In vitro antimicrobial susceptibility against human and animal strains of the Chlamydia genus

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Abstract

The in vitro activity of doxycycline, levofloxacin, moxifloxacin, azithromycin, clarithromycin, erythromycin, cotrimoxazole and rifaximin was tested against 81 strains of Chlamydia trachomatis, 12 strains of Chlamydia suis and 1 strain of Chlamydia muridarum. The ranges of MIC and MBC for C. trachomatis, C. suis and C. muridarum is described below. MIC of doxycycline ranged from 0.03 to 32 µg/ml and MBC between 0.06 and 64 µg/ml. Both the MIC and the MBC of levofloxacin ranged from 0.25 to 2 µg/ml. Both the MIC and the MBC of moxifloxacin ranged from 0.125 to 0.5 µg/ml. MIC of azithromycin ranged from 0.015 to 0.125 µg/ml and the MBC between 0.03 and 0.125 µg/ml. MIC of clarithromycin ranged from 0.015 to 0.125 µg/ml and the MBC between 0.03 and 0.25 µg/ml. MIC of erythromycin ranged from 0.25 to 1 µg/ml and the MBC between 0.5 and 2 µg/ml. MIC of cotrimoxazole ranged from 1 to 8 µg/ml and the MBC between 2 and 16 µg/ml. Both the MIC and the MBC of rifaximin ranged from 0.25 to 2 µg/ml. In contrast to strains of Chlamydia trachomatis and Chlamydia muridarum, significant resistences to doxycycline were present in all strains of Chlamydia suis.

Introduction

Chlamydia is a Gram-negative bacterium, strict intracellular parasite of eukaryotic cells. The most recent classification of Chlamydia, based on phylogenetic analysis of the rRNA, makes a distinction in the Chlamydiaceae family of two genera: Chlamydia and Chlamydophila [1,2]. Although the chlamydial genome is small (low probability of mutagenesis) and despite the peculiar life cycle (low probability of exchanging genetic material with other bacteria), some studies have reported, resistance to some antimicrobial agents (tetracycline) from C. suis (tet gene present in most strains) [3,4,5] and possible resistance from C. trachomatis [6,7], whereas for C. muridarum were not reported particular resistances [8]. The presence of the tet gene in the cryptic plasmid of C. suis could be the first case of acquisition of genetic material from bacterial intracellular parasites [3,4]. Furthermore it should be noted that the recent plasmidic mutation in C. trachomatis (deletion 377 bp), determined negative effects in the diagnosis of this pathogen, with definition of a new variant (nv) strain of C. trachomatis [9]. This, in addition to the complications that can lead the chlamydial infections (PID, ectopic pregnancy, infertility), requires monitoring of antimicrobial susceptibility of these bacteria phylogenetically related. Aim of work is the evaluation in vitro of antimicrobial susceptibility for the Chlamydia genus (C. trachomatis, C. suis and C. muridarum) to antimicrobial agents used in treatment protocols of these infections: doxycycline (DOX), levofloxacin (LVX), moxifloxacin (MXF), azithromycin (ZTM), clarithromycin (CLAR) and erythromycin (ERY) and also against cotrimoxazole (CTX) (negative control of antimicrobial sensitivity) and rifaximin (RFX) (drug with good antimicrobial activity versus C. trachomatis, as reported in other studies) [10,11].

