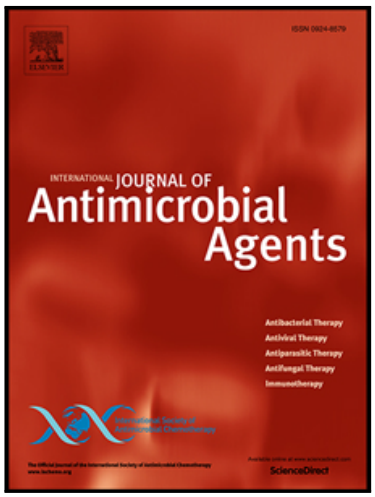

Urinary bactericidal activity of colistin and azidothymidine combinations against mcr-1 positive colistin-resistant Escherichia coli

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Highlights

- Concentrations from phase-1 study with CMS and AZT in combination with loading and maintenance dose
- Bactericidal activity in artificial urine spiked with colistin and AZT concentrations alone or combined
- Combination of colistin with AZT increased urinary bactericidal activity
- Dosage of 2 MIU CMS + AZT 100mg q12 sufficient to treat infection with colistin-susceptible strains
- for infections with colistin-resistant strains the dosage should be higher

Urinary bactericidal activity of colistin and azidothymidine combinations against *mcr-1* positive colistin-resistant *Escherichia coli*.

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Running title: urinary bactericidal activity of colistin and azidothymidine combinations

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# equal contribution

Abstract

A phase 1 clinical study was performed to assess pharmacokinetics and safety of IV administration of colistin-methanesulfonate (CMS) and azidothymidine (AZT) alone and in combination. Seven healthy subjects received 3-times (q12) 1h-IV-infusions of 4, 2 and 2 million international units (MIU) CMS co-administered with 200, 100 and 100 mg AZT, respectively. In the ex vivo study, urinary bactericidal titer (UBT) and time-kill curve determinations were performed in artificial urine, spiked with colistin-sulphate (CS) and AZT according to the median and minimum peak concentrations in urine measured after the 1st

and the 3rd dose, with four *mcr-1* positive colistin-resistant and five colistin-susceptible Gram-negative strains.

Reciprocal UBTs for the different colistin concentrations obtained in urine ranged from 1-128 and 0-2 for colistin-susceptible and colistin-resistant strains, respectively. The combination with AZT could increase the UBTs up to 2 dilution steps each for *Enterobacteriaceae* and *Acinetobacter* strains tested. In contrast, the combination had no activity against *Pseudomonas* strains. In time-kill curves the combination showed bactericidal activity against colistin-resistant strains, even when the substances alone were not bactericidal. Thus, combination of CMS with AZT shows promising synergistic activity against Gram-negative uropathogens including colistin-resistant *Enterobacteriaceae*. According to the urinary bactericidal activity, a maintenance dosage of 2 MIU CMS combined with 100 mg AZT b.i.d. may be sufficient for the treatment of urinary tract infections (UTI) caused by colistin-susceptible strains. However the dosage requires optimization for efficient treatment of UTI caused by colistin-resistant strains.

Introduction

Gram-negative bacteria, especially Enterobacteriaceae are the major cause of both community and hospital-acquired urinary tract infections (UTI). We have experienced a continuous emergence of antibiotic resistance in UTI and a shortage of newly approved antibiotics for common but clinically important indications [1]. The number of antimicrobial agents to treat infections caused by antibiotic-resistant Enterobacteriaceae declines rapidly, and the polymyxins such as colistin are considered the last resort treatment [2].

Recently studies illustrated that the combination of azidothymidine (AZT) and colistin showed synergistic antibacterial activity against *Enterobacteriaceae* strains *in vitro* and in mice [3-5]. Previously, we used serum concentrations obtained from a phase 1 study with healthy volunteers receiving a combination of colistin-methanesulfonate (CMS) and AZT [6] for *in vitro* determinations of serum bactericidal activities against colistin-resistant *Escherichia coli* strains compared to colistin-susceptible Gram-negative strains [7]. The results provided evidence that a combination of colistin and AZT potentiates the serum bactericidal effect of colistin against colistin-resistant *E. coli* strains. The aim the current study was to determine in a second part the urinary bactericidal activity of colistin and AZT combinations against four *mcr-1* positive, colistin-resistant *E. coli* strains as compared to colistin-susceptible *E. coli, Klebsiella pneumoniae, Acinetobacter baumannii* and *Pseudomonas aeruginosa* strains using urinary concentrations of both drugs obtained from the same phase 1 study [6].

