



Threats from antibiotics: A serious environmental concern

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ABSTRACT

Antibiotics are bioactive substances, used as human and animal medicines for illness prevention, disease treatment and growth promotion. They are considered to be pseudo persistent given their continuous input in the environment. Antibiotics enter agro ecosystems through several routes such as wastewater irrigation, soil application, animal manures or bio-solids which are mostly biologically active thus creating potential risks to the environment. They are present in different environmental matrices at low concentrations as residues. Antibiotic residues enter the environment primarily via urine, feces and manure from humans and animals after they have taken the medication, as well as from manufacturing wastewater. These residues contaminate the soil, surface water, and groundwater by leaching or runoff and ultimately breed antibiotic-resistant bacteria (ARB) and genes (ARG). These triune threat viz antibiotics, ARB and ARG have not been effectively removed by various treatment in wastewater treatment plants. Here, we put together existing knowledge and aim at providing in-depth knowledge to the extent to which a wide range of treatment processes determine the ultimate fate of antibiotic-bred threats (ARB and ARG) in conventional and advanced wastewater treatment. The use of antibiotics is inevitable, hence studies focusing on minimizing their discharge into the environment viz-a-viz support future regulatory measures are of great importance.

1. Introduction

Antibiotics have gained significant attention due to their widespread use in diseases treatment caused by both pathogenic and non-pathogenic bacteria. Their role in the contamination of surface water, groundwater, and environmental soil is on the increase. The pollution caused by excessive use of antibiotics is a potential threat to human health as it promotes antibiotic-resistant bacteria (ARB) breeding. ARB and antibiotic resistance genes (ARGs) have been detected in different media, such as drinking water [1], surface water [2] and soil [3]. As a result of their threat to public health, the World Health Organization (WHO) has categorized their spread as one of three most serious threats to public health in the 21st century [4]. Extensive use of antibiotics has led to the emergence of both ARB and ARG, which poses serious threat to human health [5]. In wastewater treatment plants (WWTPs), antibiotics are only moderately removed [6] thus ARG proliferation may occur and eventually spread in the environment [7,8,9]. In order to trim down the risk of ARB&ARGs emission to the environment, it is essential to study their outcome and abundance during the improved

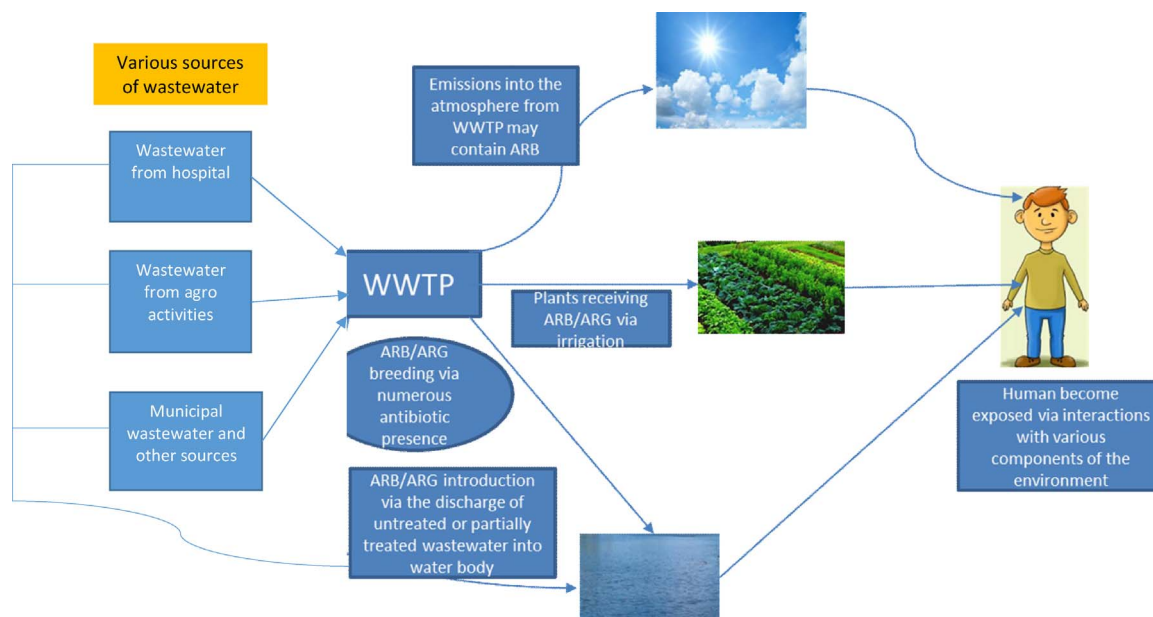
elimination of antibiotics. This review aims at considering the various studies on the occurrence as well as removal of antibiotic-resistant bacteria and genes from wastewater effluents and environmental samples, the various challenges and future prospects militating against their resistances are also discussed.

