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MultiRapid ATB NP test for detecting concomitant susceptibility and resistance of last-resort novel antibiotics available to treat multidrug-resistant Enterobacterales infections

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ABSTRACT

Background: Recently developed therapeutics against Gram-negative bacteria include the β-lactam-β-lactamase inhibitor combinations ceftazidime–avibactam (CZA), meropenem–vaborbactam (MEV), and imipenem–relebatam (IPR), and the siderophore cephalosporin cefiderocol (FDC). The aim of this study was to develop a test for rapid identification of susceptibility/resistance to CZA, MEV, IPR, and FDC for Enterobacterales in a single test for rapid clinical decision making.

Methods: The MultiRapid ATB NP test is based on the detection of glucose metabolism occurring after bacterial growth in the presence of defined concentrations of CZA, MEV, IPR, and FDC, followed by visual detection of colour change of the pH indicator red phenol (red to yellow) generated by the acidification of the medium upon bacterial growth. This test is performed in 96-well microplates. The MultiRapid ATB NP test was evaluated using 78 Enterobacterales isolates and compared to the reference method broth microdilution.

Results: The MultiRapid ATB NP test displayed 97.0% (confidence interval [CI] 92.6–98.8) sensitivity, 97.7% (CI 94.3–99.1) specificity, and 97.4% (CI 95.0–98.7) accuracy. The results were obtained after 3 h of incubation at 35 °C ± 2 °C, representing at least a 15-h gain-of-time compared with currently used antimicrobial susceptibility testing methods.

Conclusion: The MultiRapid ATB NP test provided accurate results for the concomitant detection of susceptibility/resistance to CZA, MEV, IPR, and FDC in Enterobacterales, independent of the resistance mechanism. This test may be suitable for implementation in any microbiology routine laboratory.

1. Background

Carbapenem-resistant Enterobacterales (CRE) infections are a major concern for global public health. In response to the urgent need to develop new antibiotics to treat these infections, the pharmaceutical industry has recently introduced novel antibiotics as potentially interesting therapeutic options [1,2].

Among them, the novel compounds ceftazidime–avibactam (CZA), meropenem–vaborbactam (MEV), and imipenem–relebatam (IPR), all of which are β-lactam-β-lactamase inhibitors, and cefiderocol (FDC), a broad-spectrum siderophore cephalosporin, have been approved by the U.S. Food and Drug Administration (FDA) and the European Medicines Agency (EMA). These drugs are considered last-resort antibiotics for treating infections caused by CRE [3–10].

CZA is approved for the treatment of complicated urinary tract infections (cUTIs), and hospital-associated pneumonia, and has mostly been used for the treatment of infections due to Enterobacterales producing class A carbapenemases of the KPC type [3,4]. CZA also has activity against producers of AmpC-type β-lactamases, producers of extended-spectrum β-lactamases (ESBLs), and producers of class D ß-lactamases of the OXA-48-type, but not against producers of metallo-β-lactamases (MβLs), such as NDM, IMP, and VIM enzymes [11]. MEV is being used to treat cUTIs, abdominal infections, bacteraemia, and hospital-associated pneumo-

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nia [5,6], this combination being very active against producers of KPC and cephalosporinases of the CMY type [12,13].

IPR is approved for the treatment of cUTIs (including pyelonephritis), complicated abdominal infections, and healthcare-associated pneumonia [7,8]. This drug has proven efficacy against producers of class A and C ß-lactamases [14–16], limited activity against OXA-48-producing CRE, and no activity against MβL producers [15,17]. Finally, FDC is approved for the treatment of cUTI (including pyelonephritis) and nosocomial pneumonia [9,10], and is active against most multidrug-resistant Gram-negative bacteria, including most MβL producers [9,10].

However, resistance to those novel antibiotics has been extensively reported. Mutations in β-lactamase sequences (KPC, CTX-M-14, CTX-M-15, and VEB) [18–21], overexpression of efflux pumps, mutations in penicillin-binding proteins (PBPs) [22,23], and overproduction of AmpC β-lactamases [24–26] have been associated with CZA resistance in Enterobacterales. Mutations causing defects in or loss of outer membrane porins [16,17,23,27–29] and overproduction of KPCs have been reported to be sources of resistance to CZA, MEV, and IPR [16,29–33]. Also, decreased susceptibility to FDC has been reported to be due to several mechanisms, including production of PER-like β-lactamases, NDM-like MβLs, mutations in PBP-3, and mutations in iron transport-related proteins, such as TonB-dependent siderophore receptor and siderophore genes [26,34–37].

