I. MAN AND BIOSPHERE

ANTIBIOTICS, ANTIBIOTIC RESISTANT BACTERIA AND ANTIBIOTIC RESISTANCE GENES – EMERGING POLLUTANTS IN SURFACE WATERS AND ANTHROPOGENICALLY INFLUENCED WATERS

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Abstract. The growing antimicrobial resistance of pathogenic bacteria is considered as one of the main threats to human and animal health. Despite the association of the problem mainly with clinical pathogens, there is growing evidence of a relationship with anthropogenic influence on the water. The review examines the prevalence of antimicrobial resistance among microbiomes in drinking, surface and wastewater and water-related biofilms, and summarizes available data on the content of antibiotics, antibiotic-resistant bacteria and resistance genes as pollutants of growing importance to aquatic ecosystems.

Keywords: antibiotics; antimicrobial resistance; antibiotic resistant bacteria; antibiotic resistance genes; natural waters; wastewater

INTRODUCTION

Since its discovery in the last century, antibiotics (ABs) have established themselves as the most important pharmaceuticals for the treatment of infectious diseases in humans and animals and marked the beginning of a new era in healthcare. While at the dawn of antibiotic use, pathogens have maintained their susceptibility to certain ABs for decades, nowadays the period of their effective action decreases sharply and bacteria acquire resistance to new ABs quickly, sometimes for no more than a year [3]. The acquisition of antimicrobial resistance (AMR) from previously susceptible pathogens and its increasing prevalence are becoming one of the main threats to human and animal health associated with limiting or minimizing the possibilities for effective treatment [3, 23].

The evolving of AMP can cause a decline in quality of life and significant economic consequences associated with increased health care costs and loss of workability and productivity [5]. There are around 25,000 deaths caused by infections with antibiotic resistant bacteria (RB) in the EU each year, and at least 700,000 deaths worldwide [4]. The European Center for Disease Prevention and Control and the European Food Safety Authority are alerting to high levels of AMR toward commonly used ABs among the bacterial isolates from humans, animals and food [4].

The increasing AMR among pathogens is a serious medical problem, which in recent years has grown to be an ecological issue. In the natural water and wastewater, there have been demonstrated residual quantities of ABs and a number of bacterial species that have acquired antibiotic resistance to one or

more classes of ABs. There is growing evidence that aquatic microflora represents an important reservoir of AMR, and the water pollution with ABs can facilitate the selection of resistant bacteria and the transfer of antibiotic resistance genes (ARGs) to human-associated microbiome [7, 9, 45].

Conventional wastewater treatment is insufficiently effective to remove ABs, RB and ARGs, and as a result these pollutants enter the natural waters [13, 15, 32, 53, 62, 65, 67, 69]. The so-called “Urban Water Cycle”, which includes water supply, sewerage and wastewater treatment, predetermines the social importance of the relationship between AMR and water [55, 86]. The sharing of various habitats and movement through different types of water – wastewater (WW), natural and drinking waters – determine the role of the antibiotic resistant bacteria as potential carrier and/or vectors of AMR to human [17, 55, 56].

The need of limitation measures for the spread of AMR is at the heart of various initiatives of the European Commission, as funding of research projects aimed at the evaluation of the aquatic resistome, elucidation of the mechanisms of AMR acquisition and dissemination, and searching for ways and means for limiting these processes, including the potential transfer of AMR to the human-associated microbiome [1, 2, 6]. The developed European Action Plan “One Health” against the AMR [6] emphasizes the need to extend the “One Health” approach, based on the principle of interconnection between human and animal health, to the environment, as well. The need to enrich the knowledge about AMR in environment and the ways of the prevention of AMR acquisition and dissemination is emphasized in the above action

plan, since it is already wide accepted that the environment contributes to the acquisition and dissemination of AMR among human and animals through the discharges of domestic, animal and industrial wastes [6].

