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Systematic review

Rapid phenotypic testing for detection of carbapenemase- or extended-spectrum ß-lactamase-producing Enterobacterales directly from blood cultures: a systematic review and meta-analysis

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ABSTRACT

Background: Early identification of extended-spectrum ß-lactamase (ESBL) and carbapenemase-producing Enterobacterales (CP-CRE) is critical for timely therapy. Rapid phenotypic tests identifying these resistance mechanisms from pure bacterial colonies have been developed.
Objectives: To determine the operating characteristics of available rapid phenotypic tests when applied directly to positive blood cultures.
Methods of data synthesis: Bivariate random effects models were used unless convergence was not achieved where we used separate univariate models for sensitivity and specificity.
Data sources: MEDLINE, CENTRAL, Embase, BIOSIS, and Scopus from inception to 16 March 2021.
Study eligibility criteria: Studies using any rapid phenotypic assay for detection of ESBL or CP-CRE directly from blood cultures positive for Enterobacterales, including those utilizing spiked blood cultures. Case reports/series, posters, abstracts, review articles, those with ≤5 resistant isolates, and studies lacking data or without full text were excluded.
Participants: Consecutive patient samples (main analysis) or spiked blood cultures (sensitivity analysis).
Tests: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assays (MALDI-TOF) and commercially available chromogenic or immunogenic assays.
Reference standard: Conventional laboratory methods and/or polymerase chain reaction (PCR).
Assessment of risk of bias: Quality Assessment of Diagnostic Accuracy Studies Version 2 (QUADAS-2).
Results: For detection of the ESBL phenotype the respective pooled sensitivities and specificities for consecutive clinical samples were as follows: 94% (95% CI 93–99%) and 97% (95% CI 95–100%) for MALDI-TOF/mass spectrometry ($n = 1$); and 98% (95% CI 92–100%) and 100% (95% CI 96–100%) for chromogenic assays ($n = 7$). For the CP-CRE phenotype the respective pooled sensitivity and specificities for consecutive clinical samples were as follows: 100% (95% CI 99–100%) and 100% (95% CI 100–100%) for MALDI-TOF ($n = 2$); 96% (95% CI 77–99%) and 100% (95% CI 81–100%) for chromogenic assays ($n = 4$); and 98% (95% CI 96–100%) and 100% (95% CI 100–100%) for immunogenic testing ($n = 2$).
Conclusions: Rapid phenotypic assays that can be directly applied to positive blood cultures to detect ESBL and carbapenemase production from Enterobacterales exist and, although clinical studies are limited, they appear to have high sensitivity and specificity. Their potential to facilitate patient care through timely identification of bacterial resistance should be further explored. **Olivier Del Corpo, Clin Microbiol Infect 2023;29:1516**

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Introduction

Extended-spectrum β-lactamase producing *Enterobacterales* (ESBL) and Carbapenemase-producing Carbapenem-resistant *Enterobacterales* (CP-CRE) contribute to substantial morbidity and mortality in health care settings [1]. Although new therapeutics are available in some markets, multidrug-resistant Gram-negative bacteria remain a major public health concern worldwide. Overly broad empiric therapy risks the development of resistance, whereas undertreating can lead to therapeutic failure and clinical deterioration [2]. Thus, it is important to develop diagnostic approaches for rapidly detecting multidrug-resistant organisms to allow for appropriate, timely, and targeted treatment while facilitating antimicrobial stewardship.

Several rapid phenotypic tests have been developed for the identification of ESBL and carbapenemase-producing organisms. These tests include chromogenic assays which rely on hydrolysis of β-lactam surrogates linked to chromophores that become detectable once the target is hydrolysed; matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assays (MALDI-TOF/MS) to detect hydrolysis of carbapenem or β-lactam disks; and lateral flow immunochromatographic assays which contain monoclonal antibodies targeting carbapenemases/β-lactamases on a nitrocellulose membrane. Although such tests were initially validated for use on pure growth bacterial colonies, their direct application to positive Gram-negative blood cultures promises timely and actionable clinical information. This systematic review and meta-analysis aimed to evaluate and compare the sensitivity and specificity of available rapid phenotypic tests for ESBL and CP-CRE when performed directly from blood cultures.

Methods

This analysis is reported in accordance with the Preferred Reporting Items for a Systematic Review and Meta-analysis of Diagnostic Test Accuracy Studies [3].

Search strategy

The following databases were searched for relevant studies on 16 March 2021: MEDLINE (via Ovid, 1946 to 15 March 2021; via PubMed, 1 March 2021 to 16 March 2021); CENTRAL (via Wiley, Issue 3 of 12, March 2021); Embase (via Ovid 1974 to 15 March 2021), BIOSIS (via Clarivate) and Scopus (via Elsevier). The search strategies were designed with a research librarian and peer reviewed by a second research librarian. The MEDLINE strategy (Appendix 1) was applied to all databases, with modifications to search terms as necessary. No language limits were applied with machine translation used when necessary.

Selection criteria

Studies using any rapid phenotypic assay for detection of ESBL or CP-CRE directly from blood cultures positive for Enterobacterales were screened. 'Directly from blood cultures' could include a short incubation step (<3–4 hours) such as that used for rapid MALDI identification. Studies utilizing consecutive clinical specimens were used for the primary analysis and those including spiked blood cultures were included in a supplementary analysis. Case reports, case series, posters, abstracts, review articles, and studies lacking data or without full text were excluded. Studies that had under five ESBL or CP-CRE isolates or that utilized experimental procedures (e.g. home brewed or non-commercially available solutions) were also excluded. Conventional methods (e.g. disc diffusion, broth microdilution), automated systems, and/or PCR were considered as the reference standards for bacterial identification and susceptibility.

Assessment of study bias

The risk of bias was assessed by two independent reviewers (ODC and TCL) using the QUADAS-2 tool [4]. We assessed the risk of bias and applicability concerns at the level of patient selection, index test, reference standard, patient flow, and timing of test. We reported the risk as low, high, or unclear.

Data extraction

References were imported into an EndNote library (Clarivate V20) and de-duplication was performed. Two independent reviewers screened each title and abstract (ODC and either JS or JMH or TCL) against eligibility criteria followed by full text review (ODC and TCL) of the potentially eligible studies. Disagreements were resolved by consensus or third reviewer (AL) as needed. For each study, we extracted authors, year of publication, study design, samples used, number of ESBL and CP-CRE positive samples, reference standard, assay type and method, assay run time, number of true-/false-positive and true-/false-negative ESBL or CP-CRE samples.

