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Research Article

High Prevalence of Metal Resistant Proteins in *Salmonella enterica* Multi Drug Resistant Plasmids Correlates Severe Toxicities in Water with Higher Drug Resistance Typhoid

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Abstract

Antimicrobial Resistance (AMR) and drug void have caused huge panic today. Typhoid was a serious old disease and such disease was controlled by antibiotics so far. Recent outbreaks of multi-drug resistant (MDR) salmoniasis caused serious health hazard in human and animal demanding an update on molecular biology of the status on transferable genetic elements. R-plasmids combined in F'-plasmid and the new MDR conjugative plasmids were shown abundant in *Salmonella enterica* ranging 70 - 440 kb with similarities to *Escherichia coli* and *Klebsiella pneumoniae* plasmids. Antimicrobial agents such as Tetracycline, Penicillin, Sulpha-drug and Aminoglycoside resistant determinants were abundant in > 50 plasmids analyzed and metal resistant gene clusters are predominant in most large plasmids. Among the β-lactamases, *blaTEM* was predominant including few *blaOXA, blaNDM, blaAmpC* and *blaCTX-M* whereas *blaKPC* was not detected. Among the acetyltransferase all *catA1, aacA1* and *aac-1b-cr* genes were located in association with abundant streptomycin phosphotransferases (strAB) and rarely mcr-5/9 phosphoethanolamine-lipid A transferase. Altered isomeric dihydropteroate synthase (*Sul1/2/3*) were present giving sulfamethoxazole resistance and *dhfr* gene frequently associated giving trimethoprim resistance. Metal resistant gene clusters like *SilABC* (*CusABC*), *PcoAB*, *RcnA*, *terABC* and *MerABCXT* etc. were found in many *Salmonella enterica* plasmids. Toxin genes like *HipA* and virulence genes like *spvABD* were located increasing virulence and pathogenesis. Drug efflux genes like *tetA* or *tetB* or both was abundant and *OqxB, floR, CmlA* were frequent but *QepA* and *EamA* were rarely seen. Thus, *Salmonella* metal resistant genes combined with complex antibiotic resistant genes to combat toxicities in the gut to develop so many MDR conjugative plasmids causing AMR. Such acquisition spreads salmoniasis in the live stocks (pig, cow, chicken) where toxic soil and water dominate increasing chance of MDR typhoid in human. Analysis confirmed the mutations of the metal resistant genes as observed for thousand isomers of *mdr* genes increasing PAN drug resistant microbes.

Keywords: *MDR Typhoid; Metal Resistant Genes; Water Toxicity; Gut Microbiome*

Introduction

Before 1600s we have no idea that microorganisms cause diseases and we blame ghosts, Sun and Wind. After the discovery of microscope by Anton Van Leeuwenhoek (1670s) and further pioneering works by Edward Jenner (1790s), Lewis Pasteur (1860s) and David

Koch (1880s) proved that bacteria and viruses were the culprit of many diseases like TB, Cholera, Typhoid and pox. Edward Jenner (1789) has discovered the Pox vaccination and role of viruses in diseases where antibiotics are useless [1,2]. Now we control bacteria by inhibiting central dogma processes like replication, transcription and translation as well as cell wall biosynthesis [3]. In truth, basic chemical reactions of metabolism of DNA, RNA, protein, sugar and fat are same among all life forms but molecular assembly in human's 35 trillions cells are different. Thus, understanding the molecular assembly of biological processes is vital to design drugs against deadly pathogens. Biomolecules are nanometers and could be analyzed by assembly (10^7 - 10^{15} molecules) using suitable sensitive methods like UV detection of Ethidium bromide stained DNA/RNA, Ninhydrin colour reaction of amino acids, Antigen-Antibody reaction followed by Peroxidase enzyme-mediated colour reaction (ELISA) and FITC-Fluorescence Microscopy methods. Whereas atomic structures of organic molecules were determined by absorption spectra analysis like MASS, NMR, FT-IR and Raman Spectroscopy [4].

Typhoid Fever is serious disease due to systemic infection of bacterium *Salmonella enterica* subsp *enterica* serovar *typhi* and now disease has come back due to drug-resistance [5,6]. Typhoid could be spread by eating or drinking food or water contaminated with the feces of an infected person. Risk factors include poor sanitation and poor hygiene and those travelling in the developing world. Symptoms are high fever accompanied by weakness, abdominal pain, constipation, headaches and mild vomiting. In 2000, typhoid fever caused an estimated 21.7 million illnesses and 217,000 deaths usually in the children and young adults between 5 and 19 years old mostly from south-central, Southeast Asia and sub-Saharan Africa. Reports indicated about 161,000 deaths in 2013 and 149000 in 2015 (https://www.ecdc.europa.eu/en/publications-data/typhoid-and-paratyphoid-fever-annual-epidemiological-report-2015). In the United States, about 400 cases occur each year and 75% of these are acquired while travelling internationally. According to the most recent estimates by WHO, between 11 and 21 million cases and 128 000 to 161 000 typhoid-related deaths occur annually worldwide (https://www.who.int/immunization/diseases/typhoid/en/). A typhoid vaccine can prevent about 40 to 90% of cases during the first two years. An oral live attenuated Ty21a vaccine in capsule formulation for those over six years of age but injectable polysaccharide vaccine is also available. Diagnosis is by either culturing the bacteria or detecting their DNA in the blood, stool, or bone marrow using PCR technique. During prognosis serum AST and ALT may be very high (200 - 400 U/L). The disease was treated with antibiotics such as azithromycin, fluoroquinolones, or third-generation cephalosporins. *Salmonella enterica* plasmids harbour a composite transposon that can carry multiple resistance genes, including *bla*TEM-1 (ampicillin resistance), *dfrA7* (trimethoprim resistance), *sul1+sul2* (sulfamethoxazole resistance), *catA1* (chloramphenicol resistance), and *strAB* (streptomycin resistance) genes [7-9]. This composite transposon has also been found integrated into the chromosome in some H58 *S. typhi* lineages. Many drug resistant determinants are abundant in *Salmonella enterica* plasmids isolated from different animal sources as well as water and thus AMR is a problem increasing salmoniasis in animal and typhoid in human [10]. Typhoid fever, the causative agent of *Salmonella enterica* serovar Typhi is spreading in the Asian countries due to acquisition of MDR plasmids from multidrug resistant *Escherichia coli* and *Klebsiella pneumonia* [11]. However, non-typhoidal MDR *Salmonella enterica* Serovar Typhimurium were isolated in meat foods (chicken, pork and beef) as well as milk and egg, Such MDR bacteria cause serious diarrhoea and bacteraemia and need hospitalization as happening in the Asia as well as United States due to widespread contamination in live stocks [12,13]. Other than Serovar *typhimurium*, Serovar Kentucky, Serovar Idican and Serovar enteritidis are predominant. We also found few small plasmids with one or two *mdr* genes or virulence genes or metal resistant genes suggesting toxicities of different kind prevail first generating such plasmids but now such small plasmids combined with F'-plasmids and then such *Salmonella enterica* plasmids further recombined with metal resistant genes residing in the polluted water resources. Such metal resistant genes also found in *E. coli* and *K. pneumonia* MDR plasmids [14-17].

