Diagnostic Microbiology and Infectious Disease 98 (2020) 115121

Contents lists available at ScienceDirect

Diagnostic Microbiology and Infectious Disease

journal homepage: www.elsevier.com/locate/diagmicrobio

Implementation and evaluation of methods for the optimal detection of carbapenem-resistant and colistin-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from stools

Claudine Fournier [a], Laurent Poirel [a,b,c,*], Patrice Nordmann [a,b,c,d]

[a] Medical and Molecular Microbiology, Faculty of Science and Medicine, University of Fribourg, Fribourg, Switzerland
[b] INSERM European Unit (IAME, France), University of Fribourg, Fribourg, Switzerland
[c] Swiss National Reference Center for Emerging Antibiotic Resistance (NARA), University of Fribourg, Fribourg, Switzerland
[d] Institute for Microbiology, University of Lausanne and University Hospital Centre, Lausanne, Switzerland

ARTICLE INFO

Article history:
Received 18 April 2020
Received in revised form 16 June 2020
Accepted 20 June 2020
Available online 26 June 2020

Keywords:
Colistin
Carbapenem
Optimal
Enrichment
Stools

ABSTRACT

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) and *Pseudomonas aeruginosa* (CRPA), as well as polymyxin-resistant *A. baumannii* (PMR-AB) and *P. aeruginosa* (PMR-PA), were used to test different enrichment strategies from spiked stools. Three procedures were compared, namely, direct inoculation on selective plates and plating after a 24-h enrichment step in tryptic soy broth with or without antibiotics. Selective agar plates were used including CHROMagar-*Pseudomonas* supplemented with meropenem (2 mg/L), and CHROMagar-MDR-Acinetobacter agar and CHROMagar COL-APSE plates. Use of enrichment broths significantly enhanced the recovery of CRAB, CRPA, PMR-AB, and PMR-PA. However, supplementing or not the pre-enrichment broth with antibiotics had no impact. The proposed strategy for screening multidrug-resistant nonfermenters is of low cost, is easy to implement, and might be useful for outbreak containment.

1. Introduction

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) and multidrug-resistant *Pseudomonas aeruginosa* such as carbapenem-resistant *P. aeruginosa* (CRPA) have been recently classified, respectively, as urgent and serious threats by the CDC (2019) and of priority pathogens by the WHO (2017a). Those multidrug-resistant (MDR) bacteria constitute the main sources of carbapenem-resistant Gram-negative bacilli worldwide (Gniadek et al., 2016; Nordmann and Poirel, 2019), being sources of hospital-acquired infections and causing a significant mortality and morbidity in high risk-patient populations.

Although carbapenem resistance in Enterobacterales is often related to the production of acquired carbapenemases, it is most of the time caused by permeability defects in *P. aeruginosa*, accounting for 15–20% of all *P. aeruginosa* clinical strains (Rossolini and Mantengoli, 2005; Eichenberger and Thaden, 2019).

On the other hand, resistance to carbapenems (mainly associated to class D β-lactamases) in *A. baumannii* accounts for ca. 60% of the isolates recovered from hospitalized patients worldwide (WHO, 2017b).

Owing to this high rate of carbapenem resistance in nonfermenting Gram-negatives and clinically relevant bacterial species, polymyxins are now increasingly used as last-resort antibiotics for treating corresponding infections. However, increasing occurrence of polymyxin-resistant *A. baumannii* (PMR-AB) and *P. aeruginosa* (PMR-PA) is now being reported (Potron et al., 2015; Poirel et al., 2017). To our knowledge, no guidelines have been published to optimally detect those PMR-AB and PMR-PA. However, implementation of screening strategies is required for instance in the context of outbreaks (Martischang et al., 2019).