2. Antibiotic-resistant bacteria: a silent threat in the environment

Effective wastewater treatment has been well advertised with serious focus on industries and municipal wastewater, only little attention has been given to treatment of hospital and other health care facility wastewater. Wastewater from health care facility may be a source of serious antibiotic pollution as well as platform for the growth of resistant bacteria [10]. Although antibiotics can be released into the water bodies via other sources such as the urban wastewater treatment plants, runoff from agricultural activities, indiscriminate disposal of expired and unused prescriptions as well as effluents from pharmaceutical industries. Whatever the source of antibiotic in water, the concern remains the generation of antibiotic-resistant gene (ARG) and

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Possible breeding site for ARB/ARG and their human exposure routes

Fig. 1. Possible breeding site for ARB/ARG and their human exposure routes.

antibiotic-resistant bacteria (ARB) with negative consequence of curative effects of antibiotics against human and animal pathogens [11]. ARB effects has become a global concern and their impact may vary depending on the propagation environment. In the face of resistant infection, mortality rate may increase while longer treatment or more potent antibiotic may be required for treatment in some cases [12]. Worldwide water scarcity has made treated wastewater reuse a choice to embrace, therefore identification and effective removal of ARB in wastewater before their reuse for potable or non-potable purposes is highly important. For instance, vegetables cultivated using such treated wastewater containing ARB when consumed raw or semi cooked could pose major health risk to man [13] (Fig. 1).

3. ARB in wastewater treatment plants (WWTP) and environmental samples

The wastewater goes through three treatment processes in the treatment plant. The primary treatment which aims at removal of large visible particle by trapping or sedimentation, the secondary treatment removes dissolved organic matters via aerobic decomposition while the purity of the wastewater is further enhanced via tertiary treatment process such as filtration, adsorption, chemical oxidation, ozonation and chlorination. While a broad range of pollutants can be removed at the tertiary treatment stage, very low concentration (ng/L to µg/L level) of pollutants, particularly pharmaceuticals persist through the treatment process [14]. WWTP are therefore considered the major source of antibiotic and ARB propagation into the water environment [15]. ARB existence in environmental samples does not occur as a single strain, rather, they are mixed with numerous species. Two distinct methods (Agar diffusion and growth susceptibility test) are generally employed in determining the antibiotic concentration at which the growth of bacteria is inhibited [12].

3.1. ARB occurrence in WWTP

Various studies on ARB presence in WWTP and environmental samples have been reported; Huang et al., 2012 [12] studied the average antibiotic tolerance and antibiotic resistant bacteria of effluent

from a WWTP in Beijing, China. Sedimentation followed by anaerobic-anoxic process are employed in the investigated treatment plant. Resistance of the secondary sample was tested against six antibiotics (Penicillin, Ampicillin, Cephalothin, Chloramphenicol, tetracycline and rifampicin). Their findings include high tolerance of the bacteria to Penicillin, Ampicillin, Cephalothin, and Chloramphenicol as compared with tetracycline and rifampicin. While about 99% decrease in the total heterotrophic bacteria was observed when secondary sample was exposed to tetracycline and rifampicin, less than 50% decrease was observed when sample was exposed to the other antibiotic. They inferred that

there could be prevalence of Penicillin, Ampicillin, Cephalothin, Chloramphenicol and rifampicin-resistant bacteria in the tested sample since the concentration of antibiotic required to inhibit 50% of total heterotrophic bacteria i.e. IC_{50} and highest minimum inhibitory concentration i.e. MIC (IC_{50}/MIC) ratio for these antibiotics were all greater than unity. Further investigation which used isolate of five different enterobacteria revealed the presence of multiple antibiotic resistant bacteria. Goldstein et al., 2014 [16], obtained, samples from four WWTP in the US with two from each region. These were investigated for total enterococci as well as enterococci resistant to vancomycin using standard membrane filtration for isolation of both. WWTP in region A is said to receive wastewater from domestics and hospitals while region B receives wastewater from domestics and agriculturally influenced storm water. The treatment in the two categories advances to chlorination and dichlorination levels and both the influent and effluent were investigated. Their results revealed that about 3% of the total enterococci were resistant to vancomycin in the two categories of WWTPs investigated. Although total enterococci was observed in all the samples (across treatment stages) investigated, however chlorinated effluents were found free of vancomycin resistant enterococci (VRE). It was also reported that all isolated VRE were also resistant to some other antibiotics [16]. Samples obtained from WWTP in Italy have also been investigated for presence of ARB. Wastewater sources were basically domestics and hospitals and the tertiary treatment stage used per acetic acid for disinfection. The studies focused on investigation of total coliform in both influent and effluent of the treatment plant as well as studies concerning *E. Coli* and their ampicillin and chloramphenicol

resistance. The bacterial was isolated from the collected sample using a definite diameter nitro cellulosic membrane. Removal of ARB at the biological treatment level was very negligible even when antibiotic up to 32 µg/ml was used. However, rapid decrease of ARB was observed after the disinfection with only 8 µg/ml ampicillin. About 78% of the resistant *E. Coli* were reported resistant to 32 µg/ml ampicillin [17]. Studies of a certain WWTP in Jeddah, Saudi Arabia have also been reported. Conventional aerobic activated sludge treatment and subsequently chlorination are parts of treatment methods employed in this WWTP. Bacteria isolates of the influent, effluent and chlorinated samples were tested for resistance to eight different antibiotics. Percentage of the total bacteria isolated resistant to ampicillin was found the highest (78%). Resistance to ciprofloxacin and meropenem were not as common (6.6 and 12%) respectively. Chlorination however triggered resistance of some bacteria to meropenem as about 24% of the chlorinated sample isolates was found resistant to meropenem. Non resistant bacteria were only about 10% of the total isolate [18].

