Indian Institute of Technology Delhi

DEPARTMENT OF BIOCHEMICAL ENGINEERING & BIOTECHNOLOGY

2017-18 Seminar Series

Friday, September 15, 2017

Antimicrobial Resistance

Dr. Sidharth Chopra

CSIR – Central Drug Research Institute (CDRI)
Lucknow

http://www.cdri.res.in/Profile_all.aspx?fid=4&id=1826

Antimicrobial resistance has been internationally recognized as a significant threat to healthcare systems worldwide. Due to multiple reasons including unfavorable economics, increasing emergence of drug-resistant bacteria and expensive and lengthy clinical trials, most of the big pharma has exited the discovery and development of new anti-infectives. In order to augment the non-existent to depleted drug-discovery pipeline targeting drug-resistant bacteria, our lab follows 2 complimentary approaches: conventional phenotypic whole cell screening of diverse chemical scaffold libraries as well as natural products and drug-repurposing of FDA approved drugs for new clinical uses. In my talk, I will discuss and highlight the challenges which we face along with some success stories which have come out of my lab. The talk will highlight the core issue: Drug discovery against drug-resistant microbes is a extremely interdependent, interconnected battle involving multiple expertise's against an very intelligent, ruthless and adaptable adversary.

All are welcome

Seminar will be held in DBEB COMMITTEE ROOM at Block I, Room 232 at 4 PM
For additional information, contact Seminar coordinator D. Sundar at sundar@dbeb.iitd.ac.in

Name: **Dr. Sidharth Chopra**

Address: Senior Scientist
Division of Microbiology
CSIR-Central Drug Research Institute,
Sector 10, Janakipuram Extension, Sitapur Road
Lucknow - 226031, India
Phone: +91-9792211636

E-mail: skchopra007@gmail.com

Education:

- **Senior Scientist** — Jan 2013- Present
  Division of Microbiology
  CSIR-CDRI
  Lucknow, India

- **Scientist Fellow** — May 2012- Dec 2012
  Department of Biotechnology,
  CSIR-Central Institute of Medicinal and Aromatic Plants
  Lucknow, India

- **Research Scientist** — 2008- 2012
  Centre for Infectious disease and Biodefense Research
  Stanford Research Institute International, USA

- **Postdoctoral fellow** – Microbiology and Immunology — 2004 - 2008
  Stanford University School of Medicine, USA
  Professor Gary K. Schoolnik, Advisor

- **Ph.D. in Microbiology / Biotechnology** — 2000 - 2004
  International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi, India
  Dr. Anand Ranganathan, Advisor

- **Master of Science in Microbiology** — 1997 - 1999
  Major in Microbiology
  Panjab University, Chandigarh, India

- **Bachelor of Science in Microbiology** — 1994 - 1997
  University of Delhi, New Delhi, India

Subject Area Expertise / Skills

- Microbiology with focus on Infectious diseases
- Drug discovery and development against MDR gram-positive and gram-negative bacteria including *Acinetobacter baumannii*, *Psuedomonas* sp, *Klebsiella* sp, MRSA etc.
- Cloning techniques, site directed mutagenesis, microarray design, data analysis & PCR based technologies

- Special emphasis on *Mycobacterium* species including non-tubercular mycobacteria, including gene knockouts, over-expression, transposon mutagenesis, TRASH analysis, antimicrobial susceptibility determination using MGIT.
- Protein expression and purification/interaction/bacterial two-hybrid system
- Microbial physiology and basic biochemistry including enzyme activity assays

Detailed Research and Professional background

Jan 2013-present Sr. Scientist, Division of Microbiology, CSIR-CDRI, Lucknow

I am presently engaged in Drug discovery and development against several drug resistant gram-negative and gram-positive pathogens using natural and synthetic products. I am additionally trying to understand the genetic and proteomic basis of antimicrobial resistance in bacteria.

Currently working on

1. Novel drug discovery and development against multi-drug resistant ESKAP pathogens including *A. baumannii, S. aureus, K. pneumoniae, P. aeruginosa* and *E. coli*.
2. Understanding the novel resistance mechanisms of resistance to Tigecycline, fosfomycin and Polymyxin B exhibited by *Acinetobacter baumannii* using genomics and proteomics.
3. Deciphering the role of PMQR in decreased ciprofloxacin susceptibility in *Salmonella* sp.