Data analysis

For analysis, we divided studies in groups according to type of assay used and contrasted consecutive clinical specimens (main analysis) vs. spiked samples (sensitivity analysis). Chromogenic assays included the Nordmann-Dortet-Poirel test (Hôpital de Bicêtre, France), β-Lacta (Bio-Rad, United States), CARBA-NP (Biomerieux, Francs), Blue CARBA (Biomerieux, France), and NeoRapid CARB (Rosco, United States). All MALDI-TOF-MS studies were grouped together. Lateral flow immunochromogenic studies included all those that utilized NG-test CARBA 5 (Hardy Diagnostics, United States) and RESIST-5 OOKNV (Coris BioConcept, Belgium). Finally, there was one fluorogenic study utilizing FluoroDirect (Catholic University of Korea, Republic of Korea). The results were analysed according to resistance mechanism (ESBL versus CP-CRE). With the midas [5] module in STATA version 17 (StataCorp LP, United States), we used a bivariate random effects model to calculate sensitivity and specificity for each group of assays compared with the reference standard. Where a bivariate approach was not possible (e.g. when convergence was not achieved or if there were fewer than four studies), we performed univariate analysis [6] using the metaprop_one module and a random effects generalized linear mixed model [7]. We also conducted a pre-planned subgroup analysis restricted to *Escherichia coli* and *Klebsiella pneumoniae*.

Results

Description of included studies

A total of 2986 studies were identified of which 38 were eligible for inclusion (Fig. 1). A description of the included studies is found in the supplement. Briefly, 14 studies utilized MALDI-TOF/MS [8–21], 18 used chromogenic assays [22–39], 5 used immunochromogenic assays [40–44], and one studied a fluorogenic assay [45], for the identification of ESBL or CP-CRE directly from blood cultures. Fifteen studies included only ESBL *Enterobacterales* [14–16,19,21–23,25,28,30,34–36,38,39,42], whereas 20 included only CP-CREs [8–12,17,20,26,27,29,31–33,37,41,43–46]. Three studies analysed both groups [10,18,24]. Of the 38 included studies,

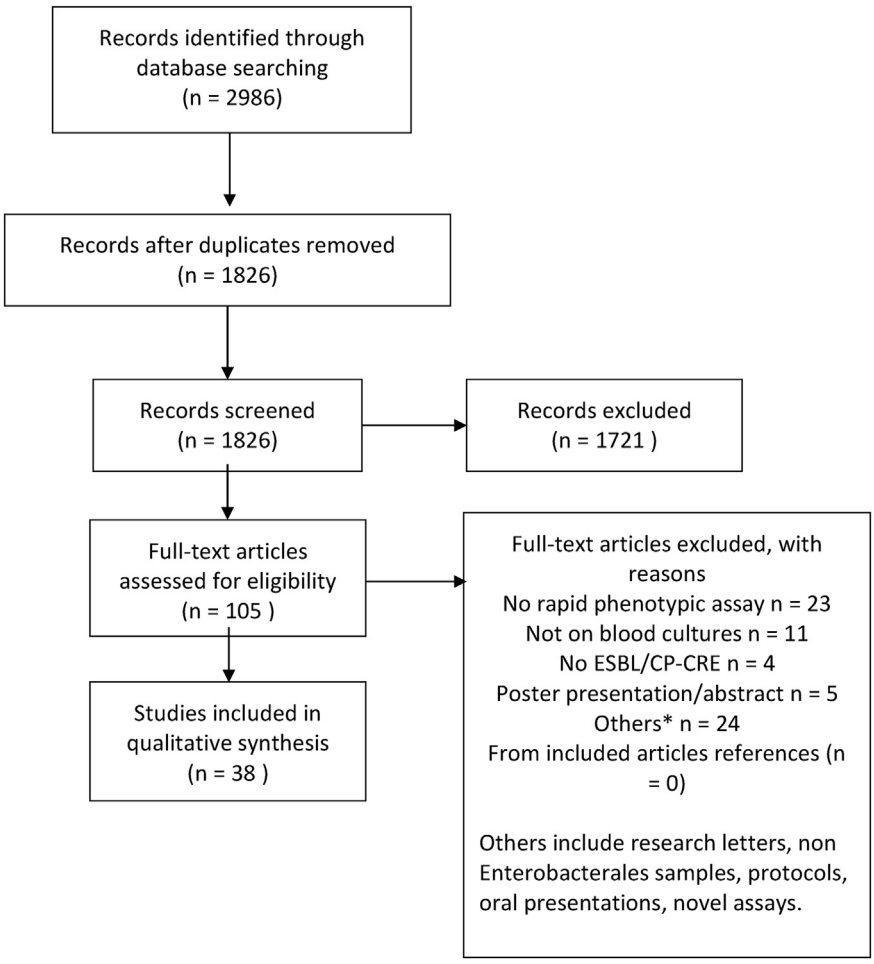

Fig. 1. Flow diagram of the process of selection of articles for inclusion in the meta-analysis.

27 provided data stratified by microorganism allowing subgroup analysis of *E. coli* and *K. pneumoniae*. Studies that utilized non-conventional/experimental phenotypic assays were excluded [47,48]. In 21 studies, bacterial isolates were identified as Enterobacterales by MALDI-TOF [9–11,14–16,20,21,23–26,28,34,36–38,40–43], one study utilized analytic profile index 20E test strips [22], five studies utilized automated systems [8,9,18,29,31], eight studies used organisms which were previously characterized on the molecular level [12,17,27,32,33,35,44,45], two studies utilized 'conventional subculture' [19,39] and one study did not provide identification methods [30]. All studies used one or more of: disc-diffusion, microdilution assays, automated methods, and/or PCR as the reference standard to characterize resistance profiles. Further details of the studies can be found in Table 1.

Quality assessment

The quality assessment using QUADAS-2 is included in the supplement. Briefly, the risk of bias and applicability concerns were low in all but two studies [11,12]. Of the 38 studies, 18 utilized consecutive blood cultures [9,10,15,21–23,25,26,29–31,34,37–39,41,43], whereas the others utilized spiked blood cultures. All but one study that utilized spiked blood cultures incubated samples until flagged positive in an automated incubator. Given the lack of universal reference standard and limited information on the clinical settings that the blood cultures were drawn, all studies were considered 'unclear' for both reference standard and patient selection bias.

Diagnostic performance of the different rapid phenotypic tests for ESBL Enterobacterales

When grouped together by resistance mechanism, there were a total of eight studies that analysed rapid phenotypic testing for ESBL Enterobacterales in consecutive clinical samples [15,22,23,25,28,34,38,39]. One study was excluded from the analysis as stratified data for spiked and clinical cultures was not available [23]. The clinical context in which the samples were drawn are not available for all seven studies; however, they were all drawn from inpatients at university hospitals. Of the seven studies, one utilized MALDI-TOF/MS and demonstrated a sensitivity and specificity of 94% (95% CI 83–99%) and 97% (95% CI 95–100%), respectively [15]. The remainder of the six studies utilized chromogenic assays with an overall sensitivity of 98% (95% CI 89–100%) and specificity 100% (95% CI 91–100%) (Fig. 2).

