Journal of Chemotherapy

ISSN: 1120-009X (Print) 1973-9478 (Online) Journal homepage: http://www.tandfonline.com/loi/yjoc20

The effect of sub-inhibitory concentrations of rifaximin on urease production and on other virulence factors expressed by Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus

Annalisa Ricci, Erika Coppo, Ramona Barbieri, Eugenio A. Debbia & Anna Marchese

To cite this article: Annalisa Ricci, Erika Coppo, Ramona Barbieri, Eugenio A. Debbia & Anna Marchese (2016): The effect of sub-inhibitory concentrations of rifaximin on urease production and on other virulence factors expressed by Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa and Staphylococcus aureus, Journal of Chemotherapy, DOI: 10.1080/1120009X.2016.1195069

To link to this article: http://dx.doi.org/10.1080/1120009X.2016.1195069

Published online: 10 Jun 2016.

Antimicrobial Original Research Paper

The effect of sub-inhibitory concentrations of rifaximin on urease production and on other virulence factors expressed by *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*

Annalisa Ricci, Erika Coppo, Ramona Barbieri, Eugenio A. Debbia, Anna Marchese

Microbiology Section "C.A. Romanzi", DISC, University of Genoa, Genoa, Italy

Rifaximin, a topical derivative of rifampin, inhibited urease production and other virulence factors at sub-MIC concentrations in strains involved in hepatic encephalopathy and the expression of methicillin resistance in *Staphylococcus aureus*. In particular, urease production was affected in all *Proteus mirabilis* and *Klebsiella pneumoniae* strains as well as in all tested *Pseudomonas aeruginosa* isolates. Other exotoxins, synthesized by *P. aeruginosa,* such as protease, gelatinase, lipase, lecithinase and DNAse were also not metabolized in the presence of rifaximin. This antibiotic inhibited pigment production in both *P. aeruginosa* and *Chromobacterium violaceum*, a biosensor control strain. Lastly, rifaximin affected haemolysin production in *S. aureus* and was able to restore cefoxitin susceptibility when the strain was cultured in the presence of sub-MICs of the drug. The present findings confirm and extend previous observations about the beneficial effects of rifaximin for the treatment of gastrointestinal diseases, since in this anatomic site, it reaches a large array of concentrations which prevents enterobacteria from thriving and/or producing their major virulence factors.

Keywords: Urease production, Quorum sensing, Methicillin resistance, Rifaximin at sub-MIC concentrations

Introduction

Rifaximin is a member of the rifamycin group and shares the same mode of action of the drugs in this class by inhibiting DNA-dependent RNA polymerase. The antibacterial spectrum of rifaximin is also similar to the parental compounds including aerobic and anaerobic, gram-positive and gram-negative bacteria.[1,2] Rifaximin is poorly absorbed after oral administration due to the presence of a pyridoimidazole moiety.[3,4] This feature, as well as its low solubility in water, allows the antibiotic to reach the faeces (approximately 97%) unchanged.[5] In fact, previous studies have shown that faecal rifaximin concentrations resulted many times greater than the minimum inhibitory concentration (MIC) for the enteropathogens responsible for many gastrointestinal pathologies,[6] such as traveller's diarrhoea, *Clostridium difficile*-associated diarrhoea, diverticular disease, hepatic encephalopathy, inflammatory bowel disease and others,[7–13] thereby reducing in many cases the duration of diarrhoea.[14,15] A similar behaviour is observed with ciprofloxacin.[15,16] A more recent study in rats demonstrated that a 10-day course of rifaximin has a modest but significant effect on stool coliforms and *Staphylococcal spp*. In particular, rifaximin *in vivo* appeared to reduce the number of resident rifampicin-resistant staphylococci without affecting the mean inhibitory concentration of rifampicin towards these bacteria.[17] Rifaximin is safe and well tolerated for long-term maintenance of remission from overt hepatic encephalopathy. In another recent study, in fact, it was demonstrated that rifaximin therapy significantly reduced the risk of overt hepatic encephalopathy recurrence and related hospitalizations during long periods of time (from six to over 24 months) in patients with cirrhosis and histories of recurrent overt hepatic encephalopathy.[12,18] Since it is necessary to assume that, given enough antibiotic and time, resistance will appear against any drug, the efficacy of rifaximin in the management of the above pathologies cannot be solely attributed to the antibacterial activity of the drug. When microorganisms are exposed to this drug for long periods of time, they might in many