2008-2012 Research Scientist at Centre for Infectious disease and Biodefense Research, Stanford Research Institute International, USA

About Stanford Research Institute International: www.sri.com

SRI International is an independent, non-profit research institute conducting client-sponsored research and development for government agencies, commercial businesses, foundations, and other organizations

My responsibilities included

1. Project leader of anti-tuberculosis drug screening National Institutes of Health (NIH) contract

Supervise day to day activities involving screening of novel chemical entities (NCE's) from various submitters world-wide as a part of $56.7 million NIH contract. The project work involves designing and conducting research experiments in the BSL-3 labs with drug-resistant *Mycobacterium tuberculosis* patient isolates. Among the experiments conducted include minimum inhibitory concentration (MIC) and bactericidal (MBC) determination, MIC against single and multiple drug-resistant strains and further pre-clinical microbiology development using

High thorugh put Resazurin microtitre plate assay (REMA). We typically handle around ~30,000 compounds per year.

This project is currently funded by NIH grant totaling $1.5 million US dollars.

Hyperlink: http://www.sri.com/news/releases/091508.html

2. Understanding the mechanisms of antibiotic resistance generation in *A. baumannii*

An important focus is to understand the generation of antibiotic resistance in *A. baumannii*. We are utilizing whole scale genomics and proteomic techniques on syngenic bacterial strains to unravel the step by step mechanism responsible for generating antibiotic resistant bacteria. The final aim of the project is to identify novel therapeutic targets for targeting drug-resistant bacteria.

3. Drug-repurposing efforts to identify new indications for FDA-approved drugs against multi-drug resistant pathogens such as *A. baumannii*, *M. abscessus* and *M. chelonae*.

Drug-resistant bacteria are on the rise while antimicrobial therapies to combat them are on the decline owing to pharmaceutical apathy. The drug-repurposing effort was initiated to identify drugs targeting nosocomial pathogens such as *A. baumannii* and emerging mycobacterial infections such as those caused by *M. abscessus* and *M. chelonae*. The currently approved therapy for *A. baumannii* is severely limited by increasing drug resistance whereas conventional approved chemotherapy for infections such as that caused by *M. abscessus* and *M. chelonae* does not guarantee eradication of infection from the host.

For *A. baumannii*, we have identified tyrothricin as being highly potent against several drug-resistant clinical isolates. This is the first ever reporting of activity against gram-negative bacteria being demonstrated by Tyrothricin as it is usually used to treat *S. aureus*. Experiments to further characterize the drug target are currently underway.

2004-08 Postdoctoral Scientist at Stanford University with Prof. Gary Schoolnik, USA

Extensive drug development work with evaluation and identification of novel Plectasin based antimicrobial peptides and their derivatives as drug candidates against Multi-drug Resistant (MDR) *Mycobacterium tuberculosis* and other pathogenic non tubercular mycobacteria (NTM's) such as *M. abscessus* and *M. kansasii* was carried out in close collaboration with Dr. Hans-Henrik Kristensen, Senior Manager, Anti-Infective Unit, Novozymes A/S, Denmark. The research included

1. Novel methods such as bacterial luciferase expressing mycobacteria were developed to allow rapid High Throughput screening and identification of hit compounds from libraries of

antimicrobial peptides.

2. These hit compounds were studied for their pattern of inhibitory activity against a large panel (~50+ strains) of globally diverse, clinical patient isolates

3. Efforts were made to identify patterns of resistance to the hit compounds leading to identification of cyclopropanated mycolic acids as being one of the resistance factors.

4. Based on identification of active compounds and concomitant Structure Activity Relation (SAR) studies, further screening of variant libraries was done to identify even better hit compounds.

5. On the basis of Dr. Chopra's findings, Novozymes was awarded US patent entitled "Use of defensin against Tuberculosis" U.S. patent application no. PCT/EP2009/052405

6. Novozymes additionally established a scientific collaboration with AstraZeneca Research and Development group in Bangalore, India which plans to carry out pre-clinical development and animal model efficacy studies.

