Antimicrobial susceptibility of *Borrelia burgdorferi* sensu lato: What we know, what we don’t know, and what we need to know

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**Summary.** Human Lyme borreliosis is a multisystem disorder that can progress in stages and is transmitted by ticks of the *Ixodes ricinus* complex infected with the spirochete *Borrelia burgdorferi* sensu lato. Today, Lyme borreliosis is regarded as the most important human tick-borne illness in the northern hemisphere. Soon after the causative agent was correctly identified and successfully isolated in 1982, antibiotic treatment was shown to be effective and since then a variety of *in vitro* and *in vivo* studies have been performed to further characterize the activity of antimicrobial agents against *B. burgdorferi* s.l. Although many antimicrobial agents have been tested for their *in vitro* activity against borreliae, the full spectrum of antibiotic susceptibility in *B. burgdorferi* s.l. has not been defined for many compounds. Moreover, our current understanding of possible antimicrobial resistance mechanisms in *B. burgdorferi* s.l. is limited and is largely founded on *in vitro* experiments on relatively few borreliial isolates. This review will summarize what is and what is not known about antimicrobial resistance in *B. burgdorferi* s.l. and will discuss open questions that continue to fuel the ongoing debate on treatment-resistant Lyme borreliosis.

**Key words:** *Borrelia burgdorferi, in vitro susceptibility, antimicrobial agents, antimicrobial resistance, spirochetes, susceptibility testing.*

**Introduction**

Human Lyme borreliosis (LB) is a multisystem disorder that can progress in stages and is transmitted by ticks of the *Ixodes ricinus* complex infected with the spirochete *Borrelia burgdorferi* sensu lato [1]. Today, LB is regarded as the most important human tick-borne illness in the northern hemisphere. In the USA the reported incidence of LB is 6.3/100,000 inhabitants [2]; in European countries the annual incidence in the normal population is estimated as 3.9–168/100,000 inhabitants [3–5].

Soon after the causative agent was correctly identified and successfully isolated in 1982, antibiotic treatment was shown to be effective on the basis of empiric treatment trials [6–8]. Since then several *in vitro* and *in vivo* studies have been performed to further characterize the activity of antimicrobial agents against *B. burgdorferi* s.l. and to determine the clinically most effective regimen for stage-dependent treatment of LB [9–11]. Clinical treatment failures are rare in LB but have been reported for almost every suitable antimicrobial agent [5, 10, 12–16]. Nevertheless, our current understanding of antimicrobial resistance in *B. burgdorferi* s.l. is limited and is largely founded on *in vitro* experiments on relatively few borreliial isolates. Moreover, sophisticated insights into the interactions between antimicrobial agents and the pathogen, as gained from animal models, are sparse and the exact mechanism of resistance to antibiotic treatment in LB patients with culture-proven treatment failure is not known. This review will summarize what is and what is not known about the *in vitro* interactions of *B. burgdorferi* s.l. with antimicrobial agents and will discuss open questions and current limits of knowledge concerning possible borreliial resistance mechanisms that continue to fuel the ongoing debate on treatment-resistant LB and post-Lyme disease syndrome.

**What we know: In vitro susceptibility testing of *B. burgdorferi* s.l. against antimicrobial agents**

*In vitro* susceptibility studies of *B. burgdorferi* s.l. have always been limited by the technical drawbacks of the test procedures and a lack of standardized methodology [11, 17, 18]. Consequently, published *in vitro* susceptibility data on the minimum inhibitory concentration (MIC) and minimum borreliacidal concentration (MBC) of antimicrobial agents for borreliae are far from being consistent because of considerable differences in test conditions; for example, variations in incubation periods (48 h–10 days), the density of the inoculum (1×10^7 borreliae/ml to ~2.5×10^7 borreliae/ml), and the criteria for correct determination of antibiotic-induced killing and growth inhibition *in vitro* [11]. No less than nine definitions of MIC and eight for MBC have been proposed in the scientific literature for susceptibility testing of borreliae [11]. For the most part, variations of common macro- and microdilution methods have been used [11, 17–24]. Testing of *B. burgdorferi* s.l. is significantly influenced by the density of the inoculum, the reading mode [17, 18, 22, 25], and the test medium [11, 18, 25–27]. The inconsistencies outlined above probably account for the wide variability of published MIC and MBC values: for example, the reported MIC of penicillin G varies from 0.003 µg/ml to
8 µg/ml and the MBC from 0.05 µg/ml to >50 µg/ml [11, 22, 28–30]. Similarly, for doxycycline, the MIC varies from 0.06 µg/ml to 2 µg/ml and the MBC from 0.25 µg/ml to 6.4 µg/ml [11, 19, 22, 28, 29]. Most importantly, the results of these studies are limited by the small numbers of isolates examined (from 2 to 30 strains), in contrast to investigations involving large numbers of common rapidly growing clinical isolates [11, 31–33], and also because susceptibility testing is rarely performed on newly isolated strains or on borreliae obtained from patients manifesting resistance to treatment. To overcome evident methodological problems and to simplify interpretation of the in vitro effectiveness of well known and newly developed antimicrobials against the *B. burgdorferi* s.l. complex, a novel standardized methodology using microdilution testing was recently introduced [29]. The susceptibility test method uses colorimetric MIC testing in conjunction with conventional MBC determination using subculture techniques, providing both ease of performance and reliability in testing large numbers of isolates against well known and newly developed antimicrobial agents under standardized experimental conditions. The MIC is determined by automated measurement of a color shift in Barbour-Stoenner-Kelly (BSK) medium in the presence of phenol red, indicating accumulation of nonvolatile acid produced by actively metabolizing spirochetes (2.5 × 10^7 borreliae/ml) over an incubation period of 72 h. The MBC is defined as the lowest concentration of the antimicrobial agent where no spirochetes can be detected microscopically after three weeks of subculture, which means 100% killing under rigorous conditions [34–36]. The effectiveness of antimicrobial substances in killing 100% of the inoculated microorganisms after 72 h is a very stringent criterion for any antibiotic and leads to higher MBCs than those obtained in time-kill studies and in investigations using less restrictive definitions of MBC [11]. Nevertheless, more stringent testing can be helpful in identifying substances that are more appropriate for antimicrobial treatment of LB.