There are many *mdr* genes located in *Enterobacteriaceae* plasmid since 1950s as shown in figure 1 [7]. First, *amp* and *tet* genes were sequenced in pBR322 in 1965 and since the application of colour di-deoxy DNA sequencing, millions plasmid sequences were deposited in GenBank. Amp gene was renamed as *bla* or beta-lactamase and now 20 different beta-lactamases classes were reported with millions of mutated isomers and most importantly ESBL and MBL multiple isomers were located in MDR single conjugative plasmid with size > 100

kb [18,19]. Similarly, *tetAB, acrAB, mexAB/CD/EF, bcr, mcr* types MFS and RND drug efflux genes were reported in *E. coli*, *P. aeruginosa*, *K. pneumonia* as well as *S. enterica* plasmids. Many metal efflux genes (*silABC, merB, rcnA*) and metal binding genes (*telC, silz*) were reported in MDR plasmids. Abundance of metal resistant gene cluster in association of *mdr* genes suggested that metal toxicity in water might be precede the antibiotic toxicity. We will describe here the different types of those genes in *Salmonella* plasmids causing recent outbreaks of salmoniasis in animals and typhoid in human [14].

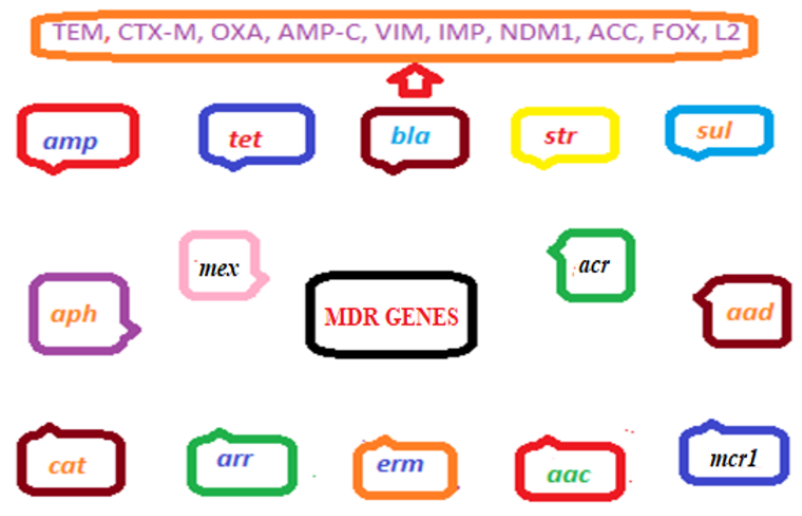

Figure 1: *Different types of mdr genes reported in Enterobacteriaceae plasmids.*

Materials and Methods

We got the plasmid sequences from NCBI Nucleotide GenBank Database by typing "*Salmonella*" and "plasmid". We retrieve sequence and searched for *mdr* genes, drug efflux genes, toxin genes and virulence genes [18-20]. BLAST search was performed to get relation among the peers (www.ncbi.nlm.nih.gov/blast). Plasmids were divided into small (3 - 15 kb), medium (15 - 49 kb), large (50 - 100 kb) and very large (> 100 kb) and plasmid may have *mdr* gene or no *mdr* gene but virulence genes. Similarly, most plasmids have both metal resistant gene and *mdr* genes but virulence genes have not detected in many plasmids Interestingly, such review was absent in pubmed [21].

Result

Table 1 showed the overall description of the plasmids describing *mdr* genes, drug efflux genes, metal resistant genes as well as other genes involved in the *Salmonella* pathogenesis. Few plasmids were found small < 50 kb and large plasmids were 200 - 400 kb in size and were sequenced between 2015 - 2019. Plasmids pHK0653, pSTm-A54650, pRH-1238, pHXY0908, P87912 and pF8475 contained nine *mdr* genes and few drug efflux genes. TetA drug efflux genes located in most plasmids irrespective of sizes as also we found strAB streptomycin phosphotransferases whereas BlaTEM was more frequent than *blaCTX-M, blaOXA1* or *blaNDM1* whereas *blaOXA23/48/58* were not detected. Sul1 isomer of dihydropteroate synthase was abundant than Sul2 and Sul3 isomers [22]. Metal resistant genes were found in many large MDR conjugative plasmids like p8025 (accession no. KP899803), pSTM6-275 (accession no. CP019647), pSH111_227 (accession no. JN983042) and pF8475 (accession no. KP899804). Mercury resistant locus was found in plasmid pF8475 (accession no.KP899804)