Therefore, to embrace the last-resort pipeline of antibiotics approved by the FDA and EMA, and available for treating infections caused by CRE, a rapid and novel test, namely the MultiRapid ATB NP test, has been developed to detect susceptibility/resistance to CZA, MEV, IPR, and FDC in Enterobacterales.

2. Methods

2.1. Bacterial strains and antimicrobial susceptibility testing

A selected set of 78 non-duplicate Enterobacterales isolates from the Swiss National Reference Centre of Emerging Antibiotic Resistance (NARA) was used for this study. There were 66 carbapenem-resistant isolates (84.6%), among which 61 produced a carbapenemase (92.4%). The main β-lactam resistance genes of those strains had been previously characterised (Table 1).

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines were used as a reference to perform the gold standard broth microdilution (BMD) method. BMD was performed using the same cation-adjusted Mueller-Hinton broth (CAMHB; AxonLab, Baden, Switzerland) without or with depletion of iron used for performing the MultiRapid ATB NP tests. The same inoculum suspension was used to perform both tests. All the minimum inhibitory concentrations (MICs) were performed in triplicate, and the reference strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC27853 were used as controls of MIC values following the quality control ranges for CZA, MEV, IPR, and FDC, in accordance with EUCAST guidelines. In addition, the reference strains *Klebsiella pneumoniae* ATCC 700603 and *K. pneumoniae* ATCC BAA-2814 were used as controls of the β-lactam inhibitor components in accordance with EUCAST recommendations. EUCAST breakpoint ranges were used to interpret the MIC results for CZA (susceptible [S] $\leq$ 8; resistant [R] $>$ 8), MEV (S $\leq$ 8: R $>$ 8), IPR (S $\leq$ 2; R $>$ 2), and FDC (S $\leq$ 2; R $>$ 2) [38,39].

2.2. MultiRapid ATB NP test

The MultiRapid ATB NP test was developed based on our previous experience of developing rapid tests for antibiotic susceptibility testing. The principle of the test is based on detecting bacterial growth in the absence or presence of antibiotics, detecting bacterial glucose metabolism, and producing a visually detectable colour change of the pH indicator (red phenol) from red to yellow, after the acidification of the medium due to bacterial growth, if any. To produce a single test for susceptibility/resistance to antibiotics, the same techniques were used as previously described for *Rapid CAZ/AVI NP test, Rapid MEV NP test, Rapid IPR NP test*, and *Rapid Cefiderocol NP test*, with some adaptations, when needed [40–43].

2.3. The Rapid NP solutions

The solution used for CZA, MEV, and IPR was prepared according to Nordmann et al. [41] and the solution used for FDC was prepared according to Nordmann et al. [43]. Cation-adjusted Mueller-Hinton broth (CAMHB; AxonLab) was used to prepare solutions for CZA, MEV, and IPR, whereas the iron-depleted cation-adjusted Mueller-Hinton broth (ID-CAMHB; chelex 100 resin, Bio-Rad, Marnes-la-Coquette, France; CAMHB, AxonLab) was used to prepare the solution for FDC [38]. For CZA, ceftazidime (Acros Organics, Thermo Fisher Scientific, Waltham, USA) and avibactam (MedChemExpress, New Jersey, USA) were used at final concentrations of 128 and 64 mg/L, respectively. For MEV, meropenem (Hui Chem, Shanghai, China) and vaborbactam (MedChemExpress) were used at 16 and 8 mg/L final concentrations, respectively. For IPR, imipenem (HuiChem) and relebactam (MedChemExpress) were used to prepare final concentrations at 12 and 4 mg/L, respectively. For FDC, cefiderocol (Shionogi, Osaka, Japan) was used at a final concentration of 64 mg/L.

2.4. Bacterial inoculum

Isolates were grown overnight on UriSelect 4 (Bio-Rad) or Mueller-Hinton (Bio-Rad) agar plates. For CZA, MEV, and IPR tests, a 0.5 McFarland scale was prepared in NaCl 0.85% and ready for use. For the FDC test, a 0.5 McFarland scale was prepared and diluted 1:1 in NaCl 0.85% before inoculation. After preparation, 50 µL of the bacterial suspensions were inoculated from 15 min to a maximum of 1 h, according to EUCAST recommendations [38].

