AAC Accepted Manuscript Posted Online 15 October 2018
Antimicrob. Agents Chemother. doi:10.1128/AAC.01618-18

AAC Research Article

Comparison of the Superpolymyxin™ and CHROMID® Colistin R screening media for the detection of colistin-resistant *Enterobacteriaceae* from spiked rectal swabs

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Running title: Screening for colistin-resistant *Enterobacteriaceae*

Keywords : polymyxin, sensitivity, specificity, MCR

Synopsis: 228 words

Text: 1833 words

ABSTRACT

The dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE), has led to the increased use of colistin, which resulted in the emergence of colistin-resistant *Enterobacteriaceae* worldwide. One of the most threatening scenarios is the dissemination of colistin-resistance in CPE, particularly the plasmid-encoded resistance MCR. Thus, it becomes now mandatory to possess reliable media to screen for colistin-resistant Gram-negative isolates, especially *Enterobacteriaceae*. In this study we evaluated the performances of the Superpolymyxin™ medium (ELITechGroup) and the CHROMID® Colistin R (bioMérieux) to screen for colistin-resistant *Enterobacteriaceae* from spiked rectal swabs. Stools were spiked with a total of 94 enterobacterial isolates (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella enterica*, *Enterobacter cloacae*), including 53 colistin-resistant isolates. ESwabs™ (Copan Diagnostics) were then inoculated with those spiked fecal suspensions and proceed as recommended by both manufacturers. The sensitivity of detection colistin-resistant Enterobacteriaceae were of 86.8 % [95 % confidence interval (CI95) 74.0 – 94.0] using both the Superpolymyxin™ medium and the CHROMID® Colistin R plates. Surprisingly, the isolates that were not detected were not the same for both media. The specificities were high for both media, at 97.9% [CI95 = 87.3% - 99.9%] for Superpolymyxin™ medium and 100% [CI95 = 90.4% - 100%] for the CHROMID® Colistin R medium. Both commercially-available media, CHROMID® Colistin R and Superpolymyxin™, provide a useful tool to screen for colistin-resistant *Enterobacteriaceae* from patient samples (rectal swabs) regardless of the level and mechanism of colistin resistance.

INTRODUCTION

Colistin and polymyxin B represent one of the few remaining treatment options for multidrug and extremely drug resistant Gram negative bacteria, especially carbapenemase-producing *Enterobacteriaceae* (CPE) (1). Uncertainty remains over the best treatment option that have to be used to manage infections caused by CPE, carbapenem in combination with amikacin, or colistin treatments have achieved therapeutic results in some cases (2). Unfortunately, due to the dissemination of CPE, the increased use of colistin led to the emergence of colistin-resistant *Enterobacteriaceae* worldwide (3). Colistin is a cationic antimicrobial peptide that interacts with the lipid A moiety of the lipopolysaccharide (LPS), disrupting the negatively charged outer-membrane of Gram-negative bacteria. In Gram-negative bacteria, the main resistance mechanisms consist on LPS modification through the addition of positively charged 4-amino-4-deoxy-L-arabinose or phosphoethanolamine. In *Enterobacteriaceae* the operons encoding enzymes involved in these modifications are *arnBCADTEF* and *pmrCAB*, respectively (4-6). Activation of the LPS-modifying genes is associated with chromosome-encoded resistance mechanisms, such as mutations in the PmrA/PmrB or PhoP/PhoQ two-component systems, or through alterations to the master regulator MgrB (5, 6). In 2016, the expression of a plasmid-encoded phosphoethanolamine transferase, named MCR-1 has been described as being involved in colistin resistance in *Enterobacteriaceae* (7). Since then eight families of *mcr* genes (*mcr-1* to *-8*) have been assigned and seven were published (7-13). One of the most threatening scenario is the wide dissemination of *mcr* in CPEs limiting again the therapeutic options. In addition, with (i) the rapid rise of *mcr* variants and (ii) the probability that an unknown number of polymyxin resistance mechanisms are as yet unidentified, the use of molecular techniques for the identification and the screening of colistin-resistant isolates is not universally possible.