3.2. Impact of ARB containing wastewater on the environment

The discharge of ARB containing wastewater/treated wastewater and antibiotics into the environment has taken their toll on the diverse components of the environment their habitat as well as various environmental samples. Wastewater and surface water samples have been investigated for various enterococci specie as well as antibiotic resistant enterococci. Wastewater collected across lakes, rivers, municipal sewage, wastewater treatment plants, stagnant water, industrial effluents and hospital sewage were analyzed for enterococci and tested for resistance to vancomycin, teicoplanin, ampicillin, trimethoprim-sulfamethoxazol, chloramphenicol, tetracycline, erythromycin and ciprofloxacin. Results revealed that about 75% of the sample had enterococci presence with 78% of surface water being polluted with enterococci. *E. faecium* was reported to be the most prevented enterococci. Over 90% of the isolate was reported to be resistant to trimethoprim-sulfamethoxazol while resistance to erythromycin and tetracycline were reported as 44 and 18% respectively. Cases of multidrug resistance were also observed for a number of species, these were found to be higher in wastewater than surface water [19]. The impact of multiple antibiotic-resistant bacteria from treated sewage effluent on fresh water habitat has been reported. The sewage tank receives wastewater basically from domestic and industrial sources. Mahmoud and co-workers [20] reported their investigations on Water, soil and snail samples collected from the pond that receives excess treated water. Isolation of total bacteria followed the membrane filtration separation techniques while isolates were tested for resistance against 12 antibiotics. Interestingly, snail was reported to have the highest bacteria count. Most of the isolated specie had resistance to ampicillin while no specie was reported to be resistant to *sulfa methoxazol* [20]. The soil samples of an active agricultural site [21] with different pattern of irrigation system have also been investigated for MRB presence. While a part of the agro site receives irrigation with untreated wastewater till the time of the investigation, another part of the site was never irrigated and irrigation was discontinued for the third part some years into their study. The soil samples were taken in three depths and multidrug resistant bacteria (MRB) as well as faecal contamination were subject of their study. While twenty four antibiotics were used in their study, ampicillin was used as the resistant strain isolation. Their results indicated high prevalence of MRB in soil sample that is still being irrigated than the soil samples for which irrigation had been discontinued while the non-irrigated soil samples showed very minute MRB presence. The depth of sample did not portray a reasonable difference in the MRB content. Coliform and salmonella was reported found across the three sampled area [21]. The resistance of *Pseudomonas aeruginosa* isolated from plant samples against a range of antibiotics have also been reported. Their results stated that *Pseudomonas aeruginosa* was resistant to thirteen antibiotics amongst which are amoxicillin, chloramphenicol, rifampicin

e.t.c while susceptible to seven other antibiotics. Their results also showed the intermediate resistance of *Pseudomonas aeruginosa* to some antibiotics (aztreonam, meropenem and imipenem) which are normally considered relevant anti pseudomonas drugs [22]. Irrigation water (from deep or shallow well) and house hold farm vegetables have been investigated for total coliform, faecal enterococci as well as *E. Coli* susceptibility to sixteen antibiotics. A nitrocellulose membrane filter with pore size of 0.45 µm was used for *E. Coli* isolation. Their results revealed high total coliform in investigated samples and more than 50% of the sample had enterococci with minimum value 2 CFU/100 ml and maximum value 62 CFU/100 ml. They reported that *E. Coli* isolated were found resistant to all antibiotic tested except piperacillin/tazobactam and resistance to streptomycin was very common in both water and vegetable samples, high resistance was also reported for tetracycline and ampicillin [23].

4. Efforts towards ARB disinfection

Wastewater reuse particularly for agricultural purposes has been well embraced worldwide [24,25]. However, several risk factors come with this water scarcity mitigation [26]. For instance, soil original physico-chemical and microbiological properties of the soil may be altered via irrigation, accumulation of chemical and biological contaminants may also occur in irrigated soil thus reducing soil productivity and posing health risks to human. Hence, an effective irrigation system must effectively eradicate challenges of contamination and health risks. Such contaminants of concerns are pathogenic bacteria and ARB. Various methods of ARB disinfection has been investigated and highlighted in Table 1;

Conclusively, most conventional wastewater treatment techniques are known to be deficient in removal of very low concentration of pollutant, only adsorption process satisfies this challenge [41,42]. The wide usage of antibiotic and their persistence through the various treatment techniques is the reason for ARB existence in wastewater and also the environment [41]. UV radiation/photocatalysis and chlorination have been well used for removal of ARB from wastewater (See some in Table 1). ARB removal is both antibiotic type and treatment method dependent. UV treatment and chlorination are the most explored methods. Challenges with UV treatment may vary through antibiotic resistant selectivity, fluence dependency and high financial demands [32]. Reactivation and regrowth of bacteria is also a major challenge recorded with the use of UV as well as chlorination. For UV irradiation, inactivation is made possible first by changes and damage of the external membrane, subsequently, cell wall damage occurs [11]. Bacteria have the ability to repair themselves at this level giving room to reactivation and regrowth. Very high irradiation will however result in serious cell wall damage. Since inactivation of these bacteria occurs via reactive oxygen specie (ROS) attacks, ROS gains access to the internal cell and consequently destroy the bacterial. Very high irradiation requires a lot of energy thus economically unfriendly.