When dealing with Enterobacterales, screening strategies include broth enrichment (CDC, 2009) followed by direct culture using corresponding selective media (e.g., CarbaSmart for carbapenem resistance). Molecular assays can also be useful for the direct detection of carbapenemase genes from stools and rectal swabs (Simner et al., 2016). Recently, we demonstrated that optimal detection of polymyxin- or carbapenem-resistant Enterobacterales may be achieved by using respective pre-enrichment steps that can be even optimized by supplementing the enrichment broth with the corresponding selective antibiotic (Sadek et al., 2020).

The aims of this study were i) to determine the optimal selective medium for the detection of CRAB, CRPA, PMR-AB, and PMR-PA; ii) to implement a selective medium for carbapenem-resistant *P. aeruginosa*; and iii) to determine whether the detection of MDR nonfermenters directly from stools could be improved by an enrichment step prior to inoculation on selective plates.

* Corresponding author. Tel.: +41-26-300-9582.
E-mail address: laurent.poirel@unifr.ch (L. Poirel).

https://doi.org/10.1016/j.diagmicrobio.2020.115121

Our evaluation of the different detection strategies focused on 4 clinically relevant MDR Gram-negatives bacilli, i.e., CRPA, CRAB, PMR-AB, and PMR-PA.

2. Material and methods

2.1. Bacterial strains

Well-characterized collections of *P. aeruginosa* and *A. baumannii* clinical strains from worldwide origin and from the Swiss National Reference Center for Emerging Antibiotic Resistance were included in the study. Resistance mechanisms had been previously characterized for each strain at the molecular levels. This collection consisted in i) carbapenemase-producing *P. aeruginosa* including producers of VIM-1 ($n = 1$), VIM-2 ($n = 6$), VIM-4 ($n = 4$), VIM-53 ($n = 3$), NDM-1 ($n = 4$), IMP-1 ($n = 1$), IMP-4 ($n = 1$), and IMP-7 ($n = 1$); ii) carbapenem-resistant but carbapenemase-negative *P. aeruginosa* including OprD porin-deficient strains ($n = 4$); iii) OprD-deficient strains overproducing their intrinsic AmpC ß-lactamase ($n = 5$); iv) strain overproducing its intrinsic AmpC ($n = 1$); v) carbapenemase-producing *A. baumannii* including producers of OXA-23 ($n = 6$), OXA-40 ($n = 2$), OXA-58 ($n = 2$), and NDM-1 ($n = 1$); vi) colistin-resistant *P. aeruginosa* with chromosomally encoded resistance mechanisms ($n = 9$); and vii) colistin-resistant *A. baumannii* with chromosomally encoded resistance mechanisms ($n = 10$).

2.2. Susceptibility testing

Firstly, antimicrobial resistance profiles for each isolate were determined by disk diffusion method on Mueller–Hinton (MH) agar plates (Bio-Rad, Cressier, Switzerland). Detection of AmpC was assessed by comparison of the susceptibility of β-lactams on MH agar plates and on MH agar plates supplemented with 2 mg/L of cloxacillin (Acros Organics, Geel, Belgium). Amoxicillin, amoxicillin/clavulanic acid, ticarcillin/clavulanic acid, piperacillin, piperacillin/tazobactam, temocillin, ceftazidime, cefotaxime, cefoxitin, aztreonam, imipenem, ertapenem, meropenem, gentamicin, amikacin, ciprofloxacin, tigecycline, trimethoprim-sulfamethoxazole, and fosfomycin (Bio-Rad) were tested by disk diffusion. MIC values for imipenem, ertapenem, and meropenem were then determined by Etest (bioMérieux) on MH agar plates overnight at the optimal temperature of 37 °C for *A. baumannii* and 30 °C for *P. aeruginosa*. Diameters of inhibition were measured by a caliper. Broth microdilution was performed for the determination of colistin (Mast Group, Amiens, France) MICs in cation-adjusted MH (Bio-Rad). MIC values were taken as the lowest concentration without visible bacterial growth. *Escherichia coli* ATCC25922 was added in the susceptibility testing of colistin as a control strain. The values of MIC and zone diameters were interpreted as susceptible, intermediate, or resistant according to EUCAST/CLSI joint guidelines (www.eucast.org; CLSI, 2018).

