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Sarir S[1], Thomas C[1], Rajagopal A[1], Zimmern P[2]
1. *University of Texas at Dallas,* ***2.*** *UT Southwestern Medical Center*

ANTIMICROBIAL PROPERTIES OF GALLIUM NITRATE AGAINST ESTABLISHED BIOFILM-FORMING URINARY PATHOGENS IN OLDER WOMEN WITH RECURRENT URINARY TRACT INFECTIONS.

Hypothesis / aims of study

Gallium nitrate is FDA approved under the name Ganite™ for IV treatment of bladder cancer [1]. Ganite™ is currently in phase II trials for its demonstrated antimicrobial activity against *Pseudomonas aeruginosa* in the lungs of cystic fibrosis patients [2]. Based on its use in respiratory infections, we assayed gallium nitrate for its microbial activity against bio-film forming uropathogenic *Escherichia coli* (UPEC), as well as lab strains of *P. aeruginosa* known to produce biofilms.

Study design, materials and methods

Strains: Urine samples from symptomatic post-menopausal women with recurrent urinary tract infections were cultured on LB agar with a threshold of $>10^6$ CFU/ml. Isolates from two symptomatic patients were characterized as bladder UPECs and designated as strains BUTI-5 and BUTI-12. Other *E. Coli* strains used included the uropathogenic strains UTI-89, CFT073, and RUTI-12 from a previous study, as well as the lab strain E. Coli B. *P. aeruginosa* strains PAO1 and Boston 4501 were included as both strains have been used in gallium studies before.

Microcidal Activity:To assay its microcidal activity, gallium nitrate was applied to sensitivity disks at a range of concentrations from 200 nmoles to 100 □moles and allowed to dry under laminar flow. Disks were then placed on Muller Hinton media plates on which bacterial strains were freshly swabbed. Zones of inhibition of bacterial growth were determined using epiluminescence (Biorad Chemidoc MP imaging system and associated ImageLab 5.2.1 software), and minimum inhibitory concerntrations (MIC) of gallium were determined by linear regression analysis of the areas of inhibition zones plotted as a function of the natural log of the gallium concentration on the disk [3].

Biofilm assays were performed using a standard crystal violet assay.

Iron Competition: To assay gallium nitrate's ability to substitute for iron in physiological conditions, pyoverdin quenching assays were performed on PAO1 and Boston 4501. Strains were grown and swabbed as described above, and sub-lethal concentrations of gallium nitrate were applied to sensitivity disks. Plates were visualized with blue LED epi-illumination and photographed through a 530/28 emission filter. Diminutions in fluorescence (black) were quantified in a manner analogous to the quantification of zones of inhibition.

All assays were performed in triplicate and reported as means +/- standard deviation.

Results

1.UPEC strains and lab strain *E. coli B* were sensitive to gallium. Inset shows a representative sensitivity plate for one of the strains (Figure 1). MICs for gallium nitrate were in the low milimolar range for all strains tested.

2. P. aeruginosa strains were sensitive to gallium nitrate, with MICs in the milimolar range, analogous to UPECs tested. The level of sensitivity seen here corresponds to reports in the literature. (Figure 2)

3. Gallium nitrate was effective in disrupting established biofilms in both UPECs and *P. aeruginosa* strains, as indicated by a lower OD_{600} absorbance (Figure 3).

4. The fluorescence of pyoverdin, a *P. aeruginosa* pigment that fluoresces in blue light upon iron binding, is known to be distrupted in low concentrations of gallium nitrate. We tested the ability of nanomolar concentrations of gallium nitrate to disrupt *P. aeruginosa* fluorescence. While gallium nitrate at these concentrations did inhibit pyoverdin fluorescence, likely due to iron competition, it had no effect on strain growth, as the bacteria grew directly to the edge of the disc as seen in the inset (Figure 4).

