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# Colistin Drug Resistant Determinant Mcr-1 Gene Spreads in Conjugative Plasmids Creating Huge Confusion for the Treatment of Multi-Drug Resistant Infections

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## ABSTRACT

*Worldwide spread of new MDR gene, Phosphoethanolamine –Lipid A transferase (Mcr-1) in superbug plasmids has threatened the treatment options of multi-drug resistant infections. Colistin is higher derivative of polymyxin that facilitates cell-membrane leakage in bacteria after binding to the membrane phospholipids. The Mcr-1 enzyme actively transfer phosphoethanolamine to membrane phospholipid-A inhibiting the asses of colistin to bind on membrane to exert its toxicity. Colistin is very active against MDR bacteria with plasmids borne or chromosomal-mediated blaTEM, blaCTX-M, blaOXA, catB3, strA/B, sul1/2, aacA1, aacC1, aadA2 and aphA4 mdr genes as well as many drug efflux genes. Although Mcr-1 gene discovered in 2015 in China but already had identified in most continents with many mutations (Mcr-1.1 to Mcr-1.8) and different isoforms (Mcr-2.1, Mcr-2.2, Mcr-3.1, and Mcr-4.1 ). TheMcr-1 plasmids are small 33kb with no other mdr gene but 60kb Mcr-1 plasmids have CTX-M, aadA2, arr3 types mdr genes. Very recently discovered high molecular weight (>200kb) conjugative plasmids however, have 4-6 mdr genes with one dozen metal drug resistant genes. Un-noticed deaths by NDM-1, MCR-1, CTX-M-15, MecA, VanA, AAC6'-1b-cr and OXA-23 superbugs are highly occurring in poor nations. Sadly, superbugs have greatly activated by acquiring 6-12 mdr genes during sexual conjugation and also mobilizing mdr genes into chromosome as well as target specific mutations of rRNA and porin genes. Thus development of alternative medicines like gene medicines (Antisense, Crisper-Cas, miRNA, SiRNA, Ribozymes), phyto-antibiotics and nano-drug carriers is urgent need to stop huge loss of life and wealth worldwide. G-20 Nations were gathered at Berlin (May 2017) and Hamburg (July 2017) and issued action plans to stop superbug horror.*

**KEYWORDS:** *Mcr-1 spread, multi-drug resistant, blaNDM-1, superbugs horror, mdr genes*

## INTRODUCTION

MDR phenomenon has crossed the border and one in every three environmental bacteria are penicillin drug resistant including >95% of the clinical isolates from human and animal [1-3]. Discovery of blaKPC-2 in 2001 and blaNDM-1 in 2009 proteins have created havoc fear among the physicians as such MDR proteins can destroy not only penicillin but also most cephalosporins and carbapenems including Beta-lactamase inhibitors but avibactam [4-6]. Antibiotic principles were discovered in 1928 and first benzyl penicillin inactivating enzymes like penicillinase was detected as early as 1940 [ 7]. Since then drug industry has always run to develop a new drug or new derivative of the existing one to overcome the drug resistant clinical isolates of Escherichia coli, Salmonella enterica, Pseudomonas aeruginosa and Klebsiella pneumoniae. R-plasmids carrying amp, tet , dhfr, sul, str, cat, aac and aph genes were sequenced in 1965 onwards and since then every day new MDR genes (Mcr-3.1, blaNDM-9, blaCTX-M-241, blaOXA-450) were deposited in the GenBank, EMBL and DDBJ databases [1-3]. Most notorious MDR genes like blaKPC was isolated in 2001 and then blaNDM in 2009 and Mcr-1 in 2015. The dangerous drug efflux genes like tetA/C were discovered in 1965, acrAB-TolC and mexAB-oprM in 1995, ermB in 2002, norA and macA/B in 2007 [8]. Such diversified drug efflux proteins (RND, MFS and MATE) kick out drug from bacterial cytoplasm increasing MIC to a level that is impossible to reach in blood to kill bacterial central dogma target enzymes like DNA polymerases, ribosomal proteins, RNA polymerases and DNA topoisomerases.

