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Review Article
Risk Assessment of Antibiotic Resistance Correlated with Antibiotic Residues in the Environment

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Abstract
The widespread use of antibiotics against bacterial infections is emerging human concern. The antibiotic resistance imposing a serious threat to the environment also presents high health risks for humans. There is still a lack of a comprehensive model to determine the risks properly. In this review, we discuss the rapidly increasing antibiotic resistance in the environment and practices to overcome the survival of bacteria against antibiotics.

Key words: Antibiotic resistance, environmental residues, waste water treatment, resistance genes, correlation

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INTRODUCTION

Anti-infection agents are a class of secondary metabolites having the ability to hinder development and endurance of different micro-organisms[1]. Today, the advancement of microbial pathogens ready to resist antibiotics is the most arising public health concern[2-5]. Due to the misuse and overuse of antibiotics now susceptible bacteria are able to develop resistance against antibiotic through many natural ways like horizontal gene transfer, chromosomal mutations etc. The various processes are involve in antibiotic resistance like conjugation, transformation and transdusction[6]. The antibiotic resistance are imposing serious problem to humans and animals as they are continuously present in the environment. Also, the metabolites of antimicrobials involve in the emergence of ARGs through many ways like agriculture runoff, wastewater treatment plants, livestock management and most important irregular junk of antibiotics in the environment[7]. Heavy metals and biocides are inducing antibiotic resistance anthropogenically in the environment leading to the survival of environmental reservoir[8]. It is also reported that non-pathogenic bacteria are capable to acquire antibiotic resistance genes from non-pathogenic bacteria[9]. Antibiotic resistance genes having the ability to transfer in pathogenic bacteria through a mechanism called horizontal gene transfer, thus, extending resistance to new bacteria[10]. There sort of processes are really complex and difficult to monitor. There is a need to develop tools to assess how anthropogenic impacts are collectively shape "resistome" systematically and objectively that affect the environment[11]. Various studies reported that aquatic environment is the key carrier of antibiotic resistance genes[12,13]. Antibiotics act as a either cytotoxic or cytostatic to the microbes. When any infectious micro-organism enters in the body antibiotics allow our immune system to eliminate them by performing actions like: by inhibiting the synthesis of bacterial cell DNA, RNA and proteins etc[14]. Antibiotics also play a vital role in the inhibition of protein synthesis of bacteria by binding to its cell wall through energy dependent transport mechanism[15]. It is reported that resistance in bacteria occur via gene level mutation which is a natural process[16]. Various doctors prescribed antibiotic for viral infections like nosocomial infection which is also the main cause of antibiotic resistance[17]. Another reason of resistance is also arise that the tone of antibiotics are also used in animals not only to treat infections, but also improve and enhance the growth[18,19].

Another reason of antimicrobial resistance also arise that the people use antibacterial soaps for personal care also many antiseptics, antimicrobial nanosilver for household cleaning purposes. The FDA is USA banned the use of antibacterial agents and other various compounds from soaps[20]. There are several reasons the most foremost is that the compound (triclosan) which was used in antibacterial soap was not efficiently applicable to reduce the infection[21]. Another reason is that long term use of triclosan lead to the development of antimicrobial resistance[22]. Bacteria develop resistance among themselves through drug efflux pumps which is a natural defense mechanism to protect them from toxic compounds. These efflux pumps are membrane proteins that expel antibiotics and other toxic compounds from the microbial cells, thereby lowering their concentration inside the cell to sub-toxic levels[21-23]. Having all the issues, we are going to discuss about how environmental residues take part in the development of antimicrobial resistance.

PREVALENCE OF RESISTANCE GENE IN ENVIRONMENT

Figure 1 represents the model that how antibiotic resistant bacteria and antibiotic resistant genes contaminate the environment. The infectious microbes that are correlated with antibiotic resistance genes in hospitals are a member of different human microbiomes[25] that are mostly present in air flow systems and hospital water[26-28]. In hospitals *de novo* resistance arise due to the presence of broad spectrum antibiotics over extended period of time for example during the treatment of consistent infections (chronic infections)[29]. Notwithstanding in-house development of resistance, pathogens carrying resistance genes may enter the medical clinics or hospital by means of contaminated patients, where they can proliferate epidemically or consolidate into another genetic background. The pervasiveness of various resistance genes empowers their combination into the equivalent hereditary background, for example through integrons, coming about multi-drug resistant pathogens, for example, MRSA. Therefore, hospitals have been under serious investigation[30]. Antibiotic resistant pathogens are found in hospital effluents such as; *P. aeruginosa enterococci* and *E. coli* carrying extended spectrum beta-lactamase[30,31]. Despite the fact that medical clinics are under broadened examination, they give generally controlled conditions to the utilization of anti-infection agents and resistance evolution which is moderately simple to follow (for example by means of testing of hospital effluent) and consequently curb.