Sorption techniques in tertiary treatment is scarcely considered, yet this can give excellent result when adsorbent are uniquely selected. For instance, nanomaterials (NMs) toxicological effects on aquatic organisms [43] and soil microorganism community [44] suggests that NMs may effectively deal with ARB in aqueous media. NMs large surface area and tiny size aids their interactions with biological specie. Removal of bacterial from aqueous media by sorption as well as bacterial capturing and inactivation using NMs have been widely reported [45–53]. Considering sorption techniques with specific attention on sorbent selection in tertiary wastewater treatment can offer great advantages. The opportunity of sorbent surface modification can enhance selective removal of a targeted ARB, about a hundred percent removal of bacteria/ARB may also be possible and reactivation/regrowth of bacterial can as well be avoided [54].

Table 1
Various methods of ARB removal as reported in literatures.

Methods of disinfection	Drug type resistant	Method effects and summary of findings	References
Chlorination	PEN, AMP, TC, CHL, RIF	Inactivation of HPC and ARB were effective at high Chlorine dosage (25 mg Cl ₂ /L). PEN- and AMP-RB decreased with increased chlorine concentration. CHL-RB however jacked to almost 100% at 10 mg Cl ₂ /L. Reactivation and regrowth of HPC and ARB after chlorination was reported. High chlorine concentration with short exposure treatment was recommended.	[27]
Solar photo-Fenton	OFX and TMP	Complete removal of OFX- and TMP-resistant bacteria was reported after 180 min of solar fenton treatment.	[28]
Solar radiation & chlorination	AMX, SMZ and CIP	For the two categories of MDR isolated, less than 0.1% of the E. Coli was left in the UWWTP. Treatment conditions of 2 mg/L chlorine dosage and 60 min contact time were used. Chlorination however did not affect the resistance of E. Coli to the selected antibiotics. Low inactivation rate was observed for the solar radiation treatment. Not more than 60% removal was reported for the two categories of MDR isolates. The authors stated that the inactivation rate obtained is quit low compared to previously reported works on E. Coli. Solar radiation had no effect on the resistance of two categories of isolates to AMX and SMZ.	[29]
UV	CEP, CIP, ERY, GENT, VAN, SD, RIF, TC, CHL	Inactivation of total heterotrophic bacteria at fluence of 5.34 MJ/cm ² CEP-, CIP-, GENT-, RIF-, TC- and CHL- resistant bacteria were completely inactivated at fluence of less or equal to 20 MJ/cm ² ERY-, SD- and VAN-resistant bacteria decreased with increasing fluence.	[30]
UV	ERY and TC	TC-resistant bacteria increased from 3% to 5% and 4% as fluence increased to 5 and 10 MJ/cm ² respectively. ERY-resistant bacteria however decreased to 22% and 12% at the same fluence respectively.	[31]
UVA/LED (Using TiO ₂ and H ₂ O ₂)	AMP and STP	ARB inactivation was faster as intensity of light increased, inactivation rate also depended on amount of photon flow and catalyst used. It was reported that persistence of H ₂ O ₂ in water resulted in residual disinfecting effects both after photocatalytic process and at the point of photoreaction. Although photoreactivation was not reported when solution was treated with UVA/LED/TiO ₂ , the ARB however gradually decreased below detection limit.	[32]
Photocatalysis (Using solar simulated N-doped TiO ₂)	CIP, CEF, TC and VAN	Irradiation gave a fast bacteria inactivation. Photocatalytic process affected CIP- and CEF-resistant E. Coli. However, no significant effect of photocatalytic treatment was recorded for TC- and VAN- resistant bacteria.	[33]
Chlorination, E-beam and ozone Using H ₂ O ₂ , S ₂ O ₈ and MPS	TC	The disinfection using ozone used only 3 mg/L ozone concentration. However, about ten times this quantity of chlorine was used to disinfect ARB. Addition of catalyst enhanced disinfection with MPS giving the best result.	[34]
Solar driven treatment and Chlorination (Using H ₂ O ₂ /sunlight, TiO ₂ /Sunlight H ₂ O ₂ /TiO ₂ /Sunlight, solar photo-fenton)	CIP, AMP and TC	TiO ₂ /sunlight Inactivation rate of MDR E. Coli was faster and used a lower energy when compared with ordinary sunlight. While complete inactivation of MDR enterococcus strain was achieved in 60 min using solar radiation/50 mg/L TiO ₂ , only half initial concentration of MDR E. Coli was inactivated. Chlorination resulted in complete inactivation of total MDR E. Coli within 15 min while H ₂ O ₂ /sunlight treatment achieved same result in 90 min. The reactivation of MDR was observed for H ₂ O ₂ /sunlight and Chlorinated samples with reactivation rate of the former lower.	[35]
UV-C	ERY	Inactivation of E. Coli was observed to be faster when compared with that of ERY-resistant E. Coli. While total E. Coli was inactivated within 45 min of UV-C irradiation, ERY-resistant E. Coli took twice this time.	[15]
Chlorination and photocatalysis (Using H ₂ O ₂ , TiO ₂ and N-TiO ₂)	CIP, AMP and TC	Total inactivation of AR E. Coli was obtained within 45 min of treatment with H ₂ O ₂ /UV. The effectiveness of N-TiO ₂ was reported to be related to methods of preparation. N-TiO ₂ prepared at -20 °C gave a better performance with total inactivation within 120 min treatment. Inactivation of AR E. Coli was achieved within 25 min contact of 1.0 mg/L chlorine dosage.	[36]
UV/H ₂ O ₂	Indigenous ARB	This technique successfully inactivates antibiotic-resistant E. Coli after 240 min of irradiation. Inactivation rate increased with H ₂ O ₂ consumption.	[37]
Ozonation	ERY, EtP	Low dissolved ozone was used for the study (0.1–0.5 mg/L). Ozonation of 15 min completely inactivated HPC as well as ERY- and EtP-resistant E. Coli.	[38]
UV/H ₂ O ₂	AMP, CIP, TC	Although relatively low consumption of H ₂ O ₂ applied. Inactivation rate was observed to increase with increased UV/H ₂ O ₂ contact time	[39]