in association with sul1/2, *strA, blaTEM*, *dhfr*, *aacC2* and *mphA mdr* genes [23]. Plasmids pHK0653, pFORC19 and pSTM6-275 have *HipA* toxin genes whereas plasmid pSE81-1706 and pFORC19 have spvA/B/D virulence genes associated with *cat*, *blaTEM* and *aph mdr* genes. The plasmid pOU1113 (accession no. AY517905) has virulence genes *spvD/trbD* but no *mdr* gene where as in plasmid pSTU288-1 (accession no. CP004058), *spvA/spvB* virulence genes were located in association with *sul1, aad, aac3', dhfr* and *cmlA2 mdr* genes giving resistant to sulfamethoxazole, streptomycin, gentamycin, kanamycin, trimethoprim and chloramphenicol. However, plasmid pA3T (accession no. KX421096) has no beta-lactamase or acetyl/phosphotransferases but accumulated OqxA and OqxB RND-type drug efflux proteins which form tripartite protein complex with oprM-type membrane protein to exclude variety of drugs. The plasmid pSH696_135 (accession no. JN983048) has many transcription factors like *merD, tetR, entR* and *flhC* regulating *mdr* genes like *blaCMY, sul1, strA/B, blaTEM* and *aadA*. Silver efflux proteins *silABC* (ARX76242/3/5) were located in small *Salmonella* plasmid pSH-01 (43kb; accession no. KY486279) and such locus was also found in many large MDR plasmids like pYU39_IncA/C (156kb; accession no. CP011429) in association with multi-drug efflux protein acrEF (protein ids. AKH10329/30), a homologue of acrAB acridine drug efflux proteins and streptomycin inactivating proteins strAB (protein ids. AKH10232/3). The *Salmonella* 249 kb plasmids pHXY0908 (accession no. KM877269) and p15-0756 (accession no. CP039857) are similar size but pHXY0908 has multiple *mdr* genes (*aph, sul1, aad, sul2, aac3', aac6'-1b-cr, cat blaOXA, arr3*) with only tellurium resistant locus whereas the other has multiple metal resistant genes locus like copper resistant locus PcoECBA (nt. 68858-74109) and silver resistant locus silPABCRSE (nt. 75407-87859) as well as tellurium resistant locus terWZABCDF (nt. 24736-30955). Abundance of metal binding proteins and metal efflux proteins in *Salmonella* plasmids indicated that such MDR bacteria suffered in the environmental water and had forced to accumulate multiple metal resistant locus where as in the human host such bacteria may lost few metal resistant genes acquiring many antibiotic resistant genes [23,24]. A similar huge accumulation of silver, copper, mercury and tellurium resistant genes were seen in large plasmid pRH-R27 (accession no. LN555650) where very rare nickel-cobalt specific efflux protein rcnA was also present (protein id. CED95467) in association with *blaVIM, aac6'-1b, sul1* and *strABmdr* genes [24]. Plasmid pCFSA300-1 appeared very similar in plasmid pHK0653 with respect to *mdr* genes (*dhfr, sul, blaOXA1, aac3', ANT3")* but citrate lyase, Adenine-Guanine phosphoribosyl transferase and carbamoyl phosphate synthase were inserted at tellurium resistant locus. Interestingly, a very small 14 kb *Salmonella typhimurium* plasmid pMG101 (accession no. AF067954) had all silver resistant genes but no other antibiotic resistant genes indicating metal resistance was primitive and likely occurred during European industry development between 1760-1850s whereas *mdr* genes were created after 1940 [19,25]. We also found a medium plasmid pSA20044414 (accession no. CP030210) with many arsenic and copper resistant genes in association of Tra conjugative proteins but no *mdr* gene was detected indicating F'-plasmid may be combined with small metal resistant plasmids like pMG101 with silver resistant locus and then small R-plasmids like pSc101 and pMB were combined to originate modern day large MDR conjugative plasmids like p87912 (accession no. CP041180) which contained sixteen *mdr* genes and two drug efflux genes like oqxA/B. Nevertheless, WGS of *Salmonella* (accession nos. CP000026, AE014613) indicated that metal resistant locus were also frequently associated with *Salmonella* genome [26]. Mcr-9 enzyme (protein id. ANV19589) was detected in plasmid p09-036813-1A_261 (261 kb; accession no. CP016526) in association of *aph6-Id, aph3"*, *dhfr*, *aac3"*, *aacA4* and tellurium, mercury and arsenic metal resistant genes. Such mutant mcr-1 was detected in many *S. enterica* isolates giving colistin resistance and more deadly *blaNDM-1* also was detected giving imipenem resistance [27,28]. *Salmonella enterica* serovar Seftenberg pNDM-SAL plasmid (accession no. KP742988) has both cephamycinase and carbapenemase and thus highly resistant to all beta-lactams and similar *Salmonella* plasmids pHS36-NDM (accession no. KU726616) and pRH-1238 (accession no. KR091911) ware sequenced [29,30].

Beta-lactamases in *Salmonella* plasmids were analyzed and major isomer was *blaTEM-1* (protein ids. AYM49671, QDG23938, QCW01640, CDR86458, CEO37446 and QEX03237) and no mutations were found (Figure 2A). Similarly, no mutation was detected in streptomycin phosphotransferases *strA* (Figure 2B) and *strB* (Figure 2C). In plasmid pSTm-A54650 (accession no. LK056646) blaOXA-1 was found in association of *blaTEM-1* similar to *blaCTX-M-65* in plasmid p87912 (accession no. CP041180) and pUo-STmRV1 (accession no. CP018220). TetA tetracycline efflux and tetracycline binding protein tetM were located in plasmid p15-6756 (accession no.

Plasmid name	Size in kb	Accession number	Mdr genes	Drug and Metal Efflux genes	Virulence genes
pGMI14-002_1	444	CP028197	blaSHV12, aac6'-II, aac3-II, arr3, mcr1, dhfr, aph6-Id	merA, terCZ, rcnA	HipA
pIMP4-SEM1	340	KX810825	blaTEM, catB, dhfr. strBA	tetA, terFECBAZW, terYX	HipA
P8025	311	KP899803	aadA1, dhfr, sul1	tetA, acrAB, merA, arsB	-
pSTm-A54650	309	LK056646	dhfr, blaOXA, catB3, tuniR, blaCTX-M15, blaTEM, strAB, sul1, aadA1, catA1	tetA, qnrS1, pcoE, rcnA, arsB, merB	TniB dcm telA
pIncH12	300	LN794248	Sul2, strAB, blaTEM, dhfr, bla-OXA30, aadA1	terXYABDEF, rcnA, arsB	vwfAB, pvuIIM
pRH-R27	299	LN555650	Sul1, strAB, aac6'-1b, blaVIM1, aadA1	TerABC, arsB, rcnA, PcoS, silAB, merTC	Dcm, dam
pFSAN096147	291	CP044256	blaTEM, qnrS1, blaLAP2	tetA, tetB, terXBCEF, rcnA, sliCB, cusF, merT, Hg R, arsHB	HipA, dcm
P280_12888	276	CP045449	Aac3-IV, aph4-Ia, sul1, ANT3"-Ia, dhfr	terXWZABCDsilESCBAP, PcoAB, merT	dcm
pSTM6-275	275	CP019647	StrA, tet, blaTEM, aadA2, dhfr	EamA, TetA, Sil,Ter, PcoA	HipA
pSa27-Tc-CIP	270	MH884653	blaTEM1, tetC, dhfr, ble	silEABCRS	virB, dcm
p09-036813-1A_261	261	CP016526	Aph6'-1a, aph3", dhfr, aac3', aacA4, mcr1	terWZABCF MerA,merT arsBA, rcnA	hipA, dcm
pA3T	253	KX421096	Ble, sul1, fosA, blaCTX-M-14, aac3-IV	OqxB/A, terB	Dam, dcm
P15-0756	249	CP039857	lnuF, ANT3", tetM, tetA, EamA	terWZABCDF PcoECBA silPABCRSE	hipA. dcm
pHXY0908	249	KM877269	aph, sul1, aad, sul2, aac3', aac6'-1b-cr, cat blaOXA, arr3,	oqxB/A, cml, floR, terE/D, terC /Y/Z	HipA
pHK0653	245	KT334335	Dhfr, sul, aad, hph, aac, blaOXA1, cat, arr3	OqxB, CmlA2, terF	HipA, Collicin1b
pJXP9	244.7	MK673549	Dhfr, aph3", estX, aad, blaCTX-M14, fosA	floR, cmlA, terFEDCBA, $terZY_1XY_2$	dcm
pSE81-1706	244	CP018656	Cat, blaTEM, aph	tetA	spvA/B/D
P87912	236	CP041180	Acc6'-1b-cr, blaOXA/CTX-M/TEM1, catB3, arr3, sul1/2, fosA3, aph3"-1b, aph6-Id, ANT3"-I, dhfr, ble, aph4-I, mph2'	oqxAB	rmtB1
pSH111_227	227	JN983042	strA/B, sph	TetA, terF, cusC	Dam, dcm
P220k	220	CP025340	ANT3", mphA2', aac3-IV, aph4-Ia,	oqxAB, cmlA1 terZXABCDF, cusAP, PcoADS, floR	dam
pHCM1	218	AL513383	blaTEM, sul1, strA/B	tetA	mucB
pCFSA300-1	209	CP033382	Sul2, dhfr, aph3"-1b, blaOXA1, ANT3"-Ia	OqxAB, terXWterZABCDF	HipA,Dcm, ArmA
pF8475	210	KP899804	Sul1/2, strA, blaTEM, dhfr, aacC2, mphA	tetB, merA/C/P/T	Dcm, dam, trhU