Correspondence to: Eugenio A. Debbia, Microbiology Section "C.A. Romanzi", DISC, University of Genoa, Largo Rosanna Benzi 10, 16132 Genoa, Italy. Email: eugenio.debbia@unige.it

DOI 10.1080/1120009X.2016.1195069

circumstances develop resistance. In any case, the topical use of rifaximin enables the drug to reach concentrations in the gastrointestinal tract which are high enough to reduce the proliferation of derivative-resistant bacterial cells, as well as interfering with their physiological functions when the level of the drug is not totally inhibitory. Rifaximin at sub-MICs has already been shown to affect several physiological functions[19], and the beneficial effects in clinical practice, in addition to its antibacterial activity, have recently been pointed out.[20] For these reasons, it appears of particular interest to evaluate the role of rifaximin against different urease-producing pathogens. In particular, *Proteus mirabilis* and *Klebsiella pneumonia*e together with *Pseudomonas aeruginosa,* the major bacterial strains responsible for hepatic encephalopathy, were exposed to sub-MICs of rifaximin to evaluate urease production.

Since many enzymes or noxious bacterial products are regulated by the quorum sensing mechanism, rifaximin was also tested against *Chromobacterium violaceum*, a biosensor of this system. Finally, any antimicrobial drug is considered an inducer of reactive oxygen species (ROS), which contribute to cell death. Drug lethality involves damage to DNA which in turn can promote mutations.[21] The role of rifaximin as a ROS inducer was then tested on MRSA, evaluating the loss of methicillin resistance in strains either susceptible or resistant to this drug.

Materials and methods

Bacterial strains

Microorganisms used in this study were isolated from clinical samples of the gastrointestinal tract and included strains from the *Enterobacteriaceae* family, *P. aeruginosa* and *Staphylococcus spp*. The *C. violaceum* strain was a generous gift from Bob McLean.[22]

Antibiotics

Rifaximin was provided by Alfa Wassermann S.p.A., Bologna. The other antibiotics and chemical compounds were obtained from commercial sources (Sigma-Aldrich, Milan, Italy) or from their respective manufacturers.

Susceptibility tests

The MIC of rifaximin and the other drugs were determined using the microdilution method in Mueller-Hinton broth with the addition of Ca^{2+} and Mg^{2+}. The microplates were incubated aerobically at 35 °C for 18–24 h according to the recommendations of the European Committee for Antimicrobial Susceptibility Testing (EUCAST, 2014) guidelines and interpretative criteria.[23] *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 29213 and *P. aeruginosa* ATCC 27853 were used as quality control strains.

Enzyme assays

Urease activity was detected in Bacto urea broth (Difco). Log-phase bacterial cultures of the tested bacteria were adjusted to 0.5 McFarland turbidity equivalents in the above broth containing graded concentrations of the tested antibiotic. The strains were incubated at 37 °C for 24 h. A medium colour change indicates a positive test for urease. Production of the other extracellular enzymes was estimated as previously described.[24] In detail, DNase production was detected on DNase plates (Difco). After 72-h incubation at 37 °C, the plates were flooded with 1N HCl and a clear zone around the growth area manifested DNase activity. Lipase synthesis was measured on Bacto Spirit blue Agar (Difco). The medium was supplemented with Bacto lipase reagent prepared by dissolving 1 ml of Tween 80 in 400 ml of warm distilled water and then adding 100 ml olive oil and agitating vigorously to emulsify. After 48-h incubation at 37 °C, the colonies of lipolytic organisms develop a clear zone around and under each colony. Gelatinase production was determined by dissolving 4 gr/l peptone, 1 gr/l yeast extract, 12 gr/l gelatin and 15 gr/l agar in distilled water. After 24-h incubation at 37 °C, the presence of gelatinase was indicated by a zone of clearing around the inoculums. Protease was detected on Mueller-Hinton agar containing 3% skimmed milk. The plates were incubated at 37 °C for two days. Afterwards, the presence of a transparent halo around the colonies indicated protease activity. Lecithinase production was tested by adding 10% egg yolk enrichment (Difco) to trypticase soy agar (Difco). Plates were incubated at 37 °C for two days. A precipitate around the inoculum spots indicated activity. Haemolysins were detected in Columbia blood agar plates with and without sub-MIC concentrations of the drug tested. The zones of haemolysis were evaluated after 48, 72 and 96 h at 37 °C.