There were a total of 11 studies that analysed rapid phenotypic testing for ESBL Enterobacterales in spiked blood cultures (including one study with mixed spiked and clinical cultures [23]) [14,16,18,19,21,24,30,35,36,42,49]. Of note, three of these studies included both ESBL and CR-CPE [18,19,24] and one study tested two different assays in ESBLs [42]. Five studies utilized MALDI-TOF/MS [14,16,18,19,21] with a pooled sensitivity of 98% (95% CI 91–100%)

Table 1
Study design

Paper	Location	Study description	Reference standard/diagnostic criteria	Assay	Resistance	TP	FP	FN	TN	Sens	Spec
Affolabi et al., 2017 [22]	Benin	Study design: Consecutive blood cultures positive for GNB from 610-bed university teaching hospital in Cotonou, Benin. Samples: GNB were obtained from the blood samples of 198 patients. A total of 175 samples contained Enterobacterales, 23 contained non-fermenting bacilli (excluded in study). 155 were ESBL.	Culture: Incubated until flagged positive by BD Bactec FX40 Identification: API 20E Susceptibility: Disk diffusion method according to EUCAST recommendations.	NDP	ESBL	155	0	0	20	1.00	1.00
Anantharajah et al., 2019 [8]	Belgium	Study design: Spiked cultures with previously characterized Gram-negative isolates intermediate or resistant to at least one carbapenem from clinical and screening samples of the Cliniques Universitaire Saint-Luc. Samples: Of 130 samples, 26 were Enterobacterales isolates of which 23 were ESBL.	Culture: Spiked (1.5×10^8 CFU/mL) and incubated in Bactec FX until flagged positive Identification: Automated Phoenix system Susceptibility: Disk diffusion method according to EUCAST. Resistance mechanism were confirmed with PCR.	STAR Carba IVD/ MALDI-TOF	CP-CRE	23	1	0	2	1.00	0.67
Arca-Suaréz et al., 2017 [23]	Spain	Study design: Consecutive blood cultures positive for Enterobacterales from bacteraemic patients and spiked blood cultures with previously characterized cryopreserved ESBL-producing Enterobacterales Samples: A total of 70 Enterobacterales from blood culture of bacteraemic patients. A total of 40 cryopreserved strains from a collection of ESBL-producing and inducible chromosomal AMPc-overproducing Enterobacterales were also included.	Cultures: Both consecutive and spiked (1.5×10^8 CFU/mL) cultures were incubated using BD BactecTM FX until flagged positive Identification: MALDI-TOF. Susceptibility: Microdilution and phenotypic tests according to EUCAST. Molecular detection of ESBL and plasmid AmpC genes was performed by multiplex PCR.	NDP	ESBL	41	3	0	68	1.00	0.96
Baer et al., 2021 [40]	Israel	Study design: Spiked blood cultures from previously characterized isolates of clinical or screening samples Samples: A total of 48 isolates, 38 CP-CRE, 10 non-CP-CRE.	Cultures: Spiked blood cultures were incubated to achieve final concentration of 6.75×10^7 CFU/mL Identification: MALDI-TOF, Susceptibility: Xpert Carba-R and PCR for the carbapenem susceptibility.	NG-test Carba 5	CP-CRE	35	0	5	8	0.88	1.00
Bianco et al., 2020 [41]	Italy	Study design: Spiked blood cultures using Enterobacterales clinical strains collected from different clinical specimens at the University Hospital Città della Salute e della Scienza di Torino from 2016 to 2019. Samples: A total of 422 isolates, of which 111 ESBL-producing bacteria, 33 AMPc-producing bacteria, and 162 CP-CRE	Cultures: Spiked (0.5 mL of 10^3 CFU/mL) and incubated till flagged positive Identification: MALDI-TOF MS Susceptibility: Disk diffusion according to EUCAST. Confirmed with PCR	Direct B-Lactam inactivation method	ESBL CP-CRE	111 161	0 0	0 1	116 116	1.00 0.99	1.00 1.00
Bianco et al., 2021 [24]	Italy	Study design: Consecutive blood cultures from CP-CRE carriers identified via rectal swabs from a 2300-bed tertiary care teaching hospital from 2019 to 2020 Samples: A total of 580 identified CP-CRE rectal carriers. Of the 580, 105 developed Gram-negative blood stream infections. From these patients, 127 Gram-negative BCs were used. 79 were CP-CRE. A total of 48 non-CPE-positive BCs (33 Enterobacterales and 15 non-fermenting species which were excluded)	Cultures: Incubated until flagged positive Identification: MALDI-TOF MS. Susceptibility: Mastdisc combi Carba plus disc system and Xpert Carba-R to identify CP-CRE	NG-test Carba 5	CP-CRE	69	0	2	30	0.97	1.00
Bianco et al., 2020 [42]	Italy	Study design: Spiked blood cultures using Enterobacterales strains collected from different Italian Hospitals from 2016 to 2019 were used. Samples: A total of 130 isolates and 30 additional pre-characterized carbapenemase-negative Enterobacterales were used as negative controls. A	Cultures: Spiked (0.5 mL of 10^4 CFU/mL) and incubated until flagged positive Identification: MALDI-TOF Susceptibility: Disc-based phenotypic according to EUCAST. Confirmed with PCR	RESIST-5 O.O.K.N.V. NG-Test Carba 5	ESBL ESBL	91 91	0 0	3 3	30 30	0.97 0.97	1.00 1.00

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Table 1 (*continued*)