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Accordingly, it becomes now mandatory to possess reliable media to screen for colistin-resistant isolates (3).

Superpolymyxin™ and CHROMID® Colistin R are ready-to-use selective agar media designed for the screening for colistin-resistance in Gram-negatives. The target microorganisms are *Enterobacteriaceae* (mostly *Escherichia coli, Klebsiella pneumoniae, Salmonella* spp. and *Enterobacter* spp.) for both media, and *Acinetobacter* spp. and *Pseudomonas aeruginosa* for the Superpolymyxin™ medium only. The CHROMID ® Colistin R is a chromogenic medium that distinguishes *E. coli* (pink), *Klebsiella* spp., *Enterobacter* spp., *Serratia* spp. (blue) and *Salmonella* spp. (colourless), while the Superpolymyxin™ contains EosinY and methylene blue dyes that help to distinguish lactose positives (purple) from lactose-non-fermenters (colourless). Both media are claimed to work on bacterial cultures, stool samples, rectal swabs (caecal samples from poultry, pigs and calves might also be used). The present study aimed to compare the performance of these media on a collection of well-characterized colistin-resistant *Enterobacteriaceae* spiked at different concentrations in stools and inoculated on swabs mimicking rectal swab samples.

RESULTS

The sensitivity for the detection of colistin-resistant *Enterobacteriaceae* were of 86.8% [50% confidence interval (CI50) = 74.0% - 94.0%] and 84.9% [CI95 = 71.8% - 92.8%] using the Superpolymyxin™ medium and the CHROMID® Colistin R plate after 24 h incubation, respectively. The sensitivity was the same after 48h incubation 86.8% [CI95= 74.0% - 94.0%]. Surprisingly, the isolates that were not detected were not the same for both media (Table 1). The specificities were high for both media, at 97.5% [CI95 = 85.6% - 99.9%] and 100% [CI95 = 89.3% - 100%] for the Superpolymyxin™ medium and the CHROMID® Colistin R medium, respectively. Overall, the CHROMID® Colistin R medium performed slightly better with *K. pneumoniae* and *Salmonella enterica* than the Superpolymyxin™ medium with sensitivities of 100% [CI50 = 85.0% - 100%] and 96.2 [CI50 = 78.4% - 99.8%] and specificities of 100% [CI50 = 80.8% - 100%] and of 87.0% [CI50 = 65.3% - 96.6%], respectively. Conversely, CHROMID® Colistin R did not detect 7/25 colistin-resistant *E. coli* while only four strains did not grow on Superpolymyxin™ (Table 1). The lack of detection was not correlated with colistin MICs, nor the presence or absence of *mcr*-like genes (Table 1). For colistin-resistant isolates detected on both media (14 *E. coli*, 24 *K. pneumoniae* and 1 *S. enterica*), the detection limit was at least 1 log lower for CHROMID® Colistin R in 69.2% (27/39) of the isolates, equivalents for both media in 20.5% (8/39) of the cases and at least 1 log better for the Superpolymyxin™ medium in 7.7% (3/39) of the tested isolates (all *E. coli*). This lower LOD of the CHROMID® Colistin R protocol might be the result of the 4 hours enrichment step in colistin supplemented broth. In order to decipher whether such enrichment step might increase the performances of the Superpolymyxin™ medium, the seven colistin-resistance isolates which did not grown on the Superpolymyxin™ medium were subjected to the enrichment step similarly to that performed for the CHROMID® Colistin R protocol. This additional step did not allow them to grow on the Superpolymyxin™ medium, suggesting that this enrichment should not be recommended for the use with this selective medium. As previously reported by Jayol *et al.* for the Superpolymyxin™ medium, the prolonged incubation from 24 to 48h did not modify the performance of the Superpolymyxin™ medium (14). Regarding the CHROMID® Colistin R, prolonged incubation to 48h of one MCR-1-producing *E. coli* isolate (strain CNR164 A5) allowed us to identify typical pink colonies that were barely undetectable at 24h of incubation. Finally, one *E. cloacae* isolate positive for *mcr-4.2* was not detected by both media. As previously described for *mcr-3* and *mcr-4* variants in CPEs (15), the presence of *mcr-4.2* do not conferred phenotypical resistance to polymyxins in this *E. cloacae* isolate (colistin MIC of 0.5 mg/L).