2000-04 Doctorate topic: *Mycobacterium tuberculosis* Aspartate decarboxylase and development of strategies for selection of inhibitors for protein targets

One aspect of my doctorate project focused on complete biochemical and structural characterization of Aspartate decarboxylase from *Mycobacterium tuberculosis* (Mtb). The crystal structure of the Aspartate decarboxylase was solved at 2.99 Å in close collaboration with Dr. K. Swaminathan, National University of Singapore to validate this target and perform targeted drug design.

The other aspect of my doctoral thesis involved the development of a novel method named "Codon Shuffling" for evolving proteins *in vitro*. In developing this method, we employed a molecular approach for generating a multitude of structurally diverse yet functionally similar proteins that have all "evolved" from the parent enzyme.

Peer Reviewed Publications

1. Kashyap DR, Vohra PK, **Chopra Sidharth**, Tewari R. Applications of pectinases in the commercial sector: A Review. *Bioresource Technology*. 2001 May; 77(3): 215-27.
2. **Chopra Sidharth**, Singh SK, Sati SP, Ranganathan A, Sharma A. Expression, purification, crystallization and preliminary X-ray analysis of the acyl carrier protein synthase (acpS) from *Mycobacterium tuberculosis*. *Acta Crystallographica (D) Biological Crystallography*. 2002

Jan; 58 (Pt 1): 179-81.

3. **Chopra Sidharth**, Pai H, Ranganathan A. Expression, purification, and biochemical characterization of *Mycobacterium tuberculosis* aspartate decarboxylase, PanD. *Protein Expression & Purification*. 2002 Aug; 25(3): 533-40.
4. **Chopra Sidharth**, Ranganathan A. Protein evolution by "codon shuffling": a novel method for generating highly variant mutant libraries by assembly of hexamer DNA duplexes. *Chemistry & Biology*. 2003 Oct; 10 (10): 917-26.
5. Rao A, **Chopra Sidharth***, Ram G, Gupta A, Ranganathan A. Application of the "codon-shuffling" method. Synthesis and selection of de novo proteins as antibacterials. *Journal of Biological Chemistry*. 2005 Jun 24; 280 (25): 23605-14. (* Equal contribution)
6. Gopalan G, **Chopra Sidharth**, Ranganathan A, Swaminathan K. Crystal structure of uncleaved L-aspartate-alpha-decarboxylase from *Mycobacterium tuberculosis*. *Proteins*. 2006, 65(4): 796-802.
7. Flores-Valdez and **Sidharth Chopra. "**Global reemergence of tuberculosis: Are host defense peptides an option to ameliorate disease burden?" *Microbial drug resistance*, 2010 Mar; 16(1):1-7.
8. **Chopra S**, Torres-Ortiz M, Hokama L, Madrid P, Tanga M, Mortelmans K, Kodukula K, Galande AK. Repurposing FDA-approved drugs to combat drug-resistant *Acinetobacter baumannii*. J Antimicrob Chemother. 2010 Dec; 65(12):2598-601.
9. **Sidharth Chopra***, Matsuyama K, Hutson K and Madrid P. Identification of antimicrobial activities amongst FDA-approved drugs for combating *Mycobacterium abscessus* and *Mycobacterium chelonae.* J Antimicrob Chemother. 2011 Jul; 66(7): 1533-6 (*First and corresponding author)
10. Malabika Sarker, **Sidharth Chopra**, Kristien Mortelmans, Krishna Kodukula, Carolyn Talcott, and Amit K. Galande. *In Silico* Pathway Analysis Predicts Metabolites That Are Potential Antimicrobial Targets. Journal of Computer Science and System Biology. (DOI

http://dx.doi.org/10.4172/jcsb.1000071)

11. Arlyn Tambo-ong, **Sidharth Chopra**, Bryan T. Glaser, Karen Matsuyama, Tran Tran and Peter B. Madrid. Mannich reaction derivatives of novobiocin with modulated physiochemical properties and their antibacterial activities. Bioorg Med Chem Lett. Oct 1; 21(19): 5697-700.

12. **Sidharth Chopra*** and Amit Galande. A Fluoroquinolone-resistant *Acinetobacter baumannii* without the Quinolone Resistance-Determining Region Mutations. Journal of Antimicrobial Chemotherapy, Nov 2011; 66(11): 2668-70. (* First and corresponding author).

