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Zidovudine plus Fosfomycin: synergistic effect against clinical isolates of multidrug-resistant *Enterobacterales*. *In vitro* and *in vivo* evidence.

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BACKGROUND

Multidrug-resistant (MDR) *Enterobacterales* are a priority health issue with few therapeutic options. “Old” antibiotics alongside non-antibiotic molecules with antibacterial properties are being re-evaluated as alternative therapies.

Recently, fosfomycin (FOF) has been reconsidered for infections sustained by MDR microorganisms.

Zidovudine (ZDV), a thymidine analogue licensed for HIV infection, has also antibacterial properties.

AIM

The aim of our study was to assess the effect of the combination ZDV + FOF against clinical isolates of MDR *Enterobacterales,* both *in vitro* and *in vivo*.

RESULTS

ZDV and FOF MICs ranged between 0.06 µg/mL - >64 µg/mL and 0.125 µg/mL - >512 µg/mL, respectively.

When tested with checkerboard assays, a synergistic effect between ZDV and FOF (FIC index ≤ 0.5) was observed in 27 isolates (75%). In the remaining isolates (25%) the combination showed an additive effect (0.5 < FIC index ≤ 1).

In 6 (85.7%) out of 7 FOF-resistant strains (MIC > 32 µg/mL), ZDV administered in combination was able to restore FOF susceptibility.

Time-kill assay confirmed the results found with checkerboard assay, showing rapid bactericidal effect against all isolates (Figure 1a-1e).

In vivo, ZDV + FOF presented greater larval survival (20-50%) than monotherapy (Figure 2a-2d).

These results were confirmed also when metallo-β-lactamase (MBL) producing isolates were tested.

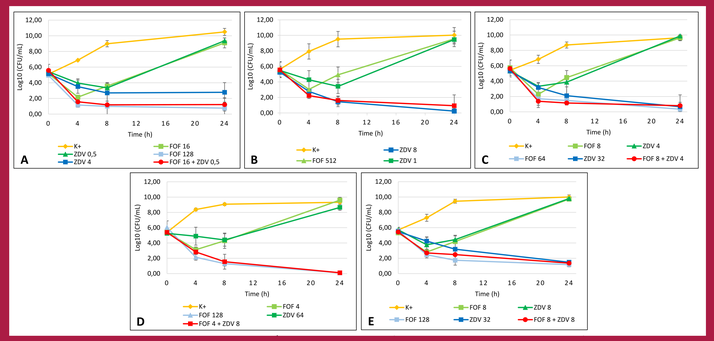

Figure 1a-1e. Time-kill assay, showing Log_{10} CFU/mL at different times (mean value and standard deviation). **1a**: *K. pneumoniae*, KPC. **1b**: *K. pneumoniae*, KPC. **1c**: *K. pneumoniae*, NDM. **1d**: *K. aerogenes*, porin loss. **1e**: *E. cloacae*, porin loss. The combination ZDV + FOF was rapidly highly bactericidal against all tested strains and no bacterial regrowth was observed even after 72 hours (data not shown). FOF susceptibility restoration occurred in all isolates when the two drugs were administered in combination.

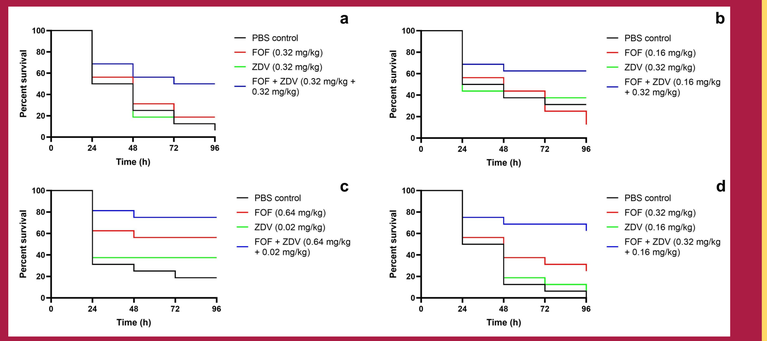

Figure 2a-2d. Survival curves for *G. mellonella* larvae treated with FOF, ZDV and a combination of both agents, post infection with multidrug resistant (MDR strains) of *E. cloacae*, porin loss (a), *K. aerogenes*, porin loss (b), *K. pneumoniae*, KPC (c) and *K. pneumoniae*, NDM (d).

MATERIALS AND METHODS

30 clinical isolates of MDR *Enterobacterales* (12 *E. coli* harbouring mcr, bla_{KPC}, bla_{OXA}, bla_{NDM}, bla_{CTXM}, bla_{HSV}, bla_{TEM}, or bla_{CMY} genes, 16 *K. pneumoniae* harbouring bla_{KPC}, bla_{OXA}, bla_{NDM}, or bla_{VIM} genes, 1 *K. aerogenes*, 1 *E. cloacae*) and 6 Shiga toxin-producing *E. coli* (STEC) were collected from different Italian hospitals.

Minimum inhibitory concentration (MIC) assay of both drugs and checkerboard assay for all isolates were performed.

FOF-resistant strains were evaluated using a time-kill assay and in an *in vivo* model of infection (*Galleria mellonella*).

CONCLUSIONS

This is the first study evaluating ZDV + FOF against clinical isolates of MDR *Enterobacterales* and STEC.

Synergistic activity was observed for ZDV + FOF in 75% of the tested MDR strains. No antagonistic effect was observed.

In vivo experiments confirmed the enhanced effectiveness of the combination compared to monotherapy. Literature data suggest that effective ZDV concentrations can be reached in human serum and urine using the actual licensed dosage, therefore the combination deserves further investigation in clinical setting.

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