Paper	Location	Study description	Reference standard/diagnostic criteria	Assay	Resistance	TP	FP	FN	TN	Sens	Spec
		total of 102 CP-CRE, 20 ESBLs, and 10 AmpC producers.									
Carvalhaes et al., 2014 [9]	Brazil	Study design: Consecutive blood cultures during selected at random in a 2-mo period. Samples: A total of 100 blood cultures flagged as positive resulting in a total of 110 bacterial isolates. Of those, 49 were Enterobacterales, 17 CP-CRE, and 32 non-CP-CRE. Non-Enterobacterales strains were excluded from the analysis.	Cultures: Incubated until flagged positive Identification: PhoenixTM automated system Susceptibility: CLSI agar dilution Confirmed with PCR	MALDI-TOF-MS	CP-RE	17	0	0	32	1.00	1.00
Compain et al., 2015 [25]	France	Study design: Consecutive blood cultures collected over a 4-mo period at Hospital Europe en Georges Pompidou, in Paris, France. Samples: Total of 108 non-repetitive blood cultures. Of those, 28 were ESBL.	Cultures: Incubated until flagged positive Identification: MALDI-TOF Susceptibility: Disc-diffusion method according to EUCAST	B-Lacta	ESBL	28	0	5	75	0.85	1.00
Coppi et al., 2017 [26]	Italy	Study design: Consecutive blood from participating centres Samples: A total of 125 isolates from blood cultures, of which, 122 gave interpretable results, whereas three isolates (one *P. aeruginosa*, one *A. baumannii* complex, and one Enterobacter cloacae) yielded insufficient growth. Of the 122 samples, 81 were Enterobacterales with 21 CP-CRE.	Cultures: Incubated until flagged positive Identification: MALDI-TOF with the VITEK® MS system. Susceptibility: AST was performed using reference broth microdilution according to CLSI guideline. Confirmed with PCR	Carba-NP	CP-CRE	21	1	0	59	1.00	0.98
Cordovana et al., 2020 [10]	Italy	Study design: Routine blood cultures flagged positive for enterobacteria Samples: A total of 407 samples positive enterobacteria of which 132 were CP-CRE	Cultures: Incubated until flagged positive Identification: MALDI Biotyper Susceptibility: Vitek2 system Confirmed with PCR	MALDI-TOF-MS (MBT STAR Carba IVD)	CP-CRE	132	0	0	275	1.00	1.00
Dortet, 2013 [27]	Switzerland	Study design: Spiked blood cultures from previously characterized strains. Samples: A total of 193 CP-CRE and 74 non-CP-CRE	Cultures: Spiked (1×10^3 CFU) and incubated until flagged positive Identification: All strains had previously been characterized for their at their molecular level.	Carba-NP	CP-CRE	189	0	4	74	0.98	1.00
Dortet et al., 2015 [28]	Paris	Study design: Consecutive blood cultures positive for GNB from 245 patients hospitalized at the Bicetre Hospital, a 950-bed hospital located in Paris. Samples: Of 245 cultures, 211 were Enterobacterales, 47 ESBL and 164 non-ESBL	Cultures: Incubated until flagged positive by BacT/Alert system Identification: MALDI-TOF. Susceptibility: AST by the disk diffusion method according to the CLSI. The double-disk synergy test was used for the phenotypic detection of ESBL producers. Confirmed by PCR	NDP	ESBL	47	0	0	164	1.00	1.00
Fernández et al., 2016 [29]	Spain	Study design: Consecutive blood cultures. Over a 3-mo period (June—August 2015), all blood cultures recovered in a Spanish hospital, which were positive for GNB by Gram staining, were included in the study. Samples: A total of 149 samples from 137 patients were selected, of which 122 were positive for Enterobacterales, with 110 corresponding to monomicrobial cultures. Of the 110 samples used, 10 were CP-CRE, 100 non-CR-CPE. An additional 19 previously characterized ESBLs were added to the study.	Culture: Incubated until flagged positive Identification: MicroScan System Susceptibility: MicroScan system for determination of the MIC to a panel of 21 antimicrobials. Confirmed by Carba-R Xpert PCR	Carba-NP	CP-CRE	18	0	1	100	0.95	1.00
Ghebremedhin et al., 2016 [11]	Germany	Study design: Spiked blood cultures Samples: A total of 63 Gram-negative bacteria, comprised of Enterobacterales and non-fermenters, were collected by the National Reference Center for Gram-negative bacteria at the Ruhr University Bochum and at the Institute in Wuppertal. Of the 63 samples, 23 were CP-CRE. The rest (2 pseudomonas	Culture: Spiked with previously characterized strains. Identification: MALDI- TOF Susceptibility: Phenotypic antimicrobial susceptibility testing by use of Phoenix automated system.	MALDI-TOF-MS	CP-CRE	22	0	1	35	0.96	1.00

		and 38 *A. baumanni* isolates were excluded). As negative control, 35 non-carbapenemase-producing, but carbapenem-resistant isolates were included.									
Giordano et al., 2019 [43]	Italy	Study design: Consecutive blood cultures from patients hospitalized at Fondazione Policlinico Universitario A. Gemelli IRCCS or the Azienda Ospedaliera San Camillo-Forlanini hospitals in Italy. Samples: A total of 484 bacterial organisms directly from clinical and spiked BCs were used. Clinical samples were collected from these samples; 237 were CP-CREs and 247 non-CP-CRE.	Cultures: Incubated until flagged positive Identification: MALDI-TOF/MS. Susceptibility: Meropenem MICs was determined using commercial broth-microdilution antimicrobial susceptibility testing panels. Confirmation by PCR	NG-test Carba 5	CP-CRE	233	0	4	247	0.98	1.00
Hoyos-Mallecot et al., 2014 [12]	Spain	Study design: Spiked blood cultures with previously characterized strains Samples: A total of 22 Enterobacterales cultures were included. Of those, 11 were ESBL and 11 non-ESBL. Of note 18 pseudomonas strains were excluded.	Cultures: Spiked (10−100 CFU) and incubated until flagged positive Susceptibility: Previously characterized stains. The presence of carbapenemase was established on the molecular level.	MALDI-TOF-MS	CP-CRE	11	2	0	9	1.00	0.82
Idelevich et al., 2018 [13]	Germany	Study design: Spiked blood cultures with isolates from routine diagnostics at the Institute of Medical Microbiology, University Hospital Münster, Germany, Samples: A total of 28 Enterobacterales, 14 CP-CRE and 14 non-CP-CRE	Cultures: Spiked (150 CFU) and incubated until flagged positive Identification: Isolates were previously characterized Susceptibility: Isolates were previously characterized	MALDI-TOF-MS DOT-MGA	CP-CRE	14	0	0	14	1.00	1.00
Jain et al., 2007 [39]	England	Study design: Consecutive blood cultures submitted to the Microbiology Department, City Hospital, Birmingham. Samples: A total of 127 blood cultures from 71 patients were studied. The HMRZ-86 assay was performed on only the 66 aerobic cultures because of limitations in the assay. A total of 20 were ESBLs and 46 non-ESBLs. A total of 2 pseudomonas samples were excluded from our study.	Cultures: Consecutive and incubated until flagged positive Identification: By standard methods Susceptibility: cefpodoxime disc susceptibility and confirmed using combined disc method cefpodoxime + clavulanic acid	Chromogenic cephalosporin HMRZ-86	ESBL	19	0	1	44	0.95	1.00
Jung et al., 2014 [14]	Germany	Study design: Consecutive blood cultures that were identified to contain Enterobacterales Samples: A total of 100 blood cultures, 20 of which were ESBL	Cultures: Incubated until flagged positive Identification: MALDI-TOF Susceptibility: BD Phoenix automated system Confirmed with PCR	MALDI-TOF-MS	ESBL	15	6	0	64	1.00	0.91
Kim et al., 2019 [45]	Korea	Study design: Spiked blood cultures utilizing various clinical samples from multiple Korean hospitals Samples: 217 previously characterized isolates of Enterobacterales. 63 CR-CPE isolates and 154 non-CPE isolates.	Cultures: Spiked (1.5×10^6 CFU) and incubated until positive Identification: Isolates were previously characterized Susceptibility: Isolates were previously characterized Confirmed with PCR	Fluore-direct	CP-CRE	62	0	1	154	0.98	1.00
Kumar et al., 2018 [30]		Study design: Consecutive Enterobacterales clinical isolates Samples: 208 Enterobacterales isolates, of which 80 were ESBL producers and 128 were non-ESBL producers. Non-fermenters and Gram-positive isolates were excluded from the study.	Cultures: No information provided Identification: No information provided Susceptibility: DDST method, after CLSI.	NDP	ESBL	80	0	0	128	1.00	1.00
Lee et al., 2018 [15]	China	Study design: Consecutive blood cultures from patients with Gram-negative bacterial bloodstream infections. Samples: 200 Gram-negative isolates. Of the samples, 126 were Enterobacterales with 32 ESBL.	Cultures: Incubated until flagged positive Identification: MALDI-TOF MS Susceptibility: Disk diffusion test Confirmed with PCR	MALDI-TOF-MS (MBT STAR-BL)	ESBL	31	0	1	94	0.97	1.00
Morales et al., 2018 [31]	Brazil	Study design: Consecutive blood cultured from patients admitted to University Hospital in Brazil. Samples: A total of 314 blood samples positive for	Cultures: Incubated until flagged positive Identification: Vitek system Susceptibility: Disc-diffusion method according to	Carba-NP Blue-Carba	CP-CRE CP-CRE	24 24	0 0	6 6	281 281	0.80 0.80	1.00 1.00

