Prevalence of faecal carriage of colistin-resistant Gram-negative rods in a university hospital in western France, 2016

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Abstract

Plasmid-mediated and chromosomally-encoded colistin resistance is increasingly being reported worldwide. We aimed to determine the prevalence of faecal carriage of colistin-resistant Gram-negative rod isolates in a university hospital in western France. From February to May 2016, rectal swabs from 653 patients hospitalized in various clinical settings were recovered and subsequently screened for colistin resistance using the SuperPolymyxin medium. Antimicrobial susceptibilities were determined according to EUCAST guidelines. Genetic detection of plasmid-mediated colistin resistance was performed by PCR. The faecal carriage with intrinsic colistin-resistant isolates was high (23 %), while the faecal carriage with Gram-negative rods showing acquired resistance was low (1.4 %). No isolate carried the plasmid-mediated *mcr-1*/*mcr-2* genes. It was noteworthy that none of the patients carrying isolates with acquired colistin resistance had previously received a colistin-based treatment, while these isolates were not multidrug resistant.

Plasmid-mediated *mcr-1* [1] and *mcr-2* genes [2] conferring colistin resistance in *Enterobacteriaceae* have been discovered recently in animals worldwide. Simultaneously, human infections and faecal carriage with *Enterobacteriaceae* isolates carrying the *mcr-1* gene were reported extensively during 2015 [3]. In France, a high prevalence of the plasmid-borne *mcr-1* gene has been reported among extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* isolates collected from the faeces of diarrheic veal calves [4]. This finding may suggest that the dissemination of *mcr-1* from animals to humans may have already occurred quite extensively.

We therefore conducted a prospective study to evaluate the prevalence of faecal carriage of acquired and intrinsic colistin-resistant Gram-negative rods among patients admitted to or hospitalized at the Pellegrin University Hospital of Bordeaux (1350 beds), which is the largest public hospital in south-west France. From February to May 2016, rectal swabs from 653 patients hospitalized in various clinical settings (emergency, intensive care unit, surgery, medical units) were screened. Colistin-resistant isolates were screened using the SuperPolymyxin medium, which contains colistin sulfate at a concentration of 3.5 mg l^{-1}, allowing the isolation of any colistin-resistant Gram-negative rods [5]. Colonies growing on this medium were identified using a MALDI-TOF mass spectrometer (Brüker, Champs-sur-Marne, France). Isolates belonging to species that are intrinsically resistant to colistin were not further investigated. Minimum inhibitory concentrations (MICs) of colistin were determined for the other isolates using the reference broth microdilution (BMD) method. The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines, 2016 [6]. All colistin-resistant isolates were subsequently screened for the *mcr-1* and *mcr-2* genes by PCR, as described previously [1, 2].

Out of the 653 rectal swabs, 150 gave isolates that are known to be intrinsically resistant species, namely *Proteus* sp. (*n*=75), *Morganella* sp. (*n*=22), *Providencia* sp. (*n*=12), *Serratia* sp. (*n*=6) and *Hafnia* sp. (*n*=35) [the latter genus was recently described as being naturally resistant to colistin (A. Jayol, personal communication)].

Nine isolates with an acquired colistin trait were recovered, including three *Escherichia coli*, two *Klebsiella pneumoniae*, one *Raoultella ornithinolytica*, two *Enterobacter cloacae* and one *Stenotrophomonas maltophilia* (Table 1). The MIC values of colistin ranged from 4 to 128 mg l^{-1} for those isolates that exhibited various profiles of resistance to the other

Table 1. Colistin-resistant *Enterobacteriaceae* isolates

Species	MIC of CS (mg l^{-1})	Additional antibiotic resistances	ESBL	Unit
E. coli	4	AMX, TIC, SXT	No	Emergency
E. coli	4	AMX, TIC, FQ, GM	No	Surgery ICU
E. coli	8	AMX, C1G	No	Neurosurgery ICU
K. pneumoniae	64	FQ, SXT	No	Medical ICU
K. pneumoniae	32	AMX, TIC, C3G, C4G, FQ, FSF, FT, GM, SXT	Yes	Surgery
R. ornithinolytica	16	No	No	Gastroenterology
E. cloacae	64	AMX, TIC, C3G, C4G, FT, GM, SXT	Yes	Medical ICU
E. cloacae	8	FT	No	Emergency
S. maltophilia	128	–	No	Medical ICU

AMX, amoxicillin; C1G, first-generation cephalosporin; C3G, third-generation cephalosporin; C4G, fourth-generation cephalosporin; CS, colistin; ESBL, extended-spectrum beta-lactamase; FQ, fluoroquinolone; FSF, fosfomycin; FT, nitrofurantoin; GM, gentamicin; ICU, intensive care unit; SXT, co-trimoxazole; TIC, ticarcillin.

antibiotics (Table 1). Only two isolates (a single *K. pneumoniae* and a single *E. cloacae*) were multidrug resistant and produced an extended-spectrum ß-lactamase. All those isolates were negative for the *mcr-1* and *mcr-2* genes. The two colistin-resistant *E. cloacae* isolates presented 'skip wells' with the BMD method, suggesting a heteroresistant phenotype. These isolates could belong to a cluster-dependent colistin-heteroresistant complex, as described recently [7].

Out of the nine patients carrying an acquired colistin-resistance trait, five were hospitalized in an intensive care unit, and two were from the community. None of the patients had previously received a colistin-based treatment, further highlighting the occurrence of colistin-resistant isolates without obvious corresponding antibiotic selection, as previously notified by Olaitan *et al* [8].

This prospective study indicates a high prevalence (23 %) of faecal carriage with intrinsic colistin-resistant Gram-negative rods, but a low prevalence (1.4 %) with isolates showing acquired resistance. No isolate carried the plasmid-mediated *mcr-1* and *mcr-2* genes, suggesting that there is currently low diffusion of these resistance determinants among human clinical samples. Similar observations were recently made during hospital- and community-based surveys in Switzerland [9]. However, single *E. coli* and *K. pneumoniae* isolates with a plasmid-borne *mcr-1* gene were isolated in 2016 from clinical infections in our hospital (A. Jayol, personal communication). Both isolates were ESBL producers and the *E. coli* one was recovered from a patient without a recent history of travel. Regular screening of plasmid-mediated colistin resistance, which is now quite easy to implement owing to the availability of a selective medium (SuperPolymyxin), may now be important to monitor and therefore possibly detect at an early stage the emergence of colistin-resistant isolates, with the ultimate goal of preventing their further spread in France.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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