2.5. Tray inoculation

The MultiRapid ATB NP test was performed in a sterile, round-based, 96-well polystyrene microplate with a lid (Sarstedt, Germany). The bacterial suspension was inoculated in separate wells, without and with antibiotics. The steps to perform the MultiRapid ATB NP test were: (1) 100 µL of antibiotic-free rapid solution prepared with CAMHB was added to wells A1–A5 (control of growth for CZA, MEV, and IPR tests); (2) 50 µL of ceftazidime (384 mg/L) and 50 µL of avibactam (192 mg/L) were added to wells B1–B5; (3) 50 µL of meropenem (48 mg/L) and 50 µL of vaborbactam (24 mg/L) were added to wells C1–C5; (4) 50 µL of imipenem (36 mg/L) and 50 µL of relebactam (12 mg/L) were added to wells D1–D5; (5) 150 µL of antibiotic-free rapid solution prepared with ID-CAMHB were added to wells E1–E5 (control of growth for FDC test); (6) 150 µL of FDC (85.3 mg/L) were added to wells F1–F5. After this step, the tray was pre-warmed for 15–30 min at 37 °C before inoculating the bacterial suspensions, to avoid delay in growth and subsequent colour change; (7) 50 µL (0.5 MacFarland) of *E. coli* ATCC 25922 (negative control) were added to wells A1, B1, C1, D1, E1, and F1; (8) 50 µL of a strain resistant to CZA, MEV, IPR, and FDC (positive control) were added to wells A2, B2, C2, D2, E2, and F2; (9) 50 µL of a first tested isolate were added to wells A3, B3, C3, D3, E3, and F3; (10) 50 µL of a second tested isolate were added to wells A4, B4, C4, D4, E4, and F4; and (11) 50 µL of NaCl 0.85% were added to wells A5, B5, C5, D5, E5, and F5 to evaluate the presence of contamination or spontaneous colour change.

Table 1
MultiRapid ATB NP test for detection of ceftazidime–avibactam, meropenem–vaborbactam, imipenem–relebactam, and cefiderocol susceptibility testing in Enterobacterales.

