Hexa G-Minus 23

HX095

Hexa G-minus 24 is an inert flat circular ring having six discs of 6 mm diameter on its projections. These discs are coated with antibiotics that aid the initial screening of ESBL producers.

Composition

Each ring contains

Antibiotic	Concentration
Aztreonam (AT)	30µg
Cefpodoxime (CPD)	10µg
Cefpodoxime/Clavulanic acid (CCL)	10/5µg
Ceftazidime (CAZ)	30µg
Ceftriaxone(CTR)	30µg
Cefotaxime (CTX)	30µg

Susceptibility Test Procedure:

1.Prepare plates with Mueller Hinton Agar (M173/M1084) for rapidly growing aerobic organisms as per Bauer-Kirby Method. For *Haemophilus* spps use Haemophilus Test Agar(M1259+FD117), for *S.pneumoniae* Muller Hinton Agar supplemented with 5% Sheep Blood is to be used, & for *Neisseria* spps : G.C.Agar +1% defined growth supplement(M434+FD025)is recommended. The medium in the plates should be sterile and should have a depth of about 4 mm.

2.Inoculate 4-5 similar colonies with a wire, needle or loop to 5 ml Tryptone Soya Broth (M011) and incubate at 35-37°C for 2-8 hours until light to moderate turbidity develops. Compare the inoculum turbidity with that of standard 0.5 McFarland (R092) (prepared by mixing 0.5 ml of 1.175% barium chloride and 99.5 ml of 0.36N sulfuric acid). Dilute the inoculum or incubate further as necessary to attain comparative turbidity. Alternatively, the inoculum can be standardized by other appropriate optical method (0.08-0.13 OD turbid suspension at 625 nm)

3.Dip a sterile non-toxic cotton swab on a wooden applicator into the standardized inoculum and rotate the soaked swab firmly against the upper inside wall of the tube to express excess fluid. Streak the entire agar surface of the plate with the swab three times, turning the plate at 60° angle between each streaking. Allow the inoculum to dry for 5 - 15 minutes with lid in place.

4.Apply the discs using aseptic technique.

5.Deposit the rings at the centre of the plate using sterile forceps

6.Incubate immediately at 35 ± 2°C and examine after 16-18 hours or longer, if necessary. For fastidious organisms incubate at appropriate temperature and time.

7.Measure the zones showing complete inhibition and record the diameters of the zones to the nearest millimeter using a calibrated instrument like zone scales (PW096/PW297)

Principle and Interpretation:

ESBLs are enzymes that mediate resistance to extended-spectrum (third generation) cephalosporins (e.g., Ceftazidime, Cefotaxime, and Ceftriaxone) and monobactams (e.g., Aztreonam) but do not affect cephamycins (e.g., Cephoxitin and Cefotetan) or carbapenems (e.g., Meropenem or Imipenem). The presence of an ESBL-producing organism in a clinical infection can result in treatment failure if one of the above classes of drugs is used. ESBLs can be difficult to detect because they have different levels of activity against various cephalosporins. Thus, the choice of which antimicrobial agents to test is critical. If an ESBL is detected, all penicillins, cephalosporins, and Aztreonam should be reported as resistant, even if in vitro test results indicate susceptibility.

CLSI has developed disk diffusion screening tests using selected antimicrobial agents (1). Strains of *Klebsiella* spp. and *Escherichia coli* that produce ESBLs may be clinically resistant to therapy with penicillins, cephalosporins, or Aztreonam,

despite apparent in vitro susceptibility to some of these agents. Some of these strains will show zone of inhibition below the normal susceptible population, but above the standard breakpoints for certain extended spectrum cephalosporins or Aztreonam. Such isolates should be considered as potential ESBL-producer if the initial screen tests results are as follows:

Zones for Cefpodoxime(10µg) ≤ 17 mm
Ceftazidime(30µg) ≤ 22 mm
Aztreonam (30µg) ≤ 27 mm
Cefotaxime (30µg) ≤ 27 mm
Ceftriaxone (30µg) ≤ 25 mm

Proteus mirabilis isolate should be considered a potential ESBL-producer if the initial screen tests results is as follows:

Zones for Cefpodoxime(10µg) ≤ 22 mm
Ceftazidime(30µg) ≤ 22 mm
Cefotaxime (30µg) ≤ 27 mm

The sensitivity of screening for ESBLs in enteric organisms can vary depending on which antimicrobial agents are tested. The use of more than one antimicrobial agent suggested for screening will improve the sensitivity of detection. Cefpodoxime and Ceftazidime show the highest sensitivity for ESBL detection.

