Recent crisis in human health is mostly due to spread of multidrug-resistant bacteria or superbugs. Molecular analysis has indicated integrons and R-plasmids (2-15 kb) have combined with F'-conjugative plasmid (62 kb) producing large MDR-Plasmids (50-500 kb) that could donate mdr genes to all bacteria present in the intestine. We used >20,000 tons antibiotics annually to treat >10 bacteria (Lactobacillus, Bifidobacterium, Streptococcus, etc.) but many gut microbes are hard to grow and study in vitro. Thus, antibiotics have produced a greater crisis that human would be extinct unless mdr genes are created and diversified to withstand the new drug derivatives discovered every year! The crisis of vitamins is obvious as bacterial plasmids have been acquiring vitamin synthesizing genes and other genes related to complex biosynthesis. Bacteria know that a further great war will be awaited.

Penicillin antibiotics discovered in 1928 by Nobel Laureate Alexander Fleming and thereafter by Dr. Selman Waksman discovered twenty antibiotics including streptomycin (1). In truth, sulfonamides was introduced in 1930, sulfa-drugs in 1940, penicillins in 1943, tetracycline in 1945-1951, streptomycin and chloramphenicol in 1949, erythromycin in 1952 and ciprofloxacin in 1965 (2,3). Sadly, first report on drug resistant bacteria appeared as early as 1940 after ten years of antibiotic discovery and 3 years early of commercialized production of Benzyl penicillin in 1943. Plasmid pBR322 was made first using restriction enzymes and DNA ligase enzymes from three natural R-plasmids followed by bacterial transformation and plasmid isolation; and we know the sequences of first mdr gene (amp) in 1965 (3). Question arises, are rapid expansion of recombinant plasmid work with amp gene and there after neo, hph, pac, geo, aac and cat genes are producing MDR bacteria even rec-strain of Escherichia coli bacteria used in RDT work! (4,5) Anyway, whole world are safe after World War II taking antibiotics in any bacterial infections and reputed Pharmaceutical Companies have generated with great capital and share market. However, in 1960 MIC of drugs were start increasing and to keep the capital market safe, drug companies were also produced 100 new derivatives of old drugs like ampicillin, oxacillin, methicillin, cefotaxime, ceftriaxone and many strong carbapenems (imipenem, doripenem, meropenem) that target bacterial peptidoglycan biosynthesis (5,6). Streptomycin and tetracycline resistance appeared between 1958-1962 followed by aminoglycosides resistance in 1980 (7). Ciprofloxacin resistance became prominent in 1985 and azithromycin resistance was vibrant between 1995-1999 (8). Most horror reported in 2009 that blaNDM1 enzyme cleaves all penicillins, cephalosporins and carbapenems and lastly Mec-1 enzyme was reported in 2016 that make resistant to colistin drug (an old 1945 toxic drug) by transferring phosphoethanolamine to lipid A of bacterial membrane (9). A great horror in Pharmaceutical Industry and many companies are sold or combined to accelerate the research on novel therapeutics like gene medicines, nanocarriers, phage therapy and enzybiotics (10,11). India uses 8 billion units of antibiotics in 2001 to 12.9 billion units in 2010 with overall 36% increase in 10 years worldwide. This suggests that it is a habit for Indian peoples to take >10 antibiotic pill per year even the infection is viral one. Sadly US peoples take average >20 antibiotic pill per year. This led to conclusion that get is the main source of mdr genes creation and multi-resistance (1,12). At least few millions peoples (mostly neonatal and elderly in the poor countries) are infected every month and expected 10 million death every year where potent drugs like ampicillin, tetracycline, chloramphenicol, azithromycin, streptomycin, ciprofloxacin, repaminic, sulfamethoxazole, etc., would not able to clear most infections like Salmonella typhi, Mycobacterium tuberculosis, Sichelia sonni, Escherichia coli, Acinetobacter baumannii and Staphylococcus aureus (13,14). But meropenem, ceftriaxone, lomoxofloxacin, linezolid, amikacin, colistin type new drug derivatives are sometime effective if the infections are not PAN drug resistant (15). It has been accepted by WHO that antibiotics have produced ugly effects on bacteria as well as human intestinal cells, those are in tight symbiosis to produce vitamins needed for >30000 enzymatic reactions called human metabolosome (16). Over use of antibiotics indeed has killed vital gut microbiota (since 1943! which are now completely replaced by enteric or commensal flora (17)). We have discussed here the anticipated side effects of antibiotic uses that have killed useful gut bacteria producing mdr genes horror of drug industry and human as well as animal health.

