Resistance to rifaximin among third-generation cephalosporin-resistant Enterobacteriaceae isolates obtained during a German multicentre hospital admission screening project - a DZIF-ATHOS cooperative study

Philipp Baumert¹, Alexander Mischnik*², Sabine Schuster³, Martina Vavra¹, Axel Hamprecht⁴, Harald Seifert⁵, Petra Gastmeier⁶, Winfried V. Kern¹

¹University Hospital & Medical Center, Freiburg, Germany
²University Medical Center Freiburg, Freiburg, Germany
³University Hospital Freiburg, Medicine, Infectious Diseases and Travel Medicine, Freiburg, Germany
⁴University Hospital Cologne, Institut Für Mikrobiologie, Immunologie Und Hygiene, German Centre for Infection Research (Dzif), Köln, Germany
⁵Institut Für Medizinische Mikrobiologie, Immunologie und Hygiene, Uniklinik Köln, Köln, Germany
⁶Institute of Hygiene and Environmental Medicine, Charité-University Medicine Berlin, Berlin, Germany

Background: Rifaximin (Rfx), a non-absorbable rifamycin, is increasingly being used for intestinal microbial suppression or decolonization making studies of the prevalence of Rfx resistance among enteric bacteria important. In a multicenter survey for hospital admission prevalence (rectal swabs) of third-generation cephalosporin-resistant Enterobacteriaceae (3GCRE) we identified 19/339 E.coli (6%), 14/39 Klebsiella spp. (36%) and 16/29 other Enterobacteriaceae (55%) with an MIC of rifaximin (Rfx) >64 µg/mL as duplicate-tested in a custom microplate dilution assay (Merlin). Since there were several isolates with discrepant results for rifaximin versus rifampicin (Rmp) MICs we retested all strains with an Rfx MIC ≥64 µg/mL (and a similar number of controls) using an in-house microdilution assay to further test for resistance. Further tests were done to characterize resistance mechanisms, and a preliminary evaluation of an agar-screen for resistance was performed.

Material/methods: Initial retesting resulted in a rate of confirmed (>64 µg/mL) Rfx resistance of (only) 5/339 E.coli (1%), 3/39 Klebsiella spp. (8%) and 0/29 other Enterobacteriaceae (<3%) without a single discrepancy between Rfx and Rmp MICs (at >64 µg/mL). We then used a novel Rfx agar screen (with 256 µg/mL, Mueller-Hinton agar) to retest all Rfx-R strains (plus negative controls and strain SA128 [no efflux phenotype, Q513L mutation in rpoB] as a positive control) and to screen a further new collection of 3GCRE (including 35 E.coli and 6 other enteric bacteria). Mutations in rpoB were determined by PCR/sequencing, and presumed efflux phenotype were assayed by measuring Rfx and Rmp MIC reversion after coincubation with PAßN (25 µg/mL).

Results: Rfx agar screen tests were positive in 5/5 and identified another 2 E.coli with confirmed Rfx-R (>256 µg/mL) whereas it was negative in 16 3GCR-E.coli control clinical isolates with Rfx MICs ranging between 2 and 64 µg/mL. Of the 7 agar-screen positive strains 3 had no efflux phenotype (versus none among controls). The Rfx agar screen also correctly identified 3/4 Klebsiella spp. with
Rfx MICs >256 µg/mL. The single agar-screen-negative *Klebsiella* strain had discrepant Rfx (>256) and Rmp (32) MICs. Mutations in *rpoB* with substitutions (L511P, D516Y, H526L, Q513L, S531F, S574F) were found in 5/7 agar-screen positive *E.coli* versus 1/16 (N450H) agar-screen-negative controls (with confirmed Rfx MIC ≤64 µg/mL) (p<0.01, Fisher’s exact test). Interestingly, substitutions at residues 513, 516, 518, 526 and 574 (as well as 626 and 723) have previously been found in association with Rfx resistance in *E.coli*, but not all have been confirmed to be causative.

**Conclusions:** High-level Rfx resistance among 3GCRE in Germany remains rare. We propose that a simple agar screen can and should be further developed for direct identification of such strains that usually carry *rpoB* mutations. Such a test if feasible as direct testing of rectal swabs/stool samples may be helpful for predicting decolonization failures.