Microbiological efficacy of ABT-773 (cethromycin) for the treatment of community-acquired pneumonia due to *Chlamydia pneumoniae*

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Nasopharyngeal specimens for culture of *Chlamydia pneumoniae* were obtained from patients with community-acquired pneumonia enrolled in a randomized study comparing the novel ketolide antibiotic ABT-773 at a dose of 150 mg once a day to 150 mg twice a day, by mouth for 10 days. *C. pneumoniae* was eradicated from the nasopharynx of 10 of 10 (100%) microbiologically evaluable patients. MICs and MBCs for 13 isolates of *C. pneumoniae* from 12 patients obtained before and after therapy were performed against ABT-773. The MIC90 and MBC90 of ABT-773 were 0.015 mg/L.

Keywords: chlamydia, *C. pneumoniae*, ketolide

Introduction

*Chlamydia pneumoniae* is a frequent cause of community-acquired respiratory tract infection, including pneumonia and bronchitis.1 However, data on treatment of respiratory infection due to *C. pneumoniae* are limited. The majority of previously published studies evaluating new macrolides/azalides and quinolones for the treatment of pneumonia have used serology alone for diagnosis, thereby limiting the evaluation of therapy. There are only three pneumonia treatment studies evaluating macrolides/azalides published to date that have utilized *C. pneumoniae* culture and assessed microbiological efficacy.5,6 We previously reported that ABT-773 (cethromycin), a new ketolide antibiotic, was the most active agent tested against *C. pneumoniae* with an MIC90 and MBC90 of 0.015 mg/L (range 0.008–0.015 mg/L).4 ABT-773 was at least four-fold more active *in vitro* against *C. pneumoniae* than telithromycin, clarithromycin and azithromycin. As part of a multicentre treatment study of community-acquired pneumonia (CAP) in adults evaluating two dosage regimens of ABT-773, we obtained nasopharyngeal swabs for *C. pneu-

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static tumour involving the lung, bronchial obstruction, a
disease such as cardiovascular, pulmonary, metabolic,
gastrointestinal, neurological or endocrine disease,
malignancy or any other abnormality other than CAP.
Furthermore, subjects needed to be assessed as suitable for
outpatient therapy such that subjects with the Fine Criteria
requiring the need for possible hospitalization were excluded.
Subjects with prior hospitalization and on antibiotics or other
drugs likely to interact with ketolides or with a history of
allergy were also excluded. Posterior nasopharyngeal (NP)
specimens were obtained for C. pneumoniae culture at
baseline, and 19–24 days after enrolment using wire-shafted,
Dacron-tipped swabs (Puritan Quality Medical Products,
Guilford, ME, USA), as described previously. The swabs
were immersed in 2 mL of transport medium (sucrose-
phosphate buffer with 20% fetal calf serum, 10 mg/L genta-
micin, 10 mg/L vancomycin and 1 mg/L amphotericin B). An
oropharyngeal swab was also obtained for detection of
C. pneumoniae DNA by PCR at baseline. Sera were obtained
at baseline and 19–24 days after enrolment for C. pneumoniae
serology.

Culture of C. pneumoniae

C. pneumoniae culture was performed at SUNY Downstate
Medical Center utilizing cycloheximide-treated HEP-2 cells
grown in 96-well microtitre plates. After 72 h incubation all
specimens were passed once. Cultures were confirmed by
fluorescent antibody staining with a Chlamydia-specific monoclonal antibody. Patient isolates were then passed five
to six times in cell culture in antibiotic-free medium.

PCR for C. pneumoniae was performed at the University of
Louisville, as described previously. Detection of anti-C. pneumoniae antibody was performed by microimmunofluorescence (MIF) assay using a commercial kit (Focus Technologies, Cypress, CA, USA). The serological diagnosis of acute C. pneumoniae infection required a four-fold increase in IgG and/or IgM in paired sera or a single
IgM ≥ 32.

ABT-773 (Abbott Laboratories) was supplied as a powder
and solubilised according to the manufacturer’s instructions.

Susceptibility studies

Susceptibility testing of C. pneumoniae was performed in cell
culture using HEP-2 cells grown in 96-well microtitre plates,
as described previously. Each well was inoculated with
0.2 mL of the organism diluted to yield 103 inclusion-forming
units (IFU)/mL, and centrifuged at 2000g for 1 h. The wells
were then aspirated and overlaid with 0.2 mL of medium con-
taining 1 mg/L cycloheximide and serial two-fold dilutions of
the test drug. After incubation at 35°C for 72 h, cultures were
fixed and stained for inclusions with fluorescein-conjugated
antibody to the lipopolysaccharide genus antigen (Pathfinder
Chlamydia Culture Confirmation System, Bio-Rad). The
MIC was the lowest antibiotic concentration at which no
inclusions were seen. The MBC was determined by freezing
the cultures at –70°C, then thawing, passing the disrupted cell
monolayers onto new cells, incubating for 72 h, then fixing
and staining as above. The MBC was the lowest antibiotic
concentration that resulted in no inclusions after passage. All
tests were run in triplicate.