ANTIBIOTICS AS EMERGING WATER POLLUTANTS

It is known that a part of the applied ABs remains unutilized during the cure treatment and together with the bacteria of human or animal origin enters the wastewater. Residual amounts of ABs are detected in the hospital and domestic wastewater, and in natural waters, although in much lower concentrations [23, 41, 52]. It was found that the concentration of different ABs in water varies from nanograms to micrograms per liter depending on the intensity of ABs use, the chemical stability of the compounds, the effectiveness of removal in the wastewater treatment processes, etc. [13, 18, 52, 60, 71]. According to data on untreated wastewater in Spain, fluoroquinolones are in the highest concentration – ciprofloxacin of 1.31 µg/L and ofloxacin of 1.56 µg/L [64], while 1.03 µg/L penicillin, 1.75 µg/L tetracycline, 0.88 µg/L sulphamethoxazole and 0.44 µg/L ciprofloxacin were detected in a Portuguese wastewater [81]. The ABs content is especially high in hospital wastewater: beta-lactams, glycopeptides and trimethoprim in concentrations from 3.67 µg/L to 53.05 µg/L are reported in Romania [80], and in Spain – 13.78 µg/L ciprofloxacin and 14.38 µg/L ofloxacin [71].

The natural waters are often polluted with substances from the group of penicillins, tetracyclines, macrolides and fluoroquinolones, these usually enter the water through the effluents. In a Spanish river water, there were found 26 types of ABs, among them, the ones with the highest content were cefotaxime (93.1 ng/L) and clarithromycin (44.7 ng/L) [71], and in river water in Pakistan: 1.1 µg/L oxitetracyclin, 1.7 µg/L trimethoprim and 2.7 µg/L sulphamethoxazole [46]. After the surface water pollution by ABs was taken into account, in 2015, the antibiotics azithromycin, clarithromycin and erythromycin were included into the Watch List of the substances, which are potentially dangerous to surface waters (according to EU Directives 2000/60/EC and 2008/105/EC). Two more antibiotic substances, ciprofloxacin and amoxicillin, were added in 2018, which shows the increased attention to the ecological problems caused by ABs pollution of the water bodies [73].

HEALTH AND ENVIRONMENTAL RISKS OF AMR

Very soon after the introduction of an antibiotic into the medical practice, it was found that target bacteria develop the capability to resist and adapt to its action. The RB developed usually more than one mechanism of resistance. That makes them resistant toward several or even all the active substances from a particular antibiotic class. The fast development and the high levels of dissemination of AMR among bacteria can be explained by: a) ineffective and unreasonable application of ABs for treatment, which causes an ecological pressure and supports the selection of RB; b) the dissemination and transfer of RB between humans, animals and environment [12, 73, 75, 96]. There is data that during the first decade of the 21st century the use of ABs for curing infection diseases has increased by 36% [55]. The excessive and sometimes improper use of ABs for therapeutic purposes in the human and veterinary medicine, and for prophylactics and growth stimulation in the animal breeding has caused acquisition of AMR in the target pathogens [38, 41, 55, 86]. The acquisition of AMR by human pathogens that used to be sensitive, such as *S. aureus*, *S. pneumoniae*, *M. tuberculosis*, *S. typhimurium*, *H. influenza*, etc., is a serious medical problem [23, 45].

There are a growing number of scientific proofs for the interrelation between the increasing AMR of bacteria and the anthropogenic impact on the aquatic ecosystems, which are an important environmental bacteria habitat [7, 12, 17, 38, 39]. As a result of human activities, such as drinking water production and its use for drinking and industrial purposes, wastewater treatment and its discharge in water bodies, the bacteria, including the resistant ones, are able to move from the polluted wastewater into the treated wastewater and the natural waters, and reach to humans. In this way, the urban water cycle contributes to the dissemination of RB and ARGs, and is a basis for a potential transmission routes of AMR from environment to humans and vice versa [15, 55, 67, 86]. ABs do not attack predominantly the pathogenic bacteria but also affect the normal microflora of the human and animal bodies and can lead to ecological imbalance and development of secondary infections. Due to the more frequent selective pressure, commensal bacteria faster develop AMR, which can subsequently transfer to pathogenic species and ensure its potential transmission to the environment [9, 45, 97].