Material & Methods

Ethics

The ethics for the phase 1 study have been described elsewhere [6-7].

Study design and urinary concentrations

Study design, sample collection and analysis as well as detailed safety information, laboratory test results and pharmacokinetic evaluations of this phase 1 study are presented elsewhere [6-7]. Maximum, median and minimum urinary concentrations of colistin and AZT obtained in the collection period 0-3 h after start of 1st, 2nd and 3rd dose are presented in table 1. Concentrations of colistin were expressed in colistin base, whose molecular weight slightly differs from colistin-sulphate (CS) (colistin base – 1155.5; CS – 1400.7; corresponding to colistin B [8-9]). Therefore, we converted the obtained colistin base concentrations to CS (factor 1.212), which were then used for spiking the artificial urine (table 1).

Bacterial strains

Four clinical *E. coli* isolates harbouring the colistin resistance gene *mcr-1* were used (table 2). In addition three carbapenem-resistant strains, *K. pneumoniae* BAA-2470, *P. aeruginosa* 1640801 and *A. baumannii* CHD102 as well as two reference strains, *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853 were included in this study.

Determination of minimal bactericidal concentrations (MBC)

MBC determinations were performed as described elsewhere [7] according to the CLSI- and EUCAST-Standards [11-13]. CAMHB (Sigma Aldrich, Darmstadt, Germany) and artificial urine (contains [g/L]: $CaCl_2$ - 0.49, $MgCl_2$*$6H_2O$ – 0.65, NaCl – 4.6, Na_2SO_4 – 2.3, Na_2 citrate · $2H_2O$ – 0.65, $Na_2C_2O_4$ – 0.02, KH_2PO_4 – 2.8, KCl – 1.6, NH_4Cl – 1.0, Urea – 25.0, gelatin - 5.0 & tryptone soya broth – 10.0; pH 6.1 [14]) were used as media. Final inocula, which were

confirmed by actual plating, ranged from 1 – 5 x 10^6 CFU/mL. All determinations were repeated at least thrice.

Determination of urinary bactericidal titers (UBTs)

UBTs corresponding to the maximal-dilution titre of urine allowing bactericidal activity were determined as described previously [15]. A two-fold serial dilution (Dilution range 1:0, 1:1 to 1:1,024) of artificial urine spiked with CS and AZT (Sigma Aldrich) was prepared in a 96-well polycarbonate plate (BioRad Laboratories, Munich, Germany). Earlier studies have shown that sticking of colistin to polycarbonate is much lower than to polystyrene (data not shown). Agent-free artificial urine was used as diluent. The final inoculum, which was confirmed by actual counting, ranged from 1.4- 3.3 x 10^6 CFU/mL. The plates were incubated at 37°C for 20 ± 2 h in ambient air. Afterwards, 3 µL of the cultured artificial urine was transferred onto IsoSensitest agar supplemented with 5% blood using a one-time inoculator. The plates were incubated overnight at 37°C. The number of colonies subsequently grown was used to determine the bactericidal endpoint. Urinary bactericidal activity was defined as a > 99.9% (> 3-log) reduction of the initially inoculated colony counts. A UBT of 0 was defined as no bactericidal activity; a UBT of 1 was used when only the undiluted artificial urine displayed bactericidal activity. UBT data were represented by reciprocals of the factor of the highest dilution showing bactericidal action. All determinations were repeated at least twice.

Time-kill curve

Time-kill curve analyses were performed as described previously elsewhere [7] by culturing the test strains (inoculum ~1*10^6 CFU/mL) in artificial urine in the presence of CS and AZT alone and in combination. Time-kill curves with colistin-susceptible strains using minimum

3rd dose concentrations were performed once. All determinations for the colistin-resistant strains were repeated thrice.

