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Transferability of the *mcr-1* Colistin Resistance Gene

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Polymyxins (colistin, polymyxin B) are last-resort antibiotics for treating infections with carbapenem-resistant bacteria. Therefore, the frequency of its use in hospital settings is on the rise worldwide. Until recently, acquired resistance to colistin was mainly due to chromosomal alterations of pathways involved in modification of the bacterial outer membrane components (lipopolysaccharides).[1,2] However, the plasmid-mediated colistin resistance gene, *mcr-1*, has been recently reported worldwide after its first identification in *Enterobacteriaceae* from the environment, animals, and humans in China.[3] The aim of this study was therefore to determine to which frequencies plasmids carrying the *mcr-1* gene could be transferred by conjugation among enterobacterial species.

Three MCR-1-producing clinical isolates were used as donors in mating-out experiments.[4] *Escherichia coli* strains Af24, Af31, and Af48 possessed plasmids of various sizes and incompatibility groups (Table 1). Mating-out assays were performed in triplicate onto Luria–Bertani (LB) agar plate, using a 1:10 donor to recipient ratio, as described elsewhere.[5] Transconjugants *E. coli* J53 were selected on LB agar plates containing colistin (1 μg/ml) and azide (100 μg/ml). Transfer frequencies were calculated as the number of transconjugants obtained per donor. *E. coli* J53 transconjugants carrying the *mcr-1* plasmids were obtained, namely Tc24, Tc31, and Tc48 (Table 1). Those plasmids were self-conjugative, although the frequency was relatively low, ranging from 10^{-6} to 10^{-7} transconjugants/donor (Table 1). These frequencies were much lower than the transfer rate reported in the pioneer work (10^{-1} to 10^{-3}) from *E. coli* SHP45 to *E. coli* C600, when determined with a 64-kb IncI2-type plasmid. Although *E. coli* Af24 harbored a plasmid of approximately the same size than that of an IncI2-type found in *E. coli* SHP45 described in the original study reporting MCR-1,[3] transfer frequency was at least 1,000-fold lower. The size and the incompatibility group of the *mcr-1*-positive plasmids did not modify their transfer rate. The IncX4 plasmid was slightly less conjugative, with a frequency observed at $7.5 \pm 1.9 \times 10^{-7}$, despite its smaller size (30 kb).

Conjugation frequencies were also determined without the influence of the chromosomal background of the donor (clinical isolate), using the three isogenic MCR-1-positive *E. coli* J53 as donors, and *E. coli* ST69 (nalidixic acid resistant), *Klebsiella pneumoniae* CIP53153, *Klebsiella oxytoca* R192, a series of *Enterobacter cloacae* recipient strains including *E. cloacae* CIP103624, and strains Enc1 to Enc5, and two *Enterobacter aerogenes* strains R232 and R233. The experiments were performed by plating the conjugation mixture onto LB agar plates supplemented with colistin (1 μg/ml) and nalidixic acid (20 μg/ml) or ampicillin (100 μg/ml). The transfer rates of the MCR-1-encoding plasmids ranged from 10^{-5} to 10^{-6} in *E. coli*, from 10^{-5} to 10^{-6} in *K. pneumoniae*, from 10^{-4} to 10^{-5} in *K. oxytoca*, and 10^{-7}–10^{-8} in *E. cloacae* (Table 2). Those results correspond to common conjugation frequencies for antibiotic resistance plasmids in *Enterobacteriaceae*, being often in the range from 10^{-5} to 10^{-6}. The *E. coli* transconjugants had minimal inhibitory concentration (MIC) values of colistin at 8–16 μg/ml for *E. coli* and at 32–64 μg/ml for *Klebsiella* spp. compared to 0.25 μg/ml for the recipient strains (Table 3). The higher MIC values obtained in *Klebsiella* spp. may be related to differential gene expression or different protein functionality in those two enterobacterial species.

Table 1. Plasmid Features of the Three MCR-1-Positive *E. coli* Clinical Isolates and Their Conjugative Frequencies in *E. coli* J53

Isolate (ST type)	*Plasmid name*	*Plasmid size (kb)*	*Incompatibility group*	*Conjugative frequency in* E. coli *J53*
E. coli Af24 (ST1007)	Af24	70	IncI2	$6.7 \pm 3.9 \times 10^{-6}$
E. coli Af31 (ST624)	Af31	150	IncHI2	$2.2 \pm 1.7 \times 10^{-6}$
E. coli Af48 (ST624)	Af48	30	IncX4	$7.5 \pm 1.9 \times 10^{-7}$

Standard deviation was calculated from three independent cultures.