Strain number	Species	Main β-lactam resistance gene	Broth microdilution (mg/L)				MultiRapid ATB NP test				
							Results				Discrepancies vs. BMD (antibiotic)
			CZA	MEV	IPR	FDC	CZA	MEV	IPR	FDC	
-	*Pseudomonas aeruginosa* ATCC 27853	-	1	0.5	0.5	0.25	-	-	-	-	-
-	*Klebsiella pneumoniae* ATCC 700603	-	1	-	-	-	-	-	-	-	-
-	*Klebsiella pneumoniae* ATCC BAA-2814	-	-	0.25	0.25	-	-	-	-	-	-
1	*Escherichia coli* ATCC 25922	-	0.25	≤ 0.125	≤ 0.125	0.5	Neg	Neg	Neg	Neg	-
2	*Escherichia coli*	CTX-M-1	≤ 0.125	≤ 0.125	≤ 0.125	≤ 0.0625	Neg	Neg	Neg	Neg	-
3	*Escherichia coli*	CTX-M-1	4	≤ 0.125	≤ 0.125	2	Neg	Neg	Neg	Neg	-
4	*Escherichia coli*	CTX-M-1	8	0.5	≤ 0.125	**8**	Neg	Neg	Neg	Pos	-
5	*Escherichia coli*	CTX-M-1	≤ 0.125	≤ 0.125	≤ 0.125	0.125	Neg	Neg	Neg	Neg	-
6	*Escherichia coli*	CTX-M-1	≤ 0.125	≤ 0.125	≤ 0.125	0.125	Neg	Neg	Neg	Neg	-
7	*Escherichia coli*	CTX-M-1	≤ 0.125	≤ 0.125	≤ 0.125	≤ 0.0625	Neg	Neg	Neg	Neg	-
8	*Escherichia coli*	CTX-M-15	0.25	≤ 0.125	≤ 0.125	0.25	Neg	Neg	Neg	Neg	-
9	*Escherichia coli*	KPC-2	0.25	≤ 0.125	≤ 0.125	0.25	Neg	Neg	Neg	Neg	-
10	*Escherichia coli*	KPC-2	0.25	≤ 0.125	≤ 0.125	0.25	Neg	Neg	Neg	Neg	-
11	*Escherichia coli*	NDM-5	**> 128**	**16**	**4**	2	Pos	Pos	Pos	Neg	-
12	*Escherichia coli*	NDM-5	**> 128**	**16**	**32**	**4**	Pos	Pos	Pos	Pos	-
13	*Escherichia coli*	NDM-5	**> 128**	**16**	**8**	**8**	Pos	Pos	Pos	Pos	-
14	*Escherichia coli*	NDM-5	**> 128**	**128**	**16**	**8**	Pos	Pos	Pos	Pos	-
15	*Escherichia coli*	NDM-5	**> 128**	**128**	**32**	**64**	Pos	Pos	Pos	Pos	-
16	*Escherichia coli*	NDM-5	**> 128**	**> 128**	**16**	**> 64**	Pos	Pos	Pos	Pos	-
17	*Escherichia coli*	NDM-5 + OXA-181	**> 128**	**32**	**8**	**8**	Pos	Pos	Pos	Pos	-
18	*Escherichia coli*	NDM-5 + OXA-48	**> 128**	**64**	**16**	**8**	Pos	Pos	Pos	Pos	-
19	*Escherichia coli*	OXA-204	0.5	≤ 0.125	0.5	≤ 0.0625	Neg	Neg	Neg	Neg	-
20	*Escherichia coli*	OXA-244	0.5	≤ 0.125	1	0.125	Neg	Neg	Neg	Neg	-
21	*Escherichia coli*	OXA-48	≤ 0.125	0.25	0.25	0.125	Neg	Neg	Neg	Neg	-
22	*Escherichia coli*	OXA-48	0.25	≤ 0.125	0.5	≤ 0.0625	Neg	Neg	Neg	Neg	-
23	*Escherichia coli*	OXA-48	0.25	≤ 0.125	≤ 0.125	1	Neg	Neg	Neg	Neg	-
24	*Escherichia coli*	VIM-1	≤ 0.125	0.5	0.5	≤ 0.0625	Neg	Neg	Neg	Neg	-
25	*Escherichia coli*	TEM-1	≤ 0.125	≤ 0.125	≤ 0.125	≤ 0.0625	Neg	Neg	Neg	Neg	-
26	*Citrobacter freundii*	CTX-M-1	0.25	0.25	≤ 0.125	1	Neg	Neg	Neg	Neg	-
27	*Citrobacter freundii*	KPC-2	4	≤ 0.125	0.5	1	Neg	Neg	Neg	Neg	-
28	*Citrobacter freundii*	OXA-181	1	4	2	**4**	Neg	Neg	Neg	Pos	-
29	*Enterobacter cloacae*	IMI-1	0.25	≤ 0.125	**32**	1	Neg	Neg	Pos	Neg	-
30	*Enterobacter cloacae*	KPC-2	4	≤ 0.125	0.125	≤ 0.0625	Neg	Neg	Neg	Neg	-
31	*Enterobacter cloacae*	NDM-1	**> 128**	2	**4**	**8**	Pos	Pos	Pos	Pos	ME (MEV)
32	*Enterobacter cloacae*	NDM-1	**> 128**	**32**	**64**	**64**	Pos	Pos	Pos	Pos	-
33	*Enterobacter cloacae*	NDM-1 + OXA-48	**> 128**	**32**	**32**	**4**	Pos	Pos	Pos	Pos	-
34	*Enterobacter cloacae*	NDM-5	**> 128**	**32**	**16**	**64**	Pos	Pos	Pos	Pos	-
35	*Enterobacter cloacae*	NDM-7	**> 128**	**16**	**32**	**4**	Pos	Pos	Pos	Pos	-
36	*Enterobacter cloacae*	OXA-48	0.5	0.25	0.25	≤ 0.0625	Neg	Neg	Neg	Neg	-
37	*Klebsiella oxytoca*	KPC-3	0.25	≤ 0.125	0.5	1	Neg	Neg	Neg	Neg	-
38	*Klebsiella oxytoca*	OXA-48	0.25	0.5	0.5	0.125	Neg	Neg	Neg	Neg	-
39	*Klebsiella pneumoniae*	CTX-M-1	2	≤ 0.125	≤ 0.125	**4**	Neg	Neg	Neg	Pos	-
40	*Klebsiella pneumoniae*	CTX-M-1	8	≤ 0.125	≤ 0.125	**8**	Neg	Neg	Neg	Pos	-
41	*Klebsiella pneumoniae*	SHV-12	**64**	2	≤ 0.125	**> 64**	Neg	Neg	Neg	Pos	VME (CZA)
42	*Klebsiella pneumoniae*	KPC-2	0.5	≤ 0.125	≤ 0.125	≤ 0.0625	Neg	Neg	Neg	Neg	-
43	*Klebsiella pneumoniae*	KPC-2	1	1	0.5	0.125	Neg	Neg	Neg	Pos	ME (FDC)
44	*Klebsiella pneumoniae*	KPC-2	1	1	0.25	0.25	Neg	Neg	Neg	Pos	ME (FDC)
45	*Klebsiella pneumoniae*	KPC-2	1	8	0.25	0.25	Neg	Neg	Neg	Neg	-
46	*Klebsiella pneumoniae*	KPC-2	2	2	≤ 0.125	0.5	Neg	Neg	Neg	Neg	-
47	*Klebsiella pneumoniae*	KPC-2	4	**16**	0.5	≤ 0.0625	Neg	Pos	Neg	Neg	-
48	*Klebsiella pneumoniae*	KPC-2 + VEB-25	**> 128**	≤ 0.125	≤ 0.125	**> 64**	Pos	Neg	Neg	Pos	-
49	*Klebsiella pneumoniae*	KPC-3	1	≤ 0.125	0.5	**4**	Neg	Neg	Neg	Pos	-
50	*Klebsiella pneumoniae*	KPC-3	2	0.5	≤ 0.125	**4**	Neg	Neg	Neg	Pos	-
51	*Klebsiella pneumoniae*	KPC-3	**16**	**16**	2	0.5	Pos	Pos	Neg	Neg	-
52	*Klebsiella pneumoniae*	KPC-11	1	≤ 0.125	≤ 0.125	**16**	Neg	Neg	Neg	Pos	-
53	*Klebsiella pneumoniae*	KPC-31	**16**	0.5	≤ 0.125	**16**	Pos	Neg	Neg	Pos	-
54	*Klebsiella pneumoniae*	KPC-41	**128**	≤ 0.125	0.25	**4**	Pos	Neg	Neg	Pos	-
55	*Klebsiella pneumoniae*	KPC-46	**64**	1	0.5	**16**	Pos	Neg	Neg	Pos	-
56	*Klebsiella pneumoniae*	KPC-49	**16**	0.5	≤ 0.125	**16**	Pos	Neg	Neg	Pos	-
57	*Klebsiella pneumoniae*	KPC-50	**> 128**	≤ 0.125	0.5	**> 64**	Pos	Neg	Neg	Pos	-
58	*Klebsiella pneumoniae*	KPC-121	**> 128**	1	0.5	**> 64**	Pos	Neg	Neg	Pos	-
59	*Klebsiella pneumoniae*	KPC-167	**64**	0.5	≤ 0.125	**16**	Pos	Neg	Neg	Pos	-
60	*Klebsiella pneumoniae*	KPC-167	**> 128**	0.5	≤ 0.125	**16**	Pos	Neg	Neg	Pos	-
61	*Klebsiella pneumoniae*	NDM-1	**> 128**	**16**	**8**	**4**	Pos	Pos	Pos	Pos	-
62	*Klebsiella pneumoniae*	NDM-1	**> 128**	**32**	**32**	**4**	Pos	Pos	Pos	Pos	-
63	*Klebsiella pneumoniae*	NDM-1	**> 128**	**128**	**32**	**4**	Pos	Pos	Pos	Pos	-