**MATERIALS AND METHODS**

Water from Ganga River was collected at the morning from Babu Ghat (Kolkata-700001) and Howrah Station River (16). About 100 μl of water was spread onto 1.5% Luria Bartoniagar plate containing different concentration of antibiotics at 2-50 μg/mL. MDR bacteria were selected in agar-plate containing ampicillin, streptomycin, chloramphenicol, tetracycline or ciprofloxacin at 50, 50, 34, 24 μg/mL, respectively. As imipenem and meropenem resistant bacteria were present low (0.08-0.2 μg/mL water), a modified method was followed. 2 ml 5X LB media was added into 10 ml River/Sea water at 2-10 μg/ml concentration and was incubated 24 h to get drug resistant bacteria population. Meropenem resistant bacteria were further selected on tetracycline, chloramphenicol and streptomycin to get superbugs. Antibiotics were purchased from HiMedia and stored at 2-50

**Key Words:** MDR-plasmids; Gut microbiota; Antibiotic void; Probiotics; Vitamin genes
mg/ml at 20°C (18, 19). Antibiotic papers were also published from HiMedia according to CLSI standard. Antibiotic papers are: A25 (ampicillin), T10 (tetracycline), AT-50 (aztreonam), COT-25 µg (Cotrimoxazole), Met-10 µg (methicillin), CAF-30 µg (cafzidomycin), LOM-15 µg (loxomacillin), VA-10 µg (vancomycin), AC-10 µg (Amikacin), TGC-15 µg (tigecycline), LZ-10 µg (linzolid), and IMP-2 µg (imipenem). The plasmid DNA was isolated from overnight culture using Alkaline-Lysis Method (20, 21). 16S rDNA gene colour Sanger’s dideoxy sequencing was performed by SeqlGenom Limited, Kerala, India. PCR amplification was performed using 1 unit Taq DNA polymerase, 20 ng DNA template, 0.25 mM dXTPs, 1.5 mM MgCl₂, for 35 cycles at 95°C/30 sec (denaturation)-52°C/50 sec(annealing)-72°C/1’ 15’ (22). The product was resolved on a 1% agarose gel in 1x TAE buffer for 2-4 h and visualized under UV light and photograph was taken. The primers for 16S rDNA amplification and mdr genes are given below. NCBI BLAST analysis was performed for bacterial specific gene analysis (http://www.ncbi.nlm.nih.gov/blast) and data was submitted to GenBank. NCBI databases were retrieved using the BLAST programmes (21-23). The complete genes are sequenced in plasmids and were analyzed by Seq-2 programme of BLAST (24). Multalin protein sequence software was used to get the nature of conserved sequences among metallo-class B β-lactamases (25). Some time, diverged sequences are manually cut and paste into align position in MS word so that it is appeared both sequences have similarity. For retrieving any nucleotide, we type the same at the NCBI port (http://www.ncbi.nlm.nih.gov/ nucleotide or Protein) and to BLAST search to type the accession number for protein or DNA into BLAST port (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch) (25-27).