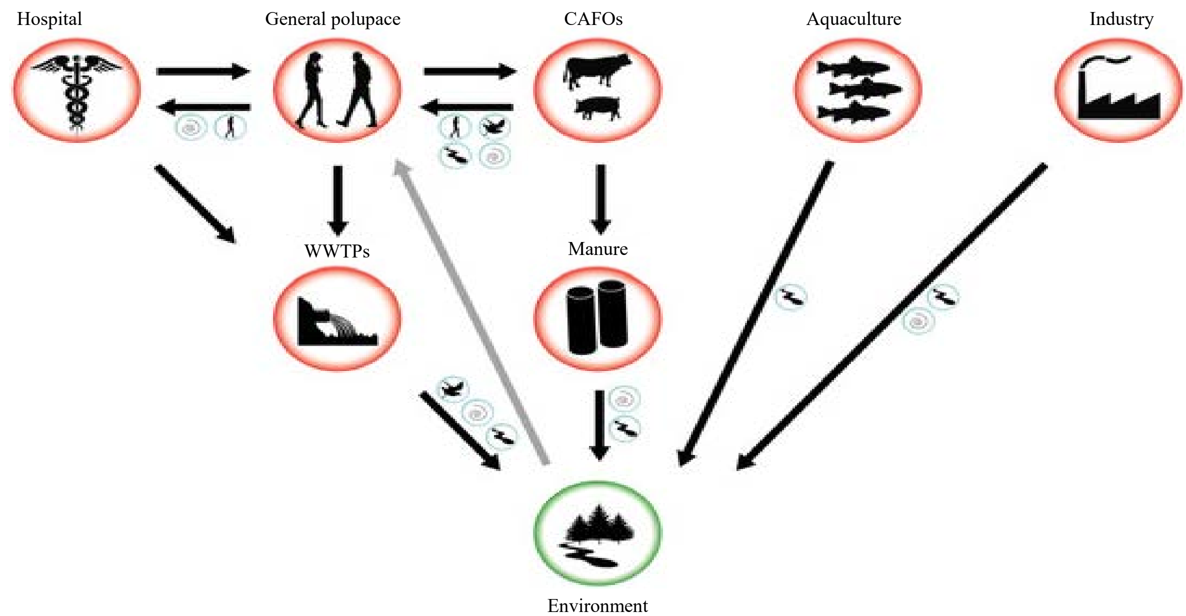

Fig. 1: Schematic flow of Antibiotic Resistance-carrying Bacteria (ARBs) and Antibiotic Resistance Genes (ARGs) from hotspots of evolution and transmission (red circles) to the environment (green circle). Blue circles indicate possible vectors that may aid transmission between specific environments including air, surface waters, humans and other animal vectors. Black arrows indicate known flows of ARBs and ARGs, grey arrow indicates a possible transmission route from a contaminated environment back to the general populace

Source: Kraemer *et al.*[24]