(*continued on next page*)

Table 1 (*continued*)

Strain number	Species	Main β-lactam resistance gene	Broth microdilution (mg/L)				MultiRapid ATB NP test				
							Results				Discrepancies vs. BMD (antibiotic)
			CZA	MEV	IPR	FDC	CZA	MEV	IPR	FDC	
64	*Klebsiella pneumoniae*	NDM-1	**> 128**	**32**	**8**	**8**	Pos	Pos	Pos	Pos	-
65	*Klebsiella pneumoniae*	NDM-1	**> 128**	**32**	**8**	**16**	Pos	Pos	Pos	Pos	-
66	*Klebsiella pneumoniae*	NDM-1	**> 128**	**16**	**16**	**> 64**	Pos	Pos	Pos	Pos	-
67	*Klebsiella pneumoniae*	NDM-4	**> 128**	**32**	**32**	**4**	Pos	Pos	Pos	Pos	-
68	*Klebsiella pneumoniae*	NDM-4 + OXA-181	**> 128**	**64**	**16**	**16**	Pos	Pos	Pos	Pos	-
69	*Klebsiella pneumoniae*	NDM-5	**> 128**	**64**	**16**	**4**	Pos	Pos	Pos	Pos	-
70	*Klebsiella pneumoniae*	NDM-5 + OXA-181	**> 128**	**128**	**64**	**4**	Pos	Pos	Pos	Pos	-
71	*Klebsiella pneumoniae*	OXA-48	0.25	**128**	**64**	**8**	Neg	Pos	Pos	Pos	-
72	*Klebsiella pneumoniae*	OXA-48	0.5	0.5	2	0.125	Neg	Neg	Neg	Pos	ME (FDC)
73	*Klebsiella pneumoniae*	OXA-48	0.5	**16**	**8**	**8**	Neg	Neg	Neg	Pos	VME (MEV/IPR)
74	*Klebsiella pneumoniae*	OXA-48	0.5	1	0.5	$\leq$ 0.0625	Neg	Neg	Neg	Neg	-
75	*Klebsiella pneumoniae*	OXA-48	1	**16**	**8**	0.125	Neg	Pos	Neg	Neg	VME (IPR)
76	*Klebsiella pneumoniae*	OXA-181	0.25	$\leq$ 0.125	0.5	0.5	Neg	Neg	Neg	Neg	-
77	*Klebsiella pneumoniae*	OXA-232	0.5	**16**	1	2	Neg	Pos	Neg	Neg	-
78	*Providencia stuartii*	NDM-1	**> 128**	1	**32**	**> 64**	Pos	Neg	Pos	Pos	-