Numerous published research data about the content of ABs, RB and ARGs in different types of water and water-associated biofilms demonstrate a high level of dissemination of AMR and the importance of the aquatic microbiota as a source of AMR in water use [10, 32, 33, 55, 70, 74, 78, 89, 97]. The presence of ARGs determinants in commensal bacteria and their ability to transmit the resistant traits to pathogens by horizontal gene transfer raise public health concern and is a reason for ARGs to be considered as emerging pollutants in aquatic ecosystems [23, 86, 94].

The assessment of the health risks for transfer of AMR from environment to people, and from water as one of its compartment, is still in its beginning stage. Following extensive discussions, a proposal was made that the ecological aspect of the development of AMR to be included into the procedure for health risk assessment [7, 9, 56]. It is considered that the potential threats are related to the possibility of emerge and evolution of clinically important AMR in water environment and the possibility it to be increased by external factors such as chemical pollutants (content of chlorine, heavy metals, etc.) or physic-chemical conditions [9, 12, 63, 67].

The observed non-direct proportionality between the abundance of RB and ARGs in environment and the associated human health risks makes it difficult to quantify the health risk of AMR [56]. But it is accepted that non-detection of particular RB and ARGs cannot be interpreted as lack of health risk, and that RB and ARGs in quantities bellow the detection limit or limit of quantification through the nowadays used analytical methods in fact can present a considerable risk to human health [56]. Therefore, it is important the polluting RB and ARGs in water to be identified and quantitatively determined using methods such as qPCR or metagenomic analyses, and the potential of water to transfer AMR to human to be evaluated. Reliable characterizing of bacterial vectors of AMR from water environment to humans may require identification at the species or strain level, as well as establishing the ability of these species to colonize and infect the human body. It is generally accepted that a particular component of the environment may pose a risk of transmission to humans only in the presence of a vector bacteria and in an amount sufficient to be able to colonize human body. Depending of the bacteria vector type it can present health risk even if it is present in a few number [9, 56].

WASTEWATER – RECEIVER AND SOURCE OF ABS AND AMR

The research on microbiota of wastewater (hospital, domestic, mixed, untreated wastewater and ones with different stage of treatment) found a number of bacterial species that exhibit acquired resistance to one or more classes of ABs. The conventional biological treatment in wastewater treatment plants (WWTP) in bio-basins (with active sludge) and biofilters (with biofilm) could not provide significant reduction of the ABs content and of the fraction of antibiotic resistant bacteria [29, 62], despite of the significant changes in the total bacteria number and in the distribution of bacterial populations with a different AMR patterns [53, 69, 71, 93]. The research data show clearly that the continuous exposition to sub-inhibitory concentrations of ABs during the processes of biological treatment creates potentially favourable conditions for selection and increase of RB, while the high bacterial density in the active sludge or biofilm stimulate cell-to-cell contacts and horizontal gene transfer [23, 41, 63]. This is why the WWTP is considered to play the role not only of a recipient but also of an important pollution source of the natural waters with ABs, antibiotic resistant bacteria and ARGs when the treated wastewater enters the receiving water body [42, 44, 53, 69, 71, 93].

Independently of the treatment scheme and the multi-fold reduction of the total number of coliforms, WWTPs are responsible for the throwing away about one milliard culturable resistant coliforms into environment each minute [55]. Carbapenem-resistant coliforms have been identified at all stages of wastewater treatment, and *E. coli* resistant to beta-lactams with extended spectrum of action and carrier of the *blaCTX-M* genes, groups 1 and 9, characteristic of clinical pathogens [72, 91].