2.3. Molecular analysis

Polymerase chain reaction (PCR) amplification of carbapenemase genes was performed for all *P. aeruginosa* and *A. baumannii* isolates using specific primers as reported (Woodford et al., 2006; Poirel et al., 2011). PCR amplification of the *mcr* genes was undergone for all colistin-resistant strains (Lescat et al., 2018; Wang et al., 2018; Yang et al., 2018; Kieffer et al., 2019). Sequencing of each PCR amplicon was outsourced (Microsynth, Balgach, Switzerland) to confirm the exact nature of each produced carbapenemase.

2.4. Preliminary studies

Preliminary studies were performed to implement the optimal screening method for the best yield of each species and their antibiotic resistance phenotype. A series of 10-fold dilutions from an original standard inoculum of 0.5 McFarland in 0.85% NaCl (~1.5×10^8 CFU/mL) was performed for 4 wild-type and 4 resistant strains belonging to each species tested in our study. These dilutions were separately inoculated on respective specific media and on LB agar plate as counting control, in biological triplicate and technical duplicate. Following an overnight incubation at the optimum temperature, colonies were counted and results from the specific media and the control LB agar media were compared. Selective media with the highest yield over all the collection of strains were considered for the spiking experiment.

The following selective media were compared: Drigalski supplemented with meropenem, CHROMagar-*Acinetobacter*-MDR (Wareham and Gordon, 2011), and modified CHROMagar-*Pseudomonas*. For CRPA isolates, no specific selective medium was commercially available. Therefore, the modified CHROMagar-*Pseudomonas* supplemented with meropenem was implemented. Different concentrations of meropenem were tested in this medium, namely, 1, 2, and 4 μg/mL (Sigma-Aldrich, Buchs, Switzerland), as described above. In addition, different concentrations of meropenem (0.1, 0.5, 1, and 2 μg/mL) supplemented in enrichment broth medium were tested, considering their concentrations being below that for the resistance breakpoint value.

For colistin-resistant nonfermenters, the following selective media were compared, namely, SuperPolymyxin (ELITech, Paris, France) (Nordmann et al., 2016; Jayol et al., 2018), CHROMID colistin R agar (bioMérieux), and CHROMagar COL-APSE (CHROMagar, Paris, France) (Abdul Momin et al., 2017), as described above. In parallel, the pre-enrichment step was evaluated with four different colistin concentrations, namely, 0.1, 0.5, 1, and 2 μg/mL, and were compared as described above.

2.5. Spiking experiments

A bacterial suspension of the tested isolates (cf. table strains) was made with an optical density at 0.5 McFarland standard in 0.85% NaCl. The primary spiked aliquot was made from 900 μL of fecal solution spiked with 100 μL of bacterial suspension. The fecal solution was obtained by suspending 5 g of fresh feces from healthy volunteers ($n = 3$) in 50 mL of distilled and sterile water. Following this primary step, serial 10-fold dilution was made from the primary aliquot in fecal suspension to obtain dilution from 10^7 to 10^1 CFU/mL. Fecal solution without the addition of bacteria was used as a control. The spiked mixture was then processed in 3 different procedures: i) to quantify the viable bacteria, 100 μL of spiked stools was plated onto screening media and incubated overnight at optimal temperature. Count of colonies were made the following day; ii) 100 μL of spiked stools was inoculated in 5 mL of tryptic soy broth (TSB) (Sigma-Aldrich, Buchs, Switzerland). Dilutions of the enrichment were streaked on selective plates. Colonies were counted after overnight incubation; iii) 100 μL of spiked stools was inoculated in 5 mL of TSB supplemented with 0.1 μg/mL colistin or meropenem 1 μg/mL. Dilution was made of the cultured TSB prior to inoculation on selective plates. Following an overnight incubation, colonies number was recorded.