CZA, ceftazidime–avibactam; MEV, meropenem–vaborbactam; IPR, imipenem–relebactam; FDC, cefiderocol; Neg, Negative; Pos, Positive; (-), no discrepancies observed; ME, major error; VME, very major error; Bold script, resistant; Normal script, susceptible; Underlined, highlights discrepancies.

Hence, each well had a final volume of 150 µL in the CZA, MEV, and IPR tests, and 200 µL in the FDC test. The final retained concentrations were 128/64 mg/L for CZA, 16/8 mg/L for MEV, 12/4 mg/L for IPR, and 64 mg/L for FDC. These final antibiotic concentrations do not correspond exactly to the breakpoint values for susceptibility/resistance as defined for detection using BMD; however, these concentrations provide the best differentiation between susceptible and resistant strains using this test.

2.6. Tray incubation and reading

The MultiRapid ATB NP test is ready to read after 3 h of incubation at 35 ± 2 °C in ambient air, covered by a lid and without agitation. To ensure carbohydrate metabolism through oxygen consumption, the tray was not sealed. Based on experience from previous works, the results were considered valid when there was (1) bacterial growth and colour change from red to yellow in the wells without antibiotics for all the strains (A1–A4 and E1–E4); (2) absence of bacterial growth for *E. coli* ATCC 25922 for all the wells with antibiotics (B1, C1, D1, and F1); (3) red-to-yellow colour change for all the wells with antibiotics for the positive control (B2, C2, D2, and F2); (4) red-to-yellow colour change for the first tested strain in the wells B3, C3, and D3 detecting resistance to CZA, MEV, and IPR, and absence of growth (i.e., remaining red) in well F3 detecting susceptibility to FDC; (5) red-to-yellow colour change for the second tested strain in wells B4 and F4, and absence of colour change in wells C4 and D4 detecting resistance to CZA and FDC but susceptibility to MEV and IPR; and (6) absence of colour change in wells with added NaCl 0.85% (A5–F5), confirming absence of contamination. Figure 1 shows a visual interpretation of the MultiRapid ATB NP test.

2.7. Data analysis

All the results were compared with those of the BMD standard reference method. Classification of major errors (MEs) and very major errors (VMEs) was used to determine discrepancies between the tests [43,44]. Sensitivity, specificity, and accuracy parameters were determined [45], and results were blindly interpreted by two laboratory members independently.

3. Results

The 78 enterobacterial isolates used to evaluate the MultiRapid ATB NP test included KPC producers [n=23 (29.5%); KPC-2, -3, -11, -31, -41, -46, -49, -50, -121, -167], NDM producers [n=19 (24.4%); NDM-1, -4, -5, -7], OXA producers [n=15 (19.2%); OXA-48, -181, -204, -232, -244], VIM producer [n=1 (1.3%)], co-producers [n=6 (7.7%); KPC-2 + VEB-25, NDM-1 + OXA-48, NDM-4 + OXA-181, NDM-5 + OXA-48, and NDM-5 + OXA-181), IMI-1 producer [n=1 (1.3%)], CTX-M producers [n=10 (12.8%); CTX-M-1 and -15], SHV producer ([n=1 (1.3%)], TEM-1 producer [n=1 (1.3%)], and the negative control without β-lactamase gene [n=1 (1.3%)]. Among the collection, 44.9% (35/78), 35.9% (28/78), 35.9% (28/78), and 56.4% (44/78) were resistant to CZA, MEV, IPR, and FDC, respectively, according with the BMD results and interpreted following EUCAST guidelines [38,39].

