The 2nd Symposium on Drug Discovery

July 2nd – 3rd, 2024 | Taipei, Taiwan

Speaker information

General Information

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Affiliation	Department of Pediatrics, School of Medicine, College of Medicine, Taipei Medical University	
Education Background	PhD in Child Health, Institute of Child Health, University College London, UK	
Professional Appointment	• Associate Dean, College of Medicine, TMU (April 2024-present) • Vice Dean, Office of Research and Development, TMU (Feb 2024-present) • Professor, Department of Pediatrics, School of Medicine, College of Medicine, TMU (Aug 2023-present) • Professor, Master Program for Clinical Genomics and Proteomics, College of Pharmacy, TMU (Aug 2023-present) • Chair of Nutrition Scientific Subcommittee, Asian Pan-Pacific Society of Pediatric Gastroenterology, Hepatology & Nutrition (APPSPGHAN) (Oct 2022-present)	
Research Interest	Salmonella virulence genes, regulation of Salmonella on host immunity, effects/mechanisms of probiotics/health foods, pathogen-host interactions using human intestinal polarized in vitro organ culture and in vitro M cells, antibiotic resistance genes in Salmonella with development of multigene panel for early detection of ampicillin-resistant Salmonella, development of novel antibiotics against ceftriaxone-resistant Salmonella, and optimized probiotics for decolonization of vancomycin-resistant enterococci (VRE)	
Website	https://medicine-en.tmu.edu.tw/portfolio-item/shiuh-bin-fang-md-phd/ https://medicine-en.tmu.edu.tw/portfolio-item/shiuh-bin-fang-md-phd/	

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Recent Publications

1. Huang WC, Chen WT, Chen YC, Fang SB, Huang TW, Chang PR, Chang YC. ***Salmonella* YqiC exerts its function through an oligomeric state.** Protein Science. 9 August 2023;e4749. https://doi.org/10.1002/pro.4749
2. Lee YJ†, Chang YC†, Lee IH, Ho KH, Fang SB, Lauderdale TL, Chen TW, Chen KC, Huang CH, Huang TW. **Persistence and dynamic structures of diverse cephalosporinase genes in nontyphoidal *Salmonella* in cross-sectional surveillance in Taiwan.** International Journal of Antimicrobial Agents. 4 August 2023;62(4):106944. (†Joint first authorship) http://doi.org/10.1016/j.ijantimicag.2023.106944
3. Fan HH†, Fang SB†*, Chang YC, Huang ST, Huang CH, Chang PR, Chang WC, Lauderdale TY, Lin PC, Cheng HY. **Effects of colonization-associated gene *yqiC* on global transcriptome, cellular respiration, and oxidative stress in *Salmonella* Typhimurium.** Journal of Biomedical Science. 1 December 2022; 29:102 (†Joint first authorship, *Correspondence author) https://jbiomedsci.biomedcentral.com/articles/10.1186/s12929-022-00885-0
4. Fang SB*, Lauderdale TY, Huang CH, Chang PR, Wang YH, Shigemura K, Lin YH, Chang WC, Wang KC, Huang TW, Chang YC. **Genotypic diversity of ciprofloxacin nonsusceptibility and its relationship with minimum inhibitory concentrations in nontyphoidal *Salmonella* clinical isolates in Taiwan.** Antibiotics. 2021 Nov 11; *10*(11), 1383; https://doi.org/10.3390/antibiotics10111383 (*Correspondence author)
5. Wang KC, Huang CH, Chang PR, Huang MT, Fang SB*. **Role of *wzxE* in *Salmonella* Typhimurium lipopolysaccharide biosynthesis and interleukin-8 secretion regulation in human intestinal epithelial cells.** Microbiological Research. 2020 Sep;238:126502 http://doi.org/10.1016/j.micres.2020.126502 (*Correspondence author)
6. Sun WS, Lee YJ, Tsai KN, Ho YH, Fang SB*. **Probiotic cocktail identified by microbial network analysis inhibits growth, virulence gene expression, and host cell colonization of vancomycin-resistant enterococci.** Microorganisms. 2020 May;8(6):816. http://doi.org/10.3390/microorganisms8060816 (*Correspondence author)
7. Fang SB*, Huang CJ, Huang CH, Wang KC, Chang NW, Pan HY, Fang HW, Huang MT, Chen CK. ***speG* is required for intracellular replication of *Salmonella* in various human cells and affects its polyamine metabolism and global transcriptomes.** Frontiers in Microbiology. 2017;8:2245. https://doi.org/10.3389/fmicb.2017.02245 (*Correspondence author)
8. Wang KC, Huang CH, Ding SM, Chen CK, Fang HW, Huang MT, Fang SB*. **Role of *yqiC* in the pathogenicity of *Salmonella* and innate immune responses of human intestinal epithelium.** Frontiers in Microbiology. 2016;7:1614. https://doi.org/10.3389/fmicb.2016.01614 (*Correspondence author)
9. Wang KC, Huang CH, Huang CJ, Fang SB*. **Impacts of *Salmonella enterica* serovar Typhimurium and its *speG* gene on the transcriptomes of *in vitro* M cells and Caco-2 cells.** PLoS One. 2016;11(4): e0153444. https://doi.org/10.1371/journal.pone.0153444 (*Correspondence author)

10. Fang SB*, Ko HY, Huang ST, Huang CH, Li LT, Chen CC, Wang KC, Pai CP, Lee HC, Fang HW*. ***Cinnamomum osmophloeum*** **extracts inhibits growth of** ***Helicobacter pylori*** **and postinfectious interleukin-8 expression in human gastric epithelial cells.** RSC Advances. 2015;5:22097-105. https://doi.org/10.1039/C4RA11026A (*Joint correspondence authors)