Results

Minimal bactericidal concentrations and urinary bactericidal titer

The MBC values of CS in CAMBH and artificial urine were 8-32 mg/L and >64 mg/L for all tested colistin-resistant *E. coli* strains, respectively (table 3). For the tested colistin-susceptible *Enterobacteriaceae* strains median MBC values in CAMBH / artificial urine range from 2 - 4 /2 – 4 mg/L, whereas median MBC values were between 1- 4/ 8 – 32 mg/L for the tested *Pseudomonas* and *Acinetobacter* strains. Median MBC values of AZT in CAMHB and artificial urine for *E. coli* ATCC 25922, *E. coli* Af48 and *K. pneumoniae* BAA-2470 ranged between 0.5 and 16 mg/L and were between 64 - >64 mg/L for all other strains tested (table 3).

To determine the urinary bactericidal activity after IV-infusions, artificial urine was spiked with CS and AZT according to the measured median and minimum urinary concentrations of the collection period 0-3 h after start of 1st and 3rd dose (table 1), alone or in combination. Since the minimum concentrations of the 1st dose corresponded closely to the median 3rd dose concentrations, only the 1st dose median as well as the 3rd dose median and minimum concentrations were tested.

UBTs for CS were positive (range 1-2) for 4/4 tested colistin-resistant *E.coli* strains using the median 1st, 3/4 using the median 3rd and 0/4 using the minimum 3rd dose concentrations. The approaches with AZT yielded reproducible results only for 2 of the 4 strains. Combination of CS with AZT showed similar or 1-2 dilution steps better results as with colistin alone for median 1st and 3rd concentrations. For colistin-susceptible strains, UBTs for

median 1st, median 3rd and minimum 3rd dose concentrations were 64-128/32-16/4-16 for Enterobacteriaceae, 8/4/1-2 for *P. aeruginosa* and 8/4/2 for *A. baumannii*. When CS was combined with AZT, UBTs were 1-2 dilution steps higher for colistin-susceptible *E. coli* and *A. baumannii* for median 1st and 3rd dose concentrations as well as for *K. pneumoniae* for median 3rd dose concentrations. Combination of minimum 3rd dose concentrations of CS and AZT showed no improvement compared to CS alone for all strains tested. Furthermore, none of the tested combinations had any benefit in killing the used *P. aeruginosa* strains (table 4).

Time-kill curve

Artificial urine spiked with concentrations of CS and AZT alone or in combination according to the obtained phase 1 median and median/minimum urinary concentrations 0-3 h after start of 1st and 3rd dose, respectively, (table 1) were also used to perform time-kill curve analysis of the *mcr-1* positive strains.

Median 1st dose CS concentration killed >99.9% of the inoculated bacteria after 30-60 min for all *mcr-1* positive strains. Furthermore, no detectable colonies were found after at the latest 4 h. Using median 3rd dose concentrations, CS decreased bacterial numbers for all *mcr-1* positive strains tested. However, a reduction below the detection limit after 24 h incubation occured only partially (1/3 repetitions for *E. coli* Af23 & CDF6, 2/3 repetitions for *E. coli* Af48). Minimum 3rd dose concentration of CS reduced not more than initially growth of the bacteria, but after 24 h incubation control levels were reached (figure 1 – 3).

AZT alone decreased the bacterial numbers within the first two to four hours for all concentrations tested. Re-growth to control levels occurred after 6 – 24 h for all strains using minimum 3rd dose concentration, for all strains except 2/3 repetitions of *E. coli* Af48 using

median 3rd dose concentration and for *E. coli* Af24, *E. coli* CDF6 as well as one repetition of *E. coli* Af23 using median 1st dose concentration (figure 1 - 3).

Combinations of median 1st dose concentrations of CS and AZT acted bactericidal within 6 h while it accelerated killing of three of the four tested strains compared to CS alone (figure 1). Using median 3rd dose concentrations, combination of CS and AZT acted bactericidal in all repetitions for all strains except *E. coli* CDF6 (figure 2). Combination of minimum 3rd dose concentrations of CS and AZT showed bactericidal activities after 24 h in triplicate only for one strain, *E. coli* Af23. For the three remaining strains, the three repetitions showed at least once a reduction of the bacterial counts under the detection limit, but also at least once a re-growth of the bacteria, after 24 h (figure 3).