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Table 1 (*continued*)

Paper	Location	Study description	Reference standard/diagnostic criteria	Assay	Resistance	TP	FP	FN	TN	Sens	Spec
		Enterobacterales were tested. 33 of the isolates were CP-CRE.	CLSI. Confirmed with real-time multiplex PCR.								
Meier and Hamprecht, 2019 [32]	Germany	Study design: Spiked blood culture, isolates from clinical microbiology laboratory of two German University Samples: 140 Enterobacterales isolates, of these, 70 were CP-CRE. In addition, 70 non-carbapenemase-producing isolates served as negative controls.	Cultures: Spiked (1.5×10^3 CFU) blood cultures, incubated until flagged positive Identification: Isolates were previously characterized Susceptibility: Isolates were previously characterized	Beta-Carba	CP-CRE	70	4	0	70	1.00	0.95
Meier and Hamprecht, 2019 [33]	Germany	Study design: Spiked blood cultures with isolates from multiple centres. Samples: 185 non-duplicate Enterobacterales. 104 isolates were CP-CRE which had been characterized in previous studies. In addition, 81 strains served as negative controls, most of them produced ESBL. In addition, 2 quality control strains without beta-lactamase activity were included.	Cultures: Spiked (1.5×10^3 CFU) blood cultures incubated until flagged positive Identification: Previously identified organisms.	Beta-Carba Carba-NP NeoRapid CARB	CP-CRE CP-CRE CP-CRE	104 103 103	4 4 7	0 1 1	77 77 74	1.00 0.99 0.99	0.95 0.95 0.91
Mizrahi et al., 2018 [34]	France	Study design: Consecutive blood cultures from the Hospital Paris Saint-Joseph. Sample: A total of 335 new episodes of blood stream infections caused by GNB for which MALDI-TOF MS was performed, were enrolled during the study. Enterobacterales were the main aetiology with 272 isolated strains. Of those 141 were used for B-Lacta testing. 27 were ESBL	Cultures: Incubated until flagged positive Identification: MALDI-TOF MS Susceptibility: Disk diffusion according to the guidelines of the Antibiogram Committee of the French Society for Microbiology.	B-Lacta	ESBL	25	2	2	113	0.93	0.98
Nordmann et al., 2012	Worldwide samples	Study design: Spiked blood cultures with isolates from various clinical origins (blood culture, urine, sputum, etc.) and of world- wide origin. Samples: 255 strains were used to evaluate the performance of the ESBL NDP test. The panel of strains used for spiking blood cultures included 64 ESBLs and 24 non-ESBL-producing Enterobacterales	Cultures: Spiked (1×10^3 CFU) and incubated until flagged positive Identification: Previously identified and characterized strains at the molecular level Susceptibility: Disk diffusion according to CLSI.	NDP	ESBL	64	0	0	24	1.00	1.00
Oviano et al., 2014 [16]	Spain	Study design: Spiked blood cultures with clinical isolates collected from intensive care unit. Samples: 128 bacterial clinical isolates were seeded in blood culture bottles. Among these 128, 94 isolates were ESBL, 22 non-ESBL, and 12 AmpC. 13 positive blood cultures from patients with real bacteraemia were also included in the study, of which, 10 were ESBL.	Cultures: Spiked (1.5×10^8 CFU) and incubated until flagged positive Identification: MALDI-TOF Susceptibility: Commercial microdilution broth according to CLSI criteria and EUCAST Confirmed with PCR testing	MALDI-TOF-MS	ESBL	103	0	1	37	0.99	1.00
Ovianio et al., 2016 [17]	Spain	Study design: Spiked blood cultures with previously characterized isolates from Spanish and German hospitals. Additional blood cultures from bacteraemic patients were included from A Coruña University Hospital in Spain. Isolates: 119 characterized non-replicate Enterobacterales, *P. aeruginosa*, and *A. baumannii* isolates, of which 81 carried a carbapenemase enzyme. 20 additional blood cultures from CP-CRE bacteraemic patients were also included. Both *P. aeruginosa* and *A. baumannii* were excluded from our analysis (24 samples) leaving 79 CP-CRE and 36 control Enterobacterales.	Cultures: Mixed spiked (1.5×10^8 CFU) and clinical cultures, both incubated until flagged positive. Identification: All spiked blood culture isolates were previously identified and characterized regarding carbapenemase, MALDI-TOF for identification of clinical cultures, Susceptibility: Confirmed with PCR for clinical isolates	MALDI-TOF-MS	CP-CRE	79	0	0	36	1.00	1.00
Pantel et al., 2018 [18]	France	Study design: Spiked blood cultures with previously characterized isolated from a Regional MDR GNB Reference Lab (the CARB-LR group) in the Occitanie Region	Cultures: Spiked (1×10^2 CFU) and incubated until flagged positive Identification: VITEKVR MS and VITEKVR 2 systems.	Accelerate PhenoTest	ESBL CP-CRE	15 43	0 0	2 7	23 23	0.88 0.86	1.00 1.00