pUo-STmRV1	197	CP018220	Aph3", aac3, ANT3", sul1, blaCTX-M	cmlA1, CuS/C, MerC/T, ArsH,	SpvA. SpvB/D
pB71	190	KP899806	aadA1, sul1	tetB	cobZ, dcm
pRH-1238	188	KR091911	mphA, sul1, aadA5, dgfr7, blaNDM1, blaCMY16, strAB, sul2	tetA, chrB	
pRH-1238	187	KR091911	Sul1/2, aad, dhfr, aac6', aph, mel, blaNDM, strB/A, blaCMY-16	tetA, floR, mel	Rhs1, vWFS
pYU39_IncA/C	156	CP011429	strA/B, sul2, ble	acrEF, silPACSER	Rhs, dcm
pSTU288-1	148	CP004058	Sul1, aad, aac, dhfr	CmlA	spvD, trbD
pNDM-SAL	146	KP742988	blaCMY4, blaNDM1	Dam, dcm	Vwf, rhs
pSH696_135	135	JN983048	Sul1, strA/B, blaCMY, blaTEM, aadA	floR, merA/D/T	-
P9134	134	KF705205	Hpt, blaTEM	tetA	pilQ
pSa18934b	133	JF274992	aph	tetA, merEA	spvABCD
pGDD25-16	130	MH316136	blaCTX-M-27, dhfr	QepA	Dcm, rmtB
R64	121	AP005147	strA/B	tetA, arsB	pilQ
pFORC19	117	CP012397	Dhfr, strA/B, aph, aac3', TunicaR	tetA, merA/C	HipA, spvA/B/D
pST1007-1B	109	MH626558	Dhfr, aad	tetB, cml, EmrE, merAT	-
pSH1148_107	107	JN983049	Sul1, aacC1, aadA1		Colicin1b
pSA20044414	93	CP030210	No mdr gene	ArsAB, PcoA, CusA	-
pSA20070548	84	CP040652	blaTEM, aadA2, ble	tetM, SilCB silA/P, merT/C	dam
pOU1113	80	AY517905	No mdr gene	-	spvAB
pQJDSal1	67	CP022964	Sul2, blaTEM1	arsABCH	virB
pSH-01	43	KY486279	QnrS1, tetA	silP/A/B/C/R/E, CusF/S	-

Table 1: *Plasmid profiles of Salmonella enterica with mdr genes and drug efflux genes.*

Note: BlaTEM is similar to amp gene of pBR322 and it lyses benzyl penicillin and ampicillin but not cefotaxime and oxacillin. TetA and TetB enzymes are ~400 aa transmembrane protein and remove tetracycline from bacterial cytoplasm giving tetracycline resistance. Such gene (tetC) was discovered first in plasmid pBR322. StrA and StrB phosphorylates streptomycin and phosphorylated streptomycin could not able to bind ribosome giving resistance. Other phosphotransferases (aph) are known to give gentamycin and kanamycin resistance. Cat enzyme acetylates chloramphenicol and acetylated chloramphenicol did not bind ribosome. AacC1 and aacA1 types acetyltransferases are abundant in plasmids causing aminoglycoside resistance. Hpt is hygromycin phosphotransferase and Arr3 is refampicin phosphotransferase. Dhfr enzyme gave resistance to trimethoprim and sul1/2/3 are altered dihydropteroate synthase enzyme giving sulphonamide resistance. spvB is Actin ADP ribosyl transferase and inactivates muscle function. Dcm is cytosine MTase and Dam is adenine methyltransferase whereas rmtB is 16S rRNA methyltransferase giving drug resistance altering rRNA structure in the ribosome. HipA is a serine-threonine protein kinase that likely phosphorylate stRNA (Glu) synthetase (GltX). CmlA is chloramphenicol efflux membrane protein and acrAB is RND-MFS drug efflux proteins and similar to OqxAB. Colicin resistance is due to a colcin1b transporter and colicin drug bind to cell membrane inhibiting mureib biosynthesis in bacteria. QepA drug efflux protein located in Salmonella plasmid pGDD25-16 gives fluroquinolone resistance as also possible for the presence of aac6'-1b-cr protein in plasmid pHXY0908 due to N-acetylation of ciprofloxacin.

CP039857) but no beta-lactamase gene was located but many metal resistant genes like *ter* locus, *sil* locus and *PcoECBA* copper resistant genes. In a pan drug resistant *S. enterica*, multiple adenyl transferases (protein ids. ALI92932, ALI92934) and acetyltransferases (protein

ids. ALI92932, ALI92954) and dihydropteroate synthases (ALI92929, ALI92944, ALI92959) were detected in its plasmid pHK0653 (accession no. KT334335).

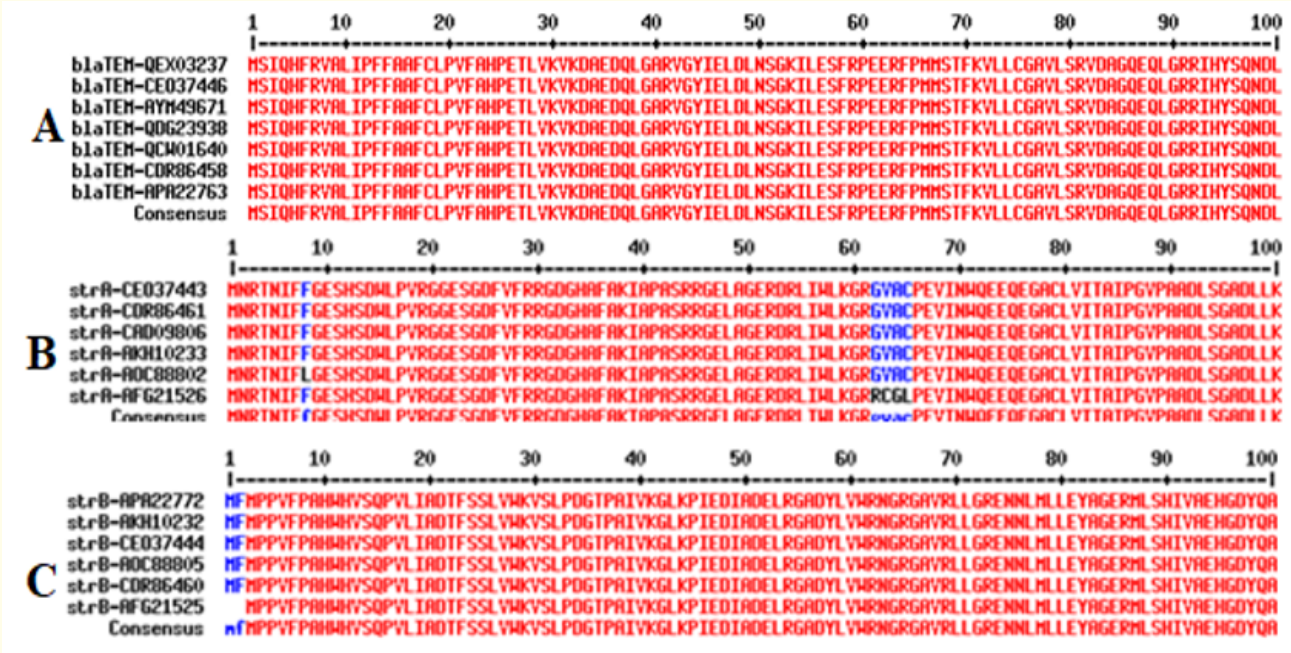

Figure 2: *Multialign of class A Beta-lactamases and streptomycin 3'/6' phosphotransferases. Part of the aligns were shown here, blaTEM-1 (A), StrA (B) and StrB (C).*

