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Non-antibiotics Reverse Resistance of Bacteria to Antibiotics

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Abstract. *Background: Most clinical isolates that exhibit a multi-drug resistant phenotype owe that resistance to over-expressed efflux pumps. Compounds that are efflux pump inhibitors (EPIs) reduce or reverse resistance to antibiotics to which the bacterial strain is initially resistant. We have evaluated non-antibiotics to reduce resistance of commonly encountered bacterial pathogens to antibiotics. Materials and Methods: The effect of non-antibiotics on the susceptibility of bacteria to antibiotics was conducted by minimum inhibition concentration determinations of the antibiotic in the absence and presence of the non-antibiotic. Results: Non-antibiotics such as chlorpromazine, amitryptiline and trans-chlorprothixene are shown to reduce or reverse resistance of a variety of bacteria to antibiotics. Conclusion: The results suggest that non-antibiotics may serve as adjuncts to conventional antibiotics for the therapy of problematic antibiotic infections caused by bacteria that owe their resistance to over-expressed efflux pumps.*

The world-wide emergence of bacteria resistant to antibiotics representative of two or more antibiotic classes has caused severe problems in the therapy of infections caused by these multidrug-resistant (MDR) bacteria (1). Although the cause of antibiotic resistance may be due to a variety of mechanisms, whenever studied, the majority of MDR clinical isolates owe their MDR phenotypes to the overexpression of efflux pumps that extrude antibiotics prior to their reaching their intended targets (2). Efflux-mediated MDR is more frequently detected in Gram-negative than in Gram-positive strains (3). Nevertheless, the rates of efflux-mediated MDR of clinical isolates is rapidly increasing, especially among methicillin-resistant *Staphylococcus aureus* (4).

Intense studies on MDR efflux pumps have resulted in the discovery of many compounds that have the ability to render MDR bacteria as susceptible as their counter wild-type reference strain (5-8) or to at least reduce resistance to antibiotics to which they were initially resistant (9). These compounds are active against MDR efflux pumps and have been termed efflux pump inhibitors (EPIs). All of these compounds express toxicity at concentrations employed for the reversal or reduction of resistance and none have yet to reach clinical trial success (1).

Phenothiazine neuroleptics such as chlorpromazine, thioridazine, promethazine, *etc*., have been shown to exhibit *in vitro* activity against a wide gamut of bacteria (10), *ex vivo* activity against MDR and XDR *Mycobacterium tuberculosis* (11) and *Staphylococcus aureus* (12), *in vivo* activity against MDR *Mycobacterium tuberculosis* (13) and virulent salmonella (14, 15). One phenothiazine in particular thioridazine, when used in combination with drugs that have had no success in establishing a positive response from XDR Mtb-infected patients, has offered a cure (16), suggesting activity of the phenothiazine on the efflux-mediated MDR mechanism of the XDR Mtb organism.

During the past decade, sporadic reports have appeared suggesting that phenothiazines have EPI activity (17). The present study investigated the ability of chlorpromazine (CPZ), amitriptyline (AMY) and *trans*-chlorprothixene (*trans*-CPT) to reduce or reverse resistance of selected clinical isolates to penicillin, tobramycin and cefuroxim in order to establish a baseline from which further studies on the efflux mechanisms of MDR bacteria would ensue.

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Key Words: Non-antibiotics, bacteria, antibiotics, reversal of resistance.

Table I. *The effect of chlorpromazine on the activity of antibiotics.*

Strain	MIC of antibiotic (μg/ml)	MIC of CPZ (μg/ml)	MIC (μg/ml) of antibiotics when CPZ is present in the medium		
			A+1/2 MIC	A+1/4 MIC	A+1/8 MIC
Corynebacteria JK AB 2227	Penicillin >200	50	1.6/3.1	>100	>100
Corynebacteria JK*	Penicillin >40050	6.2	>400	>400	
S. pneumonia 68128	Penicillin 12.5	12.5	0.003	12.5	12.5
P. aeruginosa 91	Tobramycin 1.6	600	0.2	0.4	0.4
K. pneumonia 38	Cefuroxim >100	300	6.2	25	50
E. coli 87	Ampicillin >100	150	>100	>100	>100
S. aureus D7398	Penicillin 6.2	25	0.1	1.6	3.1
	Methicillin >100	25	6.2	12.5	25
S. aureus D7391	Penicillin 1.6/3.1	25	0.1	0.2	1.6
	Methicillin 12.5	25	3.1	6.2	12.5

Materials and Methods

Bacteria. The bacteria employed in this study consist of reference strains and clinical isolates obtained from specimen of patients.

Materials. Antibiotics and agents studied for the effects on the susceptibility of bacteria to given antibiotics were generously provided by LEO, ASTRA, LILLY, GLAXO and DAK. CPZ was obtained in powder form from Sigma, (Copenhagen, Denmark), AMY from DAK and *trans* (E)-chlorprothixene T-CPT from Lundbeck A/S, (Copenhagen, Denmark). Solutions were prepared immediately before use and precautions taken to protect them from ambient light.

Methods. The bacteria employed throughout the course of this study were bacterial strains characterized and maintained at the Statens Serum Institute (SSI). These included: corynebacterium JK AB 2227, corynebacterium JK, *Streptococcus pneumonia* 68128, *Pseudomonas aeruginosa* 91, *Klebsiella pneumonia* 38, *Escherchia coli* 87, *Staphylococcus aureus* D7398, *S. aureus* D7391.

