International Journal of Antimicrobial Agents 33 (2009) 328–333

Contents lists available at ScienceDirect

International Journal of Antimicrobial Agents

journal homepage: http://www.elsevier.com/locate/ijantimicag

Spread of multidrug-resistant *Proteus mirabilis* isolates producing an AmpC-type β-lactamase: epidemiology and clinical management

Francesco Luzzaro[a], Gioconda Brigante[a], Marco Maria D'Andrea[b], Beatrice Pini[a], Tommaso Giani[b], Elisabetta Mantengoli[b], Gian Maria Rossolini[b], Antonio Toniolo[a,*]

[a] *Laboratory of Medical Microbiology, Ospedale di Circolo and University of Insubria, Varese, Italy*
[b] *Department of Molecular Biology, University of Siena, Siena, Italy*

ARTICLE INFO

Article history:
Received 16 July 2008
Accepted 11 September 2008

Keywords:
Enterobacteriaceae
Epidemiology
Antimicrobial resistance
Treatment
Outcome

ABSTRACT

A remarkable increase in *Proteus mirabilis* strains producing acquired AmpC-type β-lactamases (CBLs) has been observed at Ospedale di Circolo e Fondazione Macchi (Varese, Italy) over the last few years. The epidemiology and treatment outcome of infections associated with this unprecedented spread are reported. From 2004–2006, 2070 *P. mirabilis* isolates were investigated. Extended-spectrum β-lactamases (ESBLs) and CBL resistance determinants were identified by gene amplification and direct sequencing. Clonal relatedness was evaluated by macrorestriction analysis. Overall, 43 CBL-positive isolates were obtained from hospitalised ($n = 22$) and non-hospitalised ($n = 21$) patients (median age 78.8 years). The prevalence of CBL-positive isolates increased from 0.3% in 2004 to 4.6% in 2006, whereas that of ESBL-positive isolates remained constant (ca. 10%). CBL-positive isolates were multidrug-resistant and carried the CMY-16 determinant. All but two isolates were genetically identical or closely related. Retrospective analysis of clinical records revealed that the majority of CMY-16-positive isolates were associated with urinary tract infections. Treatment with amikacin or carbapenems was consistently effective, whereas piperacillin/tazobactam produced a clinical response in seven of nine cases. This is the first report of a rapid spread of CBL-positive *P. mirabilis* strains endowed with remarkable antimicrobial resistance. Practical methods for CBL detection are needed for the appropriate management of related infections.

© 2008 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.

1. Introduction

AmpC-type β-lactamases (CBLs), belonging to Ambler's molecular class C and group 1 of the Bush–Jacoby–Medeiros functional classification, are a large group of enzymes that degrade most β-lactams, with the exception of carbapenems and, to a lesser extent, zwitterionic oxyimino-cephalosporins (such as cefepime and cefpirome). These enzymes are poorly inhibited by clavulanic acid and penicillanic acid sulfone [1,2]. CBLs are encoded by chromosomal genes resident in some Gram-negative pathogens, but a number of them are encoded by genes associated with mobile DNA elements capable of spreading by horizontal gene transfer. Acquired CBLs, which are usually plasmid-mediated and fall into six phylogenetic groups, are emerging worldwide in various species of Enterobacteriaceae such as *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella* spp. and *Proteus mirabilis* as a mechanism of acquired resistance to third-generation cephalosporins [1,3–6]. The epidemiological impact of these enzymes remains much lower than that of classical extended-spectrum β-lactamases (ESBLs) [7]. Very little is known in terms of the clinical management and outcome of infections caused by enterobacteria producing acquired CBLs [8].

In *P. mirabilis*, the production of acquired CBLs belonging to different lineages (ACC, CMY/LAT and DHA) has been occasionally reported from Europe, the USA and Asia [7,9–12]. No major outbreaks have been reported yet. At Ospedale di Circolo e Fondazione Macchi (Varese, Italy), ESBL production has been detected in *P. mirabilis* since 1997 [13], whilst the first strain of *P. mirabilis* producing an acquired CBL (CMY-16) was detected in 2003 [14]. In the following years, a remarkable increase in resistance to third-generation cephalosporins was observed among *P. mirabilis* isolates obtained both from hospitalised and non-hospitalised patients. Here we show that the increased resistance to third-generation cephalosporins in *P. mirabilis* was associated with the clonal spread of the CBL-positive strain first isolated in 2003. Dissemination of this strain and the clinical management of infected patients are reported.