Mcr-1 gene was first reported in 2015 in colistin resistant Escherichia coli plasmids in Chinese man [ 9]. The first plasmid was ~33kb or ~63kb with no other MDR genes and contains pilus assembly and type IV secretion system genes (Tra, Trb, Trh, Tax, Pil, Rci, Vir) including Int, NicB, Rec,Topo3 , Tn3 and TnpA genes as well as few metal resistant genes like Sil, Ter and Cus [10-13]. However, the recent trend suggests that large MCR-1 containing plasmids carry other MDR genes [14-21]. It appears that bacteria exposed to any chemical can create genes to destroy it through histidine-sensitive kinases

facilitating gene rearrangement and plasmid regeneration to make mdr genes. Such rearrangement is favoured by the increase gene doses of many DNA modifying enzymes like integrases, topoisomerases, resolvases and transposases that are also mobilized into conjugative plasmids. Penicillinase destroys ampicillin, oxacillinases destroy oxacillin, cefotaximases destroy cefotaxime and carbapenemases can inactivate all penicillins, cephalosporins and carbapenem (imipenem) drugs [22]. The penem antibiotics further are inactivated by penicillin binding proteins like mecA, penA and ponA. Thus discovery of blaNDM-1 in 2009 warns the end of antibiotic era and discovery of Mcr-1 in 2015 is total disaster in drug industry as colistin act efficiently on bacteria membrane lipid with lower toxicity index in human and was very active against superbugs umtil recently. Because other potent drugs like amikacin, lomofloxacin, linezolid, isoniazid resistant genes (mexAB-oprM, macAB, aac6'-1b, arr3, aacA4) are also increasing in same conjugative plasmids of many pathogens and their is no options for treatment of PDR bacterial infections by antibiotics [23]. We will discuss the spread of Mcr-1 plasmids that has created new horror in drug industry as also happened after discovery of NDM-1 plasmids in 2009.

## RESULTS

### Characteristics of Mcr-1 Gene

Mcr-1 gene is 1626 bp and codes an enzyme of 541 amino acid. First two amino acid is Methionine and some report use second Methionine as start site and thus Mcr-1 protein is 540 amino acids (see, Fig. 1). The enzyme is phosphor ethanolamine-lipid A transferase that actively transfer phosphoethanolamine to membrane phospholipid inhibiting the asses of colistin to bind on membrane to exert its toxicity. The structure of the catalytic domain of Mcr-1 at 1.32 Å reveals the active site threonine-285 is phosphorylated and Zn+2 is present in the active site as well as at peripherally located conserved sites and binding sites for substrates lipid-A and phosphoethanolamine are located at the membrane domain [24]. The MCR-1 enzyme is 41 % and 40 % identical to the phosphoethanolamine transferases LptA and EptC, respectively, and sequence comparisons suggest the active-site residues are conserved [25-27].

### Mutational analysis of Mcr-1 genes

GenBank analysis suggested that Mcr-1 gene was abundant in plasmids of Escherichia coli, Pseudomonas aeruginosa, Salmonella enterica and Shigella sonni. Few mutations of Mcr-1 gene were reported in plasmids from many clinical isolates worldwide and described as mcr-1.2 to Mcr-1.8. A Mcr-1.9 allele was reported with C->T mutation at nucleotide 27 from ATG codon but codes Tyrosine in both cases and thus produces Mcr-1 enzyme. Table-1 was summarized the types of mutations have occurred in those mutants and also described protein ids and plasmid accession numbers. Mer-1.2 was isolated by Di Pitalo V et al from K. pneumoniae 33kb plasmid and Mcr-1.3 containing plasmid has not fully sequenced yet. Mer-1.4 was isolated by Zhao F. et al.(unpublished) in China from E. coli isolate 33kb plasmid. Mer-1.5 was isolated from E. coli (obtained from human with urinary tract infection) 62kb plasmid by Tijet N. et al. (unpublished).