DISCUSSION

Based on this study performed with spiked rectal swabs, CHROMID® Colistin R and Superpolymyxin™ selective media showed very similar performances. The main advantage of the Superpolymyxin™ medium is that it could be directly inoculated with the rectal swabs without any enrichment step (4 hours) in colistin supplemented broth as compared to the CHROMID® Colistin R. On the other hand, the main advantage of CHROMID® Colistin R lies in the use of chromogenic molecules enabling the rapid presumable identification of the growing colonies (pink for *E. coli*, blue for *Klebsiella*, *Enterobacter*, *Serratia* and white for *Salmonella*). Indeed, the morphological aspect of the colonies on the Superpolymyxin™ medium was indistinguishable between *E. coli*, *K. pneumoniae* and *Salmonella enterica* (Figure 1).). As species cannot easily be differentiated on Superpolymyxin™, clinical labs must then identify the growing colonies before reporting results. In our study, the selectivity of both media was good since no Gram-positive bacteria nor yeast grew on them.

Of note, unlike the CHROMID® Colistin R medium, which is currently limited to be used with *Enterobacteriaceae*, the Superpolymyxin™ medium was also claimed to detect colistin resistance in all Gram-negative bacteria, including *Acinetobacter* spp. and *P. aeruginosa*. Accordingly, we tested the Superpolymyxin™ medium for three colistin-resistant (all producing OXA-23 carbapenemase) and four colistin-susceptible *A. baumannii* isolates. In all three colistin-resistant isolates, a mutation of PmrB (A226T, A226V and R263H) resulted in MICs ranging from 16 to 64 mg/L. The Superpolymyxin™ medium fully detected all colistin-resistant isolates, while none of the four susceptible strains grew on the medium.

As colistin resistance is likely to increase in a near future, clinical microbiology laboratories will require, rapid and reliable screening media to identify carriers in hospital settings. Here, we have shown that both commercially available media, CHROMID® Colistin R and Superpolymyxin™, are useful tool to screen for colistin-resistant *Enterobacteriaceae* from patient samples (rectal swabs) regardless of the level and mechanism of colistin resistance.

MATERIAL AND METHODS

Susceptibility testing

MICs were determined by broth microdilution according to the guidelines of the CLSI and EUCAST joint subcommittee (16). Results were interpreted using EUCAST breakpoints as updated in 2018.

Bacterial isolates

Ninety-four enterobacterial isolates were tested, including 53 isolates exhibiting resistance to colistin (MIC > 2 mg/L). The colistin resistance mechanism of all these isolates has been characterized at the molecular level (Table 1). The tested isolates were as follows: colistin resistant isolates with colistin MICs ≥ 4 mg/L: *Escherichia coli* (n=25, including 20 isolates carrying *mcr* genes), *Klebsiella pneumoniae* (n=25, including 3 isolates carrying *mcr* genes) and *Salmonella enterica* (n=3 isolates carrying *mcr* genes), and colistin susceptible *E. coli* (n=19), *K. pneumoniae* (n=16), *Salmonella enterica* (n=5) and one *mcr-4.2* positive *Enterobacter cloacae* (Table 1). Chromosomally-encoded mutations in genes responsible for colistin resistance (*pmrA*, *pmrB*, *phoP*, *phoQ*, *mgrB*, and *crrB* genes) were also searched as described previously (17).