Overall, the MultiRapid ATB NP test showed a 97.0% (confidence interval [CI] 92.6–98.8) sensitivity, 97.7% (CI 94.3–99.1) specificity, and 97.4% (CI 95.0–98.7) accuracy (Table 2). As the number of susceptible and resistant isolates is different for each antibiotic, discrepancies were evaluated for each novel antibiotic individually. For instance, there were no MEs (false-positive) results for CZA and IPR, but one VME (2.9%; false-negative) was observed for CZA with an SHV-12-producing *K. pneumoniae* isolate presenting an MIC of 64 mg/L for CZA, and two VMEs (7.1%) for IPR with *K. pneumoniae* isolates producing OXA-48 with MICs of IPR at 8 mg/L. One ME (2.0%) and one VME (3.6%) were detected for MEV test for one *Enterbacter cloacae* isolate producing NDM-1 (MIC of MEV at 2 mg/L), and one *K. pneumoniae* isolate producing OXA-48 (MIC of MEV at 16 mg/L). For FDC, three MEs (8.8%) were observed with two *K. pneumoniae* isolates producing KPC-2 and one *K. pneumoniae* producing OXA-48, with FDC MICs of 0.125, 0.25, and 0.125 mg/L, respectively. Notably, no VME was observed for the FDC test. After final evaluation of the MultiRapid ATB NP test, the optimal reading time to obtain definitive results was defined to be 3 h after incubation at 35 °C ± 2 °C under ambient atmosphere. Results are shown in Tables 1 and 2.

4. Discussion

After the first reports of carbapenemases more than two decades ago [46,47], CRE are in their exponential phase of dissem-

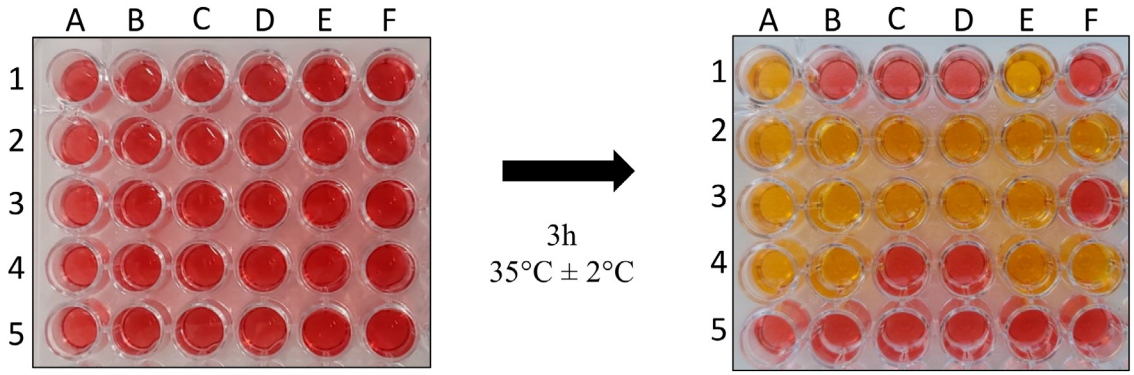

Figure 1. The MultiRapid ATB NP test. Column A presents the solution free of antibiotics and prepared with CAMHB; Column B presents the solution with ceftazidime–avibactam (CZA, 128/64 mg/L); Column C shows the solution with meropenem–vaborbactam (MEV, 16/8 mg/L); Column D shows the solution with imipenem–relebactam (IPR, 12/4 mg/L); Column E has the solution free of antibiotics and prepared with ID-CAMHB; Column F has the solution with cefiderocol (FDC, 64 mg/L). Reference strain *Escherichia coli* ATCC 25922 was inoculated in wells A1–F1; Positive control resistant to all the antibiotics was inoculated in wells A2–F2; First tested strain (resistant to CZA, MEV, and IPR) was inoculated in wells A3–F3; Second tested strain (resistant to CZA and FDC) was inoculated in wells A4–F4; and NaCl 0.85% was inoculated in wells A5–F5. Bacterial growth is shown by a colour change of the medium from red to yellow.

Table 2
The MultiRapid ATB NP test compared with the reference method broth microdilution.

MultiRapid ATB NP test	Sensitivity %	Specificity %	Accuracy %	ME % (n)	VME % (n)
Ceftazidime–avibactam	97.1	100.0	98.7	0.0 (0)	2.9 (1)
Meropenem–vaborbactam	96.4	98.0	97.4	2.0 (1)	3.6 (1)
Imipenem–relebactam	92.9	100.0	97.4	0.0 (0)	7.1 (2)
Cefiderocol	100.0	91.2	96.2	8.8 (3)	0.0 (0)
Overall	97.0	97.7	97.4	-	-

ME, major error; VME, very major error; n, number of strains

ination worldwide. CRE are a source of high morbidity and high mortality [48,49]. Therefore, developing new drugs and their companion diagnostic techniques for treating CRE is crucial.

