



# Pentamidine: a drug to consider re-purposing in the targeted treatment of multi-drug resistant bacterial infections?

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The recent emergence of plasmid-mediated colistin resistance (1) highlights a new problem in the development of bacterial antimicrobial resistance. Colistin, while an old antibiotic, is increasingly turned to as a “drug of last resort” for many recalcitrant Gram-negative infections. With the efficacy of this drug now compromised, the spread of pan-resistant organisms is a very real threat. To meet this challenge clinicians are seeking new treatment options, however with a drug development pipeline that is virtually dry, an urgent rethinking of how we employ our existing arsenal of antibiotics is required to deal with this problem. Un-orthodox combinations of existing antimicrobial drugs are increasingly being used, often with little rationale on how such combinations are selected beyond the thinking that ‘more is likely to be better’ (2). There is a clear need for a precision medicine approach that combines robust laboratory testing methods with effective dosing strategies for individual patients.

The recent paper of Stokes *et al.* (3) is notable in two ways. Firstly, the repurposing of pentamidine for the treatment of multi-drug resistant (MDR) Gram-negative infections and secondly, the rationale that took the group to make this discovery. The use of antimicrobial combinations is not a new concept. Infections such as tuberculosis, endocarditis and chronic viral infections (HIV, hepatitis B/C) are routinely treated with combinations as the standard of care. Antibiotics administered in combination may have an antibacterial effect “greater than the sum of their parts”. However, the use of antibiotics in combination to treat either multi- or pan-drug resistant (PDR) bacterial

infections poses significant problems. Enhanced activity can be readily shown *in vitro* with a myriad of combinations of drugs against a variety of organisms (4-6). Pentamidine, however, is not a traditional antibiotic. The drug is widely used in the treatment of parasitic and fungal infections, but not as an agent for the treatment of common bacterial infections. Why then has pentamidine recently been identified as a drug that could be used to treat MDR bacteria with acquired colistin resistance?

## Why pentamidine?

An early step in the pathway that ultimately led to this discovery was the observation that when incubated at low temperatures, *Escherichia coli* became susceptible to the glycopeptide antibiotic vancomycin (7). This was unexpected as vancomycin is a narrow spectrum antibiotic, usually effective only against Gram-positive bacteria. Unlike Gram-negatives, Gram-positive bacteria lack an outer membrane rendering them susceptible to a number of large hydrophilic drugs including vancomycin. The outer membrane of Gram-negative organisms like *E. coli* is usually an effective barrier against these drugs.

In an effort to elucidate why temperature would have this effect, the researchers screened a library of *E. coli* mutants, for those able to retain resistance to vancomycin under cold stress. Surprisingly many of the mutations were in genes responsible for the synthesis of components of the Gram-negative outer membrane. This implied that cold stress altered the outer membrane, making it more permeable

**Table 1** Reported pentamidine MICs against selected bacteria.

Organism	Method	MIC (µg/mL)	MBC (µg/mL)	Reference
Gram-positive bacteria				
<i>Bacillus cereus</i> ATCC 11778	Agar dilution	0.5	–	(14)
<i>Bacillus subtilis</i> ATCC 6633	Agar dilution	30	–	(14)
<i>Micrococcus luteus</i> ATCC 9341	Agar dilution	0.5	–	(14)
<i>Micrococcus luteus</i> ATCC 10240	Agar dilution	2.0	–	(14)
<i>Staphylococcus aureus</i> ATCC 6358	Agar dilution	10	–	(14)
<i>Staphylococcus aureus</i> ATCC 25923	Broth microdilution	32 [32]*	≥91 [91]*	(13)
<i>Staphylococcus aureus</i> (clinical isolate)	Broth microdilution	64 [32]*	≥128 [128]*	(13)
<i>Staphylococcus aureus</i> (MRSA)	Broth microdilution	32 [16]*	128 [64]*	(13)
<i>Staphylococcus aureus</i>	Broth microdilution	3	–	(15)
<i>Staphylococcus epidermidis</i> ATCC 29997	Broth microdilution	16 [16]*	128 [64]*	(13)
<i>Tropheryma whipplei</i>	Broth microdilution	0.125–0.25	–	(12)
Gram-negative bacteria				
<i>Coxiella burnetii</i>	qPCR	~0.6 <sup>#</sup>	–	(11)
<i>Escherichia coli</i> ATCC 8739	Agar dilution	125	–	(14)
<i>Escherichia coli</i> ATCC 10536	Agar dilution	125	–	(14)
<i>Escherichia coli</i>	Broth microdilution	200	–	(3)
<i>Escherichia coli</i>	Broth microdilution	14	–	(15)
<i>Pseudomonas aeruginosa</i> ATCC 9027	Agar dilution	500	–	(14)
<i>Pseudomonas aeruginosa</i> ATCC 15442	Agar dilution	750	–	(14)

\*, figure indicates the MIC/MBC determined in Mueller Hinton broth supplemented with calcium (25 mg/L) and magnesium (50 mg/L);

<sup>#</sup>, corresponds 73.3% reduction in *Coxiella* genome numbers as assessed by qPCR.

to vancomycin. The researchers then hypothesized that by screening for molecules able to restore susceptibility to vancomycin at low temperatures they would identify compounds that would affect outer membrane integrity and thus sensitise resistant cells to other antibiotics. Of 1,440 molecules tested, three compounds; benzalkonium chloride, diminazene and pentamidine, were found to have these effects on *E. coli*. Pentamidine was selected for further investigation.