Stool suspensions of PMR-AB and PMR-PA were directly plated on CHROMagar COL-APSE or plated after a 24-h growth either into i) 5 mL TSB or ii) 5 mL TSB supplemented with colistin (0.1 μg/mL).

For CRPA, the same procedure was used but plating i) directly onto CHROMagar-*Pseudomonas* supplemented with 2 μg/mL of meropenem, ii) after a 24-h growth into 5 mL of nonselective TSB, or iii) after a 24-h growth into 5 mL of TSB supplemented with 1 μg/mL of meropenem.

For CRAB, the procedure was identical except that the CHROMagar-*Acinetobacter*-MDR selective plates were used for screening.

All experiments were performed at least in triplicate. In addition, each dilution was inoculated on LB agar to assess the bacterial count. All results were recorded, averages of the replicate were calculated, and corresponding data were statistically analyzed by the software SPSS using Wilcoxon signed rank test. Standard deviation did not exceed 20% of the median values.

3. Results

For carbapenemase-producing *A. baumannii*, the CHROMagar-*Acinetobacter*-MDR medium showed the best performance in term of bacterial yield recovery and was therefore used.

A modified CHROMagar-*Pseudomonas* was implemented with different concentrations of meropenem to screen specifically for CR-PA. The maximal number of colonies compared to other media (e.g., Drigalski supplemented with meropenem) was observed at a concentration of 2 μg/mL of meropenem after overnight incubation. This medium showed a 100% specificity and sensitivity of with the 8 controls strains tested.

The comparison of different media for screening colistin-resistant bacteria (SuperPolymyxin, CHROMagar COL-APSE, and CHROMID colistin) showed that the CHROMagar COL-APSE medium was the most suitable selective one, with a maximal yield obtained after 18 h of incubation.

3.1. Spiking experiments

Detection of CRAB was found to be significantly improved by using a pre-enrichment step prior to plating compared to direct plating onto CHROMagar-Acinetobacter-MDR ($P < 0.05$) (Table 2). On the other hand, the use of selective broth enrichments step containing meropenem (1 or 2 μg/mL) did not significantly improve the detection of CRAB compared with nonselective broth (Table 2).

A significant benefit was observed in terms of detection of CRPA when using an enrichment step prior to inoculation of CHROMagar-*Pseudomonas* supplemented with meropenem (2 μg/mL) (Table 1) The detection rate was found to be significantly identical between selective and nonselective enrichment broth (Table 1).

An overnight pre-enrichment culture allowed to recover a higher yield of colonies on CHROMagar COL-APSE compared with direct plating from stools ($P < 0.05$) (Tables 3 and 4). However, the use of selective enrichment with colistin did not improve the detection of colistin-resistant strains ($P < 0.05$), regardless the concentration of polymyxin used.

Table 2
Evaluation of direct plating on CHROMagar *Acinetobacter* MDR agar of spiked stools, with and without an 18-h enrichment step in TSB and in TSB + meropenem (MER) at 1 μg/mL, for the detection of CRAB.

Strain	Carbapenemase determinant	MIC of meropenem (μg/mL)	*CHROMagar Acinetobacter MDR Agar* (CFU/mL)	Enrichment broth (CFU/mL)	
				TSB	TSB + MER
R2270	WT	<0.125	0	0	0
R112	WT	<0.125	0	0	0
R1386	WT	<0.125	0	0	0
R1387	WT	<0.125	0	0	0
R4	OXA-23	>128	1×10^1	5.01×10^8	1.51×10^9
R6	OXA-23	64	2.63×10^1	8.92×10^{10}	4.24×10^{11}
R163	OXA-23	>128	3.67×10^0	1.51×10^{10}	2.51×10^{10}
R3395	OXA-23	>128	7.33×10^1	1.11×10^9	3.45×10^9
R3557	OXA-23	>128	8×10^0	1.7×10^9	1.56×10^{10}
R623	OXA-23	>128	7×10^0	1.5×10^9	5.11×10^{10}
R8	OXA-58	128	7.8×10^1	4×10^9	3.18×10^{10}
R842	OXA-58	16	3.52×10^1	6.92×10^8	9.28×10^9
R14	OXA-40	>128	1.8×10^1	4.15×10^8	1.56×10^9
R2536	OXA-40	>128	3.37×10^1	1.51×10^{10}	2.51×10^{10}
R655	NDM-1	128	2.97×10^1	3.5×10^9	6.46×10^9