CLSI recommends performing phenotypic confirmation of potential ESBL-producing isolates of *K. pneumoniae, K. oxytoca, or E. coli* by testing both Cefotaxime and Ceftazidime, alone and in combination with Clavulanic acid (1). Testing can be performed by the broth micro dilution method or by disk diffusion. For disk diffusion testing, *K. pneumoniae* ATCC 700603 and *E. coli* ATCC 25922 should be used for quality control of ESBL tests (1).

However, the phenotypic confirmatory test does not detect all ESBLs. Some organisms with ESBLs contain other beta-lactamases that can mask ESBL production in the phenotypic test, resulting in a false-negative test. Currently, detection of organisms with multiple beta-lactamases that may interfere with the phenotypic confirmatory test can only be accomplished using isoelectric focusing and DNA sequencing.

If an isolate is confirmed as an ESBL-producer by the CLSI-recommended phenotypic confirmatory test procedure, all penicillins, cephalosporins, and Aztreonam should be reported as resistant. This list does not include the cephamycins (Cefotetan and Cefoxitin), which should be reported according to their routine test results. If an isolate is not confirmed as an ESBL-producer, current recommendations suggest reporting results as for routine testing. Do not change interpretations of penicillins, cephalosporins, and Aztreonam for isolates not confirmed as ESBLs.

Other isolates of *Enterobacteriaceae* such as *Salmonella* species and *P. mirabilis* , and isolates of *P. aeruginosa* produce ESBLs. Though screening of *P. mirabilis* for ESBL production is recommended only when it is deemed clinically relevant (e.g. bacteremic isolate).The decision to perform ESBL screening tests to all urine isolates should be made on an institutional basis, considering prevalence, therapy, and infection control issues

Quality Control

Appearance

Flat circular ring of inert material w/ 6 equidistant arms on the outer periphery ,each arm having a 6 mm disc at the end; each disc impregnated w/ different antibiotics ,w/ corresponding symbols & concentrations printed on the ring.

Cultural response

Average diameter of zone of inhibition is observed on Mueller Hinton Agar (M173) after 18-24 hours incubation at 35-37°C for standard cultures.

Organisms(ATCC)	Antibiotic	Std.Zone of diameter(mm)
Escherichia coli ATCC 25922	Aztreonam AT 30mcg	28 -36 mm
	Cefpodoxime CPD 10 mcg	23 -28 mm
	Cefpodoxime/Clavulanic acid CCL 10/5 mcg	17 -23 mm
	Ceftazidime CAZ 30mcg	25 -32 mm
	Ceftriaxone CTR 30mcg	29 -35 mm
	Cefotaxime CTX 30mcg	29 -35 mm

Staphylococcus aureus ATCC 25923	Cefpodoxime CPD 10 mcg	19 -25 mm
	Cefpodoxime/Clavulanic acid CCL 10/5 mcg	13 -19 mm
	Ceftazidime CAZ 30 mcg	16 -20 mm
	Ceftriaxone CTR 30 mcg	22 -28 mm
	Cefotaxime CTX 30 mcg	25 -31 mm
	Cefotaxime/Clavulanic acid CEC 30/10mcg	29 -36 mm
Pseudomonas aeruginosa ATCC 27853	Aztreonam AT 30 mcg	23 -29 mm
	Ceftazidime CAZ 30 mcg	22 -29 mm
	Ceftriaxone CTR 30 mcg	17 -23 mm
	Cefotaxime CTX 30 mcg	18 -22 mm
Klebsiella pneumoniae ATCC 700603	Aztreonam AT 30 mcg	9 -17 mm
	Cefpodoxime CPD 10 mcg	9 -16 mm
	Ceftazidime CAZ 30 mcg	10-18 mm
	Ceftriaxone CTR 30 mcg	16-24 mm
	Cefotaxime CTX 30 mcg	17-25 mm

Storage and Shelf Life

On receipt discs should always be stored at -20°C under dry conditions, along with the dessicator provided in individual pack. Use before expiry date on the label.

Reference

1.Bauer, Kirby, Sherris and Turck, 1966, Am. J. Clin. Path., 45: 493
2.Performance standards of Antimicrobial Disc Susceptibility Tests, CLSI Vol. 32 No.3, Jan 2012.

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