Journal of King Saud University – Science 34 (2022) 102275

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Journal of King Saud University – Science

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Review

Antibiotic resistance in *Salmonella:* Targeting multidrug resistance by understanding efflux pumps, regulators and the inhibitors

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ARTICLE INFO

Article history:
Received 11 December 2021
Revised 6 May 2022
Accepted 7 August 2022
Available online 10 August 2022

Keywords:
Antibiotic resistance
Efflux pump
Inhibitors
Regulators
Salmonella

ABSTRACT

An increased number of research articles in terms of infectious disease and multidrug resistance challenges reveals that antibiotic resistance is becoming a serious health issue. Multidrug resistance in *Salmonella* is acquired through efflux pumps extruding the antibiotics from the bacterial cells. Efflux pumps in bacteria help in developing resistance and tolerating antibiotics. A collective effort of efflux pumps, regulators, and other cellular components helps in multidrug resistance, pathogenicity, biofilm formation, etc. As a result, efflux pumps have become an important drug target for novel drugs and their inhibitors may help to fight the resistance to existing antibiotics. Efflux pumps recognize the substrates, and prevent their accumulation inside the cells, thus interrupting their mode of action. Drug resistance in *Salmonella* is regulated by the expression of transcriptional regulator families. In this review, the different efflux pumps, their regulators, and inhibitors are summarized to target the antibiotic resistance pattern of *Salmonella*. In addition, a special focus is made to elucidate the roles of *Salmonella* efflux pumps in virulence and biofilm formation. The detailed study of efflux pump inhibitors may also help to explore and identify novel antibiotic components or additives that enhance the drug efficacy in multidrug-resistant strains.

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https://doi.org/10.1016/j.jksus.2022.102275

1. Introduction

Salmonella is the main cause of diarrhea annually 550 million humans are infected, by 2020 million are under the age of 5. Many cases are deadly and life-threatening. The increase in antibiotic resistance to *Salmonella* has become a serious threat leading to severe problems in healthcare. According to WHO, pathogens, for new antibiotics are urgently needed. One of the highest attention needed pathogens is *Salmonella,* which is resistant to fluoroquinolone (Table 1). Recently, a report has shown that tetra and penta-resistance are found in *Salmonella* (Xiang et al., 2020). The development of resistance from multidrug (MDR), and extensive resistance (XDR) leading to pan drug resistance (PDR) may cause a post-antibiotic era in which bacterial infections cannot be controlled by antibiotics. Generally, pathogenic bacteria have mechanisms to face many risks surrounding them, the most important

Table 1
A list of pathogenic bacteria for which new antibiotics are urgently needed (World Health Organization, 2017).

Priority 1: CRITICAL	• *Acinetobacter baumannii*, carbapenem-resistant • *Pseudomonas aeruginosa*, carbapenem-resistant • *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing
Priority 2: HIGH	• *Enterococcus faecium*, vancomycin-resistant • *Staphylococcus aureus*, methicillin-resistant, vancomycin-intermediate and resistant • *Helicobacter pylori*, clarithromycin-resistant • *Campylobacter* spp., fluoroquinolone-resistant • *Salmonellae*, fluoroquinolone-resistant • *Neisseria gonorrhoeae*, cephalosporin-resistant, fluoroquinolone-resistant
Priority 3: MEDIUM	• *Acinetobacter baumannii*, carbapenem-resistant • *Pseudomonas aeruginosa*, carbapenem-resistant • *Enterobacteriaceae*, carbapenem-resistant, ESBL-producing