Conversely, anti-infection utilization by the general people is unsupervised. The development resistance genes in human microbiomes and particularly the gut microbiome, by means of in-home anti-toxins use can be a consequence of inappropriate utilization of anti-infection agents or inadequate treatment, bringing about sub-inhibitory anti-infection agents focuses *in situ*. Even when effectively utilized, around 70% of anti-microbial pass the human digestive system unaltered and are discharged through urine. These anti-infection agents, related to ARBs and ARGs are altogether joined in civil sewage and contaminate the populace[32]. Sewage from medical clinics and the general people are joined and eventually moved to WWTPs which utilize different natural and physio-chemical procedures to biodegrade sewage, decrease the quantity of pathogens and evacuate nitrogen and phosphorous before blending their release in with surface water. Sewage comprises an especially nutrient rich condition, supporting high concentration of microscopic organisms and is consistently seeded with new ARBs, ARGs and anti-infection agent's themselves[33,34]. That's why WWTPs are considered the dissemination of ARGs[35]. Independent of their application in human health, anti-infection agents are broadly utilized in the production of livestock Animals Feeding Operations (AFOs) and specifically large scale (more than 1000 animals) Concentrated Animal Feeding Operations (CAFOs), just as aquacultures[36]. Antibiotics in AFOs are not only used to treat intense diseases or long term infections, but also used to promote growth and development[37,38] prompting intensive selection on microbiomes related with livestock which bringing about manure and wastewater polluted with ARBs[39]. Manure storage decreases the total number of culturable microbes, yet regardless lagoons and manure have been demonstrated to be exceptionally advanced for ARBs[39-42]. Thus, manure is frequently utilized as fertilizer on close by crop lands, where ARGs and ARBs come into contact with the soil microbiome by means of run-off, ARGs and ARBs from excrement can arrive at both surface and ground water[37]. It is also reported that different agricultural activities to enhance the crop improvement and to control pests weeds are also involve in the resistance of microbes[43-47]. Antibiotic are also applied in extensive amount in fish farms for growth and development and prevention of different diseases. These amniotic are applied openly in ponds and open water aquaculture result is the widespread dissemination of

ARGs[48-50]. Anti-microbial resistance brought about by industrial wastes has gotten less consideration then AFOs and hospitals, but it is important to note that effluents from WWTPs enriched with the extensive concentration of antibiotics which contaminate the environment badly. Special attention should have taken towards industrial wastes to tackle the issue regarding antibiotic resistance.

DIFFERENT STRATEGIES TO TACKLE ANTIBIOTIC RESISTANCE

Removal of Antibiotic resistance through conventional wastewater treatment and advanced wastewater treatment process: Different studies reported the different ways to reduce the antibiotic resistance. As Hiller *et al.*[51] reported that the most efficient way to reduce the antibiotic resistance through advanced waste water treatment including membrane filtration, ozonation, UV-irradiation or chlorination etc (Fig. 2). These discoveries affirm that discharge from wastewater treatment plants can bring about noteworthy contributions of AMR to the aquatic environment. This discharge of pathogenic micro-organisms conveying anti-microbial resistance into surface waters may represent a potential danger to human wellbeing, particularly if accepting waterways are dependent upon downstream use, for example for recreational purposes or drinking water reflection. Another significant task is the utilization of standard scientific strategies to observe the presence of antibiotic resistance genes and to check evacuation efficiencies by various treatments. Developmental strategies empower the identification of living beings with antibiotic resistance yet are constrained to the assurance of viable and cultivable microscopic organisms. Interestingly, qPCR give a fast identification technique to antibiotic resistance genes revealing in this manner an "antibiotic resistance potential", yet are inadequate with regards to data on the intense risk, as results are not connected to pathogens. Conventional wastewater treatment plants having the capacity to lessening AMR by different orders of magnitude micro and ultra-filtration utilized in membrane bioreactors can give a productive boundary to antibiotic resistance in wastewater. Likewise progressed non-submerged membrane treatment of wastewater effluent will most likely bring about comparative execution. Key parameter for the maintenance of micro-organisms and genes is the pore size of the membrane, yet in addition the arrangement of cake layers may add to in general execution. Accessible outcomes from granular media filtration considers are of fundamental nature since significant operational parameters were frequently not indicated. The expulsion of ARGs by oxidation and sterilization is firmly identified with the method of activity of the individual oxidants. Purification forms give a proficient hindrance to pathogenic microscopic organisms regardless of whether they convey AMR. Nonetheless, it is important that ozonation of wastewater frequently designed for expulsion of trace natural synthetic compounds at which dose can't be viewed as a dependable cleansing procedure as it normally accomplishes around 1-2 log inactivation for microbes.