**Table1.** Mutational analysis of Mcr-1 enzymes in different MDR bacteria

| **Mcr-1 gene (phosphoethanolamine transferase-lipid A transferase. 541aa) in plasmids of superbugs** | | | | | |
|---|---|---|---|---|---|
| **Isomers** | **Accession** | **Bacteria /Plasmid** | **Protein id** | **Mutation** | **Source of isolation** |
| Mcr-1.1 | KP347127 | Escherichia coli , 64kb | AKF16168; 541aa | Standard | Human in China |
| Mcr-1.2 | KX236309 | K. pneumoniae, 33.3kb | ANR95875;541aa | Gln3Leu | Rectal swab of leukemic child |
| Mcr-1.3 | NG_052861 | E. coli, plasmid unnamed | WP_077064885; 541aa | Ile38Val | Chicken in China |
| Mcr-1.4 | KY463454 | E. coli plasmid 33.3 kb | ASI38452;541aa | Asp 440Asn | Sewage in China |
| Mcr-1.5 | KY471311 | E. coli plasmid 61.6kb | ARX61132;541aa | His451Tyr | Human urinary track in Argentina |
| Mcr-1.6 | KY352406 | Salmonella enterica, plasmid 47.8kb | AQK48217;541aa | Arg536His | Healthy human in China |
| Mcr-1.7 | KY829117 | E. coli, plasmid 62.1kb | ARJ33985; 541aa | Ala215Thr | Sewage in China |
| Mcr-1.8 | KY792081 | E. coli plasmid 63.8kb | ARK36096; 541aa | Gln3Arg | Healthy human fecal in Hong Kong |
| Mcr-2.1 | LT598652 | E. coli, plasmid 35kb pKP37-BE | SBV31106; 538aa | 101 mutations | Belgium, Bovine |
| Mcr-2.1 | MF176239 | Moraxella sp. Plasmid like pHNSHP45 | ASK49941 | 8 mutations to E coli Mcr-2.1 | Spain, Pig |

| | | | | | |
|---|---|---|---|---|---|
| Mcr-2.2 | MF176240 | Moraxella sp.Plasmid like pHNSHP45 | ASK49942 | 66 to Moraxella sp. Mcr-2.1 | United Kingdom, Pig |
| Mcr-3.1 ?? Mcr-1.9 | KY964067 | E. coli, plasmid 33kb, pLV23529-MCR-3 | ASK38392; 535aa | V412A & N-ter. 6aa deletion | Portugal, Swine |
| Mcr-3.1 | KY924928 | E. coli , plasmid pWJ1, 261kb | ASF81896; 541aa | 339 mutations | China, Pig |
| Mcr-4.1 | MF543359 | S. enetrica, 8kb pMCR-3445 | ASR73329, 541aa | 250 mutations to Mcr-3 | Europe, pig |

## Diverged Mcr-1 Genes

Three diverged Mcr-1 gene have sequenced so far and designated as Mcr-2.1, Mer-2.2 and Mcr-3.1. Mcr-2.1 was discovered in E. coli plasmid pKP37-BE (accession no. LT598652) from a healthy man in Belgium and the plasmid is only 35104bp that do not carry other mdr genes. Mcr-3.1 gene was discovered in E. coli 261119bp large plasmid pWJ1 (accession no. KY924928) and contained other four mdr genes like aac6"-1b-cr, blaOXA-1, catB3 and arr3. It also has tellurium resistant gene cassette (TerA/F/Z). Mcr-2.1 is 47% homology and Mcr-3.1 has 45% sequence identity to Mcr-1. We found about 101 mutations in Mcr-2.1 and >300 mutations in Mcr-3.1 as compare to Mcr-1 and such data clearly indicated that such phosphothanolamine lipid A transferase were totally different than early identified Mcr-1 enzyme (Fig. 1). If an enzyme has only <35% similarity at the amino acid level then it is totally made by different mechanism. E. coli Mcr-3 has high homology to Aeromonas sp. Mcr-3 isomer (94%). Moraxella Mcr-2.1 is very similar to E. coli Mcr-2.1 with eight mutations and Mcr-2.2 has 66 mutations with respect to Mcr-2.1. E. coli Mcr-3.1 (accession number KY964067, Protein Id. ASK38392) should be named as Mcr-1.9 being 6aa deletion at the NH2-terminus and one mutation only. A novel Mcr-4.1 gene (accession no. MF543359; protein id. ASR73329) was isolated from S. enterica (Italy isolate 2013) anwd E. coli (Spain isolate 2015, Belgium isolate 2016) demonstrating the rapid spread of Mcr-4 isomers in Europe. Salmonella R3445 genome (accession no. NC_003197.2) contains chromosomally blaTEM-1B, StrA/B, Sul2 and tetB mdr genes but a small 8.7kb plasmid pMCR3445 contains Mcr-4 gene having 82% similarity to Shewanella sp. Phosphoethanolamine transferase and 59% to Mcr-3 (Fig. 2) but minimal homology with Mcr-1 or Mcr-2. It appears the Salmonella strain-3445 will follow conjugation to make bigger plasmids as it also contains other small 4-7 kb ColE1plasmids but the high molecular plasmids may be present but has over looked during quick publication of the paper (GenBank deposited on 3rd August, 2017).

