

Review Article

Development of New Tools to Detect Colistin-Resistance among Enterobacteriaceae Strains

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The recent discovery of the plasmid-mediated *mcr-1* gene conferring resistance to colistin is of clinical concern. The worldwide screening of this resistance mechanism among samples of different origins has highlighted the urgent need to improve the detection of colistin-resistant isolates in clinical microbiology laboratories. Currently, phenotypic methods used to detect colistin resistance are not necessarily suitable as the main characteristic of the *mcr* genes is the low level of resistance that they confer, close to the clinical breakpoint recommended jointly by the CLSI and EUCAST expert systems ($S \leq 2$ mg/L and $R > 2$ mg/L). In this context, susceptibility testing recommendations for polymyxins have evolved and are becoming difficult to implement in routine laboratory work. The large number of mechanisms and genes involved in colistin resistance limits the access to rapid detection by molecular biology. It is therefore necessary to implement well-defined protocols using specific tools to detect all colistin-resistant bacteria. This review aims to summarize the current clinical microbiology diagnosis techniques and their ability to detect all colistin resistance mechanisms and describe new tools specifically developed to assess plasmid-mediated colistin resistance. Phenotyping, susceptibility testing, and genotyping methods are presented, including an update on recent studies related to the development of specific techniques.

1. Introduction

Multidrug-resistant (MDR) bacteria are of a global concern, notably with the description of carbapenemase-producing Enterobacteriaceae [1]. Colistin is an old antibiotic that regained popularity as a last resort treatment to face the worldwide emergence of these pathogens [2]. Colistin is a polycationic and bactericidal drug that targets the lipid A moiety of lipopolysaccharide (LPS), moving its cationic charges, leading to cell wall lysis and bacterial death [3]. The increasing use of colistin has led to emerging resistance, a phenomenon that represents a clinical source of worry [4]. Enterobacteriaceae are Gram-negative bacteria that are often described as the pathogens responsible for human infectious diseases, particularly the *Escherichia coli* and *Klebsiella pneumoniae* species. Until recently, all mechanisms described were of chromosomal origin, mostly mediated by the two-component systems PmrAB and PhoPQ, leading to the addition of positively charged carbohydrates

on the negatively charged lipid A (Figure 1), a phosphoethanolamine by a phosphoethanolamine transferase or a 4-amino-4-arabinose by surexpression of *arnBCADTEF* operon, leading to the loss of polymyxin affinity for the LPS [5]. In November 2015, Liu et al. reported the first plasmid-mediated gene which they named *mcr-1* [6], which encodes for a phosphoethanolamine transferase, and this was followed by the description of variants (*mcr-1.2*, *mcr-1.3*, . . .) and the genes *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7*, and *mcr-8* [7–15]. This recent discovery raised concern about the increase and spread of resistance among the Enterobacteriaceae [16] and led to new recommendations for laboratory diagnosis and clinicians [17]. Specifically, the majority of these *mcr-1* strains exhibited a low minimal inhibitory concentration (MIC) of colistin, around 4 µg/ml [6], which is close to the MIC breakpoint according to the EUCAST guidelines (susceptibility ≤ 2 µg/ml and resistance > 2 µg/ml) (<http://www.eucast.org>). Moreover, several studies have reported the detection of the *mcr-1* gene in carbapenemase-producing

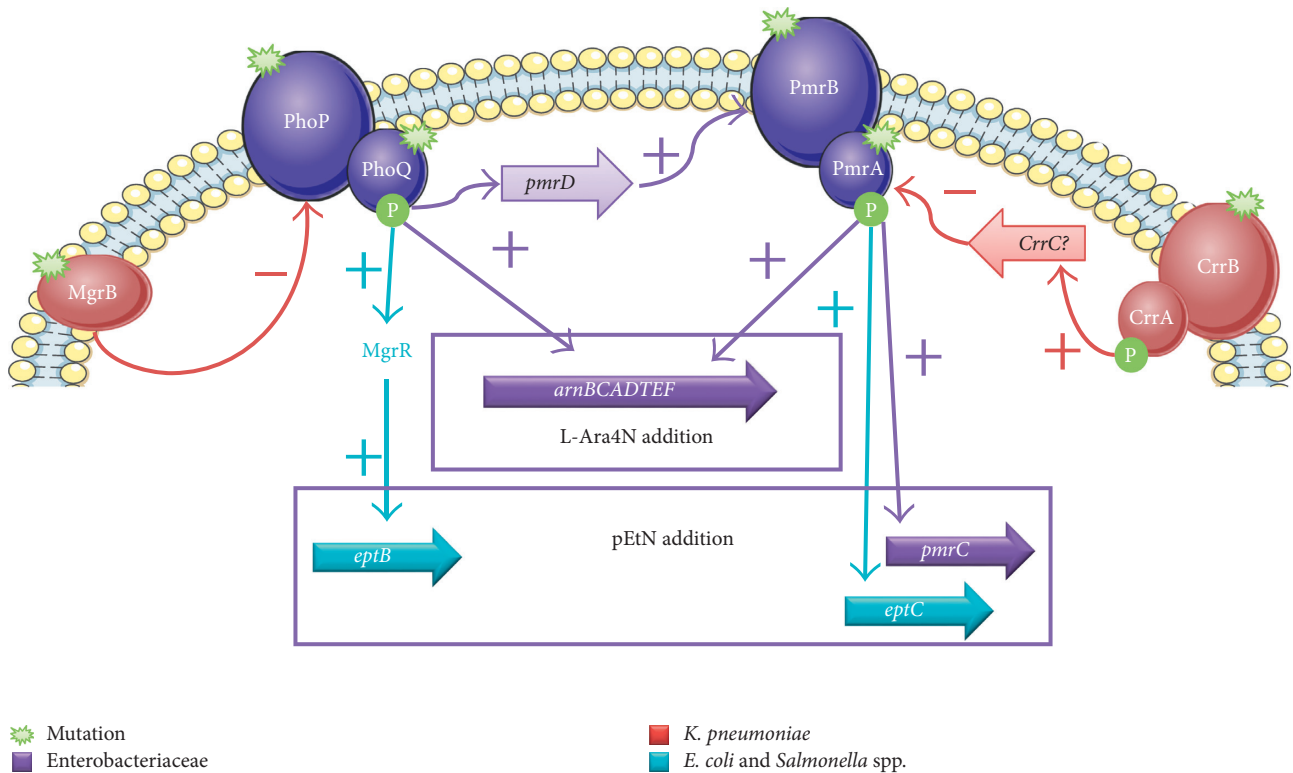


FIGURE 1: Molecular mechanisms of acquired resistance to polymyxins. L-Ara4N: 4-amino-4-arabinose; pEtN: phosphoethanolamine.

Enterobacteriaceae strains, describing coproduction with other plasmid-mediated genes (*bla_{NDM-1}*, *bla_{NDM-5}*, *bla_{NDM-9}*, *bla_{KPC-2}*, *bla_{KPC-3}*, *bla_{OXA-48}*, and *bla_{OXA-181}*) [18–24].

The emergence of antibiotic resistance of clinical interest usually conduces to the development of new tools in clinical microbiology laboratories [25]. Currently, the detection of carbapenemase-producing bacteria is well determined, combining specific culture media, phenotyping testing, antibiotic susceptibility testing, and molecular biology [26–28]. As colistin resistance is a recent global phenomenon, the implementation of rapid and reliable screening tools to detect and analyze colistin-resistant pathogens in such a way as to isolate the patient and adapt the treatment is a necessary approach [29]. Moreover, heteroresistance to colistin is a common phenomenon that is widely underestimated, requiring specific methods [30–32]. Here, we propose an overview of all the screening and analysis methods developed to assess colistin resistance among bacterial pathogens causing infectious diseases in hospitalized patients. This review summarizes the current clinical microbiology diagnosis techniques and their ability to detect all colistin resistance mechanisms, and describes new tools specifically developed to assess plasmid-mediated colistin resistance [33].

Phenotyping, susceptibility testing, and genotyping methods are presented, including an update on recent studies related to the development of specific techniques.

2. Phenotypic Detection Methods

2.1. Selective Culture Media. Culture remains the benchmark method for isolating pathogens within clinical samples, and

selective media are continuously developed to isolate specific bacteria [25]. Until recently, there was no specific culture medium for the detection of colistin-resistant strains, and current polymyxin-containing culture media were not able to detect low-level resistant strains because the concentrations of polymyxin in their composition are too high or because they contain other antimicrobial drugs [34–61] (Table 1). Therefore, some in-house media have been developed for colistin-resistant strain screening studies, including strains carrying the *mcr* genes (Supplementary Table S1). These selective culture media were developed by adding low concentrations of colistin (2 or 4 mg/L) to LB nonselective agar or a MacConkey medium, which is selective of Gram-positive contaminants [62, 63]. The chromogenic and nonselective CHROMagar Orientation medium (Biomérieux, Marcy l'Étoile, France) was also used with 4 mg/ml of colistin [64]. They were used in studies to detect the growth of colistin-resistant isolates by directly culturing samples [65–67] or following an enrichment step [68] which could also be selective with the addition of 2 mg/L of colistin to the broth medium [62, 64, 69]. Other anti-infective drugs could be added to avoid contaminants: vancomycin for Gram-positive contaminants [64, 66, 68] and/or amphotericin B for fungal pathogens [67, 68]. For some other studies, such media were developed to screen colistin resistance in bacterial isolates by subculturing them on agar with 2 mg/L of colistin: MH agar [9], COS medium [70], or MacConkey medium [65]. Wong et al. named their medium MHC1 for Mueller–Hinton colistin 1 [71]. Lastly, the selective CNA medium (colistin and nalidixic acid-containing agar), containing 10 mg/L of colistin, could

TABLE 1: Composition of polymyxin-containing agar.

Targeted bacteria	Culture medium	Antibiotics ($\mu\text{g/mL}$) targeting			References
		Gram-negative strains Polymyxins	Gram-negative strains Others	Gram-positive strains	
<i>Colistin-resistant Gram-negative strains</i> <i>VancoR Gram-positive strains</i> <i>Gram-negative strains</i>	LBJMR ^a	4 (C)		Vancomycin 50	
Colistin-resistant	SuperPolymyxin	3.5 (C)		Daptomycin 10 BM 65 Éosine 400	5 (AB) [75]
<i>Neisseria</i> sp.	Martin–Lewis agar	7.5 (C)	5 (T)	Vancomycin 4	20 (A) [34]
	Thayer–Martin agar	7.5 (C)		Vancomycin 3	2.57 (N) [35]
	MTM ^b agar	7.5 (C)	5 (T)	Vancomycin 3	2.57 (N) [36]
	NYC ^c agar	7.5 (C)	3 (T)	Vancomycin 2	20 (A) [37]
<i>Burkholderia cepacia</i>	Cepacia medium	30 (B)		Ticarcillin 100	[38]
	OPFBL ^d agar	30 (B)	Bacitracin 3		[39]
	<i>Burkholderia cepacia</i> agar	17.8 (B)	5 (GEN)	Ticarcillin 100	[38]
<i>Burkholderia cepacia</i> selective agar		71.4 (B)	10 (GEN)	Vancomycin 2.5	[40]
<i>Legionella</i> sp.	BCYE ^e selective agar with				
	GVPC ^f	9.4 (B)	Glycine 3000	Vancomycin 1	80 (CH) [41]
	CCVC ^g	16 (C)		Vancomycin 0.5 Cefalotin 4	80 (CH) [42]
	GPVA ^h	11.9 (B)	Glycine 3000	Vancomycin 1	80 (A) [43]
	PAV ⁱ	4.76 (B)		Vancomycin 0.5	80 (A) [44]
	PAC ^j	9.52 (B)		Cefamandole 2	80 (A) [45]
<i>Campylobacter</i> sp.	DGVP ^k	8 (B)	Glycine 3000	Vancomycin 1	[46]
	<i>Campylobacter</i> agar				
	Butzler	0.33 (C)	Bacitracin 338	Novobiocin 5 Cefazolin 15	50 (CH) [47]
	Skirrow	0.25 (B)	2.5 (T)	Vancomycin 5	[48]
	Blaser–Wang	0.125 (B)	2.5 (T)	Vancomycin 5 Cefalotin 15	2 (AB) [49]
	Preston	0.125 (B)	5 (T)	Rifampicin 5	50 (CH) [50]
<i>Brucella</i> spp.	<i>Brucella</i> selective medium	1 (B)	Bacitracin 500		100 (CH) [51]
<i>Vibrio</i> sp.	CPC ^l	66.34 (C)			[52]
		11.9 (B)			
<i>Gram-positive strains</i>					
<i>Streptococcus</i> sp. and Gram-positive strains	ANC ^m	10 (C)	Nalidixic acid 10		[53]
<i>Listeria monocytogenes</i>	Oxford medium	20 (C)	Fosfomycin 10	Cefotetan 2 Acridiflavine 5	400 (CH) [54]
	Modified Oxford	10 (C)		Moxalactam 15	[55]
<i>Listeria</i> spp.	PALCAM ⁿ	10 (B)		Ceftazidime 8 Acridiflavine 5	[56]
<i>Bacillus cereus</i>	MYP ^o	10 (B)			[57]
Mycobacteriaceae	Middlebrook 7H11	25 (B)	20 (T)	Carbenicillin 50	10 (AB) [58]
<i>Clostridium perfringens</i>	SPS ^p agar	10 (B)	Sulfadiazine 120		[59]
	TSN ^q agar	20 (B)	Neomycin 50		[60]
	SFP ^r agar	3.57 (B)	Kanamycin 12		[61]

B: polymyxin B; C: colistin; AB: amphotericin B; A: anisomycin; CH: cycloheximide; MB: methylene blue; N: nystatin; GEN: gentamicin; T: trimethoprim. ^aLBJMR: Lucie Bardet–Jean-Marc Rolain; ^bMTM: modified Thayer–Martin; ^cNYC: New York City; ^dOPFBL: oxidation/fermentation, polymyxin B, bacitracin, and lactose; ^eBCYE: buffered charcoal and yeast extract; ^fGPVC: glycine, polymyxin B, vancomycin, and cycloheximide; ^gCCVC: cefalotin, colistin, vancomycin, and cycloheximide; ^hGPVA: glycine, polymyxin B, vancomycin, and anisomycin; ⁱPAV: polymyxin B, anisomycin, and vancomycin; ^jPAC: polymyxin B, anisomycin, and cefamandole; ^kDGVP: dyes, glycine, vancomycin, and polymyxin B; ^lCPC: cellobiose, polymyxin B, and colistin; ^mCNA: colistin and nalidixic acid; ⁿPALCAM: polymyxin B, acridiflavine, lithium, ceftazidime, esculin, and mannitol; ^oMYP: mannitol, egg yolk, and polymyxin B; ^pSPS: sulfite, polymyxin B, and sulfadiazine; ^qTSN: trypticase, sulfite, and neomycin; ^rSFP: Shahidi-Ferguson perfringens.

detect *mcr-1*-positive isolates, one *E. coli* [72] and one *K. pneumoniae* [73], and was also used with CLED (cysteine lactose electrolyte deficient) medium (BioMérieux, Marcy

l'Étoile, France) for screening samples that had or had not been precultured on Trypticase Soy Broth ± 2 mg/L of colistin [74].

More specifically, the SuperPolymyxin medium (Elitech Microbio, Signes, France) was developed and intended to specifically detect colistin-resistant strains, including those with a low MIC of colistin and harboring the *mcr-1* gene [75]. The SuperPolymyxin medium has the advantage of facilitating the visualization of *E. coli* strains because it is composed of EMB agar, meaning that they exhibit a metallic green reflect. Its specificity is enabled by 3.5 µg/ml of colistin, 10 µg/ml of daptomycin, and 5 µg/ml of amphotericin B in its composition.