* Corresponding author. Present address: Laboratory of Medical Microbiology, Ospedale di Circolo e Fondazione Macchi and University of Insubria, Viale Borri 57, 21100, Varese, Italy. Tel.: +39 0332 278 309; fax: +39 0332 260 517.
E-mail address: antonio.toniolo@gmail.com (A. Toniolo).

0924-8579/$ – see front matter © 2008 Elsevier B.V. and the International Society of Chemotherapy. All rights reserved.
doi:10.1016/j.ijantimicag.2008.09.007

2. Materials and methods

2.1. Bacterial isolates

Consecutive non-duplicate *P. mirabilis* isolates (obtained both from inpatients and outpatients) were collected at the Microbiology Laboratory of the Ospedale di Circolo e Fondazione Macchi over a 3-year period (2004, $n = 740$; 2005, $n = 650$; and 2006, $n = 680$). Biochemical identification to species level was performed using the Phoenix Automated Microbiology System (Becton Dickinson Diagnostic Systems, Sparks, MD). The system also provided quantitative determination of drug susceptibility, including to cefotaxime and ceftazidime. Isolates showing either reduced susceptibility or resistance to cefotaxime and/or ceftazidime (minimum inhibitory concentration (MIC) ≥ 2 mg/L) were suspected of producing ESBL and/or CBL. These isolates were stored at −70 °C for further investigation.

2.2. Extended-spectrum β-lactamase detection

ESBL production was assessed by disc diffusion according to confirmatory criteria of the Clinical and Laboratory Standards Institute (CLSI) [15]. Bacteriological media and susceptibility discs were obtained from Oxoid Ltd. (Basingstoke, UK). Briefly, susceptibility to cefotaxime (30 μg) and ceftazidime (30 μg) alone and in combination with clavulanic acid was determined. Results were regarded as positive when the zone diameter around the disc containing the drug in combination with clavulanic acid was ≥5 mm larger than that around the disc containing the drug alone. In CBL-producing isolates, the presence and nature of ESBL determinants was assessed by molecular analysis, as described previously [16].

2.3. AmpC-type β-lactamase detection

CBL production was first evaluated on the basis of decreased cefoxitin susceptibility using a standard disc diffusion method. Isolates showing an inhibition zone diameter <18 mm around the cefoxitin disc (30 μg) were regarded as suspect for harbouring a CBL determinant [17]. The presence of an acquired CBL determinant and its nature were investigated by molecular methods. Briefly, *ampC*-like determinants were amplified by polymerase chain reaction (PCR) using primers specific for plasmid-mediated CBLs, including CMY/LAT, DHA/MOR, CMY/MOX, FOX, ACT and ACC genes [14]. Isolates positive for CMY/LAT determinants were subjected to further PCR analysis. The complete coding sequences were amplified using CMY/F and CMY/R primers, as described previously [14]. PCR products were purified and subjected to double-strand sequencing using amplification primers (Macrogen Inc., Seoul, South Korea). CBL-positive isolates were further investigated by the Etest method (AB BIODISK, Solna, Sweden) in order to evaluate an extensive range of MICs for the following drugs: ampicillin; piperacillin; piperacillin/tazobactam (PIP/TAZ); cefoxitin; cefotaxime; ceftriaxone; ceftazidime; cefepime; aztreonam; imipenem; meropenem; amikacin; gentamicin; ciprofloxacin; levofloxacin; and trimethoprim/sulfamethoxazole (SXT).

2.4. Genotyping by pulsed-field gel electrophoresis (PFGE)

PFGE profiles of genomic DNA from 43 CBL-positive *P. mirabilis* isolates were analysed by means of the Gene Path System (Bio-Rad Laboratories, Richmond, CA) using the restriction enzyme *Sfi*I (Promega, Madison, WI). DNA fragments were electrophoresed in 1% agarose gel in 0.5× Tris–borate–ethylene diamine tetra-acetic acid (EDTA) buffer at 14 °C, 6 V/cm for 20 h, with pulse times ranging from 2.50 s to 30 s. Bacteriophage λ concatemers (Promega) were used as DNA size markers. Clonal relationships based on PFGE patterns were interpreted according to the criteria proposed by Tenover et al. [18].

