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Antibiotic Susceptibility Patterns in *Rochalimaea quintana*, the Agent of Trench Fever

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Rochalimaea quintana, the etiological agent of trench fever, was tested by an agar dilution method for its susceptibility to the following 14 antibiotics: penicillin G, methicillin, ampicillin, cephalothin, vancomycin, doxycycline, tetracycline, erythromycin, chloramphenicol, streptomycin, kanamycin, rifampin, colistin, and amphotericin B. The MIC of each of these antibiotics was determined. The results showed that *R. quintana* is susceptible in vitro to these antibiotics, with the exception of vancomycin, kanamycin, streptomycin, colistin, and amphotericin B.

Trench fever is a nonfatal, louse-borne disease caused by *Rochalimaea quintana* (formerly *Rickettsia quintana*) and often characterized by brief febrile episodes that are recurrent over a period of weeks to months. It was first recognized in Europe during World War I (3, 4), was also encountered in Europe during World War II (3), and has subsequently been found in Mexico (9, 13) and North Africa (9). The etiological agent is a small gram-negative bacterium having some morphological, tinctorial, and metabolic properties in common with some organisms of the genus *Rickettsia* (14). However, it is cultivable on cell-free medium (14) and is not an obligate intracellular parasite, preferring a close pericellular association in the louse midgut (5) and in cell cultures (5). Both xenodiagnosis with human body lice (3) and blood culture on artificial medium (15) indicate that the bacterium may circulate in the blood for weeks to months after infection, regardless of whether clinical relapses occur. Knowledge regarding its in vivo and in vitro antibiotic susceptibility patterns is limited (6, 14). Serological studies performed in recent years on louse-infested human populations in several areas of the world indicate that trench fever is present and probably coexists with typhus fever, with which it shares some epidemiological features (14). Improved knowledge regarding the antibiotic susceptibility of *R. quintana* should prove useful in the treatment of sporadic cases or epidemics and also in devising a selective growth medium suitable for the isolation of *R. quintana* from infected body lice.

MATERIALS AND METHODS

Antibiotics. Rifampin powder (lot 100170; Calbiochem-Behring, La Jolla, Calif.) was dissolved in dimethyl sulfoxide and stored in 10% dimethyl sulfoxide. Chloramphenicol (lot 1740; Calbiochem-Behring) and doxycycline (doxycycline hyclate; concentration, 850 μg/mg; lot no. 85525-58002; gift of Pfizer Inc., New York, N.Y.) were dissolved in distilled water. All of the above antibiotics were prepared by weighing out the dry powder. All other antibiotics were in the commercial USP injection forms used for therapy: tetracycline hydrochloride (Tetracyn) and streptomycin sulfate from Pfizer Inc., New York, N.Y.; cephalothin sodium (Keflin), erythromycin gluceptate (Ilotycin), sodium penicillin G, and vancomycin hydrochloride (Vancocin hydrochloride), from Eli Lilly & Co., Indianapolis, Ind.; ampicillin (Omnipen-N), Wyeth Laboratories, Philadelphia, Pa.; methicillin sodium (Staphcillin) and kanamycin sulfate (Kantrex) from Bristol Laboratories, Syracuse, N.Y.; colistimethate sodium (Coly-Mycin M) from Warner-Lambert Co., Morris Plains, N.J.; and amphotericin B (Fungizone) from E. R. Squibb & Sons, Princeton, N.J. After reconstitution, stock solutions, which varied in concentration, were dispensed in 1.0-ml volumes into plastic vials and stored at −20°C until used, the beta-lactam antimicrobial agents being stored for no longer than 4 weeks.

***R. quintana* inoculum.** *R. quintana*, strain Fuller, had been cloned previously (10) and underwent nine passages on sheep blood agar after its recovery in 1961 from the blood of a human volunteer (10) who had been inoculated with the Osijek strain originally isolated by Mooser et al. (14) in Yugoslavia from cases of trench fever among prisoners of war. Between 1948 and 1961 it was passed an unknown number of times in human body lice. The Heliodoro strain of *R. quintana* was cultivated from an experimental human infection induced by subcutaneous injection of blood from another volunteer infected with wild-caught lice obtained in Mexico City (15). The Heliodoro strain underwent eight passages on sheep blood agar after its recovery.

A stock inoculum was prepared from each of the two strains by inoculating several drops of a cell suspension onto a series of commercially prepared chocolate agar plates (BBL Microbiology Systems, Cockeysville, Md.) and evenly distributing the suspension over the agar surface with a glass rod. The growth was harvested after 7 days by suspension in brain heart infusion broth (BBL); the cell density was adjusted optically to ca. 10^9 cells per ml (8). The suspension was dispensed in 0.3-ml quantities into sterile glass ampoules which were flame sealed, frozen in a dry ice-alcohol bath, and stored at −70°C.