For the five colistin-susceptible strains, minimum 3rd dose CS concentration was sufficient to kill all bacteria in at last 6 h (figure 4). Therefore, the higher concentrations were no longer tested. Minimum AZT concentration reduced bacterial numbers of tested E*nterobacteriaceae* in the first hours with a subsequent regrowth to control amounts, but did not affect *P. aeruginosa* and *A. baumannii* (figure 4). Combination of CS with AZT slightly accelerated killing of the A. baumannii strain tested, but was not in addition beneficial for the tested concentrations on killing of the colistin-susceptible *E. coli* and *K. pneumoniae, P. aeruginosa* strains.

All samples spiked with CS and AZT showing visible growth by turbidity after 24 h incubation were used for subsequent MIC determination of CS as well as AZT. Growing in the presence of CS or AZT did not affect the susceptibility against CS; median fold changes over MIC of control bacteria, growing without any substances added, were both 1. Furthermore, bacteria grown with CS showed no difference of AZT MIC (median fold change over control = 1.0). In contrast, 24 h growth in AZT-containing media increased AZT MIC by about 8-fold.

Discussion

This annex *ex vivo* study was carried out as part II of a phase 1 trial in healthy volunteers [6] and evaluated bactericidal activity of peak urinary concentrations obtained during IV-treatment with CMS in combination with AZT.

It could be noted that the MBC values of CS in CAMBH was several dilution steps lower than in artificial urine for *mcr-1* positive *E. coli* and *P. aeruginosa* strains. The lower pH and an increased amount of divalent cations in artificial urine compared to CAMHB contribute to this phenomenon [16-18]. For this reason, results of pharmacodynamic studies obtained in serum cannot just be extrapolated to a possible outcome in urine only by taking into account the different concentrations measured in serum and urine. Specific urodynamic studies in urine are therefore justified.

We performed determinations of bactericidal titers in artificial urine, which show only the effect after 24 h incubation, and of time-kill curves in artificial urine, showing dynamic changes of bacterial counts during 24 h incubation, using constant drug concentrations. The results showed that minimal urinary CS concentration after 2 MIU CMS infusion was sufficient to eliminate colistin-susceptible strains. This is similar to our previous results for the bactericidal activity of the concentrations obtained in the plasma [7]. However, bactericidal concentrations of CS against all tested colistin-resistant strains could only be obtained with median urinary concentrations after 4 MIU CMS infusion. For the combination of CS with AZT, our data showed that AZT potentiates the bactericidal effect of colistin against *Enterobacteriaceae*, even that of some colistin-resistant *E. coli, and A. baumannii* in artificial urine. With respect to the tested *Pseudomonas* strains, however, the combination with AZT had no advantage. These results were similar to our previous ones for serum bactericidal activity [7].

For the colistin-susceptible strains, the beneficial effect of the combination was better visible whit UBT determinations compared to time-kill curves. Minimum 3rd dose CS alone, with a concentration of 4 – 8 fold higher as the urinary MBC of the colistin-susceptible *Enterobacteriaceae*, reduced the bacterial numbers by almost 3 logs within 15 min. Therefore, no additional effect of AZT could not detected. The dilution methodology of the UBT determination allows a better presentation of positive effects.

In contrast, for the colistin-resistant strains the time-kill curve determinations gave better results compared to UBT determinations. Since UBT determinations is a one-timepoint method after 24 h incubation, an acceleration of the killing as seen in the time-kill curves could not be demonstrated. On the other hand, especially the colistin-resistant strains showed sometimes a heteroresistance against AZT. Heteroresistance describes a phenomenon where subpopulations of seemingly isogenic bacteria exhibit a range of susceptibilities to particular substance [19]. This heteroresistance was noticeable by “skip wells” (wells that exhibit no growth although growth occurs at higher concentrations) in serial dilutions even in multiple repetition. This resulted in uninterpretable UBTs for *E. coli* CDF6 and *E. coli* AF48.