Bacterial pigments

In preliminary experiments, bacterial pigment production was evaluated on Pseudomonas agar base (Biolife) plates. *P. aeruginosa* was inoculated on plates, using a 0.5 McFarland suspension, by streaking the swab over the entire agar surface. The activity of the antibiotic tested was evaluated by disc diffusion, and inhibition of pigment production was estimated by measuring the colourless halo around the disc. The same technique was used for *C. violaceum* using MH agar instead of *Pseudomonas* agar base. The plates of *P. aeruginosa* were incubated overnight at 37 °C, while those of *C. violaceum* were incubated at 30 °C. The inhibition of pigment production was also tested using MH broth, by exposing bacterial cell cultures to graded concentrations of the drug tested.

Methicillin resistance

Methicillin-resistant *S. aureus* susceptible to rifaximin and its rifaximin-resistant derivative were incubated overnight, and the bacterial concentration was then adjusted to 10^3 CFU/ml and cultured in the presence of sub-inhibitory concentrations of the antibiotic studied. After overnight incubation at 37 °C, the strains were diluted in saline buffer and plated on drug-free MH agar. After incubation

Table 1 Evaluation of sub-MIC concentrations of rifaximin on urease production in gram-negative strains in comparison with ciprofloxacin

	Rifaximin			Ciprofloxacin		
Strains	MIC (mg/L)	Used dose (xMIC)	Urease production	MIC (mg/L)	Used dose (xMIC)	Urease production
K. pneumoniae						
KPN1	64	0.5	–	128	0.5	+
KPN2	32	0.5	–	256	0.5	+
KPN3	32	0.5	–	0.12	0.5	+
KPN4	32	0.5	–	0.06	0.5	+
KPN5	32	0.5	–	0.5	0.5	+
P. mirabilis						
PMI1	64	0.5	–	256	0.5	+
PMI2	64	0.5	–	256	0.5	+
PMI3	64	0.5	–	256	0.5	+
PMI4	64	0.5	–	0.03	0.5	+
PMI5	64	0.5	–	1	0.5	+
P. aeruginosa						
PSA2	32	0.06	–	8	0.5	+
PSA3	64	0.125	–	0.015	0.5	+
PSA5	64	0.03	–	0.03	0.5	+

Notes: Results indicate no visible colour change in comparison with the control;
– and + negative and positive urease production, respectively.

Table 2 Effects of sub-MIC (0.5 × MIC) concentrations of rifaximin and ciprofloxacin on the production of exoenzymes by six clinical isolates of *P. aeruginosa*

	MIC	Protease	Gelatinase	Lipase	Lecithinase	DNAse
PSA1						
Rifaximin	256	100	18.5	48.5	54	17
Ciprofloxacin	8	0	0	37.5	50	0
PSA2						
Rifaximin	32	100	100	40	78.3	16
Ciprofloxacin	0.015	0	50	0	33.4	0
PSA3						
Rifaximin	64	100	30	34	30	16.9
Ciprofloxacin	0.03	12.5	0	0	0	0
PSA4						
Rifaximin	32	71.3	54	25.6	34.8	0
Ciprofloxacin	0.015	0	0	11.2	0	0
PSA5						
Rifaximin	64	100	39.8	59	39.4	15
Ciprofloxacin	0.015	0	0	0	0	0
PSA6						
Rifaximin	32	86.8	21.9	42	61.5	7
Ciprofloxacin	0.03	14.3	0	0	0	0

Notes: Results are expressed as per cent of inhibition of enzyme activity. Mean of at least three experiments.

for 18–20 h, colonies were replicated onto MH agar containing 4 mg/L of cefoxitin using velvets. Growth on antibiotic-containing media indicated methicillin resistance.

