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BIA
British Infection Association

Quick guide to Gram negative bacilli: antimicrobial resistance mechanisms and testing

March 2022

Useful guidance

- PHE SMI B59: Detection of Enterobacteriaceae producing extended spectrum β-lactamases
- IDSA: Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections: Version 1.0 and 2.0
- EUCAST https://www.eucast.org/resistance_mechanisms/
- PHE Detection of ESBLs and carbapenemases BMS masterclass – antibiotic resistance 2014 (bsac.org.uk)

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ESBLs

- ESBLs are enzymes that hydrolyse most penicillins, cephalosporins, and aztreonam; but not carbapenems.
- CTX-M > SHV > TEM-enzymes.
- Any G neg but most prevalent in ***Escherichia coli, Klebsiella pneumoniae,*** *Klebsiella oxytoca*, and *Proteus mirabilis*
- Level of expression and properties vary. Plus co-presence of additional resistance mechs (efflux, etc) to a broad range of antibiotics. Therefore phenotypes vary.

Screening:

- Cefpodox 10 alone

Or

- Ctx / cro **plus** caz

If I or R…. ESBL confirmation

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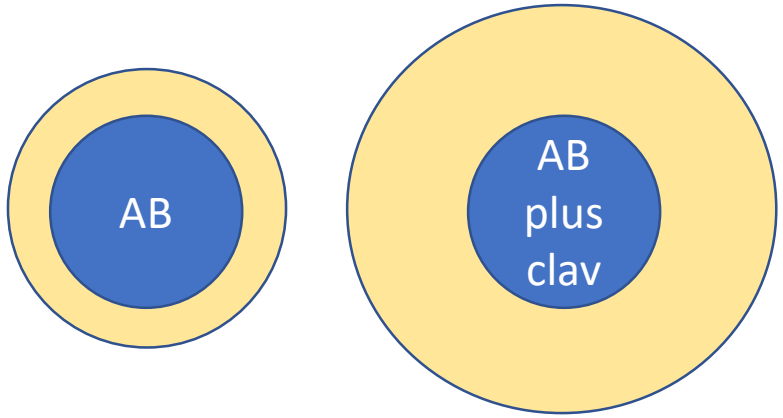
ESBL confirmation: combination disc testing

- ≥5mm increase in zone diameter with clavulanate (usually use an AMC disc);
- PHE SOP = cefpodox for all except Enterobacter spp and C freundii