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ARD68168_Mcr1    MMQHTSVWYRRSVSPFVLVASVAVFLTATANLTFFDKISQTYPIADNLGFVLTIAVVLFG-60
SBV31106_Mcr2    -MTSHHSWYRYSINPFVLMGLVALFLAATANLTFFEKAMAVYPVSDNLGFIISMAVAVMG
ARD68168_Mcr1    AMLLITTLLSSYRYVLKPVLILLLIMGAVTSYFTDTYGTVYDTTMLQNALQTDQAETKDL-120
SBV31106_Mcr2    AMLLIVVLLS-YRYVLKPVLILLLIMGAVTSYFTDTYGTVYDTTMLQNAMQTDQAESKDL
ARD68168_Mcr1    LNAAFIMRIIGLGVLPSLLVAFVKVDYPTWGKGLMRRLGLIVASLALILLPVVAFSSHYA-180
SBV31106_Mcr2    MNLAFFVRIIGLGVLPSVLVAVAKVNYPTWGKGLIQRAMTWGVSLVLLLVPIGLFSSQYA
ARD68168_Mcr1    SFFRVHKPLRSYVNPIMPIYSVGKLASIEYKKASAPKDTIYHAKDAVQATKPDMRKPRLV-240
SBV31106_Mcr2    SFFRVHKPVRFYINPITPIYSVGKLASIEYKKATAPTDTIYHAKDAVQTTKPSERKPRLV
ARD68168_Mcr1    VFVVGETARADHVSFNGYERDTFPQLAKIDGVTNFSNVTSCGTSTAYSVPCMFSYLGADE-300
SBV31106_Mcr2    VFVVGETARADHVQFNGYGRETFPQLAKVDGLANFSQVTSCGTSTAYSVPCMFSYLGQDD
ARD68168_Mcr1    YDVDTAKYQENVLDTLDRLGVSILWRDNNSDSKGVMDKLPKAQFADYKSATNNAICNTNP-360
SBV31106_Mcr2    YDVDTAKYQENVLDTLDRLGVGILWRDNNSDSKGVMDKLPATQYFDYKSATNNTICNTNP
ARD68168_Mcr1    YNECRDVGMLVGLDDFVAANNGKDMLIMLHQMGNHGPAYFKRYDEKFAKFTPVCEGNELA-420
SBV31106_Mcr2    YNECRDVGMLVGLDDYVSANNGKDMLIMLHQMGNHGPAYFKRYDEQFAKFTPVCEGNELA
ARD68168_Mcr1    KCEHQSLINAYDNALLATDDFIAQSIQWLQTHSNAYDVSMLYVSDHGESLGENGVYLHGM-480
SBV31106_Mcr2    KCEHQSLINAYDNALLATDDFIAKSIDWLKTHEANYDVAMLYVSDHGESLGENGVYLHGM
ARD68168_Mcr1    PNAFAPKEQRSVPAFFWTDKQTGITPMATDTVLTHDAITPTLLKLFDVTADKVKDRTAFI-540
SBV31106_Mcr2    PNAFAPKEQRAVPAFFWSNNTT-FKPTASDTVLTHDAITPTLLKLFDVTAGKVKDRAAFI
ARD68168_Mcr1    R-541
SBV31106_Mcr2    Q-540
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**Figure 1.** Sequence similarities between Mcr-1.1 and Mcr-2.1 enzymes.