Methods

A total of 81 C. trachomatis strains were tested. Of these, 8 were reference strain from D to K, whereas 73 were strains recently isolated from urethral or cervical swabs obtained from male and woman patients with non-gonococcal urethritis or cervicitis. The 73 strains recently isolated were typed (15 genovar D, 18 genovar E, 12 genovar F, 14 genovar G, 1 genovar H, 2 genovar I, 8 genovar J and 3 genovar K) by sequencing of the omp1 gene, as described previously [12]. A total of 12 typed strains of C. suis were tested. This strains were isolated from conjunctival swabs obteined from swines with conjunctivitis. 1 untyped strain of C. muridarum (kindly provided by Dott. Finco O., Biochemistry and Molecular Biology Unit, Novartis Vaccines and Diagnostics, Siena, Italy) were tested. Susceptibility testing was performed in LLC-MK2 cells (derived from Rhesus monkey kidney tissue) grown in 24-well plates containing a glass coverslip at the bottom. The growth medium was Eagle's Minimum Essential Medium (EMEM) supplemented with 10% heat-inactivated fetal calf serum (FCS), 10 µg/ml gentamicin, 10 µg/ml...
vancomycin, 1 µg/ml amphotericin B, 2 mM L-glutamine and 1.7 µg/ml glucose. The well plates were inoculated with a chlamydial concentration of 5000 inclusion forming unit/ml (IFU/ml), and centrifugated at 1700 g for 60 minutes. The medium was then removed and replaced with EMEM supplemented with 10% FCS, 5 µg/ml glucose, 1µg/ml cyclohexymide, and two-fold serial dilutions of each antibiotic. All test were performed in triplicate. After incubation for 72 hours at 35°C, the cultures were fixed with methanol and stained for inclusion using a fluorescein-conjugated monoclonal antibody raised against the chlamydial lipopolysaccharide antigen (Meridian Diagnostics, Cincinnati, Ohio). The Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration that prevented more than 90% chlamydial inclusion, compared with drug-free controls (Illustration 1). The Minimal Bactericidal Concentration (MBC) was measured by aspirating the antibiotic-containing medium, washing twice with phosphate-buffered saline, adding antibiotic-free medium and reincubating for 72 hours at 35°C, after which the cultures were fixed and stained as described above. The MBC was defined as the lowest concentration that resulted in more than 90% reduction of inclusion compared with drug-free controls.

Results

The 73 strains recently isolated of C. trachomatis have in vitro the same sensitivity of 8 reference strains against tested drugs, not revealing resistance. The ranges of MIC and MBC for C. trachomatis is described below. MIC of DOX ranged from 0.03 to 0.125 µg/ml and MBC between 0.06 and 0.25 µg/ml. Both the MIC and the MBC of LVX ranged from 0.25 to 0.5 µg/ml. Both the MIC and the MBC of MXF ranged from 0.25 to 0.5 µg/ml. MIC of AZM ranged from 0.015 to 0.125 µg/ml and the MBC between 0.03 and 0.25 µg/ml. MIC of CLAR ranged from 0.015 to 0.125 µg/ml and the MBC between 0.03 and 0.25 µg/ml. MIC of ERY ranged from 0.25 to 1 µg/ml and the MBC between 0.5 and 2 µg/ml. MIC of COX ranged from 1 to 8 µg/ml and the MBC between 2 and 16 µg/ml. Both the MIC and the MBC of RFX ranged from 0.25 to 1 µg/ml. The RFX it has shown good in vitro activity against C. trachomatis, while low active is CTX (Illustration 2). The 12 strains recently isolated of C. suis showed good sensitivity to LVX, MXF, ZTM, CLAR, ERY and to RFX, while low sensitivity to CTX. These strains were resistant to DOX, as reported in other studies [2,3,4] (Illustration 3). The ranges of MIC and MBC for C. suis is described below. MIC of DOX ranged from 2 to 32 µg/ml and MBC between 4 and 64 µg/ml. Both the MIC and the MBC of LVX ranged from 0.5 to 2 µg/ml. Both the MIC and the MBC of MXF ranged from 0.5 to 1 µg/ml. MIC of AZM ranged from 0.03 to 0.125 µg/ml and the MBC between 0.06 and 0.25 µg/ml. MIC of CLAR ranged from 0.3 to 0.125 µg/ml and the MBC between 0.06 and 0.25 µg/ml. MIC of ERY ranged from 0.5 to 1 µg/ml and the MBC between 1 and 2 µg/ml. MIC of COX ranged from 2 to 8 µg/ml and the MBC between 4 and 16 µg/ml. Both the MIC and the MBC of RFX ranged from 0.5 to 2 µg/ml. The strains of C. muridarum showed antimicrobial susceptibility similar to C. trachomatis and the ranges of MIC and MBC for the strain of C. muridarum is described in Illustration 3.