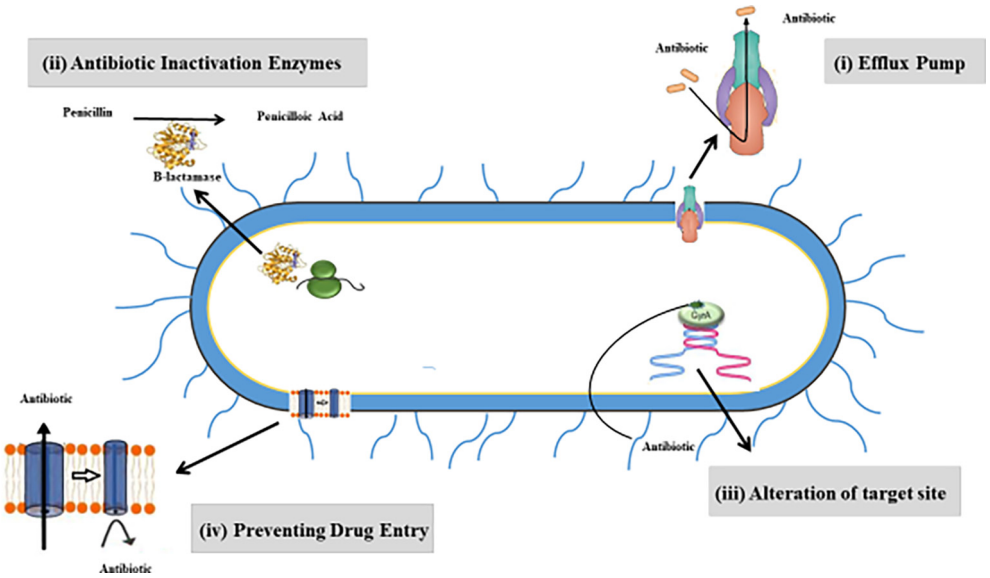

Fig. 1. Schematic diagram highlighting the main antimicrobial resistance mechanisms in pathogenic bacteria which include: i) drug efflux systems, which expel the antimicrobials outside the bacterial cell and leading to reducing their effectiveness to non-toxic levels. ii) Antibiotic inactivation enzymes, which modify or destroy the structure of antibiotics. iii) Alteration of target site, which usually occurs in the cell envelope via spontaneous mutation by chemical modification of their molecular targets. iv) Preventing drug entry through modifying the frequency, size, and selectivity of porin channels, which found in the bacterial envelope and plays a crucial role of antibiotic entry into the bacterial cell.

of which are antibiotics. These mechanisms include (i) efflux pumps, which remove the drugs from bacterial cells to the outside and causing losing their effectiveness by lowering their concentration to non-toxic levels. (ii) Antibiotic inactivation by bacterial enzymes that modify or destroy antibiotics structure. (iii) Alteration of target site via spontaneous mutation, which usually occurs in the cell envelope by modifying the chemical structure of their molecular targets. (vi) Preventing drug entry through modifying the frequency, size, and selectivity of porin channels, which are found in the bacterial envelope (Fig. 1) (Abdi et al., 2020).

The cell wall of bacteria is a complex multilayered structure that possesses complex proteins, and efflux pumps, spanning the inner membrane or cytoplasmic membrane and the outer membrane partitioned by periplasmic space. Therefore, the bacterial cell wall plays a major role in antibiotic resistance in pathogenic bacteria by preventing antibiotics and other toxic compounds, from entering the bacterial cell (Fisher and Mobashery, 2020). One of the main properties of efflux pumps is the recognition and extrusion variety of structurally diverse substrates outside the bacterial cell. This may lead to preventing the intracellular accumulation of these substances inside bacterial cells and leading to reducing their concentrations to non-toxic levels. In addition, efflux pumps prevent these substrates from reaching their sites of action inside the bacterial cell (Kornelsen and Kumar, 2021). However, the efflux pump and outer membrane barrier in bacteria are nonspecific defense mechanisms and inhibition studies in efflux pumps have proved to decrease resistance development in bacteria. The drugs enter the bacterial cell through the outer membrane via porin channels or lipopolysaccharide and reach the cytoplasm then the efflux pump expels out the harmful chemicals from the periplasm to the medium. Generally, bacteria possess five families of bacterial efflux pumps, which, are well-defined via biochemical, microbiological, and structural studies. These efflux pumps include ATP binding cassette (ABC) family, multidrug and toxic compound exporters (MATE), small multidrug resistance (SMR), resistance nodulation division proteins (RND), and major facilitator superfamily (MFS) were characterized by sequence homology (Pagès et al., 2005).