The CHROMagar COL-APSE medium was also developed to detect colistin-resistant strains and was compared to the SuperPolymyxin [76]. Its composition is not precisely described, based on commercial CHROMagar compounds containing colistin sulfate and oxazolidinone antibiotics. The CHROMagar COL-APSE medium presents the advantage to be chromogenic, with the capacity to differentiate colistin-resistant nonfermentative Gram-negative strains as well as Enterobacteriaceae.

The LBJMR medium was also developed to detect all the colistin-resistant bacteria, including those harboring *mcr-1* genes [77]. The LBJMR medium presents the advantage of being versatile, combining colistin-resistant and vancomycin-resistant bacteria screening tools, conferred by 4 µg/ml of colistin sulfate and 50 µg/ml of vancomycin. In particular, the LBJMR medium can be used to detect vancomycin-resistant enterococci (VRE), which represents another emerging field of clinical concern. Both colistin-resistant Enterobacteriaceae and VRE strains are easy to detect on the LBJMR medium with the presence of bromocresol purple and glucose: fermentative strains exhibit yellow colonies on a purple agar. Lastly, it can be used to specifically detect pathogens that are often diagnosed in cystic fibrosis patient samples.

The sensitivities of these three media were excellent to detect colistin-resistant strains.

2.2. Qualitative Detection of Colistin Resistance with Phenotypic Tests

2.2.1. Rapid NP Polymyxin Test for Enterobacteriaceae.

The rapid polymyxin NP test (Elitech, Signes, France) is based on a simple pH test, and detection of colistin resistance is obtained by a color change within 2 hours [78, 79]. The test was evaluated on 200 isolates of Enterobacteriaceae and can be used directly on blood samples [80]. A recent review proposed a diagnosis plan integrating this phenotypic test to confirm colistin resistance of Enterobacteriaceae strains after their growth on a selective medium [29], and its reliability is discussed in several studies [81, 82]. Compared to the broth microdilution (BMD) susceptibility testing method, agreements were excellent to detect *mcr-1* and *mcr-2* strains [83, 84]. The rapid polymyxin test has a good sensitivity to detect *Hafnia* sp. colistin-resistant isolates [79] but failed to detect *Enterobacter* sp. isolates, surely due to their heteroresistance to colistin [85]. This test has to be evaluated with nonfermentative colistin-resistant strains, such as *Acinetobacter baumannii* and *Pseudomonas aeruginosa*.

2.2.2. Micromax Assay for *A. baumannii*.

The Micromax assay (Halotech DNA SL, Madrid, Spain) is based on the detection of DNA fragmentation and cell wall damage in the presence of colistin [86]. Bacteria are incubated for 60 min with 0.5 µg/ml of colistin, trapped in a microgel, and then incubated with a lysis solution to remove weakened cell walls. The presence of DNA fragments is detected after staining by SYBR Gold fluorochrome and observed by fluorescence microscopy. Resistance corresponds to ≤11% of bacteria with cell wall damage. This method is rapid (3 h 30 min) and showed an excellent sensitivity for the detection of colistin resistance on the 70 *A. baumannii* tested isolates (50 susceptible and 20 resistant), but it is not specific for determining the resistance type.

2.3. Specific Phenotypic Screening Methods for the Detection of MCR-1

2.3.1. Matrix-Assisted Laser Desorption-Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS).

The detection of polymyxin-resistant bacteria by MALDI-TOF is a promising and costless approach, as the majority of clinical microbiology laboratories own the required equipment to routinely identify clinical isolates [87]. Currently, the use of MALDI-TOF for detecting the carbapenemase-producing bacteria is described, with the detection of carbapenem hydrolysis [88–90]. As a specific peak was described for lipid A at 1796.2 m/z [91], the MALDI-TOF could be used for the detection of lipid A modifications [92]. Very recently, the MALDIxin test was developed for *E. coli* strains, based on the detection of phosphoethanolamine addition on lipid A, and could specifically detect the *mcr*-positive isolates [93]. Indeed, an additional peak at 1919.2 m/z was observed for all polymyxin-resistant strains, and a second additional peak at 1821.2 m/z was observed for all the *mcr*-positives. The MALDIxin test could detect polymyxin-resistant *E. coli* and also differentiate the chromosome- and plasmid-encoded resistance in 15 minutes, and should be evaluated on other species for which phosphoethanolamine addition is involved in polymyxin resistance.

2.3.2. Inhibition of MCR-1 Activity.

Several studies on the structure of the catalytic domain of the MCR-1 protein have demonstrated that the phosphoethanolamine transferase is a zinc metalloprotein [94–96], and that zinc deprivation could reduce the colistin MIC in *E. coli* isolates [97]. Screening tests were developed to specifically detect MCR-1, based on the difference of colistin susceptibility obtained in the presence or absence of chelators of zinc ion.

The colistin-MAC test consists of the addition of dipicolinic acid (DPA) in the BMD method, leading to a colistin MIC reduction of ≥8-fold in case of MCR-1-positive strain [98]. 74 colistin-resistant Enterobacteriaceae strains were tested, and 59 of the 61 strains carrying *mcr-1*-like genes were detected by the colistin-MAC test, while the 13 *mcr*-negative strains exhibited discrepancy in results (increase, maintain, or slow decrease) giving a sensitivity of 96.7% and specificity of 100%. Interestingly, the two *mcr-1* strains that

were negative with the colistin-MAC test were *K. pneumoniae* strains.

More recently, four assays were tested, based on inhibition by EDTA [99]. The specific detection of *MCR-1* was assessed with the following tests: combined-disk method with diameter differences ≥ 3 mm, BMD with a reduction of colistin MIC of ≥ 4 -fold, modified rapid polymyxin NP test with the absence of color change, and the alteration of zeta potential $R_{ZP} \geq 2.5$. These assays were performed on 109 Enterobacteriaceae including 59 *mcr-1*-positive *E. coli* and one *mcr-1*-positive *K. pneumoniae*. The modified rapid NP test and zeta potential methods showed excellent sensitivity and specificity and could be inexpensive and simple methods to detect the presence of the *mcr-1* gene.

These tests should be performed on other species harboring the *mcr-1* gene, in particular *K. pneumoniae*, and also on strains harboring other *mcr* genes, to validate their ability.

3. New Recommendations on Polymyxins Susceptibility Testing

Polymyxin susceptibility testing is challenging, as these large and cationic molecules poorly diffuse into the reference cation-enriched Mueller-Hinton (MH2) agar, giving discrepant results, and much more since the description of the *mcr* genes that confer low MICs. Moreover, even in MH2 broth medium, the concentration of cation could largely influence the polymyxin MIC results [64], notably by interacting with the acquired resistance mechanisms of the tested isolates. Defining a reference method for colistin susceptibility testing is a priority, along with the increasing use of polymyxin as last-line therapies.

3.1. Reference Method. Broth microdilution (BMD) is the only approved method for colistin MIC determination by both the European Committee on Antibiotic Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI) [100, 101]. BMD has to be performed with colistin sulfate in untreated polystyrene plates without addition of any surfactant (polysorbate 80) (Table 2). The Mueller-Hinton broth medium has to be cation-adjusted, with a final composition of 20–25 mg/L of calcium and 10–12.5 mg/L of magnesium [102]. EUCAST and CLSI joined their recommendations on the polymyxin breakpoint for MIC of Enterobacteriaceae, *P. aeruginosa* and *Acinetobacter* spp. isolates: susceptible (*S*) if ≤ 2 $\mu\text{g/ml}$ and resistant (*R*) if > 2 $\mu\text{g/ml}$ [100, 103]. In 2017, EUCAST added a new quality control (QC) strain that has to be used to control the performances of a colistin susceptibility method: *E. coli* NCTC 18853 that harbors the *mcr-1* gene, in addition to *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 [104] (Table 2).

Dilution methods consist of adding colistin to the culture medium in such a way as to obtain twofold dilutions and are prepared according to the CLSI guide M07-A10 [101] and ISO 20776-1 standard (International Standard Organization). Broth macrodilution is performed in tubes when reference broth microdilution (BMD) is performed

in 96-well trays. Only colistin sulfate can be used and particular care is required, as the powder is expressed in IU/mg, meaning that the concentrations need to be adjusted according to the CLSI M100 and the manufacturer's instructions [103]. The antibiotic is suspended in sterile water and then diluted in MH2 broth medium before its distribution into 96-well trays. The final bacterial inoculum is 5×10^5 CFU/ml (colony-forming unit) or 5×10^4 CFU/well for the BMD method, prepared using the 0.5 McFarland standard (corresponding to approximately 1 to 2×10^8 CFU/ml) [101]. Trays are then incubated at $35 \pm 1^\circ\text{C}$ for 18 ± 2 hours [100, 102]. Results are read visually or with a spectrophotometer.

Broth microdilution is a time-consuming and fastidious way to assess MIC in clinical routines [105, 106]. Many errors can occur, such as an incorrect colistin concentration or dilution. This technique is not well suited to clinical microbiology routines and needs to be automated. Moreover, this method exhibits limitations for assessing heteroresistance. Indeed, the presence of resistant subpopulations can give uninterpretable results due to the presence of skipped wells and has been described for the *Enterobacter* species, as presented in a study of 114 *Enterobacter cloacae* [107]. Population analysis profiling is recommended to confirm heteroresistance [108]. For now, heteroresistance to polymyxins is not correlated with the presence of *mcr* genes.

3.2. Comparative Evaluations of Polymyxin Susceptibility Testing Methods. Evaluating antimicrobial susceptibility testing (AST) methods is performed using a comparison with the reference method, as per the ISO 20776-2 standard [109]: a categorical agreement (CA) is obtained when the strain is in the same clinical category (R, I, S), and an essential agreement (EA) is obtained when the MIC is within plus or minus one doubling dilution from the reference MIC. A very major error (VME) corresponds to a false susceptibility result and is calculated using the resistant strains tested, and a major error (ME), in the case of false resistance, is calculated on the number of susceptible strains. Finally, a minor error (MiE) occurs when a strain is classified as Intermediate (*I*) instead of *S* or *R*, or *S* or *R* instead of *I*. A reliable method will obtain the following scores: CA $\geq 90\%$, EA $\geq 90\%$, VME $\leq 3\%$, and ME $\leq 3\%$ [109]. The results of all the comparative studies performed on colistin susceptibility testing are summarized in Table 3 (in Table S2 for polymyxin B). MIC50 and MIC90 correspond to the MIC that inhibits 50 or 90%, respectively, of the tested strains of the same species.

The surfactant polysorbate 80 was previously added to trays to limit polymyxin adherence to polystyrene and is not yet recommended; however, it could induce VME and *mcr* strains might not be detected [31, 110–112]. Albur et al. demonstrated that the polystyrene trays used also have an influence: using tissue-culture-coated round-bottom trays gave a 5.3-fold increase in MIC values compared to non-coated V-bottom trays [113], for the material used [106] (Table S3). A very recent study compared polystyrene coated trays to glass coated trays and also showed very few differences (Table 3) [114].

TABLE 2: Joint EUCAST-CLSI recommendations on colistin susceptibility testing.

Reference method	Broth microdilution		
Preparation according to ISO 20776-1 standard [102]	(i) Cation-adjusted Mueller-Hinton medium (MH2)		
	(ii) Colistin sulfate		
	(iii) Polystyrene trays without pretreatment		
	(iv) Absence of polysorbate 80 or any surfactant		
MIC breakpoint ($\mu\text{g/ml}$)	Enterobacteriaceae	<i>P. aeruginosa</i>	<i>Acinetobacter</i> spp.
EUCAST [100]	$S \leq 2$ and $R > 2$	$S \leq 2$ and $R > 2$	$S \leq 2$ and $R > 2$
CLSI [103]	ECV*: WT ≤ 2 and NWT ≥ 4	$S \leq 2$ and $R \geq 4$	$S \leq 2$ and $R \geq 4$
Quality control [104] ($\mu\text{g/ml}$)	<i>E. coli</i> ATCC 25922	<i>P. aeruginosa</i> ATCC 27853	<i>E. coli</i> (<i>mcr-1</i>) NCTC 18853 [#]
Target	0.5–1	1–2	4
Range	0.25–2	0.5–4	2–8

*Epidemiological cutoff values: clinical data and PK/PD are not sufficient to evaluate a clinical breakpoint for the following species: *E. aerogenes*, *E. cloacae*, *E. coli*, *K. pneumoniae*, and *R. ornithinolytica*. WT: wild type; NWT: non-wild type. [#]Recommended by EUCAST; MIC must be $4 \mu\text{g/ml}$ and only occasionally 2 or $8 \mu\text{g/ml}$.

Until 2013, many comparative studies used agar dilution (AD) as the reference method for polymyxins susceptibility testing, a method that differs from the BMD only because the polymyxins are added to a solid MH2 medium [31, 32, 115–126]. Compared to BMD, agar dilution generally gave concordant results for colistin and polymyxin B [31, 110, 127, 128]. VMEs were very uncommon with AD, and this pointed to the AD's potential role in screening, as it presents the advantage to test several strains on the same plates [117, 129]. A recent study compared agar dilution to broth macro- and microdilution on 8 strains and concluded that agar dilution was the most reproducible method, with an excellent distribution of colistin in agar, but that colistin-containing agar plates could be only stored for 7 days [130].

Diffusion methods based on the antibiotic diffusion in agar, whether with the Kirby–Bauer disk diffusion [131] or with the gradient strips, are not reliable for polymyxin testing and should not be used as a large number of studies have obtained high rates of VMEs or poor EA [32, 120, 125, 128, 132–135]. Some studies showed good results but contained only susceptible strains [136–138]. The influence of MH2 agar composition was assessed: agreement was not affected with agar dilution, but important differences were highlighted with Etest (BioMérieux, Marcy l'Étoile, France) [31, 139]. The advantage of the agar diffusion method is the detection of heteroresistance: colonies present within the inhibition zone correspond to resistant subpopulations [140]. One study compared disc diffusion to Etest method, and a large rate of minor errors occurred [141]. The ColiSpot test consists of replacing the disk of colistin by a drop of a calibrated colistin solution ($8 \mu\text{g/ml}$). Colistin resistance is revealed in the absence of the inhibition zone. This technique was evaluated with 89 colistin-resistant and 52 colistin-susceptible strains and was developed for veterinarian laboratories [142].

3.3. Commercial Devices Based on Broth Microdilution Reference Method. Several commercial devices based on BMD reference methods were developed to easily assess the reference method by offering ready-to-use systems. Their advantage is the elimination of critical preparation steps of MH2 medium and antibiotic dilutions. These systems were used to detect *mcr-1* strains and were evaluated by EUCAST,

giving correct results, with essential agreement ranging from 82% to 96%, and few MEs or VMEs (http://www.eucast.org/ast_of_bacteria/warnings/) [143].

3.3.1. UMIC Colistine (Biocentric, Bandol, France). UMIC colistine consists of unitary tests composed of 12-well polystyrene strips, one for growth control and 11 containing dehydrated colistin, with concentrations ranging from 0.06 to $64 \mu\text{g/ml}$, provided with unitary MH2 tubes. Inoculation is performed simply, after diluting the 0.5 McFarland suspension by 200-fold into the MH2 tubes, by distributing $100 \mu\text{L}$ of this diluted suspension into the 12 wells of the strip, leading to the rehydration of the antibiotic. The strips are then incubated at $35 \pm 1^\circ\text{C}$ using the UMIC box to avoid any desiccation. One comparative evaluation on 71 *A. baumannii* isolates and one on 92 Gram-negative isolates including 76 Enterobacteriaceae highlighted the reliability of the UMIC colistine kit [144, 145].