Speaker information

Speech Topic and Abstract

Title:
Identification of compounds against ceftriaxone-resistant nontyphoidal *Salmonella* by Ultra-High Throughput Screening and exploration of the antibacterial effect and potential mechanism of its derivatives

Abstract:
Objectives: For the past three decades, ceftriaxone-resistant (CRO-R) nontyphoidal *Salmonella* (NTS) are emerging worldwide over 43 countries reporting cases with the resistance rates from 0.2% to 56.5%, particularly from high-prevalence regions including Africa, India, Pakistan, Bangladesh, and Southeast Asia (Al kraiem AA. 2018). Taiwan CDC reported an increased rate of cefotaxime resistance in NTS to 7.2% in 2016. WHO announced in 2017 that third generation-resistant *Enterobacteriaceae*, including CRO-R NTS, are one of the priority pathogens for research and development of new antibiotics. Therefore, the objective of this study is to identify new compounds as potential antibiotics against CRO-R NTS.

Methods: First, a recombinant GFP-cloned CRO-R NTS strain (ciprofloxacin-susceptible ceftriaxone-resistant isolate NHRI-172) was generated for quantitative confocal microscopy in High Content Screening. Then, an *in vitro* invasion assay was established using 96-well plates by infecting monolayers of 4-5 day old Caco-2 cells with CRO-S (*Salmonella* Typhimurium SL1344) and CRO-R (NHRI-172) strains for 2h, followed by 1h with gentamicin and subsequent removal of extracellular bacteria, and another 1h with ciprofloxacin (CIP), ceftriaxone (CRO). This validated *in vitro* model was later applied to Ultra-High Throughput Screening (uHTS) using 1536-well plates and robotics, with library compounds added in the last one hour for screening 22,677 chemical compounds which are mainly FDA-approved drugs, known bioactive compounds, and nature products. Second, derivatives of the target compounds were generated for determining their minimum inhibitory concentrations (MICs) in *S*. Typhimurium SL1344 (SL1344), its *yqiC* (*ubiK*, related to ubiquinone (UQ)/menaquinone (MK) synthesis)-deleted mutant (Δ*yqiC*) and CRO-R strain (NHRI-172) using CLSI microdilution method. Third, adenosine triphosphate (ATP) assays were conducted for quantifying ATP production in SL1344 treated with 1/64x, 1/8x, 1/4x, 1/2x, 1x, and 2xMIC of the target compound derivative in comparison with ATP production in SL1344 not treated with the derivative (control). Fourth, RNA samples were extracted from mid-log cultures of NHRI-172 and SL1344 for quantitative Reverse Transcription PCR (qRT-PCR) to quantify the mRNA expression levels of the 23 *ubi*/*men* genes relative to housekeeper 16S rRNA. The relative mRNA expression levels were compared between NHRI-172 and SL1344. The experiments were done in triplicate. Student's *t*-test was used for statistical analysis. A *p* value of <0.05 was regarded as statistically significant.

Results:
Our uHTS identified naphthoquinone-imidazolium (sepantronium bromide, YM155) from the 86 groups of the 451 active compounds in the library. At least 22 derivatives of YM155 were generated with their chemical structures characterized by NMR, Mass and Infrared (IR) Spectrometry. Compared with the MICs of the parent compound YM155 in SL1344 (>128 *μ*g/mL), CRO-R NHRI-172 (>128 *μ*g/mL), and Δ*yqiC* (32 *μ*g/mL), the MICs of two YM155 derivatives showed their remarkably lower MICs for SL1344 (2 *μ*g/mL), CRO-R NHRI-172 (8 *μ*g/mL), and Δ*yqiC* (<0.25 *μ*g/mL) in AST-062 ($C_{31}H_{45}BrN_2O_2$) as well as SL1344 (1 *μ*g/mL), CRO-R NHRI-172 (2 *μ*g/mL), and Δ*yqiC* (<0.25 *μ*g/mL) in AST-064 ($C_{46}H_{60}Br_2N_4O_4$). Our ATP assays showed that 1/2x, 1x, and 2xMICs, but not 1/8xMIC and 1/4xMIC, of AST-062 significantly decreased ATP concentrations in SL1344 at 2h when compared with control (p <0.01). The qRT-PCR analysis showed that most of the *ubi* genes (13 in 14: p <0.05 in *ubiI*; p <0.01 in *ubiC*, *ubiA*, *ubiX*, *ubiG*, *ubiH*, *ubiE*, *ubiK*, *ubiD*, *ubiT*, *ubiU*, *ubiV*, and *ubiJ*) and the *men* genes (7 in 9: p <0.01 in *menF*, *menC*, *menE*, *menB*, *menI*, *menA*, and *menG*) are significantly downregulated in CRO-R NHRI-172 when compared with CRO-S SL1344 .

Conclusions: At least two new derivative compounds (AST-062 and AST-064) were generated from the uHTS-identified naphthoquinone-imidazolium and showed significant antibacterial effect on CRO-R NTS. Their inhibitory effects possibly work through compromising bacterial ATP production and involve biosynthesis of menaquinone and ubiquinone.

Keywords: Ceftriaxone-resistant nontyphoidal *Salmonella* (CRO-R NTS), Ultra-High Throughput Screening (uHTS), naphthoquinone-imidazolium (sepantronium bromide, YM155), minimum inhibitory concentration (MIC), ubiquinone (UQ), menaquinone (MK)