2. Pathogenicity of *Salmonella*

Salmonella (Enterobacteriaceae) Gram-negative rods, facultative anaerobe associated with foodborne illness, causing Salmonellosis (Jajere, 2019). Two broader species namely *Salmonella enterica* and *S. bongori* has been described and more than 2600 serovars belonging to the former species are reported as animal and human pathogen. *Salmonella enterica* serovar Typhimurium causes food poisoning known to be the second-largest cause of bacterial diarrhea (Sutkuviene et al., 2013). Multidrug-resistant *Salmonella* infections lead to a twofold higher mortality rate than normal strains responsible for 1–4% worldwide. Upon entry to the host body, *Salmonella* raises the acid tolerance response (ATR) to tolerate low pH in the stomach as well as the internal pH homeostasis of the bacterial cell. *Salmonella enterica* as an intracellular pathogen enters the M cells to cause gastrointestinal diseases or systemic diseases by crossing epithelial cells with specialized antigen sampling cells (M cells) depending on the strains or serovars (Buckley et al., 2006). The host cells undergo cytoskeletal changes that help in the entry of *Salmonella*. The capability to enter the host cells and cause infections are mediated by *Salmonella* Pathogenicity Islands (SPI) in specific virulent phenotypes. These genes are attained through horizontal gene transfer (Zha et al., 2019). The entry is mediated by the genes present in *Salmonella* Pathogenicity Island (SPI-1 contains at least 29 genes), by infecting the macrophages, stimulating mucosal inflammation, and disseminating from lymph nodes to phagocytes, spleen, and liver (Lou et al., 2019). Type secretion system (T3SS) that functions like a molecular syringe encoded by SPI1 and SPI2, helps in the effector protein secretion required for invasion of epithelial cells in the intestine that increases secretion and inflammation. The effector proteins help in translocating the proteins required for intracellular survival, multiplication, and causing infections (Srikanth et al., 2011). T3SS (Inv/Spa) helps in delivering proteins such as SopE, SipA, SipC, and StpP from *Salmonella* cytosol into host cells that help in cytoskeletal reshuffling (Groisman and Mouslim, 2000). Further, for the survival and growth of *Salmonella* SPI-2, 3 and 4 are required. During the enteric phase of disease inflammation and chloride secretion are mediated by SPI-5 (Marcus et al., 2000). Chronic infections of the gallbladder are caused by *Salmonella* that invades the liver or from the biliary tract. Further, it forms biofilms in gallstones and causes reinfection of the gastrointestinal tract, and transmits through the fecal route to new hosts (Crawford et al., 2010). Persistent *Salmonella* infection may cause irritable bowel syndrome and cancer by impairing the mucosal barrier, T3activation leading to host immune disorders and unbalancing the microbial community (Zha et al., 2019).

3. Multi-drug resistance in *Salmonella*

Fluoroquinolones were included from 1980 in Europe and further in 1995 in the USA for the treatment of salmonellosis in veterinary medicine (Bager and Helmuth, 2001). The resistance development in *Salmonella* had been reported by chromosomal mutation and exclusively by epidemic dissemination of mutant strain by the clonal spread. In recent years, ceftriaxone and azithromycin became the secondary drug of choice for treating typhoid due to the emergence of fluoroquinolone-resistant strains (Klemm et al., 2018). For animals and poultry, antibiotics are incorporated into feeds and water to control infections. The feeding rate varies and is subjected to under-dosing of drugs leading to an increase of selective pressure on *Salmonella* strains and inducing the development of resistant strains. Further, even for clinical Salmonellosis, these drugs such as fluoroquinolones are not widely used for treatment again unintentionally force *Salmonella* to acquire resistance (Dekker et al., 2019). In contrast, Salmonellosis in human are treated with fluoroquinolones, and the resistance is acquired by the mutation in the quinolone determining region (QRDR) of *gyr*A, *gyr*B, *par*C, and *par*E in chromosomal DNA. The plasmid-mediated quinolone resistance (PMQR) is coded by qnr determinants namely *qnr*A, *qnr*B, *qnr*C, *qnr*D, and *qnr*S and *aac (6′)-ib-cr* and quinolone extrusion *qep*A and *oqx*AB (Cui et al., 2019). Carbapenems and colistin are considered as the drug of choice for multidrug resistant *Salmonella*, however, carbapenemase and plasmid-mediated colistin resistance *mcr-1- Salmonella* emerged as a threat to these drug options (Liu et al., 2016). Recently developed multidrug resistant in *Salmonella* Typhimurium may cause life-threatening diseases and cannot be treated with common antibiotics. Salmonella entrica serovar Typhimurium DT104 showed multidrug resistance to ampicillin, chloramphenicol, florfenicol, streptomycin, sulfonamides, and tetracycline by the genes (43 kb) present in *Salmonella* genomic island I when compared with the *Salmonella enterica* serovar Typhimurium LT2 (Baucheron et al., 2004). Moreover, MDR strains of *Salmonella* have shown resistance to colistin, carbapenem, and tigecycline (Dawan and Ahn, 2020). New therapeutic strategies such as targeting the efflux pump and other resistance mechanisms are needed to fight against the developing multidrug resistance in *Salmonella*. Sequential antibiotic therapy and antibiotic rotation that reduces side effects have gained attention in past years. However, these approaches may favor the cross resistance to different groups of

