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Screening protocol for colistin resistance using CHROMagar™ COL-APSE medium

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Soe Yu Naing[1]

[1]Utrecht University

Soe Yu Naing: Project Manager | PhD Candidate

Soe Yu Naing
Utrecht University

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We use this protocol and it's working

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Abstract

Colistin resistance presents a major challenge in combating multidrug-resistant Gram-negative bacteria. To address this concern, a screening protocol employing the selective CHROMagar COL-APSE medium has been developed. This medium enables differentiation between colistin-susceptible and colistin-resistant *E. coli* strains based on growth characteristics and distinct colony coloration. The method is well-suited for screening large numbers of isolates, making it practical for routine surveillance efforts. Its applicability across both low- and middle-income countries (LMICs) and high-income countries (HICs) enhances its global relevance, offering a cost-effective and scalable tool for antimicrobial resistance monitoring. Additionally, a tailored spot-test protocol was developed to complement the CHROMagar screening, providing a rapid and adaptable approach for preliminary identification of resistant isolates.

Materials

- CHROMagar™ COL-APSE medium plates prepared according to the manufacturer's protocol (Appendix: Manufacture leaflet) Plates can be stored for up to 1 month under refrigeration (2/8 °C) if properly prepared and protected from light and dehydration
- fresh 24-hour cultures of the E.coli isolates grown on blood agar
- MCR-1 control strain for quality control purposes.
- Tubes with sterile saline NaCl (0.85%) to prepare 0.5 McFarland suspension
- Vortex
- Densitometer for measuring bacterial density
- Sterile wooden-cotton swab

1.Background

1 Colistin resistance has become a significant concern in the fight against multidrug-resistant Gram-negative bacteria. To address this issue, a screening protocol utilizing CHROMagar™ COL-APSE medium has been developed. This selective agar medium allows for the differentiation of colistin-susceptible and colistin-resistant strains based on growth and distinctive colony colors.

2. Aim

2 To efficiently screen a large batch of *E. coli* isolates obtained from fecal samples for colistin resistance using CHROMagar™ COL-APSE medium.

3. Materials needed

3
- CHROMagar‰ COL-APSE medium plates prepared according to the manufacturer's protocol

Plates can be stored for up to 1 month under refrigeration (2/8 °C) if properly prepared and protected from light and dehydration
- fresh 24-hour cultures of the *E.coli* isolates grown on blood agar
- MCR-1 control strain for quality control purposes.
- Tubes with sterile saline NaCl (0.85%) to prepare 0.5 McFarland suspension
- Vortex
- Densitometer for measuring bacterial density
- Sterile wooden-cotton swab

4. Procedures

4 Note: If the agar plate has been refrigerated, allow to warm to room temperature before inoculation

4.1 Work with batches of strains as a total of 8 strains can be tested per plate

4.2 Include 1 positive control and 1 negative control strain on a plate on the day of testing regardless of the number of test strains

4.3 Make a pie of 8 on CHROMagar plates and label them carefully

4.4 Prepare 0.5 McFarland bacterial suspension from fresh 24-hour cultures of the *E.coli* isolates, grown on blood agar

4.5 Use a wood-cotton swab to immerse into a 0.5 McFarland bacterial suspension, and make sure to remove
excess liquid by pressing and turning the swab against the inside of the tube

4.6 Use the wood-cotton swab to spot inoculate the bacterial suspension on CHROMagar plates
(8 grid as a pie)

4.7 Incubate in aerobic conditions at 35-37 °C for 18-24 hours

4.8 On the next day, check the results and take a photograph of the plates

4.9 Record positive and negatives strains and include the results of the control strains

4.10 If the results of the control strains are not correct, all strains of that day has to be repeated

4.11 In case of any doubt or possible contamination, retest the strain(s)

The scheme for colistin resistance screening using selective CHROMagar COL-APSE medium to detect *E. coli* isolates resistant to colistin