**In vitro antimicrobial susceptibility pattern of the *B. burgdorferi* s.l. complex**

Although several antimicrobial agents have been tested for their in vitro activity against borreliae, the full spectrum of antibiotic susceptibility in *B. burgdorferi* s.l. has not been defined for many compounds. Spirochetes have been tested against a large number of antimicrobial agents using the standardized methodology outlined above [11, 36] and a summary of in vitro data on the susceptibility pattern of *B. burgdorferi* s.l. to well known and recently introduced antimicrobial agents is given in Table 1. The colorimetric MIC<sub>50</sub> values for classic β-lactams, carbapenems, macrolides, tetracyclines, quinolones, and glycopeptides against borreliae are in close agreement with MICs available from other investigators using comparable micro- and macrodilution methods [12, 17-19, 23, 24, 37, 38]. Mezlocillin, piperacillin, ceftriaxone, azithromycin, telithromycin, and cethromycin had the greatest in vitro activity, exhibiting low values for MIC<sub>50</sub> (≤0.03 µg/ml) and MBC<sub>50</sub> (≤2 µg/ml) against the isolates tested. For all the other antimicrobial agents the MIC<sub>50</sub> values were ≥0.06 µg/ml.

*B. burgdorferi* s.l. is susceptible in vitro to a variety of antimicrobial agents, but its susceptibility profile is difficult to predict because it differs from that of common gram-negative bacteria (Table 1). Whereas β-lactams such as ceftriaxone and piperacillin are highly active against *B. burgdorferi* s.l., this is not true for aminoglycosides, ofloxacin, ciprofloxacin, and aztreonam. Interestingly, although vancomycin and linezolid do not usually show in vitro activity against common gram-negative bacteria, they show significant antibiotic effects against *B. burgdorferi* s.l. [35, 37, 38]. In addition, the activity of compounds belonging to a defined class of substances (e.g. cephalosporins or quinolones) against *B. burgdorferi* s.l. does not fit with the classic grouping of these antimicrobial agents according to their spectrum of activity against gram-negative and gram-positive bacteria. For example, among the cephalosporins, the group II compound cefuroxime is highly active against borreliae, whereas loracarbef is not [39]. Also, ceftriaxone, cefotaxime, cefdinir, and cefixime are effective, but other group III agents such as cefamet pivoxil, cefbuten, and cefpodoxime-proxetil are ineffective in vitro. These findings resemble those of cephalosporins against *Leptospira*, which also show variable activity independent of the cephalosporin groups [39]. Similarly, the finding of greater susceptibilities of *B. burgdorferi* s.l. to class III and IV fluoroquinolones such as sparafloxacin and gemifloxacin (Table 1) − derivatives showing enhanced activity against gram-positive organisms and anaerobes − points to the fact that in vitro susceptibility of borreliae probably does not resemble that of common gram-negative bacteria [36, 37, 39, 40]. For the borrellial DNA-gyrase, it must be borne in mind that the naturally occurring protein in *B. burgdorferi* s.l. and the homologous C-terminal region in *E. coli* GyrA are biochemically distinct, sharing only 24% identity at the amino acid level [41]. Moreover, the GyrA C-terminal domain in *E. coli* is acidic with a predicted isoelectric point of 4.0, whereas the naturally occurring 34 kDa protein from *B. burgdorferi* s.l. is basic, with a predicted isoelectric point of 9.1 [41]. These differences may explain the lower activity of class I and II quinolones against borreliae in comparison with common gram-negative bacteria such as *E. coli*. Observations on the somewhat uncommon susceptibility pattern of borreliae are possibly due to different binding affinities of antimicrobial agents to the corresponding borrellial target proteins such as penicillin binding proteins, ribosomes, and DNA-topoisomerases or may result from the fact that the unique cell envelope of these spirochetes has features in common with both gram-positive and gram-negative bacteria [39].

**In vitro interactions of recently developed antimicrobial agents with *B. burgdorferi* s.l.**

During the last decade, novel antimicrobial agents such as the ketolides, everninomycins, oxazolidinones, and broad spectrum fluoroquinolones have been introduced and may become therapeutic alternatives for a variety of infections in adults. Nonetheless, relatively little is known to date about the in vitro pharmacodynamic interactions of new drugs such as ketolides and fluoroquinolones with *Borrelia* species. Owing to the limited in vitro activity of the earlier quinolones against borreliae, in particular nalidixic acid and pefloxacin, the fluoroquinolones have not been recommended as drugs of choice for the treatment of LB and some authors report borreliae in general to be re-