

TITLE: Sodium Salicylate Polymyxin Test: an easy disk diffusion for screening to *Klebsiella pneumoniae*

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ABSTRACT:

Broth microdilution (BMD) is the only methodology available for testing the susceptibility for polymyxins, however, this is an expensive and laborious technique. Disk diffusion (DD) is an easy and low-cost method but is not recommended to polymyxins due to false susceptibility results. Sodium salicylate (SS) is a salt that inhibits the production of *K. pneumoniae* capsules. This study aims to perform a modified disk diffusion method using sodium salicylate to determine polymyxin B susceptibility to *K. pneumoniae*. For this purpose, 26 clinical isolates of *K. pneumoniae* were submitted to a polymyxin B disk diffusion method. In parallel, to improve the test, 5 µL of 1% sodium salicylate solution was added directly under the disc, before incubation. The susceptibility category was compared with the results obtained by cation-adjusted broth microdilution (BMD). CLSI 2016 break points (≤ 11 mm: R and ≥ 12 mm: S) were used in disk diffusion interpretation, while BRCast, 2021 was used in BMD interpretation. Additional D-test-based evaluation was performed to prove no synergic or antimicrobial effect of SS with polymyxin B. Categorical Agreement (CA), Major Error (ME), and Very Major Error (VME) were determined according to FDA guidelines. The polymyxin disc diffusion added to SS (SSDD) showed improved accuracy compared to the original disk diffusion test, increasing their CA from 64% to 92%. ME rates were reduced from 32% to 8%, and VME rates were reduced from 4% to 0. The use of SS in DD reduced the mucoid appearance of clinical isolates of *K. pneumoniae*. This mucoid effect is one of the factors that difficult to read the inhibition halo for polymyxin B, leading to misinterpretation. The excellent categorical agreement observed for the SSDD is a promising advance in screening of polymyxin B susceptibility test for *K. pneumoniae*. SS is low-cost salt and the direct addition to the disk allows its use without the need for changes in commercially available media and the use of ready-made plates. In conclusion, we perform an easy and low-cost test for screening of polymyxin B resistance, for *K. pneumoniae* isolates for use in the clinical routine.

KEYWORDS: Polymyxins Susceptibility Test, Sodium Salicylate Salt, *Klebsiella pneumoniae*, Disk Diffusion.