Spiked-rectal swabs

Bacterial suspensions of strains with an optical density of 0.5 McFarland (inoculum of $\sim 10^{8}$ CFU/mL) were serially diluted in water and ten fold dilutions of pure solution to 10^{-3} dilution were used to spike liquid stools from healthy volunteers (1g in 1 mL of sterile water), as previously described (18). The bacterial suspensions that were used to spike stools from healthy volunteers were verified by concomitant inoculation of Mueller-Hinton agar with 10 µL of the 10^{-4} suspension diluted to in water. Ten microliters of bacterial suspension were added to 90 µL of stool. The totality (100 µL) of this spiked stool was then absorbed on the Eswab™ and introduced into 1 ml AMIES transport medium (Copan Diagnostics, Murrieta, CA, USA) to mimic a true rectal swabs. Each ESwab™ containing stool with each dilution of bacteria was then performed according to the recommendations of both manufacturers (Figure S1). Briefly, ten microliters of the inoculated AMIES medium were transferred to the Superpolymyxin™ agar (ELITechGroup, Puteaux, France) and spread with a plate spreader without enrichment step. The CHROMID® Colistin R agar plates (bioMérieux, La Balmes-Les-Grottes, France) were inoculated after an enrichment step as follows: 200 µl of each inoculated AMIES suspension were introduced into 10 mL of Brain Heart Infusion medium (BHI, bioMérieux) supplemented with one disc of colistin (10 µg) and incubated for four hours at 37°C before seeding in dials of 50 µL.

Determination of the limit of detection (LOD)

The lowest detection limit (LOD) correspond the minimum number of bacteria that must be present in the sample to obtain a growth on the selective medium. In contrast to other studies that evaluated the performance of selective media with cultured bacteria (14, 19, 20), our study was performed on inoculated rectal swabs. This involves further dilution of the spiked stool sample in the Eswab™ AMIES buffer (Figure S1). As indicated by the manufacturer of the Superpolymyxin™ medium (ELITechGroup), the threshold value of the susceptible strains could not be greater than 5 x 10^6 CFU/ml (directly from a bacterial suspension) because susceptible bacteria could benefit from an inoculum artifact to grow on the selective medium. Accordingly, the threshold for the LOD value was set at ≥ 1 x 10^6 CFU/mL in the Eswab™ AMIES buffer corresponding to an initial concentration of 1 x 10^8 CFU/ml in the spiked stool (Figure S1, Table 1). A fecal suspension without addition of bacterial strain was used as negative control. In addition, ten randomly selected strains were tested by a second experimenter to assess reproducibility. In all cases results were identical between all experimenter.

Statistical analysis

The sensitivity and specificity values were calculated with their respective confidence interval 95% (95%CI) using the free software vassarStats: (Website for statistical Computation on http://vassarstats.net/).

AKNOWLEDGMENTS

This work was partially supported by bioMérieux and ELITechGroup.

We would like to thank Pr. Glupczynski, Dr. Pierre Bogaerts and Pr. Richard Bonnet for providing some well-characterized colistin-resistant *E. coli* and *K. pneumoniae* isolates.

CONFLICT OF INTEREST

None to declare

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Table 1. Limit of detection of colistin resistant *Enterobacteriaceae* on CHROMID® Colistin R and Superpolymyxin™ media.