The membrane permeabilization by colistin [20] could increase the entry of AZT into the bacterial cells. The antibacterial activity of AZT can be traced back to the inhibition of the replicative DNA-synthesis after incorporation of azidothymidine-triphosphate [3-4]. This effect is often only temporary. Mutations resulting in a lack of thymidine kinase lead to an increased resistance against AZT [3-4]. Increased AZT MIC values of the *mcr-1* positive *E. coli* strains after growing in the presence of the deoxyribonucleoside analogue during the time-kill curve experiments lead to the assumption that mutations are selected which enhanced the resistance against AZT. But, no cross-resistance to CS was seen. Rather, the combination

of CS and AZT potentiate killing of *mcr-1* positive *E. coli*, especially at lower concentrations after 3rd dosage compared to CS alone. Compared to the *Enterobacteriaceae*, AZT alone did not affect the growth of the *Pseudomonas* and *Acinetobacter* strains tested, as these bacteria often naturally lack a thymidine kinase [21-22]. Nevertheless, our data suggested a beneficial effect of AZT on colistin activity against the *A. baumannii* strain tested.

Our results revealed that a low dose of 2 MIU CMS infusion combined with 100 mg AZT twice daily seems to be sufficient for treatment of UTI caused by colistin-susceptible strains. The combination also showed improved killing of some colistin-resistance *E. coli* strains in the urine compared to 2 CMS infusion alone. However, especially at the minimum concentrations, the killing effect could not be reproduced for all strains tested. Therefore the dosage for treatment of complicated UTI caused by colistin-resistant *E. coli* probably needs to be increased. Due to a possible elevated risk of nephrotoxicity [23], CMS should be increased carefully. For AZT no nephrotoxicity has been described so far [24].

Conclusions

In this part II *ex vivo* study, combination of CMS with AZT shows promising activity against Gram-negative uropathogens, including colistin-resistant *E. coli*. According to the urinary bactericidal activity a dosage of 2 MIU CMS plus 100 mg AZT is sufficient for the treatment of UTI with colistin-susceptible strains. In contrast, for UTI caused by colistin-resistant *E. coli*, the combination of 2 MIU CMS with 100 mg AZT showed good indications that AZT potentiates the bactericidal effect of colistin. However, a higher dosage should be tested in well-designed clinical studies.

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Declarations

Funding: The study originated from and was supported by Helperby Therapeutics-Group Ltd, London, UK.

Competing Interests: ML: Collaborator – Helperby Therapeutics. KGN: Investigator - Enteris Biopharma; Scientific Advisor (Review Panel or Advisory Committee) - Bionorica, Enteris Biopharma, Helperby Therapeutics, Leo Pharma, MerLion, MSD Sharp & Dohme, OM Pharma, Paratek, Rosen Pharma, Roche, Zambon; Speaker's Bureau - Bionorica, Daiichi Sankyo, Leo Pharma, OM Pharma, Rosen Pharma, Zambon. YH: Grant Investigator – Helperby Therapeutics. AC: Shareholder (excluding diversified mutual funds) - Helperby Therapeutics. FMEW: Collaborator – Helperby Therapeutics; Investigator - Enteris BioPharma; Scientific Advisor (Review Panel or Advisory Committee) - Bionorica, Enteris BioPharma, Helperby Therapeutics, Leo Pharma, MerLion, OM Pharma, Rosen Pharma, Achaogen, AstraZeneca, Janssen, MSD, Shionogi, Pfizer.

