# Synergistic Activity of Colistin and Zidovudine (AZT) **Combinations against Colistin-Resistant Enterobacteriaceae**

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# Introduction

- Novel strategies are required to combat the increasing rates of antimicrobial resistance among gram-negative pathogens
- One rapid and attractive strategy is to investigate the therapeutic advantages exhibited by combinations of known antimicrobials
- Azidothymidine (AZT, zidovudine) is an antiretroviral thymidine analog that is typically used in combination with other drugs to treat or prevent HIV infection by inhibiting reverse transcriptase activity
- AZT also exhibits bactericidal antimicrobial activity against many Enterobacteriaceae species
- Colistin is a lipopeptide that is clinically used to treat infections caused by highly resistant isolates of some groups of gram-negative bacteria including Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii
- Previous work demonstrated that AZT-colistin combinations exhibited *in vitro* synergy against many antibiotic-resistant Enterobacteriaceae isolates and exhibited superior activity to either agent alone in a mouse peritoneal infection model
- This study confirmed and extended previous results on the *in vitro* antimicrobial potency, spectrum, and synergy of colistin-AZT combinations against recent Enterobacteriaceae isolates using Clinical and Laboratory Standards Institute (CLSI) reference testing methods

# Materials and Methods

- The *in vitro* antimicrobial activities of colistin, AZT, AZT-colistin (1:2), AZT-colistin (1:1), and AZT-colistin (2:1) combinations were measured against a set of 333 Enterobacteriaceae clinical isolates
- 277 randomly selected Enterobacteriaceae clinical urinary tract infection isolates (Table 1) recently recovered from patients in Europe and the United States
- A partially overlapping set of 33 Enterobacteriaceae isolates (Table 2) with a colistin-resistant phenotype
- Additional isolates were from Enterobacteriaceae species that are intrinsically resistant to colistin (data not shown)
- AZT, ceftriaxone, and meropenem were purchased from United States Pharmacopeia, and colistin sulfate was purchased from Sigma-Aldrich
- CLSI broth microdilution and quality control methodologies were followed
- Cation-adjusted Mueller Hinton broth was the test medium
- European Committee on Antimicrobial Susceptibility Testing (EUCAST) interpretive criteria were used to categorize Enterobacteriaceae isolates as colistin-susceptible (MIC value,  $\leq 2 \text{ mg/L}$ ) or colistin-resistant (MIC value, >2 mg/L)
- Because AZT exhibited a trailing inhibition of growth phenotype against some isolates, all MIC values were measured in 2 ways
- As the lowest concentration of compound that completely inhibited growth (indicated by 100%)
- As the lowest concentration of compound that significantly inhibited growth (indicated by SR, for significant reduction)
- Although this study evaluated AZT-colistin *in vitro* activity at 3 fixed ratios rather than in full checkerboard panels,  $\Sigma$ FIC index values could be calculated from the available data using the following equation:

FIC index =  $\left(\frac{\text{MIC}(\text{drug A})_{\text{combinationAB}}}{\text{MIC}(\text{drug A})_{\text{alone}}}\right) + \left(\frac{\text{MIC}(\text{drug B})_{\text{combinationAB}}}{\text{MIC}(\text{drug B})_{\text{alone}}}\right)$ 

- $\Sigma$ FIC index values were interpreted using 3 categories
- Synergy:  $\Sigma$ FIC value ≤0.5 – Indifference:  $\Sigma$ FIC value >0.5 to  $\leq$ 4 – Antagonism: ∑FIC value >4

# Results

- AZT alone exhibited a trailing inhibition of growth phenotype for some isolates (Figure 1) that was largely absent for AZT-colistin combinations (Figure 2)
- The overall colistin resistance rate was 4% for the randomly selected Enterobacteriaceae isolates from species intrinsically susceptible to colistin (Table 1) – The isolate set was 20.9% resistant to ceftriaxone and 0.7% resistant to
- meropenem (Table 1) Against this isolate set, the MIC<sub>50/90</sub> values for AZT and colistin were 2/16 mg/L and 0.25/0.25 mg/L using the SR reading criterion, respectively, and the MIC  $_{50/90}$ values for AZT-colistin combinations were not significantly improved relative to colistin alone (Table 1)
- In contrast, all tested AZT-colistin combinations exhibited more potent MIC<sub>50/90</sub> values than AZT or colistin alone against the subset of Enterobacteriaceae isolates with acquired colistin resistance (Table 2)
- The isolate set was 57.6% resistant to ceftriaxone and 12.1% resistant to meropenem (Table 2)
- The AZT and colistin  $MIC_{50/90}$  values were 1/32 mg/L and 16/>64 mg/L, respectively, against this isolate set using the SR reading criterion, but the MIC<sub>50/90</sub> values for AZT-colistin (1:1) were 0.25/1 mg/L This effect was observed with both MIC reading criteria
- In total, 25 of 33 (76%) colistin-resistant isolates exhibited at least 1 instance of synergy among the tested AZT-colistin combinations (Table 3) - For many isolates, synergy was observed with more than 1 of the tested AZTcolistin combinations
- AZT did not improve the activity of colistin against isolates of Enterobacteriaceae species that are intrinsically resistant to colistin (data not shown)