		Isolates: A total of 99 Enterobacterales isolates were included of which, 17 were ESBLs and 33 CP-CRE.	Susceptibility: E-test VR method Confirmed with PCR								
Prod'hom et al., 2015 [36]	Switzerland	Samples: Spiked blood cultures from the Lausanne and University Hospital Center. Isolates: 45 spiked blood cultures were tested for ESBL production. 35 were ESBL-producing Enterobacterales	Cultures: Spiked (15 CFU) and incubated until flagged positive. Identification: MALDI-TOF used for Susceptibility: AST-N242 cards phenotypic tests, double disks synergy tests, cefepime ± clavulanate E-tests, and cefotetan ± cloxacillin E-test were used. Confirmed with PCR	Beta-Lacta	ESBL	33	5	2	138	0.94	0.97
Roncarati et al., 2021 [19]	Italy	Study design: Clinical blood cultures positive for Gram-negative rods, unclear from where. Isolates: A total of 185 blood cultures positive for Gram-negative rods. Of which 40 were ESBLs and 50 CR-CPE.	Cultures: Incubated until flagged positive Identification: MALDI-TOF Susceptibility: Double-disk synergy test for the detection of ESBL and combination disk test for CP-CRE in accordance with EUCAST.	MALDI-TOF-MS	ESBL CP-CRE	39 48	1 0	0 2	145 136	1.00 0.96	0.99 1.00
Seco et al., 2019 [37]	Brazil	Study design: Consecutive blood cultures from a clinical microbiology laboratory Samples: A total of 100 consecutive, one per patient, aerobic, and anaerobic blood bottles were included in this study. Of which 95 were Enterobacterales with 14 CP-CRE	Cultures: Incubated until flagged positive. Identification: MALDI-TOF-MS Susceptibility: PCR/sequencing for resistance.	Carba-NP Blue-Carba	CP-CRE CP-CRE	14 14	0 41	0 0	86 45	1.00 1.00	1.00 0.52
Takissian et al., 2019 [44]	France	Study design: Spiked blood cultures with previously identified isolates Isolates: A total of 205 isolates. 132 CP-CRE and 58 non-carbapenemase producers.	Cultures: Spiked (1.5×10^8 CFU) and incubated until flagged positive. Previously characterized strains at the molecular level	NG-test Carba 5	CP-CRE	129	0	3	58	0.98	1.00
Walewski et al., 2015 [38]	France	Study design: Prospective study using all positive Gram-negative blood cultures collected at the Hôpital Européen Georges Pompidou and Hôpital Necker Enfants-Malades [28]. Isolates: 132 blood cultures positive for GNB were tested. These included 7 control cultures inoculated with *E. coli*, *K. pneumoniae*, *Enterobacter cloacae*, and *Citrobacter freundii*. Of the 125 patient cultures, 104 were monomicrobial and 19 multimicrobial. Overall, there were 129 enterobacterial isolates, 23 ESBL. 23 strict aerobic GNB isolates belonging to Pseudomonas, Acinetobacter, Achromobacter, and Aeromonas species which were excluded in this study.	Cultures: Incubated until flagged positive Identification: MALDI-TOF MS Susceptibility: Standard disc diffusion and double-disc synergy tests for detection of ESBLs were carried out according to the recommendations of the Comité de l'Antibiogramme, Société Française de Microbiologie	B-Lacta	ESBL	22	0	1	106	0.96	1.00
Yu et al., 2018 [20]	China	Study design: Spiked blood cultures with clinical isolates collected from Xinhua Hospital. Including bacterial isolates from different body parts. Isolates: 385 blood culture bottles contained Enterobacterales strains from 218 patients. A total of 72 CP-CRE and 313 non-CR-CRE	Cultures: Spiked (3×10^5 CFU) and incubated until flagged positive Identification: MALDI-TOF Susceptibility: AST with Vitek 2 Confirmed with PCR and DNA sequencing	MALDI-TOF-MS	CP-CRE	62	0	10	313	0.86	1.00
Carolis et al., 2017 [21]	Italy	Study design: Prospective blood cultures collected at the Università Cattolica del Sacro Cuore. Only *E. coli* and *K. pneumoniae* isolates were included in rapid phenotypic testing. Isolates: A total of 93 blood cultures that were detected as positive for *E. coli* (58 isolates) and *K. pneumoniae* (35 isolates). Of which 38 were ESBL-producing bacteria.	Cultures: Incubated until flagged positive Identification: MALDI-TOF Susceptibility: AST was performed using Vitek 2 AST cards N201. The MIC results were interpreted according to the EUCAST breakpoints. Confirmed with PCR.	MALDI-TOF-MS	ESBL	33	1	5	54	0.87	0.98

AmpC, AmpC β-lactamases; AST, antibiotic susceptibility testing; CFU, colony forming units; CLSI, clinical and laboratory standard institute; CP-CRE, carbapenemase-producing Enterobacterales; DDST, direct disc susceptibility testing; ESBL, extended-spectrum beta lactamase; EUCAST, European Committee on Antimicrobial Susceptibility Testing; FN, false negative; FP, false positive; GNB, Gram-negative bacilli; MALDI-TOF/MS, matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry assays; MIC, minimum inhibitory concentration; NDP, Nordmann-Dortet-Poirel; PCR, polymerase chain reaction; TN, true negative; TP, true positive.