Results

Susceptibility tests and urease production

In Table 1, the minimal inhibitory concentrations of rifaximin and the comparative agent ciprofloxacin are reported against the strains tested. All strains showed MIC values for rifaximin ranging from 32 to 64 mg/L, while fluoroquinolone MICs exhibited values >64 mg/L for resistant strains and lower than 1 mg/L for susceptible isolates.

Urease production was inhibited by rifaximin in all *Enterobacteriaceae* at a dose of 0.5 × MIC. In the three *P. aeruginosa* able to produce the above enzyme, urease synthesis was affected by rifaximin levels ranging from 0.03 to 0.125 × MIC. The comparative agent, ciprofloxacin, did not demonstrate any inhibition of urease synthesis in all the strains tested.

Exoenzyme production

The fact that rifaximin was able to inhibit urease production in *P. aeruginosa* prompted us to evaluate whether rifaximin was able to affect the synthesis of several exoenzymes produced by this pathogen. Table 2 summarizes the results obtained after exposing six *P. aeruginosa* isolates to sub-MIC concentrations of rifaximin and ciprofloxacin. Significant inhibition of protease synthesis (71.3–100%) was observed in all strains when treated with rifaximin, while the exoenzyme was only inhibited in two of the six isolates when exposed to ciprofloxacin (12.5 and 14.3%). Gelatinase production was inhibited by the presence of rifaximin, from 18.5 to 100%, compared to the positive

Table 3 Inhibition of pigment production in *P. aeruginosa* and in *C. violaceum* exposed to rifaximin and ciprofloxacin at the indicated sub-MIC concentrations

	Rifaximin		Ciprofloxacin	
Strain	MIC (mg/L)	Used dose (× MIC)	MIC mg/L	Used dose (× MIC)
PSA1	256	0.03	8	0.5
PSA2	32	0.125	0.015	0.25
PSA3	64	0.125	0,03	–
PSA4	32	0.25	0.015	–
PSA5	64	0.015	0.015	–
PSA6	32	0.03	0.03	–
CVI	256	0.03	0.03	0.5

control. Ciprofloxacin affected the production of this metabolite in one strain. In the presence of sub-inhibitory concentrations of rifaximin, lipase activity decreased from 25.6 to 59% while ciprofloxacin only affected the production of this protein in two strains (11.2 and 37.5%). Lecithinase synthesis was reduced from 30 to 78.3% in all strains studied, the comparative agent, ciprofloxacin, only affected the activity of this molecule in two strains (33.4 and 50%). DNase activity decreased in the presence of sub-MIC concentrations of rifaximin, which ranged from 0 to 17% in five of the six strains analysed. No effect was observed for ciprofloxacin in the above isolates.

Bacterial pigments

In *P. aeruginosa,* the pigment pyocyanin is another potential virulent factor for this opportunistic pathogen. Since pigment production is under the control of the quorum sensing system, *C. violaceum*, a biosensor of this process was included in the tests to evaluate the efficacy of rifaximin as an inhibitor of the quorum sensing process.

As reported in Table 3, pyocyanin synthesis was affected in all *P. aeruginosa* strains tested at very low concentrations of rifaximin; this characteristic was also observed in *C. violaceum*. The comparative antibiotic ciprofloxacin inhibited pigment production in two strains of *P. aeruginosa* as well as in *C. violaceum* (Table 3).

Methicillin resistance

In a preliminary approach, MRSA strains cultured in the presence of sub-inhibitory concentrations of rifaximin were plated in drug-free medium and in Mueller-Hinton agar containing 4 mg/L of cefoxitin. The comparative evaluation of the CFU/ml in both media revealed that the number of colonies in the drug-free medium was at least double the amount registered in the cefoxitin containing medium. However, when the colonies grown in the drug-free medium were replicated in the antibiotic-containing medium, only a small percentage (from 0 to 3.1%) was found to be susceptible to cefoxitin. This result suggested that rifaximin, at sub-inhibitory concentrations, interfered with the expression of methicillin resistance during the growth of the bacterial cells. When the bacteria were plated on antibiotic-containing medium, they did not have enough time to restore the methicillin resistance phenotype and were inhibited by cefoxitin present in the medium. On the contrary, the bacterial cells plated on drug-free medium had enough time to restore the antibiotic resistance mechanism that resulted operative when the colonies were replicated on antibiotic-containing medium. To verify this point, a cefoxitin disc susceptibility test was carried out on plates containing sub-MICs of rifaximin employing microorganisms both resistant and susceptible to this drug. After incubation, the strains grown in the presence of sub-MICs of rifaximin resulted susceptible to cefoxitin (Figure 1). In several separate experiments, susceptibility to methicillin was found in 0 to 1.7% of bacterial cells of Rif-R *S. aureus* grown in the presence of 0.5xMIC of rifaximin and from 0 to 2% in Rif-S *S. aureus* after growing for several generations in 0.25 × MIC of rifaximin (Table 4). A sample of bacteria randomly chosen among the methicillin-susceptible isolates was tested for the presence of the PTA gene (*S. aureus* house keeping gene) using PCR. All cells showed the presence of the PTA gene and were susceptible to methicillin.