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Table 1 (continued)

Methods of disinfection	Drug type resistant	Method effects and summary of findings	References
UV radiation (Using H ₂ O ₂ and TiO ₂)	MTC	Removal of ARB by TiO ₂ only is negligible. ARB inactivation was however significant when UV irradiation was combined with TiO ₂ treatment. Both methicillin-resistant bacteria and multidrug-resistant bacteria were effectively inactivated.	[40]

Note: Cephalexin (CEP), Ciprofloxacin (CIP), Erythromycin (ERY), Gentamicin (GENT), Vancomycin (VAN), Sulfadiazine (SD), Rifampicin (RIF), Tetracycline (TC), Chloramphenicol (CHL), Cefuroxime (CEF), Ethylparaben (EtP), Penicillin (PEN), Ampicillin (AMP), Ofloxacin (OFX), Trimethoprim (TMP), Methicillin (MTC), Streptomycin (STP), Amoxicillin (AMX), Sulphamethoxazole (SMZ), Peroxymonosulphate (MPS), Total heterotrophic bacteria (HPC).

5. Antibiotics resistance genes: abundance and diversity in waste water treatment plant

Wrong use and high use of antibiotics is one major promoter of antibiotic resistance. Due to the importance of antibiotics in various applications, the abundance of resistance bacteria and genes continues to increase [55]. Studies indicating such abundance in WWTP as well as other environmental samples are presented here.

5.1. ARG occurrence in WWTP

Five municipal WWTPs in Central eastern Tunisia have been investigated for abundance of ARGs. Only one of the investigated WWTPs employs a tertiary UV disinfection treatment while three used conventional activated sludge process as secondary treatment technique. Influent and effluent water samples were investigated and six ARGs including bla_{CTX-M} were selected for the study. Presences of the ARGs selected were observed in all the samples. However, bla_{CTX-M} and qnrS genes were absent in two of the WWTPs investigated. Sul1 and int1 genes concentrations seems to be unaffected by treatments across the WWTPs. WWTP using UV treatment still had its effluent rich in bla_{CTEM}, qnrA and sul1 genes [56].

Two wastewater treatment plants in China, one receiving wastewater basically from domestic and the other receiving industrial and domestic wastewater have been investigated for ARG presence. Disinfection techniques employed within the two systems is chlorination and the contact dose was reported to be 5 mg/L. Samples were collected across ten points including the dewatered sludge, thirty ARGs were considered for this study from which twenty three were detected in high quantity. Both sewage and sludge had high sulfonamide (sul1 and sul2) while quinolone resistant genes were found to be of lowest quantity. While they achieved significant decrease in ARG after treatment, dewatered sludge harbors quit a high volume of ARG and this could be a serious threat to the soil environment [57]. Studies reporting the effects of various pH on the behavior and distribution of tetracycline resistant gene (TRG) have been conducted and reported. Studied pH ranged between 4 and 10 and five genes were quantified via real time quantitative. Their findings reveals that acidic condition aids TRG propagation as the quantity of assessed TRG increased at low pH compared with neutral and influent sludge. The alkaline media however reduced the quantity of TRG [58]. Studies investigating the fate of ARGs (sul and tet) in a long term fermented sludge. Samples obtained from secondary sedimentation tank of municipal wastewater treatment tank in Shanghai have been reported. Alkaline fermentation accounted for better removal of ARG than the usual conventional methods. All investigated ARG were found in abundance in dried sludge, this however greatly reduced when sludge was fermented at pH of 10. Their conclusion hinges on environmental protection via ARG removal from sludge by fermentation [59]. Zhang et al. [60], have also reported the possible characterization of ARG in activated sludge subjected to pressure of some common antibiotics. Proliferation of ARG carrying bacteria was strong under the selected antibiotics thus increasing number of ARGs in activated sludge when tetracycline and sulfamethoxazole were introduced. Statistical analysis showed that all

identified tet genes (A, C, G, K and M) and sul1 was reported to be strongly correlated with int1 [60].