RESULTS AND DISCUSSION

Multidrug-resistant bacteria were contaminated in world water as PM10 particulate (200-400 µg/m³) in air carry bacterial spores and during rain drop anywhere. Table 1 shows the recently isolated plasmids from MDR pathogens carrying multiple mdr genes (amp, blaNDM1, blaOXA, tet, mexAB, arr3, cat, sul, dfrh, penA, acrAB, aacC1, aacA2, aphA4, aacA4, merI). Indeed such GenBank search indicated a rapid acquisition of multiple mdr genes in Escherichia coli, Mycobacterium tuberculosis, Acinetobacter baumanii and Salmonella typhi, etc., is shocking and now >35000 species gut microbiota were antibiotic resistant Figure 1 for EM structures of 1-2 µm superbugs). The antibiotics were available in 1940s onwards for all and we ingested at least 20 great antibiotics (ampicillin, tetracycline, chloramphenicol, erythromycin, oxacillin, sulfamethoxazole, streptomycin, etc.) between 1950-1960. Benzyl penicillin was discovered in 1928 and presence of penicillin resistant bacteria was detected in 1940 but during World War I and World War II Benzy lpenicillin was only available to soldiers to stop infections of injuries (27). Figure 2 shows how new derivatives of penicillin were made following the genesis of new beta-lactamases that cleave beta-lactam ring and inactivate the drug. Drug industry is always run to discover new derivatives of penicillin, streptomycin was inactivated by strA and strB enzymes that phosphorylate and inactivate streptomycin. Similarly, acetylating enzymes (CAT, AAC) activate chloramphenicol and aminoglycoside antibiotics as well as ciprofloxacin and those drugs usually prevent protein synthesis in the ribosome (10). Figure 3 panel C shows the blaTEM (panel A) and blaCTXM (panel B) genes from isolated plasmids of superbugs that resistant to many drugs (ampicillin, tetracycline, cephalosporin, azithromycin) (18). The situation is so frustrating that drug companies merged for more basic research as drug development needs >2 billion dollars and ten years research for clinical study. Tetracycline drug was used due to tetEF protein and tetG protein that has strong affinity for tetracycline. Streptomycin was inactivated by strA and strB enzymes that phosphorylate and inactivate streptomycin. Similarly, acetylating enzymes (CAT, AAC) inactivate chloramphenicol and aminoglycoside antibiotics as well as ciprofloxacin and those drugs usually prevent protein synthesis in the ribosome (10). Figure 3, panel C shows the presence of AAC6'-1b genes in multidrug-resistant bacteria isolated in Kolkata water bodies. Moreover, mutations in the gyrAB and parC protect MDR bacteria against fluoroquinolones (norfloxacin, lomofloxacin). Arm3 gene ribosylates rifampicin and inactivate it preventing to act on bacterial RNA polymerase. Figure 4 shows the structure of a conjugative MDR plasmid (pK-109-R) with many mdr genes activated by many transposons and many Tra genes. We are yet unable to fully sequence large plasmids of our isolates from India. Such study clearly indicated that MDR bacteria are abundant as have described worldwide with activation of mdr genes and probably other genes involved in vitamin synthesis (28). Figure 5 shows the activation of pigmentation production in Pseudomonas aeruginosa DB-1_mdr (accession no.KY769875) induced by Beta-lactam drug (lane 3). Such study is important as WHO has reported the escalated unnecessary of antibiotic use worse the condition of patients increasing drug MIC due to over expression of Beta-lactamases (27). We previously showed that acrAB, mer and tetC drug transporters were activated in Kolkata superbugs (18, 29, 30). The analysis showed a escalated antibiotic use has killed gut microbiota that synthesized 20 vitamins and bio-molecules needed for >30,000 enzymatic reactions favouring normal human metabolosome (glycolysis, TCA cycle, electron transport in the mitochondrial membrane and metabolism of fat, protein and nucleic acids). We have raised the strong voice that we must use multi-vitamin complex after each antibiotic dose and for regeneration of the gut bacteria, we must use probiotic (bifidobacterium) capsule. Sadly, many gut bacteria are hard to grow in vitro as live in a tight symbiotic relation with intestinal cells that secrete interleukins and cytokines. High quality research from US Human Microbiome Project (HMP), European Metagenomics of the Human Intestinal Tract (MetaHIT) and others have demonstrated the beneficial functions of the normal gut flora (>35000 species) on health. Figure 6 shows a complex relation between gut microbiota and intestinal luminal cells (30). So repeated dose of antibiotics is not good and vigilance of probiotic use must be increased as I am advised amoxicillin+cavulinate but not vitamins or probiotics are prescribed recently. The situation in poor countries (Africa, Latin America and Asia) is more severe. Thus we have presented a relation among superbugs, antibiotics, probiotics and vitamins in Figure 7. We cannot ignore any one of the four parameters as (i) 95% clinical bacteria are now mupilillin drug resistant, (ii) critical messages have generated between bacteria and intestinal cells to make more mdr genes in plasmids, (iii) mdr genes have moved into bacterial chromosome, (iv) many integrons, transposons and IS-elements assembled in MDR plasmids and gut bacteria received these from mutations from US Human Microbiome Project (HMP) and European Metagenomics of the Human Intestinal Tract (MetaHIT) and vitamins are available and cost effective. Finally, Pharmaceutical companies are well ahead in new drug development and perhaps new medical technological advances are in the TABLE 1