3.3.2. MIC Strip Colistin (MERLIN Diagnostika Bornheim-Hersel, Germany). MIC Strip Colistin also consists in unitary 12-well strips with concentrations of dehydrated colistin ranging from 0.06 to $64 \mu\text{g/ml}$, and Micronaut-S is a panel composed of different antibiotics on standard 96-well trays. Those systems can be automated with Micronaut ASTroID that concomitantly performs dilution for antimicrobial susceptibility testing (AST) and deposits on the MALDI-TOF target, simultaneously identifying the same colony being tested.

3.3.3. Sensitest Colistin (Liofilchem, Roseto degli Abruzzi, Italy). It consists of a compact panel of 4 tests containing 7 twofold dilutions of dehydrated colistin (0.25 – $16 \mu\text{g/ml}$). It showed excellent correlation with BMD when tested on 353 isolates, including 259 Enterobacteriaceae, 83 harboring the *mcr-1* gene [146]. Recently, a combined Sentitest colistin/piperacillin-tazobactam was developed, with the same design, providing a unitary test for testing both antibiotics, with colistin concentrations ranging from 0.008 to $128 \mu\text{g/ml}$.

TABLE 3: Comparison of different colistin susceptibility testing methods to detect colistin resistance in Gram-negative clinical isolates.

Bacterial species	Reference method	MIC breakpoint	MIC range; % resistant	MIC50 ($\mu\text{g/ml}$)	MIC90 ($\mu\text{g/ml}$)	Methods	CA $\geq 90\%$	EA $\geq 90\%$	ME $\leq 3\%$	VME $\leq 3\%$	MiE	References
42 <i>A. baumannii</i> isolates	BMD	$S \leq 2 \mu\text{g/ml}$	0.5–4 $\mu\text{g/ml}$	1 $\mu\text{g/ml}$	2 $\mu\text{g/ml}$	BMD in glass-coated plates	92.8	100	0	100		
		$R > 2 \mu\text{g/ml}$	0.07%			Vitek 2 ² AST-N2812	78.5	92.8	15.4	100		[114]
353 isolates (83 <i>mcr-1</i>)	BMD	$S \leq 2 \mu\text{g/ml}$	ND	ND	ND	Sensitest	98.9	96	1.46	0.93		[146]
		$R > 2 \mu\text{g/ml}$	38.8%			Phoenix 100 ³ NMIC-417	96.8	ND	0.6	10		[146]
14 <i>E. coli</i> isolates	BMD	$S \leq 2 \mu\text{g/ml}$	0.25–128 $\mu\text{g/ml}$; 48%	2	16	Sensititre ¹ SEMPAl	94.7	96	10.2	0		[143]
18 <i>K. pneumoniae</i> isolates 21 <i>P. aeruginosa</i> isolates 22 <i>Acinetobacter</i> spp. isolates	BMD	$R > 2 \mu\text{g/ml}$				Micronaut-S	89.3	96	15.4	5.6		
						Micronaut-MIC	90.7	99	12.8	5.6		
						Etest, Oxoid MH	81.3	71	5.1	33.3		
						Etest, BBL MH	78.7	43	2.6	41.7		
						Etest, MHE	85.3	47	5.1	25		
117 <i>A. baumannii</i> isolates	BMD	$S \leq 2 \mu\text{g/ml}$	≤ 0.5 – $\geq 16 \mu\text{g/ml}$; 24.8%	≤ 0.5	8	Vitek 2 AST-XN05	89.7	88.9	1.1	37.9		[129]
		$R > 2 \mu\text{g/ml}$				Phoenix 100 NMIC/ID-96 AD	88.9	91.5	1.1	41.4		
							87.2	93.2	15.9	3.4		
123 Enterobacteriaceae isolates (14 <i>mcr-1</i> and 1 <i>mcr-2</i>)	BMD	$S \leq 2 \mu\text{g/ml}$	0.12–128 $\mu\text{g/ml}$; 67.5%			Phoenix 100 NMIC-93	91.8	ND	0	12.1		[83]
		$R > 2 \mu\text{g/ml}$				Rapid NP	98.3	NA	2.5	1.2		
15 <i>Hafnia alvei</i> isolates	BMD	$S \leq 2 \mu\text{g/ml}$	0.125–32 $\mu\text{g/ml}$; 96%	8	16	DD	4	NA	0	100		[79]
		$R > 2 \mu\text{g/ml}$				Etest	76	32	0	25		
10 <i>Hafnia paralvei</i> isolates	BMD	$S \leq 2 \mu\text{g/ml}$				Phoenix NMIC-93	100	NA	0	0		
		$R > 2 \mu\text{g/ml}$				Rapid NP	100	NA	0	0		
76 Enterobacteriaceae isolates (21 <i>mcr-1</i>)	BMD	$S \leq 2 \mu\text{g/ml}$	0.06– $>64 \mu\text{g/ml}$; 32.9%	0.25	16	Vitek 2 AST N315	88.2	93.9	0	36		[147]
		$R > 2 \mu\text{g/ml}$				Sensititre GNX3F Etest	90.1	89.5	11.8	4		
						92.1	75	5.9	12			
						88.2	NA	15.8	4			

TABLE 3: Continued.

Bacterial species	Reference method	MIC breakpoint	MIC range; % resistant	MIC50 ($\mu\text{g/ml}$)	MIC90 ($\mu\text{g/ml}$)	Methods	CA $\geq 90\%$	EA $\geq 90\%$	ME $\leq 3\%$	VME $\leq 3\%$	MiE	References
172 Gram-negative isolates	AD	S $\leq 2 \mu\text{g/ml}$ R $> 2 \mu\text{g/ml}$	0.5–64; 31.4%			Vitek 22 AST-N032 (n = 32) Etest (n = 137)	82	75.2	0	57.4		[32]
115 <i>A. baumannii</i> isolates	BMD	S $\leq 2 \mu\text{g/ml}$ R $> 2 \mu\text{g/ml}$	≤ 0.06 –512 $\mu\text{g/ml}$; 19.1%	≤ 0.06	32	Etest	98.2	16.5	0	1.7		[138]
501 <i>P. aeruginosa</i> isolates (401 CF)	AD	S $\leq 4 \mu\text{g/ml}$ R $> 4 \mu\text{g/ml}$	≤ 0.5 – $\geq 16 \mu\text{g/ml}$; 17.8%	2	4	BMD 24 h	96		1.2	26.5		[121]
50 <i>A. xylooxidans</i> isolates		R $> 4 \mu\text{g/ml}$		4	≥ 16	BMD 48 h	93.6		3.9	18.0		
50 <i>S. maltophilia</i> isolates				8	≥ 16							
70 <i>S. maltophilia</i>	AD	S $\leq 2 \mu\text{g/ml}$ R $> 2 \mu\text{g/ml}$	0.12–32 $\mu\text{g/ml}$; 24.3%	2	4	DD 10 μg ; R ≤ 8 ; S ≥ 11 mm Etest	71.2	NA	0	93.7	5.7	[125]
200 Gram-negative isolates	BMD	S $\leq 2 \mu\text{g/ml}$ R $> 2 \mu\text{g/ml}$	≤ 1 – $> 128 \mu\text{g/ml}$; 15%			DD 10 μg ; R ≤ 10 ; S ≥ 14 mm	94	NA	0	21.8	1.5	[127]
(i) 60 <i>A. baumannii</i> isolates		R $> 2 \mu\text{g/ml}$		≤ 1	2	DD 10 μg ; R ≤ 8 ; S ≥ 11 mm	95		0	31.2	1	
(ii) 80 <i>P. aeruginosa</i> isolates				≤ 1	≤ 1							
(iii) 12 <i>S. maltophilia</i> isolates				≤ 1	32							
35 representatives	BMD	S $\leq 2 \mu\text{g/ml}$ R $> 2 \mu\text{g/ml}$	≤ 1 – $\geq 128 \mu\text{g/ml}$; 40%			AD	97.1	91.4	47.6	0		[127]

CA: categorical agreement; EA: essential agreement; VME: very major error; ME: major error; MiE: minor error; AD: agar dilution; BMD: broth microdilution; DD: disk diffusion; MH: Mueller-Hinton. Italic values indicate the number of errors and not the percentage when a too few number of strains tested, where R for VME or S for ME. ¹Sensititre panels: ≤ 0.25 – $> 4 \mu\text{g/ml}$ except for SEMPAL. ²Vitek 2 reagent cards: ≤ 0.5 – $\geq 16 \mu\text{g/ml}$ except for AST-N038 (≤ 2 , 4, and $> 4 \mu\text{g/ml}$) and AST-N032 (1–4 $\mu\text{g/ml}$). ³Phoenix 100 cards: ≤ 1 – $> 4 \mu\text{g/ml}$. ⁴MicroScan-dried Gram-negative breakpoint combo panel type 42 ≤ 2 and $> 4 \mu\text{g/ml}$.
*Prediffusion test: discs were removed after 2 h of incubation.

3.3.4. *The Sensititre System* (Thermo Fisher Scientific, Waltham, MA, USA). It presents different antibiotics on 96-well trays with a customizable plate layout. Inoculation, incubation, and reading (based on fluorescence) steps can be automated. Chew et al. [147] recently evaluated a Sensititre GNX3F panel containing both polymyxin B and colistin (0.25–4 mg/L) and presented a sensitivity of 95.2% and 100% in detecting the 21 *mcr-1* isolates tested, respectively.

3.4. *Automated Systems*. Automated systems were developed to shorten result timeframes by increasing sensitivity, and also to avoid manipulation bias [148], with incubation and real-time reading. However, by combining several antibiotics, the number of concentrations tested is limited, and they cannot give a real MIC (Table 3).

3.4.1. *MicroScan WalkAway* (Beckman Coulter, San Diego, CA, USA). It uses standard trays that are manually inoculated, and reading is based on fluorometry, with results obtained in 3.5–7H. It is not available on polymyxin B, and essential agreement cannot be evaluated between techniques as the NM44 panel proposes only ≤ 4 and ≥ 4 $\mu\text{g/ml}$ for colistin. In the recent study of Chew et al., this panel was able to detect all *mcr-1* tested isolates and presented only one VME on 76 Enterobacteriaceae isolates tested. It also evaluated 213 *Acinetobacter* species and presented 99.1 % categorical agreement against the agar dilution method [115].

3.4.2. *Vitek 2* (BioMérieux, Marcy l'Étoile, France). It is a semiautomated system that uses reagent cards containing dehydrated antibiotics and other reagents in a 64-well format. It combines rapid identification and AST using an extrapolated growth algorithm. Various comparative studies performed to evaluate Vitek 2 have returned discrepant results with high rates of VME. In their recent evaluation, Chew et al. [147] demonstrated the efficacy of Vitek 2 in assessing both polymyxin B and colistin MIC with only one VME for each and 96.1% and 93.9% EA, respectively, but it was only able to detect *mcr-1* strains with polymyxin B.

3.4.3. *BD Phoenix™* (Becton Dickinson, Le Pont de Claix, France). It performs identification and AST in parallel in 84-well specific plates. Reading is based on an oxidation-reduction indicator in 6–16 hours. One study showed excellent agreement on 109 *P. aeruginosa* strains, but only colistin-susceptible strains were tested [149]. Vourli et al. [129] have shown concerning results testing *Acinetobacter baumannii* strains with very high VME rates (41.4%) despite the study including 24.8% of colistin-resistant strains. This was explained by the majority of errors occurring near the breakpoint (2 instead of 4 $\mu\text{g/ml}$). Lastly, in the study by Jayol et al. [83], the Phoenix system was able to detect all *mcr*-carrying bacteria, even those with a colistin MIC of 4 $\mu\text{g/ml}$, but the high rates of VMEs obtained prove its inability to assess heteroresistance.

4. Genotyping and Molecular Screening

4.1. *PCR Amplification and Sequencing to Detect Gene Mutations*. Molecular biology methods are the most sensitive for determining antibiotic resistance by assessing the presence of resistance genes or mutations conferring resistance. These methods are complementary to the phenotypic techniques and confirm the resistant status of bacterial isolates. The main mutations for Enterobacteriaceae species are located on genes coding the two-component systems PmrA/PmrB and PhoP/PhoQ (Figure 1). Specifically, mutations in the *mgrB* gene—the negative feedback regulator of PhoPQ—notably with the presence of insertional sequences, appeared to be the main resistance mechanism observed in *K. pneumoniae* strains. These colistin resistances are not based on drug-modifying enzymes or the acquisition of a resistance gene which could be easily detected. Screening of potential mutations on these chromosomal genes is done by amplification and sequencing, takes 3 days, and requires that all genes are tested. Sequenced amplicons are then compared by the BLAST tool against the NCBI database to screen possible mutations compared to wild-type genes.

4.2. *Real-Time qPCR to Detect the Presence of mcr Genes*. The discovery of the acquired gene *mcr-1* justifies the use of molecular detection with RT-PCR, a rapid quantitative technique to detect the presence of the gene. A systematic screening of the gene in colistin-resistant strains was performed [150] (Figure 2). For such purposes, scientists have used the primers of the original study [6], or have designed their own primers for standard PCR [132, 151–160] or RT-PCR, based on SYBR Green assays [64, 151, 161], TaqMan probe [66, 72, 152, 162], or other FAM-labelled probe [9, 71, 163, 164] or HEX-labelled probe [165] (Table 4).

Xavier et al. designed primers to screen *mcr-2* [7], giving a 567 bp product [166]. Some designed their own primers for standard PCR [167–169], and one study developed a TaqMan assay for qPCR [170]. Interestingly, three studies went further by designing a universal primer to detect both *mcr-1* and *mcr-2* genes by standard PCR [166, 171] and a generic primer and a probe to detect them by qPCR [74], but these have not yet been tested on other *mcr* genes. Lastly, primers were designed for detecting *mcr-3* [10], *mcr-4* [11], and *mcr-5* genes [12] by standard PCR (Table 4). A recent study described a multiplex SYBR Green real-time PCR assay for the simultaneous detection of *mcr-1*, *mcr-2*, and *mcr-3* genes [172]. Finally, a multiplex PCR assay for detection of the five *mcr* genes: *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, and *mcr-5*, was developed in order to obtain sequential amplicons with a size difference of 200 bp, allowing their fast and simultaneous detection on agarose gels [173].