A study by Guo et al., 2017, [61] used Illumina high-throughput sequencing to investigate the broad-spectrum profiles of both ARGs and mobile genetic elements (MGEs) in activated sludge and anaerobically digested sludge from a full-scale wastewater treatment plant. A pipeline for identifying antibiotic resistance determinants was developed that consisted of four categories: gene transfer potential, ARG potential, ARGs pathway and ARGs phylogenetic origin. The metagenomic analysis showed that the activated sludge and the digested sludge exhibited different microbial communities and changes in the types and occurrence of ARGs and MGEs. But in total, 42 ARGs subtypes were identified in the activated sludge, while 51 ARG subtypes were detected in the digested sludge. Additionally, MGEs including plasmids, transposons, integrons (int1) and insertion sequences (e.g. ISSsp4, ISMSa21 and ISMba16) were abundant in the two sludge samples [61]. The co-occurrence pattern between ARGs and microbial taxa showed by network analysis indicated some environmental bacteria (e.g. Clostridium and Nitrosomonas) might potentially harbour multiple ARGs. The finding improves our understanding of WWTPs as hotspots of ARGs and MGEs, thereby preventing their release into the downstream environment.

5.2. Impact of ARG containing wastewater on the environment

Like ARB, the impact of ARG on the environment has been observed [62]. ARG release into the environment can be a source of serious risk to human as it interacts with components of the environment which serves as source of development to human. Investigations showing the presence of this threat in the environment are of great importance. Various studies on ARG abundance in environmental samples have been reported. The influent, activated sludge, effluent as well as water samples from the canal receiving treated effluents from Lucun WWTP have been investigated for the abundance of bacteria and ARGs. The sewage treatment process was reported to be effective as it eliminated over 70% of pathogens in the influent wastewater. ARG was also greatly decreased by the biological wastewater treatment technique to the tune of 6.82% and 5.72% for activated sludge and final effluent respectively. The impact of WWTP on the water body was observed by increased ARG particularly at the downstream of the river [63]. Such environmental impact of antibiotic pollution viz-a-viz resistant genes hazard has been reported by some German researchers [64]. Clinical wastewater (influent and effluent) and water samples from rivers receiving treated water as well as ground water and rain water overflow basin were investigated for bacteria prevalence as well as resistant gene presence. The interrelation between methicillin-resistant staphylococci and abundance of mecA gene as well as molecular study approach gave uniqueness to their work. Downstream of one of the river was reported to have high loads of bacteria and ARG with comparable values to that of wastewater effluents. Clinical wastewater was found to be greatly characterized by high level bacteria as well as ARG. Based on the method employed, the entire bacteria population was determined without discrimination. Clinical wastewater was also reported to contain very high concentration of antibiotic thus a platform for antibiotic-resistant bacteria/gene evolution. The ARG in surface water was

reported to be high with 71% abundance being the least. The prevalence of vanA resistant gene was reported for rain basin overflow while blavim-1, vanA and ampC frequently occurred in ground water sample [64]. The occurrence of ARG in river water and river sediments have been investigated and reported. Samples as much as a hundred and thirty nine were collected over twenty four months period. Six clinically relevant ARG whose abundance in the studied river has been previously reported was used in this study. The persistency of ARG in sediment was also investigated. One or more ARG was detected in all of the water samples analyzed with sul1 being the most common followed by bla_{TEM} and then qnrS and qnrA respectively. One or more ARG was all also detected in all sediment they analyzed; they reported sul1 and bla_{TEM} took the lead in abundance. Based on the persistency studies, they concluded that sediment may serve as reservoir for ARG [65]. Soil samples obtained from twelve parks in Victoria, Australia were investigated for ARG abundance. Six of these twelve parks were irrigated with reclaimed water while portable water was used for irrigation of the other six parks. The parks irrigated with reclaimed water had higher ARG enrichment compared to ones irrigated with portable water. About fifteen ARG were detected in each of the parks. They concluded that qPCR array techniques vividly identified reclaimed water irrigation to have had impact on the abundance, diversity as well as composition of ARGs in the investigated park [66].

Jia et al. [67] used high-throughput sequencing based metagenomic approaches to characterize the tempo-spatial changes of antibiotic resistance, bacteria community and their correlations in pig farming wastewater and its receiving river. A total of 194 ARG subtypes within 14 ARG types were detectable in all the samples, and their total relative abundance increased in the river water after receiving wastewater discharge, while decreased in the downstream river water. The wastewater discharge evidently increased bacteria diversity and induced bacteria community shift in the receiving river water. Variation partitioning analysis indicated that bacteria community played a more important role in the resistome alteration than mobile genetic elements. This study may help to understand the correlations among antibiotic resistance, microbiota and environmental conditions in the wastewater receiving river water. The dependent existence of ARGs and subtypes was vividly displayed by the high correlation coefficient and the network analysis of co-occurrence of ARG is adapted as Fig. 2.

6. Efforts towards ARG removal

The use of treated wastewater in agriculture is widely acceptable thus the unavoidable human daily interaction with her environment has become one major exposure route to antibiotic resistant genes (ARG) (See Fig. 1). It is therefore important that careful and serious attention be paid to the spread of ARG via wastewater reuse processes hence effective treatment technology for ARG removal must be established. Such studies which aimed at establishing efficient/novel technologies to effectively address this issue have been reported.