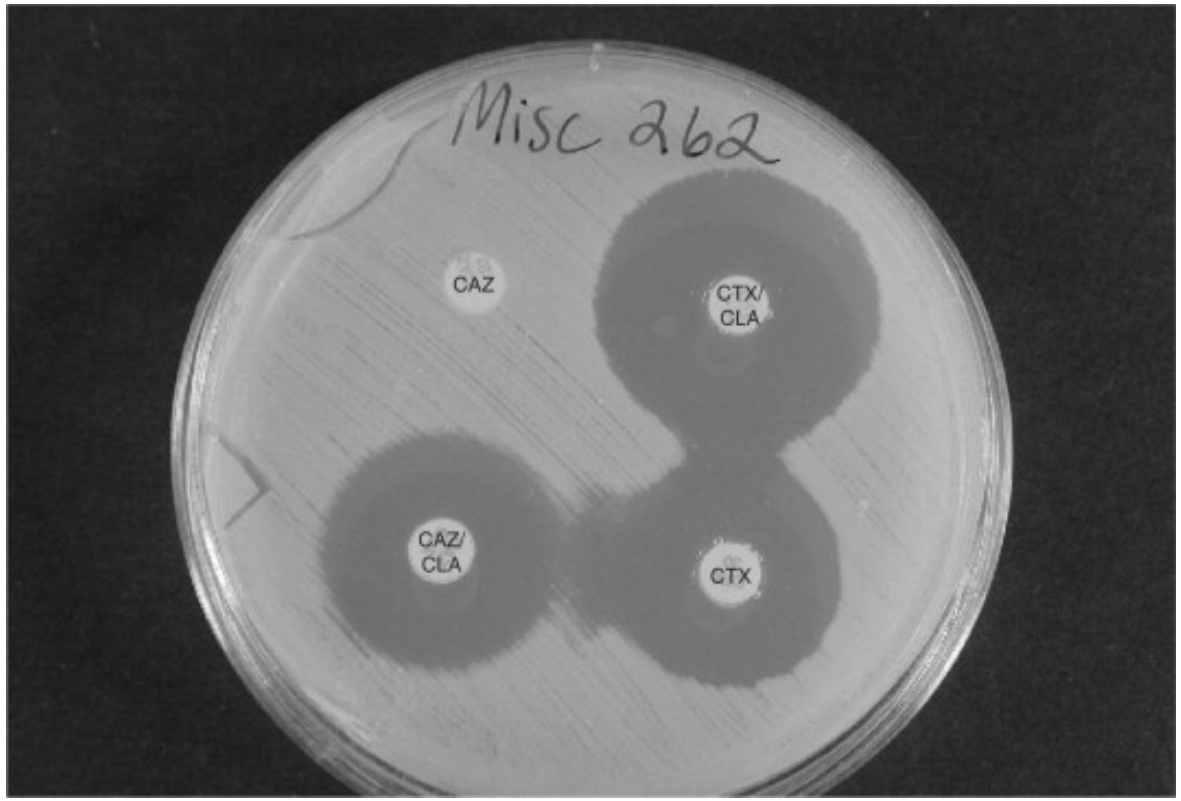


Image c/o https://doi.org/10.1016/j.clinmicnews.2008.04.004

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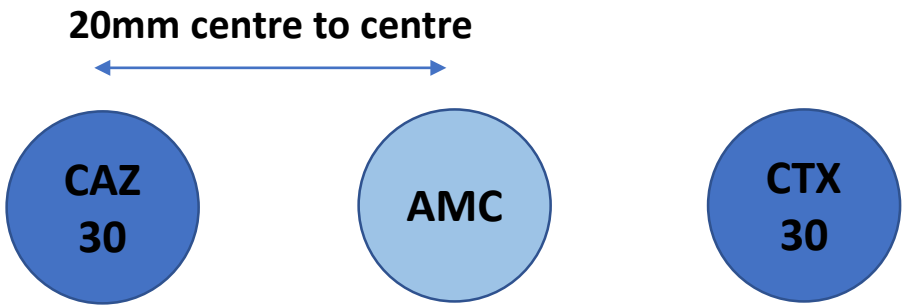
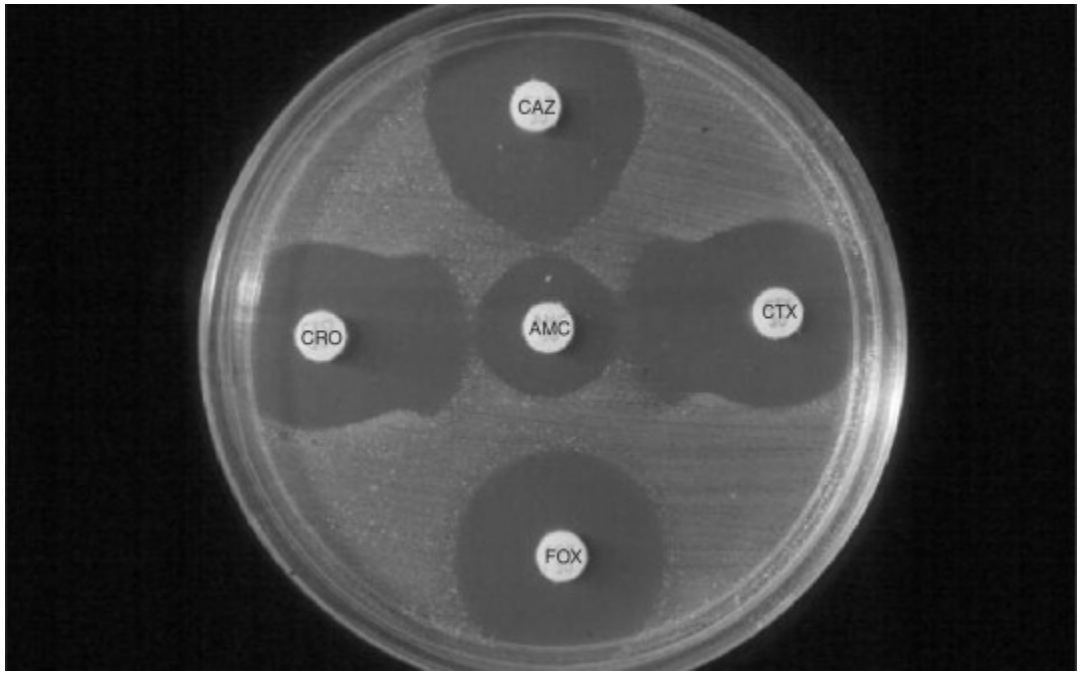
ESBL confirmation: Double-disc synergy