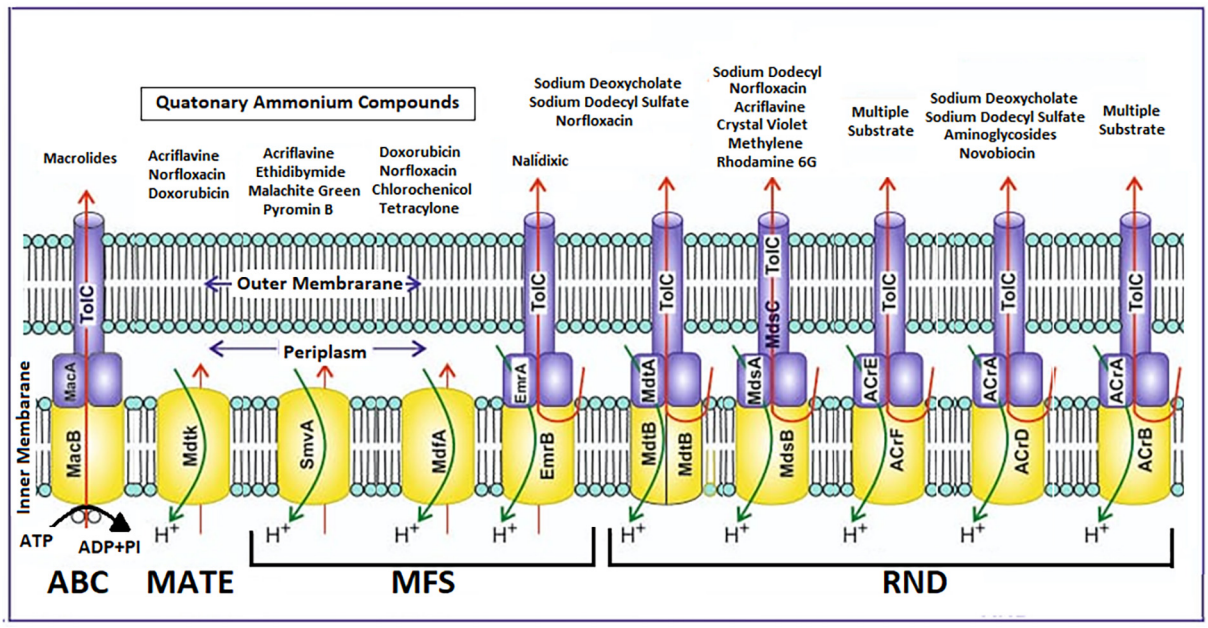

Fig. 2. Schematic diagram describing representative structures of the main efflux pump systems of *Salmonella* including transporters, efflux pumps, and substrates. These transporters are the adenosine triphosphate (ATP)-binding cassette (ABC) superfamily, the multi-drug and toxic compound extrusion (MATE) family, the major facilitator superfamily (MFS) family, and the resistance nodulation-cell division (RND) family. All these transporters use electrochemical gradients to drive their transport processes, except the ABC transporters, which utilize ATP to drive substrate transport processes.

antibiotics (Vestergaard et al., 2016). The cross resistance evaluation in different strains of *Salmonella* Typhimurium under sequential antibiotic treatment showed that effective therapeutic strategies, appropriate selection, and antibiotic therapy helps in preventing cross resistance. (Dawan and Ahn, 2020).