Herein is proposed a novel rapid test, the MultiRapid ATB NP test, for detecting susceptibility/resistance to the novel molecules CZA, IPR, MEV, and FDC. Overall, the test shows 97–98% sensitivity and specificity for all those molecules. Time to obtain results is less than 3 h, which represents a gain of around 15 h compared with the common susceptibility tests, including the reference standard BMD. Overall, the strains for which a VME was detected may correspond to slow metabolism characteristics that will not be observed for tests with a longer turnaround time for interpreting the results, such as the BMD (16–24 h). One of the VMEs was observed in a *K. pneumoniae* with a borderline MIC of MEV (16 mg/L). The test showed one ME for MEV and three for FDC. Of note, the ME for MEV was observed in an NDM-producing *E. cloacae* strain, which is understandable clinically as MEV has no activity against MβL producers [12].

The MultiRapid ATB NP test offers reliable results and a variety of novel antibiotic options. This test offers the possibility of rapid antibiotic stewardship. For instance, *E. coli* isolate (strain N ° 11) carrying an NDM-5 MβL displayed a resistance phenotypic profile with MICs $>$ 128 mg/L, 16 mg/L, and 4 mg/L for CZA, MEV, and IPR, respectively. The isolate remained susceptible to FDC, in which case this drug could be proposed for adequate therapy (Figure 1).

Another example worthy of mention is the *K. pneumoniae* isolates producing KPC variants (KPC-31, -49, -121) [50,51] that were resistant to CZA and co-resistant to FDC, but remained susceptible to MEV and IPR, giving only two interesting options for treatment. Conversely, 28.2% (22/78) of isolates were resistant to all four antibiotics tested, resulting in a lack of an immediate treatment option, and reinforcing the need for the development of novel antibiotics to treat infections caused by CREs. Of note, all the isolates that were resistant to all four antibiotics produced an NDM-like enzyme, which is not inhibited by avibactam, vaborbactam, or relebactam [15].

The MultiRapid ATB NP detects phenotypic susceptibility/resistance to CZA, MEV, IPR, and FDC independent of the resistance mechanisms of the isolate. This feature distinguishes the test from molecular and immunological techniques that focus on detecting specific resistance traits. Compared with these techniques, the novel rapid test proposed herein has the practical advantage of being effective even when considering clinical isolates that present resistance due to combined mechanisms, such as modification in β-lactamase structure, overexpression of β-lactamase, structural changes of PBPs, and outer membrane defects.

The concentrations of the MultiRapid ATB NP test do not correlate with the EUCAST breakpoint values for each compound due to an inoculum effect. The final inoculum concentration in the MultiRapid ATB NP test for CZA, MEV, and IPR (1.5×10^8 cells) or for FDC (7.5×10^7 cells) is higher than with the BMD inoculum (1.5×10^6 cells), enabling the strains to grow faster. Therefore, to avoid false-positive results it was necessary to increase the concentration of the antibiotics to provide an optimal condition for the test. Limitations of the current study include the small sample size and the limited isolates showing borderline MICs.

5. Conclusion

The MultiRapid ATB NP test provided accurate results for the concomitant detection of susceptibility/resistance to CZA, MEV, IPR, and FDC in Enterobacterales. This novel rapid test is based on the phenotypic detection of susceptibility/resistance to these antibiotics within 3 h. The MultiRapid ATB NP test will be further evaluated in routine clinical microbiology laboratories to validate the test in different settings and geographic regions. Finally, as for cancer therapies, the time has come to use companion diagnostics such as this rapid test to optimise management of infected patients with MDR Enterobacterales.

Author Contributions

All the author contributions are described as follows: Formal analysis: Otávio Hallal Ferreira Raro and Maxime Bouvier; Investigation: Auriane Kerbol, Maxime Bouvier, and Otávio Hallal Ferreira Raro; Methodology: Maxime Bouvier and Otávio Hallal Ferreira Raro; Validation: Auriane Kerbol, Laurent Poirel, Maxime Bouvier, Otávio Hallal Ferreira Raro and Patrice Nordmann; Writing - original draft: Otávio Hallal Ferreira Raro and Maxime Bouvier; Conceptualisation: Patrice Nordmann; Supervision: Patrice Nordmann and Laurent Poirel, Writing – review and editing: Laurent Poirel and Patrice Nordmann, Funding acquisition: Patrice Nordmann.

Declarations

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Data sharing

The datasets generated for this study are available on request to the corresponding author.

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