Bacterial counts are average. Standard deviations did not exceed 20% of the mean.

Table 1
Evaluation of direct plating of carbapenem-resistant *P. aeruginosa* isolates on CHROMagar *Pseudomonas* + 2 mg/L of meropenem agar of spiked stools, with and without an 18-h enrichment step in TSB and TSB + meropenem (MER) at 1 μg/mL.

Strain	Carbapenem resistance determinant[a]	MIC of MER (μg/mL)	CHROMagar *Pseudomonas* + MER Agar (CFU/mL)	Enrichment broth (CFU/mL)	
				TSB	TSB + MER
N382	WT	<0.125	0	0	0
N414	**VIM-2**	4	1.4×10^1	2.28×10^{11}	1.13×10^{13}
N926	**VIM-2**	128	2.63×10^1	3×10^6	2.75×10^9
N1073	**VIM-53**	>128	5.2×10^1	3.3×10^{10}	2.49×10^{10}
N1053	**VIM-53**	>128	5.4×10^1	3.7×10^{10}	5.5×10^{10}
N922	**VIM-2**	>128	6.8×10^2	4.2×10^{10}	4.1×10^{10}
N792	**VIM-1**	>128	1.76×10^2	3.13×10^{10}	2.94×10^{10}
N650	**VIM-53**	>128	4.64×10^2	2.15×10^{11}	5.76×10^{11}
N16	**VIM-4**	>128	1.38×10^3	3.7×10^{10}	1.28×10^{11}
N17	**VIM-4**	64	7.3×10^1	4.86×10^{10}	2.17×10^{10}
N18	**VIM-4**	128	4.32×10^2	2.8×10^{10}	2.9×10^{10}
N19	**VIM-4**	64	1.92×10^2	2.56×10^{10}	1.98×10^{10}
N419	**VIM-2**	8	2.8×10^1	2.4×10^{10}	8.96×10^{10}
N310	**VIM-2**	>128	4×10^2	3.58×10^{10}	8.96×10^{10}
N342	**VIM-2**	>128	1.4×10^2	1.36×10^{11}	1.2×10^{11}
N1090	**NDM-1**	>128	2.3×10^1	1.81×10^{11}	8.72×10^{11}
N682	**NDM-1**	>128	5.56×10^2	1.54×10^8	5.03×10^9
N899	**NDM-1**	64	1.92×10^2	3.45×10^{10}	4.6×10^{10}
N885	**NDM-1**	>128	8.4×10^1	7.15×10^{10}	6.4×10^{10}
R66	**IMP-1**	>128	3×10^0	1.5×10^{11}	$5.15x \times 10^{10}$
N687	**IMP-4**	>128	5.3×10^1	1.37×10^9	1.75×10^{10}
N1029	**IMP-7**	>128	1.61×10^2	8.19×10^{10}	7.17×10^{10}
N705	AmpC overexpression	128	1.6×10^1	1.96×10^9	1.46×10^{10}
R128	OprD deficient + AmpC overexpression	128	1.67×10^1	2.35×10^{11}	7.32×10^9
N928	OprD deficient + AmpC overexpression	>128	1.52×10^2	1.84×10^8	2.72×10^8
N104	OprD deficient + AmpC overexpression	128	1.1×10^1	2.91×10^9	1.53×10^{12}
N323	OprD deficient + AmpC overexpression	>128	7×10^0	7.5×10^8	3.84×10^9
N538	OprD deficient + AmpC overexpression	>128	4.96×10^2	1.37×10^{10}	1.6×10^{10}
N693	OprD deficient	4	2×10^0	8.7×10^{11}	3.25×10^{12}
N34	OprD deficient	18	6.1×10^1	8.93×10^{10}	1.83×10^{12}
N968	OprD deficient + AmpC overexpression	32	2.7×10^1	6.47×10^{11}	2.44×10^{11}
N151	OprD deficient	4	7.3×10^1	1.4×10^{11}	2.08×10^{11}