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ASF81896_Mcr3.1   MPSLIK-IKIVPLMFFLALYFAFMLNWRGVLHFYEILYKLEDFKFGFAISLPILLVAALN-59
ASR73329_Mcr4.1   MISRFKTLSVNQFTFITALFYVAIFNLPLFGIVRKGIEKQPEVDPLFIASMPLFLTFALS-60
ASF81896_Mcr3.1   FVFVPFSIRYLIKPFFALLIALSAIVSYTMMKYRVLFDQNMIQNIFETNQNEALAYLSLP-119
ASR73329_Mcr4.1   FLFSIFTVKYLLKPFFIVLTLLSSSVFFAAVQYNVVFDYGMIENTFQTHPAEALMYVNLA-120
ASF81896_Mcr3.1   IIWVVTIAGFIPAILLFFVEIEYEEKWFKGILTRALSMFASLIVIAVIAALYYQDYVSVG-179
ASR73329_Mcr4.1   SITNLLLTGLLPSYLIYKADIHYQP-FFKELLHKLAFMLLMFVGIGIVAFFYYQDYAAFV-179
ASF81896_Mcr3.1   RNNSNLQREIVPANFVNSTVKYVYNRYLAEPIPFTTLGDDAKRDTNQ—SKPTLMFLVVG -237
ASR73329_Mcr4.1   RNNSELRRYIVPTYFVSSASKYLNEHYLQTPMEYQQLGLDAKNASRNPNTKPNLLVVVVG-239
ASF81896_Mcr3.1   ETARGKNFSMNGYEKDTNPFTSKSGGVISFNDVRSCGTATAVSVPCMFSNMGRKEFDDNR-297
ASR73329_Mcr4.1   ETARSMSYQYYGYNKPTNAHTQNQG-LIAFNDTSSCGTATAVSLPCMFSRMGRADYDPRR-298
ASF81896_Mcr3.1   ARNSEGLLDVLQKTGISIFWKENDGGCKGVCDRVPNIEIEPKDHPKFCDKNTCYDEVVLQ-357
ASR73329_Mcr4.1   ANAQDTVIDVLSHSGIKVQWFDNDSGCKGVCDQVENLTIDLKSDPKLCSGQYCFDQVLLN-358
ASF81896_Mcr3.1   DLDSEIAQMKG-DKLVGFHLIGSHGPTYYKRYPDAHRQFTPDCPRSDIENCTDEELTNTY-416
ASR73329_Mcr4.1   KLDKILAVAPSQDTVIFLHIIGSHGPTYYLRYPPEHRKFIPDCPRSDIQNCSQEELINTY-418
ASF81896_Mcr3.1   DNTIRYTDFVIGEMIAKLKTYEDKYNTALLYVSDHGESLGALGLYLHGTPYQFAPDDQTR-476
ASR73329_Mcr4.1   DNTILYTDFILSEVVNKLKGKQDMFDTAMLYLSDHGESLGEKGMYLHGAPYSIAPKEQTS-478
ASF81896_Mcr3.1   VPMQVWMSPGFTKEKGVDMACLQQKAADTRYSHDNIFSSVLGIWDVKTSVYEKGLDIFSQ-536
ASR73329_Mcr4.1   VPMLAWVSNDFSQDNQLNMTCVAQRAEQGGFSHDNLFDSLLGLMNVKTTVYQSQLDIFAP-538
ASF81896_Mcr3.1   CRNVQ-541
ASR73329_Mcr4.1   CR--Y-541
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**Figutre 2.** Sequence similarity between Mcr-3.1 and Mcr-4.1. N

## MCR-1 Gene Containing Plasmids

Escherichia coli 33kb plasmids pMCR-1-IHIT35346, pECMCR-1101, pMCR_WVHEC1604-IncX4 and pICBEC7Pmcr are early plasmids carrying only Mcr-1 mdr gene and 99% similar. Medium sized plasmids (55-65kb) like E. coli pMCR-M19855, pJIE2288-1, pOM97-mcr, pBA76-MCR-1are very similar carrying Mcr-1 gene. The genetic backbone of K. pneumoniae 33kb plasmid pmcr1_IncX4 is different than E. coli plasmids but Shigella sonni 62kb plasmid pEG430-1 is similar to K. pneumoniae plasmid pMCR_1511. Salmonella enterica Taiwan isolate 43kb pC214 plasmid has sul2 (protein id. APY21940) and cmlA1 chloramphenicol transporter (protein id. APY21944) including Mcr-1 gene (protein id. APY21931) flanked by ISAPI1 transposase (protein id. APY21930). E. coli Bahrain isolate 64kb pBA76-MCR-1 plasmid (accession no. KX013540) has blaCTX-M-64 gene (protein id. ANS54751) including ISEcp1 transposase, NickB, Topo3, PilB/B/N, VirB, Rci, and Tra proteins. However, E. coli Argentina isolate has 60kb plasmid pMCR_M19855 (accession no. KY471315) has Mcr-1 gene (protein id. ARX61484) flanked by PAP2 super family protein but no mdr gene detected.