Ethical Approval: Not required

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Figure Legends

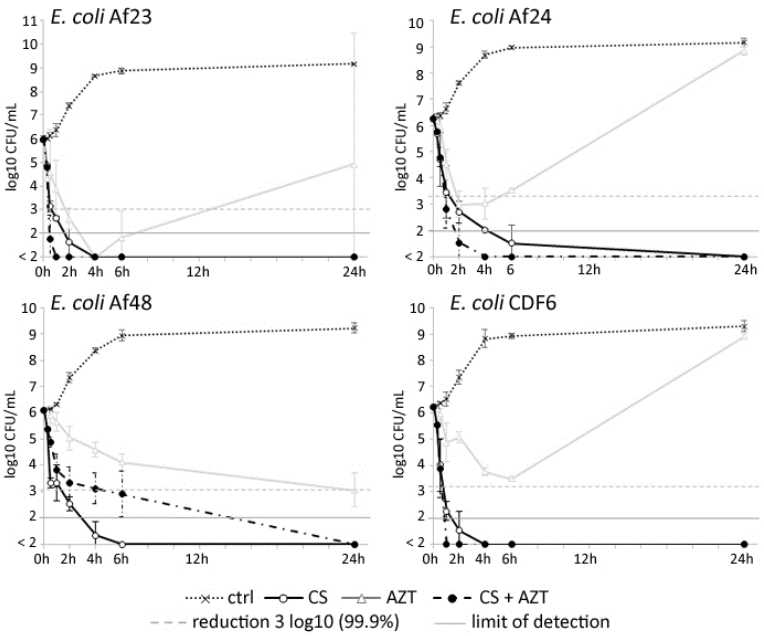

Figure 1 Time-kill curves using median urinary concentrations after 1st dose (0-3 h urine collection period)

Bacteria were inoculated with ~1*10^6 CFU/mL in artificial urine. After adding colistin sulphate (CS) and azidothymidine (AZT) alone or in combination according to the median urinary concentrations after 1st dose bacteria were incubated at 37°C and 180 rpm. Ctrl – control without any addition. Error bars indicate standard deviations.

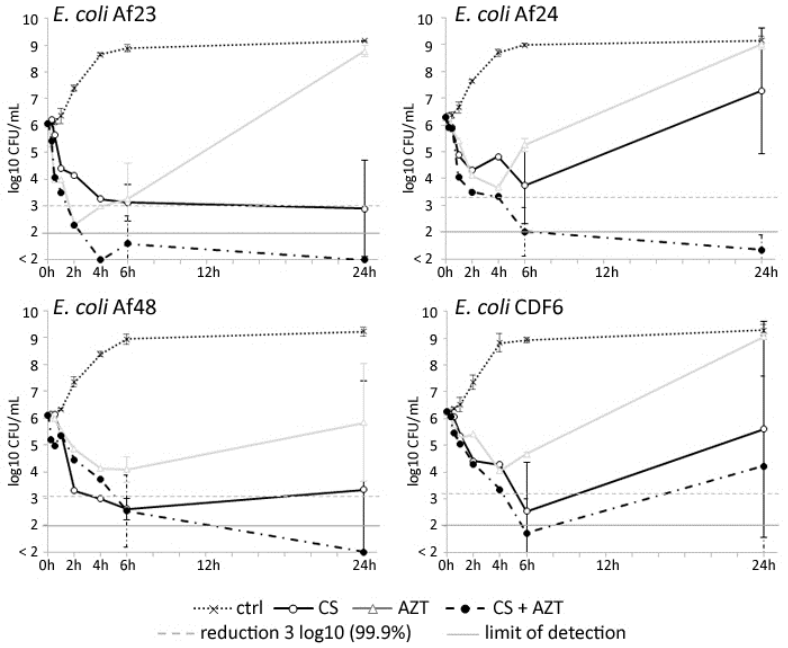

Figure 2 Time-kill curves with median urinary concentrations after 3rd dose (24-27 h urine collection period)

Bacteria were inoculated with ~1*10^6 CFU/mL in artificial urine. After adding colistin sulphate (CS) and azidothymidine (AZT) alone or in combination according to the median urinary concentrations after 3rd dose bacteria were incubated at 37°C and 180 rpm. Ctrl – control without any addition. Error bars indicate standard deviations.

