May 06, 2021

Salmonella spp. antibiotic susceptibility testing by the Kirby-Bauer disk diffusion method

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1 *Works for me* dx.doi.org/10.17504/protocols.io.bpypmpvn

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EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243681

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Delgado-Suárez EJ, Palós-Guitérrez T, Ruíz-López FA, Pérez CFH, Ballesteros-Nova NE, Soberanis-Ramos O, Méndez-Medina RD, Allard MW, Rubio-Lozano MS (2021) Genomic surveillance of antimicrobial resistance shows cattle and poultry are a moderate source of multi-drug resistant non-typhoidal *Salmonella* in Mexico. PLoS ONE 16(5): e0243681. doi: 10.1371/journal.pone.0243681

DOI

dx.doi.org/10.17504/protocols.io.bpypmpvn

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0243681

PROTOCOL CITATION

Enrique Delgado 2021. Salmonella spp. antibiotic susceptibility testing by the Kirby-Bauer disk diffusion method. **protocols.io** https://dx.doi.org/10.17504/protocols.io.bpypmpvn

MANUSCRIPT CITATION please remember to cite the following publication along with this protocol

Delgado-Suárez EJ, Palós-Guitérrez T, Ruíz-López FA, Pérez CFH, Ballesteros-Nova NE, Soberanis-Ramos O, Méndez-Medina RD, Allard MW, Rubio-Lozano MS (2021) Genomic surveillance of antimicrobial resistance shows cattle and poultry are a moderate source of multi-drug resistant non-typhoidal *Salmonella* in Mexico. PLoS ONE 16(5): e0243681. doi: 10.1371/journal.pone.0243681

CREATED

Nov 23, 2020

LAST MODIFIED

May 06, 2021

PROTOCOL INTEGER ID

44783

MATERIALS TEXT

1. Soy tripticase agar (TSA), BIOXON

2. Soy tripticase broth (TSB), BIOXON

3. Muller-Hinton agar (MH), BD

4. BD BBL™ Sensi-Disc™ antimicrobial susceptibility test discs. The choice of antimicrobials to use in the test may vary across research projects. The table below shows the panel of antimicrobials used in our lab up to 2019, and their respective concentrations, for *Salmonella* AST.

Antimicrobial	Concentration, ug
Amikacin	30
Gentamicin	10
Carbenicillin	100
Amoxicillin-clavulanic acid	20/10
Cefotaxime	30
Ceftriaxone	30
Cefepime	30
Imipenem	10
Ertapenem	10
Meropenem	15
Chloramphenicol	30
Trimethoprim-sulfamethoxazole	1.25/23.75
Ciprofloxacin	5
Tetracycline	30

5. Quality control organisms: *Escherichia coli* ATCC 8739, *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923 y *Pseudomonas aeruginosa* ATCC 9027

Refreshment of pure isolates 2d

1 Pick growth from pure isolates of samples to be analyzed and from quality control organisms to be included in the AST test. Transfer to sterile assay tubes containing 10 mL of TSB and place the tubes in a shaking incubator at 35 ºC / 18-24 h / 150 rpm.

2 Using a sterile loop, collect TSB broth and streak onto TSA agar plates. Incubate TSA agar plates at 35 ºC / 18-24 h.

Inoculum standarization and plating in Muller-Hinton (MH) agar

3 Prepare assay tubes containing 4 mL of 0.85-0.9% (w/v) NaCl solution. Sterilize tubes by autoclaving at 121 ºC / 20 min.

4 Using a sterile cotton swab, pick growth from an isolated colony on the TSA agar plate and mix it in the tube containing the saline solution. Discard the used cotton swab.