Tetracycline resistant drug efflux proteins tetA and tetB have 60% homology (Figure 3D) but no mutations was detected among the tetA proteins (Figure 3A) or tetB proteins (Figure 3B) butno mutation was detected in the RND drug efflux protein OpxB (Figure 3C). Chloramphenicol/florfenicol efflux MFS transporter (FloR) was found in many plasmids and few mutations were present (Figure 4). *Salmonella enterica* fluoroquinolone MFS drug efflux transporter QepA (protein id. AWW22306, plasmid pGDD25-21) and macrolide MFS efflux protein (Msr-family ABC-F type like Mel; protein id. AKN19296, plasmid pHXY0908) and chloramphenicol drug efflux protein (protein id. AXY98896, plasmid pST1007-1B; Bcr-cflA family) were rarely detected. In *Salmonella* plasmids, arsB (A) and arsC (B) arsenic metal efflux genes were found and mutations were detected (Figure 5) as well as silver efflux genes like *sil*ABC (Figure 6). Mercuric reductase (merA) and multi-copper oxidase (PcoA) were abundant and many mutations were observed in PcoA but merA was conserved among the plasmids (Figure 7). RcnA Ni^{++}/Co^{++} transporters of *Salmonella enterica* plasmids (protein ids. AVS55158, AZM67488, QEX03304, CEO37522, CDR86475) were identical as also found in other bacterial species like *E. coli, E. cloacae, S. enterica, K. oxytoca, S. marcescens* but I327F mutation in *C. freundii* whereas Other *Klebsiella* species like *K. pneumoniae, K. aerogenes* and *K. quasipneumoniae* have more mutated forms (a new lineage) of RcnA transporter (Figure 8). Further in *K. quasipneumoniae* plasmid-mediated RcnA four amino acids (GHDH) insertion was detected at 234 amino acid position where as a four amino acid deletion (AEHD) at amino acid position 230 of RcnA of *Klebsiella aerogenes*.

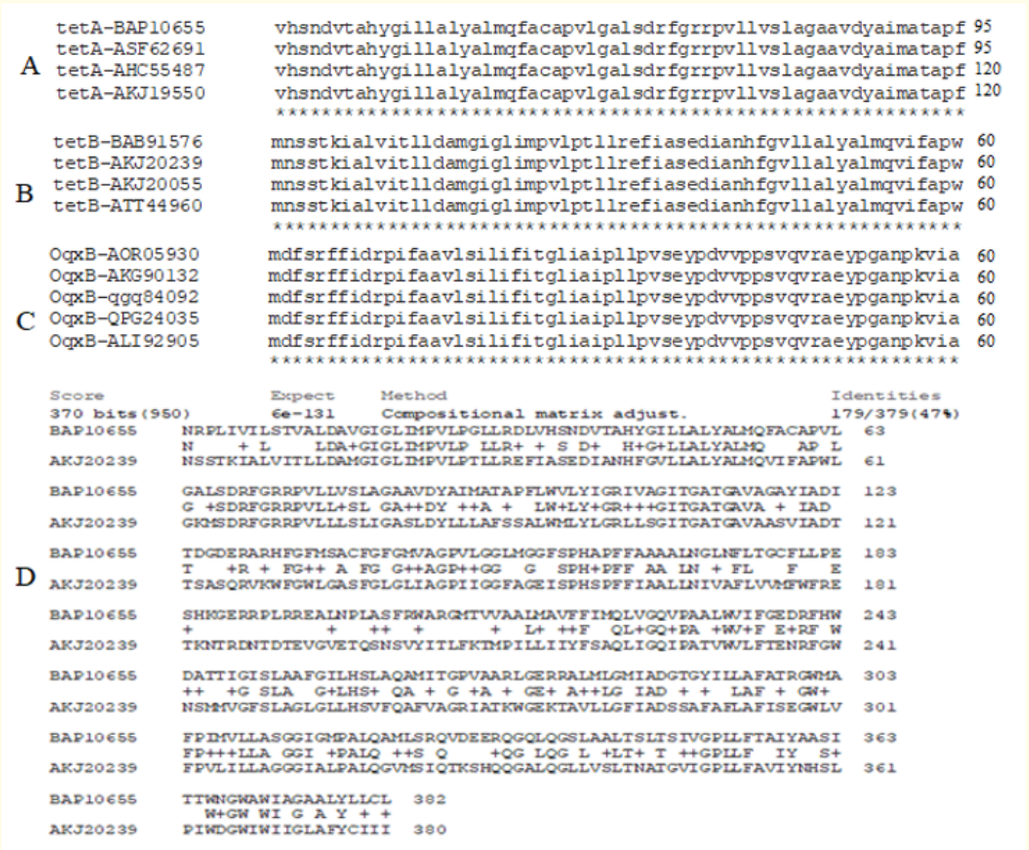

Figure 3: *Multialign analysis of plasmid-mediated TetA (A), tetB (B) tetracycline drug efflux proteins and OqxB (C) drug efflux protein of Salmonella enterica. Amino acids 201 - 240 and 404 - 420 have difference between tetA and tetB as demonstrated by BLAST Seq-2 align (D). No mutations among tetA, tetB and OqxB were detected in Salmonella plasmids.*

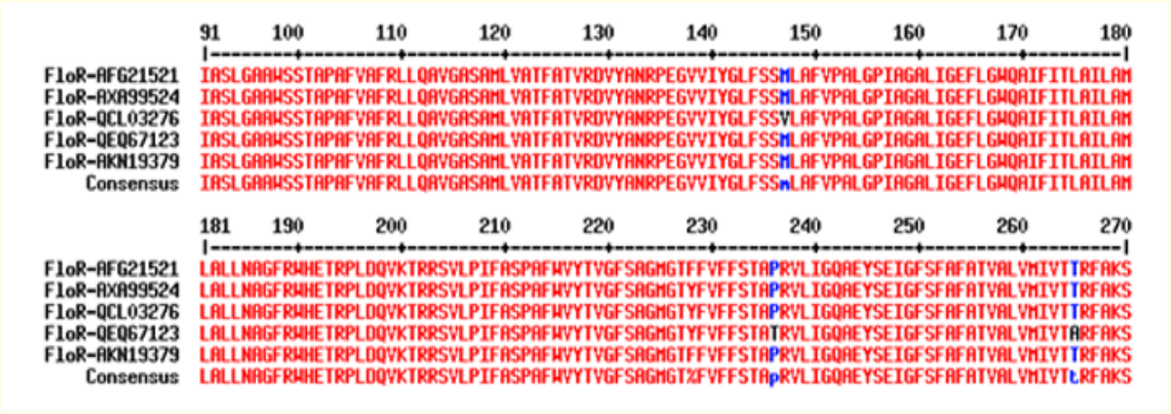

Figure 4: *Multialign sequence analysis of plasmid-associated FloR protein (chloramphenicol/florfenicol efflux MFS transporter) showing mutations. FloR protein AFG21596 of S. enterica serovar Heidelburg has more mutations.Other chloramphenicol MFS drug efflux transporter CmlA family has only 25% sequence similarity to FloR (protein ids. ALI92933 and AXH26379).*
Part of the alignment was shown due to page limit.

