CFAES **Food Animal Health Research Program**

Small molecule targeting outer membrane lipopolysaccharide transporter complex (LptD/E) reduces avian pathogenic *E. coli* (APEC) infection in poultry

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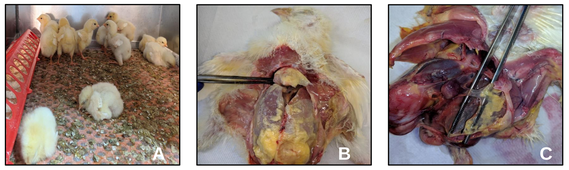
INTRODUCTION

- Avian pathogenic *E. coli* (APEC), a most common bacterial pathogen of poultry, causes colibacillosis in chickens[1].
- Colibacillosis results in high morbidity and mortality in chickens, decreased meat and eggs production, and increased condemnation of carcasses at slaughter[1].
- APEC has been also reported as a food-borne human uropathogen transmitted through consumption of contaminated poultry products[2].
- Antibiotics are routinely used to treat APEC infections; however, the emergence of antibiotic-resistant APEC strains[3] and increased restrictions placed on the use of antibiotics in food-producing animals[4] necessitate the development of newer therapies.



Colibacillosis in one-week-old chickens. Clinical signs (A; dull and depressed, reduced appetite, and respiratory distress) and lesions (B: perihepatitis, pericarditis & C: air-sacculitis) caused by APEC in chickens.

HYPOTHESIS

- High throughput screening of bioactive small molecule (SM) library can identify new antimicrobial(s) effective in treating APEC infection in poultry.

OBJECTIVE

- To identify, evaluate, and develop anti-APEC SM antimicrobial(s) with novel antibacterial target(s) as an alternative to antibiotics.

METHODS

1. Bioactive SM library (ChemBridge) was screened, which identified 40 small molecules inhibitory to APEC growth at 100 µM[5].
2. Eleven SMs, which were bactericidal to APEC, were selected and evaluated for efficacy and toxicity *in vitro* in cultured epithelial and macrophage cells and in wax moth larva model[5].
3. Eight SMs, that are non-toxic and effective *in vitro* and in larva model, were evaluated in commercial broiler chickens by administering orally in non-natural subcutaneous (s/c) APEC infection model (**Fig. 1**).
4. Candidate drug, GI-7, was identified, dose was optimized for drinking water delivery (**Fig. 2**), and tested in field simulated conditions using natural oral APEC infection model (**Fig. 3**).
5. The antibacterial target of GI-7 was identified by performing bacterial cytological profiling (BCP), gene and protein expression-based studies and LC-MS/MS analysis (**Fig. 4**).

Screening of small molecule library → Identify APEC growth inhibitors → Evaluate *in vitro* and in chickens → Candidate drug → Test in field simulated conditions

RESULTS

❑ GI-7 most effectively reduced the mortality, APEC load, and APEC lesions in chickens

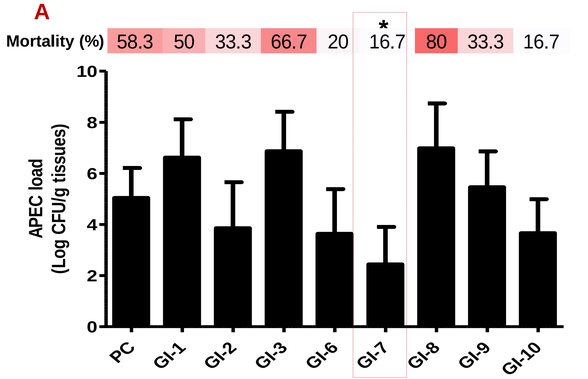


Fig 1. (A) Mortality and APEC load in SMs treated groups as compared to untreated (PC) group. (B) APEC lesions in GI-7 and PC groups, Arrows (yellow- perihepatitis and orange- pericarditis). SMs were administered orally (1 mg/kg), twice a day, from day 4 to day 8. Chickens (n=6/group) were infected subcutaneously (s/c) with rifampicin resistant (Rif^r) APEC O78 (1 x 10^7 CFU/chicken) at day 5. Mortality was recorded from day 5 to day 12. All live chickens were euthanized and necropsied at day 12 to measure the APEC load in the internal organs by plating on MacConkey agar plates containing 50 µg/ml rifampicin and the APEC lesions were scored as previously described[6], *$P<0.1$, Mann-Whitney U test.

❑ GI-7 when administered at 60 mg/L in drinking water resulted in effective anti-APEC effect in chickens

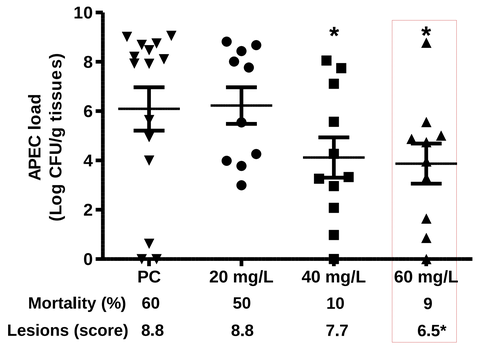


Fig 2. Mortality, APEC load and APEC lesions in chickens treated with GI-7 at different doses as compared to untreated (PC) group. GI-7 was administered in drinking water at 20, 40, and 60 mg/L doses from day 4 to day 10. Chickens (n=11/group) were infected and mortality, APEC load, and APEC lesions were measured/scored as described earlier, *$P<0.05$, Student's t-test.