Removal of ARGs by membranes ranging from micro-filtration to reverse osmosis process: According to the Fig. 3, it has been reported by Slipko *et al.*[52] that free DNA

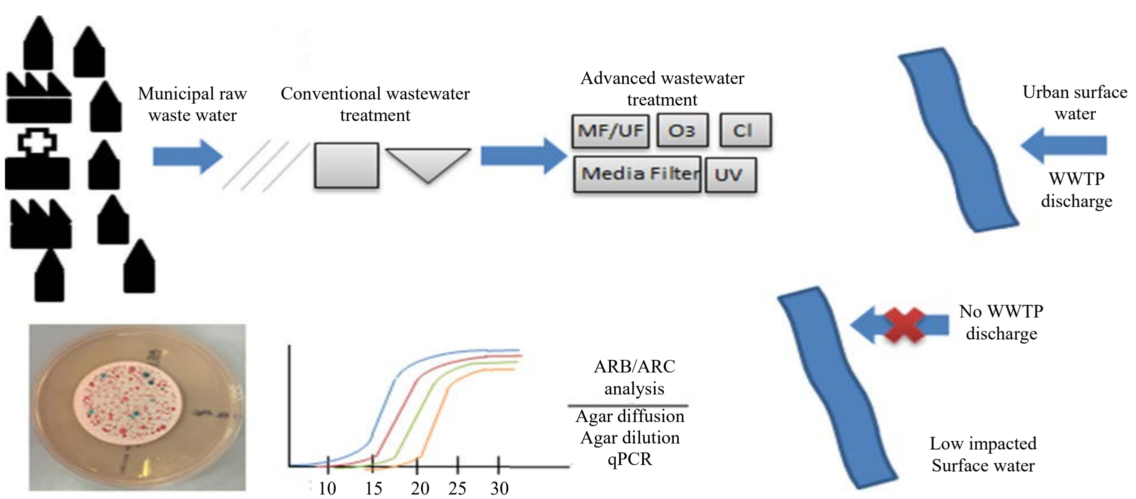

Fig. 2: Removal of antibiotic resistance through conventional wastewater treatment and advanced wastewater treatment

Source: Hiller *et al.*[51]