4.3. *PCR to Detect Plasmid Carrying mcr Genes*. *mcr-1* is a 1626-base pair-long gene located on a 2607 bp common region flanked on both ends by an IS*AplI* insertion sequence (IS) in some plasmids [174]. This sequence may form a composite transposon that can potentially move as one complete unit [155, 175, 176]. This insertion sequence appears

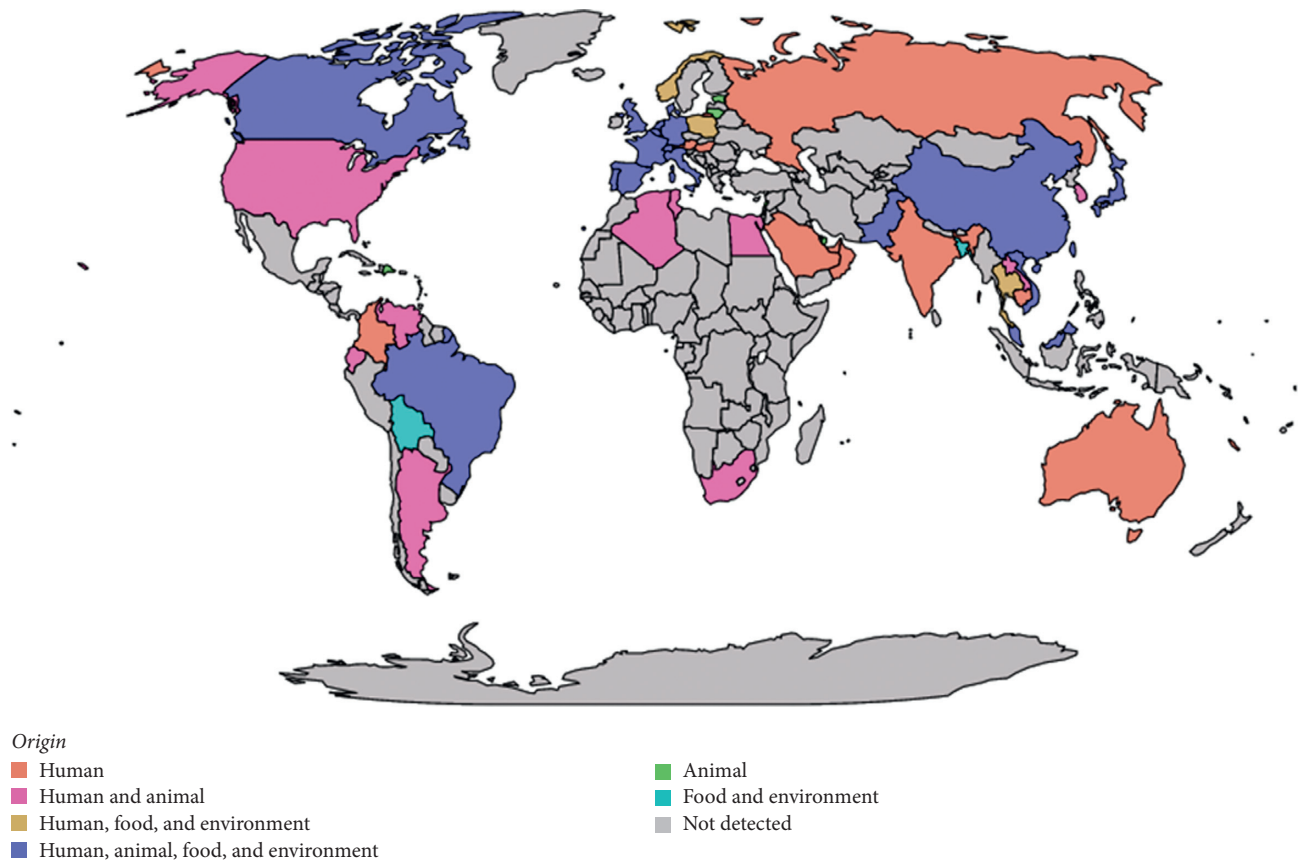


FIGURE 2: Worldwide dissemination of the *mcr-1* gene. The map was performed with Magrit mapping application (<http://magrit.cnrs.fr>).

to be a key component of the mobilome, and its presence is not systematic. Furthermore, only the upstream region can contain *ISApI1* [165]. Li et al. identified the ability of the Tn6330 transposon (*ISApI1-mcr-1-orf-ISApI1*) to generate circular *ISApI1-mcr-1-orf* [177]. Specific primers were developed to screen the upstream presence of this IS transposon by PCR and Sanger sequencing [178–180]. Others have also designed their own system to directly screen on plasmid carrying *mcr*-gene type IncX4 [9, 181], but these methods also exhibit limitations, as a wide distribution has been observed for *mcr-1* among different plasmids (IncI2, IncX4, IncHI2, IncY, IncF, IncP, IncH1, and IncX3), demonstrating the great ability of *mcr* genes to spread.

4.4. Microarray. Microarray technology allows scientists to analyze dozens of genes at the same time. The Check-MDR CT103 microarray system (Check-Points, Wageningen, the Netherlands) was developed to screen the presence of extended-spectrum beta-lactamase (ESBL) genes (TEM and SHV) and carbapenemase genes (OXA-48, KPC, NDM. . .) in the same assay and can assay 24 samples at the same time, with an effective detection in 6.5 hours. Recently, a study evaluated this assay for detecting *mcr* genes: sensitivity and specificity were excellent for *mcr-1* and its variants (from *mcr-1.2* to *mcr-1.7* and *mcr-2* genes), but it was not able to detect the new gene *mcr-3* [182]. *mcr-4* has not been assayed yet.

4.5. Loop-Mediated Isothermal Amplification (LAMP). The Eazyplex SuperBug *mcr-1* kit (Amplex Biosystems GmbH, Giessen, Germany) was developed to assess the presence of the *mcr-1* gene within 20 minutes [183]. It was effective on 104 microbial strains but needs to be assayed directly on clinical samples. As it was developed before the description of the other *mcr* genes, it can only detect the *mcr-1* gene and the *mcr-1.2* variant.

More recently, another LAMP-based assay was developed to detect *mcr-1* gene and evaluated as a screening tool on 556 multidrug-resistant Enterobacteriaceae [184]. Seven isolates were positive by both standard PCR and LAMP-based assay (6 *E. coli* and 1 *K. pneumoniae*). The results can be assessed by chromogenic visualization. This test constitutes a rapid, specific, and cost-effective tool that exhibits a higher sensitivity than PCR (10-fold). It has to be assayed on clinical samples; as for now, only spiked tools were used.

4.6. Novel Approach with Direct Resistome Analysis. Genomic screening is an alternative approach for studying resistance and providing a better understanding of the behavior of bacterial isolates [185]. The development of next-generation sequencing has led to lower costs, reduced screening delay, and increased sequencing speeds combined with updated databases providing access to a large amount of information. The *mcr-1* gene was initially discovered by whole

TABLE 4: Continued.

Targeted genes	Analyte	Method	Primer sequences	Cycle (nb: steps)	Product (bp)	Study	
31 colistin-resistant isolates	MCR1_22,763_Pb1 FAM-TGGTCTCGG/ZEN/ C TTGGTGGTCTGTAGGGC-3IABKfq	Std	MCR1_22,810_R1: 5'-CCCCAAACCAATGATACGCAT-3'	45: 15 s, 95°C; -1 min, -60°C	309	[157]	
			<i>mcr-1_F</i> : 5'-ATGATGCAGCATACTTCTGTGTGG-3' <i>mcr-1_R</i> : 5'-GTGGGGTCTTGTACTTTGTCC-3'				
122 faecal samples	TaqMan probe: 5'- TTGACCGGACCGCCCAATCTTA-3' FAM	[6]				[63]	
48 <i>E. coli</i> and 27 KP strains			<i>mcr-1-F1</i> : 5'-ATGATGCAGCATACTTCTGTG-3'			[153,	
			<i>mcr-1-R1</i> : 5'-TCAGCGGATGAATGCGGTG-3'			[155]	
			CLR5-F1: 5'-ATGATGCAGCATACTTCTGTGTGG-3'			[156]	
			CLR5-R1: 5'-TCAGCGGATGAATGCGGTGC-3'				
			CLR5-F: 5'-CGGTCAAGTCCGTTTGTTC-3' <i>Mcr1-Rv2</i> : 5'-CCAGCGTATCCAGCACATTT-3'			[176]	
136 colistin-resistant isolates	Std		MCR2-IF: 5'-TGTTCCTTGTGCGGATTGGA-3'	33: 95°C, 3 min; 65°C, 30 s; 72°C, 1 min	567	[7]	
			MCR2-IR: 5'-AGATGGTATTGTTGGTTGCTG-3'			[157]	
1200 isolates						[163]	
<i>mcr-2</i>	Std		<i>MCR-2-F(EcoRI)</i> : 5'- AACCGAATTCATGACATCACATCCTCTTG-3'			[164]	
			<i>MCR-2-R (SalI)</i> : 5'- CCGGTCGACTTACTGGATAAAATGCCGCGC-3'				
			<i>Mcr-2 full Fw</i> : 5'-ATGACATCACATCCTCTTGG-3'			34: 95°C, 1 min; 52°C, 30 s; 72°C, 1 min	[165]
			<i>Mcr-2 full Rv</i> : 5'-TTACTGGATAAAATGCCGCGC-3'				[165, 166]
436 cultures	TaqMan <i>mcr-2</i> _Probe ROX- ACTGATTATGGGTGCGGTGACGAG- BHQ-2		<i>Mcr-2_fwd</i> : TTGTCGTGCTGTTATCCTAATCG <i>Mcr-2_rev</i> : CCGTGCCATAAAGTATCGGTAAA	30: 95°C, 15 s; -60°C, 1 min		[167]	
1200 isolates	Std		<i>mcr1-2 universal F</i> : ACTTATGGCAGGGTCTATGATAC	30: 94°C, 30 s; 58°C, 30 s; 72°C, 2 min	1311	[163]	
			<i>mcr1-2 universal R</i> : CCGCGGTGACATCAAACA				
<i>mcr-1</i> and <i>mcr-2</i>	Std		<i>MCR-1/2-Fw</i> : 5'-TAT CGC TAT GTG CTA AAG CC-3'		715 bp	[168]	
			<i>MCR-1/2-Rv</i> : 5'-TCT TGG TAT TTG GCG GTA TC-3'				
			<i>Mcr-generic fw</i> : GCCAAATACCAAGAAAATG <i>Mcr-generic rev</i> : TTATCCATCACGCCCTTTT				
621 faecal samples	TATCAGGCCACAAGATAC		<i>MCR3-F</i> : 5'-TTGGCACTGTATTTTGCATTT-3' <i>MCR3-R</i> : 5'-TTAACGAAATTTGGCTGGAACA-3'	30: 95°C, 30 s; 50°C, 30 s; 72°C, 45 s	98 bp	[71]	
580 <i>E. coli</i> strains	Std				542	[10]	

TABLE 4: Continued.

Targeted genes	Analyze	Method	Primer sequences	Cycle (nb: steps)	Product (bp)	Study
<i>mcr-1</i> , <i>mcr-2</i> , and <i>mcr-3</i>	25 isolates: 17 <i>mcr-1</i> and 8 <i>mcr-3</i> 20 samples	SYBR Green	<i>mcr1</i> -qf: AAAGACGGGTACAAGCAAC MCR-1	40: 95°C, 30 s; 60°C, 30 s; 72°C, 30 s	213	[169]
			<i>mcr1</i> -qr: GCTGAACATACAGGGACAG			
			<i>mcr2</i> -qf: CGACCAAGCCGAGTCTAAGG MCR-2			
			<i>mcr2</i> -qr: CAACTGCGACCAACACACTT			
			<i>mcr3</i> -qf: ACCTCCAGGTGAGATTGTTCCA MCR-3			
<i>mcr3</i> -qr: GCGGTTTCACCAACGACCAGAA						
<i>mcr-4</i>	125 isolates	Std	<i>Mcr-4</i> FW: ATTGGGATAGTCGGCTTTTT <i>Mcr-4</i> RV: TTACAGCCAGAAATCATATCA		487	[11]
<i>mcr-5</i>	12 Salmonella paratyphi B isolates	Std	<i>MCR5</i> _fw: 5'-ATGCGGTTGTCTGCATTTAATC-30'	30: 95°C, 30 s; 50°C, 95 s; 72°C, 95 s	1644	[12]
			<i>MCR5</i> _rev: 5'-TCATTTGTGGTTGTTCCTTTTCTCG-3'			
<i>mcr-1</i> , <i>mcr-2</i> , <i>mcr-3</i> , <i>mcr-4</i> and <i>mcr-5</i>	49 <i>E. coli</i> and Salmonella isolates	Std	<i>mcr1</i> _fw: AGTCCGTTTGTCTGTGGC	25: 94°C, 30s; 58°C, 90s; 72°C, 60s	320	[170]
			<i>mcr1</i> _rev: AGATCCTTGGTCTCGGGCTTG			
			<i>mcr2</i> _fw: CAAGTGTGTGGTCGGAGTT			
			<i>mcr2</i> _rev: TCTAGCCCGACAAGCATAAC			
			<i>mcr3</i> _fw: AAATAAAAAATTGTTCCGCTTAIG			
<i>mcr3</i> _rev: AATGGAGATCCCGGTTTTT						
<i>mcr4</i> _fw: TCACITTCATCACTGGGTTG						
<i>mcr4</i> _rev: TTGGTCCATGACTACCAATG						
<i>mcr5</i> _fw: ATGCGGTTGTCTGCATTTAATC						
<i>mcr5</i> _rev: TCATTTGTGGTTGTCTCTTTCTG						

Std: standard; KP: *K. pneumoniae*.

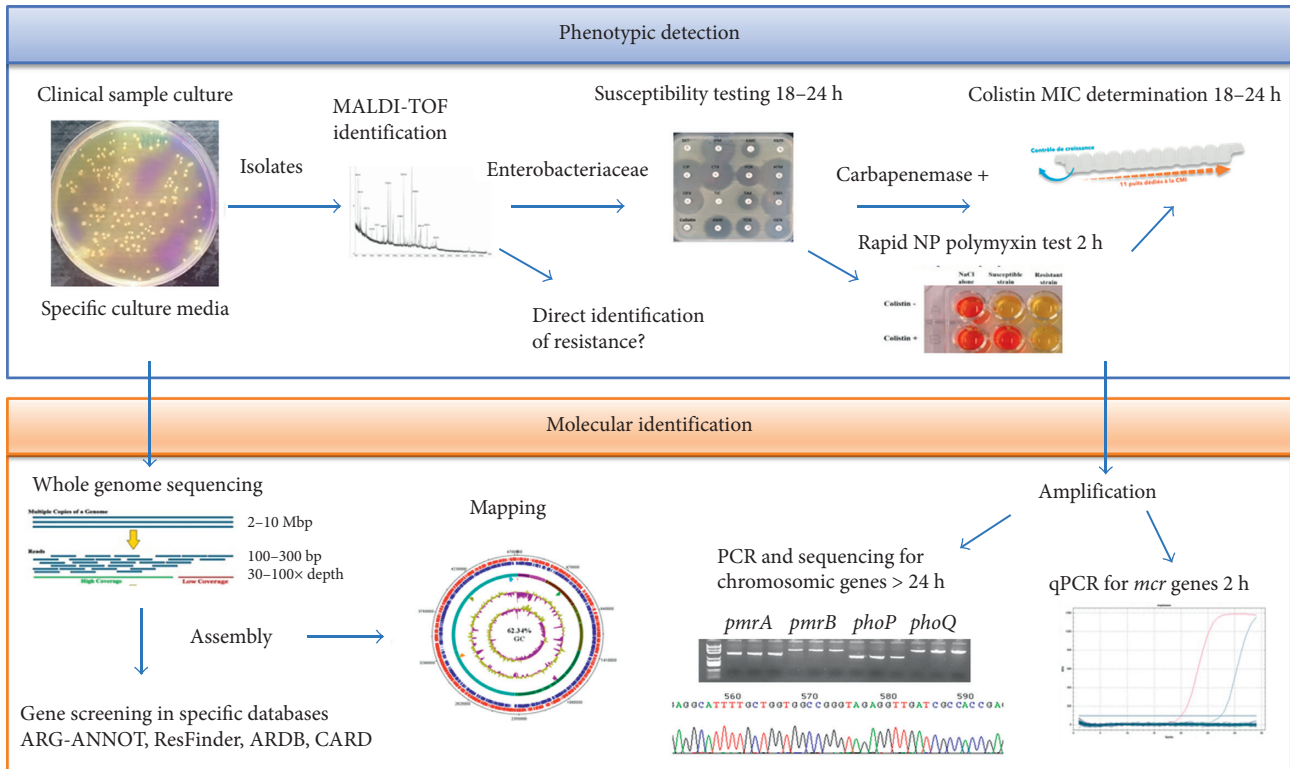


FIGURE 3: Complementarity of phenotypic and genotypic methods in detection and analysis of colistin-resistant bacteria.

TABLE 5: Comparison of detection methods for polymyxin resistance.