2.5. Clinical data

Clinical records of hospitalised patients with infections caused by CBL-positive *P. mirabilis* were reviewed retrospectively. The following data were collected: age; sex; diagnosis at admission; ward of admission; site of infection; specimen source; underlying disease(s); treatment; and patient outcome. Data on antimicrobial treatment (drug, dosage and duration) were collected taking into account both the period preceding and that following the isolation of CBL-positive *P. mirabilis*. Treatment outcome was defined as: complete response (resolution of signs and symptoms of infection with microbiological evidence of eradication); clinical response (resolution of signs and symptoms in the absence of microbiological evaluation); failure (absence of resolution or worsening of signs and symptoms with or without microbiological evidence of persisting infection); or not assessable (incomplete records or death occurring within 72 h of diagnosis). With regard to non-hospitalised patients, the following data were collected: age; sex; site of infection; specimen source; residence in a nursing home; and hospital admission(s) in the preceding 2 years.

3. Results

3.1. Evolution of β-lactam resistance during the study period

From 2004–2006, 258/2070 (12.5%) *P. mirabilis* isolates showed reduced susceptibility or resistance to cefotaxime and/or ceftazidime, with an increasing prevalence over the 3-year period: 10.8% in 2004 (80/740), 11.2% in 2005 (73/650) and 15.4% in 2006 (105/680).

Based on CLSI criteria, 215 of the 258 isolates were confirmed as ESBL-producers (the nature of ESBL determinants was not established). The prevalence of these isolates remained constant over the study period (10.5% in 2004, 9.7% in 2005 and 10.9% in 2006). Among the 215 ESBL-positive isolates, 5 showed reduced susceptibility or resistance to cefoxitin (inhibition zone diameter <18 mm) and were taken as suspected CBL-producers. Molecular analysis failed to demonstrate acquired CBL determinants. The remaining 43 of the 258 isolates did not exhibit an ESBL phenotype according to CLSI criteria, and were cefoxitin-resistant (inhibition zone diameter ≤14 mm). Molecular analysis showed that all 43 of the latter strains carried the recently described bla_{CMY-16} acquired CBL gene [14]. Three of the isolates (VA-1395/05, VA-0186/06 and VA-0197/06) also encoded the TEM-92 ESBL. Fig. 1 summarises the procedures used to investigate *P. mirabilis* isolates and the numbers of ESBL-positive and/or CBL-positive strains.

Compared with that of ESBL-positive isolates, the prevalence of CMY-16-positive isolates increased significantly over time (0.3% in 2004, 1.5% in 2005 and 4.6% in 2006). Thus, CMY-16-producers represented the major reason for the increase in third-generation cephalosporin resistance observed from 2004 to 2006.

PFGE macrorestriction analysis revealed that 41 of the 43 isolates were identical or closely related to the CMY-16-positive isolate detected in 2003 (VA-1017/03). Three of the clonally related isolates carried the TEM-92 determinant in addition to CMY-16. The remaining two isolates only produced the CMY-16 enzyme and were not genetically related to the VA-1017/03 isolate (Fig. 2).

