Detection of *mcr-1* colistin resistance gene in polyclonal *Escherichia coli* isolates in Barcelona, Spain, 2012 to 2015

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Colistin resistance was detected in 53 of 10,011 *Escherichia coli* (0.5%) by prospective phenotypic testing of consecutive clinical isolates in a single hospital in Barcelona, Spain (2012–15). The *mcr-1* gene was retrospectively identified by PCR and sequencing in 15 of 50 available isolates. Each isolate had a unique PFGE pattern except for two. This clonal diversity supports the hypothesis of horizontal dissemination of the *mcr-1* gene in the local study population.

Following the report on the plasmid-mediated colistin resistance gene *mcr-1* in China [1], several authors have reported the detection of this gene in *Escherichia coli* isolates of animal origin [1–5]. Currently there have been few reports of detections in humans and these involve mainly multidrug-resistant (MDR) Gram-negative bacilli [3,5–7]. To date, *mcr-1* has been detected in at least five European countries in animals and humans, and often in association with recent travel to Asia [3,5–7]. In this context, we describe *mcr-1* detection in unselected clinical isolates of *E. coli* in Barcelona in samples from 2012 to 2015.

Laboratory investigation

A total of 10,011 *E. coli* were isolated between January 2012 and December 2015 from clinical specimens in our institution, a tertiary referral teaching hospital covering an area of 407,902 inhabitants in Barcelona, Spain. Only one isolate per patient was included. Isolates from colonisation screenings were not considered. Antibiotic susceptibility testing was performed by disc diffusion according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) [8]. As a first approach to screen colistin resistance, a 10 µg disc of colistin was used. Isolates displaying an inhibition zone ≤12 mm (n = 61) were selected for further testing of minimal inhibitory concentration (MIC) by gradient diffusion (Etest, bioMérieux, France). Both diffusion methods were performed on Mueller Hinton agar (bioMérieux, France). MIC results of colistin

were interpreted following the EUCAST breakpoints for *Enterobacteriaceae* [9]. Resistance to colistin was detected in 53 *E. coli* isolates (0.5%). Of these, 40 were isolated from urine specimens, eight from blood cultures and the remaining five from other clinical specimens. The average age of the patients with infections caused by colistin-resistant *E. coli* was 70.9 years (range: 6–99 years). The male:female ratio was 1:2.

By amplification and Sanger sequencing, we searched for the presence of the *mcr-1* gene in our collection of colistin-resistant *E. coli* isolates (only 50 isolates were available). The amplification of *mcr-1* was performed as described by Liu et al. [1]. This gene was detected in 15 isolates; the amplified fragments had 100% sequence homology with the previously described *mcr-1* [1].

The patients' average age was 62 years (range: 6–97), eight of them were male and seven were female. Patients were not epidemiologically linked (Table). One patient was referred from a nursing home, and nine had had at least one hospital admission during the previous year. No travel abroad was recorded in any of the patients. The rate of positivity corresponded to 0.15% of the total of *E. coli* isolates within the period studied. Seven *mcr-1*-harbouring isolates were not MDR according to international definitions [10]. Only two were extended-spectrum beta-lactamase carriers and one had an AmpC overproduction profile (Table). Tested by Etest, the MIC to colistin ranged from 4 mg/L to 12 mg/L. The *mcr-1*-positive isolates were typed by pulsed-field gel electrophoresis (PFGE); each isolate had a unique PFGE pattern except for two.

Discussion

Colistin is one of the last resorts to treat infections caused by MDR Gram-negative bacilli. Resistance to colistin is rarely reported in *E. coli*, especially in non-MDR isolates from humans [11]. Until recently, this resistance was considered to be based solely on

TABLE

Characteristics of *Escherichia coli* isolates harbouring *mcr-1* and epidemiological data of the patients, Barcelona, 2012–15 (n = 15)

