Time-kill Assay and Etest Evaluation for Synergy with Polymyxin B and Rifampin against Escherichia coli Carrying the mcr-1 Gene

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#38 - ID Week 2018

Abstract

Introduction

The mobile colistin resistance gene (mcr-1), first isolated from an E. coli in a pig in China, was reported by Liu et al in 2015. The mcr-1 gene has been found in various parts of the USA (Fig.1) and can render bacteria resistant to the polymyxins, a last resort drug for multidrug-resistant Gram-negative infections. Being plasma mediated, the gene is mobile, hence able to move from one bacterium to another possibly making multidrug-resistant bacteria also resistant to the polymyxins. This increases the need for new therapeutic approaches. In 2018 Macnair et al showed potentiation of rifampin by polymyxin E (colistin) against mcr-1 positive E. coli using the checkerboard method. We evaluated the combination of polymyxin B and rifampin against 5 mcr-1 positive E. coli using time-kill assay (TKA) and an Etest method.

Methods

- Five clinically unique mcr-1 positive E. coli isolates were obtained from the CDC Antimicrobial Resistance Bank.
- MICs for polymyxin B (PO) and rifampin (RI) were determined in triplicate by Etest and broth microdilution (mean values used).
- Eucast defines PO MIC >2μg/ml as resistant. No Eucast or CLSI breakpoint available for RI.
- Synergy testing with Etest was performed in triplicate (mean value used) by a MIC/MIC method (Pankey et al 2013, *Diag Microbiol Infect Dis*) (Fig. 2). Summation fractional inhibitory concentration (FIC) was calculated for each isolate at 24h:
  - FIC PO + MIC PO with RI
  - FIC PO + MIC PO + MIC RI
  - FIC PO + MIC RI with PO

- Synergy testing by TKA was performed with each drug at 1/2MIC. Synergy was defined as ≤2 log10 decrease in CFU/mL after 24h by the comparison compared to the most potent agent alone (Pillai et al 2005, *Antibiotics in Laboratory Medicine*).

Results

- Etest MICs (μg/ml):
  - polymyxin B, 3-6
  - rifampin, >32
- Broth microdilution MICs (μg/ml): polymyxin B, 2-4
  - rifampin, 6 to >48
- The combination of polymyxin B and rifampin against the mcr-1 positive E. coli isolates demonstrated 100% synergy using both the MIC:MIC Etest method and the time-kill assay (1/2MIC) (Table).

Conclusions

- Synergy was seen using polymyxin B plus rifampin against 5/5 mcr-1 positive E. coli isolates by both the MIC:MIC Etest method and time-kill assay (1/2MIC).
- 100% synergy with each drug at 1/2MIC may enable the use of a lower amount of polymyxin B and rifampin against mcr-1 positive E. coli.
- Further studies with additional mcr-1 (as well as mcr-2-5) positive isolates, including other bacterial genera are needed.
- In vitro synergy may or may not correlate clinically.

Table. MICs (μg/ml) determined by Etest and broth microdilution (BMD) and synergy testing by Etest and time-kill assay for E. coli carrying the mcr-1 gene.

<table>
<thead>
<tr>
<th>MICs</th>
<th>E. coli, mcr-1+ (μS)</th>
<th>Polymyxin B MIC*</th>
<th>Rifampin MIC*</th>
<th>Synergy testing</th>
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<tbody>
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<td></td>
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<tr>
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</tbody>
</table>

*postdetermined in triplicate, mean value used. ZFIC, summation fractional inhibitory concentration; SYN, synergy.