Bacterial counts are average. Standard deviations did not exceed 20% of the mean.
[a] Carbapenemase enzymes are in bold.

Table 3
Evaluation of direct plating on CHROMagar COL-APSE agar plates of spiked stools, with and without an 18-h enrichment step in TSB, and selective enrichment in TSB + colistin (CST) at 0.1 μg/mL for the detection of PMR-PA.

Strain	Resistance mechanism	MIC of colistin (μg/mL)	CHROMagar COL-APSE Agar (CFU/mL)	Enrichment broth (CFU/mL)	
				TSB	TSB + CST
N4	WT	<0.125	0	0	0
N14	WT	<0.125	0	0	0
R128	WT	0.5	0	0	0
R2565	WT	0.25	0	0	0
N69	Chromosomal	8	2.76×10^2	9.2×10^8	2.7×10^8
N76	Chromosomal	4	2.71×10^3	3.97×10^{10}	2.95×10^{10}
N142	Chromosomal	4	7.6×10^1	$5.63 \times ^{10}$	1.02×10^{10}
R3419	Chromosomal	32	1.72×10^2	1.37×10^{10}	6.03×10^9
R3421	Chromosomal	16	1.1×10^2	5.2×10^9	1.8×10^9
R3422	Chromosomal	8	8.1×10^2	2×10^9	5.41×10^9
R3425	Chromosomal	8	2.53×10^3	3.52×10^9	1.24×10^{10}
R3418	Chromosomal	4	8.96×10^2	1×10^9	1.41×10^{10}
R142	Chromosomal	8	5.2×10^1	1.96×10^{10}	1.82×10^{10}

Bacterial counts are average. Standard deviations did not exceed 20% of the mean.

4. Discussion

By evaluating the performances of detection of CRPA, PMR-PA, CRAB, and PMR-AB, remarkable differences were observed between direct plating and precultures in nonselective/selective broth enrichment. These data correspond to the first evaluation of optimal selection of CRAB, CRPA, PMR-AB, and PMR-PA. Simner et al. (2016) previously observed a lack of significant difference between direct plating and pre-enrichment with carbapenem-resistant organisms, but this study was performed on a small panel of strains. Indeed, only KPC producers were tested, and only MacConkey with or without ertapenem disks and CHROMID CARBA selective plates were evaluated.

The use of enrichment broth culture with meropenem did not significantly improve the detection of CRAB compared to nonselective enrichment step. To our knowledge, this study is the first comparison of methods for the specific detection of CRAB. Also, selective enrichment did not significantly improve the detection of CRPA, PMR-AB, and PMR-PA compared to nonselective enrichment. The detection limit was between 10 and 100 CFU/mL in stools, corresponding to the usual fecal carriage load of MDR bacteria (Louie et al., 2015; Islam et al., 2019). To our knowledge, no study had been previously performed to compare screening protocols for the detection of PMR-AB and PMR-PA, although there is a real need for detection tools with regard to carbapenem-resistant nonfermenters, to eventually detect all carbapenem-resistant organisms and limit their spread, as underscored by Gniadek et al. (2016).

Table 4
Evaluation of direct plating on CHROMagar COL-APSE agar plates of spiked stools, with and without an 18-h enrichment step in TSB, and selective enrichment in TSB + colistin (CST) at 0.1 μg/mL for the detection of PMR-AB.