| Generation of multidrug resistant plasmid with diversified mdr genes in superbugs |
|------------------|------------------|------------------|------------------|
| pS121-1a         | CP022170         | 192 kb           | mexC, aac6'-1b, cmaA5, ANT3'-1a, dfrh, aac6'-1b-cmr, OXA1, sul1, mphE 2', floR |
| pEGY1mcr-1       | CP023143         | 229 kb           | Aac3'-2a, EmAa, TelA/BF, tetA, mcr-1, ANT3'-1a, cma, sul1, floR |
| P1688-KPC        | MF168402         | 146 kb           | CTX-M-65, TEM-1, SHV-12, KPC-2, MerA/C, RmtB |
| PSHE-CTX-M       | CP022359         | 193 kb           | CTX-M-15, aac3'-2a, CusA, tetA, aph3'-1d, aph3'-1b, sul1, (Vit) |
| pECA2155-KPC     | CP019001         | 272 kb           | KPC, mphA, sulA, aac6II-cma, ahp4-1d, Cmy2, MFT, tetA, study, Ter2, OXA-1, aac6', sulA, XA/C |
| pKP13f           | CP022170         | 205 kb           | Sul1, QacE, TEM-1, OXA-9, aac3', su2, CTX-M-2, aac3'-1a, floR |
| pAUSDUM8141      | CP022697         | 149 kb           | MFS, suf1, catA2, TedT-1, TEM-1, aac3'-1a |
| pKON-L6S         | NC_012654        | 246 kb           | CatA1, dfrh, suf1, mphA, MFS, ABL, SII/E/A/B, a disaggregate, ab3/B/C/S |
| pRJ119-NDM1      | KM630695         | 335 kb           | Ble, TEM, cobS, aac6', su1, cuaA3, dfrh, qnr, tetA, NDM1, ahp3', sulA,sulH |
| pDZ176           | KC534357         | 501 kb           | Ter2, OXA-10, MFS, TEM-8, ble, catB3, aac6', IMP-9, neo |
| pKPx-2           | AP012056         | 141 kb           | Aac3'-6', CatB4, tetA, su2, OXA-1, CTX-M, TEM-1, strAB, qnrB |
| pKH409080        | KM877269         | 249 kb           | Aad, floR, hph, aac6'3', OXA-1, catB3, arr3, sul1, cma |

Aeromonas salmonidica
Escherichia coli
Klebsiella pneumoniae
Shewanella buctiari
Escherichia coli
Klebsiella pneumoniae
Salmonella enterica
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Figure 1) Electron microscopy of few multidrug-resistant bacteria which are very small (1-2 µm) but deadly due to accumulation of 5-15 mdr genes and drug efflux genes in plasmids and chromosome(s) with many target mutations. The result is 100 strong antibiotics fail to kill in vivo due to increase of MIC or total lysis and/or inactivation of drugs due to acetylation, phosphorylation, ribosylation and adenylation.

Figure 2) Demonstration of Great War between discovery of new drug derivatives of penicillin (β-lactams) and gradual generation superior beta-lactamases that destroy beta-lactams. Last drugs meropenem and avibactam are still safest in any bacterial infection. Sadly, in India still we use amoxicillin + clavulinate, methicillin, cefotaxime + sulbactam and ceftriaxone + ciprofloxacin against diarrhoea, TB, pneumonia and UTI. More sadly, β-lactam inducible bla genes (blaAmp-C) has appeared which means more drug you use more mdr genes will produce causing sepsis shock and death.