In spite of the faecal indicator value of *E. coli* and *Enterococcus spp.* [21, 22, 32, 34, 37, 66] and a high potential of multidrug resistance (MDR), a significant scientific interest is focused on the autochthonic water bacteria (*Aeromonas*, *Acinetobacter* and other), which can play an important role in the process of dissemination of AMR because of their abundance [54, 70, 77, 86].

Many studies of AMR of water bacteria are based on cultural methods, which comprise a little part of the bacterial community and cannot take into account the real resistance level among aquatic microbiota [41, 82]. It is thought that ABs that are highly active on proliferating cells do not necessary impact on viable but not culturable (VBNC) cells, which

demonstrate effective antibiotic resistance because of their low metabolic activity, and are able to recover and maintain their virulence [64]. In view of this, the latest scientific research on AMR is focused on the genetic determinants of resistance (ARGs) and the mechanisms of gene transfer, studied by qualitative and quantitative molecular methods [8, 31, 48, 74, 85, 94, 95], as well on the contribution of integrons class 1 in the transfer of ARGs [29, 35, 50, 51].

The discharge of treated wastewater appears to be a significant point source of ARGs along the river [57, 71, 76]. It was proven that in spite the moderate effectiveness of the wastewater treatment in WWTP, the ARGs undergo various dynamic changes depending on the technology scheme, and the origin and pollution of wastewaters [53]. The reduction is not the same for all ARGs [64] and the treated wastewater and the disposed sludge can contain significant levels. For many ARGs, significant positive correlations with the ABs concentration in water were found: between ciprofloxacin/ofloxacin and *qnrS*; cefazolin/ cefotaxim and *blaTEM*; clarithromycin and *ermB*; sulphamethoxazole and *sulI* [33]; tetracycline and *tetB* and *tetW* [43].

The research data reveal the role of WWTP as a “hot spot” for acquisition and dissemination of ABR, where during the treatment processes, the bacteria can develop tolerance through new mutations or transfer of the available ARGs into new bacterial hosts [14]. The ubiquitous distribution of RB, which can share habitats and hosts, has the potential to promote the wide spreading of ARGs. Therefore, the family of ARGs, such as *blaTEM*, *blaSTX*, *blaSHV* (for resistance to beta-lactams), *acc-lbq*, *qnrS*, *sulI* (plasmid-mediated quinolones resistance) and *tetX*, *tetQ*, *tetS*, *tetO*, *tetM* (resistance to tetracycline), beside their clinic importance, can be found not only in wastewater, but also in surface water, aquacultures and wild animals [26]. The validity of these tendencies for different WWTPs, different bacterial communities and different classes of ABs needs a deeper study so that the influence of these underestimated pollutants on environmental and human health to be elucidated. The increasing AMR calls for a better understanding of the ecology of the antibiotic resistant bacteria, their selection and dissemination in the environment, as well. The various effectiveness of removal of ABs, antibiotic resistant bacteria and ARGs in WWTPs depending on the type of pollutants, water and treatment facilities, makes it necessary to collect information on each different pollutant and particular water object [12, 87].

AMR IN SURFACE WATER

The dissemination of antibiotic resistant bacteria in surface water is determined by its role as a receiver of WW with various level of treatment [18, 21, 23]. The water bodies are recognized as an important reservoir of ABs, RB and ARGs that facilitate the transfer of ARGs between pathogenic and nonpathogenic bacteria and can contribute maintaining AMR in environment [9, 75, 96].

The numerous comparative studies of the bacterial community upstream and downstream from the effluent receiving point demonstrated a significant increase of the population of antibiotic resistant bacteria and a change in the model of AMR [18, 20, 35], and an increase of ARGs and integron class 1 [33]. For example, the information about Dambovită River (Romania) confirmed the trend of quite lower levels of AMR of the *E. coli*, *Enterobacter* and *Klebsiella* isolates from the river water than of the ones isolated from the WW, and an increase in the AMR level along the river under the influence of the received influents of treated WW, as well [27].

