The Effect of AcrAB-TolC MDR Pump and Media on Susceptibility to Antibiotics

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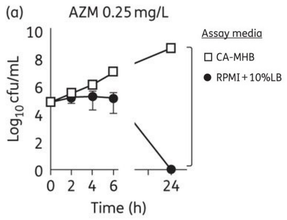
Abstract

Microbroth dilution, a common method for antibiotic susceptibility testing, is used to determine the minimal inhibitory concentration (MIC). Most susceptibility testing occurs in rich bacterial media that does not mirror the environment of a patient. Previously, it was shown that a Gram-negative pathogen was susceptible to Azithromycin in RPMI, a common media used to propagate eukaryotic cells, but was resistant in MHB, a nutrient rich bacterial media. Similarly, preliminary experiments revealed there was an increase in antibiotic susceptibility of *E. coli* when cultured using RPMI compared to other rich bacterial media. The purpose of this study was to determine if the observed trend of increased susceptibility of *E. coli* to Azithromycin in RPMI occurs with other antibiotics and if the susceptibility is the result of down-regulation of the AcrAB-TolC MDR pump during growth in RPMI. To determine this, we found the minimal inhibitory concentration (MIC) of different antibiotics targeting a variety of cellular pathways in two medias (LB and RPMI) using two different strains of *E. coli.* The wild type strain contained the AcrAB-TolC MDR pump, whereas the *ΔtolC* strain had a deletion of the pump. These two strains were chosen to determine if the AcrAB-TolC pump had an effect on susceptibility of *E. coli* to different antibiotics. We determined that MICs were generally higher in the wild type strain compared to the *ΔtolC* strain. In addition, there are two independent pairs antibiotics that have similar targets, and their MICs increase in RPMI. Meanwhile, kanamycin did not have a drastic increase or decrease in MIC, so it is not an AcrAB-TolC substrate and is not affected by media. In conclusion, we determined that the difference in susceptibility is not based solely on the AcrAB-TolC MDR pump and MIC testing with RPMI might reveal previously unknown susceptibilities potentially useful in a clinical setting.

Effect of RPMI on Azithromycin Susceptibility



Killing activity of Azithromycin in bacterial vs Eukaryotic media, Kumaraswamy, et al (2016).

Growth in RPMI sensitizes *Stenotrophomonas maltophilia* to Azithromycin compared to rich bacterial media.

The effect of Azithromycin in RPMI is also applicable in *E. coli*.

AcrAB-TolC Multidrug Resistance Efflux Pump

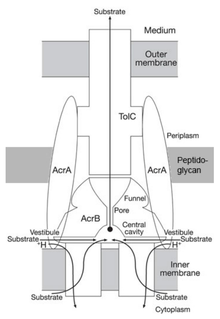


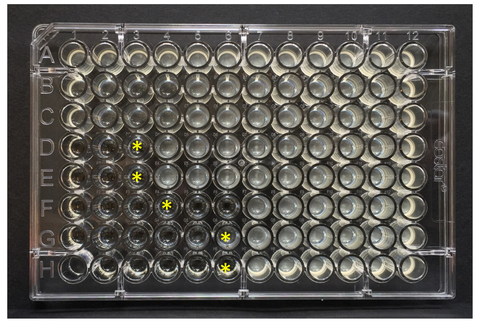
Diagram of the tolC pump, adapted from Murakami, et al(2002).

The AcrAB-TolC efflux pump is a multidrug resistance efflux pump (MDR pump) that spans the inner and outer membranes of *E. coli*.

- Deletion of the AcrAB-TolC MDR pump increases susceptibility to antibiotics.
- A study testing the susceptibility of 35 compounds concluded that 24 of the 35 compounds increased in susceptibility when tested on strains with deletion of the AcrAB-TolC pump.
- Azithromycin sensitivity is similar in RPMI between the wild type and *tolC* deletion versus a seven fold difference in LB.

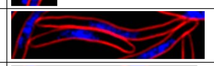
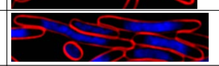
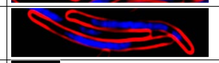
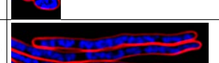
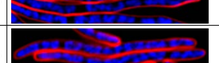
Is the increased susceptibility of *E. coli* to Azithromycin in RPMI broadly applicable to other antibiotics and are these a result of a decreased expression of the AcrAB-TolC MDR pump?

Determining MICs via Microbroth Dilution



- Example of minimal inhibitory concentration (MIC) determination utilizing microbroth dilution of antibiotics
- *= MICs
- 2-fold serial dilution of antibiotics across each row.
- MIC= the lowest concentration that inhibits growth.