Method	Principle	Time	Manual (M); automated (A)	Detection	ColR	<i>mcr</i>	HR	MIC
Phenotypic								
Selective agar	Selective growth	18 h	M	+	-	+	-	-
Rapid polymyxin NP	pH change	4 h	M	+	-	-	-	-
Micromax	Cell wall lysis detection by fluorescence	3 h	M/A	+	-	-	-	-
MALDI-TOF MS	Specific peak detection	1 h	A	+	+	-	-	-
<i>MCR-1</i> inhibition	Chelation with	18 h	M	+	+	±	+	+
Colistin MAC	Dipicolinic acid							
EDTA assays	EDTA							
AST								
BMD (UMIC, Micronaut-MIC, Sensitest, Micronaut-S, and Sensititre)	Growth inhibition	18 h	M/A	+	-	±	+	+
Agar diffusion	Measure of growth inhibition zone	18 h	M					
Disk diffusion				-	-	+	-	-
Gradient strip				-	-	+	+	+
ColiSpot				+	-	ND	-	-
Agar dilution	Growth inhibition	18 h	M	-	-	+	+	+
Automatized system	Growth detection							
MicroScan	Fluorimetry	3.5–7 h	A	+	-	-	-	-
Vitek 2	Algorithm	4–10 h	A	+	-	-	-	-
Phoenix	Oxidoreduction	6–16 h	A	+	-	-	-	-
Genotypic								
Standard PCR	Amplification	3 h	A	+	+	-	-	-
RT-PCR	Amplification	1 h	A	+	+	-	-	-
LAMP (Eazyplex, etc.)	Amplification	20 min	A	+	-	-	-	-
Microarray	DNA hybridization	6.5 h	A	+	-	-	-	-
NGS								
Whole-genome sequencing								
Illumina		4–56 h	A	+	+	-	-	-
PacBio RS II		0.5–3 h	A	+	+	-	-	-

ColR: colistin resistance; HR: heteroresistance; +: yes; -: no; ±: sometimes.

genome sequencing during an active livestock monitoring program in China [6]. A considerable number of retrospective studies analyzing previously recorded genomic sequences have since been carried out, showing the global dispersion of the gene [9, 10, 20, 23, 24, 72, 74, 157, 158, 174, 177, 180, 186–204] (Figure 2).

The technologies used to completely sequence the bacterial genome are Illumina (Illumina Inc., San Diego, CA, USA), which produces short sequences (300 bp) and requires several days, and PacBio RS II (Pacific Biosciences, Menlo Park, CA, USA), which produces a single real-time molecule producing long sequences (60kb) in a few hours [205]. The use of Illumina sequencers is not suitable for covering bacterial genomes with multiple repetitive elements because too many sequence pieces are obtained after assembly, whereas PacBio RS II delivers a single sequence without missing regions [174]. Sekizuka et al. performed a hybrid analysis using the two technologies to analyze three Inc2 plasmids and found that they were highly conserved with the exception of the shufflon region, meaning that the combination of the two methods enables to analyze rearrangements in highly recombinant regions [174].

The sequences obtained are assembled, the genome is annotated, and then a mapping is carried out against a reference plasmid, in general pHNSHP45 for *mcr-1* [6]. The aligned sequences are then compared to one of the resistance gene databases: Antibiotic Resistance Gene-Annotation [206], ResFinder [207], Comprehensive Antibiotic Resistance Database [208], and Antibiotic Resistance Genes Database [209]. They could also be compared to the plasmid genome, with GenEpid-J [210] or PlasmidFinder, which enabled the discovery of the *mcr-4* gene [11] and presents the advantage of screening multiple genes and detecting the coexistence of several genes including carbapenemases. Lindsey et al. proposed a whole protocol for plasmid sequencing [211]. More specifically, PointFinder was developed to detect chromosomal point mutations associated with antimicrobial resistance [212].

Whole genome sequencing combined with new bioinformatic tools improves our ability to detect several resistance genes at the same time [186, 205] but presents the same limitations than PCR: new genes are not recognized by these techniques, which require the continuous updating of databases [175, 213] that should be merged into a single reference database [213].

5. Conclusion

The recent description of plasmid-mediated colistin-resistant genes has generated concern among the global scientific community about the lack of new antibiotics to treat infections caused by multidrug-resistant pathogens. This concern was raised by the worldwide screening that demonstrated the global spread of bacterial strains harboring the *mcr-1* gene from diverse human and animal origins. Thus, it is necessary to implement an adapted protocol to effectively detect colistin-resistant strains in clinical microbiology laboratories.

Phenotypic methods indicate to the microbiologist the presence of polymyxin-resistant strains but do not define the mechanism involved and the risk of transmission. Molecular methods are rapid and more sensitive but are specific to the resistance genes examined and faced with the large number of molecular mechanisms conferring resistance to polymyxins, should only be used to screen *mcr* genes in clinical microbiology laboratories. Genomic analysis enables the complete screening of resistance genes in genetically identified bacteria from clinical samples but remains an *in silico* study which enables predictions but not resistance observation, as the presence of a resistance gene in a genome does not mean that the corresponding isolate is resistant, supported by studies that identified polymyxin-susceptible bacteria carrying the *mcr-1* gene [92, 165, 213]. Thus, phenotypic and molecular methods are complementary in detecting colistin-resistant pathogens in order to analyze the behavior of the clinical isolate, and it is important to carry them out in parallel [148] (Figure 3). All these techniques and their detection characteristics are summarized in Table 5.

In conclusion, these new techniques need to be combined for a complete understanding of colistin resistance, in particular for strains carrying *mcr* genes, so clinicians can rapidly adapt treatments or isolate the carrier patient in the hospital.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

Table S1: home-made polymyxin-containing agar. Table S2: comparison of different polymyxin B susceptibility testing methods to detect polymyxin B resistance in Gram-negative clinical isolates. Table S3: other comparative AST methods. (*Supplementary Materials*)

References

- [1] K. Jeannot, A. Bolard, and P. Plésiat, “Resistance to polymyxins in Gram-negative organisms,” *International Journal of Antimicrobial Agents*, vol. 49, no. 5, pp. 526–535, 2017.
- [2] S. Biswas, J. M. Brunel, J. C. Dubus, M. Reynaud-Gaubert, and J. M. Rolain, “Colistin: an update on the antibiotic of the 21st century,” *Expert Review of Anti-infective Therapy*, vol. 10, no. 8, pp. 917–934, 2012.
- [3] R. E. Hancock and D. S. Chapple, “Peptide antibiotics,” *Antimicrob Agents Chemother*, vol. 43, no. 6, pp. 1317–1323, 1999.

- [4] A. O. Olaitan and J. Li, "Emergence of polymyxin resistance in Gram-negative bacteria," *International Journal of Antimicrobial Agents*, vol. 48, no. 6, pp. 581-582, 2016.
- [5] A. O. Olaitan, S. Morand, and J. M. Rolain, "Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria," *Frontiers in Microbiology*, vol. 5, p. 643, 2014.
- [6] Y. Y. Liu, Y. Wang, T. R. Walsh et al., "Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study," *Lancet Infectious Diseases*, vol. 16, no. 2, pp. 161-168, 2016.
- [7] B. B. Xavier, C. Lammens, R. Ruhel et al., "Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016," *Eurosurveillance*, vol. 21, no. 27, pp. 6-11, 2016.
- [8] V. Di Pilato, F. Arena, C. Tascini et al., "*mcr-1.2*, a new *mcr* variant carried on a transferable plasmid from a colistin-resistant KPC carbapenemase-producing *Klebsiella pneumoniae* strain of sequence type 512," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 9, pp. 5612-5615, 2016.
- [9] Y. Q. Yang, Y. X. Li, T. Song et al., "Colistin resistance gene *mcr-1* and its variant in *Escherichia coli* isolates from chickens in China," *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 5, pp. e01204-e01216, 2017.
- [10] W. Yin, H. Li, Y. Shen et al., "Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*," *MBio*, vol. 8, no. 3, article e00543-17, 2017.
- [11] A. Carattoli, L. Villa, C. Feudi et al., "Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016," *Eurosurveillance*, vol. 22, no. 31, p. 30589, 2017.
- [12] M. Borowiak, J. Fischer, J. A. Hammerl, R. S. Hendriksen, I. Szabo, and B. Malorny, "Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B," *Journal of Antimicrobial Chemotherapy*, vol. 72, no. 12, pp. 3317-3324, 2017.
- [13] M. AbuOun, E. J. Stubberfield, N. A. Duggett et al., "*mcr-1* and *mcr-2* (*mcr-6.1*) variant genes identified in *Moraxella* species isolated from pigs in Great Britain from 2014 to 2015," *Journal of Antimicrobial Chemotherapy*, vol. 72, no. 10, pp. 2745-2749, 2017.
- [14] Y.-Q. Yang, Y.-X. Li, C.-W. Lei, A.-Y. Zhang, and H.-N. Wang, "Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*," *Journal of Antimicrobial Chemotherapy*, vol. 73, no. 7, pp. 1791-1795, 2018.
- [15] X. Wang, Y. Wang, Y. Zhou et al., "Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*," *Emerging Microbes and Infections*, vol. 7, no. 1, 2018.
- [16] J. M. Rolain and A. O. Olaitan, "Plasmid-mediated colistin resistance: the final blow to colistin?," *International Journal of Antimicrobial Agents*, vol. 47, no. 1, pp. 4-5, 2016.
- [17] Ministère de la santé, *Recommandations pour la prévention de la transmission croisée des bactéries hautement résistantes aux antibiotiques émergentes*, 2014.
- [18] J. Sun, R. S. Yang, Q. Zhang et al., "Co-transfer of *bla*_{NDM-5} and *mcr-1* by an IncX3-X4 hybrid plasmid in *Escherichia coli*," *Nature Microbiology*, vol. 1, p. 16176, 2016.
- [19] C. C. Lai, Y. C. Chuang, C. C. Chen, and H. J. Tang, "Co-existence of MCR-1 and NDM-9 in a clinical carbapenem-resistant *Escherichia coli* isolate," *International Journal of Antimicrobial Agents*, vol. 49, no. 4, 2017.
- [20] J. F. Delgado-Blas, C. M. Ovejero, L. Abadia-Patino, and B. Gonzalez-Zorn, "Coexistence of *mcr-1* and *bla*_{NDM-1} in *Escherichia coli* from Venezuela," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 10, pp. 6356-6358, 2016.
- [21] O. C. Conceição-Neto, C. A. M. Aires, N. F. Pereira et al., "Detection of the plasmid-mediated *mcr-1* gene in clinical KPC-2-producing *Escherichia coli* isolates in Brazil," *International Journal of Antimicrobial Agents*, vol. 50, pp. 282-284, 2017.
- [22] M. Tação, S. R. Tavares, P. Teixeira et al., "*mcr-1* and *bla*_{KPC-3} in *Escherichia coli* sequence type 744 after meropenem and colistin therapy, Portugal," *Emerging Infectious Diseases*, vol. 23, no. 8, pp. 1419-1421, 2017.
- [23] R. Beyrouthy, F. Robin, A. Lessene et al., "MCR-1 and OXA-48 in vivo acquisition in KPC-producing *Escherichia coli* after colistin treatment," *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 8, article e02540-16, 2017.
- [24] S. Pulss, T. Semmler, E. Prenger-Berninghoff, R. Bauerfeind, and C. Ewers, "First report of an *Escherichia coli* strain from swine carrying an OXA-181 carbapenemase and the colistin resistance determinant MCR-1," *International Journal of Antimicrobial Agents*, vol. 50, no. 2, pp. 232-236, 2017.
- [25] J. D. Perry, "A decade of development of chromogenic culture media for clinical microbiology in an era of molecular diagnostics," *Clinical Microbiology Reviews*, vol. 30, no. 2, pp. 449-479, 2017.
- [26] P. Nordmann and L. Poirel, "Strategies for identification of carbapenemase-producing Enterobacteriaceae," *Journal of Antimicrobial Chemotherapy*, vol. 68, no. 3, pp. 487-489, 2013.
- [27] L. Dortet, L. Brécard, L. Poirel, and P. Nordmann, "Rapid detection of carbapenemase-producing Enterobacteriaceae from blood cultures," *Clinical Microbiology and Infection*, vol. 20, no. 4, pp. 340-344, 2013.
- [28] P. Nordmann, L. Poirel, A. Carrère, M. A. Toleman, and T. R. Walsh, "How to detect NDM-1 producers," *Journal of Clinical Microbiology*, vol. 49, no. 2, pp. 718-721, 2011.
- [29] I. Caniaux, A. van Belkum, G. Zambardi, L. Poirel, and M. F. Gros, "MCR: modern colistin resistance," *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 36, no. 3, pp. 415-420, 2017.
- [30] M. E. Falagas, G. C. Makris, G. Dimopoulos, and D. K. Matthaiou, "Heteroresistance: a concern of increasing clinical significance?," *Clinical Microbiology and Infection*, vol. 14, no. 2, pp. 101-104, 2008.
- [31] J. R. Lo-Ten-Foe, A. M. G. A. de Smet, B. M. W. Diederer, J. A. J. W. Kluytmans, and P. H. J. van Keulen, "Comparative evaluation of the VITEK 2, disk diffusion, estest, broth microdilution, and agar dilution susceptibility testing methods for colistin in clinical isolates, including heteroresistant *Enterobacter cloacae* and *Acinetobacter baumannii*," *Antimicrobial Agents and Chemotherapy*, vol. 51, no. 10, pp. 3726-3730, 2007.
- [32] T. Y. Tan and S. Y. Ng, "Comparison of Etest, Vitek and agar dilution for susceptibility testing of colistin," *Clinical Microbiology and Infection*, vol. 13, no. 5, pp. 541-544, 2007.
- [33] L. Bardet, *Development of New Tools for Detection of Colistin-Resistant Gram-Negative Bacteria*, Aix-Marseille Université, Marseille, France, 2017.
- [34] J. E. Martin and J. S. Lewis, "Selective culture screening for penicillinase-producing *Neisseria gonorrhoeae*," *The Lancet*, vol. 2, no. 8038, pp. 605-606, 1977.