There are a lot of published data on AMR of the microbiota in European aquatic ecosystems – in Spain [16, 36, 71], Germany [74, 76], Sweden [13], Switzerland [19, 20, 28], Serbia [25] and Romania [30, 59]. As part of European joint Danube survey, the acquired AMR among the isolates of *E. coli* and *Pseudomonas spp.* was studied in six river water sampling locations. It was found an increase of the number of resistant coliforms, including ones with MDR, along the river under the influence of the point sources of pollution and the river tributaries. More than 50% of the isolated *E. coli* strains were resistant to one or two ABs, while all the isolated species *Pseudomonas* had low AMR, without any clinical importance [93]. However, a finding raised an alarm – there were found phenotypes for production of extended spectrum beta-lactamases and resistance to carbapenems among *E. coli*, *Enterobacter spp.* and *Klebsiella spp.* Because of the human-generated AMR often detected in the coliforms from Danube River and the AMR patterns related to intensive hospital treatments, the data was regarded as supporting the assumed mechanisms of AMR transfer from clinically relevant pathogens to the environmental microbiota [49, 93].

The surveillance data of the prevalence of AMR among clinically relevant pathogens in Europe shows regional differences due to health habits, healthcare and lifestyle in the individual countries [4]. Regional differences are also observed in the prevalence of RB and ARGs in surface waters.

Significant levels of resistance to ABs with long-term use and prevalence of ARGs among aquatic microbiota have been reported in the rapidly developing countries, such as China, India, and Pakistan [46, 57, 91]. Bacteria isolated from river waters in Pakistan show varying degrees of resistance to chloramphenicol, ampicillin, streptomycin, tetracycline and kanamycin depending on ABs residuals. The high levels of the *sulI* and *dfrA1* genes, as well as *intI1* (class 1 integron), correlate with the high ABs contamination of water [46]. Despite the low concentrations of ABs measured in river waters, Berglund *et al.* (2015) observed an increase in ARGs and integron class 1 downstream, most likely related to genetic contamination by inflowing wastewater [13]. The studies of Koczura *et al.* (2012) demonstrate the contribution of treated effluents from WWTPs to the increased frequency of integron-positive *E. coli* isolates in the river waters after the discharge point, as well as to the increased AMR level [50]. Similarly, the waters of the Ter River have significantly higher values of the *ermB*, *qnrS* and *sulI* genes downstream the river after the source of pollution (the WWTP of Girona, Spain) [71]. The data for a Romanian river confirm the discussed trend for influence of inflowing treated wastewater on the AMR level in the river flow: no ARGs were detected in the bacterial isolates from upstream river, while after the effluent discharge the *blaCMY*, *blaNDM*, *tetD*, *sulI* and *qnrB* genes were detected in river downstream [59]. Studies of river waters in Germany and Australia have revealed widespread dissemination of genes for resistance to sulfonamide, trimethoprim, macrolide, beta-lactams and chloramphenicol. The most common are genes to sulphonamides resistance *sulI* and *sulII* (77-100%) and trimethoprim *dfrA1* (43-55%). The resistance gene *ermB* (to macrolide) is more prevalent in Germany (68%), and the *cat* (to chloramphenicol) and *ampC* (beta-lactams) genes are more commonly found in Australia [76].

In surface waters, a prerequisite for a significant environmental and health risk is the emergence and the increasing prevalence of carbapenemase-producing *Enterobacteriaceae* expressing enzymes such as KPC-2 (carbapenemase produced by *K. pneumoniae*) and NDM-1 (New Delhi metallo-beta-lactamase) [39]. Extended spectrum beta-lactamase-producing (ESBL) enterobacteria have been detected in more than one-third of the 58 rivers and lakes studied in northern Switzerland, and carbapenemase-positive *K. pneumoniae* has been isolated from one water source. The identified

enterobacteria strains with ESBL phenotype are predominantly carriers of the *CTX-M* gene and less frequently of *SHV-12*, and belong to the species *E. coli*, *K. pneumoniae*, *Raoultella planticola*, *Ent. cloacae* and *Ent. amnigenus* [97].