Strain	Resistance mechanism	MIC of colistin (μg/mL)	CHROMagar COL-APSE Agar (CFU/mL)	Enrichment broth (CFU/mL)	
				TSB	TSB + CST
R3390	WT	0.5	0	0	0
N298	WT	0.25	0	0	0
N313	WT	<0.125	0	0	0
N370	WT	<0.125	0	0	0
R3393	Chromosomal	8	1.3×10^1	7.88×10^9	1.28×10^9
R3395	Chromosomal	64	3.13×10^2	1.09×10^{10}	2.16×10^9
R3396	Chromosomal	4	3.95×10^1	5.95×10^8	3.15×10^{10}
R3397	Chromosomal	16	1.5×10^1	3.08×10^9	4.9×10^8
R3398	Chromosomal	128	1.57×10^2	2.08×10^{10}	1.91×10^{10}
R3400	Chromosomal	32	1.5×10^0	1.04×10^{10}	9.2×10^{10}
R3401	Chromosomal	32	1.35×10^1	2.67×10^{10}	3.15×10^{10}
R3402	Chromosomal	32	1.3×10^1	1.14×10^{10}	4.9×10^8
R3403	Chromosomal	>128	5×10^0	1.63×10^{10}	1.91×10^{10}
R3804	Chromosomal	16	3×10^1	5.41×10^{10}	9.2×10^{10}

Bacterial counts are average. Standard deviations did not exceed 20% of the mean.

Currently, no selective medium for the screening of CRPA only was available. A screening medium was implemented here for the detection of CRPA possessing variable resistance mechanisms, such as efflux pump overproduction, porin deficiency, and/or AmpC overexpression, but also carbapenemase-producing *P. aeruginosa.* This strategy was found optimal here with a sensitivity of 93% when testing clinical isolates (data not shown).

Hence, we present here clear evidence of the benefit of enrichment steps in terms of sensitivity of detection of colistin- and carbapenem-resistant nonfermenters. In the context of an outbreak, we may propose the following strategy, which has to be to performed in parallel: i) direct plating of the stools on the selective medium and ii) inoculating an enrichment broth to be further plated on the selective medium, eventually improving the sensitivity of detection of those MDR nonfermenters, if no growth could be observed with direct plating. That strategy might be efficient for saving time, and to detect all carbapenem-resistant isolates, regardless of the corresponding resistance mechanism (carbapenemase or not).

As stated by the French guidelines (Rémic, 2018), screening method to detect *A. baumannii* and *P. aeruginosa* require 2 sampling sites, namely, the gut (rectal swab or stools) and the respiratory tract (bronchoalveolar aspirate or throat swab). We therefore propose that our proposed screening strategy could be implemented for those 2 sample types either in the context of outbreaks but also for performing prospective surveys.

Acknowledgments

This work was funded by the Swiss National Science Foundation (projects numbers FNS-407240_177381 and FNS-407240_177382).

Authors' statement

CF, LP, and PN designed the study. CF performed the experiments. CF drafted the manuscript. LP and PN wrote the final version of the manuscript.

References

Abdul Momin MHF, Bean DC, Hendriksen RS, Haenni M, Phee LM, Wareham DW. CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens. J Med Microbiol 2017;66:1554–61.

Center for Diseases Control and Prevention. Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, *Klebsiella* spp. and *E. coli* from rectal swabs. U.S. Department of Health and Human Services, CDC; 2009.

Centers for Disease Control and Prevention. Antibiotic resistance threats in the United States, 2019. U.S. Department of Health and Human Services, CDC; 2019.

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing; 28th informational supplement (M100-S28). CLSI; 2018.

Eichenberger EM, Thaden JT. Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. Antibiotics 2019;8:37.

Gniadek TJ, Carroll KC, Simner PJ. Carbapenem-resistant non–glucose-fermenting gram-negative bacilli: the missing piece of the puzzle. J Clin Microb 2016;54:1700–10.