			Colistin resistance		Lowest detection limit (CFU/mL)[a]				
					in Eswab™ AMIES buffer		in spiked stools		
Species	**Name**	**Colistin MIC (mg/L)**	**Plasmid / Chromosome**	**Mechanism**	**CHROMID® Colistin R**	**Superpolymyxin™**	**CHROMID® Colistin R**	**Superpolymyxin™**	**Ref.**
Colistin-resistant Enterobacteriaceae (n=53)									
Escherichia coli	CNR 111 J7	16	Chr	PmrB mutations (D14N, S71C, V83A)	1 x 10^2	1 x 10^4	1 x 10^4	1 x 10^6	(21)
	CNR 20160039	4	Chr	unknown	1 x 10^2	> 1 x 10^6	1 x 10^4	> 1 x 10^8	(21)
	CNR 20160235	8	Chr	MgrB mutation (V8A)	1 x 10^5	> 1 x 10^6	1 x 10^7	> 1 x 10^8	(21)
	CNR 1728	8	Chr	PmrB mutation (G160E)	1 x 10^6	1 x 10^4	1 x 10^8	1 x 10^6	(21)
	41489	4	P	*mcr-1*	1 x 10^3	1 x 10^5	1 x 10^5	1 x 10^7	(21)
	J53 + *mcr-1**	8	P	*mcr-1*	1 x 10^6	1 x 10^4	1 x 10^8	1 x 10^6	(21)
	CNR20140385	4	P	*mcr-1*	> 1 x 10^6	1 x 10^4	> 1 x 10^8	1 x 10^6	(21)
	S08-056	4	P	*mcr-1*	1 x 10^4	1 x 10^3	1 x 10^6	1 x 10^5	(21)
	CNR 117 G7	4	P	*mcr-1*	> 1 x 10^6	1 x 10^4	> 1 x 10^8	1 x 10^6	(22)
	CNR 121 G9	4	P	*mcr-1*	1 x 10^6	1 x 10^5	1 x 10^8	1 x 10^7	(23)
	R12 F5	4	P	*mcr-2*	1 x 10^3	> 1 x 10^6	1 x 10^5	> 1 x 10^8	(11)
	CNR 1745	4	P	*mcr-1*	> 1 x 10^6	1 x 10^4	> 1 x 10^8	1 x 10^6	(21)
	CNR 1604	4	P	*mcr-1*	1 x 10^6	1 x 10^4	1 x 10^8	1 x 10^6	(21)
	CNR 1790	4	P	*mcr-1*	1 x 10^3	1 x 10^3	1 x 10^5	1 x 10^5	(21)
	CNR 1859	4	P	*mcr-1*	1 x 10^3	1 x 10^3	1 x 10^5	1 x 10^5	(21)
	CNR 1886	4	P	*mcr-1*	1 x 10^2	1 x 10^4	1 x 10^4	1 x 10^6	(21)