4. Efflux system in *Salmonella*

The efflux pumps porins and the active role is documented. The multiple target gene mutation in *Salmonella* contributed largely to the multiple drug resistance by activation of efflux pumps. Numerous efflux pump encoding genes were identified in *Salmonella* sp. Ten or more genes in chromosomal and plasmid DNA encoding for efflux pump were identified (Nishino, 2016). The drug-resistant genes responsible for drug transportation and the functioning of efflux pumps have been documented. Most of these transporters have a wide substrate range to withstand a large set of antibiotics within the small genome of bacterial cells (Nishino et al., 2007). Additionally, it helps in quorum sensing regulated virulence (Szemeredi et al., 2020) biofilm formation, colonization, cell invasion, adhesion, and heavy metal resistance (Ebbensgaard et al., 2020). ArcAB is an important efflux transporter and OmpF porin is considered a major influx that selectively allows antibiotics inside the cells (Piddock, 2019). Efflux pump AcrAB-ToIc is overexpressed during the exposure to antimicrobial agents by using transcriptional activators such as RamA, MarA, SoxRS, and Rob. In the absence of RamA and presence of soxR transcriptional activators *Salmonella* can respond to the selective antibiotic pressure and develop mutation (Grimsey et al., 2020). This overexpression may lead to mutations showing multidrug resistance. However, *Salmonella* susceptible to antibiotics and chemical agents were reported to have defects or mutations in the ArcAb operon (Alav et al., 2020). The amino acid residue substation in the AcrB drug-binding pocket formed an alternate substrate binding specificity and increased the susceptibility to other antibiotics (Blair et al., 2015). The mutations in local repressor genes, global regulatory gene and transport gene promotor region, and upstream insertion elements in transporter genes are found responsible for the overexpression of efflux pumps (Piddock et al., 2010).

The drug efflux system in *Salmonella* consists of ten pumps (Fig. 2) belonging to four different exporter families. ToIc is the major outer membrane channel helping in the extrusion of toxic antibiotics from the cells. Several research has been done to identify the expression of these pumps and their physiological properties in multidrug resistant strains of *Salmonella*. There are some more proteins belonging major facilitator superfamily (MFS) that help in the extrusion of solutes and other toxic components from the cells in which the proper physiological properties are well studied (Kumar et al., 2020). Therefore, detailed research is needed to reveal the contribution of pumps in developing multidrug resistance.

4.1. Multidrug and toxic compound exporters (MATE)

This is a secondary transport system that utilizes an electrochemical gradient of cations for drug transportation. MdtK is the MATE type efflux pump in *Salmonella* Typhimurium, and it is substrate-specific and pumps out the antimicrobial drug such as aminoglycosides, fluoroquinolones, cation drugs from the cells (Nishino et al., 2009). It can transport drugs with unrelated chemical structures and is called multidrug exporters (Kuroda and Tsuchiya, 2009). It has 12 transmembrane proteins and the protein structure remains similar to MSF, however, there are differences in the aminoacid sequences (Farhat et al., 2020). It utilizes sodium gradients to eject the toxic compounds from the cells.

4.2. ATP binding cassette (ABC)

ABC family are the transporters responsible for the uptake and efflux of components by the cells. Nucleotide-binding and transmembrane components of different or sometimes the same proteins form this domain. The permeases with six transmembrane regions are connected as dimers in the cytoplasmic membrane from the pores for transport (Nishino et al., 2009).

5. Resistance nodulation division proteins (RND)

In *Salmonella* RND proteins are located in the inner membrane and with the help of other proteins and outer membrane channel plays a major role in the drug efflux system with the tripartite system (AcrAB-ToIC). It transports substrates such as antibiotics, dyes, detergent and host metabolites. The substrates for AcrAB-ToIC are acriflavine and ethidium bromide is captured by AcrAB and extruded across the outer membrane through the ToIC channel. Even this RND helps in the virulence of *Salmonella*, and the virulence is affected if some components of AcrAB-ToIC are altered. The absence of RND protein components makes *Salmonella* susceptible to drugs and overexpression of these genes leads to multidrug resistance (Higgins et al., 2020). Therefore the studies on these proteins are gaining importance to fight the multidrug resistance and decrease the virulence of *Salmonella* (Blair and Piddock, 2009).