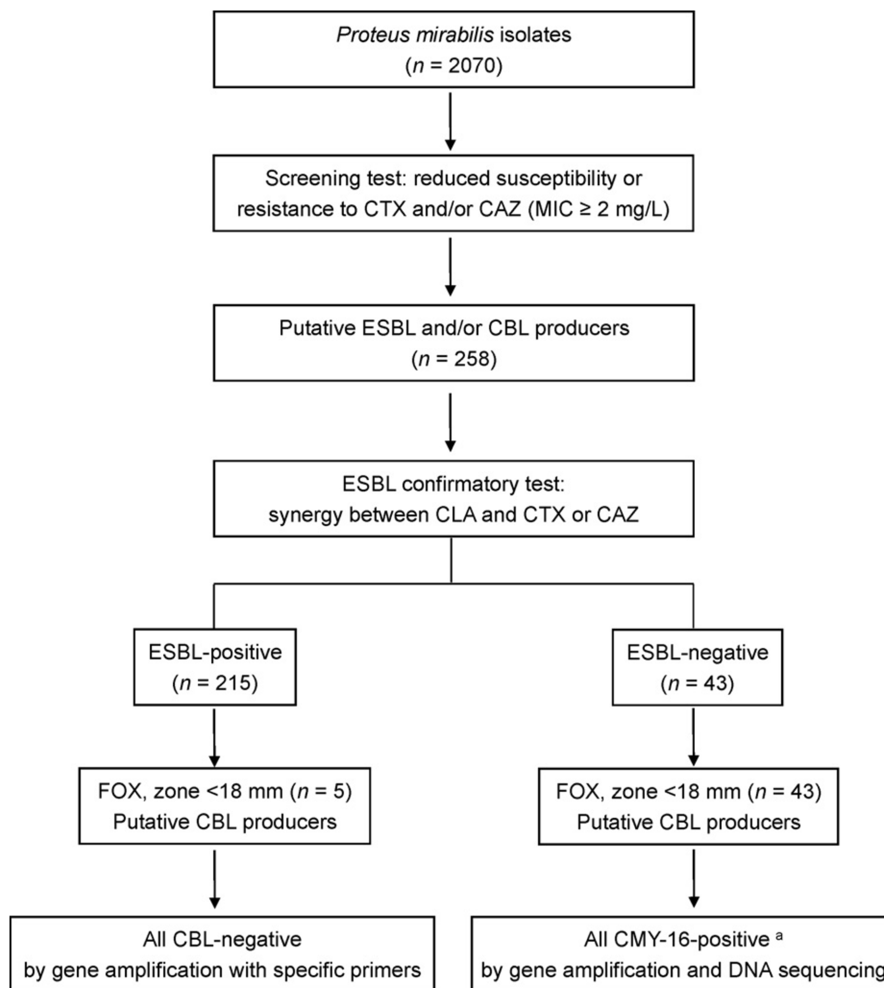

Fig. 1. Number of isolates and procedures used for the selection of extended-spectrum β-lactamase (ESBL)- and AmpC-type β-lactamase (CBL)-positive *Proteus mirabilis* isolates. CTX, cefotaxime; CAZ, ceftazidime; CLA, clavulanic acid; FOX, cefoxitin. [a]Three of CMY-16-positive isolates co-produced the TEM-92 ESBL.

3.2. *In vitro susceptibility to clinically relevant drugs*

CMY-16-positive isolates showed homogeneous β-lactam resistance phenotypes. As shown in Table 1, they were consistently intermediate or resistant to most β-lactams, including ampicillin, piperacillin, cefoxitin, cefotaxime, ceftazidime and ceftriaxone. In contrast, all strains remained susceptible to PIP/TAZ, cefepime, aztreonam, imipenem and meropenem. The same profile was also retained by the three isolates co-producing the TEM-92 ESBL, although elevated cefepime MICs were observed (8 mg/L vs. 2–4 mg/L). With regard to non-β-lactam drugs, CMY-16-positive isolates were consistently resistant to SXT and fluoroquinolones but susceptible to amikacin and gentamicin (with the exception of the three isolates co-producing TEM-92 that were also characterised by high-level gentamicin resistance) (Table 1).

3.3. *Patient population and source of specimens producing the CMY-16 enzyme*

The mean ± standard deviation age of the 43 patients with infections caused by CMY-producing isolates was 75.1 ± 15.3 years (median 78.8 years), i.e. similar to that of patients infected by ESBL-positive strains (mean 75.9 ± 16.6 years; median 75.6 years). In contrast, younger patients (mean 57.6 ± 27.6 years; median 65.4 years) were infected with ESBL- and/or CBL-negative *P. mirabilis* strains. The female/male ratio was 1.1 among patients infected by

Table 1
Resistance phenotype of CMY-16-positive *Proteus mirabilis* isolates.

Drug	Resistance phenotype[a]	MIC (mg/L)
Ampicillin	R	≥128
Piperacillin	R	≥128
PIP/TAZ	S	2/4–4/4
Cefoxitin	R	32–64
Cefotaxime	R	≥128
Ceftriaxone	I–R	16–32
Ceftazidime	R	32–64
Cefepime	S	2–4[b]
Aztreonam	S	1–2
Imipenem	S	2–4
Meropenem	S	0.064–0.25
Amikacin	S	4–8
Gentamicin	S–I	4–8[b]
Ciprofloxacin	R	>32
Levofloxacin	R	>32
SXT	R	>2/38

MIC, minimum inhibitory concentration; I, intermediate; R, resistant; S, susceptible; PIP/TAZ, piperacillin/tazobactam; SXT, trimethoprim/sulfamethoxazole.
[a] According to Clinical and Laboratory Standards Institute criteria [15].
[b] Isolates VA-1395/05, VA-0186/06 and VA-0197/06 (which co-produced the TEM-92 extended-spectrum β-lactamase) had a cefepime MIC of 8 mg/L. These isolates also showed high-level resistance to gentamicin (MIC ≥ 128 mg/L).