13. **Sidharth Chopra**; Matsuyama, Karen; Tran, Tran; Malerich, Jeremiah; Lun, Shichun; Guo, Haidan; Maiga, Miriama; Wan, Baojie; Franzblau, Scott; Bishai, William; Madrid, Peter. Gyrase B as validated drug target in *Mycobacterium tuberculosis*. J Antimicrob Chemother. 2012 Feb; 67(2): 415-21.

14. Malabika Sarker, Carolyn Talcott, Peter Madrid, **Sidharth Chopra**, Barry A. Bunin, Gyanu Lamichhane, Joel S. Freundlich and Sean Ekins. Combining Cheminformatics Methods and Pathway Analysis to Identify Molecules with Whole-Cell Activity against *Mycobacterium tuberculosis*. Pharm Res. 2012, Aug; 29(8):2115-27.

15. **Sidharth Chopra**, Koolpe GA, Tambo-Ong AA, Matsuyama KN, Ryan KJ, Tran TB, Doppalapudi RS, Riccio ES, Iyer LV, Green CE, Wan B, Franzblau SG, Madrid PB. Discovery and Optimization of Benzotriazine Di-N-Oxides Targeting Replicating and Non-replicating *Mycobacterium tuberculosis*. J Med Chem. 2012 Jul 12; 55(13): 6047-60.

16. **Chopra, Sidharth**; Matsuyama, Karen; Tran, Tran; Madrid, Peter. Systematic discovery of synergistic novel antibiotic combinations targeting multidrug resistant *Acinetobacter baumannii.* International Journal of Antimicrobial Agents, 2012. Oct; 40(4): 377-9.

17. Kurnellas MP, Brownell SE, Su L, Malkovskiy AV, Rajadas J, Dolganov G, **Chopra Sidharth**, Schoolnik GK, Sobel RA, Webster J, Ousman SS, Becker RA, Steinman L, Rothbard JB. Chaperone activity of small heat shock proteins underlies therapeutic efficacy in experimental

autoimmune Encephalomyelitis**.** J Biol Chem. 2012 Sep 6.

18. Kumar K, **Chopra Sidharth**. New drugs for methicillin-resistant *Staphylococcus aureus*: an update. J Antimicrob Chemother. 2013 Jul;68(7):1465-70.

19. **Chopra Sidharth**, Ramkissoon K, Anderson DC. A systematic quantitative proteomic examination of multidrug resistance in *Acinetobacter baumannii*. J Proteomics. 2013 Jun 12;84:17-39.

20. Madrid PB, **Chopra Sidharth**, Manger ID, Gilfillan L, Keepers TR, Shurtleff AC, Green CE, Iyer LV, Dilks HH, Davey RA, Kolokoltsov AA, Carrion R Jr, Patterson JL, Bavari S, Panchal RG, Warren TK, Wells JB, Moos WH, Burke RL, Tanga MJ. A systematic screen of FDA-approved drugs for inhibitors of biological threat agents. PLoS One. 2013;8(4):e60579.

21. **Chopra Sidharth**. Could repurposing existing drugs be an efficient protective method against microbial biologic threats? Future Microbiol. 2013 Aug; 8:951-2.

22. **Sidharth Chopra***, Ellen D Beaulieu, Jeremiah P Malerich. Natural Products as Anti-Infective Agents.2014. http://ebooks.benthamscience.com/book/9781608058600/chapter/120358/ (* First and corresponding author).

23. Thakare, R, Soni, I, Dasgupta,A, **Chopra Sidharth**. Delamanid for the treatment of pulmonary multidrug-resistant tuberculosis. 2014. Drugs of Today (Spain).51: 2; 117-123.

24. Pandey, Shilpika, Gaur, Sarthak, Topno, Neha, C**hopra**, Sidharth and Dasgupta, Arunava. Cofactor Biosynthetic Pathways in *Mycobacterium tuberculosis* as Potential Drug Targets. Current Respiratory Medicine Reviews. 2014. 10, 2: 97-108.

25. Pandey, Shilpika, Soni, Isha, Topno, Neha, Dasgupta, Arunava and **Sidharth Chopra**. Current approaches for new TB drugs. Current Respiratory Medicine Reviews. 2014. 10, 2: 88-96.

