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# Rifaximin combined with polymyxins: A potential regimen for selective decontamination of multidrug-resistant bacteria in the digestive tract?

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ABSTRACT

Selective decontamination of the digestive tract (SDD) using combinations of oral non-absorbable antibiotics has been proposed as a means of preventing multidrug-resistant (MDR) infections. The minimum inhibitory concentrations (MICs) of rifaximin (RIFAX) were determined against 262 Gram-negative and Gram-positive bacterial isolates by broth microtitre assay. Rifampicin (RIF) was used as a comparator in the analysis. Synergistic interactions between RIFAX and polymyxin B (PMB) were assessed by using the chequerboard method and calculating the fractional inhibitory concentration index (FICI). The antimicrobial activities of both RIFAX and RIF were similar with little variation in the overall MIC distributions for Gram-negative non-fermenters and Gram-positive bacteria. However, against Enterobacteriaceae higher MICs (>16 mg/L) were observed for RIFAX than for RIF (50% vs 27%). Amongst the 262 isolates tested, 100 could be considered resistant to RIFAX. Overall, the combination of RIFAX and PMB was more active against all of the isolates tested compared with either drug alone, with reductions of 2–11 doubling dilutions in individual MICs. Potent synergy was observed with the RIFAX + PMB combination using FICI criteria (FICI range 0.02–0.5). The data presented here suggest that combination therapy may be significantly more effective against isolates with RIFAX and/or PMB resistance and could be considered as part of a SDD regimen aimed at reducing enteric carriage of MDR pathogens in colonised and infected patients.


## 1. Introduction

Many bacterial infections arise endogenously from the normal flora of the gastrointestinal tract. Acquisition and colonisation of the human gut by multidrug-resistant (MDR) strains either in hospitals, through travel, due to antibiotic exposure or in early life may have significant implications for treatment. Selective decontamination of the digestive tract (SDD) using combinations of oral non-absorbable antibiotics has been proposed as a means of preventing MDR infections [1]. A systematic review concluded that the use of SDD can reduce the incidence of respiratory tract infections as well as overall in-hospital mortality [2]. Pooled data from 36 clinical trials involving 6914 patients demonstrated that both systemic and topical regimens reduce the rate of respiratory tract infections and lower mortality in patients receiving treatment in intensive care units (ICUs). Other evidence suggests that SDD regimens have the potential to prevent between 2000 and 3000 deaths per annum in individuals hospitalised in the UK alone [3].

Although a number of antimicrobials have been used in SDD trials (polymyxins, glycopeptides, aminoglycosides, amphotericin), there is little consensus on the optimum combination of drugs to use, particularly in individuals colonised with MDR strains. There is a need to identify a regimen that may be effective against those colonised with bacteria belonging to the ESKAPE group of pathogens (*Enterobacter*, *Staphylococcus aureus*, *Klebsiella*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterococcus*) [4]. These

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organisms are responsible for the majority of nosocomial infections and display high levels of antimicrobial resistance, including the production extended-spectrum β-lactamases (ESBLs) and resistance to carbapenems [5].

Rifaximin (RIFAX) is a semisynthetic rifamycin derivative. It is poorly absorbed in the human gut and is licensed to treat travel-associated diarrhoea due to enterotoxigenic strains of *Escherichia coli* and other enteric pathogens [6]. It is effective in reducing bacterial overgrowth in the small intestine [7] and has also been used successfully in the treatment of *Clostridium difficile* infection [8]. It has orphan drug status in the adjunctive treatment of hepatic encephalopathy [7].

Polymyxins, which have activity primarily against Gram-negative bacteria, are also poorly absorbed and have been used in SDD regimens [9]. In SDD, polymyxin E (colistin) in combination with either oral gentamicin or neomycin has been shown to be effective in the eradication of ESBL-producing Enterobacteriaceae and carbapenem-resistant *Klebsiella pneumoniae* [10,11] from colonised patients. There is also evidence that polymyxins significantly enhance the activity of other antimicrobials that have little or no activity alone. Colistin combined with rifampicin (RIF) has also been proposed as a combination therapy for the treatment of systemic MDR *A. baumannii* and *P. aeruginosa* infections [12,13].

In this study, we investigated the in vitro activity of RIFAX combined with polymyxin B (PMB) against a diverse collection of strains that would need to be targeted in any regimen based on exploiting the properties of these drugs in any future SDD therapy aimed at tackling the problem of MDR bacteria.

## 2. Methods

### 2.1. Bacterial isolates, antimicrobials and media

Bacterial type strains were obtained from the National Collection of Type Cultures (NCTC), Public Health England (Colindale, UK). Clinical isolates were sourced from Barts Health NHS Trust (London, UK) and the existing collection held at Queen Mary University London (Antimicrobial Research Group). *Staphylococcus aureus* strains with reduced susceptibility to glycopeptides (GISA) were obtained from the Network for Antimicrobial Resistance on *Staphylococcus aureus* (Network for Antimicrobial Resistance in *Staphylococcus aureus*, USA). Identification and routine susceptibility testing of clinical isolates was performed according to standard laboratory protocols. RIFAX was purchased from Santa Cruz Biotechnology Inc. (Heidelberg, Germany), polymyxin B sulphate was from VWR International Ltd. (Leighton Buzzard, UK) and RIF was from Sigma–Aldrich (Dorset, UK). All bacterial culture media were sourced from Oxoid Ltd. (Basingstoke, UK) or Sigma–Aldrich.