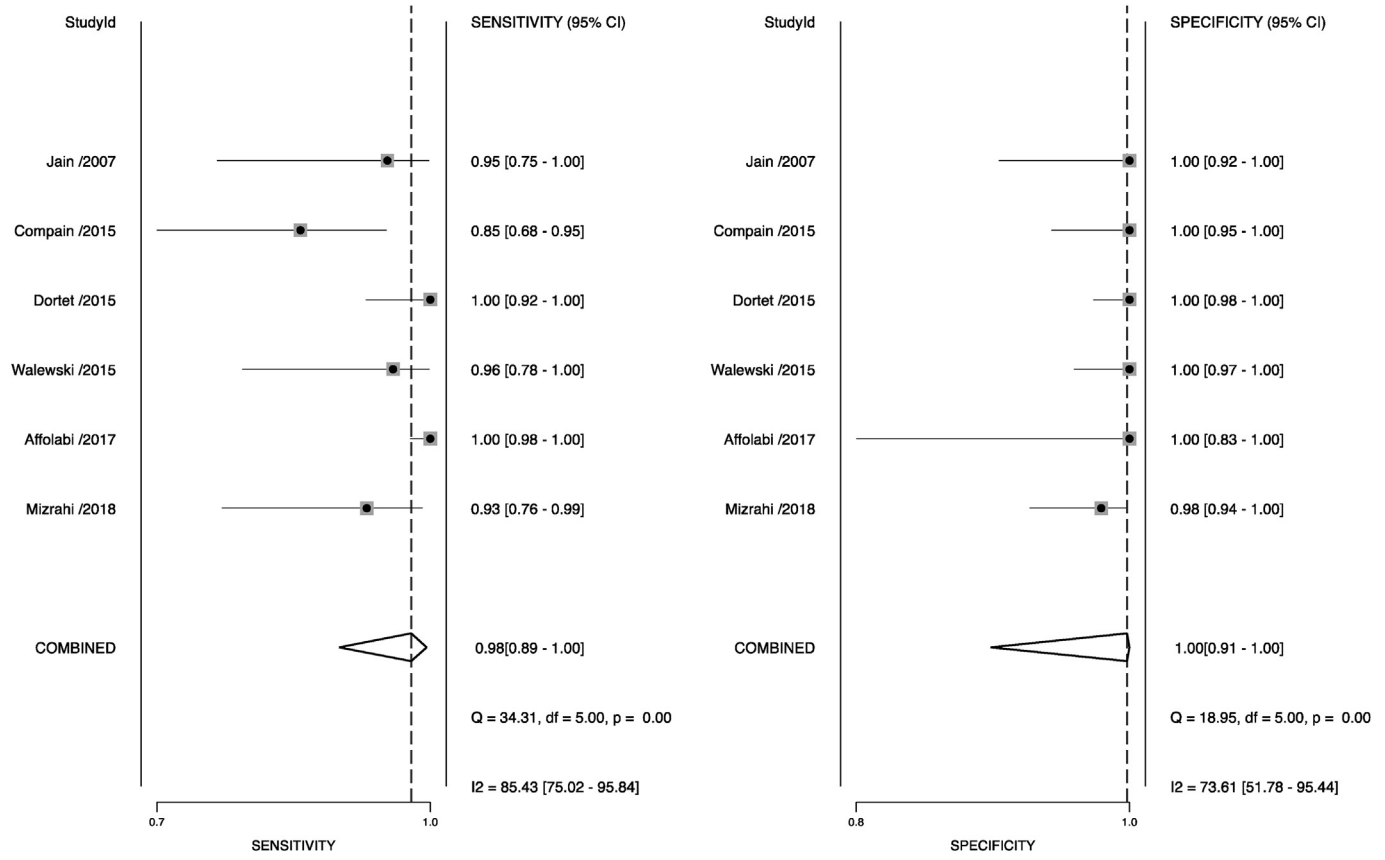

Fig. 2. Forest plot of sensitivity and specificity for the detection of ESBL Enterobacterales utilizing chromogenic testing directly from consecutive blood cultures. Circles in the squares represent the point estimate of each study, whereas the horizontal line represents the 95% CI. The dotted vertical line represents the average point estimate and the diamond shape represents the 95% CI of the average estimate.

and specificity of 99% (95% CI 93–100%) (Fig. S1). Five studies [23,30,35,36,42] utilized chromogenic assays which had an overall pooled sensitivity of 100% (95% CI 99–100%) and specificity of 99% (95% CI 97–100%) (Fig. S2) and two studies [24,42] utilized immunogenic methods with an overall specificity and specificity of 97% (95% CI 94–99%) and 100% (95% CI 97–100%), respectively (Fig. S3).

Diagnostic performance of the different rapid phenotypic tests for CP-CRE

There were a total of eight studies that analysed rapid phenotypic testing for CP-CRE in consecutive clinical samples [9,10,26,29,31,37,41,43], two of which tested two different assays [31,37]. The clinical context in which the samples were drawn are not available for the included studies; however, they were all drawn from inpatients at university hospitals. Of these studies, two utilized MALDI-TOF/MS [9,10] with a pooled sensitivity and specificity of 100% (95% CI 99–100%) and 100% (95% CI 100–100%), respectively (Fig. S4). There were four studies that utilized chromogenic assays [26,29,31,37] with a pooled sensitivity of 96% (95% CI 77–99%) and specificity of 100% (95% CI 96–100%) (Fig. 3). Finally, there were two studies [41,43] that utilized immunogenic assays with a pooled sensitivity of 98% (95% CI 96–100%) and specificity of 100 (95% CI 100–100%) (Fig. S5).

There were a total of 15 studies that analysed rapid phenotypic testing for CP-CRE in spiked blood cultures [8,11,12,17–20,24,27,32,33,40,44–46]. Of these, three studies included both ESBL and CP-CRE [18,19,24] and three studies utilized multiple assays for detection of CR-CPE [31,33,37]. A total of eight studies utilized MALDI-TOF/MS [8,11,12,17–20,46] with a pooled sensitivity of 98% (95% CI 89–100%) and specificity of 100% (95% CI 82–100) (Fig. S6). A total of four studies utilized chromogenic assays [27,32,33,42] with a pooled sensitivity of 99% (98% CI 96–100%) and specificity of 97% (95% CI 92–99%) (Fig. S7). Finally, two studies utilized immunogenic assays [40,44] with a pooled sensitivity and specificity of 96% (95% CI 93–99%) and 100% (95% CI 99–100%) (Fig. S8).

Subgroup of ESBL and CP-CRE E. coli and K. pneumoniae

There were a total of 11 studies included in this subgroup analysis. For detection of ESBL-producing *E. coli* and *K. pneumoniae* directly from consecutive blood samples. One study [15] utilized MALDI-TOF/MS with a sensitivity and sensitivity of 97% (95% CI 83–99%) and specificity of 100% (95% CI 95–100%). Five studies utilizing chromogenic assays with a pooled sensitivity and specificity of 99% (95% CI 95–100%) and 100% (95% CI 93–100%). No studies utilized immunogenic assays.

For detection of CP-CRE *E. coli* and *K. pneumoniae* directly from consecutive blood samples, there were two studies [9,10] that utilized MALDI-TOF/MS with a pooled sensitivity of 100% (95% CI 100–100%) and specificity of 100% (95% CI 99–100%) (Fig. S9) and two studies [29,37] that utilized chromogenic assays with a pooled sensitivity of 96% (95% CI 77–100%) and specificity of 100% (95% CI 97–100%) (Fig. S10). One study [41] utilized immunogenic assay with a sensitivity and specificity of 98% (95% CI 93–99%) and 100% (95% CI 81–100%).

