

TECHNICAL REPORT

Microbiologic Properties of Flucytosine 500 mg two capsules mixed with BASSA-GEL™ against selected pathogens was assessed and the results are conveyed here.

Executive Summary: Flucytosine 500 mg capsules (2 capsules) mixed with BASSA-GEL™ (“DRUG”) was tested against the identified pathogens and the results of these tests are reported as follows. Should there be only a “blue-line” reported that means the DRUG was so effective against the pathogen that the detection limit was below the assay of the experiment.

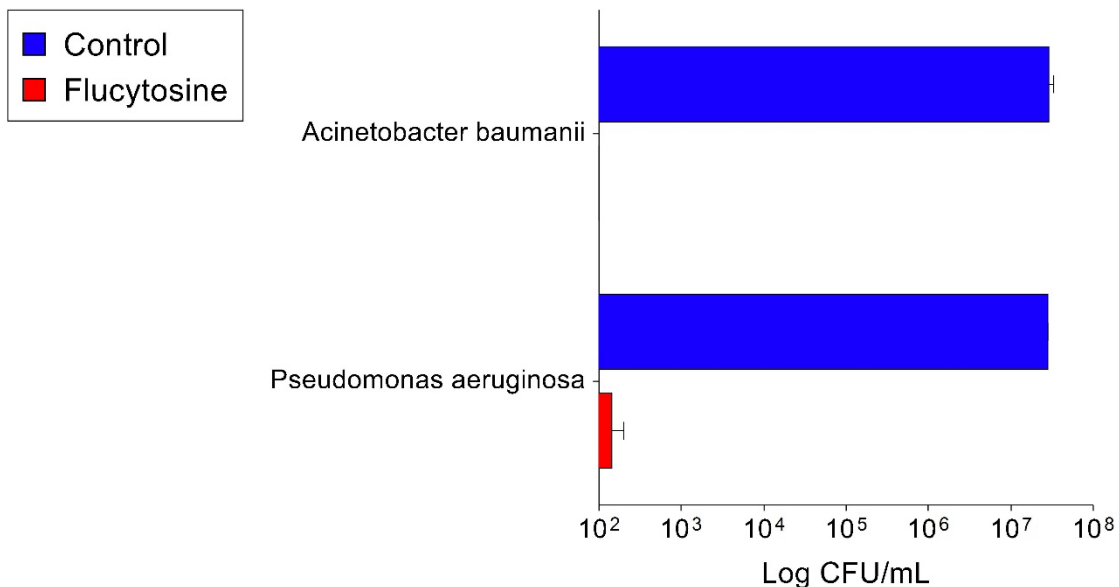
Methods overview: Methods for this laboratory study were adapted from Bearden *et al* and from FDA Docket No. FDA-1975-N-0012.^{1,2} All experiments were performed using the commercially available formulations. Reductions in bacterial counts between agents were determined.

Methods and Results:

Bacterial strains: Pathogens selected are defined in ATCC or CDC AR strains (Table 1, page 2).^{3,4}

Antimicrobial agents: Flucytosine 500 mg capsules (NDC 42494-0340-01) – 2 capsules mixed with BASSA-GEL™

Experiment: Pre-sterilized discs were saturated with $1 \times 10^{7-8}$ CFU/mL of bacterial culture, allowed to incubate for 24 hours to mimic *ex vivo* wound infection, exposed to the gel/drug solution or positive control (phosphate buffer saline, PBS), and then incubated aerobically at 37°C for 24 hours. After this time, disks were washed, diluted, and then cultured onto blood agar plates for colony forming unit (CFU/mL) counts using serial dilution spread plate technique. The results are reported below (mean log CFU/mL \pm standard error). As stated above in the executive summary, should there be only a “blue-line” reported that means the DRUG was so effective against the pathogen that the detection limit was below the assay of the experiment.



Interpretation: Flucytosine with BASSA-GEL™ was tested in a model mimicking a bandaged wound. The experiment demonstrated significant reductions in bacterial species tested.

Table 1. Organisms Included in Testing

| Organism | ATCC number |
|--------------------------------|--------------------|
| <i>Acinetobacter baumannii</i> | BAA747 |
| <i>Pseudomonas aeruginosa</i> | 27853 |

References

1. Bearden, D.T., Allen, G.P. & Christensen, J.M. Comparative in vitro activities of topical wound care products against community-associated methicillin-resistant *Staphylococcus aureus*. *J Antimicrob Chemother* **62**, 769-72 (2008).
2. Huang, D.B., Okhuysen, P.C., Jiang, Z.D. & DuPont, H.L. Enteroaggregative *Escherichia coli*: an emerging enteric pathogen. *Am J Gastroenterol* **99**, 383-9 (2004).
3. Rezzoagli, C., Wilson, D., Weigert, M., Wyder, S. & Kummerli, R. Probing the evolutionary robustness of two repurposed drugs targeting iron uptake in *Pseudomonas aeruginosa*. *Evol Med Public Health* **2018**, 246-259 (2018).
4. Imperi, F., Fiscarelli, E.V., Visaggio, D., Leoni, L. & Visca, P. Activity and Impact on Resistance Development of Two Antivirulence Fluoropyrimidine Drugs in *Pseudomonas aeruginosa*. *Front Cell Infect Microbiol* **9**, 49 (2019).