The loss of methicillin resistance was also evaluated by growing MRSA in the presence of sub-MICs of ciprofloxacin. In MRSA ciprofloxacin-resistant strains, methicillin resistance was not lost. When the same experiments were carried out employing ciprofloxacin susceptible strains, methicillin resistance was lost from 0.4 to 2.4% (Table 4).

Haemolysin production was also affected both in rifaximin-susceptible and rifaximin-resistant MRSA strains grown in the presence of 0.5 × MIC of rifaximin (data not shown).

Discussion

The present findings demonstrate that sub-inhibitory concentrations of rifaximin inhibit the synthesis of several extracellular products in different gram-negative microorganisms and in *S. aureus*. In particular, urease production was affected in all *P. mirabilis* and *K. pneumoniae* strains as well as in all tested *P. aeruginosa* isolates. Other exotoxins such as protease, gelatinase, lipase, lecithinase and DNAse, synthesized by *P. aeruginosa,* were also not metabolized in the presence of rifaximin. Pigment production was inhibited in both *P. aeruginosa* and *C. violaceum*, a biosensor control strain, by this antibiotic. Finally, in *S. aureus,* rifaximin affected haemolysin production and was able to restore cefoxitin susceptibility when the strain was cultured in the presence of sub-MICs of the drug.

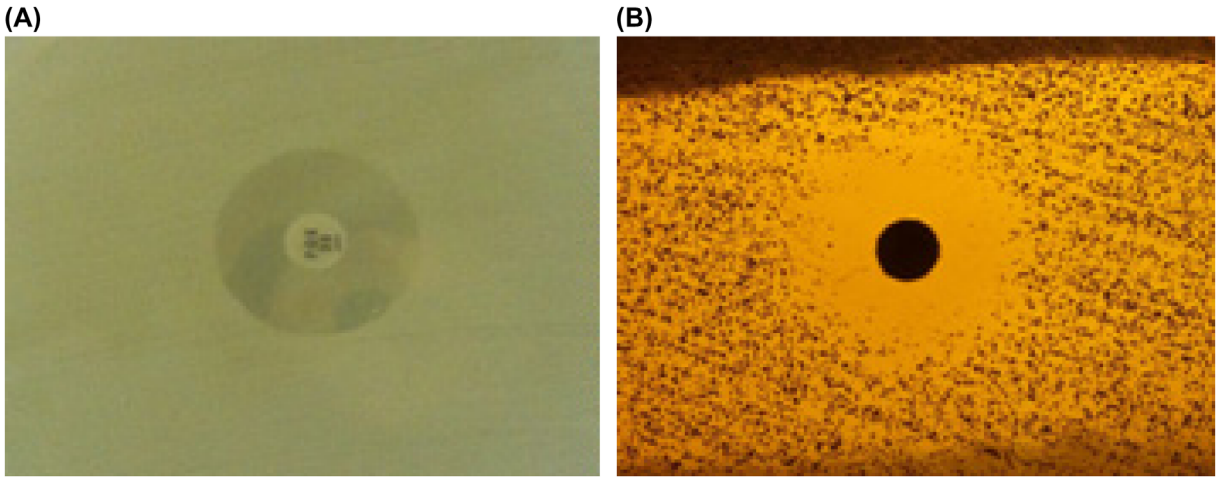

Figure 1 Susceptibility of MRSA to cefoxitin in the presence of a sub-MIC of rifaximin. (A) susceptible to rifaximin; (B) resistant to rifaximin.