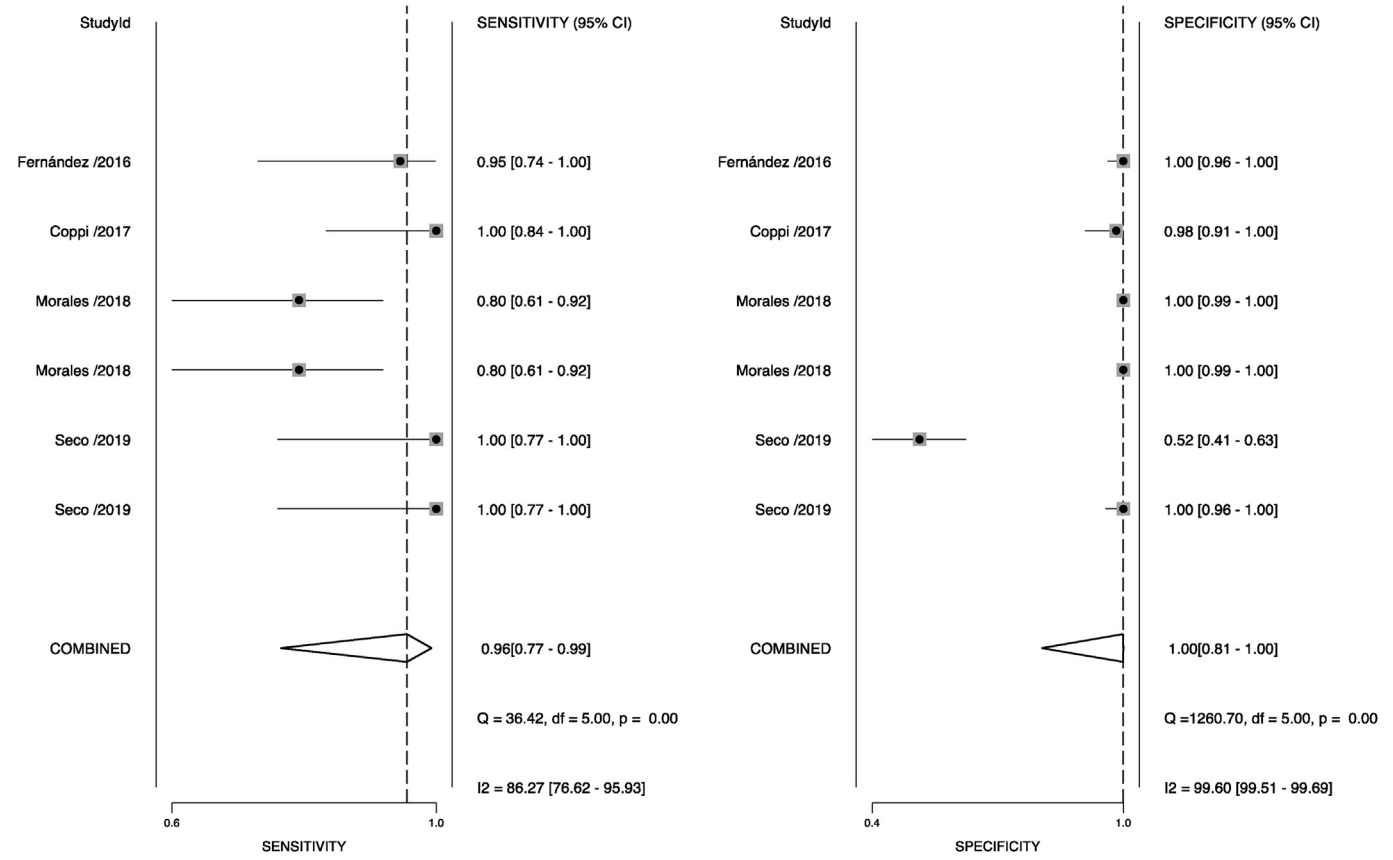

Fig. 3. Forest plot of sensitivity and specificity for the detection of CP-CRE Enterobacterales utilizing chromogenic testing directly from consecutive blood cultures. Circles in the squares represent the point estimate of each study, whereas the horizontal line represents the 95% CI. The dotted vertical line represents the average point estimate and the diamond shape represents the 95% CI of the average estimate.

Discussion

With multi-drug resistant Gram-negative infections becoming increasingly common, the utilization of rapid diagnostic techniques coupled with antimicrobial stewardship interventions has the potential to reduce both morbidity and mortality from undertreatment while also preventing collateral damage from overly broad empiricism. Unfortunately, commonly employed methods for the detection of ESBLs and CP-CRE such as disk diffusion tests, the modified Hodge test, and the modified carbapenem inactivation method are significantly limited by their long turnaround time [50]. Recently, real-time PCR and DNA microarray assays have become commercially available and deployed in many clinical laboratories; however, they are limited by cost, the requirement of a high degree of expertise, the potential for disconnect between genotype and phenotype, and the inability to detect novel unidentified resistance genes [51]. This meta-analysis has shown that phenotypic diagnostic tests offer the potential for rapid detection of ESBL and CP-CRE mediated resistance directly from blood cultures with high sensitivity and specificity.

MALDI-TOF/MS, chromogenic, immunochromogenic, and fluorogenic assays each demonstrated over 95% pooled sensitivity and specificity to detect the ESBL and CP-CRE phenotypes. The ability to perform these assays directly on the positive blood cultures bypasses the need for pure bacterial colony growth. Depending on the organism and strain, this can reduce the time to recognition of the resistance mechanism by more than 12–24 hours minimizing the risk of over and under treatment during the critical earliest phase of sepsis. Subgroup analysis of these rapid phenotypic tests in *E. coli* and *K. pneumoniae*, the most common Gram-negative pathogens in clinical blood cultures, also demonstrated high specificity and sensitivity. A deployment strategy that focused efforts specifically on these organisms could be a manageable and justifiable first step in the clinical laboratory.

Most studies included (52.6%, 28 total) utilized spiked blood cultures for the detection of ESBL or CP-CRE via rapid phenotypic testing. It is important to note that the initial inoculum utilized for spiking blood cultures ranged from 1×10^3 to 1.5×10^8 colony forming units per millilitre (CFU/mL) and all but one study incubated the spiked blood cultures until flagged positive. Given that the average CFU/mL in blood cultures that flag positive is 8.6×10^8 CFU/mL, the bacterial load at time of rapid phenotypic testing should have been similar throughout studies and mirror real life clinical specimens [52]. Although more consecutive blood sample studies are indeed needed, the results from rapid phenotypic testing in spiked blood culture validates the potentially high sensitivity and specificity of these phenotypic assays.

There are several additional limitations to our study. First, we focused on studies including exclusively Enterobacterales, limiting the applicability in clinical settings in which the bacterial order is unknown at the time of assay use. Using 'Hot Chocolate' or 'Smudge' protocols to identify the bacteria with MALDI-TOF directly from the same positive blood culture [53], delays in identification can be minimized and remaining growth re-used for phenotypic detection. Secondly, local epidemiology will dictate the prevalence of resistance and, therefore, the positive and negative predictive values for the assays. This is an important lens through which the application of rapid phenotypic tests must be considered. Furthermore, if third-

generation cephalosporin or carbapenem resistance is present but not due to drug hydrolysis, the performance of these assays in predicting resistance will deteriorate. Similarly, as new ß-lactamases and carbapenemases are identified, the operating characteristics of the assays may need to be re-evaluated to ensure continued adequate negative predictive values. Thirdly, the clinical context in which these blood cultures were taken is missing from the included studies which precludes a more in-depth understanding of how they perform in specific clinical circumstances. Fourthly, the studies included did not have the same reference standards for resistance testing which may lead to discrepant results. Although most studies utilized disc-diffusion assays according to European Committee on Antimicrobial Susceptibility testing criteria, there were a total of 12 studies which utilized automated or PCR methods for susceptibility testing. Finally, given the limited number of studies, we have broadly grouped together assays that use slightly different methods involving a shared underlying mechanism. The operating characteristics of individual assays will need to be assessed in any deployment.

Conclusion

Several phenotypic assays exist that can be employed to detect ESBL and carbapenemase production directly from blood culture specimens containing Enterobacterales. Despite the limited clinical studies evaluating their performance in this context, these assays appear to have high sensitivity and specificity. Such assays could potentially be incorporated in laboratory workflows to increase the appropriateness of empiric therapy and to support antibiotic stewardship and quality improvement initiatives. The impact of such deployments on important patient outcomes remains to be fully realized and further study in this area is encouraged.

Author contributions

Conceptualization—ODC, TCL; methodology—ODC, TCL; validation—ODC, JS, JMH, TCL, AL; formal analysis—ODC, TCL; investigation—ODC, JS, JMH, TCL, AL; resources—TCL, AL; data curation—ODC, TCL, AL; writing—original draft—ODC, TCL, AL; writing—review and editing—all authors; visualization—ODC, TCL; supervision—TCL, AL; project administration—ODC; funding—TCL.

Transparency declaration

The authors declare that they have no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cmi.2023.09.007.

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