Mcr-1 large IncHI2 plasmid p538 (accession no. KX129782) has many mdr genes like blaCTX-M-1 (protein id. ANR95458), cmlA1 (protein id. ANR95465), aadA2 (protein id. ANR95466) and sul1 (protein id. ANR95449) including Mcr-1 gene (protein id. ANR95472). It also contained trhC/H/N, terA/C/Z, MerC/E etc. genes involved in metal resistance as in 209kb plasmid pH22613 (accession no. KX129784) but no mdr gene was detected in the later but Mcr-1(protein id. ANR95768, nt. 170653-172278) (Zurfluh K et al. 2016). Escherichia coli 225kb plasmid pMR0516mcr has different backbone as compare to MCR-1 plasmids pSA26-MCR-1 (240kb; accession no. KU743384) and pS38 (248kb; accession no. KX129782). The E. coli pSA26-MCR-1 plasmid contained MFS transporter, mphA macrolide efflux (protein id. ANC48121), aadA2 streptomycin adenyltransferase , aac-Sat1 streptothricin acetyltransferase, blaTEM-1, sul3 (protein id. ANC48130), aadA1 streptomycin 3"-O-adenyl transferase (protein Id. ANC48133), aph(3')-aminoglycoside phosphotransferase (protein id. ANC48134) and strA/B streptomycin phosphotransferase, floR chloramphenicol resistant (protein id. ANC48140), tetA (protein id. ANC48145) including Mcr-1 gene (protein id. ANC48188). Mcr-4.1 plasmid is very small (8.7kb) and has no mdr gene although such bacteria has acquired many chromosomal mdr genes (39).

## DISCUSSION

The Mcr-1 colistin resistanr gene appeared in a similar way to other mdr genes had emerged like amp, tet, strAB, catB3, aacA4, dhfr, sul1/2 and aacC2 due to huge use of antibiotics in a irregular manner for a longer period. But we have no choice as the rapid increase in the prevalence of ESBL pathogens and recurrence infections have promoted physicians to prescribe combination therapy. Development of CRE pathogens similarly has forced physicians to use colistin in human infections and in animal production facilities knowing inevitable risk of emerging resistance. Escherichia coli was highly contaminated with Mcr-1 plasmids worldwide [28]. The mcr-1 gene was detected with other mdr genes blaCTX-M-15, blaTEM-1, qnrS, and aac(6')-Ib-cr in carbapenem resistant Enterobacter aerogenes GB68 and Enterobacter cloacae GB38isolates [29]. Sadly, the plasmid-borne mcr-1 gene in ESBL-producing Enterobacteriaceae was detected in river water and vegetables in Switzerland [30]. More sadly

ESBL and MBL gram(-) bacteria were detected in Germany [10]. Kolkata water bodies (Ganga River, Digha sea and Rain water) are also contaminated with ESBL and MBL bacteria and huge blatEM-1, blaCTX-M-15, aac6'-1b-cr , tetC, acrAB, mcr, blaNDM-1 genes and others are activated [31]. IncI2 plasmids pHN1122-1, pSTH21, pHNY2, pCTXM64_C0967 with blaCTX-M isomers may be progenitor of Mcr-1 plasmids like pHNSHP45 and pA31-12 that are isolated in China [16]. Ye et al. recently identified three diversified plasmids (pE15004, pE15015 and pE15017) carrying single MCR-1 colistin resistance gene in the isolates from gut microbiota of diarrheal patients having similarity to the Inc plasmid pHNSHP45 [28].

## CONCLUSION

The spread of Mcr-1 gene in conjugative plasmids is huge and it appears the number of such clinical isolates will increase similar to ~550 of blaOXA and ~200 blaCTX-M isomers that destroy all cephalosporins [32-37 ]. Wonder drugs like vancomycin, amikacin, colistin, rifampicin, linezolid and imipenem resistant genes have highly created in conjugative plasmids of Kolkata Ganga River water [ 31]. Yao X et al (Lancet Correspondence, 2016) has reported E. coli strains from meat samples from United States harbouring two deadly mdr genes NDM-9 and MCR-1 including others. Such superbug isolates are resistant to ampicillin, cefotaxime, streptomycin, fosfomycin, neomycin, tetracycline (64µg/ml), ciprofloxacin (32µg/ml), co-trimethoxazole, tetracycline (64µg/ml), gentamycin, amikacin, imipenem (8µg/ml) and colistin (8µg/ml) with highest MIC (100-200µg/ml) but only inhibited by tigecycline (0.5µg/ml). Treatmen t of such infection is complex with repeated exposure of different antibiotics doses and will create more rearrangement in genes under pressure to create new isomers like Mcr-3.1 and Mcr-4.1 which is only 35% similarity to Mcr-1.1. Gene medicines, phyto-antibiotisc and nano-drugs carrier are welcomed by medical authorities [38].

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