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ESBL confirmation: Gradient test (double-ended e tests)

- CTX vs CTX/L
- and
- CAZ vs CAZ/L
- Phantom zone, deformation of elipse OR MIC reduced ≥3log dilutions by clavulanate.

- Muller-Hinton agar.
- 0.5 McFarland suspension in saline
- Incubate inverted 35^{o} 16-20h
- Confluent lawn of growth needed

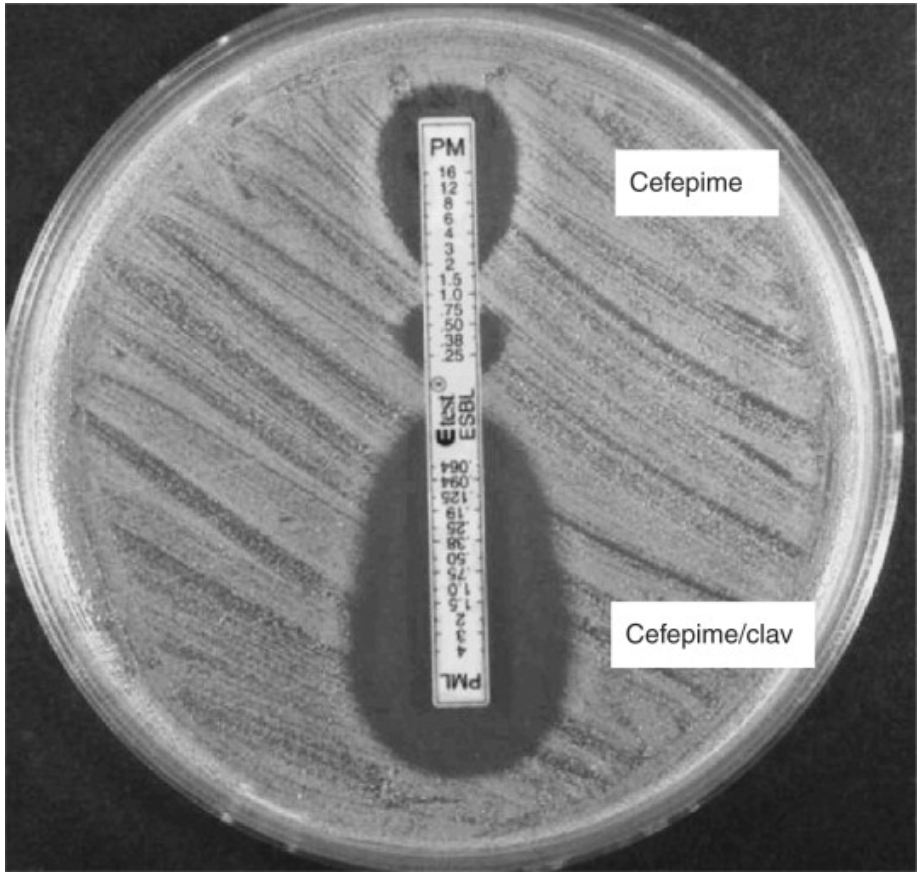

Image c/o https://doi.org/10.1016/j.clinmicnews.2008.04.004

Images c/o AB Biodisk Etest ESBL instructions

Different growth-inhibition patterns:

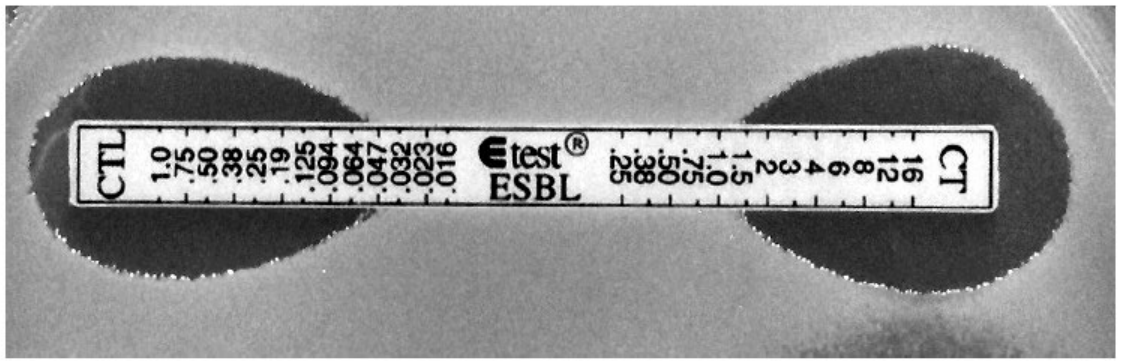

Figure 4. Clear cut ESBL positive:
MIC CT/CTL =1.5/0.047 =32

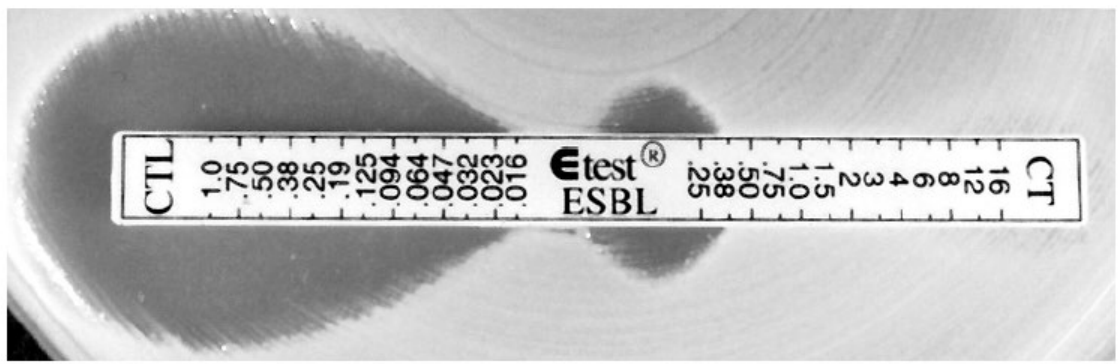

Figure 5. A "rounded" phantom inhibition zone below CT indicative of ESBL.