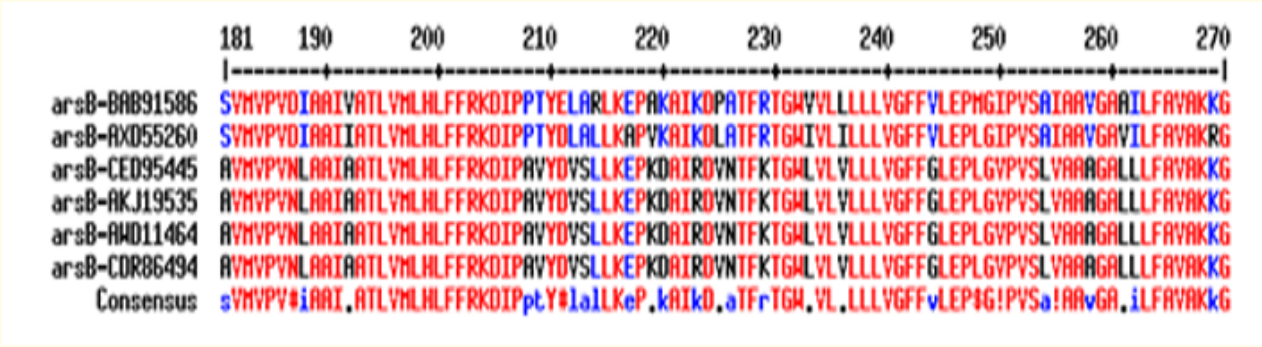

Figure 5A: *Multialign of Salmonella plasmid-associated ArsB transporter. Few mutations were detected with two plasmids types where, LF vs IL at 9 amino acid, KST vs RGA at 137 amino acid, PA vs VN at 119 amino acid and AY vs PI at 208 amino acid are predominant. Proteins ids. CED95445, AKJ19535 and AWD11464 are one type cluster.*

Only part of the alignment shown where mutations were clustered.

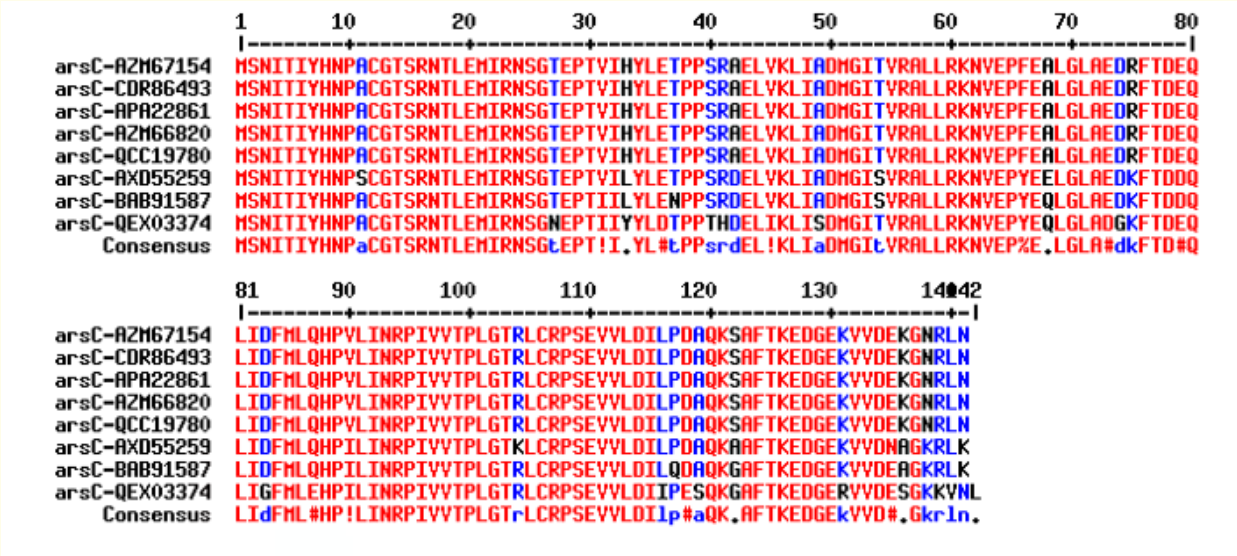

Figure 5B: *Detection of mutations in Salmonella enterica plasmid-associated ArsC protein. In major substitution was detected in QEX03374 at 40 (SRA vs THD), at 74 (DR vs GK) and point mutations at 27, 49,68, 83, 104, 119, 131, 136 and 142 amino acids. Point mutations are in AXD55259; A vs S at 11, H vs L at 33 A vs E at 68, R vs K at 75 and R vs K at 104 amino acids.*

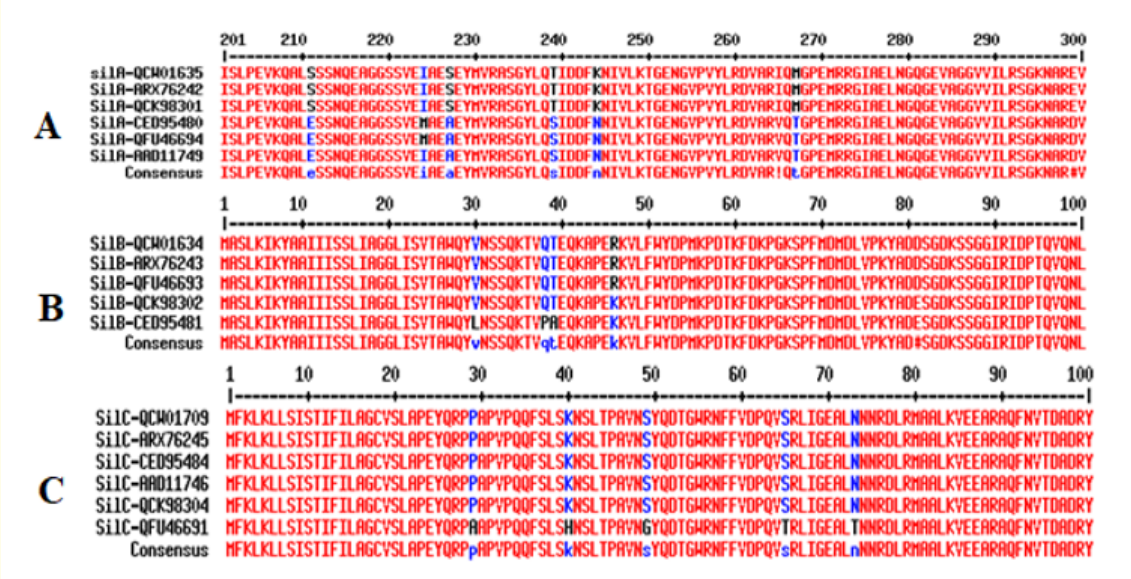

Figure 6: *Mutations of plasmid-associated SilA/B/C of Salmonella enterica. Parts of the SilA (A), SilB (B) and SilC (C)protein alignments were shown with multiple mutations. SilA protein id. AAD11749, SilB protein id. CED95481 and SilC protein id. QFU46691 have more mutations. Such genes were also assigned as CusA, CusB and CusC as Ag+/Cu+ double resistance was observed.*