### 2.2. Antimicrobial susceptibility testing

Minimum inhibitory concentrations (MICs) of RIFAX were assessed by broth microdilution (BMD) assay in cation-adjusted Mueller–Hinton II broth (CA-MHB) according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines [14]. The MIC of RIFAX was determined against 262 bacterial isolates, including 200 Gram-negative isolates [*E. coli*, $n = 27$; *K. pneumoniae*, $n = 38$; miscellaneous Enterobacteriaceae (*Serratia marcescens*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Morganella* spp. and *Citrobacter* spp.), $n = 35$; *A. baumannii*, $n = 36$; *Stenotrophomonas maltophilia*, $n = 32$; and *P. aeruginosa*, $n = 32$] and 62 Gram-positive bacterial strains [*S. aureus* (meticillin-susceptible *S. aureus*, meticillin-resistant *S. aureus* (MRSA) and vancomycin-intermediate-resistant *S. aureus*), $n = 28$; *Streptococcus* spp. (*Streptococcus pyogenes*, *Streptococcus agalactiae* and *Streptococcus sanguinis*), $n = 9$; *Enterococcus faecalis* and glycopeptide-resistant *E. faecium*, $n = 25$].

As no clinical breakpoints have been proposed by either EUCAST or the Clinical Laboratory Standards Institute (CLSI) to infer susceptibility to RIFAX, the MIC of RIF was also determined for each isolate and was used as a comparator in the analysis of RIFAX MIC distributions.

### 2.3. Rifaximin and polymyxin B synergy studies

The potential for synergy between RIFAX and PMB was investigated against 31 type strains and MDR isolates with defined mechanisms of resistance, including *E. coli*, *K. pneumoniae*, *A. baumannii*, *S. marcescens*, *S. maltophilia*, *P. aeruginosa*, *E. cloacae* and *E. aerogenes* producing KPC, VIM-2/4, NDM and OXA-23/48/181 carbapenemases, *S. aureus (mecA/vraSR)* and enterococcal isolates (*vanA/B)* with meticillin resistance and reduced susceptibility to glycopeptides, and antibiotic-susceptible strains of *S. pyogenes* (Table 1).

Activity of the RIFAX + PMB combination was then assessed in chequerboard assays by BMD in CA-MHB. The MICs of each drug alone and in combination were recorded and interactions were assessed by calculation of the fractional inhibitory concentration index (FICI) [15]. An FICI of $\leq 0.5$ was defined as synergy, FICIs of $>0.5$ to $\leq 4.0$ were deemed intermediate/additive, and an FICI of $>4.0$ was considered antagonistic.

As there are no established breakpoints for defining susceptibility to RIFAX, a breakpoint value of $\leq 16$ mg/L suggested by the French Society for Microbiology (SFM) [16] for determining susceptibility/resistance of *Acinetobacter* spp. to RIF was used in the interpretation of MICs. For Gram-positive bacteria, the EUCAST RIF staphylococcal/streptococcal species-specific breakpoint of MIC $\leq 0.06$ mg/L [14] was used to infer susceptibility. The EUCAST breakpoint of $\leq 2$ mg/L for colistin sulfate was used in the interpretation of susceptibility to PMB for all Enterobacteriaceae and other Gram-negative species [14].

## 3. Results and discussion

The antimicrobial activities of RIFAX and RIF were similar with little variation in the overall MIC distributions for Gram-negative non-fermenters (GNNFs) and Gram-positive bacteria (Figs. 1 and 2). However, against Enterobacteriaceae higher MICs (>16 mg/L) were observed for RIFAX than for RIF (50% vs 27%). Amongst the 262 isolates tested, 100 could be considered resistant to RIFAX (GNNFs, $n = 23$; Enterobacteriaceae, $n = 50$; and Gram-positives, $n = 27$) based on the breakpoints for *A. baumannii* [16], *S. aureus* and *Streptococcus* spp. [14].

Analysis of MIC distributions revealed that the majority of GNNFs (Fig. 2) had a RIFAX MIC between 1 mg/L and 16 mg/L. This is comparable with current data on the antimicrobial activity of RIF against *A. baumannii* [17]. Both $MIC_{50}$ and $MIC_{90}$ values were lowest for *S. maltophilia* isolates (RIF/RIFAX $MIC_{50} = 8/8$ mg/L, $MIC_{90} = 16/64$ mg/L). The MIC ranges for *P. aeruginosa* (RIF/RIFAX $MIC_{50} = 16/16$ mg/L, $MIC_{90} = 32/128$ mg/L) were narrower than those for *A. baumannii* (RIF/RIFAX $MIC_{50} = 2/1$ mg/L, $MIC_{90} = 256/256$ mg/L).

Against Enterobacteriaceae, the MIC distribution was narrower than that observed with GNNFs, with the majority of strains requiring 4–256 mg/L (RIF/RIFAX $MIC_{50} = 16/16$ mg/L, $MIC_{90} = 32/256$ mg/L). This is not surprising as resistance to both RIF and RIFAX has been previously reported in *E. coli* owing to chromosomal mutations in *rpoB* and active efflux of the antibiotic [18,19].