Antibiotics Utilized in this Study

Antibiotic	BCP Category	Target	*E. coli lptD4213* Microscopy phenotype (from Nonejuie, et al.)
Chloramphenicol	P1	50S Ribosome (inhibits peptidyl transferase)	
Kanamycin	P2	30S Ribosome (promotes mistranslation)	
Puromycin	P3	50S Ribosome (premature chain termination)	
Naladixic acid	D1	DNA gyrase A	
Novobiocin	D2	DNA gyrase B	
Daunorubicin	D3	DNA intercalation	
Mitomycin C	D4	DNA crosslinker	
Vancomycin	C1	Binds D-Ala-D-Ala terminus	
Mecillinam	C2	Penicillin-binding proteins	
Cephalexin	C3	Penicillin-binding proteins	
Ampicillin	C3	Penicillin-binding proteins	
Nigericin	M1	K^+ ionophore	
CCCP	M3	Protonophore	
Cerulenin	L1	Binds fatty acid synthase	

MIC Results for LB and RPMI

LB and RPMI MIC◊ Results					
		Wildtype		*ΔtolC*	
	BCP category	LB	RPMI+10% LB	LB	RPMI+10% LB
Azithromycin	N/A	3.13	0.73	0.47	0.38
Chloramphenicol	P1	7.29	6.25	1.27	0.88
Kanamycin	P2	3.13	3.39	3.65	2.60
Puromycin	P3	150.00	58.33	1.95	1.69
Naladixic acid	D1	4.69	54.17	0.23	7.33
Novobiocin	D2	216.67	>1000	1.35	5.47
Daunorubicin	D3	>20	>20	2.08	1.88
Mitomycin C	D4	4.00	0.44	0.03	0.01
Vancomycin	C1	>100	>100	>100	>100
Mecillinam	C2	0.78	50.00	0.46	1.38
Cephalexin	C3	7.29	20.83	10.42	26.56
Ampicillin	C3	93.75	177.08	25.00	15.63
Nigericin*	M1	>50	>50	>50	>50
CCCP*	M3	75.00	100.00	6.25	9.38
Cerulenin	L1	66.67	37.50	7.81	2.34

◊MICs in μg/ml unless otherwise indicated
*MICs in μM

Wildtype vs *ΔtolC* and LB vs RPMI MIC Comparisons

MIC Comparisons (fold increase or decrease)**					
	BCP category	LB wt/Δ tolC	wt LB/RPMI	Δ tolC LB/RPMI	RPMI wt/Δ tolC
Azithromycin	N/A	6.7	4.3	1.2	1.9
Chloramphenicol	P1	5.7	1.2	1.5	7.1
Kanamycin	P2	-1.2	-1.1	1.4	1.3
Puromycin	P3	76.8	2.6	1.2	34.5
Naladixic acid	D1	20.4	11.6	32.0	7.4
Novobiocin	D2	160.3	<-4.5	4.0	>183
Daunorubicin	D3	>9.6	N/A	1.1	>10.6
Mitomycin C	D4	144.2	9.1	2.7	42.0
Mecillinam	C2	1.7	64.0	3.0	36.4
Cephalexin	C3	-1.4	2.9	2.6	-1.3
Ampicillin	C3	3.8	-1.9	1.6	11.3
CCCP	M3	12.0	-1.3	-1.5	10.7
Cerulenin	L1	8.5	1.8	3.3	16.0

	>10 fold increase
	2-10 fold increase
	>10 fold decrease
	2-10 fold decrease

**fold change=MIC1/MIC2 if <1 fold change =-(MIC2/MIC1)

Conclusions

- MICs are generally higher in wildtype vs *ΔtolC* (including for RPMI).
- Two sets containing pairs of antibiotics which have similar targets showed an increase in MIC in RPMI.
  - Mecillinam and cephalexin (PBP inhibitors).
  - Naladixic acid and novobiocin (DNA gyrase inhibitors).
- Kanamycin is not a AcrAB-TolC substrate and MIC was unaffected by culture media.
- The difference in susceptibility in LB vs RPMI is not solely due to the AcrAB-TolC pump.

References

1. Murakami, S., Nakashima, R., Yamashita, E., and Yamaguchi, A. (2002) Crystal structure of bacterial multidrug efflux transporter AcrB, *Nature 419*, 587-593.
2. Nonejuie, P., Burkart, M., Pogliano, K., and Pogliano, J. (2013) Bacterial cytological profiling rapidly identifies the cellular pathways targeted by antibacterial molecules, *Proc Natl Acad Sci U S A 110*, 16169-16174.
3. Kumaraswamy, M., Lin, L., Olson, J., Sun, C. F., Nonejuie, P., Corriden, R., Dohrmann, S., Ali, S. R., Amaro, D., Rohde, M., Pogliano, J., Sakoulas, G., and Nizet, V. (2016) Standard susceptibility testing overlooks potent azithromycin activity and cationic peptide synergy against MDR Stenotrophomonas maltophilia, *J Antimicrob Chemother 71*, 1264-1269.

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