6. Major facilitator superfamily (MFS)

This comprises membrane proteins that are involved in symport, antiport, or uniport of various substrates and solutes such as sugars, ions, drugs, and metabolites by using proton motive force or electrochemical gradient. Further, the superfamily consists of two families DHA12 and DHA14 with 12 and 14 transmembrane proteins (Li and Nikaido, 2004). These transporters are considered a largest group among the exporter families found in bacteria. It transports H+/drug or solutes from the cell by using proton motive force (PMF) as an energy source (Andersen et al., 2015). EmrAB is an important pump in *Salmonella* for developing drug resistance. EmrA, is a membrane fusion protein that transports the substrates across the inner and outer membrane. This pump has been reported to develop resistance to triclosan, novobiocin, sodium deoxycholate, and nalidixic acid (Rensch et al., 2014). MdfA is another pump in the MFS family conferring resistance to tetracycline, norfloxacin, and doxorubicin (Yamasaki et al., 2016). This pump also helps in the alkaline adaptation by transporting Na^+ or K^+/H^+ in addition to drug/H+ transport (Lewinson et al., 2004). Some genes responsible for resistance in *Salmonella* are encoded in the integrin sites of SGI-1. The *floR* (gene for *chloramphenicol*/florfenicol resistance), *tetG* (tetracycline resistance), and *qnr* (fluoroquinolone resistance) belong to the MF superfamily (Braibant et al., 2005). For tetracycline resistance TetA, TetB and TetG are responsible for *Salmonella* and belong to the family DHA12. This also confers tigecycline resistance (Akiyama et al., 2013). TetA gene is also considered a supplementary gene to the *ramR* that regulates the ramA responsible for multidrug resistance in *Salmonella* (Tawyabur et al., 2020).

7. Regulators modulating the resistance

Utilizing multiple transporters for multidrug resistance by the bacterial cell depends upon the regulation of the transporter expression. Multidrug transporter is expressed under a specific and rich transcriptional control. Multiple transcriptional regulators repress or activate the genes for controlling the expression of AcrAB-ToIC and MDR pumps in *Salmonella* (Nikaido et al., 2011). Cellular activity, growth factors, and environmental conditions may transform the expression of these regulators. A list of regulators is given in Table 2. Efflux pump inhibition methods such as dissipation of proton motive force, chemical inhibition, or deletion of efflux pump are activated by RamA, which increases the expression of *acrAB* (Lawler et al., 2013). Thereby cells may increase the efflux pump activity that compensates for low activity by using the expression of regulators to overcome the inhibitors (Marshall et al., 2020). Even for expression of AcrAB efflux pump multilevel regulations are needed, in which AcrR is essential with the help of other regulators such as SoxS, Rob, and MarA. Mutations in acrR overexpress the acrAB in *Salmonella* lead to multidrug resistance For transcriptional activators marA and soxS expression acrB, acrF and acrD are jointly regulated. Integration of IS1 or IS 10 elements in the acrEF operon in the upstream region increases the acrEF expression (Olliver et al., 2005). Earlier acrAB acrD and acrEF genes were known to regulate multidrug transport. Later positive regulation of multidrug efflux pump by mdtABC and arcD genes with the help of BaeR signal transduction system was identified (Nishino et al., 2007).

The resistance of *Salmonella* is regulated by transcriptional regulator families. For example TetR for tetracycline, AraC/Xyls that regulate both transcriptional activators and repressors, signal transduction systems (PhoPQ), mer-R for mercury resistance, Mar-R regulator for multiple drug resistance, LysR-type regulators (LeuO) and histone-like protein regulators involved in repression of new genes that are responsible for antibiotic resistance (Shaheen et al., 2020). Nonantibiotic compounds such as dephostatin help in reducing both virulence and Polymyxin resistance by disrupting the signaling of SsrA-SsrB and PmrB-PmrA two-component regulatory system and enhancing the susceptibility to colistin (Tsai et al., 2020). The EmrAB genes responsible for the functioning of a pump in the major facilitator family is regulated by EmrR (Andersen et al., 2015). The MFS efflux pump genes such as tetA, tetB, and tetG are controlled by the TetR (Colclough et al., 2019). The expression of AcrAB-ToIC MDR efflux pumps is regulated by multiple transcriptional regulators by repressing or activating the promotor of efflux pump genes based on environmental or cellular activity. The transcriptional activator RamA, which increases the acrAB expression is sensitive to inhibition of efflux activity by deletion of genes or using chemicals, or by indulging proton motive force (Nikaido et al., 2011). This may be the response to inhibitors to increase the action of the pump to pump the chemicals from the cells.

8. Virulence and efflux pumps

The efflux pumps present in bacteria are not only helping in extruding the drugs from the cells but also helps in maintaining the physiological activities of the cells including virulence (Blanco et al., 2016). In *Salmonella* efflux pumps are essential to overcome the lethal effects of bile salts and host defense

Table 2
Transcriptional regulators and its functions studied in *Salmonella*.