All cultures performed in this study consisted of the seeding of the identified bacteria at an inoculum of 10^5 cells corresponding to 10^5 colony forming units (CFU) in 100 μl of saline into 10 ml of oxoid-serum broth (Difco) with and without the agents, singly or in combination. All cultures were incubated at 37˚C for 16 hours.

Determination of the minimal inhibitory concentration (MIC) for each of the antibiotics, agents (non-antibiotics) and antibiotics in combination with different concentrations of the agents (non-antibiotics) were performed through the use of the tube dilution procedure (13). The MIC was recorded as the minimum concentration of the drug that completely inhibited growth (no visual turbidity). All evaluations were conducted in duplicate.

The determination of the effect of the non-antibiotics CPZ, AMY and T-CPT on the MIC of specific antibiotic-bacteria combinations was conducted at a concentration of these respective non-antibiotic compounds that corresponded to concentrations equivalent to 1/2, 1/4 and 1/8 of their MICs for that bacterium-drug combination. At all times, this concentration was sub-inhibitory since no effect on the final growth of the organism could be detected when compared to its corresponding control culture.

Results

The MIC for each of the antibiotics or non-antibiotics for the bacteria employed in this study are provided in Tables I-III. The effects of the non-antibiotics on the MICs of antibiotics considered to be significant are highlighted. Briefly, as shown in Tables I-III, whereas combinations of CPZ and T-CPT at one-half their MIC reduce the MIC of penicillin, cefuroxim and tobramycin against non-beta-lactamase producing corynebacteria, *P. aeruginosa, S. pneumonia, K. pneumonia* and *S. aureus*, similar combinations of the non-antibiotics with ampicillin had no effect on the MIC of ampicillin against *E. coli*. AMY had no effect on the MIC of penicillin against corynebacterium. Because reversal of antibiotic resistance by an agent is now known to be due to the inhibition of an efflux pump that extrudes the antibiotic prior to it reaching its intended target, we may surmise that the restoration of antibiotic susceptibility by the co-presence of the non-antibiotic known to act as an EPI, is due to the inhibition of an efflux pump by the said agent.

Table II. *The effect of amitriptyline on the activity of antibiotics*.

Strain	MIC of antibiotic (μg/ml)	MIC of CPZ (μg/ml)	MIC (μg/ml) of antibiotics when CPZ is present in the medium		
			A+1/2 MIC	A+1/4 MIC	A+1/8 MIC
Corynebacteria	Penicillin				
JK AB 2227	>200	25	>100	>100	>100
Corynebacteria	Penicillin				
JK	>400	100	>200	>400	>400
S. pneumonia 68128	Penicillin				
	12.5	100	0.03	1.6	6.2
P. aeruginosa 91	Tobramycin				
	1.6	>200	0.4	0.8	0.8
K. pneumonia 38	Cefuroxim				
	>100	200	25	50	100
E. coli 87	Ampicillin				
	>100	>200	>100	>100	>100
S. aureus D7398	Penicillin				
	6.2	100	0.05	0.2	1.6
	Methicillin				
	>100	100	3.1	6.2	12.5
S. aureus D7391	Penicillin				
	1.6	100	0.1	0.2	0.4
	Methicillin				
	12.5	100	1.6	3.1	6.2

Note: None of the strains produced β-lactamases.

Table III. *The effect of trans(e)chlorprothixene on the activity of antibiotics*.

Strain	MIC of antibiotic (μg/ml)	MIC of CPZ (μg/ml)	MIC (μg/ml) of antibiotics when CPZ is present in the medium		
			A+1/2 MIC	A+1/4 MIC	A+1/8 MIC
Corynebacteria	Penicillin				
JK AB2227	>200	25	3.1	>100	>100
Corynebacteria	Penicillin				
JK	>400	50	6.2	>400	>400
S. pneumonia 68128	Penicillin				
	12.5	12.5	0.003	12.5	12.5
P. aeruginosa 91	Tobramycin				
	1.6	>200	0.8	3.1	>1.6
K. pneumonia 38	Cefuroxim				
	>100	200	12.5	12.5	50
*E. coli*87	Ampicillin				
	>100	>200	>100	>100	>100
S. aureus D7398	Penicillin				
	6.2	25	0.1	1.6	6.2
	Methicillin				
	>100	25	1.6	6.2	12.5
*S. aureus*D7391	Penicillin				
	1.6	25	0.05	0.2	0.8
	Methicillin				
	12.5	25	0.2	3.1	6.2

Discussion

CPZ, AMY and T-CPT were able to reduce or reverse resistance of Gram-positive and Gram-negative bacterial strains to antibiotics to which these strains were initially resistant. The ability of non-antibiotics to render MDR bacteria is a clinically relevant observation inasmuch as these agents have been in safe use for many decades and their use as adjuvants for therapy of MDR bacterial infections mediated by over-expressed efflux pumps is promising. As of the time of this writing, there is much interest in the potential of medicinal compounds for adjunct use. However, because these agents are no longer under patent protection and they present no economic advantage to pharmaceutical companies, there is resistance to their development for therapy of infectious disease. Nevertheless, if the example of the situation involving extensive drug resistant tuberculosis (XDR-TB), an essentially terminal condition, and the successful therapy of unresponsive XDR-TB with the non-antibiotic thioridazine (18), the message that non-antibiotics offer a potential to serve as adjuncts for the therapy of MDR infections is being heard. Hopefully, we will see clinical trials for therapy of problematic MDR bacterial infections with non-antibiotics in the near future (16).

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