26. **Sidharth Chopra** and A Dasgupta. ERAVACYCLINE Antibacterial agent Treatment of intra-abdominal infection Treatment of complicated urinary tract infection. DRUGS OF THE FUTURE. 2014. 39 (4), 247-256

27. Sashidhara KV, Rao KB, Kushwaha P, Modukuri RK, Singh P, Soni I, Shukla PK, **Chopra Sidharth**, Pasupuleti M. Novel Chalcone-Thiazole Hybrids as Potent Inhibitors of Drug Resistant *Staphylococcus aureus*. ACS Med Chem Lett. 2015 May 29;6(7):809-13.

28. Soni I, Chakrapani H, **Chopra Sidharth**. Draft Genome Sequence of Methicillin-Sensitive *Staphylococcus aureus* ATCC 29213. Genome Announc. 2015 Sep 24;3(5).

29. Soni I, De Groote MA, Dasgupta A, **Chopra Sidharth**. Challenges facing the Drug Discovery pipeline for Non-tuberculous mycobacteria. J Med Microbiol. 2016 Jan;65(1):1-8.

30. Singh AK, Karaulia P, Chopra S, Dasgupta A. Draft Genome Sequence of *Mycobacterium fortuitum* Isolated from Murine Brain. Genome Announc. 2016 Mar 31;4(2). pii: e00191-16

31. Uttam B. Karale, Saradhi Kalari, Jala Shivakumar, Vitthal B. Makane, dattatraya A. Babar, Ritesh Thakare, Nagendra Babu Bathini, **Sidharth Chopra** and Haridas B. Rode . Ligand-free Pd-catalysed decarboxylative arylation of Imidazo[1,2-a]pyridine-3-carboxylic acids with Aryl bromides ***RSC Adv.***, 2016, Accepted Manuscript. **DOI:** 10.1039/C6RA12166G.

32. Misra R, Thakare R, Amrin N, Prasad KN, **Chopra Sidharth**, Dhole TN. Antimicrobial susceptibility pattern and sequence analysis of DNA gyrase and DNA topoisomerase IV in *Salmonella enterica* serovars Typhi and Paratyphi A isolates with decreased susceptibility to ciprofloxacin. Trans R Soc Trop Med Hyg. 2016 Aug;110(8):472-9. doi: 10.1093/trstmh/trw051

33. Singh AK, Karaulia P, Yadav P, Narender T, Singh SP, Sashidhara KV, Pandey AK, **Chopra Sidharth**, Dasgupta A. Identification of lipid metabolism-targeting compounds active against drug-resistant *M. tuberculosis*. J Glob Antimicrob Resist. 2016 Dec;7:26-27. doi: 10.1016/j.jgar.2016.07.003.

34. Das S, Dasgupta A, **Chopra Sidharth**. Drug repurposing: a new front in the war against Staphylococcus aureus. Future Microbiology, 2016 Aug, Vol 11, 1091-99.

35. Kulkarni, A.; Soni, I.; Dharmaraja, A.; Sankar, R.; Thakare, R.; **Chopra, Sidharth**, Chakrapani, H. Synthesis and biological evaluation of indole-based 2-Aryl-2,3-epoxy-1,4-naphthoquinones as methicillin-resistant staphylococcus aureus (MRSA) inhibitors. INTERNATIONAL JOURNAL OF INFECTIOUS DISEASES, 2016, Vol 45, Pg 100.

36. Nair, Ratheesh S.; Deepthi, Ani; Adarsh, K.; **Chopra, Sidharth**. A comparative study on the isolation and cerium (IV) ammonium nitrate mediated oxidative transformation of oleanolic acid and ursolic acid. INDIAN JOURNAL OF CHEMISTRY SECTION B ORGANIC CHEMISTRY INCLUDING MEDICINAL CHEMISTRY, 2016, Vol 55 (5), Pg 598-603.

37. Thakare, R.; Dasgupta, A.; **Chopra, Sidharth**. LEFAMULIN: Pleuromutilin antibacterial Treatment of pneumonia and ABSSI. DRUGS OF THE FUTURE, 2016, Vol 41(3), Pg 157-167.