Date of isolation	Isolation site	Classification of infection ^a	Colistin MIC (mg/L)	Antimicrobial resistance pattern
27/12/2012	Blood	Community-acquired	8	AMP-SXT
09/01/2013	Sputum	Hospital-acquired (haematology)	4	AMP-CTX-CAZ-FEP CIP-SXT (ESBL)
26/02/2013	Blood	Community-acquired	4	AMP-SXT
01/03/2013	Blood	Hospital-acquired (oncology)	12 ^b	AMP-GEN-TOB
07/03/2013	Blood	Healthcare-associated	6	AMP-CTX-CAZ-FEP (ESBL)
12/03/2013	Sputum	Healthcare-associated	12	AMP-AMC-CTX-CAZ-SXT
08/06/2013	Urine	Community-acquired	4	AMP-NAL
07/07/2013	Blood	Community-acquired	4 ^b	AMP-GEN-TOB
01/11/2013	Sputum	Hospital-acquired (recovery room)	4	AMP-CIP
22/05/2014	Urine	Hospital-acquired (neurosurgery)	4	AMP-NAL-SXT
22/08/2014	Urine	Healthcare-associated	6	AMP-NAL-GEN-TOB-SXT
06/10/2014	Surgical wound	Healthcare-associated	8	AMP-CIP-GEN-TOB
14/03/2015	Urine	Healthcare-associated	4	AMP-CIP-GEN-TOB-SXT
29/03/2015	Urine	Hospital-acquired (cardiology)	4	AMP-CIP-GEN-SXT
16/06/2015	Urine	Healthcare-associated ^c	4	AMP-NAL

AMP: ampicillin; AMC: amoxicillin/clavulanic acid; CAZ: ceftazidime; CIP: ciprofloxacin; CTX: cefotaxime; FEP: cefepime; GEN: gentamicin; MIC: minimum inhibitory concentration; NAL: nalidixic acid; SXT: trimethoprim-sulfamethoxazole; TOB: tobramycin; ESBL: extended spectrum beta-lactamase.

^a Classification of the infection according to the place of acquisition. When hospital-acquired, the hospital ward where the clinical specimen was taken is shown in brackets.

^b Isolates sharing the same PFGE pattern.

^c This patient was referred from a nursing home.

genomic mutations in several genes involved in the synthesis of lipopolysaccharide [12]. Since Liu et al. reported plasmid-mediated colistin resistance in *E. coli* isolates [1], the whole scenario has changed and the possibility of horizontal gene transfer needs to be considered. These plasmids carry the *mcr-1* gene coding for a phosphoethanolamine transferase, an enzyme related to changes in lipid A [1]. Despite the large amount of information on *mcr-1* obtained in only a few months, the real prevalence of this gene in clinical isolates is not yet known. Most reports are retrospective, mainly refer to faecal carriers and describe scattered colistin-resistant isolates randomly collected [3,5-7,13]. We here describe *mcr-1* prevalence in colistin-resistant clinical isolates of *E. coli*. As a limitation, no other mechanisms of colistin resistance were searched for in the present study. However, the high percentage of *mcr-1* among our colistin-resistant isolates is noteworthy.

The clonal diversity shown in the present report supports the hypothesis of horizontal dissemination of *mcr-1* gene-related colistin resistance in *E. coli* isolated from our urban patient population in Barcelona. Colistin is not always tested in non-MDR *E. coli* isolates of human origin. This may explain why the previous reports describing *mcr-1* in humans mainly referred to MDR *E. coli* isolates [3,5-7]. Technical variability among methods for colistin susceptibility testing is notorious

[14]. Given the discrepancies between the international committees and the lack of colistin breakpoints for *Enterobacteriaceae* in CLSI, we considered it convenient to apply a screening method. Although disc diffusion is not recommended to test colistin susceptibility, it was useful for an initial screening followed by confirmation using a MIC method.

The fact that seven of 15 *mcr-1*-harbouring strains were not MDR may not seem clinically relevant. However, horizontal spread is important epidemiologically. Screening of colistin resistance in human isolates of *Enterobacteriaceae* should be encouraged in order to know the real extent of a problem that may get worse given the constant exchange of resistance genes across microbiomes (i.e. food animals, the environment and human populations). The broad veterinary use of colistin and the increasing reports of colistin resistance in *Enterobacteriaceae* isolates from food animals are a matter of concern [15]. Spain is one of the European countries with larger use of polymyxins in veterinary medicine [16]. This fact may correlate with the high rates of colistin-resistant *Salmonella* spp. isolates in farm animals previously reported in our country [17]. The use of colistin in humans varies depending on the type of institution involved and their corresponding antimicrobial policy. In our hospital, it has increased 14-fold (0.10 to 1.47 defined daily dose /100 occupied bed-days) from 2007 to 2014.

Considering that the emerging plasmid-mediated resistance to colistin has already spread across microbiomes and considering the selective pressure that the veterinary use of this antibiotic may exert, action is urgent at a global level. Otherwise we may soon face a situation without useful antibiotics to treat infections caused by MDR Gram-negative bacteria.

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Conflict of interest

None declared.

Authors' contributions

NP, AR and BM conceived and designed the study; NP and AR performed the antimicrobial susceptibility tests; JR and ME performed the molecular assays; MT collected the epidemiological data; NP, AR, MT, PC and BM wrote the manuscript.

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