A novel treatment technology for ARG removal from municipal wastewater intended for reuse in agriculture has been reported [68]. This study which was built on an established knowledge of effectiveness of Sequencing Batch Biofilter Granular Reactor (SBBGR) in pathogens removal aimed at understanding the fate of ARGs and establishing their removal technology. Thirty liters wastewater was used for the laboratory studies. Subsequently, a pilot scale studies which used 3001 of wastewater was carried out. Although nine selected genes representing resistance to five antibiotics were selected for their work; ampC, mecA for beta-lactam, ermB for macrolide-lincosamide-streptogramin B, sul1, sul2 for sulfonamide, tetA, tetO, tetW for tetracycline and vanA for glycopeptide, only four of these was confirmed in the influent samples of the treatment plant. Their result revealed that SBBGR is more effective in the removal of enteric bacteria than ARGs as high ARGs concentration was recorded for the effluent after SBBGR treatment. In validating the fact that different gene usually exhibit different ecology,

sul2 removal was very low compared with other ARGs in the two systems i.e the laboratory and the pilot scale study system. Tertiary treatment techniques viz ultraviolet light (UV) radiation, sand filtration and peracetic acid (PAA) treatments was further applied and their result showed that only sand filtration enhanced ARG removal. The graphical summary of their work is presented here as Fig. 3.

Studies focusing on the removal of microbes and antibiotic resistant genes from a local wastewater treatment plant which uses conventional aerobic sludge activation followed by chlorination treatment techniques has been reported. Sampling was done at undefined intervals across eleven months. Influent, chlorinated and effluent wastewater were collected for analysis. About three hundred bacteria isolates were used and their reports revealed that only 10% of this huge volume was found nonresistant while about 20% of the total isolates were multidrug resistant. Chlorinated effluent was reported to contain some ARGs although at a lower concentration when compared with the influent wastewater. Chlorination also results in the enrichment of some bacteria, specifically is the bacteria containing tetZ genes [18].

The potential of coagulants in ARG removal was investigated. Coagulants used in the study were added at a dosage of 6–30 mg/L. The study selected five genes for quantification (two sul and three tet genes). The concentration of various ARG investigated reduced after coagulation using FeCl₃ and polyferric chloride (PFC) with various effects depending on types and dosage. Dosage increase did not affect ARG removal using FeCl₃ but removal of individual gene varies. They concluded that ARG removal using coagulants such as PFC and FeCl₃ gives an excellent result [55]. Reports on the use of UV/H₂O₂ for ARG removal has also been reported. Effluent obtained from urban wastewater treatment plant (UWTPs) was used for the said studies. Studies was however carried out in the laboratory. UWTPs samples used were exposed to catalyzed UV radiation for up to 240 minutes. Although total coliform was successfully inactivated, selected ARGs were still found abundance in wastewater after 240 min [37]. Studies using ozonation and UV 254 nm for ARG removal were also reported. The two samples used were synthetic wastewater and urban treated wastewater. Microbial inactivation was found to increase with increased irradiation contact time and vanA went below detection limit after 15 min of ozonation. Above 98% ARG removal was recorded for UWTP samples [69]. Relative abundance of ERY resistant gene in the face of *E. faecium* inactivation using peracetic has also been reported. ermB abundance was not affected by disinfection treatment, regrowth experiment and various system conditions. Their conclusion encouraged cross disciplinary examinations/studies for proper understanding of complex ecological system as well as efficient disinfection treatment design [70]. Secondary effluent of a WWTP which uses anoxic-aerobic activated sludge technology for treatment have been investigated for ARG and possible removal via fenton and UV/H₂O₂ treatment. Five genes (Sul1, tetX, tetG, int1 and 16rRNA genes) were identified as original gene copies of the wastewater. ARG removal was found to increase with increase in Fe²⁺/H₂O₂ molar ratio and maximum was observed at 0.5 Fe²⁺/H₂O₂ molar ratio. Investigating concentration effects, they reported increase in genes removal with respect to increased concentration. While more removal of int1 was obtained at 0.01 mol L⁻¹, other genes had more removal at 0.03 mol L⁻¹. Acidic media favoured ARG removal and increase in reaction time also aided ARG removal. UV radiation reduced targeted genes with increased radiation time. Concentration of 0.01 mol L⁻¹ was reported to give maximum log reduction and maximum ARG removal was observed at 3.5 pH. They concluded that fenton oxidation is a better technique in ARG removal than UV/H₂O₂ process [71]. Primary and secondary activated sludge collected from municipal sewage treatment works have been investigated for ARG occurrence as well as possible removal and enrichment in aerobic digester. Post digestion secondary activated sludge had lower ARG abundance than the primary activated sludge. Quinoline resistant genes were greatly removed from primary activated sludge to a tune of over 90% while inconsistency in the removal/enrichment exists for other