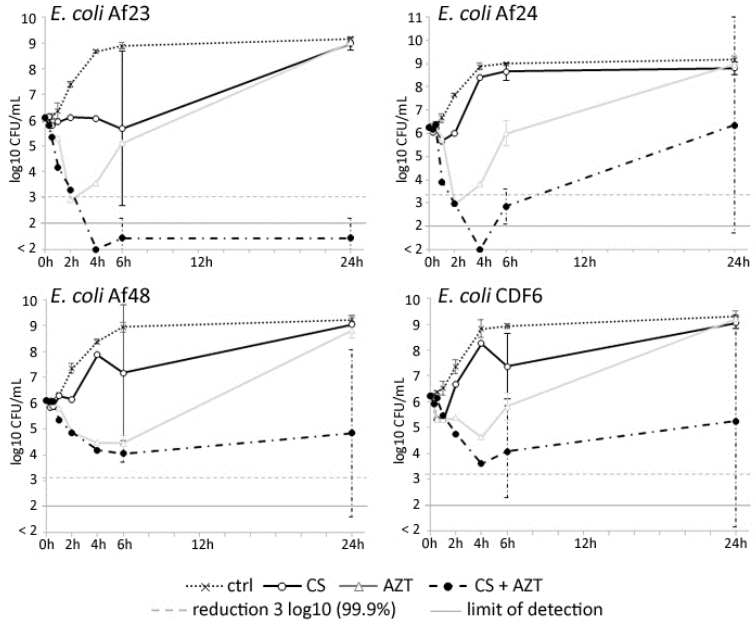

Figure 3 Time-kill curves with minimum urinary concentrations after 3rd dose (24-27 h urine collection period)

Bacteria were inoculated with ~1*10^6 CFU/mL in artificial urine. After adding colistin sulphate (CS) and azidothymidine (AZT) alone or in combination according to the minimum urinary concentrations after 3rd dose bacteria were incubated at 37°C and 180 rpm. Ctrl – control without any addition. Error bars indicate standard deviations.

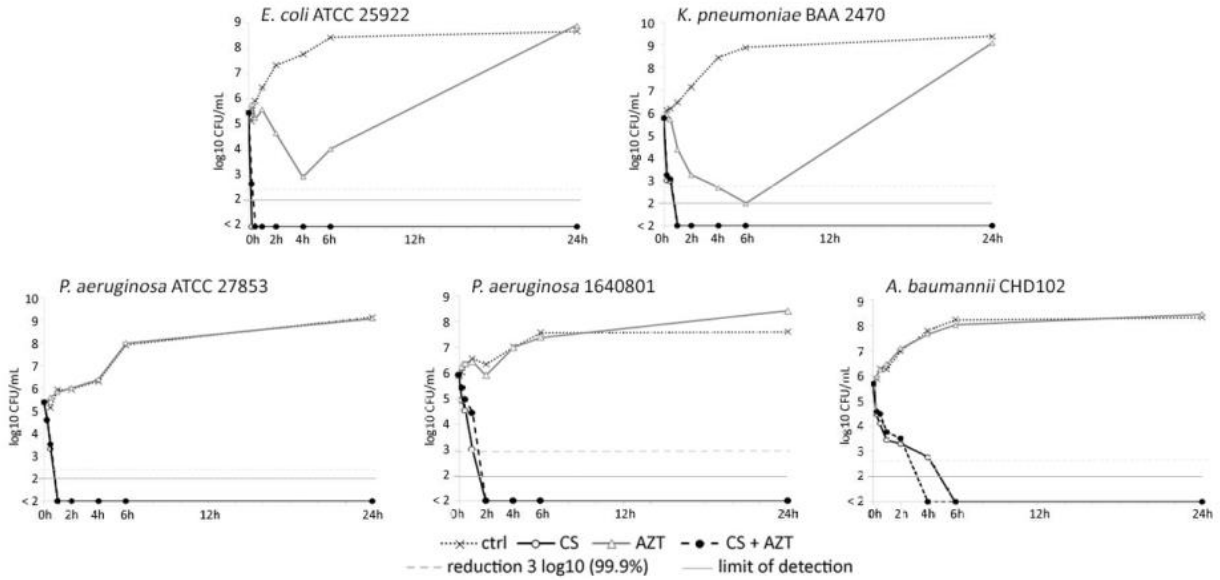

Figure 4 Time-kill curves of colistin-susceptible strains with minimum urinary concentrations after 3rd dose (24-27 h urine collection period)

Bacteria were inoculated with ~1*10^6 CFU/mL in artificial urine. After adding colistin sulphate (CS) and azidothymidine (AZT) alone or in combination according to the minimum urinary concentrations after 3rd dose bacteria were incubated at 37°C and 180 rpm. Ctrl – control without any addition.