Preparation of antibiotic assay plates. The antibiotic assay medium was GC agar with hemoglobin (1%) and IsoVitaleX (1%) (BBL). The GC agar base and the hemoglobin were prepared separately by the recommended procedure (BBL) and stored at 6°C until needed. The assay medium was prepared by adding an appropriate amount of membrane filter-sterilized antibiotic to the hemoglobin solution and

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then adding IsoVitaleX. Amphotericin B and rifampin were not filtered but were diluted in sterile water. The hemoglobin and the agar base were then combined and poured into petri dishes (100 by 15 mm).

Inoculation of assay plates. The inoculum was thawed quickly and diluted serially in brain heart infusion broth (BBL) so that there were ca. 2,000 to 3,000 CFU/ml. Three 0.05-ml spots (each ca. 3 cm in diameter) were applied to each plate with a micropipette. Two plates were used for each antibiotic concentration. The plates were then incubated in 95% air–5% CO_2–95% humidity in a water-jacketed incubator for 14 days.

Measurement of growth inhibition. The colonies were counted under a dissecting microscope at ×26 magnification. An average of the counts from the six spots for each antibiotic concentration was determined, the results were expressed as a percentage of the counts from the control plates, and a $\log_{10}$ dose-response (% growth) curve was constructed for each antibiotic. The following descriptors of growth inhibition were determined from the log dose-response curves: (i) the minimum concentration causing complete inhibition of colony formation (MIC); (ii) the concentration causing 90% inhibition of colony formation; and (iii) the concentration causing 50% inhibition of colony formation.

RESULTS

Table 1 presents the antibiotic susceptibilities for strains Fuller and Heliodoro. *R. quintana* was observed to be susceptible to representatives of antibiotic classes that act by inhibiting peptidoglycan, protein, and nucleic acid synthesis. Both strains were susceptible to all members of the penicillin and cephalosporin groups tested, but they were resistant to vancomycin.

Both Fuller and Heliodoro strains showed a similar pattern of resistance to representatives of antibiotic classes that act by inhibiting ribosome function. Both strains were susceptible to doxycycline, tetracycline, chloramphenicol, and erythromycin, although three- and fivefold-greater levels of resistance were observed against doxycycline and tetracycline, respectively, in the Heliodoro strain. Both strains showed a moderate degree of resistance to the aminoglycosides kanamycin and streptomycin. Both Fuller and Heliodoro strains were quite susceptible to rifampin, an inhibitor of nucleic acid function. Only the Fuller strain was tested against colistin and amphotericin B, inhibitors of membrane function, and it was resistant to both. Figure 1 shows dose-response curves for the Fuller strain against antibiotics representative of inhibitors of peptidoglycan synthesis, ribosome function, and nucleic acid synthesis. The reponses for all antibiotics were similar, with the response occurring over approximately a 10-fold range of concentration.

DISCUSSION

By monitoring the growth of the Fuller strain of *R. quintana* in blood broth cultures by examining Macchiavello-stained smears, Vinson and Fuller (14) have demonstrated that growth is completely inhibited by ≤2 μg of tetracycline hydrochloride per ml and ≤2 U of penicillin G per ml and is partially inhibited by 100 μg of streptomycin per ml. Referring to unpublished studies of Vinson and Sawyer, Vinson (13) has reported that *R. quintana* growth is inhibited by chloramphenicol.

The present study provides quantitative data on the in vitro inhibition of growth of extracellular *R. quintana* by a series of antibiotics with different modes of action. The results not only have practical relevance to the therapy of trench fever, but also, because antibiotics are selective probes of microbial processes, the results help define the nature of this interesting organism. Thus, the organism is susceptible to most of those antibiotics which inhibit peptidoglycan synthesis, except for vancomycin, whose action is limited almost entirely to gram-positive organisms (1, 2). Although peptidoglycan has not been specifically isolated from any rickettsia, diaminopimelic acid and muramic acid, compounds peculiar to peptidoglycan, have been demonstrated in whole cells of *R. quintana* and typhus rickettsiae (7, 11, 12, 19). Moreover, penicillin G in culture medium induces typical spheroplast formation in intracellular *Rickettsia prowazekii* and *Rickettsia rickettsii* in cell cultures (17). Antibiotics which inhibit bacterial protein synthesis at the ribosomal level (tetracyclines, chloramphenicol, erythromycin, streptomycin, and kanamycin) (1, 2) and at the level of mRNA transcription (1, 2) inhibit the growth of *R.*

TABLE 1. Comparison of antibiotic susceptibility patterns of the Fuller and Heliodoro strains of *R. quintana*