- [35] J. D. Thayer and J. E. Martin, "Improved medium selective for cultivation of *N. gonorrhoeae* and *N. meningitidis*," *Public Health Reports*, vol. 81, no. 6, pp. 559–562, 1966.
- [36] J. E. Martin and A. Lester, "Transgrow, a medium for transport and growth of *Neisseria gonorrhoeae* and *Neisseria meningitidis*," *HSMHA Health Reports*, vol. 86, no. 1, pp. 30–33, 1971.
- [37] Y. C. Faur, M. H. Weisburd, M. E. Wilson, and P. S. May, "A new medium for the isolation of pathogenic *Neisseria* (NYC medium). I. Formulation and comparisons with standard media," *Health Laboratory Science*, vol. 10, no. 2, pp. 44–54, 1973.
- [38] P. H. Gilligan, P. A. Gage, L. M. Bradshaw, D. V. Schidlow, and B. T. DeCicco, "Isolation medium for the recovery of *Pseudomonas cepacia* from respiratory secretions of patients with cystic fibrosis," *Journal of Clinical Microbiology*, vol. 22, no. 1, pp. 5–8, 1985.
- [39] D. F. Welch, M. J. Muszynski, C. H. Pai et al., "Selective and differential medium for recovery of *Pseudomonas cepacia* from the respiratory tracts of patients with cystic fibrosis," *Journal of Clinical Microbiology*, vol. 25, no. 9, pp. 1730–1734, 1987.
- [40] D. A. Henry, M. E. Campbell, J. J. LiPuma, and D. P. Speert, "Identification of *Burkholderia cepacia* isolates from patients with cystic fibrosis and use of a simple new selective medium," *Journal of Clinical Microbiology*, vol. 35, no. 3, pp. 614–619, 1997.
- [41] P. J. L. Dennis, C. L. R. Bartlett, and A. E. Wright, "Comparison of isolation methods for *Legionella* spp.," in *Proceedings of the 2nd International Symposium*, pp. 294–296, American Society, Washington, DC, USA, 1984.
- [42] C. A. Bopp, J. W. Sumner, G. K. Morris, and J. G. Wells, "Isolation of *Legionella* spp. from environmental water samples by low-pH treatment and use of a selective medium," *Journal of Clinical Microbiology*, vol. 13, no. 4, pp. 714–719, 1981.
- [43] A. Ta, J. Stout, V. Yu, and M. Wagener, "Comparison of culture methods for monitoring *Legionella* species in hospital potable water systems and recommendations for standardization of such methods," *Journal of Clinical Microbiology*, vol. 33, no. 8, pp. 2118–2123, 1995.
- [44] J. Stout, V. L. Yu, R. M. Vickers, and J. Shonnard, "Potable water supply as the hospital reservoir for Pittsburgh pneumonia agent," *The Lancet*, vol. 1, no. 8270, pp. 471–472, 1982.
- [45] P. H. Edelstein, "*Legionella*," in *Manual of Clinical Microbiology*, 9th edition, Washington, DC, USA, 2007.
- [46] P. R. Murray, E. Baron, J. H. Jorgensen, M. L. Landry, and M. A. Pfaller, *Manual of Clinical Microbiology*, American Society for Microbiology, Washington, DC, USA, 9th edition, 2007.
- [47] J. P. Butzler, P. Dekeyser, M. Detrain, and F. Dehaen, "Related vibrio in stools," *Journal of Pediatrics*, vol. 82, no. 3, pp. 493–495, 1973.
- [48] M. B. Skirrow, "*Campylobacter* enteritis: a 'new' disease," *British Medical Journal*, vol. 2, no. 6078, pp. 9–11, 1977.
- [49] W. L. Wang, M. Blaser, and J. Cravens, "Isolation of *Campylobacter*," *British Medical Journal*, vol. 2, no. 6129, p. 57, 1978.
- [50] F. J. Bolton and L. Robertson, "A selective medium for isolating *Campylobacter jejuni/coli*," *Journal of Clinical Pathology*, vol. 35, no. 4, pp. 462–467, 1982.
- [51] L. M. Jones and W. J. B. Morgan, "A preliminary report on a selective medium for the culture of *Brucella*, including fastidious types," *Bulletin of the World Health Organization*, vol. 19, no. 1, pp. 200–203, 1958.
- [52] C. Vanderzant and D. F. Splittstoesser, *Compendium of Methods for the Microbiological Examination of Foods*, American Public Health Association, Washington, DC, USA, 1992.
- [53] P. D. Ellner, C. J. Stoessel, E. Drakeford, and F. Vasi, "A new culture medium for medical bacteriology," *American Journal of Clinical Pathology*, vol. 45, no. 4, pp. 502–504, 1966.
- [54] G. D. W. Curtis, W. W. Nichols, and T. J. Falla, "Selective agents for *Listeria* can inhibit their growth," *Letters in Applied Microbiology*, vol. 8, no. 5, pp. 169–172, 1989.
- [55] W. H. Lee and D. McClain, "Improved *Listeria monocytogenes* selective agar," *Applied and Environmental Microbiology*, vol. 52, no. 5, pp. 1215–1217, 1986.
- [56] P. van Netten, I. Perales, A. van de Moosdijk, G. D. W. Curtis, and D. A. A. Mossel, "Liquid and solid selective differential media for the detection and enumeration of *L. monocytogenes* and other *Listeria* spp.," *International Journal of Food Microbiology*, vol. 8, no. 4, pp. 299–316, 1989.
- [57] D. A. Mossel, M. J. Koopman, and E. Jongerius, "Enumeration of *Bacillus cereus* in foods," *Applied Microbiology*, vol. 15, no. 3, pp. 650–653, 1967.
- [58] M. L. Cohn, R. F. Waggoner, and J. K. McClatchy, "The 7H11 medium for the cultivation of mycobacteria," *American Review of Respiratory Disease*, vol. 98, no. 2, pp. 295–296, 1968.
- [59] R. Angelotti, H. E. Hall, M. Foter, and K. M. Lewis, "Quantification of *Clostridium perfringens* in foods," *Applied Microbiology*, vol. 10, no. 3, pp. 193–199, 1962.
- [60] R. Marshall, "Rapid technique for the enumeration of *Clostridium perfringens*," *Applied Microbiology*, vol. 13, no. 4, pp. 559–563, 1965.
- [61] S. A. Shahidi and A. R. Ferguson, "New quantitative, qualitative, and confirmatory media for rapid analysis of food for *Clostridium perfringens*," *Applied Microbiology*, vol. 21, no. 3, pp. 500–506, 1971.
- [62] R. J. Meinersmann, S. R. Ladely, J. R. Plumblee et al., "Colistin resistance *mcr-1*-gene-bearing *Escherichia coli* strain from the United States," *Genome Announcements*, vol. 4, no. 5, p. e00898-16, 2016.
- [63] M. R. Fernandes, Q. Moura, L. Sartori et al., "Silent dissemination of colistin-resistant *Escherichia coli* in South America could contribute to the global spread of the *mcr-1* gene," *Eurosurveillance*, vol. 21, no. 17, pp. 1–6, 2016.
- [64] V. Donà, O. J. Bernasconi, S. Kasraian, R. Tinguely, and A. Endimiani, "A SYBR[®] green-based real-time PCR method for improved detection of *mcr-1*-mediated colistin resistance in human stool samples," *Journal of Global Antimicrobial Resistance*, vol. 9, pp. 57–60, 2017.
- [65] D. F. Monte, A. Mem, M. R. Fernandes et al., "Chicken meat as a reservoir of colistin-resistant *Escherichia coli* strains carrying *mcr-1* genes in South America," *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 5, p. e02718-16, 2017.
- [66] C. J. H. von Wintersdorff, P. F. G. Wolffs, J. M. van Niekerk et al., "Detection of the plasmid-mediated colistin-resistance gene *mcr-1* in faecal metagenomes of Dutch travellers," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 12, pp. 3416–3419, 2016.
- [67] S. Buess, M. Nüesch-Inderbinnen, R. Stephan, and K. Zurfluh, "Assessment of animals as a reservoir for colistin resistance: no MCR-1/MCR-2-producing Enterobacteriaceae detected

- in Swiss livestock," *Journal of Global Antimicrobial Resistance*, vol. 8, pp. 33-34, 2017.
- [68] K. Zurfluh, R. Stephan, A. Widmer et al., "Screening for fecal carriage of MCR-producing Enterobacteriaceae in healthy humans and primary care patients," *Antimicrobial Resistance & Infection Control*, vol. 6, no. 1, p. 28, 2017.
- [69] Y. Hu, Y. Wang, Q. Sun et al., "Colistin-resistance gene *mcr-1* in children's gut flora," *International Journal of Antimicrobial Agents*, vol. 50, no. 4, pp. 593-597, 2017.
- [70] Y. Wang, G. B. Tian, R. Zhang et al., "Prevalence, risk factors, outcomes, and molecular epidemiology of *mcr-1*-positive Enterobacteriaceae in patients and healthy adults from China: an epidemiological and clinical study," *Lancet Infectious Diseases*, vol. 17, no. 4, pp. 390-399, 2017.
- [71] S. C. Y. Wong, H. Tse, J. H. K. Chen, V. C. C. Cheng, P. L. Ho, and K. Y. Yuen, "Colistin-resistant Enterobacteriaceae carrying the *mcr-1* gene among patients in Hong Kong," *Emerging Infectious Diseases*, vol. 22, no. 9, pp. 1667-1669, 2016.
- [72] M. Payne, M. A. Croxen, T. D. Lee et al., "*mcr-1*-positive colistin-resistant *Escherichia coli* in traveler returning to Canada from China," *Emerging Infectious Diseases*, vol. 22, no. 9, pp. 1673-1675, 2016.
- [73] Y. Caspar, M. Maillot, P. Pavese et al., "*mcr-1* colistin resistance in ESBL-producing *Klebsiella pneumoniae*, France," *Emerging Infectious Diseases*, vol. 23, no. 5, pp. 874-876, 2017.
- [74] E. M. Terveer, R. H. T. Nijhuis, M. J. T. Crobach et al., "Prevalence of colistin resistance gene (*mcr-1*) containing Enterobacteriaceae in feces of patients attending a tertiary care hospital and detection of a *mcr-1* containing, colistin susceptible *E. coli*," *PLoS One*, vol. 12, no. 6, Article ID e0178598, 2017.
- [75] P. Nordmann, A. Jayol, and L. Poirel, "A universal culture medium for screening polymyxin-resistant Gram-negative isolates," *Journal of Clinical Microbiology*, vol. 54, no. 5, pp. 1395-1399, 2016.
- [76] M. H. F. Abdul Momin, D. C. Bean, R. S. Hendriksen, M. Haenni, L. M. Phee, and D. W. Wareham, "CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens," *Journal of Medical Microbiology*, vol. 66, no. 11, pp. 1554-1561, 2017.
- [77] L. Bardet, S. Le Page, T. Leangapichart, and J. M. Rolain, "LBJMR medium: a new polyvalent culture medium for isolating and selecting vancomycin and colistin-resistant bacteria," *BMC Microbiology*, vol. 17, no. 1, pp. 1-10, 2017.
- [78] P. Nordmann, A. Jayol, and L. Poirel, "Rapid detection of polymyxin resistance in Enterobacteriaceae," *Emerging Infectious Diseases*, vol. 22, no. 6, pp. 1038-1043, 2016.
- [79] A. Jayol, M. Saly, P. Nordmann, A. Ménard, L. Poirel, and V. Dubois, "*Hafnia*, an enterobacterial genus naturally resistant to colistin revealed by three susceptibility testing methods," *Journal of Antimicrobial Chemotherapy*, vol. 72, no. 9, pp. 1-5, 2017.
- [80] A. Jayol, V. Dubois, L. Poirel, and P. Nordmann, "Rapid detection of polymyxin-resistant Enterobacteriaceae from blood cultures," *Journal of Clinical Microbiology*, vol. 54, no. 9, pp. 2273-2277, 2016.
- [81] Y. D. Bakthavatchalam and B. Veeraraghavan, "Challenges, issues and warnings from CLSI and EUCAST working group on polymyxin susceptibility testing," *Journal of Clinical and Diagnostic Research*, vol. 11, pp. DL03-DL04, 2017.
- [82] C. G. Giske and G. Kahlmeter, "Colistin antimicrobial susceptibility testing—can the slow and challenging be replaced by the rapid and convenient?," *Clinical Microbiology and Infection*, vol. 24, no. 2, pp. 93-94, 2017.
- [83] A. Jayol, P. Nordmann, P. Lehours et al., "Comparison of methods for detection of plasmid-mediated and chromosomally encoded colistin resistance in Enterobacteriaceae," *Clinical Microbiology and Infection*, vol. 51, no. 2, pp. 3726-3730, 2017.
- [84] L. Poirel, Y. Larpin, J. Dobias et al., "Rapid polymyxin NP test for the detection of polymyxin resistance mediated by the *mcr-1/mcr-2* genes," *Diagnostic Microbiology and Infectious Disease*, vol. 90, no. 1, pp. 7-10, 2017.
- [85] S. Simar, D. Sibley, D. Ashcraft, and G. Pankey, "Evaluation of the rapid polymyxin NP test for polymyxin B resistance detection using *Enterobacter cloacae* and *Enterobacter aerogenes* isolates," *Journal of Clinical Microbiology*, vol. 55, no. 10, pp. 3016-3020, 2017.
- [86] M. Tamayo, R. Santiso, F. Otero et al., "Rapid determination of colistin resistance in clinical strains of *Acinetobacter baumannii* by use of the Micromax assay," *Journal of Clinical Microbiology*, vol. 51, no. 11, pp. 3675-3682, 2013.
- [87] J. Osei Sekyere, U. Govinden, and S. Y. Essack, "Review of established and innovative detection methods for carbapenemase-producing Gram-negative bacteria," *Journal of Applied Microbiology*, vol. 119, no. 5, pp. 1219-1233, 2015.
- [88] B. Ghebremedhin, A. Halstenbach, M. Smiljanic, M. Kaase, and P. Ahmad-Nejad, "MALDI-TOF MS based carbapenemase detection from culture isolates and from positive blood culture vials," *Annals of Clinical Microbiology and Antimicrobials*, vol. 15, no. 1, p. 5, 2016.
- [89] J. Hrabák, E. Chudáčková, and C. C. Papagiannitsis, "Detection of carbapenemases in Enterobacteriaceae: a challenge for diagnostic microbiological laboratories," *Clinical Microbiology and Infection*, vol. 20, no. 9, pp. 839-853, 2014.
- [90] P. Seng, M. Drancourt, F. Gouriet et al., "Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry," *Clinical Infectious Diseases*, vol. 49, no. 4, pp. 543-551, 2009.
- [91] G. Larrouy-Maumus, A. Clements, A. Filloux, R. R. McCarthy, and S. Mostowy, "Direct detection of lipid A on intact Gram-negative bacteria by MALDI-TOF mass spectrometry," *Journal of Microbiological Methods*, vol. 120, pp. 68-71, 2016.
- [92] J. O. Sekyere, U. Govinden, L. A. Bester, and S. Y. Essack, "Colistin and tigecycline resistance in carbapenemase-producing Gram-negative bacteria: emerging resistance mechanisms and detection methods," *Journal of Applied Microbiology*, vol. 121, no. 3, pp. 601-617, 2016.
- [93] L. Dortet, R. A. Bonnin, I. Pennisi et al., "Rapid detection and discrimination of chromosome- and MCR-plasmid-mediated resistance to polymyxins by MALDI-TOF MS in *Escherichia coli*: the MALDIxin test," *Journal of Antimicrobial Chemotherapy*, pp. 1-9, 2018, In press.
- [94] V. Stojanoski, B. Sankaran, B. V. V. Prasad, L. Poirel, P. Nordmann, and T. Palzkill, "Structure of the catalytic domain of the colistin resistance enzyme MCR-1," *BMC Biology*, vol. 14, no. 1, p. 81, 2016.
- [95] M. Hu, J. Guo, Q. Cheng et al., "Crystal structure of *Escherichia coli* originated MCR-1, a phosphoethanolamine transferase for colistin resistance," *Scientific Reports*, vol. 6, no. 1, p. 38793, 2016.

