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A novel carbapenem resistance screening method (Carba Plate) shows high specificity and sensitivity against carbapenem resistant Enterobacterials: One Health Sample to Solution strategy

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Background: Carbapenem resistant organisms (CROs) pose an enormous threat to human health as mentioned by World Health Organization (WHO) who has enlisted CRO as one among the major pathogens with critical priority. Detection of carbapenem resistance is not yet accessible in terms of daily or routine use by the hospitals of Low- and middle-income countries because the present methods are inadequate in terms of time required, sophistication, facility required and over all the need for specific and trained human resource for this purpose. The present work designed a sample to solution carbapenem resistance screening method (Carba Plate), optimized and validated its efficacy.

Material and methods:

- 189 isolates from clinical, environment and clinical-environmental origin of *Escherichia coli* (n=125), *Klebsiella pneumoniae* (n=64) which were carbapenem non susceptible was selected for the study.
- *E. coli* ATCC25922 and 25 susceptible isolates were used as negative control. Isolates streaked onto a inhouse developed test medium containing chromogenic substrate of β-galactosidase and carbapenem antibiotic was incubated at 37°C for 16 hrs.
- After overnight incubation, 2 µl of beta-lactamase substrate solution is added over an isolated colony and a color change predicts carbapenem resistance (IP published Application no. 202531039691 A).
- Further Carba NP test and Kirby Bauer disc diffusion was done to calculate specificity and sensitivity of the newly designed media.

Figure 1: *E. coli* grown on MacConkey agar

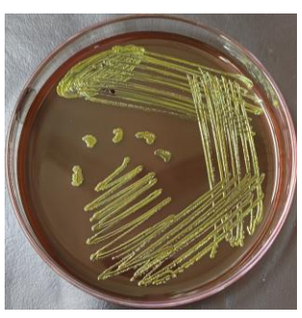

Figure 2: *E. coli* grown on Eosin Methylene Blue agar

Figure 3: Microscopic view of *Enterobacterial*

Results: All the carbapenem non susceptible isolates could be identified by the new screening method and the test was found to be 100% sensitive and specific comparing with disc diffusion method whereas Carba NP test failed to detect non carbapenemase mediated resistance. Formation of red coloration upon addition of the beta-lactamase substrate indicated carbapenemase production. Also, the blue color and colorless colonies could differentiate between lactose and non-lactose fermenters.

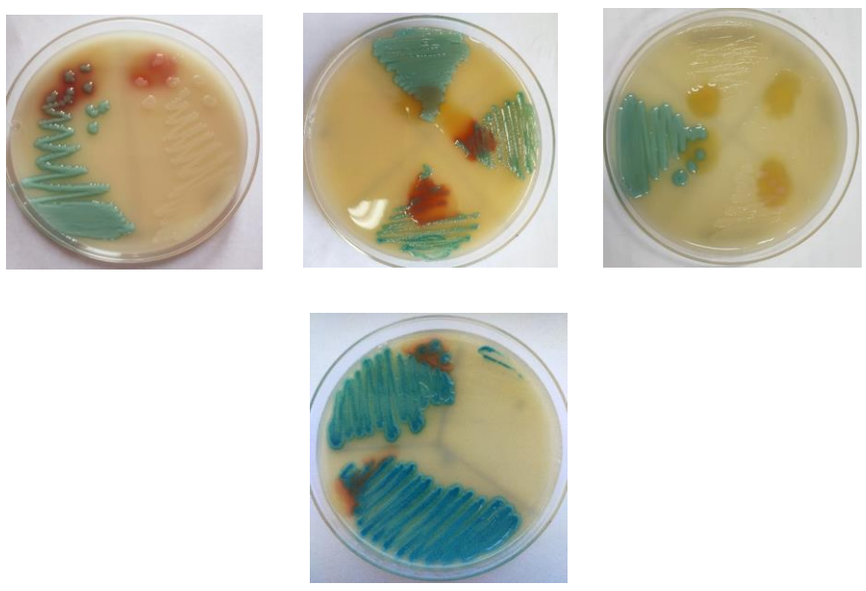

Figure 4: Carbapenem resistance screen agar test pilot validation

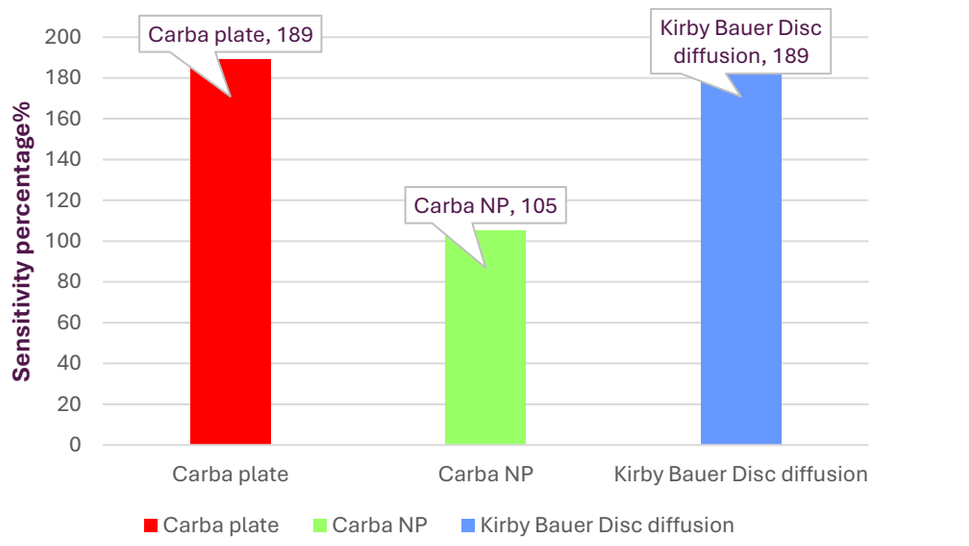

Figure 5:Carbapenem resistance screen agar test with clinical isolates (n=189) Carba NP test and Kirby Bauer Disc diffusion

Conclusion: The present screen agar-based screening method simultaneously differentiates between lactose and non-lactose/ non fermenting bacteria and also carbapenemase or non carbapenemase mediated resistance. The method is simple, deployable in routine diagnostic laboratories of resource limited countries. The screening method can holistically detect carbapenem resistance in all settings in a sample to solution approach thereby reducing diagnostic intervention by 24 hrs.

Reference:

- Novais A, Brilhante M, Pires J, Peixe L. 2015. Evaluation of the recently launched Rapid Carb Blue kit for detection of carbapenemase producing Gram negative bacteria. J Clin https://doi.org/10.1128/JCM.01170-15
- Poirel, L., and Nordmann, P. (2015). Rapidec Carba NP Test for rapid detection of carbapenemase producers. J. Clin. Microbiol. 53, 3003–3008. doi: 10.1128/JCM.00977-15

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