	TOP 10 + *mcr-5**	8	P	*mcr-5*	1 x 10^{3}	1 x 10^{5}	1 x 10^{5}	1 x 10^{7}	(21)
	4222	4	P	*mcr-1*	1 x 10^{2}	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	(21)
	4070	4	P	*mcr-1*	1 x 10^{4}	1 x 10^{3}	1 x 10^{6}	1 x 10^{5}	(21)
	979	4	P	*mcr-1*	1 x 10^{3}	1 x 10^{3}	1 x 10^{5}	1 x 10^{5}	(21)
	6383	4	P	*mcr-1.5*	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	(21)
	1724	4	P	*mcr-1*	1 x 10^{4}	1 x 10^{3}	1 x 10^{6}	1 x 10^{5}	(21)
	1670	4	P	*mcr-1.5*	1 x 10^{5}	1 x 10^{5}	1 x 10^{7}	1 x 10^{7}	(21)
	36070	8	P	*mcr-3.2*	1 x 10^{5}	1 x 10^{5}	1 x 10^{7}	1 x 10^{7}	(24)
	CNR 164 A5	4	P	*mcr-1*	[b]1 x 10^{5}	$\geq$ 1 x 10^{6}	1 x 10^{7}	$\geq$ 1 x 10^{8}	This study
Klebsiella pneumoniae	CNR 20140042	16	Chr	MgrB N42Y and K43I	1 x 10^{3}	1 x 10^{3}	1 x 10^{5}	1 x 10^{5}	This study
	CNR 20140661	64	Chr	MgrB Q30 stop	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	This study
	CNR 20151119	64	Chr	MgrB L4 stop	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	This study
	CNR 20150622	64	Chr	MgrB Y41 stop	1 x 10^{2}	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	This study
	CNR 20150777	128	Chr	MgrB Y41 stop	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	This study
	CNR 20150944	64	Chr	MgrB modified sequence since AA 42	1 x 10^{2}	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	This study
	CNR 20150309	64	Chr	MgrB modified sequence since AA 37	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	This study
	CNR 20150675	64	Chr	*mgrB* truncated in *orf* by IS*10*	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	This study
	CNR 20140483	32	Chr	*mgrB* truncated in *orf* by IS*1F*-like	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	This study
	CNR 20140563	64	Chr	*mgrB* truncated in *orf* by IS*1R*	1 x 10^{2}	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	This study
	CNR 20150050	32	Chr	*mgrB* truncated in promoter by IS1R	1 x 10^{3}	1 x 10^{3}	1 x 10^{5}	1 x 10^{5}	This study
	CNR 20140591	64	Chr	*mgrB* truncated in *orf* by IS5-like	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	This study
	CNR 20140550	32	Chr	*mgrB* truncated in promoter by IS903D	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	This study
	CNR 20151285	32	Chr	*mgrB* truncated in *orf* by IS*903*-like	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	This study
	S14-002	64	Chr	*mgrB* truncated in promoter by IS*Kpn*14	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	This study
	CNR 20140101	32	Chr	*ΔmgrB*	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	This study
	CNR 2015007	32	Chr	*ΔmgrB*	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	This study
	CNR 20150066	16	Chr	*ΔmgrB*	1 x 10^{3}	$\geq$ 1 x 10^{6}	1 x 10^{5}	$\geq$ 1 x 10^{8}	This study
	CNR 20151223	32	Chr	*ΔmgrB*	1 x 10^{2}	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	This study
	S15	64	Chr	*mgrB* truncated in *orf* by IS*Kpn*25	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	(25)
	CNR 1630	64/32	Chr	*mgrB* truncated in *orf* by IS5	1 x 10^{2}	1 x 10^{5}	1 x 10^{4}	1 x 10^{7}	This study
	CNR 1861	16	Chr	PmrB mutation (T157P)	1 x 10^{3}	1 x 10^{4}	1 x 10^{5}	1 x 10^{6}	This study
	CNR 1601	32	Chr + P	*mcr-1* + *mgrB* truncated in *orf* by IS5	1 x 10^{2}	1 x 10^{4}	1 x 10^{4}	1 x 10^{6}	This study
	CNR 1732	4	P	*mcr-1*	1 x 10^{3}	1 x 10^{3}	1 x 10^{5}	1 x 10^{5}	This study

	CNR 1853	4	P	*mcr-1*		1×10^{3}	1×10^{3}	1×10^{5}	1×10^{5}	This study
Salmonella enterica										
Paratyphi B d-tartrate + (biotype java)	201610686	8	P	*mcr-1*		1×10^{3}	1×10^{5}	1×10^{5}	1×10^{7}	This study
Typhimurium	CNR 1776	8	P	*mcr-1*		1×10^{3}	$\geq 1 \times 10^{6}$	1×10^{5}	$\geq 1 \times 10^{8}$	This study
Paratyphi B d-tartrate + (biotype java)	13-SA01718	8	P	*mcr-5*		1×10^{3}	$\geq 1 \times 10^{6}$	1×10^{5}	$\geq 1 \times 10^{8}$	(8)
Colistin-susceptible Enterobacteriaceae (n=41)										
Escherichia coli	TOP 10	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608071881	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608072264	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608073733	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608073228	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608078635	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608078858	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608062671	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1608064819	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	2H6	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	LAN 10.48	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	VER 9.39	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1F1	0.25				$\geq 1 \times 10^{6}$	1×10^{6}	$\geq 1 \times 10^{8}$	1×10^{8}	(21)
	1A6	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	1A8	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	2A1	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	2D9	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	2C4	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
	2D5	0.25				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	(21)
K. pneumoniae	1609056413	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	This study
	1609061149	1				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	This study
	2 E8	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	This study
	2 I4	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	This study
	2 F1	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	This study
	2 I5	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	This study
	3 B4	0.5				$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{6}$	$\geq 1 \times 10^{8}$	$\geq 1 \times 10^{8}$	This study