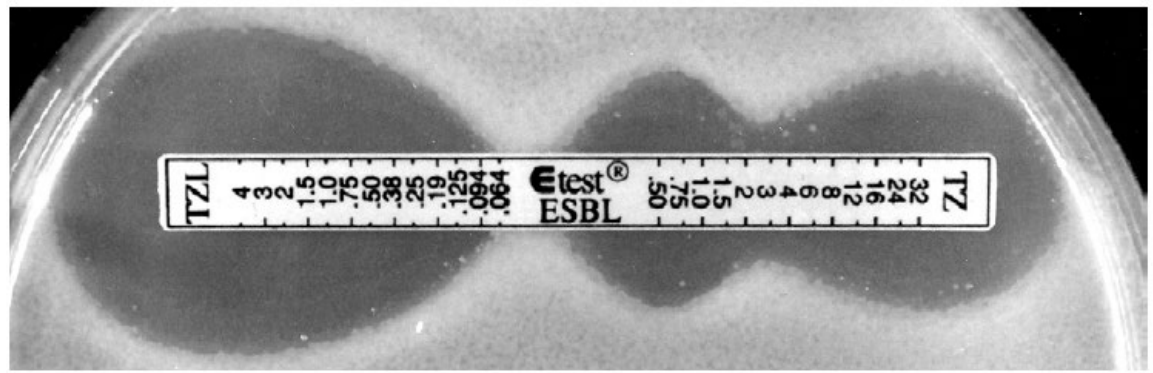

Figure 6. Deformation of the TZ inhibition ellipse indicative of ESBL.

Figure 7. When MIC values are above the test ranges, result is Non-Determinable (ND).

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Amp C βlactamase-producing Enterobacterales

- Class C serine β lactamases
- May be
- Inducible chromosomal
- Constitutive chromosomal (stable de-repression)
- Plasmid-mediated *ampC* (generally constitutive production)
- Inducible is the problem!
- May result in sufficient enzyme in periplasmic space to reduce MIC. Appears initially susceptible on testing, but then becomes non-susceptible after initiating tx. Only a few doses may be enough.

- Which bugs to suspect?
- *Enterobacter cloacae, Klebsiella aerogenes*, and *Citrobacter freundii* are at moderate to high risk for clinically significant inducible AmpC production.
- SO
- Avoid tx with cro, tzp or caz
- USE
- (Cefepime or) carbapenems

IDSA: Read the results as they stand for….

- other organisms historically presumed to be at risk for the development of clinically significant *ampC* expression, such as *Serratia marcescens, Morganella morganii, and Providencia species.*

- They are unlikely to overexpress *ampC* based on both *in vitro* analysis and clinical reports.

- When *S. marcescens*, *M. morgannii*, or *Providencia* spp. are recovered from clinical cultures, the panel suggests selecting antibiotic treatment according to susceptibility testing results.