### Table 1 Antimicrobial activity of AZT, colistin, and AZT-colistin combinations tested against 277 randomly selected Enterobacteriaceae isolates from species intrinsically susceptible to colistin

		EUCAST°				
Antimicropial agent <sup>*</sup>	MIC 50	MIC <sub>90</sub> <sup>b</sup>	Range	% <b>S</b>	%	% <b>R</b>
AZT 100%	4	>64	0.06 to >64			
Colistin 100%	0.25	0.25	0.12 to >64	96.0		4.0
AZT-colistin (1:1) 100%	0.25 / 0.25	0.25 / 0.25	≤0.008 to 16			
AZT-colistin (1:2) 100%	0.12 / 0.25	0.12 / 0.25	0.06 to 8			
AZT-colistin (2:1) 100%	0.25 / 0.12	0.5 / 0.25	0.06 to 32			
AZT SR	2	16	≤0.03 to >64			
Colistin SR	0.25	0.25	0.12 to >64			
AZT-colistin (1:1) SR	0.25 / 0.25	0.25 / 0.25	≤0.008 to 16			
AZT-colistin (1:2) SR	0.12 / 0.25	0.12 / 0.25	0.03 to 8			
AZT-colistin (2:1) SR	0.25 / 0.12	0.5 / 0.25	0.03 to 32			
Ceftriaxone	0.06	>32	≤0.015 to >32	79.1	0.0	20.9
Meropenem	0.03	0.06	≤0.015 to	98.9	0.4	0.7

Criteria as published by EUCAST 2018.

- The MIC<sub>50/90</sub> values for all 3 AZT-colistin combinations agreed within 2-fold

, zidovudine; 100%, 100% reading criterion; SR, significant reduction reading criterion; S, susceptible; I, intermediate; R, resistant. MIC values were read using 2 criteria: 100% inhibition of growth and significant reduction of growth. <sup>b</sup> For AZT-colistin combinations, the individual concentrations of AZT and colistin are shown.

rganisms included: Citrobacter freundii species complex (10), C. koseri (6), Enterobacter aerogenes (10), E. cloacae species complex (19), Escherichia coli (156), Klebsiella oxytoca (11), K. pneumoniae (65)

### Table 2 Antimicrobial activity of AZT, colistin, and AZT-colistin combinations tested against 33 colistin-resistant Enterobacteriaceae isolates

		MIC (mg/L)	EUCAST°			
Antimicropial agent <sup>a</sup>		MIC <sub>90</sub> <sup>b</sup>	Range	% <b>S</b>	%	
AZT 100%	2	>64	0.25 to >64			
Colistin 100%	16	>64	4 to >64	0.0		
AZT-colistin (1:1) 100%	0.5 / 0.5	2/2	0.12 to 16			
AZT-colistin (1:2) 100%	0.25 / 0.5	1/2	0.06 to 8			
AZT-colistin (2:1) 100%	0.5 / 0.25	2 / 1	0.12 to 32			
AZT SR	1	32	0.06 to >64			
Colistin SR	16	>64	4 to >64			
AZT-colistin (1:1) SR	0.25 / 0.25	1/1	0.12 to 16			
AZT-colistin (1:2) SR	0.25 / 0.5	1/2	0.06 to 8			
AZT-colistin (2:1) SR	0.5 / 0.25	2 / 1	0.06 to 32			
Ceftriaxone	32	>32	0.06 to >32	42.4	0.0	
Meropenem	0.03	16	≤0.015 to >32	81.8	6.1	

0% reading criterion; SR, significant reduction reading criterion; S, susceptible; I, intermediate: R, resistant <sup>a</sup> MIC values were read using 2 criteria: 100% inhibition of growth and significant reduction of growth. <sup>o</sup> For AZT-colistin combinations, the individual concentrations of AZT and colistin are shown. Criteria as published by FUCAST 2018

Organisms included: Enterobacter aerogenes (1), E. cloacae (2), E. cloacae species complex (7), Escherichia coli (8), Klebsiella oxytoca (1), K. pneumoniae (14)