Transcriptional regulator	Regulation	Reference
TetR	Tetracycline resistance	Miruka et al., 2011
AraC/Xyls	Regulates both transcriptional activators and repressors	Shaheen et al., 2020
PhoPQ	Signal transduction systems	Dalebroux and Miller, 2014
Mer-R	Mercury resistance	Brown et al., 2003
Mar-R regulator	Multiple drug resistance	Hünnefeld et al., 2019
LeuO	LysR-type regulators	Dillon et al., 2012

mechanisms (Pasqua et al., 2019). Mutant strains of *Salmonella* lacking one or more efflux pumps are unable to adapt to the host environment with bile resistance (Urdaneta and Casadesús, 2018). In a recent study the genomes of African iNTS isolates of *Salmonella enterica* serovar Typhimurium, were analyzed and found the multilateral pump MacAB with TolC, which is upregulated during macrophage infection. This also helps *Salmonella* to colonize in the gut and survive in presence of antimicrobial peptides. Earlier studies on the inactivation of acrB, acrAB, and acrEF resulted in attenuation of *Salmonella* Typhimurium (Blair et al., 2015). The compounds that are inhibiting the efflux pump not only favor an alternative approach to multidrug resistance treatment, but also opens helps in anti-virulence and anti-biofilm that offers clinical benefit.

9. Role of efflux pump in *Salmonella* in biofilm formation

Biofilms are also formed with the help of an efflux pump. It helps in transport of extra polymeric substances and quorum quenching molecules supportive to make biofilms. It regulates the genes responsible for biofilm formation and extrudes metabolic intermediates, harmful chemicals, and antibiotic molecules. Further, it stimulates the aggregation of cells in the formation of biofilms. Biofilm is one of the major factor contributing toward the tolerance and resistance to antibiotic resistance by directly extruding the antibiotics or indirectly forming the biofilm (Reza et al., 2019). Inhibition of the efflux system by using efflux pump inhibitors and interrupting the biofilm formation may reverse the antibiotic resistance in bacteria. It potentiates antibiotic efficiency. *Salmonella* forms biofilms on abiotic and biotic surfaces including gall stones and epithelial cells and causes reinfection. These biofilms formed by *Salmonella* on food products are one of the major causes of food borne illness. It is more difficult to treat these biofilms due to their high tolerance to antibiotics than the planktonic cells. The MIC and MBC values of the antibiotics become 100–1000 higher due to biofilm formation and endure in host making the treatment difficult. Biofilm formation are controlled by many factors including genes *csqD*, *adrA,* and *bapA* for matrix formation and adhesion (Gonzalez-Escobedo and Gunn, 2013). Efflux pumps in *Salmonella* plays an important role in formation of biofilm. The biofilm ability as decreased after the deletion of efflux pumps including acrD, acrEF, emrAB, macAB, mdfA, mdsABC, mdtBC, mdtK and tolC from *Salmonella* enterica serovars (Baugh et al., 2012). AcrD transports the regulatory molecules controlling the genes in biofilm formation. The RND acrD is also involved in transcriptomic variations according to the habitat associated with environmental sensing, stress tolerance, pathogenicity and metabolic pathways. MacAB-TolC has been reported to involve in efflux of protoporphyrin, an intermediate biomolecule that chelates transition metals to form metallophorphyrins for various biological functions (Turlin et al., 2014). It confirms the functional role of efflux pumps in biofilm formation. MdsABC also helps in *Salmonella* to defend chemicals, to overcome oxidative stress and macrophage mediated killing that prevents biofilm formation. The over expression of MdsABC led to increase in 1-palmitoyl-2-stearoyl-phosphatidylser ine (PSPS) that helps in host cell death and survival of *Salmonella* in phagocytic cells (Song et al., 2015). MATE superfamily MdtK extrudes antibiotics and some antimicrobial peptide components from PhoP/PhoQ system which possess antimicrobial activity. Dipeptides efflux function was also reported in *Salmonella* (Kapach et al., 2020). MdtK is 90% similar in both *Salmonella* and *E.coli* and found in all Enterobacteriaceae groups (Nishino et al., 2006). MdtK pump may play an important role in the efflux of dipeptides in cells of *Salmonella* by preventing the accumulation of antimicrobial dipeptides through quorum sensing. The formation of curli, the cell surface protein responsible for matrix forma-