### What is pentamidine?

Pentamidine is an aromatic diamidine drug. It has activity against a number of microorganisms including protozoa (*Trypanosoma brucei*, *Leishmania* spp., and *Babesia* spp.) and fungi (*Pneumocystis jirovecii*) (8). Diamidines were investigated during the 1940's and 1950's as potential

antibacterial compounds (9,10), but were seldom used clinically due to adverse side effects and the abundance of other antimicrobials that were then available. Although they have not been widely used as antibacterials they are known to have activity *in vitro* against a number of bacterial pathogens, including *Coxiella burnetii* (11), *Tropheryma whipplei* (12) and *Staphylococcus aureus* (13). Table 1 shows some reported pentamidine MICs against a range of different bacterial species.

The mechanism of action of pentamidine remains largely unknown but evidence suggests that it binds nucleic acids with the positively charged amidinium ions directing it towards the negatively charged phosphate backbone. Pentamidine has been shown to bind to the minor groove of DNA (16) and is AT selective, with most efficient binding observed at sites containing runs of 5 or more AT base pairs (17). Pentamidine has also been found to block

mitochondrial translation in eukaryotes by binding to tRNA (*Saccharomyces cerevisiae*) (18).

Stokes and colleagues (3) demonstrated an entirely new mechanism of action accounting for its antimicrobial activity against *E. coli*. Using atomic force microscopy, they observed undulations in the outer membrane of pentamidine-treated *E. coli* cells, suggesting direct interaction of the drug with components of the outer membrane. This was supported by the observation that pentamidine treated cells also released lipopolysaccharide (LPS), suggesting that the integrity of their membrane had been compromised.

As the outer membrane of Gram-negative bacteria is an impermeable barrier to many antibiotics (19), any disruption may render the cell susceptible to other antibiotic agents, particularly those which are normally excluded. Indeed, this type of synergy has been demonstrated previously with other outer membrane targeting agents such as colistin and polymyxin B (20,21). Interestingly, the addition of  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  antagonised the low temperature activity of vancomycin on *E. coli* (7). Likewise, pentamidine did not potentiate rifampicin in the presence of elevated  $\text{Mg}^{2+}$  concentrations and, moreover, pentamidine activated the PhoPQ system, which responds to  $\text{Mg}^{2+}$  starvation (22). These observations suggest that pentamidine may function in a manner similar to polymyxins: disruption of the stability of the outer membrane LPS by interference with the bridging provided by divalent cations.

Synergy against a variety of Gram-negative pathogens was demonstrated when pentamidine was combined with rifampicin, novobiocin or erythromycin, again representing types of drugs usually used for treating Gram-positive bacteria. Notably the combinations were not effective against *Proteus* or *Morganella* species. The reason for this has yet to be determined although these species are considered intrinsically resistant to polymyxins. The combination of pentamidine and rifampicin however was able to overcome the resistance to polymyxins imparted by the *mcr-1* gene. This suggests that pentamidine is active via a method which is insensitive to MCR mediated changes to the lipid A moiety of bacterial LPS and might be efficacious as a treatment for organisms with acquired colistin resistance.

Finally, the authors showed that these pentamidine combinations were not just active *in vitro*, but also *in vivo*. Pentamidine and novobiocin were able to successfully treat mice with an experimental *Acinetobacter baumannii* infection, with both colistin-susceptible (100% survival rate) and colistin-resistant (91% survival rate) strains. Furthermore, they demonstrated that the doses of each individual

antibiotic could be significantly reduced below the levels required for inhibition *in vitro*. The doses required to treat these animals were at concentrations which should be safely tolerated by the animals and significantly lower than equivalent human therapeutic doses.

The combination of pentamidine with conventional antibiotics is promising. Obviously translating these laboratory results into clinical outcomes and treatment regimens will require further investigation. Anecdotally, pentamidine may have successfully cleared a *S. aureus* infection in a patient being treated for a presumed *P. jirovecii* pneumonia (13). It remains to be seen whether this combination of agents will be well tolerated in humans and improve the outcome of MDR infections.

While the prevalence of antibiotic resistant organisms continues to increase worldwide, the number of drugs we have to treat them remains constant. There is an urgent, and currently unmet, need for new therapeutic agents. The paper of Stokes *et al.* introduces not only a new potential antibacterial agent, but more importantly highlights a novel approach for identification of new antimicrobials, enabling the repurposing of existing drugs.

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