Table 4 Effects of sub-MIC of rifaximin and ciprofloxacin on the expression of methicillin resistance in S. aureus

	MIC (mg/L)	Used dose (mg/L)	Methicillin[a]	Methicillin[b]
MRSA1				
Rifaximin	0.004	0[c]	0	R
Ciprofloxacin	0.5	0[c]	0	R
MRSA1				
Rifaximin		0.002	0–1.7	S
Ciprofloxacin		0.25	0.4–2.4	R
MRSA1				
Rifaximin		0.001	0–2.3	S
Ciprofloxacin		0.125	0.3–2–1	R
MRSA2				
Rifaximin	>1024	0[c]	0	R
Ciprofloxacin	512	0[c]	0	R
MRSA2				
Rifaximin		1024	0–2.2	S
Ciprofloxacin		256	0.6–3.1	R
MRSA2				
Rifaximin		512	0.2–1.8	R
Ciprofloxacin		128	0.5–1.2	R

[a]Per cent of cefoxitin-susceptible strains found by the replica plate method.
[b]Recovery of cefoxitin susceptibility evaluated by disc susceptibility test in plates that contained the indicated sub-MIC dose of the antibiotic.
[c]Control, no drug.
Note: Mean of at least three experiments.

Considering these observations, it is important to underline the role of urease as a general microbial virulence factor[25] and in particular in patients affected by overt hepatic encephalopathy. Urea is produced during the degradation of amino acids and then distributed in the human body. Many microorganisms use urea as a nitrogen source through the activity of the enzyme urease that converts urea into ammonia and carbamic acid, which then spontaneously reacts with water to form carbonic acid. In physiological conditions, the carbonic acid dissociates and the ammonia forms ammonium causing an increase in pH that can interfere with host functions.[25] In patients with impaired liver function, the concentration of ammonium cannot be reduced, leading to the systemic accumulation of this toxin, a factor that is correlated with the establishment of hepatic encephalopathy. Recent studies have demonstrated that rifaximin is well tolerated and has beneficial effects on patients affected by hepatic encephalopathy treated for long periods of time (from six to over 24 months) also reducing the risk of recurrence of this pathology and related hospitalisations.[12,18] Rifaximin should have a double advantage in comparison with other topically used drugs: the antibacterial activity against urease-producing bacteria reduces their proliferation and number in the gastrointestinal tract and interferes with urease synthesis which deprives these pathogens of their major virulence factor. This should explain the beneficial effects of rifaximin reported in the above-mentioned studies. This is also of special interest and should be taken into account when an oral antibiotic is used for the treatment of enteric diseases. Protease, lipase, gelatinase, lecithinase and DNase production also decreased in *P. aeruginosa* cultured in the presence of sub-inhibitory concentrations of rifaximin. *P. aeruginosa* is a clinically

significant opportunistic pathogen involved in nosocomial infections due to its large number of extracellular products which play a significant role in its pathogenic process. In particular, pathogenesis of *P. aeruginosa* is a multifactorial process involving invasive as well as toxic mechanisms provided by a large array of extracellular products such as those tested here, which enables the spread of the pathogen from the initial site of colonization to distant tissues and facilitates invasion inducing necrotic processes in tissues, or kills phagocytes.[26] Sub-inhibitory concentrations of rifaximin also reduced and inhibited pigment production. Pyocyanin curtails mammalian cell respiration, disrupts the beating of respiratory epithelial cilia and inhibits both epidermal cell growth and lymphocyte proliferation. Pigment production, like many of the virulence factors studied here, is under control of the quorum sensing system.[27–29] Therefore, rifaximin, by inhibiting pyocyanin in *P. aeruginosa,* appears to interfere with quorum sensing activity. This feature is also confirmed with the results obtained with *C. violaceum,* the biosensor of this system. This appears to be another striking observation of the study, as quorum sensing is, in fact, widely used by bacterial pathogens to coordinate the expression of several multiple virulence factors. This finding should suggest that rifaximin is able to interfere with quorum sensing systems in microorganisms other than those studied here, giving the beneficial effects observed in many pathologies treated with this drug.