The wastewater discharge exerts also polluting influence on the bottom sediments of the receiving rivers and lakes, which is related to the increased load with antibiotic resistant bacteria and ARGs [16, 19]. In the river sediments, it was found rather higher concentrations of ARGs and extracellular DNA (eDNA) [58], the last supporting a longer occurrence and abundance of ARGs [58, 78], as well the dissemination of AMR. There is evidence for releasing eDNA with antibiotic resistance through WWTP effluent, as well a possible bacterial transformation by ARGs transfer to environmental bacteria [58].

AMR IN WATER-ASSOCIATED BIOFILMS

It is well known that in water-associated biofilms, the attached bacteria show higher tolerance to antimicrobial agents compared to free-living cells in water [10, 77]. Hence, to gain the same level of bacterial reduction, higher concentrations and longer contact time are needed. The specific defense mechanisms of biofilm that hinders the penetration of ABs, the lower bacterial growth rate etc., act synergistically with the mechanisms responsible for the conventional AMR, such as the presence of ARGs in the bacterial genome and of mobile gene elements (MGE) [10, 40, 77]. A lot of the research data support the hypothesis that biofilms in natural and wastewater can provide optimal conditions for acquisition and dissemination of the AMR determinants, related to the high cell density within the biofilm, the close cell-to-cell contact and the accumulation of MGE [9, 10, 74]. The reported higher conjugative activity in the biofilms compared to free-living bacteria in water, the higher frequency of mutations, as well as the higher horizontal gene transfer reveal the role of biofilms for acquisition and transfer of ARGs [23, 40].

The inducing of antibiotic resistance depends on the ABs present and can be specific or non-specific, with the most universal non-specific mechanism being overregulation of the efflux pumps. A significant part of the development of antibiotic resistance can occur when bacteria are exposed to non-lethal concentrations of ABs. In addition, at sub-inhibitory concentrations, the ABs can induce biofilm formation and thus support the susceptible bacteria [10, 33, 88].

The effluents inflow in receiving water bodies favors the dissemination of antibiotic resistant bacteria and ARGs in the benthic river biofilms after the point of wastewater discharge [28, 68, 74, 89]. Under the influence of the human activities and the intensive livestock breeding along the river, there exist river stretches with biofilms characterized by high level of resistance to the ABs used in human and veterinary medicine [88, 89]. A complex study of the distribution of various ARGs in river waters found the greatest abundance in biofilms, followed by sediments and the lowest values in water, suggesting the contribution of eDNA to the abundance of ARGs in the biofilms and the sediments [92].

AMR IN DRINKING WATER

The presence of antibiotic resistant bacteria and ARGs in the water sources and the purified drinking water is an emerging problem in the water supply industry, in spite of the fact that some of the resistance phenotypes are innate and the transfer of AMR to human-associated bacteria is considered to be unlikely [82]. Nevertheless, the risk of antibiotic resistant bacteria and ARGs reaching the water consumers is the main reason for the research of AMR in drinking water. Bacteria of the genera *Sphingobium*, *Sphingomonas*, *Pseudomonas*, *Acinetobacter* and non-fecal *Enterobacteriaceae*, capable of withstanding the action of various ABs has been proven in drinking water [61, 83, 84, 85, 90]. Resistant coagulase-negative staphylococci, mainly *S. pasteurii* and *S. epidermidis*, can be found in purified drinking water and tap water after its transport in the water supply network [28].

The changes that raw water undergoes in drinking water purification plants (DWPPs) and during its transportation through the water supply network to the end user's tap focus the researches on the evolution and the spread of AMR among the drinking water microbiome [11, 47, 48, 61, 85]. The contribution of the individual stages of water purification in DWPPs, including the filtration processes with sand or granular activated carbon, has been clarified [79, 94]. The action of chlorine as a disinfectant was assessed, but also as a factor in the acquisition of AMR [47, 61], including MDR.