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Table 2. Conjugative Transfer Frequencies of *mcr*-1-Positive *E. coli* J53 Transconjugants to *E. coli* ST69, *Klebsiella pneumoniae* CIP53153, *Klebsiella oxytoca* R192 *Enterobacter cloacae* CIP103624, and *Enterobacter aerogenes* R232

	E. coli *ST69*	K. pneumoniae *CIP53153*	K. oxytoca *R192*	E. cloacae *CIP103624*	E. aerogenes *R232*
Tc24	$2.8 \pm 1.7 \times 10^{-5}$	$6.0 \pm 7.4 \times 10^{-6}$	$1.2 \pm 0.43 \times 10^{-4}$	10^{-7}	$1.3 \pm 0.43 \times 10^{-3}$
Tc31	$5.6 \pm 3.8 \times 10^{-6}$	$6.5 \pm 1.4 \times 10^{-5}$	$1.9 \pm 3.3 \times 10^{-4}$	10^{-7}	$1 \pm 1.8 \times 10^{-4}$
Tc48	$4.9 \pm 2.3 \times 10^{-5}$	$1.7 \pm 0.41 \times 10^{-6}$	$2.8 \pm 4.3 \times 10^{-5}$	10^{-7}	$5.7 \pm 5 \times 10^{-3}$

Standard deviation was calculated from three independent cultures.

Table 3. MIC Values of Colistin (μg/ml) for *E. coli* Clinical Strains and Transconjugants Producing MCR-1

	Clinical strain	E. coli *J53*	*Tc* E. coli *J53*	E. coli *ST69*		
Plasmid	*Donor*	*Transconjugant*	*Donor*	*Transconjugant*	K. pneumoniae *CIP53153*	K. oxytoca *R192*
Af24	8	8	8	8	32	64
Af31	8	8	8	16	64	64
Af48	8	8	8	8	32	64

All recipient strains have a minimal inhibitory concentration (MIC) value of 0.25 μg/ml for colistin.

Since the occurrence of the *mcr-1* gene was reported in *E. cloacae*,[6] and since our preliminary experiments indicated failed to detect conjugation into that species, we conducted additional experiments to evaluate the conjugation efficiency in additional strain backgrounds. Six *E. cloacae* and two *E. aerogenes* strains were therefore tested as recipients. A very low conjugation frequency (between 10^{-7} and 10^{-8} depending on the strains) was obtained with the different *E. cloacae* recipient strains. A higher conjugation rate was found with the different *E. aerogenes* strains (between 10^{-3} and 10^{-4}).

The transfer frequency of the IncI2-type plasmid carrying the *mcr-1* gene reported in the pioneer study was surprisingly high, ranging from 10^{-1} to 10^{-3} from *E. coli* to *E. coli*. Here our results do not support those latter observations, with conjugation frequencies being lower in the different enterobacterial backgrounds used. Regardless of their incompatibility types, plasmids carrying the *mcr-1* gene could be transferred to several enterobacterial species (at least to *E. coli*, *K. pneumoniae*, *K. oxytoca*, and *E. aerogenes*) at a conjugation rate varying from 10^{-4} to 10^{-6}.

The study further confirms that the *mcr-1* gene may efficiently spread among most enterobacterial species, with variable frequencies depending on the recipient strains/species, rather than on the plasmid types. Low rates of transconjugants obtained in *E. cloacae* might be linked to a deleterious effect of *mcr-1* gene expression in that species, considering that MCR-1 is a phosphoethanolamine transferase modifying the lipopolysaccharides structure, therefore modifying the bacterial surface.

There is a need to monitor the spread of the *mcr-1* gene between *E. coli* on one hand and multidrug-resistant *K. pneumoniae* strains on the other hand, since it may possibly give rise to pandrug-resistant isolates such as those producing MCR-1 and carbapenemases.[7]

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Disclosure Statement

No competing financial interests exist.

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