Staphylococci are important agents of both nosocomial and community infections. The major therapeutic threat of these organisms is the presence of the methicillin-resistant trait, which occurs not only in strains circulating in hospital settings but also in outpatients. These microorganisms are particularly difficult to eradicate since not only do they exhibit resistance to all β-lactam antibiotics but also to other classes of antimicrobials.

In this context, rifaximin appears to affect this therapeutic threat, in fact a small portion of the bacterial strains exposed to rifaximin lost, in a definitive manner, this antibiotic-resistant trait, and about half of the bacterial population exhibited a methicillin-susceptible phenotype. Although there is no evidence about the exact mechanism conferring susceptibility to cefoxitin in MRSA, it is reasonable to suppose that rifaximin interferes with the synthesis of messenger RNA leading to a wrong mecA gene product. Considering the clinical impact of this phenomenon, this result represents further evidence for circumventing methicillin resistance in staphylococci;[30] in addition, this feature should provide the proof that as long as rifaximin is present in an anatomic site where there is *S. aureus*, the methicillin-resistant trait is not expressed. In conclusion, rifaximin at sub-inhibitory concentrations inhibits the production of a lot of virulence and pathogenicity factors. The present results confirm and extend previous observations about the beneficial role of rifaximin in the treatment of many gastrointestinal pathologies irrespective of a mere inhibition of bacterial growth.[19,20] Lastly, given its efficacy and safety in the treatment of various gastrointestinal pathologies, additional indications have been suggested and reviewed.[31]

Contributors

Study concept, design, funding availability, drafting and revising of manuscript were done by EAD and AM; production, collection and analysis of experimental data were done by AR, EC and RB.

Acknowledgements

We are indebted to Jennifer McDermott for English assistance, Bob McLean who kindly provided *C. violaceum*, Alfa Wassermann S.p.A Bologna, Italy, for supplying rifaximin and Mirko Lucchetti who helped at various stages of this project.

Funding

This work was supported by the Università degli Studi di Genova.

References

1 Gillis GC, Brogden RN. Rifaximin: a review of its antibacterial activity, pharmacokinetic properties and therapeutic potential in conditions mediated by gastrointestinal bacteria. Drugs. 1995;49:467–84.
2 Jiang ZD, Dupont HL. Rifaximin: in vitro and in vivo antibacterial activity – a review. Chemotherapy. 2005;51(Suppl 1):67–72.
3 Alvisi V, D'Ambrosi A, Lo Ponte A, Pazzi P, Greco A, Zangirolami A, *et al*. Rifaximin, a rifampicin derivative for use in the treatment of intestinal bacterial infectious in seriously disabled patients. J Int Med Res. 1987;15:49–56.
4 Hoover WW, Gerlach EH, Hoban DJ, Eliopolus GM, Pfaller MA, Jones RN. Antimicrobial activity and spectrum of rifaximin, a new topical rifamycin derivative. Diagn Microbiol Infect Dis. 1993;16:111–8.
5 Jiang ZD, Ke S, Palazzini E, Riopel L, Dupont HL. In vitro activity and fecal concentration of rifaximin after oral administration. Antimicrob Agents Chemother. 2000;44:2205–6.
6 Sierra JM, Ruiz J, Navia MM, Vargas M, Vila J. In vitro activity of rifaximin against enteropathogens producing traveler's diarrhoea. Antimicrob Agents Chemother. 2001;45:643–4.
7 Frieri G, Pimpo MT, Scarpignato C. Management of colonic diverticular disease. Digestion. 2006;73:58–66.
8 Lanas A, Scarpignato C. Microbial flora in NSAID-induced intestinal damage: a role for antibiotics? Digestion. 2006;73:136–50.
9 Johnson S, Schriever C, Galang M, Kelly CP, Gerding DN. Interruption of recurrent *Clostridium difficile*-associated diarrhea episodes by serial therapy with vancomycin and rifaximin. Clin Infect Dis. 2007;44:846–8.
10 Shen B, Remzi FH, Lopez AR, Queener E. Rifaximin for maintenance therapy in antibiotic-dependent pouchitis. BMC Gastroenterol. 2008;8:26.
11 Pimentel M. Review of rifaximin as treatment for SIBO and IBS. Expert Opin Investig Drugs. 2009;18:349–58.
12 Bass NM, Mullen KD, Sanyal A, Poordad F, Neff G, Levy CB, *et al*. Rifaximin treatment in hepatic encephalopathy. N Engl J Med. 2010;362:1071–81.
13 Guslandi M. Rifaximin in the treatment of inflammatory bowel disease. World J Gastroenterol. 2011;17:4643–6.
14 DuPont HL, Jiang ZD, Okhuysen PC, Ericsson CD, de la Cabada FJ, Ke S, *et al*. A randomized, double – blind, placebo-controlled trial of rifaximin to prevent travelers' agents. Ann Intern Med. 2005;142:805–12.
15 Taylor DN, Bourgeois AL, Ericsson CD, Steffen R, Jiang ZD, Halpern J, *et al*. Randomized, double-blind, multicenter study of rifaximin compared with placebo and with ciprofloxacin in the treatment of travelers' diarrea. Am J Trop Med Hyg. 2006;74:1060–6.