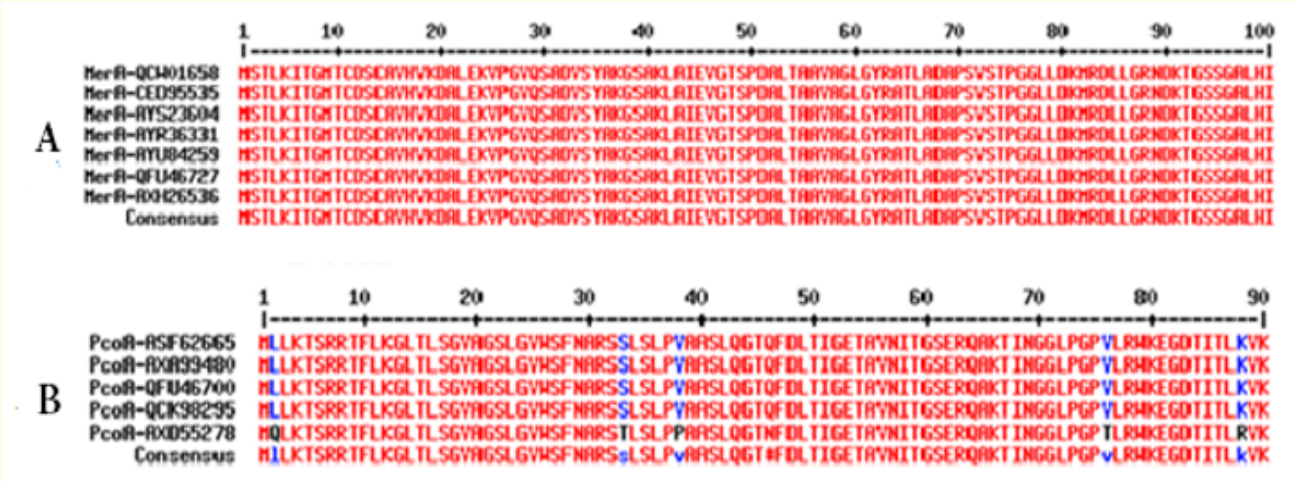

Figure 7: *Multialign of Mercuric reductase (A) and Copper oxidase (B) showing MerA has no mutation but PcoA has mutations.*

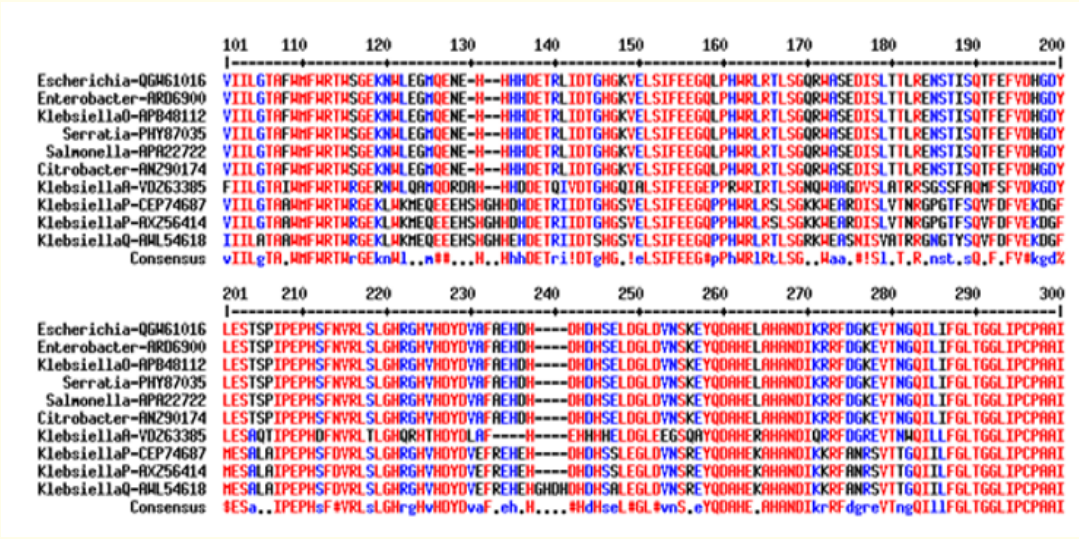

Figure 8: *Multialign analysis of plasmid-mediated Ni^{++}/Co^{++} Transporter RcnA showing mutations with varying efflux strengths. Part of align was showed where maximum mutations were found.*

Discussion

Analysis suggested that most *Salmonella enterica* have acquired MDR plasmids and many of them accumulated also toxin genes and virulence genes increasing pathogenesis (Table 1). Rahman., *et al*. has recently demonstrated by WGS that *gyr*AB mutations and increased in non-H58 *Salmonella typhi* (genotype 4.3.1) may be a threat to South Asian population [31]. However, *blaTEM*, *catA1*, *dhfrA7, sul1*, *sul2, strAB* and gyrase A subunit mutations were detected whereas our review demonstrated the presence of blaOXA1, *blaCMY* and *blaNDM* like deadly beta-lactamases (Table 1 and figure 1 and 2). Holt., *et al*. demonstrated the emergence of IncHII MDR plasmids in *Salmonella typhi* and our search demonstrated the existence of IncH1 (pA3T, pHK0653), IncF (pSH696_135, pFORC19), IncCI1 (p9134, pSH1148_107), and IncFII (pGDD25-16) [32]. We detected extended spectrum β-lactamases in few plasmids (pRH-1238, pGDD25-16 and pA3T) as reported recently [28,33]. Whole genome sequencing (WGS) of *Salmonella enterica* were done considerably and we analyzed few sequence data to check the presence of *mdr* genes and drug efflux genes [34] (Figure 2 and 3). Heavy metals ($Co^{++}/Ni^{++}/Cr^{++}$) transporter like czcCB and chrAB were not detected in *Salmonella enterica* but widely distributed in *Acinetobacter* sp and *Pseudomonas* sp (protein ids. MPS58401, KHV65566, APW48833 and APW48831/32 but *Salmonella enterica* RcnA Co^{++}/Ni^{++} transporter (protein ids. CDR86475, APA22722, AVS55158) may perform the similar protection from heavy metals (Figure 8).