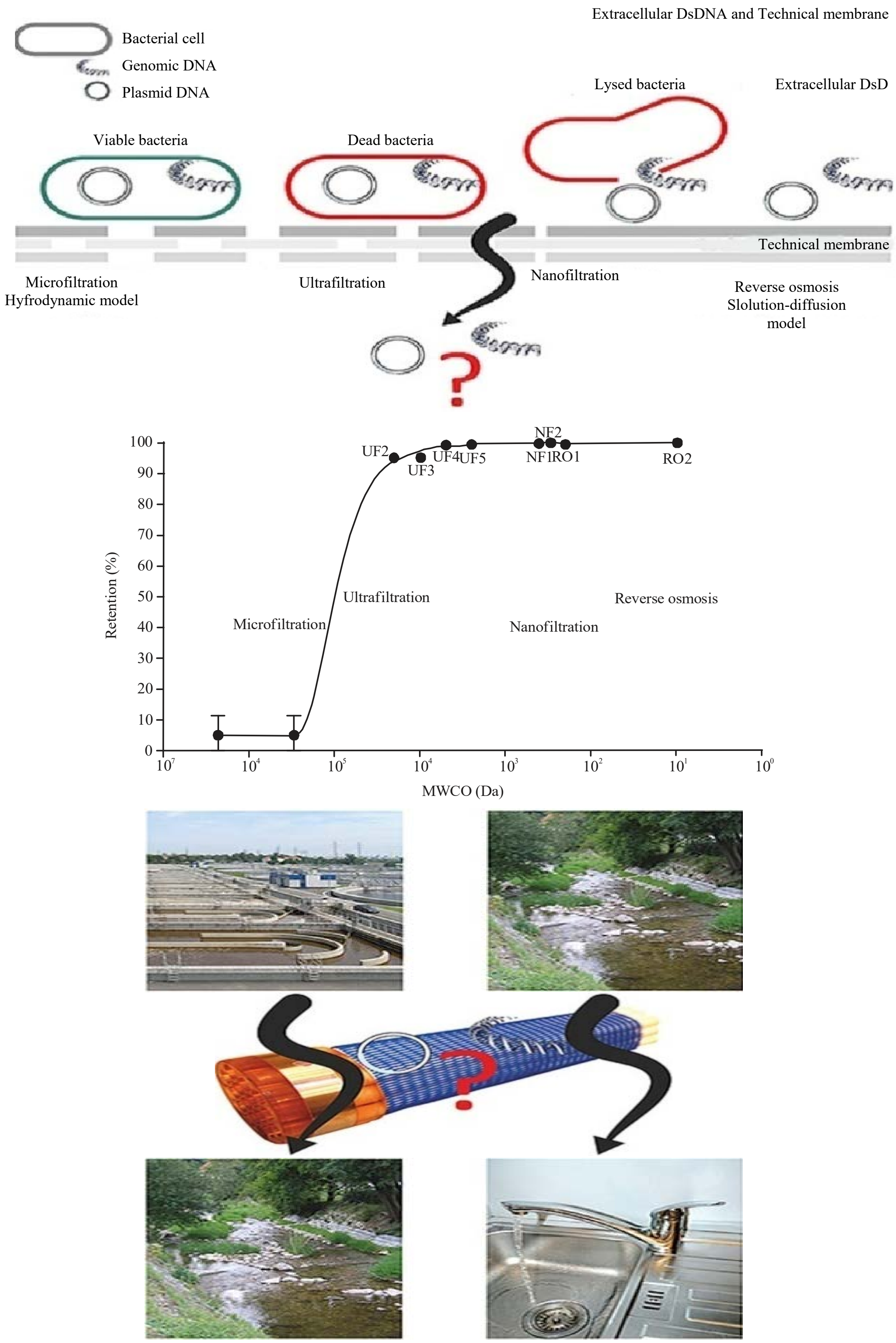

Fig. 3: Removal of extracellular free DNA and antibiotic resistance genes from water and wastewater by membranes ranging from microfiltration to reverse osmosis

Source: Slipko *et al.*[52]

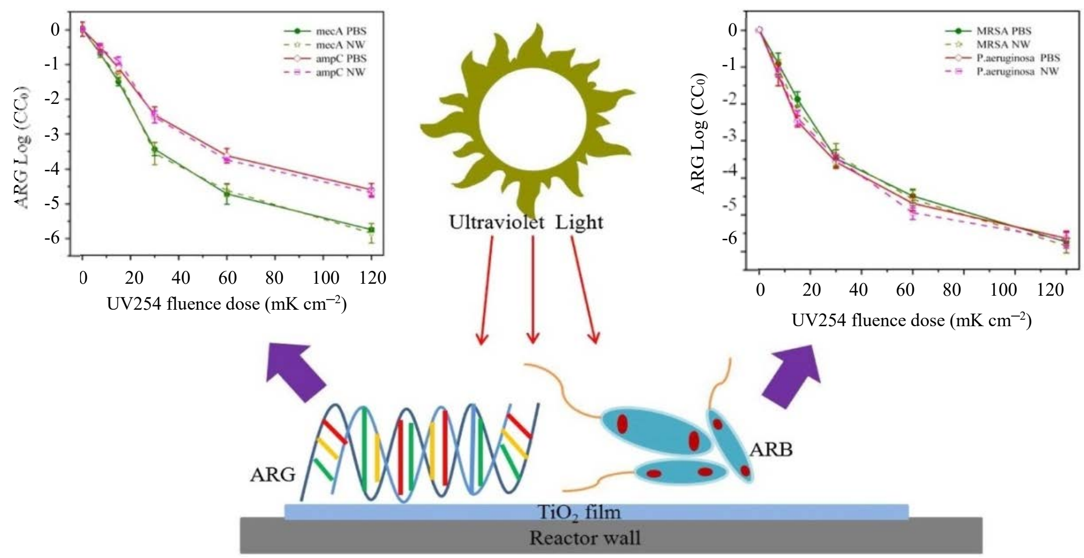

Fig. 4: Antibacterial resistance removal by photocatalysis of H_2O_2 and/or TiO_2 under UV irradiation
Source: Guo *et al.*[53]

demonstrated a capacity to penetrate through the pores of tested ultrafiltration membranes, which were altogether smaller than its size, because of the prolongation or distortion of the molecules in the joining flow field. Also, free DNA has the ability to permeate through the nano-filtration and reverse osmosis membrane. Maintenance of free DNA molecules was predominantly rely on size prohibition. Moreover, adsorption of free DNA particles on the surface of neutral membrane play a vital role in counteraction of penetration of free DNA particles. Adsorbed particles blocked further entry through the film and dribe back the particles moving toward the layer. While in negatively charged membranes, adsorption was decreased because of repulsion between free DNA and the membrane surface, which resulted in lower retention values. This study reported that molecular weight cut off above 5000 Da and not negatively charged, may be helpful in the effective reduction of the dissemination of ARGs.

