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SYSTEMATIC REVIEW OF LABORATORY-BASED METHODS FOR DETECTING EXTENDED-SPECTRUM BETA-LACTAMASE (ESBL)-PRODUCING ENTEROBACTERIACEAE

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Abstract

Rapid and accurate laboratory detection of extended spectrum β lactamase (ESBL) producing Enterobacteriaceae is essential for antimicrobial stewardship and infection control. We systematically reviewed phenotypic and genotypic laboratory methods and rapid assays for ESBL detection across clinical specimens and isolates. Searches identified 12 original studies evaluating chromogenic media, confirmatory disc, Etest approaches, automated systems, MALDI-TOF hydrolysis assays, and lateral-flow immunoassays (LFIA) for CTX-M enzymes. Across chromogenic media, sensitivity typically exceeded 85-95% with notable gains after selective pre-enrichment, albeit at a trade-off in specificity. Combination disc tests and ESBL-Etest configurations performed well, especially when cefepime-clavulanate was incorporated, which improved detection in Enterobacter spp. Automated platforms (Vitek 2) achieved rapid, high agreement versus reference standards in large series. MALDI-TOF-based β-lactam hydrolysis and CTX-M LFIAs provided actionable same-shift results directly from positive blood culture fluid or colonies, facilitating earlier targeted therapy. However, co-production of AmpC or K1 β-lactamases and species-specific traits can mask ESBL phenotypes, underscoring the need for method combinations or reflex molecular testing in selected contexts. Overall, an algorithm that integrates selective culture (with pre-enrichment where appropriate), a clavulanate-based confirmatory test, and a rapid ESBL, CTX-M assay yields timely, reliable detection to inform clinical and infection-prevention decisions.

Keywords: ESBL; Enterobacteriaceae; Laboratory Diagnosis; Chromogenic Media; Combination Disc Test; Etest; Vitek 2; MALDI-TOF; Lateral-Flow Immunoassay; CTX-M.

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INTRODUCTION

Extended spectrum β lactamase (ESBL) producing Enterobacteriaceae compromise third-generation cephalosporins and are linked to worse outcomes and carbapenem overuse. Routine laboratories accurately distinguish ESBL producers from organisms with other resistance mechanisms (AmpC overproduction, inhibitor-resistant β-lactamases), which mask synergy signals and mimic ESBL phenotypes [1]. Contemporary practice depends on harmonized breakpoints and standardized workflows from EUCAST and CLSI; EUCAST v15.0 (2025) is the primary reference for clinical MIC, disk diffusion interpretation in Europe and includes notes on confirmatory testing and reporting [2]. Clinical guidance documents, including IDSA 2024 AMR guidance for ESBL-E infections, emphasize the stewardship impact of fast and reliable ESBL recognition to guide carbapenem-sparing therapy when appropriate [3].

A variety of laboratory approaches exist. Chromogenic ESBL screening media (chromID ESBL, Brilliance ESBL, CHROMagar ESBL) enable color-based presumptive identification and colony selection within 18-24 hours; performance is generally high, yet false positives may occur with Pseudomonas aeruginosa, AmpC-overproducing Enterobacter spp., or Klebsiella oxytoca with chromosomal K1 [4]. Pre-enrichment broths (nonselective or selective with low-level cephalosporin) can substantially increase sensitivity for low-burden carriage detection, particularly in stool or rectal swabs, though at a cost of time and potential specificity loss [5-7].

Clavulanate based confirmatory methods, double disc synergy, combined disc tests, and ESBL Etest configurations, remain robust mainstays, with optimized variants (cefepime-clavulanate) improving detection in Enterobacter spp [1,8].

Vitek 2 system provide rapid classification with good concordance to molecular references in large isolate cohorts [9]. Rapid phenotypic and immunologic assays accelerated detection directly from positive blood cultures: the Rapid ESBL NP test (biochemical hydrolysis with inhibitor) and NG-Test cut turnaround to minutes and have shown high predictive values in prospective evaluations [10-12]. MALDI TOF based β lactam hydrolysis extends same-shift confirmation for cephalosporin hydrolysis, including ESBL producers [13].

Surveillance frameworks (ECDC EARS-Net; regional guidance) and periodic EUCAST updates reinforce the need for validated algorithms tailored to specimen type and local epidemiology [2,6,14]. This review synthesizes evidence from original laboratory evaluations of ESBL detection methods and contextualizes these findings within current guidance and rapid testing advances to propose pragmatic diagnostic pathways.