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AmpC detection

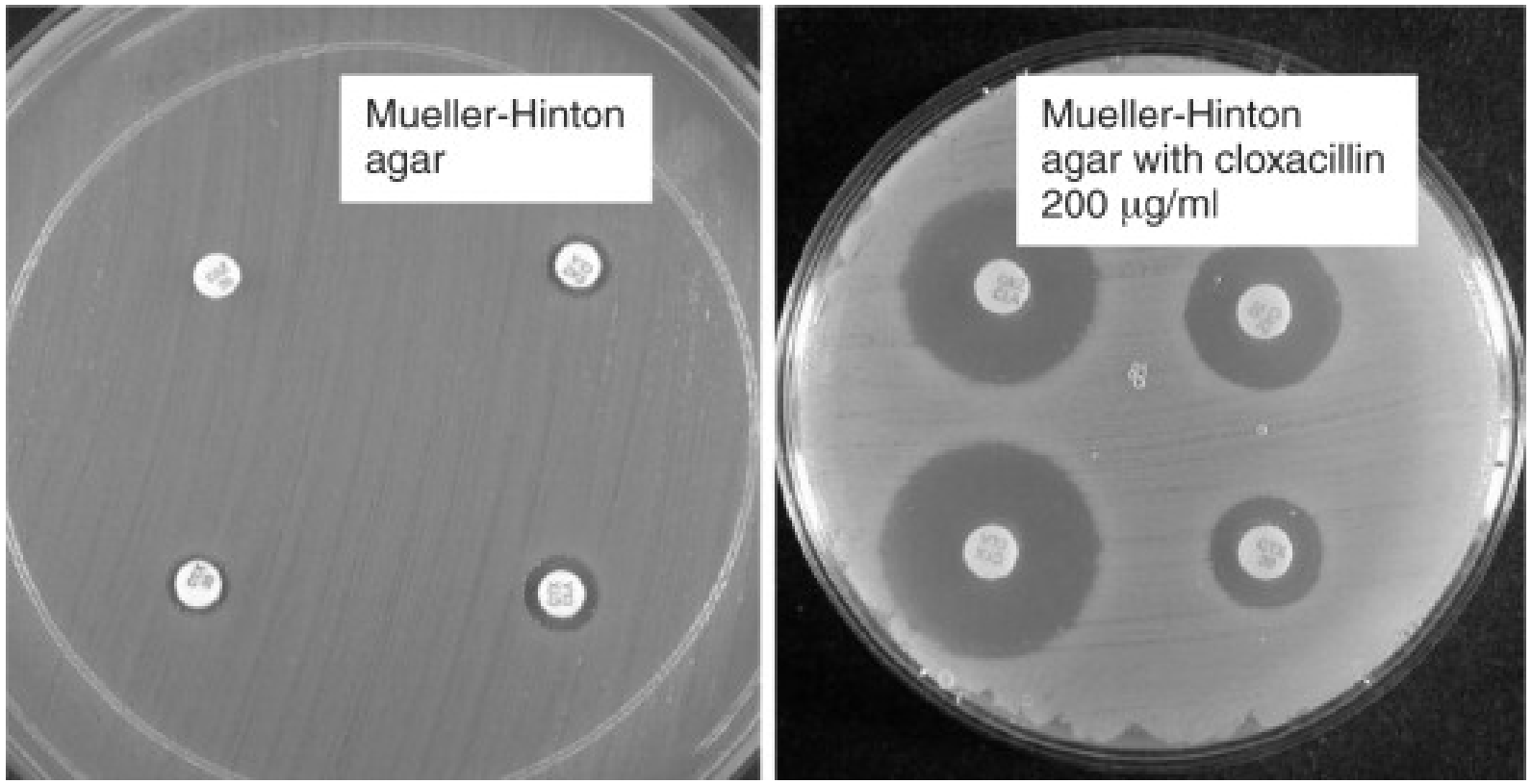
Suspect if:

- CTX R or CAZ R **AND** FOX R
- Then look for cloxacillin synergy:

- AmpC confers cefoxitin resistance (MIC >8mg/L; zone size<19mm).

- Cloxacillin inhibits AmpC. As does boronic acid.

AmpC AND ESBL?



- Use cloxacillin in combination with clavulanate in discs;
- Or incorporate cloxacillin into the MH agar.

Image c/o https://doi.org/10.1016/j.clinmicnews.2008.04.004

Carbapenemases.....

- Intrinsic carbapenemase resistance in *Stenotrophomonas maltophilia* and *Aeromonas* species.
- Most non-fermenters naturally resistant to ertapenem
- *Serratia* species and *Proteeae* intrinsic low-level resistance to imipenem (but not to other carbapenems)

Acquired:

- Class A eg KPC. Hydrolyse carbapenems effectively. Partially inhibited by clavulanate
- Class B = metallo-carbapenemases eg NDM, VIM and IMP. Require zinc ions in active site. Inactivated by chelators such as EDTA.
- Class D eg OXA-48-like.

2 confounders

Not all carbapenemase-producing organisms are resistant to carbapenems

Not all carbapenem resistance is due to carbapenemase production

“Higher MICs are observed when carbapenemase producers also lack major porins.
Among strains with lower MICs and without porin loss there is potential for carbapenemase producers to spread undetected.”

Given this and the diversity of enzyme type plus variable phenotypic expression, there is no single mechanism to reliably detect all carbapenemases.

PHE UK SMI Detection of carbapenemases

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Preliminary detection of carbapenem resistance

Screening swabs:

- UK SMI recommends the use of commercial chromogenic agar for the detection of carbapenemase-producing Enterobacterales.
- An alternative (less preferred) option is to use MacConkey or CLED with an ertapenem disc and a screening cut off of 27mm.

Clinical isolates:

- Use meropenem as indicator carbapenem
- If cannot incorporate mero in 1st line panel, then must use co-amoxiclav
- If AMC R or mero I/S, do full susceptibility testing