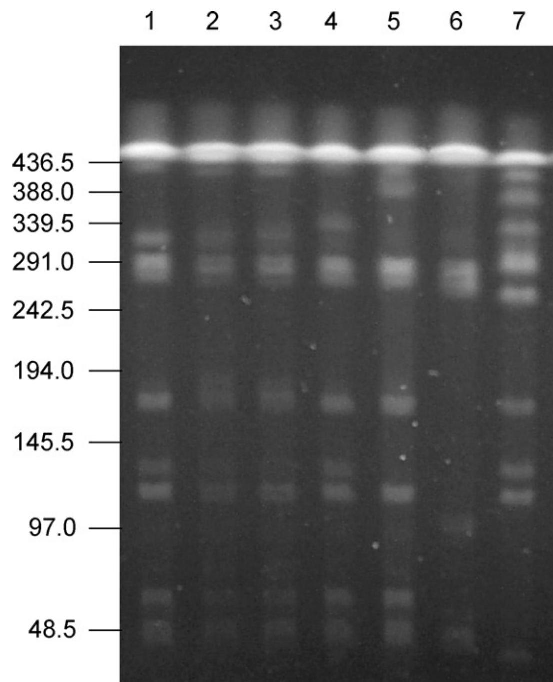

Fig. 2. Pulsed-field gel electrophoresis (PFGE) of genomic DNA digested with *Sfi*I of representative *Proteus mirabilis* isolates. Phage λ concatemers were used as size markers. Molecular sizes (in kb) are indicated to the left. Lane 1, VA-1017/03 (obtained in 2003) [14]; lanes 2 and 3, VA-0574/05 and VA-1395/05 (obtained in 2005), representative of isolates that showed a PFGE profile identical to that of VA-1017/03; lanes 4 and 5, VA-0070/06 and VA-0414/06 (obtained in 2006), representative of isolates that showed PFGE profiles closely related to that of VA-1017/03; and lanes 6 and 7, VA-0832/05 and VA-0887/05 (obtained in 2005), the two CMY-16 isolates unrelated to VA-1017/03.

CMY-16-positive strains vs. 1.6 in those infected by ESBL-positive isolates, and 2.1 in patients infected by *P. mirabilis* strains not carrying ESBL or CBL determinants.

Isolates carrying the CMY-16 determinant were obtained both from hospitalised ($n = 22$) and non-hospitalised ($n = 21$) patients. In all cases, isolates were most frequently obtained from urine samples (72.8% and 90.5%, respectively). The remaining isolates were obtained from the lower respiratory tract (13.6% inpatients, no outpatients), skin and soft-tissue infections (9.1% inpatients, 9.5% outpatients) and bloodstream infections (4.5% inpatients, no outpatients).

Among hospitalised patients, CMY-16-positive isolates were most frequently detected in medical wards (geriatrics, $n = 8$; internal medicine, $n = 4$; pneumology, $n = 1$; nephrology, $n = 1$; infectious diseases, $n = 1$; oncohaematology, $n = 1$), but also from Intensive Care Units ($n = 2$) and surgical wards ($n = 3$). One isolate was obtained from the blood of a patient admitted to the emergency room.

Sixteen of 21 non-hospitalised patients were resident in long-term care facilities (LTCFs), whereas the remaining five were regarded as true outpatients. Thirteen of the LTCF patients had been admitted to the hospital during the preceding 2 years (nine with multiple hospitalisations in the same geriatric ward). Prior hospital admissions were also documented in three of the five outpatients. These observations suggest that the CMY-16-positive strain may have initially spread from within the geriatric ward.

3.4. Antimicrobial treatment and outcome

Underlying co-morbidities, antimicrobial treatment and outcome were assessable in only 15 of the 22 inpatients. As shown in Table 2, different drugs were used for treating infections caused by CMY-positive strains. Antimicrobial treatment included PIP/TAZ ($n = 9$), imipenem ($n = 3$), meropenem ($n = 1$), amikacin ($n = 3$), gentamicin ($n = 2$), ciprofloxacin ($n = 2$) and SXT ($n = 1$). Overall, drugs ineffective in vitro (i.e. ciprofloxacin and SXT) failed to resolve infections, whereas carbapenems and amikacin (consistently active in vitro) were clinically effective. PIP/TAZ produced a clinical response in seven of nine cases. The overall mortality was low (2/15 cases) and was not directly attributable to infection.

4. Discussion

Acquired CBLs are emerging resistance determinants in Enterobacteriaceae and their increasing occurrence is of clinical and microbiological concern. Until now, acquired CBLs have been mainly reported from *K. pneumoniae* and *E. coli* [3,4,8,19], but only occasionally in other species that are devoid of resident CBLs (i.e. *K. oxytoca*, *Salmonella* spp. and *P. mirabilis*) [20]. With regard to *P. mirabilis*, recent investigations consistently indicated a very low prevalence of these enzymes: 0% in Korea [21]; 0.7% in Switzerland [22]; and 1.4% in the USA [7]. To our knowledge, this is the first report of the spread in a clinical setting of CBL-positive *P. mirabilis* strains that, over a 3-year period, reached the remarkable prevalence of ca. 5% and accounted for one-third of *P. mirabilis* isolates resistant to third-generation cephalosporins.