METHODS

We conducted a systematic search of PubMed, MEDLINE and domain literature for original laboratory evaluations of ESBL detection methods in Enterobacteriaceae, Enterobacterales (phenotypic culture, susceptibility, confirmatory disc, Etest, automated systems, MALDI-TOF hydrolysis, and rapid ESBL, CTX-M assays), along with high-

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quality reviews, guidelines for context. Search strings combined terms for (extended-spectrum beta-lactamase, Enterobacteriaceae, Enterobacterales,) and method keywords (chromogenic agar, pre-enrichment, double-disc synergy, combined disc, Etest, Vitek 2, MALDI-TOF, NG-Test CTX-M, Rapid ESBL NP). Eligible original studies evaluated diagnostic performance (sensitivity, specificity, predictive values, accuracy) or operational impact of laboratory methods on clinical specimens or clinical isolate collections. We excluded non-ESBL targets (carbapenemase-only assays), purely epidemiological prevalence studies without method evaluation, and non-laboratory point-of-care contexts.

Screening proceeded in two stages (title, abstract, then full text) against inclusion criteria. Data were extracted on design, specimens, isolates, index method, reference, comparator(s), key performance metrics, time to result, and notable pitfalls (masking by AmpC or K1).

Risk of bias was considered qualitatively based on sampling strategy, blinding, and appropriateness of reference standards. Given heterogeneity in index tests, specimens, and thresholds, we synthesized findings narratively without meta-analysis.

For contextual discussion and practice implications, we included authoritative reviews and current guidance (EUCAST breakpoint tables and methods notes; IDSA AMR guidance; ECDC, EARS-Net reporting protocol) to align laboratory performance with reporting and stewardship considerations [2-7,10].

The final dataset comprised 12 original laboratory studies used in the Results and 10 discussion, introduction sources used to frame interpretation and practice (listed in the Reference section). All references are verifiable peer-reviewed articles or official guidance; where needed, article details (journal, year, volume, pages, DOI) were retrieved from PubMed, official publisher pages.

RESULTS

1. Chromogenic ESBL Media

chromID ESBL performance. In a clinical evaluation of chromID ESBL medium, Réglier-Poupet et al. found the medium to be both sensitive and specific for rapid presumptive identification of ESBL-producing Enterobacteriaceae, while noting common false-positive scenarios (P. aeruginosa, AmpC overproducers, K. oxytoca with K1) requiring confirmatory testing [4].

Brilliance ESBL agar. Huang et al. evaluated Brilliance ESBL agar for stool carriage screening and reported high sensitivity, selectivity, emphasizing its suitability as a screening medium in clinical labs [15].

Head-to-head comparison and pre-enrichment. In nursing-home stools (n=298), Blane et al. compared chromID ESBL vs Brilliance ESBL, demonstrating comparable sensitivity, selectivity; pre-enrichment with cefpodoxime and 48-h incubation improved sensitivity but reduced selectivity, underscoring the trade-off between yield and false positives [16].

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Multiple studies showed pre-enrichment improves recovery of ESBL carriers: Murk et al. doubled detection using TSB pre-enrichment for rectal, throat swabs [5], and Jazmati et al. confirmed significant gains in clinical stools with selective and nonselective broths [6]. These data support enrichment when colonization burden is low or screening sensitivity is prioritized.

2. Phenotypic Confirmatory Tests (Double-Disc, Combined Disc, Etest)

Modified double-disc synergy (MDDT). Kader et al. assessed an MDDT incorporating cefepime-clavulanate, finding higher sensitivity than NCCLS combination discs and demonstrating that AmpC co-production can mask traditional ESBL signals; cefepime synergy helped unmask ESBLs [8].

Stürenburg et al. evaluated the cefepime clavulanate ESBL Etest strip versus cefotaxime- and ceftazidime-clavulanate strips in a mixed Enterobacteriaceae panel, showing the cefepime-clavulanate configuration improved sensitivity and was effective for Enterobacter spp., though specificity fell in K. oxytoca K1 producers [17].

In a six-method comparison on 206 clinical isolates, Singh & Singh reported the combined disc test had the best agreement with the MIC reference standard and that Vitek 2 provided acceptable performance with faster turnaround than conventional methods [18].

Garrec et al., in a nine-method comparison, indicated how two-step strategies improve accuracy, especially in the presence of Amp C, and that method choice and thresholds matter by species and enzyme background [19].

3. Automated Systems

Spanu et al. evaluated 1,129 Enterobacteriaceae isolates; the Vitek 2 ESBL classification showed 98.1% sensitivity and 99.7% specificity versus molecular references with a median 7.5 h turnaround, supporting its role in routine, rapid ESBL identification and rule-out [20].

4. Rapid Assays from Positive Blood Cultures, Colonies

Boattini et al. prospectively compared three rapid approaches directly on Gram-negative positive blood cultures (n=142; E. coli,K. pneumoniae).

Rapid ESBL NP, NG-Test CTX-M MULTI (LFIA), and direct ESBL Etest all showed high predictive values; NG-Test CTX-M yielded results in 15-20 min and Rapid ESBL NP in 40-45 min, enabling same-shift stewardship action [11].

In a quasi-experimental study, Boattini et al. showed that direct CTX-M detection accelerated targeted therapy adjustments and improved early antimicrobial management for E. coli bacteremia [12]. Bianco et al. validated NG-Test CTX-M MULTI directly from positive blood cultures, reporting rapid, accurate CTX-M detection that complements organism ID for early escalation, de-escalation [21].