Removal of antibiotic resistant bacteria through photocatalysis of H_2O_2 and/or TiO_2 under UV irradiation: Guo *et al.*[53] reported the removal of ARB and ARGs from aqueous solution by potential alternative method and observed that approximately 4.5-5.0 and 5.5-5.8 log ARB reductions were achieved by TiO_2 under 6 and 12 mJ cm^{-2} UV254 fluence dose, respectively (Fig. 4). For ARGs, 5.8 log mecA reduction and 4.7 log ampC reduction were achieved under 120 mJ cm^{-2} UV254 fluence dose in the presence of TiO_2. Also, reported that high concentration of H_2O_2 increasing the capability of removal of both ARB and ARGs. Intracellular and extracellular form of resistance also removed by the photocatalysis of TiO_2.

Removal of ARB and ARGs by UV-activated persulfate: There is another process, UV-activated persulfate (UV/PS), which is an advanced oxidation process for the Removal of ARB and ARGs reported by Zhou *et al.*[54] (Fig. 5). It was reported that the inactivation efficiency by UV/PS process of Macrolides Resistant Bacteria (MRB), Sulfonamides Resistant Bacteria (SRB), Tetracyclines Resistant Bacteria (TRB) and Quinolones Resistant Bacteria (QRB) reached at 96.6, 94.7, 98.0 and 99.9% in 10 min. The UV/PS likewise indicated noteworthy expulsion productivity on ARGs. The decrease of total ARGs arrived at 3.84 orders of magnitude in UV/PS which is more than that in UV by 0.56 log. Especially, the evacuation of Mobile Genetic Elements (MGE) which may support the horizontal gene transfer of ARGs among various microbial accomplished 76.09% by UV/PS. High-throughput sequencing uncovered that UV/PS changed the microbial network. The extents of proteobacteria and actinobacteria that posture human wellbeing dangers were 4.25 and 1.6% not as much as UV, individually. Co-occurrence demonstrated that ARGs were differentially contributed by bacterial taxa. In UV/PS framework, hydroxyl radical (•OH) and sulfate radical (SO_4•$^-$) added to the evacuation of microscopic organisms and ARGs. This examination gave a new technique for UV/PS to evacuate ARGs and ARB for wastewater treatment.

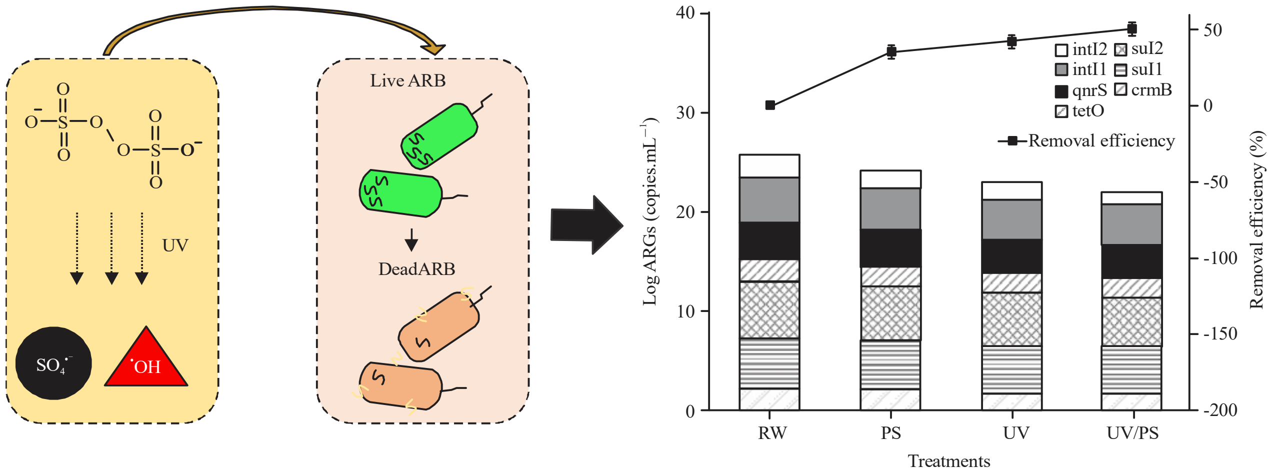

Fig. 5: Removal of antibiotic resistance through UV-activated persulfate

Source: Zhou *et al.*[54]