Studying the resistance of bacteria to ABs and chlorine, Khan *et al.* (2016) found a significant correlation between chlorine tolerance and the minimum inhibitory concentration to the studied ABs. Chlorine-resistant bacteria are more resistant to tetracycline, amoxicillin, and sulfamethoxazole, and survive longer than susceptible bacteria [47].

A number of independent studies showed a higher dissemination of bacteria that were resistant toward some classes of ABs in purified drinking water compared to the water source [11, 61, 79, 85, 86, 90], and also a bigger part of isolates with MDR phenotype. These phenomena were connected with a possible selective effect of the disinfection [47, 48] or an increase of resistant bacteria as a result of re-growth in water or biofilm [74, 90]. There is insufficient evidence to suggest that the water source is the most likely source of AMR in purified drinking water [61, 84], and the complicated restructuring processes in bacterial communities as a result of the disinfection, make it difficult to establish the origin of AMR. The conventional water disinfection can reduce the number of some bacteria below the detection limit; however, there is evidence for increased levels of ARGs and increased AMR in some ubiquitous bacteria [28, 61, 90]. For example, the widely spread in drinking water members from the family *Sphingomonadaceae* have a rich and diverse profile of AMR but unclear significance for AMR dissemination [94].

The latest molecular studies of AMR in drinking water focus on the genetic determinants of antibiotic resistance and the mechanisms of gene transfer, including, at the processes of water purification [29, 31, 48, 74, 85, 94]. The participation of class 1 integrons in the transfer of ARGs is also studied [29, 31, 35, 48]. In a study of 27 ARGs in the drinking water in its way from the water source to the consumers, Su *et al.* (2018) found a lower abundance of different ARGs in the purified drinking water compared to water sources, dominated by *floR* and *sulI* genes [79]. The detection of the plasmid-mediated genes *blaTEM* and *blaSHV* in drinking water supply systems, unlike of their absence in the outlet water from the DWPP, suggests that the spread of at least some determinants of beta-lactams resistance occurs in drinking water distribution systems, and is related to water-associated biofilm and/or secondary bacterial growth [90]. The drinking water supply systems could be an incubator for the growth of antibiotic resistant bacteria and an important reservoir for the dissemination of AMR among opportunistic pathogens [30, 31]. Despite the reported data on the bacterial resistance in drinking water to the "old" ABs and dissemination of respective ARGs [28, 61, 83, 84, 90], there are also studies that indicated a low content or even absence of RB and ARGs in the drinking water [11, 25, 48].

CONCLUSION

Antimicrobials set the beginning of a new era in healthcare, but nowadays, the period of their effective action has decreased dramatically and the pathogenic bacteria develop resistance to newly developed antibiotics quickly, sometimes for less than a year. The increasing antimicrobial resistance among clinically important pathogens is a serious medical problem with significant health and economic consequences for society. In recent years, the antimicrobial resistance has grown into an ecological challenge. There is increasing evidence that antibiotics remained unutilized in treatment become emerging pollutants of wastewaters and natural waters, and that the aquatic microbiota represents an important reservoir of acquired AMR to one or more classes of ABs. The human health risks are related to emerge and evolution of clinically important antibiotic resistance in environment, and the possibility of being increased by exogenous factors.

The research interest and the numerous published scientific studies on the mechanisms of acquisition of AMR and the dissemination of antibiotic resistant bacteria and ARGs in different types of water show the up-to-date character and comprehensiveness of the problem. There are not legal norms for maximum admissible level of ABs, antibiotic resistant bacteria or ARGs in drinking, natural and waste water, nevertheless, the potential health and ecological risk of acquisition and dissemination of AMR through water requires reliable information on bacterial resistome and on the effectiveness of the different technological approaches, as well as public mechanisms for restricting the dissemination of AMR to human-associated microbiota. However, protection of natural waters is an important societal challenge because of the human-water relationship and the need for water for drinking, irrigation, recreation and sports, with a quality that does not endanger human health.

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