Additional evaluations (urine workflows and broader clinical panels) confirm high sensitivity, specificity for CTX-M groups 1, 2, 8, 9, 25, with operational simplicity [22].

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5. MALDI-TOF B-Lactam Hydrolysis

Kawamoto et al. evaluated MALDI-TOF hydrolysis assays (MBT STAR-BL) to detect cephalosporin hydrolysis, successfully identifying ESBL producers and offering a molecular-free, rapid phenotypic confirmation pathway where MALDI-TOF is available [13].

6. Synthesis of Method Performance and Pitfalls

Across studies, screening chromogenic agars offer fast colony selection and high sensitivity; pre-enrichment increases yield for low-level carriage but reduce specificity and adds time. Confirmatory clavulanate-based tests (combined disc, DDST, Etest) is robust; cefepime-clavulanate improves detection with Enterobacter spp. but generate false positives in K1-producing K. oxytoca [8,17,19]. Automated systems (Vitek 2) provide practical throughput with strong accuracy and fast TAT [20]. Rapid ESBL, CTX-M tests (Rapid ESBL NP, NG-Test CTX-M) enable actionable decisions within minutes on positive blood cultures and compare favorably with direct Etest strategies [11-12,21-22]. MALDI-TOF hydrolysis adds same-shift phenotypic confirmation without PCR [13].

Screen with chromogenic medium (with pre-enrichment where sensitivity is critical); confirm with a clavulanate-based test; deploy rapid CTX-M LFIA or ESBL NP directly from positive blood cultures to speed therapy; consider MALDI-TOF hydrolysis as an advanced, rapid phenotypic confirmation, and use reflex molecular testing selectively (discordant phenotypes, outbreaks, epidemiology).

DISCUSSION

This review consolidates performance data for key laboratory methods and aligns them with current guidance. Phenotypic detection hinges on synergy with β-lactamase inhibitors; careful method selection is necessary because AmpC overproduction and K. oxytoca K1 can obscure or mimic ESBL phenotypes [1]. EUCAST breakpoint updates and method notes (2025) emphasize interpreting MIC,disk diffusion with contextual notes and, when indicated, confirmatory testing using disks with or without clavulanate [2,23]. IDSA's 2024 guidance underscores that reliable ESBL recognition informs carbapenem-sparing regimens (piperacillin-tazobactam non-inferiority limitations) and stewardship decisions, making time-to-result a critical laboratory outcome [3].

For colonization screening (rectal, stool), chromogenic agars are efficient first-line tools; consistent with earlier studies, pre-enrichment substantially raises sensitivity for low-burden carriage but can reduce specificity and lengthen workflows [5-7]. Laboratories should tailor enrichment to screening goals (high-risk units, outbreak response) and local prevalence. Where Enterobacter spp. are frequent, cefepime-clavulanate-based confirmation mitigates AmpC masking [8,17,19]. Automated systems (Vitek 2) remain a pragmatic choice for routine labs, balancing accuracy and throughput [20]. In bloodstream infection, rapid methods directly from positive blood culture, Rapid ESBL NP and NG-Test CTX-M MULTI, consistently demonstrated high predictive values with 15-45 min turnaround, and prospective data show measurable impact on antimicrobial management

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[11-12]. While CTX-M is the dominant ESBL family globally, labs must remain aware of non-CTX-M ESBLs; Rapid ESBL NP has the advantage of detecting any ESBL hydrolysis rather than only CTX-M epitopes [11]. MALDI-TOF hydrolysis offers a complementary, rapid, phenotypic route without molecular infrastructure, though it requires instrumentation and expertise [13].

Surveillance and reporting frameworks (ECDC, EARS-Net) and method guidance provide the scaffolding for standardized detection and data quality, and they stress harmonized interpretive criteria across labs [6,24]. Recent reviews also highlight cephalosporin, β-lactamase inhibitor pipeline agents and evolving breakpoints, reinforcing the link between accurate ESBL detection and therapeutic decision-making [25]. The included studies vary in isolate selection, prevalence, and reference standards, limiting direct meta-analysis. Some evaluations focus on specific specimen types (stool, blood culture) or dominant ESBL families (CTX-M).

CONCLUSION

Laboratories can achieve fast and reliable detection of ESBL-producing Enterobacteriaceae by combining complementary methods. Chromogenic media enable efficient screening; selective pre-enrichment boosts sensitivity in low-burden specimens. Clavulanate-based confirmatory tests (combined disc, Etest), particularly with cefepime-clavulanate, address masking by AmpC and improve detection in Enterobacter spp. Automated systems provide rapid routine classification, while rapid ESBL NP and NG-Test CTX-M deliver same-shift answers directly from positive blood cultures, improving therapy timeliness. Where available, MALDI-TOF hydrolysis adds rapid phenotypic confirmation. Aligned with EUCAST, IDSA guidance, this layered algorithm supports stewardship and infection control.

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