

# AZD0914: A New Spiropyrimidinetrione DNA Gyrase Inhibitor - Effect of Inoculum Size, Incubation Time/Temperature, pH, Atmosphere, Urine, Serum and Combination/Synergy Testing on the *In Vitro* Antibacterial Activity of AZD0914

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## Introduction

A medical need exists for novel antibacterial compounds to treat infections caused by multi-drug resistant (MDR) organisms including those caused by *Staphylococcus aureus* (MRSA), *Streptococcus pneumoniae*, vancomycin-R enterococci, and *Neisseria gonorrhoeae*. AZD0914 is a novel spiropyrimidinetrione bacterial DNA gyrase/topoisomerase IV inhibitor with potent *in vitro* antibacterial activity against fluoroquinolone-resistant and MDR Gram-positive and fastidious Gram-negative bacterial species. This study evaluated the effect of inoculum size, incubation time/temperature, pH, atmosphere, urine, serum, and combination/synergy testing on the *in vitro* antibacterial activity of AZD0914.

## Materials and Methods

**Compound:** The compound structure for AZD0914 is listed in Figure 1.

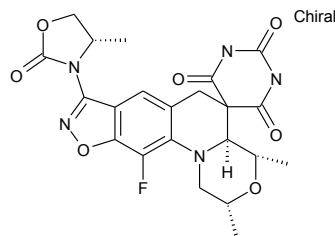
**Experimental procedures:** Broth microdilution Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) testing of AZD0914 and levofloxacin was conducted according to CLSI guidelines (M07-A09, M26-A, M100-S23).

**Data analysis:** MIC values were determined visually and reported as the lowest concentration of drug that completely inhibited growth of the organism. MBC values were determined by plating the entire well contents onto a fresh 5% sheep blood agar plate, incubating overnight and counting colonies. The MBC was defined as a  $\geq 3 \log_{10}$  reduction in viable organism counts within 8-fold the MIC in 24 hours.

## Results

AZD0914 MIC values for *S. aureus*, *S. pyogenes* and *S. pneumoniae* were either unchanged or increased 2-fold as the inoculum was raised from  $10^5$  to  $10^7$  CFU/ml. Against these same strains, AZD0914 was bactericidal regardless of inoculum level (Table 1). A larger MIC increase ( $\geq 32$ -fold) was observed for *H. influenzae* as the inoculum was raised from  $10^5$  to  $10^7$  CFU/ml. AZD0914 MICs remained within 2-fold over a temperature range of 30-37°C. MICs were unaffected (within 2-fold) by incubation in aerobic, anaerobic or 5% CO<sub>2</sub> atmospheres (Table 2). The effect of pH on the *in vitro* activity of AZD0914 was also determined. Compared to physiological pH (~7.4), AZD0914 was generally 2-fold more active at acidic pH (5.0 to 6.5) and 2-fold less active at basic pH (8.0). This trend was more difficult to observe for *S. pneumoniae* and *H. influenzae* due to the lack of growth of these cultures at pH values of 5.0-6.0 and above 8.0. AZD0914 MICs generated in the presence of 50% human urine or 50% human serum remained within 2-fold and 4-fold, respectively, of those obtained in cation adjusted Mueller Hinton broth. MICs for AZD0914 were unchanged in varying concentrations of divalent cations. In checkerboard/synergy combination testing of AZD0914 with 16 comparator antimicrobial agents (aztreonam, amoxicillin, amoxicillin-clavulanic acid, azithromycin, ceftazidime, ceftriaxone, cefixime, clindamycin, gentamicin, levofloxacin, linezolid, meropenem, metronidazole, rifampin, tetracycline and vancomycin) versus the 7 strains listed in Table 1 showed only additivity/indifference. No antagonism or synergy was observed.

Figure 1  
AZD0914



## Results

Table 1: Effect of Inoculum Concentration on AZD0914 MICs and MBCs (µg/ml)

Organism	Strain #	Inoculum Conc. (CFU/mL)	AZD0914			Levofloxacin
			MIC	MBC	Cidal/Static	MIC
<i>Staphylococcus aureus</i>	ATCC 29213	$1.2 \times 10^7$	0.25	0.5	Bactericidal	0.25
		$1.2 \times 10^5$	0.25	0.25	Bactericidal	0.125
		$1.2 \times 10^3$	0.25			0.125
<i>S. aureus</i> MRSA	USA300	$1.7 \times 10^7$	0.5	1	Bactericidal	1
		$1.7 \times 10^5$	0.25	0.5	Bactericidal	1
		$1.7 \times 10^3$	0.25			0.5
<i>S. aureus</i> MRSA Levofloxacin-R	USA100	$1.0 \times 10^7$	0.25			16
		$1.0 \times 10^5$	0.25	0.25	Bactericidal	8
		$1.0 \times 10^3$	0.125			8
<i>S. aureus</i> MRSA Levofloxacin-R	ARC517	$1.0 \times 10^7$	0.5	1	Bactericidal	8
		$1.0 \times 10^5$	0.25	0.5	Bactericidal	4
		$1.0 \times 10^3$	0.25			4
<i>Streptococcus pyogenes</i>	ARC838	$1.0 \times 10^7$	0.5	1	Bactericidal	1
		$1.0 \times 10^5$	0.125	0.25	Bactericidal	0.5
		$1.0 \times 10^3$	0.125			0.25
<i>S. pneumoniae</i>	ATCC 49619	$1.0 \times 10^7$	0.25	0.5	Bactericidal	1
		$1.0 \times 10^5$	0.125	0.25	Bactericidal	0.5
		$1.0 \times 10^3$	0.125			0.5
<i>Haemophilus influenzae</i>	ATCC 49247	$1.4 \times 10^7$	0.5	0.5	Bactericidal	0.03
		$1.4 \times 10^5$	$\leq 0.015$	$\leq 0.015$	Bactericidal	$\leq 0.015$
		$1.4 \times 10^3$	$\leq 0.015$			$\leq 0.015$

Table 2: Effect of Incubation Temperature and Atmosphere on AZD0914 MICs (µg/ml)

Strain	Culture #	30°C	35°C	37°C	35°C (CO <sub>2</sub> )	35°C (Anaerobic)
<i>S. aureus</i>	ATCC 29213	0.25	0.25	0.25	0.125	0.125
<i>S. aureus</i>	USA300	0.25	0.25	0.25	0.125	0.125
<i>S. pyogenes</i>	ARC838	0.125	0.125	0.125	0.125	0.125
<i>S. pneumoniae</i>	ATCC 49619	0.125	0.125	0.25	0.125	0.125
<i>H. influenzae</i>	ATCC 49247	0.25	No data	No data	0.25	0.25

## Conclusions

- AZD0914 was bactericidal against the strains tested regardless of inoculum concentration.
- AZD0914 MICs were unchanged over a temperature range of 30-37°C and in various atmospheres.
- AZD0914 MICs were 2-fold lower at acidic pH and somewhat higher at basic pH.
- AZD0914 remained active in 50% human urine or serum and in varying concentrations of divalent cations.
- AZD0914 showed only additivity/indifference in *in vitro* combination/synergy testing.