Highly toxic metal ions like Ag^{+}, AsO^{-2}, $AsO_4(^{-3})$, Cd^{+2} Co^{+2}, $CrO_4(^{-2})$, Cu^{+2}, Hg^{2+}, Ni^{+2}, Pb^{2+}, $TeO_3(^{-2})$, Tl^{+} and Zn^{2+} were modulated in bacteria by various mechanisms like metal efflux (*SilABC* for Ag^{+}/Cu^{+2} or *Czc* for Cd^{+2}/Co^{+2}) and enzyme-mediated transformations like oxidation-reduction (mercuric reductase, multicopper oxidase), metal-binding proteins (*silE*, metallothionine, chaperone *copZ*) or methylation-demethylation to control intracellular concentrations of heavy metal ions that may be inhibitory sulphydryl complexes with enzymes. Resistance to inorganic mercury, Hg^{++} as well as organomercurials, such as CH_3Hg^{+} and phenylmercury required *mer* locus involving a series of metal-binding and membrane transport proteins as well as the enzymes mercuric reductase and organomercurial lyase [35-37]

(See figure 5A and 5B and figure 6). A high frequency resistant *Salmonella*, *Pseudomonas* and *Bacillus* genera bacteria to mercury (10 mg/L; < 10 ppm) and other heavy metals were reported in environmental water resources where co-resistance were detected to ampicillin, chloramphenicol, tetracycline and streptomycin (60 - 80%) as well as 40% resistant to all four drugs.

In this review, we have presented the molecular view of MDR plasmids in different Serovar of *Salmonella enterica*, analyzing the GenBank database. Such molecular biology technology rely on Drug Selection of *Salmonella enterica*, Plasmid Isolation from MDR bacteria and Di-Deoxy Sanger DNA sequencing of the Plasmid DNA following GenBank submission (www.ncbi.nlm.nih.gov/genbank). Arsenic-Antimony toxicities were balanced by arsABCH locus in bacteria and arsenic resistant genes were located in few *Salmonella* plasmids like pRH-R27, pIncH12, pFSAN096147 and pSA20044414 but AarsB arsenic transporter were very abundant in *E. coli* (protein id. MHS90779), *K. pneumoniae* (protein id. ARR90324) and *E. cloacae* (protein id. VAL63027) (Figure 5). Arsenate reductase (arsC) is glutaredoxin-dependent small enzyme (protein id. WP_000065805) and arsH is arsenic-binding protein (protein id. WP_000130816) whereas arsA is metal efflux-mediated ATPase (protein id. WP_0011057014), all involved in arsenic resistance.

Tellurium resistance locus (terXYABDEFW) is abundant in large *Salmonella* plasmids like pIMP4-SEM1, pIncH12, pFSAN096147, p09-036813-1A_261, pJXP9, pCFSA300-1, p280_12888 and p200k. TerC protein (346aa) mediates tellurium ion efflux and also abundant in *E. coli* plasmids (pTE63) with association of terB and terE [38]. TeO_3^{-2} resistance determinants found in extra-chromosomal elements include IncHI-2 and pMER610 plasmids [39]. The unique structure of the *Klebsiella pneumoniae* TerB protein (151 AA residues, KP-TerB) has recently been determined [40]. TehA/B type genes have been found in *Salmonella enterica* serovar *typhi* (CAD01716 and CAD01717), *S. enterica* serovar *typhimurium* (NP_460568 and NP_460567) as well as in *Shigella sp* (YP_403356 and YP_689244) and *Haemophilus influenzae* (YP_248222 and YP_249313) [39,41,42].

Abundance of tellurium resistance genes is obscure as it is not an essential element like zinc but its applications in electronics, optics, batteries and mining industries have expanded during the last few years, leading to an increase in environmental contamination [43,44]. Gold ores containing Tellurium are calaverite ($AuTe_2$), sylvanite ($AgAuTe_4$), and nagyagite [$AuPb(Sb, Bi)Te_2$-$3S_6$] and thus gold use increase may correlates its abundance in water. $TeO_3^{(-2)}$ may cause garlic like smell of dimethyltellurite on ingestion of bismuth salt contaminated with tellurite [38]. Whereas, in another mechanism of detoxification, $TeO_3^{(-2)}$ was reduced to $Te^{(0)}$ causing precipitation because $TeO_3^{(-2)}$ was very toxic to bacteria at < 1 µg/L concentration. $TeO_3^{(-2)}$ could also be reduced chemically to lower oxidation states by glutathione or by other reduced thiol-containing proteins (metallothionine) with drastic decrease in the concentration of antioxidant molecules such as glutathione and cysteine causing a phenotype of higher $TeO_3^{(-2)}$ tolerance. In this context, mutants of cysteine biosynthetic pathway have shown highly sensitive to tellurite [40,45]. Prevalence of *mdr* genes and metal resistant genes were also demonstrated in many *Salmonella* sp isolated from food animals [46-48]. The genetic exchange and acquisition of *mdr* genes were happed in the gut and thus gut microbiome placed a central role in shaping both *mdr* and metal resistant genes [49]. WGS of *Salmonella* has showed the existence of MDR-islands in *Salmonella* genome and thus virulence and multi-resistance will be more prominence in *Salmonella* infections [50-53] (Figure 6-8 and table 1).

Conclusion

We explained the recent salmoniasis outbreaks in India as well as abroad due to over expression of plasmid-mediated *mdr* genes, drug efflux genes as well as metal resistant genes which have acquired when *Salmonella* spends its life in the contaminated water originated due to huge expansion of metal industry, coal industry as well electronics industry. We presented small plasmids with only metal resistant genes or drug resistant genes or toxin genes. However, combination of such plasmids with 62.5 kb F' conjugative plasmids created large *mdr* conjugative plasmids accumulating different genes that might not necessary for drug resistance. The localization of complete metal resistant operons like *sil, cus, mer* and *ter* with 5 - 15 metal resistant genes in large plasmids indicated that live stocks (pig, chicken, goat)

grew in the metal contaminated soil and water with poor hygienic condition [54]. Such report thus confirmed the spread of animal salmoniasis and *Salmonella enterica* could be located in cow milk and chicken meat. *Salmonella typhi* plasmids also analyzed to dictate same notion indicating the passage of the organisms in zoonotic reservoirs has to be carefully studied. Never the less we have authenticated the metal resistant proteins as well as their relation to transposons with *mdr* genes like *blaTEM1, blaNDM1, blaCTX-M15, strAB, mcr5/9, dhfr, sul1/2* and drug efflux genes like *tetA, tetB, floR* and *oqxB* [55]. Thus, this report is a valuable source of drug resistant and metal resistant proteins and their symbiotic relation with respect to co-passage of *Salmonella enterica* to intestine (to make gut microbiome) and water resources. We are studying the metal resistant bacteria in lakes near Midnapore City where clusters of metal and steel industries are accumulating at the side of the Bombay Road and Kangsabati River of West Bengal, India.

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