This large outbreak was mostly produced by dissemination of a single *P. mirabilis* clone producing the CMY-16 enzyme that was first detected at Ospedale di Circolo e Fondazione Macchi in 2003 [14]. The clonal dissemination was not only limited to inpatients of different hospital wards but also involved non-hospitalised patients, most of whom were LTCF residents and had previous admissions to the same geriatric ward. This observation indicates that the latter facilities may be responsible for the dissemination of resistant strains, as in the case of ESBL-producers [23,24]. The investigated strains appeared to have the potential for spreading across different health facilities and even to the community. The clonal nature of the outbreak suggests that suitable infection control measures could have been effective at containing the dissemination. It is also worth noting that clinical microbiology laboratories do play an important role in identifying outbreaks of this type and in monitoring the clinical outcome [25].

Treatment outcome could only be evaluated for a subgroup of patients. CMY-positive *P. mirabilis* strains were mostly associated with urinary tract infections in aged patients carrying a variety of co-morbidities. Among the drugs active in vitro, carbapenems and amikacin appeared consistently effective for these infections, whilst PIP/TAZ was ineffective in some cases. However, it should be noted that failures were observed among patients treated with low dosages of this drug. As expected, the use of drugs inactive in vitro (e.g. ciprofloxacin and SXT) resulted in clinical failure. Based on these observations and on a recent analysis of the clinical outcome of infections due to CBL-positive *K. pneumoniae* [8], carbapenems should be regarded as the drugs of choice for serious infections caused by CBL-positive organisms, as in the case of ESBL-associated infections [26,27]. Amikacin may be a useful option, taking into account its toxicity. The role of PIP/TAZ remains controversial. Nevertheless, satisfactory responses obtained with PIP/TAZ suggest that this drug combination may represent a valuable option in non-life-threatening infections.

From a microbiological point of view, the spread of plasmid-mediated AmpC-type enzymes highlights the need to establish criteria for detecting and reporting CBL production. Our data indicate that cefoxitin resistance in *P. mirabilis* combined with resistance to an oxyimino-cephalosporin (i.e. ceftazidime or cefotaxime) is a valuable indicator for suspecting CBL

Table 2
Antimicrobial treatment of CMY-associated infections and related outcome.

Isolate	Reason for admission	Age (years)	Sex	Date of admission	Date of isolate collection	Ward	Source of isolate	Antimicrobial agent	Daily dose	Duration (days)	Treatment outcome	Patient outcome
VA-0177/05	Removal of prosthetic devices	58	F	13 Jan. 2005	02 Feb. 2005	Neuro ICU	Urine	Amikacin	750 mg	12	Complete response	Transferred on Day 34 to rehabilitative LTCF
VA-1088/05	Prostatic resection	76	M	24 Aug. 2005	25 Aug. 2005	Urology	Urine	Gentamicin	80 mg × 3	6	Clinical response	Discharged on Day 9
VA-1162/05	Liver failure	92	M	22 Sept. 2005	23 Sept. 2005	Geriatrics	Urine	PIP/TAZ	2.25 g × 3	10	Failure	Transferred on Day 30 to nursing home
								Imipenem	500 mg × 2	8	Complete response	
VA-0104/06	COBP exacerbation	85	M	13 Jan. 2006	31 Jan. 2006	Geriatrics	Urine	PIP/TAZ	2.25 g × 3	12	Failure	Death on Day 59 not attributable to infection
								Imipenem	1 g × 3	2	Not assessable	
VA-0201/06	Cerebral thrombosis	90	F	02 Feb. 2006	28 Feb. 2006	Geriatrics	Urine	PIP/TAZ	2.25 g × 3	5	Clinical response	Discharged on Day 35
VA-0299/06	Pulmonary carcinoma	47	F	26 Mar. 2006	30 Mar. 2006	Medicine	Urine	PIP/TAZ	2.25 g × 3	7	Clinical response	Discharged on Day 17
VA-0313/06	Cerebral ischaemia	91	F	05 Mar. 2006	05 Apr. 2006	Geriatrics	Urine	Ciprofloxacin Gentamicin PIP/TAZ	400 mg × 2 120 mg × 2 2.25 g × 3	6 7 11	Failure Failure Complete response	Discharged on Day 72
VA-0361/06	Wasting	91	F	31 Mar. 2006	19 Apr. 2006	Geriatrics	Urine	Amikacin	500 mg × 2	11	Complete response	Transferred on Day 37 to nursing home
VA-0366/06	Pulmonary embolism	69	F	19 Apr. 2006	21 Apr. 2006	General ICU	Urine	Ciprofloxacin PIP/TAZ	400 mg × 2 2.25 g × 3	6 6	Failure Clinical response	Discharged on Day 15
VA-0492/06	Cerebral ischaemia	69	M	13 May 2006	29 May 2006	Medicine	Urine	PIP/TAZ	4.5 g × 3	6	Clinical response	Transferred on Day 25 to nursing home
VA-0493/06	Respiratory failure	91	M	27 May 2006	29 May 2006	Pneumology	Urine	PIP/TAZ	2.25 g × 3	13	Clinical response	Discharged on Day 13
VA-0530/06	Respiratory failure	60	M	17 Apr. 2006	07 June 2006	Pneumology	Bronchoaspirate	Amikacin	500 mg × 2	10	Clinical response	Transferred on Day 66 to nursing home
VA-0678/06	Hyperpyrexia in post-neurosurgical patient	66	M	10 Aug. 2006	14 Aug. 2006	Emergency room	Blood	Meropenem	1 g × 3	11	Complete response	Death on Day 77 not attributable to infection
VA-0777/06	Aplastic anaemia	67	M	30 Aug. 2006	04 Sept. 2006	Medicine	Urine	PIP/TAZ	4.5 g × 3	7	Complete response	Discharged on Day 16
VA-0833/06	Cerebral ischaemia	79	M	10 Sept. 2006	29 Sept. 2006	Medicine	Urine	SXT Imipenem	480 mg 500 mg × 2	4 8	Failure Clinical response	Discharged on Day 25