Antibiotic	MIC (μg/ml) for strain:					
	Fuller			Heliodoro		
	50%[a]	90%	100%	50%	90%	100%
Ampicillin	0.021	0.080	0.12	0.040	0.082	0.10
Penicillin G	0.024	0.035	0.040	0.024	0.044	0.050
Methicillin	0.22	0.40	0.70	NT[b]	NT	NT
Cephalothin	1.8	3.0	5.0	3.5	5.4	6.0
Vancomycin	3.6	6.2	10.0	3.0	5.2	7.0
Doxycycline	0.021	0.036	0.040	0.095	0.115	0.12
Tetracycline	0.040	0.068	0.20	0.35	0.82	1.0
Chloramphenicol	0.48	0.70	1.0	0.52	0.66	0.90
Erythromycin	0.026	0.036	0.040	0.033	0.040	0.070
Kanamycin	5.6	7.2	10.0	7.6	11.3	13.0
Streptomycin	3.8	4.6	6.0	8.2	10.0	12.0
Rifampin	0.016	0.028	0.060	0.008	0.022	0.060
Colistin	18.0	55.0	70.0	NT	NT	NT
Amphotericin B	55.0	80.0	100.0	NT	NT	NT

[a] Percentage of growth inhibition compared with control (100%).
[b] NT, Not tested.

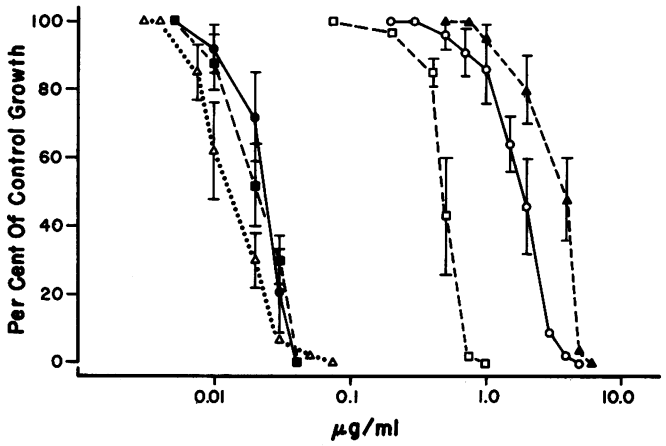

FIG. 1. Dose-response curves of *R. quintana* to a representative group of antibiotics. Symbols: ●, penicillin; ○, cephalothin; ■, doxycycline; □, chloramphenicol; ▲, streptomycin; △, rifampin. ——, Cell wall inhibitor; ---, ribosome inhibitor; . . ., nucleic acid inhibitor. Points plotted are mean values ± 1 standard deviation.

quintana. Finally, *R. quintana* showed a high degree of resistance to amphotericin B, which acts on the plasma membrane of fungi, but not bacteria, through an ergosterol receptor (1, 2).

Comparison of the antibiotic susceptibility patterns of *R. quintana* with those of members of the genus *Rickettsia* is complicated by the fact that, whereas the former is tested in the extracellular state, the latter must be tested while growing within host cells. The host cell plasma membrane is undoubtedly permeable to different antibiotics to greater or lesser degrees; hence, the extracellular antibiotic concentration may not be an accurate measure of the concentration to which the intracellular organisms are actually exposed. Thus, extracellular *R. quintana* was extremely susceptible to the action of penicillin G, whereas the inhibition of growth of intracellular *R. prowazekii* required ca. 500-fold-higher concentrations of penicillin G in the culture medium (18). On the other hand, the MICs of the tetracyclines, chloramphenicol, and erythromycin for *R. quintana* and *R. prowazekii* were comparable (18). Finally, the MIC of rifampin for *R. quintana* was about 10-fold higher than that for *R. prowazekii* (18).

Published observations on the treatment of trench fever with antibiotics are extremely sparse and generally unsatisfactory. The variable duration of the acute febrile episodes and the variable number of relapses make it difficult to evaluate the effects of antibiotic treatment except in large-scale controlled trials with very long observation periods. However, it is significant that Mohr and Weyer (6) report that the usually long, sustained bacteremia in late relapses, as detected by xenodiagnosis with body lice, became negative within 1 week after beginning treatment with chlortetracycline (one patient) or tetracycline (three patients). Vinson et al. (15) report similar findings in patients treated with chloramphenicol.

Since the tetracyclines and chloramphenicol are usually considered primarily bacteriostatic, it would be important to know whether clinical relapses and bacteremia recur later in treated patients. It is conceivable that such drugs would have only a questionable therapeutic effect and a transient epidemiological effect by temporarily interrupting louse transmission, as discussed by Wisseman et al. (16, 17) in their work on louse transmission relative to louse-borne typhus. A bactericidal drug, which would eradicate the organism, would seem to be preferable for both therapeutic and epidemiological objectives (17). The extreme susceptibility of extracellular *R. quintana* to penicillin G offers the possibility that a practical, inexpensive bactericidal drug may be available and that therapeutic trials might be warranted. However, the pitfalls of direct application of in vitro susceptibility tests to clinical situations are well known. Although *R. quintana* exists in a pericellular state in both the louse gut and cell culture (5), the exact state in which it exists in infected human subjects is unknown. It appears to be cell associated in the blood of patients with trench fever (4) and of experimentally infected monkeys (9), but it is unknown whether it is, in reality, extracellular on the surface of blood elements or whether the organisms are intracellular as, for example, within circulating phagocytic cells. The nature of the cell association could determine whether a drug like penicillin G would be effective.

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