Results

Nasopharyngeal specimens for C. pneumoniae were obtained
from 335 patients, 12 (3.6 %) were culture positive for
C. pneumoniae. The mean age of the C. pneumoniae-positive
patients was 49 ± 13.8 years (range 34–70); seven were male
and five were female. Two culture-positive patients were
excluded from the final analysis because they were negative at
baseline, but positive at the third follow-up visit, 19–24 days
after the initiation of treatment. One of these patients, a
52-year-old man, was found to have pulmonary tuberculosis.
The other patient, a 70-year-old man, had serological evi-
dence of acute Legionella pneumophila infection. He initially
responded to treatment and was felt to have a new respiratory
infection at the time of evaluation 23 days after enrolment,
when he was found to have a positive C. pneumoniae culture.
Both these patients had stable anti-C. pneumoniae IgG titres
and no detectable anti-C. pneumoniae IgM.

C. pneumoniae was eradicated from the nasopharynx of all
10 (100%) microbiologically evaluable patients; all were
clinical cures. Five patients each were treated with either the
once a day or twice a day dosing schedule. Eight of the
C. pneumoniae-positive patients had other pathogens iso-
lated from sputum, including Haemophilus influenzae (one),
H. parainfluenzae (seven) and Moraxella catarrhalis (one).
C. pneumoniae DNA was detected by PCR in only two of the
10 patients who were culture positive at baseline. Of the
10 evaluable patients, two had anti-C. pneumoniae IgM ≥ 32;
five had a four-fold rise in IgM with no detectable or stable
IgG, two had a four-fold rise in IgG and one patient had a four-
fold rise in IgM and IgG.

In vitro susceptibility testing of 13 isolates of C. pneumo-
niae from the 12 patients in this study, obtained before and
after therapy, was performed against ABT-773 (one subject
had two samples sent). The MIC90 and MBC90 were 0.015 mg/L
(range 0.008–0.015 mg/L).
Microbiological efficacy of ABT-773 against *C. pneumoniae*

**Discussion**

ABT-773 was very efficacious in the eradication of *C. pneumoniae* from the nasopharynx of adults with pneumonia. Both dosage regimens were equally effective; however, the numbers were too small for a meaningful statistical analysis. These results compare very favourably with prior experience of macrolides/azalides. Results of several treatment studies that performed *C. pneumoniae* culture demonstrated that 10–14 days of treatment with erythromycin and clarithromycin or 5 days of azithromycin has ~80% efficacy in the eradication of *C. pneumoniae* from the respiratory tract of children and adults with pneumonia.2,3

The *C. pneumoniae* isolates obtained from the patients in this study were highly susceptible to ABT-773, with a MIC<sub>90</sub> and MBC<sub>90</sub> of 0.015 mg/L, which was identical to our earlier report. ABT-773 also appears to be more active than telithromycin, another ketolide. We previously reported a MIC<sub>90</sub> and MBC<sub>90</sub> of telithromycin for 19 isolates of *C. pneumoniae* of 0.25 mg/L. Unlike ABT-773, there was wide inter-isolate variation in the MICs and MBCs, which ranged from 0.031 to 2.0 mg/L. Similar data were reported by Miyashita et al., who tested 20 isolates of *C. pneumoniae* and found a MIC<sub>90</sub> and MBC<sub>90</sub> of 0.125 mg/L (range 0.031–0.25 mg/L). There are no data on the efficacy of telithromycin for the eradication of *C. pneumoniae* from the respiratory tract of patients with pneumonia. However, *in vitro* activity may not always predict *in vivo* efficacy. For example, clarithromycin is 10- to 100-fold more active than erythromycin, but it was not more effective in the eradication of *C. pneumoniae*.2

As access to culture is limited and there are no Food and Drug Administration-approved, commercially available, standardized nucleic amplification tests for *C. pneumoniae*, diagnosis in many treatment studies has been based on serology. Unfortunately, use of serology provides only a clinical endpoint and does not allow for the assessment of microbiological efficacy. In addition, *C. pneumoniae* serology is also not standardized and there are problems with both inter- and intra-laboratory reproducibility, especially with the MIF assay. Studies of children, and to a lesser extent in adults, have often found a poor correlation between serology and the identification of the organism by culture and/or PCR.2,3,9,10 Two studies in children found that >70% culture-positive patients with pneumonia were seronegative. Although the 10 evaluable culture-positive patients in this study met the study criteria for serological diagnosis of acute *C. pneumoniae* infection, the Centers for Disease Control and Prevention (CDC) recently proposed several modifications of these criteria. They include a single IgM ≥ 16 and/or a four-fold rise in IgG only. However, the use of a single IgM could be misleading as 10–18% of asymptomatic, culture-negative adults may have anti-*C. pneumoniae* IgM ≥ 16. Results of two adult treatment studies found that only 30–40% of patients with culture-documented *C. pneumoniae* infection met the serological definition for acute infection with the MIF test recently proposed by the CDC. The remaining patients had either no detectable antibody or stable IgG titres.9,10

In addition to having excellent activity against a wide range of bacteria involved in respiratory infection, including erythromycin-resistant *Streptococcus pneumoniae*,11 ABT-773 has also been demonstrated to have excellent *in vitro* activity against extracellular and intracellular *L. pneumophila*.12 The results of this initial study support further studies of the clinical and microbiological effectiveness of ABT-773 for the treatment of CAP, including infections due to *C. pneumoniae*.

**References**