ICU, Intensive Care Unit; LTCF, long-term care facility; PIP/TAZ, piperacillin/tazobactam; COBP, chronic obstructive bronchopneumonopathy; SXT, trimethoprim/sulfamethoxazole.

production. However, current phenotypic methods are not conclusive in this regard and molecular methods remain indispensable [17].

It is worth noting that the simultaneous production of CBLs and ESBLs (as observed in three isolates) complicates the phenotypic detection of ESBLs. In our experience, combination disc tests failed to demonstrate ESBL production in *P. mirabilis* strains co-producing TEM-92 and CMY-16. ESBL determinants could only be demonstrated by molecular methods. In conclusion, expression of plasmid-mediated CBLs in *P. mirabilis* considerably limits therapeutic options. Practical laboratory methods are needed for the detection of these determinants and the management of related infections.

Funding: This work was supported by grants from the Ministry of Education (Rome, Italy) and the Ministry of Health (Rome, Italy).

Competing interests: None declared.

Ethical approval: Not required.

References

[1] Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type β-lactamases. Antimicrob Agents Chemother 2002;46:1–11.

[2] Jacoby GA, Munoz-Price LS. The new β-lactamases. N Engl J Med 2005;352:380–91.

[3] Alvarez M, Tran JH, Crow N, Jacoby GA. Epidemiology of conjugative plasmid-mediated AmpC β-lactamases in the United States. Antimicrob Agents Chemother 2004;48:533–7.

[4] Deshpande LM, Jones RN, Fritsche TR, Sader HS. Occurrence of plasmidic AmpC type β-lactamase-mediated resistance in *Escherichia coli*: report from the SENTRY Antimicrobial Surveillance Program (North America, 2004). Int J Antimicrob Agents 2006;28:578–81.

[5] Zaidi MB, Leon V, Canche C, Perez C, Zhao S, Hubert SK, et al. Rapid and widespread dissemination of multidrug-resistant *bla*$_{\text{CMY-2}}$ *Salmonella* Typhimurium in Mexico. J Antimicrob Chemother 2007;60:398–401.

[6] Literacka E, Empel J, Baraniak A, Sadowy E, Hryniewicz W, Gniadkowski M. Four variants of the *Citrobacter freundii* AmpC-type cephalosporinases, including novel enzymes CMY-14 and CMY-15, in a *Proteus mirabilis* clone widespread in Poland. Antimicrob Agents Chemother 2004;48: 4136–43.

[7] Moland ES, Hanson ND, Black JA, Hossain A, Song W, Thomson KS. Prevalence of newer β-lactamases in Gram-negative clinical isolates collected in the United States from 2001 to 2002. J Clin Microbiol 2006;44:3318–24.