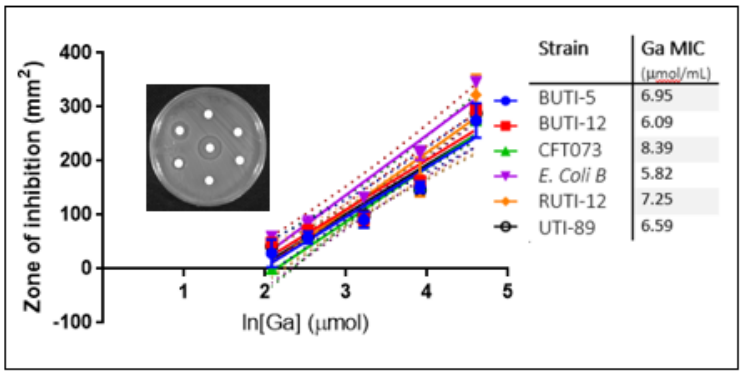

Figure 1 UPECs are sensitive to $Ga(NO_3)_3$

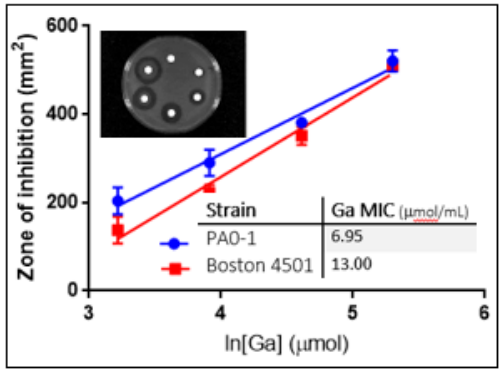

Figure 2 *P. aeruginosa* are sensitive to $Ga(NO_3)_3$

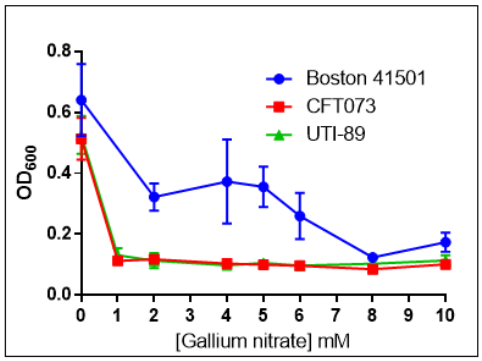

Figure 3 $Ga(NO3)_3$ inhibits biofilms

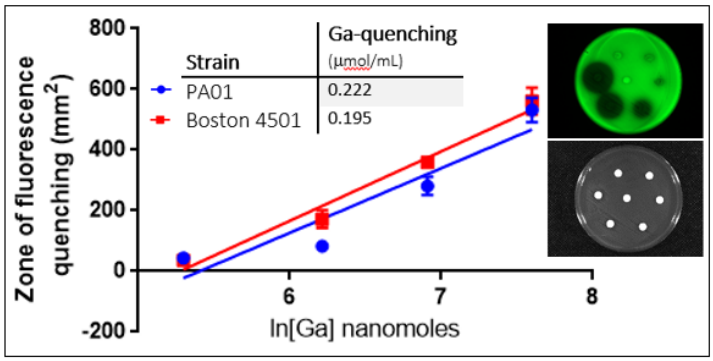

Figure 4 $Ga(NO3)_3$ interferes with *P. aeruginosa* fluorescence

Interpretation of results

To our knowledge, this is the first demonstration of the microcidal activity of gallium nitrate on clinical isolates of uropathogenic *E. Coli*. Gallium nitrate also disrupted biofilm formation in these strains. The observation that gallium nitrate uniformly inhibited the growth and biofilm formation of a number of different strains tested, including UPEC strains, an *E. Coli* lab model strain, and two pathogenic *Pseudomonas* strains, is suggestive of the possibility that gallium nitrate might be effecting a pathway that is conserved among gram negative bacteria. Gallium nitrate is known to act on transcriptional pathways involved in detecting iron limitation in *P. aeruginosa*, as well as interfering with its iron-scavenging siderophores. While our results seem to support the published claims about the involvement of gallium nitrate in iron-dependent pathways in pseudomonads, gallium nitrate seems to have greater efficacy against biofilm formation in UPECs than in *P. aeruginosa*.

Concluding message

In the search for alternatives to conventional antibiotic therapy against common uropathogens, these *in vitro* experiments demonstrated gallium nitrate's potential as a novel tool in the management of recurrent urinary tract infections in women.

References

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Disclosures

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