- [96] G. Ma, Y. Zhu, Z. Yu, A. Ahmad, and H. Zhang, "High resolution crystal structure of the catalytic domain of MCR-1," *Scientific Reports*, vol. 6, no. 1, p. 39540, 2016.
- [97] P. Hinchliffe, Q. E. Yang, E. Portal et al., "Insights into the mechanistic basis of plasmid-mediated colistin resistance from crystal structures of the catalytic domain of MCR-1," *Scientific Reports*, vol. 7, p. 39392, 2017.
- [98] M. Coppi, A. Cannatelli, A. Antonelli et al., "A simple phenotypic method for screening of MCR-1-mediated colistin resistance," *Clinical Microbiology and Infection*, vol. 24, no. 2, pp. 201.e1–201.e3, 2017.
- [99] F. Esposito, M. R. Fernandes, R. Lopes et al., "Detection of colistin-resistant *mcr-1*-positive *Escherichia coli* by use of assays based on inhibition by EDTA and zeta potential," *Journal of Clinical Microbiology*, vol. 55, no. 12, pp. 3454–3465, 2017.
- [100] European Committee on Antimicrobial Susceptibility Testing (EUCAST), *Breakpoint Tables for Interpretation of MICs and Zone Diameters*, Vol. 8, EUCAST, Växjö, Sweden, 2018.
- [101] CLSI, Clinical and Laboratory Standards Institute, *M07–A10: Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard*, Vol. 35, CLSI, Wayne, PA, USA, 10th edition, 2015.
- [102] ISO 20776-1:2006, *Clinical Laboratory Testing and In Vitro Diagnostic Test Systems—Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices—Part 1: Reference Method for Testing the In Vitro Activity of Antimicrobial Agents against Rapidly Growing Aerobic Bacteria Involved in Infectious Diseases n.d.*, <https://www.iso.org/standard/41630.html>.
- [103] Clinical and Laboratory Standards, *M100–S27: Performance Standards for Antimicrobial Susceptibility Testing*, Clinical and Laboratory Standards, Wayne, PA, USA, 2017.
- [104] European Committee on Antimicrobial Susceptibility Testing (EUCAST), *Routine and Extended Internal Quality Control for Mic Determination and Disk Diffusion as Recommended by EUCAST*, Vol. 8, EUCAST, Växjö, Sweden, 2018.
- [105] K. H. Jerke, M. J. Lee, and R. M. Humphries, "Polymyxin susceptibility testing: a cold case reopened," *Clinical Microbiology Newsletter*, vol. 38, no. 9, pp. 69–77, 2016.
- [106] R. M. Humphries, "Susceptibility testing of the polymyxins: where are we now?," *Pharmacotherapy*, vol. 35, no. 1, pp. 22–27, 2015.
- [107] D. Landman, J. Salamera, and J. Quale, "Irreproducible and uninterpretable polymyxin B MICs for *Enterobacter cloacae* and *Enterobacter aerogenes*," *Journal of Clinical Microbiology*, vol. 51, no. 12, pp. 4106–4111, 2013.
- [108] A. Poudyal, B. P. Howden, J. M. Bell et al., "In vitro pharmacodynamics of colistin against multidrug-resistant *Klebsiella pneumoniae*," *Journal of Antimicrobial Chemotherapy*, vol. 62, no. 6, pp. 1311–1318, 2008.
- [109] ISO 20776-2:2007, *Clinical Laboratory Testing and In Vitro Diagnostic Test Systems—Susceptibility Testing of Infectious Agents and Evaluation of Performance of Antimicrobial Susceptibility Test Devices—Part 2: Evaluation of Performance of Antimicrobial Susceptibility Test Devices n.d.*, 2018, <https://www.iso.org/standard/41631.html>.
- [110] K. Dafopoulou, O. Zarkotou, E. Dimitroulia et al., "Comparative evaluation of colistin susceptibility testing methods among carbapenem-nonsusceptible *Klebsiella pneumoniae* and *Acinetobacter baumannii* clinical isolates," *Antimicrobial Agents and Chemotherapy*, vol. 59, no. 8, pp. 4625–4630, 2015.
- [111] C. A. Sutherland and D. P. Nicolau, "To add or not to add polysorbate 80: impact on colistin MICs for clinical strains of Enterobacteriaceae and *Pseudomonas aeruginosa* and quality controls," *Journal of Clinical Microbiology*, vol. 52, no. 10, p. 3810, 2014.
- [112] H. S. Sader, P. R. Rhomberg, R. K. Flamm, and R. N. Jones, "Use of a surfactant (polysorbate 80) to improve MIC susceptibility testing results for polymyxin B and colistin," *Diagnostic Microbiology and Infectious Disease*, vol. 74, no. 4, pp. 412–414, 2012.
- [113] M. Albur, A. Noel, K. Bowker, and A. MacGowan, "Colistin susceptibility testing: time for a review," *Journal of Antimicrobial Chemotherapy*, vol. 69, no. 5, pp. 1432–1434, 2014.
- [114] L. Singhal, M. Sharma, S. Verma et al., "Comparative Evaluation of Broth Microdilution with Polystyrene and Glass-Coated Plates, Agar Dilution, E-Test, Vitek, and Disk Diffusion for Susceptibility Testing of Colistin and Polymyxin B on Carbapenem-Resistant Clinical Isolates of *Acinetobacter baumannii*," *Microbial Drug Resistance*, 2018.
- [115] S. Y. Lee, J. H. Shin, K. Lee et al., "Comparison of the Vitek 2, MicroScan, and Etest methods with the agar dilution method in assessing colistin susceptibility of bloodstream isolates of *Acinetobacter* species from a Korean University Hospital," *Journal of Clinical Microbiology*, vol. 51, no. 6, pp. 1924–1926, 2013.
- [116] S. M. Maalej, M. R. Meziou, F. M. Rhimi, and A. Hammami, "Comparison of disc diffusion, Etest and agar dilution for susceptibility testing of colistin against Enterobacteriaceae," *Letters in Applied Microbiology*, vol. 53, no. 5, pp. 546–551, 2011.
- [117] B. Behera, P. Mathur, A. Das et al., "Evaluation of susceptibility testing methods for polymyxin," *International Journal of Infectious Diseases*, vol. 14, no. 7, pp. e596–e601, 2010.
- [118] T. Y. Tan, L. S. Y. Ng, and K. Poh, "Susceptibility testing of unconventional antibiotics against multidrug-resistant *Acinetobacter* spp. by agar dilution and Vitek 2," *Diagnostic Microbiology and Infectious Disease*, vol. 58, no. 3, pp. 357–361, 2007.
- [119] T. Y. Tan, "Comparison of three standardized disc susceptibility testing methods for colistin," *Journal of Antimicrobial Chemotherapy*, vol. 58, no. 4, pp. 864–867, 2006.
- [120] S. M. Moskowitz, E. Garber, Y. Chen et al., "Colistin susceptibility testing: evaluation of reliability for cystic fibrosis isolates of *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia*," *Journal of Antimicrobial Chemotherapy*, vol. 65, no. 7, pp. 1416–1423, 2010.
- [121] M. Hogardt, S. Schmoltdt, M. Götzfried, K. Adler, and J. Heesemann, "Pitfalls of polymyxin antimicrobial susceptibility testing of *Pseudomonas aeruginosa* isolated from cystic fibrosis patients," *Journal of Antimicrobial Chemotherapy*, vol. 54, no. 6, pp. 1057–1061, 2004.
- [122] M. Richter and R. Rosselló-Móra, "Shifting the genomic gold standard for the prokaryotic species definition," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 106, no. 45, pp. 19126–19131, 2009.
- [123] F. Boyen, F. Vangroenweghe, P. Butaye et al., "Disk diffusion is a reliable method for testing colistin susceptibility in porcine *E. coli* strains," *Veterinary Microbiology*, vol. 144, no. 3–4, pp. 359–362, 2010.
- [124] A. Nemeč and L. Dijkshoorn, "Variations in colistin susceptibility among different species of the genus

- Acinetobacter*,” *Journal of Antimicrobial Chemotherapy*, vol. 65, no. 2, pp. 367–369, 2010.
- [125] A. C. Nicodemo, M. R. E. Araujo, A. S. Ruiz, and A. C. Gales, “In vitro susceptibility of *Stenotrophomonas maltophilia* isolates: comparison of disc diffusion, Etest and agar dilution methods,” *Journal of Antimicrobial Chemotherapy*, vol. 53, no. 4, pp. 604–608, 2004.
- [126] F. W. Goldstein, A. Ly, and M. D. Kitzis, “Comparison of Etest with agar dilution for testing the susceptibility of *Pseudomonas aeruginosa* and other multidrug-resistant bacteria to colistin,” *Journal of Antimicrobial Chemotherapy*, vol. 59, no. 5, pp. 1039–1040, 2007.
- [127] A. C. Gales, A. O. Reis, and R. N. Jones, “Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: review of available interpretative criteria and quality control guidelines,” *Journal of Clinical Microbiology*, vol. 39, no. 1, pp. 183–190, 2001.
- [128] J. A. Hindler and R. M. Humphries, “Colistin MIC variability by method for contemporary clinical isolates of multidrug-resistant Gram-negative bacilli,” *Journal of Clinical Microbiology*, vol. 51, no. 6, pp. 1678–1684, 2013.
- [129] S. Vourli, K. Dafopoulou, G. Vrioni, A. Tsakris, and S. Pournaras, “Evaluation of two automated systems for colistin susceptibility testing of carbapenem-resistant *Acinetobacter baumannii* clinical isolates,” *Journal of Antimicrobial Chemotherapy*, vol. 72, no. 9, pp. 2528–2530, 2017.
- [130] A. Turlej-Rogacka, B. B. Xavier, L. Janssens et al., “Evaluation of colistin stability in agar and comparison of four methods for MIC testing of colistin,” *European Journal of Clinical Microbiology & Infectious Diseases*, vol. 37, no. 2, pp. 345–353, 2017.
- [131] A. W. Bauer, W. M. Kirby, J. C. Sherris, and M. Turck, “Antibiotic susceptibility testing by a standardized single disk method,” *American Journal of Clinical Pathology*, vol. 45, no. 4, pp. 493–496, 1966.
- [132] L. J. Rojas, M. Salim, E. Cober et al., “Colistin resistance in carbapenem-resistant *Klebsiella pneumoniae*: laboratory detection and impact on mortality,” *Clinical Infectious Diseases*, vol. 314, p. ciw805, 2016.
- [133] L. R. R. Perez, “Evaluation of polymyxin susceptibility profile among KPC-producing *Klebsiella pneumoniae* using Etest and MicroScan WalkAway automated system,” *APMIS*, vol. 123, no. 11, pp. 951–954, 2015.
- [134] A. Lat, S. A. Clock, F. Wu et al., “Comparison of polymyxin B, tigecycline, cefepime, and meropenem MICs for KPC-producing *Klebsiella pneumoniae* by broth microdilution, Vitek 2, and Etest,” *Journal of Clinical Microbiology*, vol. 49, no. 5, pp. 1795–1798, 2011.
- [135] I. M. van der Heijden, A. S. Levin, E. H. De Pedri et al., “Comparison of disc diffusion, Etest and broth microdilution for testing susceptibility of carbapenem-resistant *P. aeruginosa* to polymyxins,” *Annals of Clinical Microbiology and Antimicrobials*, vol. 6, no. 1, p. 8, 2007.
- [136] P. Piewngam and P. Kiratisin, “Comparative assessment of antimicrobial susceptibility testing for tigecycline and colistin against *Acinetobacter baumannii* clinical isolates, including multidrug-resistant isolates,” *International Journal of Antimicrobial Agents*, vol. 44, no. 5, pp. 396–401, 2014.
- [137] M. Sinirtaş, H. Akalin, and S. Gedikoğlu, “Investigation of colistin sensitivity via three different methods in *Acinetobacter baumannii* isolates with multiple antibiotic resistance,” *International Journal of Infectious Diseases*, vol. 13, no. 5, pp. e217–e220, 2009.
- [138] L. A. Arroyo, A. Garcia-Curiel, M. E. Pachón-Ibáñez et al., “Reliability of the E-test method for detection of colistin resistance in clinical isolates of *Acinetobacter baumannii*,” *Journal of Clinical Microbiology*, vol. 43, no. 2, pp. 903–905, 2005.
- [139] R. Girardello, P. J. M. Bispo, T. M. Yamanaka, and A. C. Gales, “Cation concentration variability of four distinct Mueller-Hinton agar brands influences polymyxin B susceptibility results,” *Journal of Clinical Microbiology*, vol. 50, no. 7, pp. 2414–2418, 2012.
- [140] L. Bardet, S. Baron, T. Leangapichart, L. Okdah, S. M. Diene, and J. M. Rolain, “Deciphering heteroresistance to colistin in a *Klebsiella pneumoniae* isolate from Marseille, France,” *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 6, p. e00356-17, 2017.
- [141] I. Galani, F. Kontopidou, M. Souli et al., “Colistin susceptibility testing by Etest and disk diffusion methods,” *International Journal of Antimicrobial Agents*, vol. 31, no. 5, pp. 434–439, 2008.
- [142] E. Jouy, M. Haenni, L. Le Devendec et al., “Improvement in routine detection of colistin resistance in *E. coli* isolated in veterinary diagnostic laboratories,” *Journal of Microbiological Methods*, vol. 132, pp. 125–127, 2017.
- [143] E. Matuschek, J. Åhman, C. Webster, and G. Kahlmeter, “Antimicrobial susceptibility testing of colistin—evaluation of seven commercial MIC products against standard broth microdilution for *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp.,” *Clinical Microbiology and Infection*, 2017.
- [144] P. Plésiat, K. Jeannot, and P. Triponney, “Évaluation comparative de la détermination du test de sensibilité à la colistine UMIC (Biocentric) chez *Pseudomonas aeruginosa*,” in *Proceedings of the 36ème Réunion Interdiscip. Chim. Anti-Infectieuse*, Paris, France, December 2016.
- [145] L. Bardet, L. Okdah, S. Baron, S. Le Page, and J. Rolain, “Évaluation du test UMIC Colistine de détermination de la CMI,” in *Proceedings of the 37ème Réunion Interdiscip. Chim. Anti-Infectieuse*, Paris, France, December 2017.
- [146] E. Carretto, F. Brovarone, G. Russello et al., “Clinical validation of the Sensitest Colistin, a broth microdilution based method to evaluate colistin MICs,” *Journal of Clinical Microbiology*, vol. 56, no. 4, 2018.
- [147] K. L. Chew, M. V. La, R. T. P. Lin, and J. W. P. Teo, “Colistin and polymyxin B susceptibility testing for carbapenem-resistant and *mcr*-positive Enterobacteriaceae: comparison of Sensititre, MicroScan, Vitek 2, and Etest with broth microdilution,” *Journal of Clinical Microbiology*, vol. 55, no. 9, pp. 2609–2616, 2017.
- [148] T. Cimmino, S. Le Page, D. Raoult, and J. M. Rolain, “Contemporary challenges and opportunities in the diagnosis and outbreak detection of multidrug-resistant infectious disease,” *Expert Review of Molecular Diagnostics*, vol. 16, no. 11, pp. 1163–1175, 2016.
- [149] T. Giani, M. I. Morosini, M. M. D’Andrea, M. García-Castillo, G. M. Rossolini, and R. Cantón, “Assessment of the Phoenix™ automated system and EUCAST breakpoints for antimicrobial susceptibility testing against isolates expressing clinically relevant resistance mechanisms,” *Clinical Microbiology and Infection*, vol. 18, no. 11, pp. E452–E458, 2012.

