significantly improved ZOIs for SE and CA strains ($p < 0.05$). There were no significant ZOI differences between carriers for PSA strains; by D5, no inhibition was observed for PSA with any material. For CA, bioceramics tended to yield larger ZOIs. BOAT demonstrated optical density inhibition for all strains except MRSA exposed to G alone and CA strains.

Conclusion: Both bioceramics and PMMA beads acted as effective carriers; however, greater antimicrobial elution and biofilm eradication during early phases (D1–D5) were observed with bioceramics, while PMMA showed superior activity at later stages (D5–D15).

Keywords: Biofilm, Osteomyelitis, Antimicrobial, Local treatment, Biomaterials.

https://doi.org/10.1016/j.bjid.2026.104660

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DETECTION OF AMPC BLACMY AND BLAACT VARIANTS USING QPCR-HRM IN ENTEROBACTERIACEAE

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Introduction/Objective: AmpC β-lactamase confers resistance to several antibiotics, including penicillins, second- and third-generation cephalosporins, and monobactams, but does not hydrolyze cefepime or carbapenems. Its origin may be chromosomal, inducible in some species after cephalosporin exposure, or plasmid-mediated (pAmpC), characterized by constitutive and abundant expression in different bacteria. This study aimed to develop specific primers for pAmpC gene identification using qPCR-HRM, a technique that combines the sensitivity of qPCR with High-Resolution Melting analysis, enabling differentiation of resistance genes based on melting temperature (Tm), including the detection of mutant variants.

Methods: A total of 148 clinical *Enterobacteriaceae* isolates from hospitals and clinics in Rio de Janeiro (2021–2024) were analyzed. Bacterial identification was performed using VITEK-2 and MALDI-TOF. Antimicrobial susceptibility was determined by VITEK-2 and disk diffusion according to BRCAST (2024). Forty-six β-lactam-resistant isolates were screened for pAmpC genes using conventional PCR, followed by sequencing and analysis in PubMLST and Institut Pasteur MLST databases.

Results: Three pAmpC-producing strains were confirmed: two *Escherichia coli* and one *Klebsiella pneumoniae*, carrying CMY-44, CMY-69, and ACT-5 genes, respectively. These variants had not been previously reported in Brazil. qPCR-HRM showed Tm values of 82.0°C (± 0.5) for CMY in *Salmonella enterica*, 84.0°C (± 0.5) in *E. coli*, and 86.0°C (± 0.5) for ACT in *K. pneumoniae*, allowing clear distinction between CIT and EBC targets.

Conclusion: qPCR-HRM proved to be a rapid (2 h), sensitive, specific, cost-effective, and efficient tool for detecting and differentiating pAmpC genes, even in the presence of mutations. The observed prevalence was 6.52% among β-lactam-resistant strains. These findings underscore the importance of broader surveillance studies to monitor pAmpC epidemiology in Brazil.

Keywords: *Enterobacteriaceae*, AmpC, qPCR-HRM, antimicrobial resistance.

https://doi.org/10.1016/j.bjid.2026.104661

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DIAGNOSTIC STEWARDSHIP: CLINICAL AUDIT OF BLOOD CULTURE REQUESTS MANAGED BY CLINICAL PHARMACY

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Introduction: Early microbiological diagnosis of bloodstream infections is essential to guide therapy, limit unnecessary antibiotic use, and reduce selective pressure. In 2024, the FDA reported a global shortage of BACTEC (BD) blood culture vials, requiring contingency and diagnostic stewardship measures integrated with antimicrobial stewardship programs.

Objective: To describe the design and implementation of a contingency plan based on prospective audit and feedback by the clinical pharmacy team to optimize the use of blood culture vials during the shortage, and to assess its impact on clinical processes and outcomes.

Methods: Conducted in a tertiary pediatric hospital (369 beds; 60% public system coverage), this study established a multidisciplinary technical group (pharmacists, infectious disease specialists, microbiologists, and administrators) to: review literature on blood culture yield and clinical indications; create a clinical decision flowchart for blood culture collection; provide multidisciplinary training led by pharmacists to improve request assessment; and perform prospective audits of requests in real time, evaluating justification, number of vials, requesting units, withholding unjustified requests, and discussing uncertain cases within the task group. Sepsis kits remained exempt from prior authorization.

Results: Historical monthly consumption averaged 938 blood culture collections. A retrospective analysis of 144 requests over 12 days revealed 56.3% noncompliance (routine or admission requests without justification). During the