### Table 3 Synergism of AZT-colistin combinations against a subset of colistin-resistant Enterobacteriaceae

		MIC value (mg/L) (ΣFIC value)										
				100% reading c		Significant reduction reading criterion <sup>a,b</sup>						
Collection number Species		AZT	Colistin	AZT-colistin (1:1)	AZT-colistin (1:2)	AZT-colistin (2:1)	AZT	Colistin	AZT-colistin (1:1)	AZT-colistin (1:2)		
1004447	Enterobacter cloacae species complex	8	>64	0.25 / 0.25	0.5 / 1	0.5 / 0.25	8	>64	0.25 / 0.25	0.5 / 1		
1024263	Klebsiella pneumoniae	8	32	0.5 / 0.5 (0.08)	2 / 4 (0.38)	1 / 0.5 (0.14)	8	32	0.5 / 0.5 (0.08)	2 / 4 (0.38)		
1033266	Enterobacter cloacae species complex	8	>64	0.25 / 0.25	0.12 / 0.25	0.5 / 0.25	2	>64	0.25 / 0.25	0.12 / 0.25		
996443	Enterobacter cloacae species complex	16	64	0.25 / 0.25 (0.02)	0.12 / 0.25 (0.01)	0.5 / 0.25 (0.04)	16	64	0.25 / 0.25 (0.02)	0.12 / 0.25 (0.01)		
960660	Klebsiella pneumoniae	64	64	8 / 8 (0.25)	2 / 4 (0.09)	4 / 2 (0.09)	64	64	4 / 4 (0.13)	2 / 4 (0.09)		
953366	Klebsiella pneumoniae	>64	32	16 / 16	8 / 16	32 / 16	>64	32	16 / 16	8/16		
989319	Escherichia coli	>64	4	2/2	1/2	1/0.5	4	4	0.5 / 0.5 (0.25)	1 / 2 (0.75)		
991175	Enterobacter cloacae species complex	>64	>64	0.5 / 0.5	1/2	0.5 / 0.25	2	>64	0.5 / 0.5	0.5 / 1		
1021931	Enterobacter cloacae species complex	>64	>64	0.25 / 0.25	0.12 / 0.25	0.5 / 0.25	32	>64	0.25 / 0.25	0.12 / 0.25		
1040346	Klebsiella pneumoniae	>64	16	0.25 / 0.25	0.12 / 0.25	0.25 / 0.12	>64	16	0.25 / 0.25	0.12 / 0.25		

 $\Sigma$ FIC. sum of fractional inhibitory concentrations: AZT, zidovudine Shaded areas indicate AZT-colistin combinations that exhibited synergism at the corresponding MIC values.  $\circ$   $\Sigma$ FIC index values could not be calculated when 1 or both MIC values were off-scale. Only a subset of the tested colistin-resistant isolates is shown. A total of 76% (25/33) of the tested isolates exhibited synergism for at least 1 AZT-colistin combination.

Figure 1 Comparison of AZT MIC values read at 100% inhibition and significant reduction against 333 Enterobacteriaceae solates tested in this



AZT, zidovudine; 100%, 100% reading criterion; SR, significant reduction reading criterion. MIC values were read using 2 criteria: 100% inhibition of growth and significant reduction (SR) of growth.

Figure 2 Comparison of AZT-colistin (1:1) MIC values read at 100% inhibition and significant reduction against 333 Enterobacteriaceae isolates tested in this study



64														
4														
2													3	
6												2	3	
-										1	1			
)									1					
								4						
5							14	1	1	1				
25						197	2							
L2					59	10								
)6				2	4									
)3				1										
15														
800	1													
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	
						AZT	-colistin (	1:1) 1009	% MIC (m	g/L)				

AZT, zidovudine; 100%, 100% reading criterion; SR, significant reduction reading criterion MIC values were read using 2 criteria: 100% inhibition of growth and significant reduction (SR) of growth.

L00.0	

AZT-colistin (2:1)
0.5 / 0.25
1 / 0.5 (0.14)
0.5 / 0.25
0.5 / 0.25 (0.04)
4 / 2 (0.09)
32 / 16
1 / 0.5 (0.38)
0.5 / 0.25
0.5 / 0.25

0.25 / 0.12

	30
6	3
1	4
	8
1	6
3	3
	6
	7
	4
	1
64	>64



## Conclusions

- AZT alone (but not AZT-colistin combinations) exhibited a trailing inhibition of growth MIC phenotype for some Enterobacteriaceae isolates
- Two reading criteria were used to confirm the following MIC and synergy results
- Against 33 Enterobacteriaceae isolates with acquired colistin resistance, the  $MIC_{50/90}$ values for the AZT-colistin (1:1) combination (0.5/2 mg/L) were significantly lower than AZT (2/>64 mg/L) or colistin (16/>64 mg/L) alone
- The MIC<sub>50/90</sub> values for the AZT-colistin (1:2) and AZT-colistin (2:1) combinations were similar to those observed for AZT-colistin (1:1)
- AZT-colistin combinations did not significantly improve the MIC<sub>50/90</sub> values for the randomly selected isolate set, which exhibited a low level of colistin resistance
- Synergy was observed for AZT-colistin combinations against 25 of 33 (76%) of the tested colistin-resistant isolates
- Because full checkerboard panels were not evaluated, the actual frequency of in vitro synergy within the isolate set may be even higher than observed in this
- These results support the further exploration of AZT-colistin combinations for the treatment of colistin-resistant Enterobacteriaceae

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