-Hameg A, Lucet JC, Régnier B (2003) Epidemiology of *Pseudomonas aeruginosa* and risk factors for carriage acquisition in an intensive care unit. J Hosp Infect 53:274–282