[8] Pai H, Kang C-I, Byeon J-H, Lee KD, Park WB, Kim HB, et al. Epidemiology and clinical features of bloodstream infections caused by AmpC-type-β-lactamase-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother 2004;48:3720–8.

[9] Bret L, Chanal-Claris C, Sirot D, Chaibi EB, Labia R, Sirot J. Chromosomally encoded AmpC-type β-lactamase in a clinical isolate of *Proteus mirabilis*. Antimicrob Agents Chemother 1998;42:1110–4.

[10] Bidet P, Verdet C, Gautier V, Bingen E, Arlet G. First description of DHA-1 *ampC* β-lactamase in *Proteus mirabilis*. Clin Microbiol Infect 2005;11:591–2.

[11] Girlich D, Karim A, Spicq C, Nordmann P. Plasmid-mediated cephalosporinase ACC-1 in clinical isolates of *Proteus mirabilis* and *Escherichia coli*. Eur J Clin Microbiol Infect Dis 2000;19:893–5.

[12] Yong D, Lim Y, Song W, Choi YS, Park DY, Lee H, et al. Plasmid-mediated, inducible AmpC β-lactamase (DHA-1)-producing Enterobacteriaceae at a Korean hospital: wide dissemination in *Klebsiella pneumoniae* and *Klebsiella oxytoca* and emergence in *Proteus mirabilis*. Diagn Microbiol Infect Dis 2005;53:65–70.

[13] Endimiani A, Luzzaro F, Brigante G, Perilli M, Lombardi G, Amicosante G, et al. *Proteus mirabilis* bloodstream infections: risk factors and treatment outcome related to the expression of extended-spectrum β-lactamases. Antimicrob Agents Chemother 2005;49:2598–605.

[14] D'Andrea MM, Nucleo E, Luzzaro F, Giani T, Migliavacca R, Vailati F, et al. CMY-16, a novel acquired AmpC-type β-lactamase of the CMY/LAT lineage in multifocal monophyletic isolates of *Proteus mirabilis* from Northern Italy. Antimicrob Agents Chemother 2006;50:618–24.

[15] Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Seventeenth informational supplement. M100-S17. Wayne, PA: CLSI; 2007.

[16] Perilli M, Dell'Amico E, Segatore B, de Massis MR, Bianchi C, Luzzaro F, et al. Molecular characterization of extended-spectrum β-lactamases produced by nosocomial isolates of Enterobacteriaceae from an Italian nationwide survey. J Clin Microbiol 2002;40:611–4.

[17] Doi Y, Paterson DL. Detection of plasmid-mediated class C β-lactamases. Int J Infect Dis 2007;11:191–7.

[18] Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 1995;33:2233–9.

[19] Woodford N, Reddy S, Fagan EJ, Hill RL, Hopkins KL, Kaufmann ME, et al. Wide geographic spread of diverse acquired AmpC β-lactamases among *Escherichia coli* and *Klebsiella* spp. in the UK and Ireland. J Antimicrob Chemother 2007;59:102–5.

[20] Beceiro A, Bou G. Class C β-lactamases: an increasing problem worldwide. Rev Med Microbiol 2004;15:141–52.

[21] Song W, Kim J-S, Kim H-S, Yong D, Jeong SH, Park MJ, et al. Increasing trend in the prevalence of plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking chromosomal *ampC* gene at a Korean university hospital from 2002 to 2004. Diagn Microbiol Infect Dis 2006;55:219–24.

[22] Adler H, Fenner L, Walter P, Hohler D, Schultheiss E, Oezcan S, et al. Plasmid-mediated AmpC β-lactamases in Enterobacteriaceae lacking inducible chromosomal *ampC* genes: prevalence at a Swiss university hospital and occurrence of the different molecular types in Switzerland. J Antimicrob Chemother 2008;61:457–8.

[23] Bonomo RA. Multiple antibiotic-resistant bacteria in long-term care facilities: an emerging problem in the practice of infectious diseases. Clin Infect Dis 2000;31:1414–22.

[24] Crossley K. Long-term care facilities as sources of antibiotic-resistant nosocomial pathogens. Curr Opin Infect Dis 2001;14:455–9.

[25] Simor AE. The role of the laboratory in infection prevention and control programs in long-term care facilities for the elderly. Infect Control Hosp Epidemiol 2001;22:459–63.

[26] Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657–86.

[27] Yang K, Guglielmo BJ. Diagnosis and treatment of extended-spectrum and AmpC β-lactamase-producing organisms. Ann Pharmacother 2007;41: 1427–35.