Discussion

Except C. suis (resistance to DOX) were not detected in this study, resistances to the drugs tested (Table 1). The 4th strain of C. suis is distinguished from other strains for higher sensitivity at lower dilutions of MIC90 and MBC90 of the drugs tested: DOX MIC 0.03 µg/ml and MBC 4 µg/ml, LVX MIC and MBC 0.5 µg/ml, MXF MIC and MBC 0.5 µg/ml, ZTM MIC 0.03 µg/ml and MBC 0.06 µg/ml, CLAR MIC 0.03 µg/ml MBC 0.06 µg/ml, ERY MIC 0.5 µg/ml and MBC 1 µg/ml, CTX MIC 2 µg/ml MBC 4 µg/ml, RFX MIC and MBC 0.5 µg/ml. The strains of C. trachomatis, therefore did not present resistance to drugs used in therapeutic protocols and did not present significant genetic mutations in bacterial DNA, as reported previously [12]. It should be noted that against C. trachomatis in vitro the activity of DOX was also assessed in patients with chlamydial urethritis or cervicitis, from which these strains were isolated, through the use of this drug in all cases of infection. So all patients treated with DOX have eradicated the infection in 100% of cases. All patients were followed four times for one year after the diagnosis of infection through free medical visits (Sexually Transmitted Diseases centre of Policlinico S. Orsola-Malpighi of Bologna, Italy) and through use of direct diagnostic laboratory techniques, culture (LLC-MK2 cells) and RT-PCR (Abbott RealTime CT/NG, Delkheneim, Germany) and through indirect diagnostic laboratory techniques, ELISA (VIRION/SERION ELISA classic Chlamydia IgA/IgG, Würzburg, Germany) and microimmunofluorescence (home-made) [13]. Furthermore the data obtained show that among the genovar of C. trachomatis, the genovar F was sensitive to the lower dilutions of MIC90 and MBC90 of the drugs tested: DOX MIC 0.03
μg/ml and MBC 0.06 μg/ml, LVX MIC and MBC 0.25 μg/ml, MXF MIC and MBC 0.25 μg/ml, ZTM MIC 0.03 μg/ml and MBC 0.06 μg/ml, CLAR MIC 0.015 μg/ml MBC 0.03 μg/ml, ERY MIC 0.5 μg/ml and MBC 1 μg/ml, CTX MIC 2 μg/ml MBC 4 μg/ml, RFX MIC and MBC 0.25 μg/ml. Conversely the genovar G showed the highest MIC and MBC: DOX MIC 0.06 μg/ml and MBC 0.125 μg/ml, LVX MIC and MBC 0.5 μg/ml, MXF MIC and MBC 0.5 μg/ml, ZTM MIC 0.03 μg/ml and MBC 0.06 μg/ml, CLAR MIC 0.03 μg/ml MBC 0.06 μg/ml, ERY MIC 1 μg/ml and MBC 2 μg/ml, CTX MIC 4 μg/ml MBC 8 μg/ml, RFX MIC and MBC 1 μg/ml.

Conclusion(s)

It should be stressed the good in vitro activity shown by RFX against the three species of Chlamydia object of this study. Further in vivo studies should be made, however, to confirm this finding and to assess the potential clinical applicability. This molecule, a derivative of rifampicin, could be used in the chlamydial topical treatment, but also in gastro-intestinal tract infections of veterinary interest incurred from C. suis, as a function of the low cost of this molecule and fewer side effects compared to macrolides and quinolones. The rational use of this drug is also linked to what is shown in an other study: rifaximin reduced plasmid transfer from donor to recipient strains by >99% [14]. Finally the drug most active, in vitro, in this study against the Chlamydia genus with the lower dilutions of MIC90 and MBC90 was CLAR. COX was a good negative control of antimicrobial susceptibility, because as the sulphonamides, COX has little antimicrobial activity against genus Chlamydia. Although the three species of the Chlamydia genus are phylogenetically related, C. suis differs for sensitivity to antimicrobial agents by the other two species (C. trachomatis and C. muridarum), which between them were similar. The study also shows that, as the quinolones, the RFX has a coincident value of MIC and MBC against the Chlamydia genus. Therefore the equivalent concentrations at the MIC of these antimicrobial agent kill the chlamydial strains (bactericidal activity).

Reference(s)

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Illustrations

Illustration 1

C. trachomatis inclusions at the Zeiss UV microscope (40X): A drug free control and B MIC90 of LVX

Illustration 2

In vitro MIC90 (µg/ml) and MBC90 (µg/ml) modal values against 81 genovar of C. trachomatis
Illustration 3

In vitro MIC90 (µg/ml) and MBC90 (µg/ml) modal values against C. suis and C. muridarum

<table>
<thead>
<tr>
<th>DRUGS</th>
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<th>C. muridarum (1)</th>
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