5

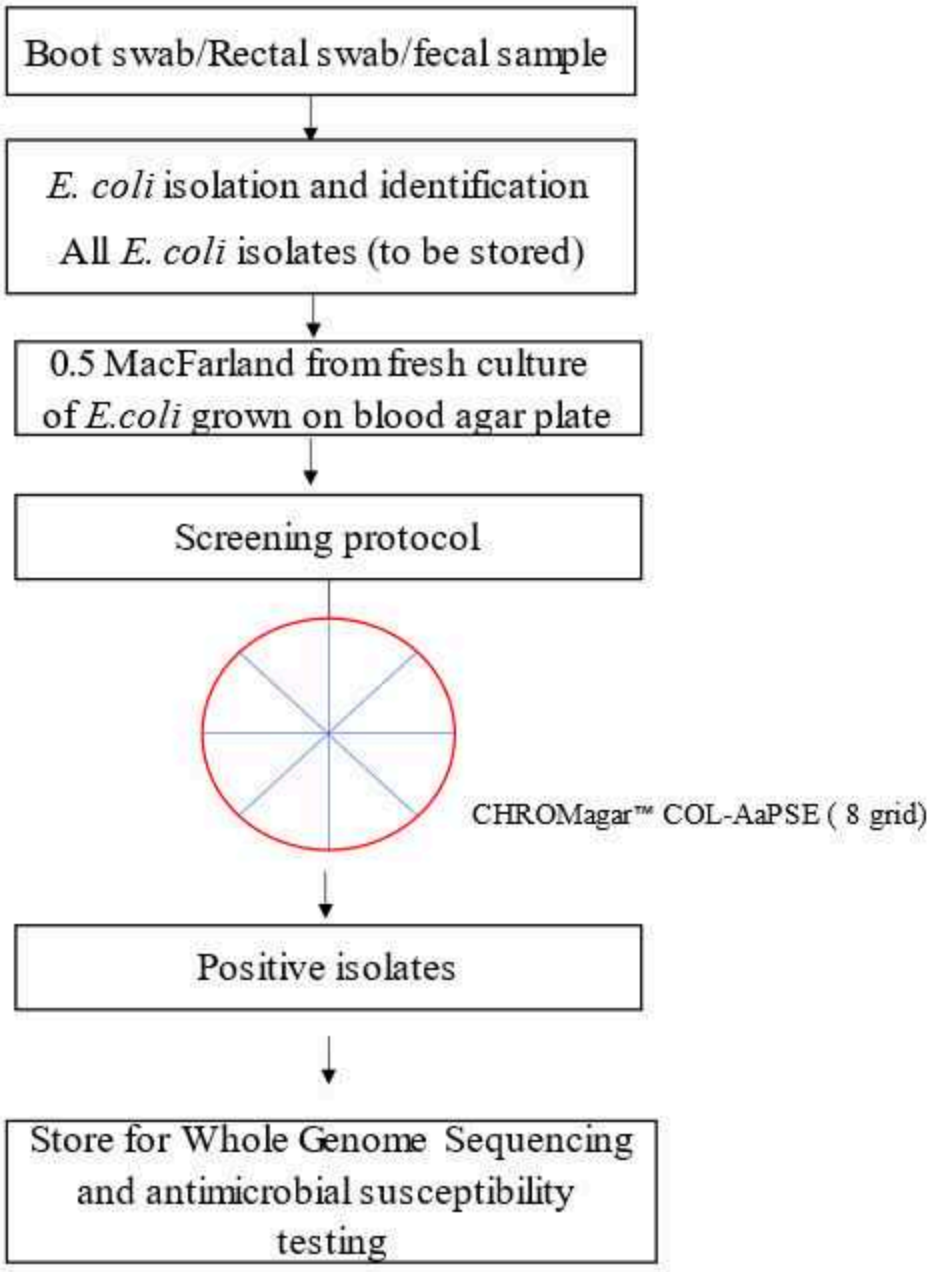

Figure 1. Screening protocol using a selective CHROMagar COL-APSE medium to detect E. coli isolates resistant to colistin.

5. Interpretation of colistin resistance

6 Growth of *E.coli* with dark pink to reddish color (as in figure below) is considered positive. Absence of growth is considered negative.

Example of colony appearance of colistin-resistant *E.coli*

6. Quality Control

7 Please perform Quality Control according to the use of the medium and the local QC regulations and norms. Good preparation of the medium can be tested, isolating the following ATCC strain: *MCR*-1 *E. coli* NCTC 13846 displaying typical colony appearance of dark pink to reddish.

Please perform the calibration of densitometer regularly.

7. Video Protocol

8 **COINCIDE VideoProtocol ColistinScreening Chromagar_Naing**

Protocol references

Abdul Momin MHF, Bean DC, Hendriksen RS, Haenni M, Phee LM, Wareham DW. CHROMagar COL-APSE: a selective bacterial culture medium for the isolation and differentiation of colistin-resistant Gram-negative pathogens. J Med Microbiol. 2017;66(11):1554-1561. doi:10.1099/jmm.0.000602

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