- [150] R. L. Skov and D. L. Monnet, "Plasmid-mediated colistin resistance (*mcr-1* gene): three months later, the story unfolds," *Eurosurveillance*, vol. 21, no. 9, p. 30155, 2016.
- [151] S. Bontron, L. Poirel, and P. Nordmann, "Real-time PCR for detection of plasmid-mediated polymyxin resistance (*mcr-1*) from cultured bacteria and stools," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 8, pp. 2318–2320, 2016.
- [152] S. Chabou, T. Leangapichart, L. Okdah, S. Le Page, L. Hadjadj, and J. M. M. Rolain, "Real-time quantitative PCR assay with Taqman[®] probe for rapid detection of MCR-1 plasmid-mediated colistin resistance," *New Microbes and New Infections*, vol. 13, pp. 71–74, 2016.
- [153] J. Quan, X. Li, and Y. Chen, "Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study," *The Lancet Infectious Diseases*, vol. 17, no. 4, pp. 400–410, 2017.
- [154] S. S. Elnahriry, H. O. Khalifa, A. M. Soliman et al., "Emergence of plasmid-mediated colistin resistance gene *mcr-1* in a clinical *Escherichia coli* isolate from Egypt," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 5, pp. 3249–3250, 2016.
- [155] L. Falgenhauer, S. E. Waezsada, Y. Yao et al., "Colistin resistance gene *mcr-1* in extended-spectrum beta-lactamase-producing and carbapenemase-producing Gram-negative bacteria in Germany," *Lancet Infectious Diseases*, vol. 16, no. 3, pp. 282–283, 2016.
- [156] H. Ye, Y. Li, Z. Li et al., "Diversified *mcr-1*-harbouring plasmid reservoirs confer resistance to colistin in human gut microbiota," *mBio*, vol. 7, no. 2, p. e00177-16, 2016.
- [157] A. Walkty, J. A. Karlowisky, H. J. Adam et al., "Frequency of MCR-1-mediated colistin resistance among *Escherichia coli* clinical isolates obtained from patients in Canadian hospitals (CANWARD 2008–2015)," *CMAJ Open*, vol. 4, no. 4, pp. E641–E645, 2016.
- [158] R. Gao, Y. Hu, Z. Li et al., "Dissemination and mechanism for the MCR-1 colistin resistance," *PLoS Pathogens*, vol. 12, no. 11, p. e1005957, 2016.
- [159] A. Cannatelli, T. Gianì, A. Antonelli, L. Principe, F. Luzzaro, and G. M. Rossolini, "First detection of the *mcr-1* colistin resistance gene in *Escherichia coli* in Italy," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 5, pp. 3257–3258, 2016.
- [160] G. D. Wright, "Antibiotic resistance in the environment: a link to the clinic?," *Current Opinion in Microbiology*, vol. 13, no. 5, pp. 589–594, 2010.
- [161] I. Lekunberri, J. L. Balcázar, and C. M. Borrego, "Detection and quantification of the plasmid-mediated *mcr-1* gene conferring colistin resistance in wastewater," *International Journal of Antimicrobial Agents*, vol. 50, no. 6, pp. 734–736, 2017.
- [162] A. Irrgang, N. Roschanski, B. A. Tenhagen et al., "Prevalence of *mcr-1* in *E. coli* from livestock and food in Germany, 2010–2015," *PLoS One*, vol. 11, no. 7, Article ID e0159863, 2016.
- [163] R. H. T. Nijhuis, K. T. Veldman, J. Schelfaut et al., "Detection of the plasmid-mediated colistin-resistance gene *mcr-1* in clinical isolates and stool specimens obtained from hospitalized patients using a newly developed real-time PCR assay," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 8, pp. 2344–2346, 2016.
- [164] D. Yang, Z. Qiu, Z. Shen et al., "The occurrence of the colistin resistance gene *mcr-1* in the Haihe River (China)," *International Journal of Environmental Research and Public Health*, vol. 14, no. 6, p. 576, 2017.
- [165] E. Snesrud, A. C. Ong, and B. Corey, "Analysis of serial isolates of *mcr-1*-positive *Escherichia coli* reveals a highly active IS *Apl1* transposon," *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 5, p. e00056-17, 2017.
- [166] N. Lima Barbieri, D. W. Nielsen, Y. Wannemuehler et al., "*mcr-1* identified in avian pathogenic *Escherichia coli* (APEC)," *PLoS One*, vol. 12, no. 3, Article ID e0172997, 2017.
- [167] J. Sun, Y. Xu, R. Gao et al., "Deciphering MCR-2 colistin resistance," *mBio*, vol. 8, no. 3, p. e00625-17, 2017.
- [168] N. Liassine, L. Assouvie, M. C. Descombes et al., "Very low prevalence of MCR-1/MCR-2 plasmid-mediated colistin resistance in urinary tract Enterobacteriaceae in Switzerland," *International Journal of Infectious Diseases*, vol. 51, pp. 4–5, 2016.
- [169] S. Simmen, K. Zurfluh, M. Nüesch-Inderbinen, and S. Schmitt, "Investigation for the colistin resistance genes *mcr-1* and *mcr-2* in clinical Enterobacteriaceae isolates from cats and dogs in Switzerland," *ARC Journal of Animal and Veterinary Sciences*, vol. 2, no. 4, pp. 26–29, 2016.
- [170] N. Roschanski, L. Falgenhauer, M. Grobbel et al., "Retrospective survey of *mcr-1* and *mcr-2* in German pig-fattening farms, 2011–2012," *International Journal of Antimicrobial Agents*, vol. 50, no. 2, pp. 266–271, 2017.
- [171] L. Poirel, A. Jayol, and P. Nordmann, "Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes," *Clinical Microbiology Reviews*, vol. 30, no. 2, pp. 557–596, 2017.
- [172] J. Li, X. Shi, W. Yin et al., "A multiplex SYBR Green real-time PCR assay for the detection of three colistin resistance genes from cultured bacteria, feces, and environment samples," *Frontiers in Microbiology*, vol. 8, pp. 1–5, 2017.
- [173] A. Rita Rebelo, V. Bortolaia, J. S. Kjeldgaard et al., "Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, *mcr-1*, *mcr-2*, *mcr-3*, *mcr-4* and *mcr-5* for surveillance purposes," *Eurosurveillance*, vol. 23, no. 6, 2018.
- [174] T. Sekizuka, M. Kawanishi, M. Ohnishi et al., "Elucidation of quantitative structural diversity of remarkable rearrangement regions, shufflons, in IncI2 plasmids," *Scientific Reports*, vol. 7, no. 1, p. 928, 2017.
- [175] M. Doumith, G. Godbole, P. Ashton et al., "Detection of the plasmid-mediated *mcr-1* gene conferring colistin resistance in human and food isolates of *Salmonella enterica* and *Escherichia coli* in England and Wales," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 8, pp. 2300–2305, 2016.
- [176] N. Stoesser, A. J. Mathers, C. E. Moore, N. P. J. Day, and D. W. Crook, "Colistin resistance gene *mcr-1* and pHNSHP45 plasmid in human isolates of *Escherichia coli* and *Klebsiella pneumoniae*," *Lancet Infectious Diseases*, vol. 16, no. 3, pp. 285–286, 2016.
- [177] R. Li, M. Xie, J. Lv, E. Wai-Chi Chan, and S. Chen, "Complete genetic analysis of plasmids carrying *mcr-1* and other resistance genes in an *Escherichia coli* isolate of animal origin," *Journal of Antimicrobial Chemotherapy*, vol. 72, no. 3, pp. 696–699, 2017.
- [178] K. Veldman, A. van Essen-Zandbergen, M. Rapallini et al., "Location of colistin resistance gene *mcr-1* in Enterobacteriaceae from livestock and meat," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 8, pp. 2340–2342, 2016.

- [179] J. Campos, L. Cristino, L. Peixe, and P. Antunes, "MCR-1 in multidrug-resistant and copper-tolerant clinically relevant *Salmonella* 1,4,[5],12:i:- and *S. Rissen* clones in Portugal, 2011 to 2015," *Eurosurveillance*, vol. 21, no. 26, p. 30270, 2016.
- [180] M. F. Anjum, N. A. Duggett, M. AbuOun et al., "Colistin resistance in *Salmonella* and *Escherichia coli* isolates from a pig farm in Great Britain," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 8, pp. 2306–2313, 2016.
- [181] L. Poirel, N. Kieffer, A. Brink, J. Coetze, A. Jayol, and P. Nordmann, "Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates in South Africa," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 7, pp. 4394–4397, 2016.
- [182] O. J. Bernasconi, L. Principe, R. Tinguely et al., "Evaluation of a new commercial microarray platform for the simultaneous detection of β -lactamase and *Mcr-1*/*-2* genes in Enterobacteriaceae," *Journal of Clinical Microbiology*, vol. 55, no. 10, pp. 3138–3141, 2017.
- [183] C. Imirzalioglu, L. Falgenhauer, J. Schmiedel et al., "Evaluation of a LAMP-based assay for the rapid detection of plasmid-encoded colistin resistance gene *mcr-1* in Enterobacteriaceae isolates," *Antimicrobial Agents and Chemotherapy*, vol. 61, no. 4, p. e02326-16, 2017.
- [184] D. Zou, S. Huang, H. Lei et al., "Sensitive and rapid detection of the plasmid-encoded colistin-resistance Gene *mcr-1* in Enterobacteriaceae isolates by loop-mediated isothermal amplification," *Frontiers in Microbiology*, vol. 8, pp. 1–7, 2017.
- [185] J. L. Martinez, T. M. Coque, and F. Baquero, "What is a resistance gene? Ranking risk in resistomes," *Nature Reviews Microbiology*, vol. 13, no. 2, pp. 116–123, 2015.
- [186] Y. Wang, R. Zhang, J. Li et al., "Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production," *Nature Microbiology*, vol. 2, p. 16260, 2017.
- [187] A. Gundogdu and O. U. Nalbantoglu, "Humans as a source of colistin resistance: in silico analysis of public metagenomes for the *mcr-1* gene in the gut microbiome," *Erciyes Medical Journal*, vol. 38, no. 2, pp. 59–61, 2016.
- [188] D. P. Thanh, H. T. Tuyen, T. N. T. Nguyen et al., "Inducible colistin resistance via a disrupted plasmid-borne *mcr-1* gene in a 2008 Vietnamese *Shigella sonnei* isolate," *Journal of Antimicrobial Chemotherapy*, vol. 71, no. 8, pp. 2314–2317, 2016.
- [189] R. J. Meinersmann, S. R. Ladely, J. L. Bono et al., "Complete genome sequence of a colistin resistance gene (*mcr-1*)-bearing isolate of *Escherichia coli* from the United States," *Genome Announcements*, vol. 4, no. 6, p. e01283-16, 2016.
- [190] M. F. Kluytmans-van den Bergh, P. Huizinga, M. J. Bonten et al., "Presence of *mcr-1*-positive Enterobacteriaceae in retail chicken meat but not in humans in the Netherlands since 2009," *Eurosurveillance*, vol. 21, no. 9, p. 30149, 2016.
- [191] S. Malhotra-Kumar, B. B. Xavier, A. J. Das et al., "Colistin-resistant *Escherichia coli* harbouring *mcr-1* isolated from food animals in Hanoi, Vietnam," *Lancet Infectious Diseases*, vol. 16, no. 3, pp. 286–287, 2016.
- [192] P. McGann, E. Snesrud, R. Maybank et al., "*Escherichia coli* harboring *mcr-1* and *bla*_{CTX-M} on a novel IncF plasmid: first report of *mcr-1* in the USA," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 7, pp. 4420–4421, 2016.
- [193] O. J. Bernasconi, E. Kuenzli, J. Pires et al., "Travelers can import colistin-resistant Enterobacteriaceae, including those possessing the plasmid-mediated *mcr-1* gene," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 8, pp. 5080–5084, 2016.
- [194] M. R. Fernandes, J. A. McCulloch, M. A. Vianello et al., "First report of the globally disseminated IncX4 plasmid carrying the *mcr-1* gene in a colistin-resistant *Escherichia coli* sequence type 101 isolate from a human infection in Brazil," *Antimicrobial Agents and Chemotherapy*, vol. 60, no. 10, pp. 6415–6417, 2016.
- [195] M. Corbella, B. Mariani, C. Ferrari et al., "Three cases of *mcr-1*-positive colistin-resistant *Escherichia coli* bloodstream infections in Italy, August 2016 to January 2017," *Eurosurveillance*, vol. 22, no. 16, p. 30517, 2017.
- [196] S. Guenther, L. Falgenhauer, T. Semmler et al., "Environmental emission of multiresistant *Escherichia coli* carrying the colistin resistance gene *mcr-1* from German swine farms," *Journal of Antimicrobial Chemotherapy*, vol. 72, pp. 1289–1292, 2017.
- [197] M. Fritzenwanker, C. Imirzalioglu, K. Gentil, L. Falgenhauer, F. M. Wagenlehner, and T. Chakraborty, "Incidental detection of a urinary *Escherichia coli* isolate harbouring *mcr-1* of a patient with no history of colistin treatment," *Clinical Microbiology and Infection*, vol. 22, no. 11, pp. 954–955, 2016.
- [198] J. A. Ellem, A. N. Ginn, S. C. A. Chen, J. Ferguson, S. R. Partridge, and J. R. Iredell, "Locally acquired *mcr-1* in *Escherichia coli*, Australia, 2011 and 2013," *Emerging Infectious Diseases*, vol. 23, no. 7, pp. 1160–1163, 2017.
- [199] Y. Qian, J. B. Bulitta, C. A. Peloquin, and P. N. Holden, *Crossm Polymyxin Combinations Combat Era*, 2017.
- [200] S. B. Jørgensen, A. Søråas, L. S. Arnesen, T. Leegaard, A. Sundsfjord, and P. A. Jenum, "First environmental sample containing plasmid-mediated colistin-resistant ESBL-producing *Escherichia coli* detected in Norway," *APMIS*, vol. 125, no. 9, pp. 10–12, 2017.
- [201] T. He, R. Wei, L. Zhang et al., "Characterization of NDM-5-positive extensively resistant *Escherichia coli* isolates from dairy cows," *Veterinary Microbiology*, vol. 207, pp. 153–158, 2017.
- [202] K. Rutherford, J. Parkhill, J. Crook et al., "Artemis: sequence visualization and annotation," vol. 16, no. 10, pp. 944–945, 2000.
- [203] A. O. Olaitan, S. Chabou, L. Okdah, S. Morand, and J. M. Rolain, "Dissemination of the *mcr-1* colistin resistance gene," *Lancet Infectious Diseases*, vol. 16, no. 2, p. 147, 2016.
- [204] Y. Zhang, K. Liao, H. Gao et al., "Decreased fitness and virulence in ST10 *Escherichia coli* harboring *bla*_{NDM-5} and *mcr-1* against a ST4981 strain with *bla*_{NDM-5}," *Frontiers in Cellular and Infection Microbiology*, vol. 7, p. 242, 2017.
- [205] A. C. Schürch and W. van Schaik, "Challenges and opportunities for whole-genome sequencing-based surveillance of antibiotic resistance," *Annals of the New York Academy of Sciences*, vol. 1388, no. 1, pp. 108–120, 2017.
- [206] S. K. Gupta, B. R. Padmanabhan, S. M. Diene et al., "ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes," *Antimicrobial Agents and Chemotherapy*, vol. 58, no. 1, pp. 212–220, 2014.
- [207] E. Zankari, H. Hasman, S. Cosentino et al., "Identification of acquired antimicrobial resistance genes," *Journal of Antimicrobial Chemotherapy*, vol. 67, no. 11, pp. 2640–2644, 2012.
- [208] A. G. McArthur, N. Waglechner, F. Nizam et al., "The comprehensive antibiotic resistance database," *Antimicrobial Agents and Chemotherapy*, vol. 57, no. 7, pp. 3348–3357, 2013.

- [209] B. Liu and M. Pop, "ARDB-antibiotic resistance genes database," *Nucleic Acids Research*, vol. 37, pp. 443–447, 2009.
- [210] S. Suzuki, M. Ohnishi, M. Kawanishi, M. Akiba, and M. Kuroda, "Investigation of a plasmid genome database for colistin-resistance gene *mcr-1*," *Lancet Infectious Diseases*, vol. 16, no. 3, pp. 284–285, 2016.
- [211] R. L. Lindsey, D. Batra, L. Rowe et al., "High-quality genome sequence of an *Escherichia coli* o157 strain carrying an *mcr-1* resistance gene isolated from a patient in the United States," *Genome Announcements*, vol. 5, no. 11, p. e01725-16, 2017.
- [212] E. Zankari, R. Allesøe, K. G. Joensen, L. M. Cavaco, O. Lund, and F. M. Aarestrup, "PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens," *Journal of Antimicrobial Chemotherapy*, vol. 72, no. 10, pp. 2764–2768, 2017.
- [213] M. J. Ellington, O. Ekelund, F. M. Aarestrup et al., "The role of whole genome sequencing in antimicrobial susceptibility testing of bacteria: report from the EUCAST Subcommittee," *Clinical Microbiology and Infection*, vol. 23, no. 1, pp. 2–22, 2017.