16 DuPont HL, Jiang DZ, Ericsson CD, Adachi JA, Mathewson JJ, DuPont MW, *et al*. Rifaximin versus ciprofloxacin for the treatment of traveler's diarrhea: a randomized, double-blind clinical trial. Clin Infect Dis. 2001;33:1807–15.
17 Kim MS, Morales W, Hani AA, Kim S, Kim G, Weitsman S, *et al*. The effect of rifaximin on gut flora and *Staphylococcus* resistance. Dig Dis Sci. 2013;58:1676–82.
18 Mullen KD, Poordad F, Rossaro L, Jamal M, Talwalkar J, Huang S, *et al*. Long-term efficacy and survival in patients treated with the gut-selective antibiotic rifaximin (550 mg BID) for the maintenance of remission from overt hepatic encephalopathy. J Hepatol. 2011;54:S49.
19 Debbia EA, Maioli E, Roveta S, Marchese A. Effect of rifaximin on bacterial virulence mechanisms at supra and sub-inhibitory concentration. J Chemother. 2008;20:61–9.
20 Calanni F, Renzulli C, Barbanti M, Viscomi GC. Rifaximin: beyond the traditional antibiotic activity. J Antibiot (Tokyo). 2014;67:667–70.
21 Dwyer DJ, Collins JJ, Walker GC. Unraveling the physiological complexities of antibiotic lethality. Annu Rev Pharmacol Toxicol. 2015;55:313–32.
22 McLean RJC, Pierson LS, Fuqua C. A simple screening protocol for the identification of quorum signal antagonists. J Microbiol Methods. 2004;58:351–60.
23 European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters Version 4.0 [software]. 2014 Jan 14.
24 Molinari G, Guzman CA, Pesce A, Schito GC. Inhibition of *Pseudomonas aeruginosa* virulence factors by sub-inhibitory concentrations of azithromycin and other macrolide antibiotics. J Antimicrob Chemother. 1993;31:681–8.
25 Rutherford JC. The emerging role of urease as a general microbial virulence factor. PLoS Pathog. 2014;10:e1004062.
26 Gellatly SL, Hancock RE. *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. Pathog Dis. 2013;67:159–73.
27 Venturi V. Regulation of quorum sensing in *Pseudomonas*. FEMS Microbiol Rev. 2006;30:274–91.
28 Fulghesu L, Giallorenzo C, Savoia D. Evaluation of different compounds as quorum sensing inhibitors in *Pseudomonas aeruginosa*. J Chemother. 2007;19:388–90.
29 Castillo-Juárez I, Toshinari M, Ayerim Mandujano-Tinoco E, Tomàs M, Perez-Eretza B, Garcia-Contreras SJ, *et al*. Role of quorum sensing in bacterial infections. World J Clin Cases. 2015;3:575–98.
30 Gonzales PR, Pesesky MW, Bouley R, Ballard A, Biddy BA, Suckow MA, *et al*. Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA. Nat Chem Biol. 2015;11:855–61.
31 Rivkin A, Gim S. Rifaximin: new therapeutic indication and future directions. Clin Ther. 2011;33:812–27.