Figure 3) Amplification of few beta-lactamase mdr genes from superbugs isolated from Kolkata water bodies. Panel A, blaTEM specific primers; panel B, blaCTX-M specific primers and panel C, aac6'-1b specific primers showing acetylating enzyme other than catB3 also has present.
So phage therapy is in the centre stage (31). New antibiotics against cancer, diabetes and mental disorders have been discovered, but none against infectious bacterial pathogenesis. So we hope gene therapeutics will be advanced like ribozyme therapy, Casper-Case technology (32,33) and toxic drugs will be delivered by nano-carriers like fullerenes, DNA cages and disialoglycopeptides (34). We have MDR horror now but we will overcome the antibiotic Dark Age as Nobel Laureates Alexander Fleming and Selman Waksman did in 1928 and onwards.

**CONCLUSION**

Diversified MDR genes are created highly in bacterial plasmids and it appears most bacteria have received transferable small, medium and large plasmids with 5-15 \( mdr \) genes and 10-60 transposons and IS-elements (35). WHO and G-20 Nations recently gathered in Germany for making One Nation platform and united research programmes. Indian NAP-AMR (The National Action Plan on Antimicrobial Resistance 2017-2021) has pinpointed five main areas to curve superbug horror: (i) improving awareness and understanding of MDR, (ii) improving surveillance and antibiotic stewardship, (iii) developing new drugs, (iv) developing biomarkers for MDR, and (v) developing vaccines for MDR.
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of AMR through effective education and surveillance, (ii) reducing infection by increasing preventive measures, (iv) reducing the use of antimicrobials in health, food animals and agriculture (v) promoting for AMR research and drug innovations and (vi) strengthening India’s leadership on AMR and International collaboration (assessed on October, 2017). Practically, we have frightened to see the MDR plasmid’s size and genes are incorporated and reported a *Vibrio cholera* strain 2012EL-2176 harboring IncA/C2 plasmid containing blaCMY-2, blaCTX-M-2, blaTEM-1, floR, aac(3)-IIa, strA/B, sul1/2, dfrA1, dfrA27, tetA, mphA, mdr-genes and also resistant to ciprofloxacin due to mutation in gyrA (S83I)/parC (S85L) has also seen in plasmid pMRV150 (accession no. EU116442). *Bacillus thuringiensis* plasmid pBMB293 (Accession no. CP007615, 294 kb) has no mdr gene but genes for enterotoxins (protein id. AIM34697), dipterans toxin (protein id. AIM34741) and reverse transcriptase, DNA polymerase β, DNA topoisomerase III and type II secretion system. Similarly, *Bacillus anthracis* plasmid pX01 (accession no. CM002399; 171 kb) has tox gene (protein id. AFL55645, 809 aa) and also in pBMB293 plasmid. More and more genes in plasmids will be evident and likely phage therapy comes of age (36-38) (Table 1). Small non-coding RNAs (miRNA) have potential to therapeutic applications as in cancer but its role in MDR bacteria remains elusive (39-41). Many unknown small proteins are located in such plasmids and all have indicated alarming signal. In fact, our work has not completed yet and we argue that others work on MDR bacterial mechanism of pathogenesis is also premature. So much diversities among the mdr genes in single MDR plasmid, then by studying 2-3 genes in clinical samples is nothing conclusive (44)! The MDR plasmids have 20-50 unknown genes and we first to show that such genes actually vitamin synthesizing genes, DAF domain proteins and proteins involved in complex biomolecules synthesis (28). We anticipate GenBank data submission should be given priority check-up and all original colour data should be submitted. We have to minimize research cost and also our mission will be truthful and fruitful research to reduce the drug cost! In India, we now know antibiotic is dangerous but do not know repeated doses of antibiotics how could be dangerous without taking vitamins and probiotics. Mostly antibiotic drug sensitivity tests of blood, urine and stool must mandatory before taking oral antibiotics. This is first paper that questioned many problems of modern MDR research and quick publication. We do understand that funding sources are not enough but G-20 Nations agreed in common research agenda. Our research first has shown that contamination of this Earth with MDR bacteria occurs during rain (17). We believe this article will promote awareness on diseases as neonatal and elderly should not bath in any raw water (river, sea and pond). We hope this paper and other related papers (10) and reviews (45) will change the concept of diagnosis, treatment options and quality health in the era of superbug horror and antibiotic horror. To reduce the expenditure, one nation research programmes will be adopted and all medical discoveries must be patented by United Nations or WHO.

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