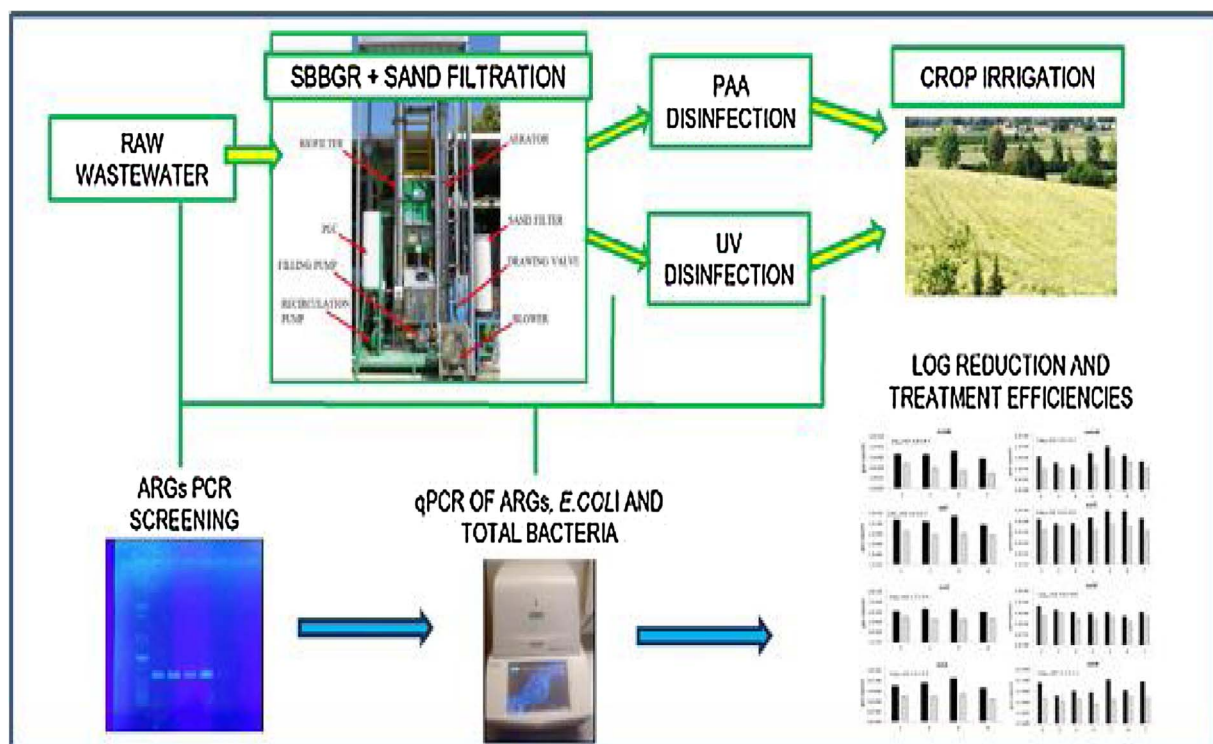


Fig. 3. Antibiotic resistance genes removal by a technological treatment solution for water reuse in agriculture [68].

membrane filter decreased, ARG reduction was said to be high and beyond expected. Natural colloidal material present in waste also aid ARG removal. Their conclusion pointed to the fact that membranes are promising technology for the treatment/removal of ARG [75]. The studies reporting the variation of bla_{TEM}, qnrS and tetW in water treatment by advanced oxidation process using UV/H₂O₂. qnrS and tetW was reported to be slightly present or below detection limit. They concluded that no removal was achieved for bla_{TEM} [39].

7. Concluding remarks and future trends

Attempts to tone down, put off and manage antibiotic-resistant bacteria and genes are a modern worldwide challenge. The growing threats from antibiotic resistance in man and animals calls for immediate attention. In spite of the little knowledge and understanding on risk of the antibiotics occurrence in the environment, their protracted effect in both aquatic and terrestrial systems cannot be ignored. Although various inactivation techniques have been investigated by environmentalists in order to establish a perfect way of preventing the release of antibiotic resistant specie into the environment. Most of these techniques have their attendant challenges. For instance, UV gives great inactivation results, however, possibility of specie regrowth and photoreactivation is considered a major challenge. Radiation dose versus total inactivation as well as economic disadvantages are also some of UV shortcomings.

Several of the reported works also indicated high removal/inactivation of resistant specie. This may not be the way out of the threats these resistant specie pose. The quantity of resistant specie left in water or released into the environment does not really determine risk associated. The propagation of these resistant specie are uncertain hence can greatly multiply once they overcome disinfection stress. Various measures that must be put in place to curb the attendant risks of ARB and ARG persistence in the ecosystem are:

1. A methodical and broad inspection of contaminated antibiotic waters is necessary

2. The improvement and adherence to specific guidelines on the analysis of antibiotics in environmental samples becomes imperative in order to obtain dependable results.
3. Future research should be tailored to support legislative measures in combating antibiotic resistance.
4. The general norm of reuse of treated wastewater in different countries is an important subject considering environmental sustainability, economic efficiency, and food security.
5. Improving the knowledge and benefits of wastewater reuse is needed to achieve enhanced integrated water resources management.
6. A thorough investigation of new trends in the development of antibiotic resistance in various sectors needs to be investigated.
7. Sensitization and education of the general community and other health related professionals about the sensible use of antibiotics are vital to ensure its effectiveness.
8. Prevention and management of infection and antibiotic resistance should be investigated in order to control infection. The connection between the utilization of antibiotics and surfacing of its resistance should be looked into.
9. A study on new efficient antibiotics or other treatment technologies should be conducted
10. In order to surmount the increased emergence of antibiotic by-products, different proactive approaches in pharmaceutical research and development, and collaboration by a wide range of stakeholders are sacrosanct.

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