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First Detection of the *mcr-1* Colistin Resistance Gene in *Escherichia coli* in Italy

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Polymyxins are old antibiotics that have recently regained popularity for treatment of severe infections caused by extensively drug-resistant (XDR) Gram-negative bacterial strains (1). As a likely consequence, emergence of polymyxin resistance is being increasingly reported in the clinical setting, especially among carbapenem-resistant *Klebsiella pneumoniae* isolates (2, 3). Acquired resistance to polymyxins is generally associated with chromosomal mutations (4, 5), but a new plasmid-mediated transferable resistance determinant, the *mcr-1* gene, encoding a phosphoethanolamine transferase, has been described recently (6). The *mcr-1* gene was originally detected in *Enterobacteriaceae* (mostly *Escherichia coli*) of animal and human origin in China (6) and subsequently also elsewhere (7–15), suggesting a broader distribution. In this communication, we report on the first detection of *mcr-1* in colistin-resistant (COL-R) *E. coli* isolates from Italy.

A retrospective analysis of the laboratory databases from the clinical microbiology laboratories of two Italian hospitals (Florence, central Italy; Lecco, northern Italy) revealed overall stable and low rates of colistin resistance among *E. coli* clinical isolates in the period 2012 to 2015 (Table 1). In both laboratories, routine susceptibility testing had been performed with the Vitek 2 system (bioMérieux, Marcy l'Etoile, France), and interpretation had been performed according to the EUCAST breakpoints (www.eucast.org).

Eleven isolates that had been reported as colistin resistant (COL-R) were available for investigation, and nine of them were confirmed to be COL-R by reference broth microdilution (16). The COL MIC was 8 μg/ml in all cases.

The presence of *mcr-1* was screened for by PCR and sequencing as previously described (6), using also an additional pair of primers (CLR5-F1, 5′-ATGATGCAGCATACTTCTGTGTGG; CLR5-R1, 5′-TCAGCGGATGAATGCGGTGC) targeting the extremities of the *mcr-1* gene. Multilocus sequence typing (MLST) was carried out as described previously (17).

Eight of the nine COL-R *E. coli* isolates were positive for the *mcr-1* gene. In the sequenced region (positions 25 to 1576), with reference to the *mcr-1* coding sequence (accession no. KP347127), the nucleotide sequences from all isolates were identical to that previously reported (6). Isolates positive for *mcr-1* were detected from both centers, from inpatients in different wards, and also from two outpatients. Two *mcr-1*-positive isolates (LC-902/14 and LC-279/13) exhibited a multidrug-resistant phenotype, including expanded-spectrum cephalosporins, fluoroquinolones, and trimethoprim-sulfamethoxazole, and produced extended-spectrum-β-lactamase activity (Table 2).

TABLE 1 Rates of colistin resistance among *E. coli* clinical isolates from two different Italian settings

Year	Florence		Lecco	
	Total no. of tested isolates	No. (%) of COL-R isolates	Total no. of tested isolates	No. (%) of COL-R isolates
2012	6,241	50 (0.8)	1,995	17 (0.9)
2013	8,927	62 (0.7)	2,456	19 (0.8)
2014	10,636	64 (0.6)	2,534	9 (0.4)
2015	9,373	66 (0.7)	2,368	24 (1)

The *mcr-1*-positive isolates belonged to several different sequence types (STs) (Table 2), some of which have not been previously associated with *mcr-1*. No clear epidemiological relationships could be traced among the *mcr-1*-positive isolates detected in each setting.

Taken together, the present findings revealed that *E. coli* strains carrying the *mcr-1* gene are circulating in Italy, in the clinical setting, with a multifocal distribution and have done so at least since 2013. The fact that *mcr-1* was detected in the majority of the COL-R *E. coli* isolates available for investigation suggests that it plays an important role as a polymyxin resistance determinant in this species. Emergence of *mcr-1* in clinical isolates of *E. coli* is alarming, and a broader surveillance of this resistance determinant would be advisable, although colistin resistance in *E. coli* from clinical specimens remains very uncommon and no increasing trends were recently observed in our settings.

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TABLE 2 Features of the *mcr-1*-positive *E. coli* clinical isolates[a]

Isolate	Isolation date	Source	Ward	ST	MIC mg/liter (S/I/R)[b]										
					AMC	CTX	CAZ	FEP	PIP/TAZ	ERT	IMP	MEM	GEN	CIP	SXT	COL
LC-279/13[c]	June 2013	Urine	Neurology	354	8 (S)	8 (R)	2 (I)	2 (I)	≤4 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤1 (S)	>4 (R)	>320 (R)	8 (R)
LC-705/14	August 2014	Urine	Medicine	131	8 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤4 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤1 (S)	≤0.25 (S)	>320 (R)	8 (R)
LC-902/14[c]	October 2014	Urine	Outpatient	602	4 (S)	>64 (R)	≤1 (S)	2 (I)	≤4 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤1 (S)	>4 (R)	>320 (R)	8 (R)
LC-968/14	November 2014	Surgical wound	Intensive care unit	10	16 (R)	≤1 (S)	≤1 (S)	≤1 (S)	8 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤1 (S)	>4 (R)	>320 (R)	8 (R)
LC-17/15	January 2015	Urine	Neurosurgery	95	4 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤4 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤1 (S)	≤0.25 (S)	>320 (R)	8 (R)
FI-4531	November 2015	Urine	Outpatient	648	8 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤4 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤1 (S)	≤0.25 (S)	>320 (R)	8 (R)
FI-4592	November 2015	Urine	Orthopedics	804	4 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤4 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	>16 (R)	≤0.25 (S)	>320 (R)	8 (R)
FI-4451	November 2015	Urine	Medicine	117	≤2 (S)	≤1 (S)	≤1 (S)	≤1 (S)	≤4 (S)	≤0.5 (S)	≤0.25 (S)	≤0.25 (S)	≤1 (S)	≤0.25 (S)	≤20 (S)	8 (R)

[a] Colistin MICs were determined by reference broth microdilution; susceptibility to other agents was determined with the Vitek 2 system. Isolates indicated as LC were from Lecco, and those indicated as FI were from Florence.

[b] Abbreviations for antimicrobial agents are as follows: AMC, amoxicillin-clavulanic acid; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; PIP/TAZ, piperacillin-tazobactam; ERT, ertapenem; IMP, imipenem; MEM, meropenem; GEN, gentamicin; CIP, ciprofloxacin; SXT, trimethoprim-sulfamethoxazole; COL, colistin. S, susceptible; I, intermediate; R, resistant.

[c] Extended-spectrum-β-lactamase (ESBL)-producing isolate.

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