❑ GI-7 showed efficacy superior than currently used antibiotic sulfadimethoxine when tested in field simulated conditions

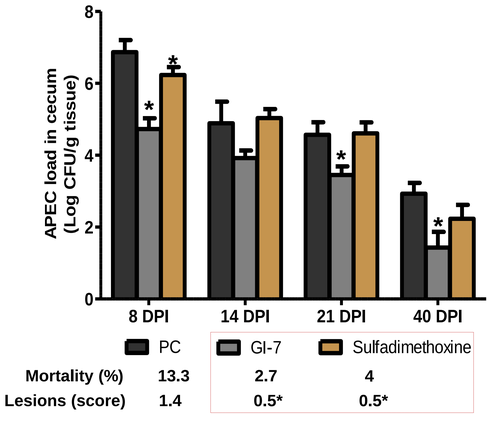


Fig 3. Mortality, APEC load and APEC lesions in chickens treated with GI-7 and sulfadimethoxine as compared to untreated (PC) group. GI-7 was administered at 60 mg/L; whereas, sulfadimethoxine was administered at its therapeutic dose (0.05%), from day 2 to day 8, in drinking water of chickens. Chickens (n=75/group) were infected at day 2 orally (1x10^9 CFU/chicken) similar to natural infection and raised on built-up floor litter until day 42 similar to poultry farms. APEC load was quantified at 8, 14, 21, and 40 days post-infection (DPI). Mortality and APEC lesions were scored as described earlier, *$P<0.05$, Student's t-test.

❑ GI-7 affects the APEC outer membrane by most likely targeting lipopolysaccharide transporter complex (LptD/E)

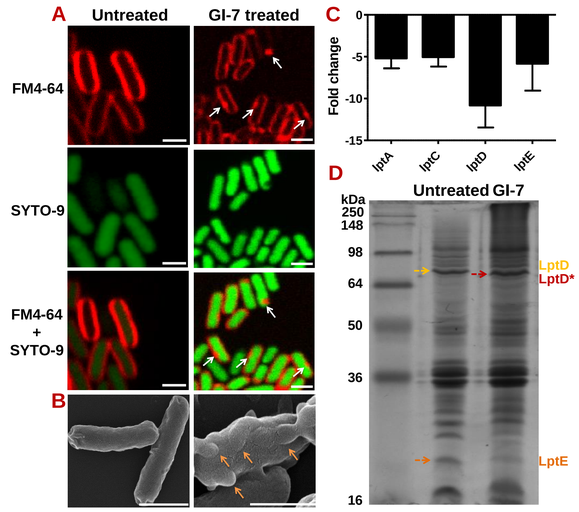


Fig 4. **(A)** Confocal microscopy images (FM4-64: membrane stain and SYTO-9: nuclear stain) of untreated and GI-7 treated APEC. Membrane bleb-like structures (white arrows) were observed with GI-7 treatment which is characteristic to antibacterials targeting Lpt complex (LptABCDEFG)[7]. **(B)** Scanning electron microscopy images of untreated and GI-7 treated APEC. Membrane blebs (orange arrows) were observed with GI-7 treatment. **(C)** GI-7 treatment downregulated the expression of *lptD/E* as determined by RT-qPCR ΔΔCt method. **(D)** GI-7 treatment depleted the level of LptE and induced the formation of non-functional LptD intermediate (LptD*; slightly faster mobility compared to LptD[8]) as determined by SDS-PAGE (membrane proteins fraction) followed by LC-MS/MS analysis of excised gel fragments.

CONCLUSION AND FUTURE DIRECTIONS

- ❑ GI-7 can represent a novel anti-APEC therapeutic; thereby, can be developed as an alternative to currently used antibiotics.
- ❑ Our future studies will be focused on validating the antibacterial target of GI-7 and developing formulations to advance GI-7 into field applications.

BIBLIOGRAPHY

[1]Mellata M. 2013, Foodborne Pathogens and Disease 10:916-932.
[2]Liu et al. 2018. MBio 9.
[3]Gyles CL. 2008. Animal Health Research Reviews 9:149-58.
[4]Van Boeckel et al. 2015. Proceedings of the National Academy of Sciences 112:5469.
[5]Kathayat et al. 2018. Scientific Reports 8:15329.
[6]Antao et al. 2008. Microbial Pathogenesis 45:361-9
[7]Vetterli et al. 2018. Science Advances 4:eaau2634.
[8]Chng et al. 2012. Science 337:1665-1668

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