Table 3
Efflux pump inhibitors in *Salmonella.*

Compound	Target efflux pump	Substrate	Reference
Compound (OU33858)	MacAB	Macrolids	Yamagishi et al., 2020
Trimethoprim	AcrAB-TolC	Ciprofloxacin	Piddock et al., 2010
Cathinone			
Carbonyl cyanide m chlorophenylhydrazone, norepinephrine and trimethoprim	AcrAB-TolC	Ciprofloxacin	Rushdy et al., 2013
Tetraphenylphosphonium and ethidium cations	AcrAB-TolC	Polymyxin B and Gramicidin D	Sutkuviene et al., 2013
Phe-Arg-β-naphtylamide	AcrAB-TolC OqxAB	Tigecycline	Chen et al., 2017
Polyaminoisoprenyl derivatives	AcrAB-TolC	Erythromycin Doxycycline Nalidixic acid Chloramphenicol	Lieutaud et al., 2020
Hexadecanoic acid Ethyl ester Cyclopropane 1-(1-hydroxy-1-heptyl) 2-methylene-3- pentyl from *Artemisia tournefortiana*	AcrAB-TolC	Ciprofloxacin	Khosravani et al., 2020
Chlorpromazine	AcrAB-TolC	Chloramphenicol, tetracycline, nalidixic acid and triclosan	Bailey et al., 2008
Trans-cinnamaldehyde, β- resorcyclic acid carvacrol thymol eugenol		Ampicillin Chloramphenicol Streptomycin Sulfamethoxazole Tetracycline	Johny et al., 2010
Pyranopyridine	AcrAB-TolC	Fluoroquinolones, B-lactams Chloramphenicol Minocycline Erythromycin Linezolid	(Aron and Opperman, 2016

tion in biofilm is also exaggerated in efflux mutants, where the transport of activators and regulators for *csgB* and *csgD* curli genes were affected (Barnhart and Chapman, 2006). The four families of efflux pumps reported in *Salmonella* are important in biofilm formation. However, RND family is predominant and molecules on quorum sensing in *Salmonella* were not reported.

10. Inhibitors of efflux pumps

Exploring the efflux pump inhibitors helps to overcome the problem of multidrug resistance in bacteria. The outer membrane (OM) permeability barrier with the help of an efflux pump is the non-specific protection mechanism that increases antibiotic resistance. Studies on components inhibiting the efflux mechanism can increase the antibiotic susceptibility thereby preventing the development of resistance to antibiotics (Table 3). Novel approaches for identifying the natural components and inhibitors of efflux pump allow the antibiotics to accumulate inside the bacterial cell for effective treatment (Farhat et al., 2020). Several efflux pump inhibitors have been reported to date and the mechanism of these inhibitors are not completely understood. Some molecules act as a competitive to the substrates and export it and some depolarize the plasma membrane making it susceptible to the antibiotic. Further, the efflux pump inhibitors restore the activity of the drug inside the cells and inhibit the growth of multidrug resistant strains. The compounds or molecules help in inhibiting the efflux pump regulate the overexpression of the efflux pump help the antibiotic attach to the target receptors interrupt the assembly of efflux pumps, competitive or non-competitive inhibition of antibiotic binding, hindering the pores of efflux pumps and inhibiting the energy source of efflux pump activity (Aparna et al., 2014). Heterocyclic nitrogen containing compounds that could act as *EPI* were tested in *Salmonella* Typhimurium with an overexpressed AcrAB-ToIC efflux pump. The strains in which efflux pump acrAB inactivated were hyper-susceptible to antibiotics such as ciprofloxacin chloramphenicol, and erythromycin. There was a 2–4-fold decrease in the MIC values in presence of trimethoprim. Theobromine and norepinephrine separately tested with ciprofloxacin also showed synergetic activity in *Salmonella* strains (Piddock et al., 2010).

11. Conclusion

Multidrug efflux pump in *Salmonella* helps in antibiotic resistance leading to untreatable bacterial infections. Understanding the role of efflux pumps their regulators and inhibitors may help in identifying novel target and control resistance in *Salmonella*. Identifying the efflux inhibitor molecules that can enhance the effect of antibiotics may also restore the efficacy of drugs to fight against multidrug-resistant strains of *Salmonella*. Modern technologies and molecular approaches may help to discover drug targets and novel molecules that can fight against the development of antibiotic resistance and treat *Salmonella* infections.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

The author extends his appreciation to the Deanship of Research, Shaqra University, Kingdom of Saudi Arabia.

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