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Decoding co-production of extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL): a phenotypic observation in routine testing

Dr. Gopal Agrawal[1], Dr. Sarfaraz Ahmed[2], Dr. Nishu Sharma[3]

Professor and Head, Associate Professor, Senior Resident
Department of Microbiology, Indira Gandhi Government Medical College, Nagpur, Maharashtra, India

Antimicrobial resistance among Gram-negative bacilli remains a major challenge in clinical microbiology [1]. We report a phenotypic observation suggestive of the co-production of extended-spectrum β-lactamase (ESBL) and metallo-β-lactamase (MBL) based on routine antimicrobial susceptibility testing.

The culture plate (Figure 1) shows antibiotic susceptibility testing performed using the Kirby–Bauer disk diffusion method on a Mueller–Hinton agar plate [2,3].

The plate features several antibiotic disks, including amoxicillin–clavulanate (AMC), cefotaxime (CTX), aztreonam (AT), imipenem (IPM), imipenem–EDTA (IE), ceftazidime–avibactam (CZA), meropenem–boronic acid (MRB), cefoxitin (CX), and a cefoxitin–cloxacillin screening disk (CXX).

A prominent feature on this plate is the extension of the aztreonam inhibition zone towards the amoxicillin–clavulanate (AMC) disk. This double-disk synergy test (DDST) observation is positive for the production of extended-spectrum β-lactamases (ESBLs) by the bacterial isolate [4,5].

Furthermore, another critical feature observed on the plate is the interaction involving imipenem (IPM) and imipenem–EDTA (IE). There is a distinct enhancement of the inhibition zone around the IE disk compared to the IPM disk alone. The restoration of imipenem susceptibility in the presence of EDTA phenotypically suggests the production of MBL by this isolate [6].

However, no synergy was seen between CTX and AMC, which can be attributed to MBL co-production. This suggests that in case of ESBL and MBL co-production, this confirmatory testing can fail and we should carefully observe the other patterns of enzyme production like AMC–AT combination [7,8].

The CLSI-recommended ceftazidime/ceftazidime–clavulanate (CAZ/CAC) combined disk test also failed to detect ESBL [3]. Further, ESBL and MBL were confirmed by molecular testing.

These findings highlight a classic diagnostic setup for detecting complex, overlapping antibiotic resistance mechanisms. The AMC–AT synergy and the larger IE zone indicate co-production of ESBL and MBL enzymes, necessitating careful interpretation to guide appropriate antimicrobial therapy [8].

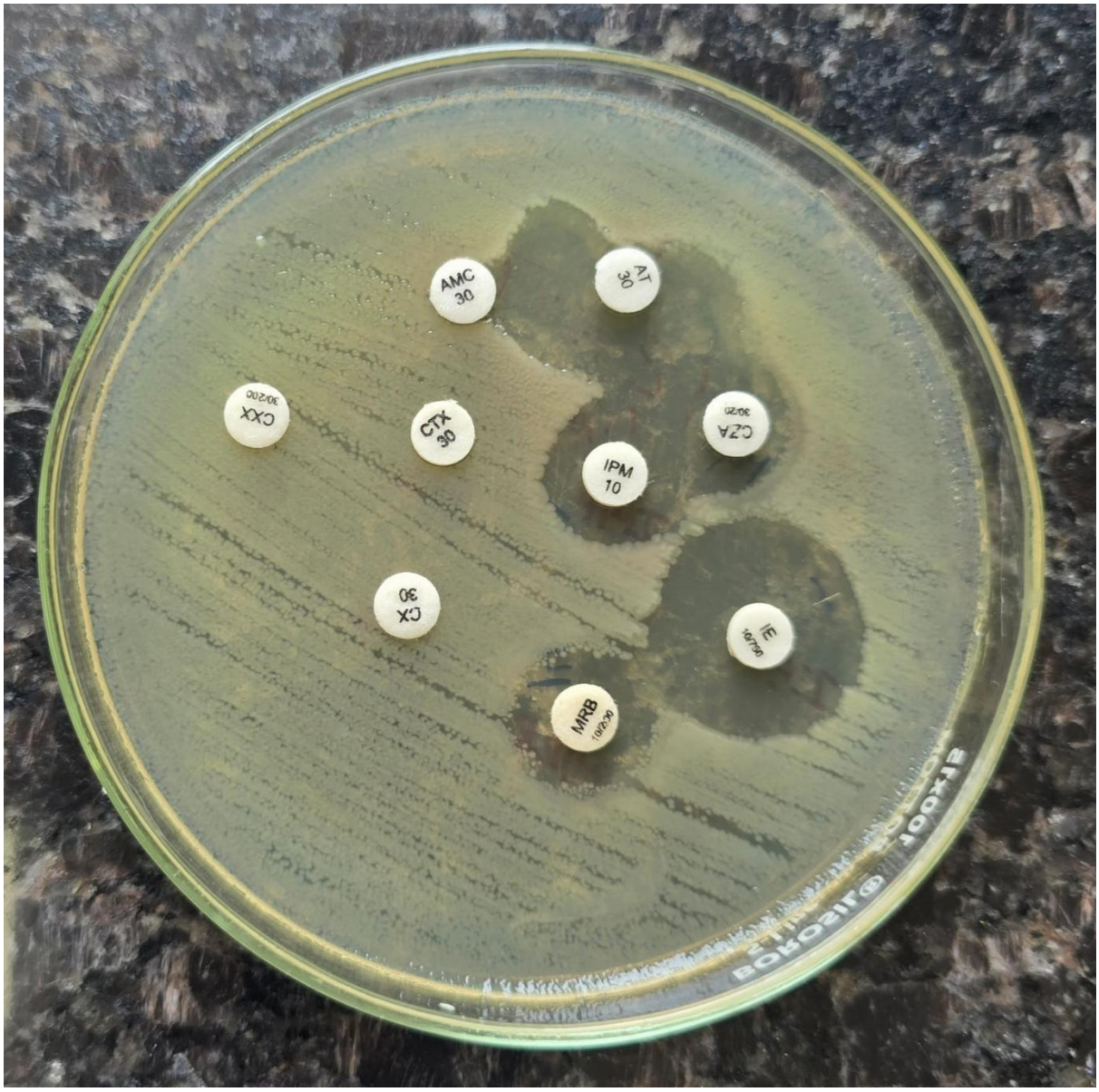


Figure 1. Kirby–Bauer disk diffusion on Mueller–Hinton agar showing phenotypic co-production of ESBL and MBL by *Klebsiella pneumoniae* (pus isolate). The AT disk inhibition zone is distorted and extended towards the AMC disk (keyhole effect), a positive double-disk synergy test for ESBL. The inhibition zone around IE is clearly larger than that around IPM, indicating EDTA-mediated restoration of imipenem activity (MBL). No synergy is seen between CTX and AMC.

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