38. Thakare, R.; Dasgupta, A.; **Chopra, Sidharth.** VABORBACTAM. DRUGS OF THE FUTURE, 2016, Vol 41(5), Pg 157-167.

Funding received

- Novozymes A/S: Awarded $88,000 for Post-doctoral Research at Stanford University School of Medicine
- Council for Scientific and Industrial Research, India (CSIR)-UGC-NET Fellowship: Awarded fellowship for pursuing doctoral studies

Patents:

International patents:

Inventors: Hans-Henrik Kristensen Hogenhaug, Gary K. Schoolnik, **Sidharth Chopra** and

Dorotea Raventos Segura

Title: USE OF DEFENSINS AGAINST TUBERCULOSIS

U.S. patent application no. PCT/EP2009/052405

Indian patents:

Inventors: Ranganathan, Anand, **Chopra, Sidharth**

Title: Conscious evolution of proteins.

Priority Date: 16 April 2003.

Indian Patent Application no. 609/DEL/2003

Completed NIH Research Support

Project Code: HHSN266200600011C/N01-AI-60011

Duration: 02/08 – 1/13

Funding agency: Department of Microbiology and Infectious Diseases - DMID/NIAID/NIH

Project Title: Microbiology Activity Screening for TB Drug Candidates

Role: Project Leader

Funding amount: US $1.5 million

Project Description: This contract involves novel drug screening against *M. tuberculosis* and further pre-clinical development of identified inhibitors. The mandate is to handle ~30,000 compounds per year.

Project Code: 1U01AI082070-01

Duration: 9/09–9/11

Role: Co-PI

Funding agency: NIH/NIAID

Project Title: Dual Action Inhibitors of Gyrase Beta and Histidine Kinases as Broad Spectrum Antibacterials

Funding amount: US $1.0 million

Project Description: This project involves screening of multiple compound libraries eg Sigma, Chembridge etc. to identify dual inhibitors of gyrase B and histidine kinases as broad spectrum anti-bacterials. Currently, we have identified several very promising inhibitors in the nano-molar range and are progressing them through pre-clinical development program. Also, there are animal studies underway to validate the *in vivo* activity of this class of compounds.

Project Code: 1R56AI090817-01

Duration: 08/10-9/11

Role: Co-PI

Funding agency: NIH/NIAID

Project Title: Development of Benzotriazine Oxides as Therapeutics for Drug Resistant Tuberculosis

Funding amount: US $1.0 million

Project Description: This project involves screening and identification of benzotriazine oxides as novel therapeutics for drug-resistant tuberculosis. These novel SRI compounds have been identified as being extremely potent against *M. tuberculosis,* even against MDR strains. The lead compound is being channeled through the pre-clinical developmental pathway involving determination of toxicity and its mode of action determination. Also, there are animal studies underway to validate the *in vivo* activity of this class of compounds.

Peer Review of Manuscripts from

1. Biomed Central Microbiology
2. Journal of Antimicrobial Chemotherapy
3. Drug Discovery Today
4. PLoS One
5. Journal of Infectious diseases
6. PLoS Medicine
7. International Journal of Tuberculosis and Lung Disease
8. Molecular Microbiology
9. Journal of Clinical Microbiology
10. Antimicrobial and Agents Chemotherapy
11. Journal of Bacteriology

Conferences and Posters

9. **Sidharth Chopra** and Anand Ranganathan. **"**Studies on L-Aspartate decarboxylase of *Mycobacterium tuberculosis*". Poster presented at International Congress on Infectious diseases (ICID) held from March 11-14th, 2002 in Singapore.
10. **Sidharth Chopra** and Anand Ranganathan. **"**L-Aspartate decarboxylase of *Mycobacterium*

tuberculosis: a validated drug and vaccine target". Poster presented at the First International Conference on "TB Vaccines for the World" held from 17-19th of September 2003 in Montreal, Canada.

11. Gopalan Gayathri, **Sidharth Chopra**, Anand Ranganathan & K. Swaminathan. "Structural investigations of L-Aspartate decarboxylase from *Mycobacterium tuberculosis*". Poster presented at Third International Conference on Structural Biology and Functional Genomics, Singapore, December 2-4, 2004.
12. Alka Rao, **Sidharth Chopra,** Geeta Ram, Anand Ranganathan. "Synthesis And Selection of Antibacterials using Codon-Shuffling As Method For De Novo Protein Design". Poster presented at 14th International Conference on Intelligent Systems for Molecular Biology, Fortaleza, Brazil, August 6-10, 2006.

List of Referees: Available upon request