Short Communication

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Archives of Clinical Microbiology
ISSN 1989-8436

2021
Vol. 12 No. 5:173

In vitro Activity of The Quinoline Derivatives RD-3 and ER-2 Against Clinical Isolates of *C. pneumoniae*

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Citation: Rao SS, Raghunathan M, Manian RD, Ramesh E, Raghunathan R (2021) *In vitro* Activity of The Quinoline Derivatives RD-3 and ER-2 Against Clinical Isolates of *C. pneumoniae*. Arch Clin Microbial Vol.12 No.5: 173.

Received: August 25, 2021; **Accepted:** September 08, 2021; **Published:** September 15, 2021

Description

RD-3 and ER-2 are both, investigational quinoline derivative drugs that have previously shown potent activity against Gram-positive and Gram-negative organisms [1], and clinical isolates of *M. pneumoniae* [2,3]. *C. pneumoniae* is an obligate intracellular pathogen that causes respiratory tract infections and community-acquired pneumonia, in adults and children [4]. Antibiotics that are commonly used to treat *C. pneumoniae* are macrolides, quinolones and tetracycline. In this study we examined the activity of RD-3 and ER-2 against clinical isolates and standard strain AR-39 of *C. pneumoniae* and compared the activities against azithromycin, doxycycline, moxifloxacin, levofloxacin.

Twenty one *C. pneumoniae* strains were used in this study. AR-39 was obtained from the Washington Research Foundation, Seattle WA, USA. Wild type strains were isolated from nasopharyngeal swabs specimens collected from patients with acute respiratory tract infections at different hospitals in Chennai. The clinical isolates were stained with *C. pneumoniae* specific monoclonal antibody.

The comparator agents, azithromycin, doxycycline, moxifloxacin, and levofloxacin were from Sigma-Aldrich (St. Louis, MO,USA).

Antimicrobial powders were used according to the manufacturer's protocol. Working dilutions of the drugs were prepared fresh on the day of the assay.

Susceptibility testing of *C. pneumoniae* was performed as described [5]. The HEp-2 cells were grown in 96 well microfiber plates containing Eagle's essential medium and 10% heat-inactivated fetal calf serum. The wells were inoculated with 0.1 ml of the test strain that was diluted to yield 10^3 to 10^4 Inclusion forming units per ml. The plates were centrifuged at 1,700 × g for 1 hr and incubated at 35°C for 1 hr. The wells were aspirated and 0.2 ml of media containing 1microgram of cycloheximide per ml was dispensed into each well with serial two fold dilution of the test drug. After incubation in 5% CO_2 at 35°C for 72 h, the cultures were stained with fluorescent isothiocyanate-conjugated monoclonal antibody (Bio-Rad, Hercules, CA. Minimum Inhibitory Concentration (MIC) was defined as the lowest concentration at which no inclusions were found. Minimum Bactericidal Concentration (MBC) was determined by aspirating the antibiotic-containing medium, washing wells twice with phosphate-buffered saline and adding antibiotic-free medium. The infected cells were frozen at 70°C, thawed and then passed onto new cells incubated for 72 h, fixed and then stained as described above. MBC was the lowest antibiotic concentration which showed no inclusions after passage. All tests were performed in triplicates. The MIC and MBC of *C. pneumoniae* against different drugs are given in (**Table 1**). RD-3 was most active with an MIC_{90} and MBC_{90} of 0.125 mg/L (range 0.03-0.125 mg/L). ER-2 was better than doxycycline and azithromycin with an MIC_{90} of 0.25mg/L and MBC_{90} of 0.25mg/L. Doxycycline and azithromycin were superior to moxifloxacin and levofloxacin with an MIC_{90} of 0.25mg/L (range 0.06-0.25 mg/L), and MBC_{90} of 0.25 mg/L (range 0.06-0.25 mg/L).

Table 1: Activities of RD-3 and other antibiotics against clinical isolates of *C. pneumoniae.*

	MICs mg/L			MBCs mg/L	
	Range	MIC50	MIC90	Range	MBC90
RD-3	0.03-0.125	0.06	0.125	0.03-0.125	0.125
ER-2	0.06-0.125	0.06	0.25	0.06-0.25	0.25
Doxycycline	0.06-0.25	0.125	0.25	0.06-0.5	0.25
Azithromycin	0.06-0.25	0.125	0.25	0.06-0.6	0.25
Moxifloxacin	0.125-0.5	0.25	0.5	0.25-0.5	0.5
Levofloxacin	0.25-0.5	0.25	0.5	0.5-1	1

Due to the rise in antimicrobial resistance in alarming speed there is a need for alternative drugs [6,7].

Conclusion

The results presented in this study and our previous studies indicate that RD-3 and ER-2 could be effective in the treatment of *C. pneumoniae* infections; however its clinical application will depend on its toxicity and pharmacokinetic properties.

Acknowledgement

This study was funded partially by University of Madras. Azithromycin, levofloxacin and moxifloxacin were gifts from Department of Microbiology, University of Madras.

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