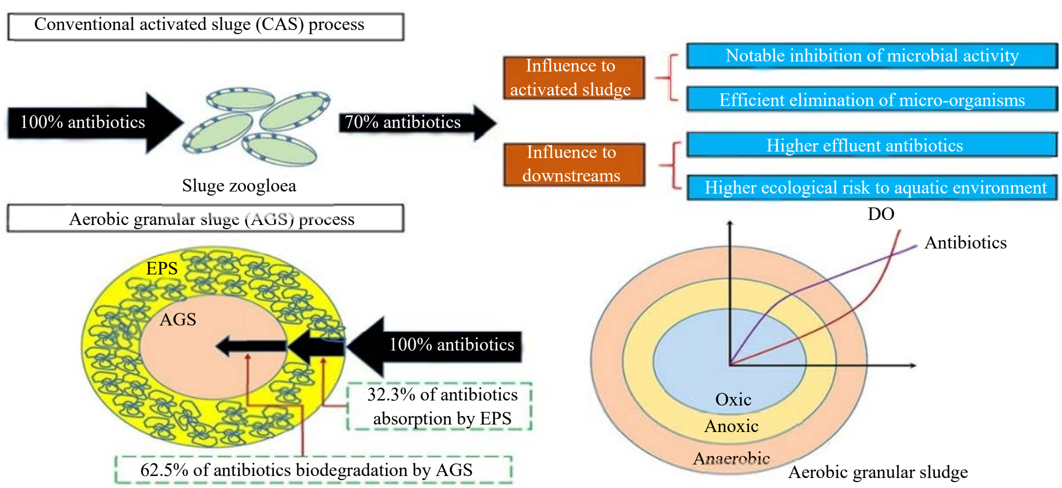

Fig. 6: Piggery wastewater treatment by aerobic granular sludge: Granulation process and antibiotics and antibiotic-resistant bacteria removal and transport

Source: Wang *et al.*[55]

Removal of antibiotic resistance by conventional activated sludge process: Wang *et al.*[55] reported that Aerobic Granular Sludge (AGS) was prevailing in the bioreactors at day 45 and the moderately high protein content from firmly bound extracellular polymeric substances (TB-EPS) encouraged high-aerobic granulation and maintained biomass stabilization. The protein substance in EPS and TB-EPS were emphatically associated with relative hydrophobicity, in this way improving the adsorption limit among hydrophobic particles. The compound oxygen request (COD), NH_3-N and absolute N expulsion efficiencies were 98.0, 97.0 and 92.4%. Five anti-infection agents, including kanamycin, tetracycline, ciprofloxacin, ampicillin and erythromycin were inspected in piggery wastewater, with focuses up to the fixation scope of 29.4-44.1 μg L^{-1} and the all out anti-toxins evacuation rate came to up to 88.4$\pm$4.5%. An aggregate of 5.2% of the all out anti-infection agents were released from bioreactors and 62.5% of the absolute anti-infection agents were degraded and 32.3% of complete anti-toxins were adsorbed by oxygen consuming granules. The nearness of anti-infection agents once in a while showed an effect on AGS development and the moderately high microbial action of vigorous granules was advantageous to anti-infection agent's expulsion. The ARB evacuation rate expanded up to 89.4$\pm$3.3%, however, a lot of ARB was enhanced in oxygen consuming granules (Fig. 6).

CONCLUSION

Antibiotic resistance is the current public health concern causing various threats to human beings and environmental pollution is the main cause of resistance. Different harmful microbes who are not only resistance to single antibiotic but against multiple antibiotics. It is also noted that the effects of antibiotic pollution are expected to be especially disruptive in aquatic environments. There are numerous ways to combat the antimicrobial resistance as discussed like UV radiation, conventional waste water treatment process osmosis etc. More efficient polices should be introduced to combat the resistance is highly required.

SIGNIFICANCE STATEMENT

The current study highlighted that how the antibiotic polluting our environment day by day. Different ways are also described to eradicate the antibiotic resistance. Further study is needed to generate different practices for the removal of antibiotic resistance from the different area of environment because if this threat remains the same our future population will affected badly.

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