Islam MA, Amin MB, Roy S, Asaduzzaman M, Islam MR, Navab-Daneshmand T, et al. Fecal colonization with multidrug-resistant *E coli* among healthy infants in rural Bangladesh. Front Microbiol 2019;10:640.

Jayol A, Poirel L, André C, Dubois V, Nordmann P. Detection of colistin-resistant Gram-negative rods by using the SuperPolymyxin medium. Diagn Microbiol Infect Dis 2018;92:95–101.

Kieffer N, Royer G, Decousser JW, Bourrel AS, Palmieri M. Ortiz de la Rosa JM, et al. *mcr-9*, an inducible gene encoding an acquired phosphoethanolamine transferase in *E. coli*, and its origin. Antimicrob Agents Chemother 2019;63:e00965. 19.

Lescat M, Poirel L, Nordmann P. Rapid multiplex polymerase chain reaction for the detection of *mcr-1* to *mcr-5* genes. Diagn Microbiol Infect Dis 2018;92:267–9.

Louie TJ, Byrne B, Emery J, Ward L, Krulicki W, Nguyen D, et al. Differences of fecal microflora with *Clostridium difficile* therapies. Clin Infect Dis 2015;60:S91–7.

Martischang R, Buetti N, Balmelli C, Saam M, Widmer A, Harbarth S. Nation-wide survey of screening practices to detect carriers of multi-drug resistant organisms upon admission to Swiss healthcare institutions. Antimicrob Resist Infect Control 2019;8:37.

Nordmann P, Poirel L. Epidemiology and diagnostics of carbapenem resistance in gram-negative bacteria. Clin Infect Dis 2019;69:S521–8.

Nordmann P, Jayol A, Poirel L. A universal culture medium for screening polymyxin-resistant Gram-negative isolates. J Clin Microbiol 2016;54:1395–9.
Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 2011;70:119–23.
Poirel L, Jayol A, Nordmann P. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 2017;30:557–96.
Potron A, Poirel L, Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: mechanisms and epidemiology. Int J Antimicrob Agents 2015;45:568–85.
Rémic. Référentiel en microbiologie médicale, Société Française de Microbiologie, 6e éd; 2018.
Rossolini GM, Mantengoli E. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. Clin Microb Infect 2005;11:17–32.
Sadek M, Poirel L, Nordmann P. Optimal detection of extended-spectrum β-lactamase producers, carbapenemase producers, polymyxin-resistant Enterobacterales, and vancomycin-resistant enterococci from stools. Diagn Microb Infect Dis 2020;96:114919.
Simner PJ, Martin I, Opene B, Tamma PD, Caroll KC, Milstone AM. Evaluation of multiple methods for detection of gastrointestinal colonization of carbapenem-resistant organisms from rectal swabs. J Clin Microbiol 2016;54:1664–7.
Wang X, Wang Y, Zhou Y, Li J, Yin W, Wang S, et al. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. Emerg Microbes Infect 2018;7:122.
Wareham DW, Gordon NC. Modifications to CHROMagar Acinetobacter for improved selective growth of multi-drug resistant *Acinetobacter baumannii*. J Clin Pathol 2011;64:164–7.
Woodford N, Ellington MJ, Coelho JM, Turton JF, Ward ME, Brown S, et al. Multiplex PCR for genes encoding prevalent OXA carbapenemases in *Acinetobacter spp*. Int J Antimicrob Agents 2006;27:351–3.
World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research development of new antibiotics. WHO; 2017a.
World Health Organization. Guidelines for the prevention and control of carbapenem-resistant *Enterobacteriacae*, *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in health care facilities. WHO; 2017b.
Yang YQ, Li YX, Lei CW, Zhang AY, Wang HN. Novel plasmid-mediated colistin resistance gene *mcr-7.1* in *Klebsiella pneumoniae*. J Antimicrob Chemother 2018;73:1791–5.