CHAPTER 1

Introduction

_Salmonella_, a Gram-negative bacterium, is one of the important microbes responsible for both typhoid and nontyphoid infections. It belongs to the _Enterobacteriaceae_ family. _Salmonella_ are sub-divided into six groups and approximately 2500 serovars have been identified, 99 % of which are responsible for disease (Bopp et al., 2003). _Salmonella_ can cause different syndromes, for example gastroenteritis, enteric fever, and septicemia. _Salmonella enterica_ serovar typhimurium is a food-borne pathogen which leads to salmonellosis and gastrointestinal infections. Approximately 93.8 million patients have been reported with gastroenteritis, and approximately 155,000 deaths of nontyphoid patients are reported annually around the globe (Majowicz et al., 2010). Non-typhoid _Salmonella_ infections are emerging in both developing and developed countries because of global trade and food exchange (Wong et al., 2011; Fisher and Threlfall, 2005; Esaki et al., 2004). Environmental risk factors, for example poor sanitation, overpopulation, and food and water contamination, can lead to salmonellosis (Crump and Mintz, 2010).

Antimicrobial agents, for example ampicillin, chloramphenicol, and co-trimoxazole, with or without tetracycline, were used in the twentieth century for treatment of salmonella infection (Sirinavin and Garner, 2000). The inhibition of topoisomerase enzymes, DNA Gyrase is the mode of action for all members of this class of drug (Hawkey, 2003). The heterotetramer DNA gyrase functions by introducing negative supercoiling in DNA by reactions of breakage and rejoining. Quinolones render themselves toxic to bacterial cells by stabilizing DNA breaks formed by gyrase and arresting rejoining (Willmott et al., 1994). Thus, replication of DNA is affected leading to death of the cell (Fig. 1.1). Increased isolation of multi-drug-resistant species now limits use of these drugs, however (Pillai and Prakash, 1993; Saha et al., 1992). Subsequently, two broad ranges of antibacterial agents, the quinolones and fluoroquinolones, have been regarded as the drugs of choice in treatment, owing to resistance that appeared during the 1980s (Hassing et al., 2013, 2011). These antimicrobial agents are highly active,
particularly against Enterobacteriaceae (Satish et al., 2012; Ronald and Low, 2003; Robert et al., 2001). However, the efficacy of antimicrobial therapy has been substantially affected by an increase in the number of quinolone-resistant salmonella isolates (Piddock et al., 2010; Soto et al., 2003). The frequent use of fluoroquinolones, particularly ciprofloxacin, has led to the global emergence of nalidixic acid-resistant salmonella strains (WHO, 2012; Menezes et al., 2011; Mohanty et al., 2006). Most importantly, isolates with nalidixic acid resistance have reduced susceptibility to ciprofloxacin, resulting in increased treatment failure (Oteo et al., 2000). The global increase in the prevalence of *Salmonella* strains with resistance to nalidixic acid is a major health concern. Literature evidence indicates that quinolone resistance of *Salmonella* spp. is mostly attributed to mutations in the quinolone resistance-determining regions (QRDR) of the target enzymes DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE) (Giraud et al., 2006; Velge et al., 2005; Piddock, 2002; Cloeckaert and Chaslus-Dancla, 2001). In particular, for DNA gyrase, a single mutation resulting in amino acid substitution at position 83 (Ser to Phe or to Tyr) or at position 87 (Asp to Asn or Gly) has been most frequently reported in nalidixic acid resistance isolates (Baucheron et al., 2014). The evidence also indicates that the S83F mutation occurs most frequently and is observed to result in high nalidixic acid resistance (Zheng et al., 2009; Cloeckaert and Chaslus-Dancla, 2001).

![Fig. 1.1 Gyrase Mechanism of Action](image)

It has been reported that binding of nalidixic acid to the gyrase/topoisomerase IV-DNA complex inhibits DNA replication. This action is responsible for the bacteriostatic and bactericidal property of quinolones, in particular nalidixic acid. Mutations in the DNA gyrase and topoisomerase IV may confer resistance
to nalidixic acid, and it has been shown that altered structures of these enzymes prevent binding of quinolones (Michael et al., 2006; Ruiz, 2003). Lamentably, the mechanism of resistance to nalidixic acid at the molecular level is not completely understood.

Therefore, new and more effective derivatives for the treatment of gastroenteritis are urgently required. Development of efficient drugs via the conventional drug discovery process is challenging in that it requires a high level of resource consumption and is a lengthy and risky venture (Paul et al., 2010). In light of these challenges, the repurposing or identification of novel indications for existing drugs is a viable strategy for drug development. Most importantly, this approach utilizes a bioinformatics and system biology approach to directly compare host responses to drug and pathogen (Law et al., 2013). There are many good examples of drugs for which new indications were identified serendipitously (Ashburn and Thor, 2004); thalidomide is a prime example. It was originally developed for the treatment of morning sickness during pregnancy but caused an epidemic of severe birth defects in children exposed to the drug in utero. It was subsequently withdrawn from the market but was later accidentally found to be uniquely effective in treating severe complications of leprosy and multiple myeloma. It is now marketed for this use under the trade name Thalomid. Furthermore, bupropion was originally developed to treat depression but was also found to be effective in smoking cessation (Boguski et al., 2009). Recently, much literature has highlighted the success of drug repurposing strategies (Gibson et al., 2015; Gold et al., 2012). For instance, discovery of antibiotic properties from the existing anticancer, antifungal, anthelmintic, and anti-inflammatory drugs was also reported in recent literature. Of note, 5-Florouracil, an anticancer drug, shows significant inhibitory activity against several Gram-negative and Gram-positive bacteria. Ciclopirox, an antifungal drug, inhibits the lipopolysaccharide (LPS) coat of Gram-negative bacteria (Brown, 2015; Rangel-Vega et al., 2015).

Despite recent advances in in vitro and in vivo repurposing, these approaches are considerably less feasible when screening large libraries (Veljkovic et al., 2015). Conversely, computational drug repurposing can be extremely helpful in screening large libraries in a shorter timeframe (Jin and Wong, 2014). In particular, target-based
drug repurposing approach is helpful in screening large library of compounds within a few days. Additionally, the information hidden in gene expression profiles was also exploited to capture similarity in drug mode of action. This enables the screening of 1309 drugs efficiently within a few days against *S. typhimurium*. Most importantly, the successful discovery of a potential drug with target-based approach is much higher than blinded screening method (Jin et al., 2012; Swamidass, 2011). In view of this background information, the present study was undertaken to emphasize on molecular level understanding of the characteristics of nalidixic acid resistance due to single mutation S83F, D87N, D87Y and A119S and double mutation S83F and D87G by employing various computational techniques. Alongside, virtual screening was performed to identify DNA gyrase inhibitors. Subsequently, the screened candidate was experimentally validated for its antibacterial activity. Hopefully, the results obtained in our study are certainly helpful not only for understanding the mechanism of drug resistance but also to aid in the design of potential inhibitor especially for the treatment drug-resistant *S. typhimurium* strains.
Objectives of Our Study

- Molecular level understanding of resistance to nalidixic acid in *Salmonella Enteric* serovar typhimurium associates with the S83G, S83F, S83Y, D87G, D87N, D87Y, A119S sequence types.
- Identification of potential therapeutics to conquer drug resistance in *Salmonella* Typhimurium: Drug repurposing strategy using DrugBank and Mantra 2.0 databases.
- Computer assisted virtual screening with help of Asinex database to find potent lead molecule to overcome drug resistance: Ligand based pharmacophore screening.

**Fig. 1.2** Work Plan of the study