TABLE 1 Urinary concentrations of colistin and AZT obtained 0-3 h after dosage

	Colistin [mg/L]		AZT [mg/L]
	CB	≙CS	
		1st dose	
minimum	55	66.6	41.5
median	106	128.3	70.4
maximum	776	939	893
		2nd dose	
minimum	37.8	45.7	24.9
median	54.9	66.4	46.4
maximum	107	129.5	99.2
		3rd dose	
minimum	14.9	18	11.8
median	46.9	56.7	39
maximum	217	262.6	444

CB – colistin base; CS – colistin sulphate; AZT - azidothymidine

TABLE 2 Features of the *mcr-1* positive *E. coli* isolates

Strain	*mcr-1* positive plasmid type/ plasmid size (ca. kb)	Sequence type	Source	Co-Resistances	Co-resistance gene	Country of Isolation	Reference
E. coli Af23	IncI2/ 70	10	Human blood	AMX-CIP-SXT-TET	bla_{TEM-1}	ZA	[10]
E. coli Af24	IncI2/ 65	1007	Human pus	AMX-CIP-SXT-TET-CHL	bla_{TEM-1}	ZA	[10]
E. coli Af48	Incx4/ 30	624	Human urine	AMX-CEF-CIP-SXT-TET-KAN	bla_{CMY-2}	ZA	[10]
E. coli CDF6	IncHI2/ 150	446	Human urine	AMX-CTX-CIP-SXT-TET	bla_{CTX-M}	CH	-

AMX – Amoxicillin; CEF - cephalothin; CHL – Chloramphenicol; CIP – Ciprofloxacin; CTX – cefotaxime; KAN – Kanamycin;

SXT – Trimethoprim/Sulfamethoxazole; TET – Tetracycline,

ZA – South Africa; CH – Switzerland;

TABLE 3 Minimal bactericidal concentrations (MBC) of colistin and azidothymidine in CAMHB and artificial urine

	Ec Af23	Ec Af24	Ec Af48	Ec CDF6	Ec ATCC 25922	Kp BAA 2470	Pa ATCC 27853	Pa 1640801	Ab CDH102
MBC median (range) [mg/L] in CAMHB									
CS	8 (4-64)	16 (16-32)	12 (8-32)	32 (8-32)	2 (1-2)	4 (2-8)	4 (2-8)	2 (1-4)	1 (1)
AZT	>64 (>64)	>64 (>64)	16 (16-128)	>64 (32->64)	2 (1-4)	1 (1-2)	>64 (>64)	>64 (>64)	>64 (>64)
MBC median (range) [mg/L] in artificial urine									
CS	>64 (>64)	>64 (>64)	>64 (>64)	>64 (>64)	2 (0.5 - 4)	4 (2 – 8)	32 (16 – 32)	16 (16 – 32)	8 (8)
AZT	>64 (>64)	>64 (>64)	4 (1 – 32)	64 (2- >64)	2 (1 – 2)	0.5 (0.5-2)	>64 (>64)	>64 (>64)	>64 (>64)

CS – colistin-sulphate; AZT – azidothymidine; Ec – *E. coli*; Kp – *K. pneumoniae*; Pa – *P. aeruginosa*; Ab – *A. baumannii*

TABLE 4 Reciprocal urinary bactericidal titers (UBTs) at median and/or minimum urinary concentrations (UC) after the 1st dose (0-3h urine collection period) and 3rd dose (24-27h urine collection period)

Reciprocal UBTs									
	Ec Af23	Ec Af24	Ec Af48	Ec CDF6	Ec ATCC 25922	Kp BAA 2470	Pa ATCC 27853	Pa 1640801	Ab CDH102
Median UC 1st dose									
CS	1	1	2	2	128	64	8	8	8
AZT	1	0	*	*	32	256	0	0	0
CS+AZT	4	1	*	*	256	256	8	8	16
Median UC 3rd dose									
CS	1	0	1	1	16	32	4	4	4
AZT	0	0	*	*	32	32	0	0	0
CS+AZT	4	1	*	*	128	128	4	2	8
Minimum UC 3rd dose									
CS	0	0	0	0	4	16	1	2	2
AZT	0	0	*	*	16	32	0	0	0
CS+AZT	0	0	*	*	16	32	1	1	2

*Interpretation of azidothymidine alone and in combination was not possible due to incoherent data; CS – colistin-sulphate; AZT – azidothymidine; Ec – *E. coli*; Kp – *K. pneumoniae*; Pa – *P. aeruginosa*; Ab – *A. baumannii*