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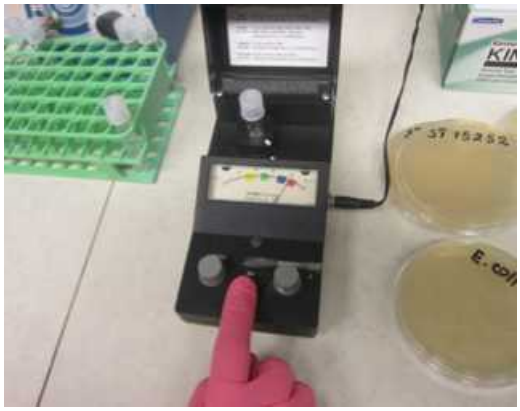
5 Adjust inoculum turbidity to 0.5 McFarland units with the aid of a spectrophotometer. Use an automated pipette to add more saline solution (if turbidity is higher) or a second cotton swab to add more growth (if turbidity is lower). Standardized inoculum shall be processed immediately (or within the first 15 min post-standardization).



6 Bring 150-mm MH agar plates and SensiDics to room temperature before inoculation. To avoid leaving the standardized inoculum standing for more than 15 min, place MH agar plates in an incubator at 35 ºC before starting inoculum standarization. Likewise, take the Sensidiscs out of the refrigerator 2-3 h before standardizing inoculum and let them stand on the benchtop. In that way, both the plates and the disks will be at room temperature timely.

7 Use a sterile cotton swab and dip it into the tube containing the standardized inoculum. Squeeze the cotton swab against the tube's walls to avoid bringing excess liquid to the MH plate. Streak the 150-mm MH plate horizontally, making sure the whole agar surface is covered. Then, rotate the MH plate 60 º and streak again. Rotate the MH plate 60 º a second time and streak again.

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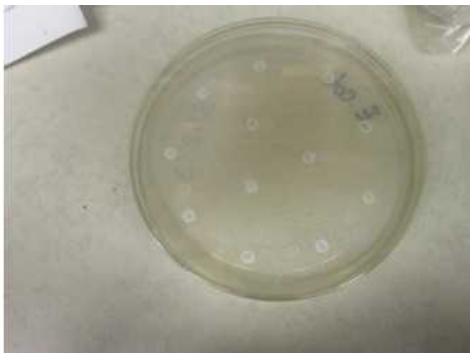
8 Place the discs cartridges in the disk dispenser. Open a MH plate and place it below the dispenser. Press the dispensing knob firmly and all the way down to dispense the disks. After dispensing, visually check the plates to make sure all the disks are in full contact with the MH agar surface. If necessary, use a sterile tweezer to press the top of the disk until the whole disk area touches the agar. Close the MH plate and let it stand for 2-3 min before incubation.



Disk shall be dispensed within 15 min post streaking.

9 Invert MH plates and incubate at 35 ºC / 16-18 h. Incubation shall start not later than 15 min after dispensing the disks.

10 Read the inhibition zone diameter around each disk with a ruler and against a dark background. Record results for each sample and for quality control organisms. The inhibition zones of quality control organisms must comply with CLSI quality control ranges for nonfastidious organisms. Otherwise, the test must be repeated.

11 Compare results with CLSI clinical breakpoints and classify each strain as susceptible, intermediate (I) or resistant (R). The table below summarizes the clinical breakpoints of the antimicrobial panel used in our lab according to the 30 Edition of CLSI M100 Supplement.

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Antimicrobial	Concentration, ug	Zone diameter breakpoint, mm (R)	Zone diameter breakpoint, mm (I)
Amikacin	30	≤14	15-16
Gentamicin	10	≤12	13-14
Carbenicillin	100	≤19	20-22
Amoxicillin-clavulanic acid	20/10	≤13	14-17
Cefotaxime	30	≤22	23-25
Ceftriaxone	30	≤19	20-22
Cefepime	30	≤18	19-24
Imipenem	10	≤19	20-22
Ertapenem	10	≤18	19-21
Meropenem	15	≤19	20-22
Chloramphenicol	30	≤12	13-17
Trimethoprim-sulfamethoxazole	1.25/23.75	≤10	11-15
Ciprofloxacin	5	≤20	21-30
Tetracycline	30	≤11	12-14

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