	3 B7	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
	1 B6	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
	CNR 173 F9	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
	1 C9	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
	1 E3	1			> 1 x 10^6	1 x 10^4	> 1 x 10^8	1 x 10^6	This study
	2 B1	1			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
	CNR 173 E3	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
	2 C6	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
	2 D2	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
Salmonella enterica									
4,12:i:-	201604739	1			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
Enteritidis	201608919	1			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
Typhimurium	201606509	1			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
Enteritidis	201607559	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
Veneziana	201610299	0.5			> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study
Enterobacter cloacae	CNR 131 G4	0.5	P	*mcr-4.2*	> 1 x 10^6	> 1 x 10^6	> 1 x 10^8	> 1 x 10^8	This study

Sensitivity (%) : 86.8 (CI 95 % 74.0 – 94.0) for both media after 48h incubation

Specificity (%) : 100 (CI 95% 89.3 -100) and 97.5 (CI95% 85.6 – 99.9) respectively for CHROMID® Colistin R and Superpolymyxin™

[a] Underligned CFU counts are considered as negative results.

[b] P, Plasmid; Chr, Chromosome

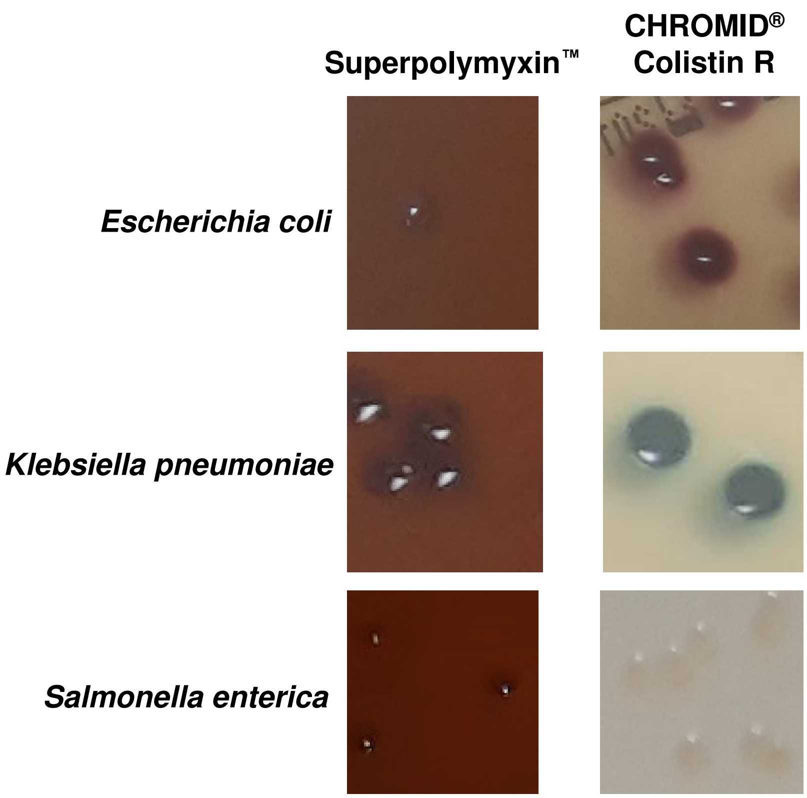
[c] After 48h incubation (no colony at 24h)

Ref., reference number

LEGEND OF THE FIGURE

Figure 1. Morphological aspect of colonies of *E. coli*, *K. pneumoniae* and *Salmonella enterica* grown on Superpolymyxin™ and CHROMID® Colistin R media.

Figure 1.


Superpolymyxin™
CHROMID®
Colistin R
Escherichia coli
Klebsiella pneumoniae
Salmonella enterica