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1 **Antibiotic Resistance Genes in the Human Impacted Environment: a One Health**
2 **Perspective**

3

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21 **Abstract**

22 Antibiotic resistance and its environmental component is gaining more attention as part of
23 combating the growing healthcare crisis. The One-Health framework, promulgated by many global
24 health agencies, recognizes that antimicrobial resistance is a truly inter-domain problem in which
25 human health, animal agriculture and the environment are the core and interrelated components. This
26 prospectus presents the status and issues relevant to the environmental component of antibiotic
27 resistance, namely the needs for advancing surveillance methodology: the environmental reservoirs
28 and sources of resistance, namely urban wastewater treatment plants, aquaculture production systems,
29 soil receiving manures and biosolids and the atmosphere which includes longer range dispersal. Much
30 recent work has been done describing antibiotic resistance genes in various environments; now
31 quantitative, mechanistic and hypothesis driven studies are needed to identify practices that reduce
32 real risk and maintain effectiveness of our current antibiotics as long as possible. Advanced
33 deployable detection methods for antibiotic resistance in diverse environmental samples are needed
34 to provide the surveillance information to identify risk and define barriers that can reduce risk. Also
35 needed are practices that reduce antibiotic use and thereby reduce selection for resistances, as well as
36 practices that limit dispersal or destroy antibiotic resistant bacteria or their resistance genes that are
37 feasible for these varied environmental domains.

38 Key words: Antibiotic Resistance, Transmission, Agriculture, One-Health

39 **1 Introduction**

40 The overuse and misuse of antibiotics for human therapy and livestock production around the
41 world over the past decades have increased antimicrobial resistance in diverse environments
42 (Berendonk *et al.*, 2015; Garbisu *et al.*, 2018). Exposure to antibiotic resistant bacteria (ARB) and
43 antibiotic resistance genes (ARGs) in the environment can increase the prevalence of resistance
44 determinants in the human microbiome (Leonard *et al.*, 2015). In December 2017, the United Nations
45 Environment Programme identified environmental antibiotic resistance as the top of six emerging
46 issues of concern (UNEP, 2017). Together with climate change, water stress and environmental
47 degradation, widespread antibiotic resistance should be regarded as one of the global challenges
48 humans face in this century.

49 ARGs have been widely identified in various environments, including wastewater and sludge
50 (Manaia *et al.*, 2018), livestock farms and soil (Zhu *et al.*, 2013), river water and sediment (Muziasari
51 *et al.*, 2017), drinking water (Ma *et al.*, 2017a), glacier environments (Segawa *et al.*, 2013) and even
52 the Antarctic (Wang *et al.*, 2016). The use of antibiotics in therapy and livestock production has
53 greatly promoted the development and spread of antibiotic resistance making impacted environments
54 into large reservoirs of ARGs (Larsson *et al.*, 2018; Manaia *et al.*, 2018). Furthermore, the prevalence
55 of mobile genetic elements, e.g. transposons, integrons and plasmids, could promote the horizontal
56 gene transfer of ARGs to other bacteria including human pathogens, exacerbating the antimicrobial
57 resistance issue (Gillings *et al.*, 2015; Ma *et al.*, 2017b).

58 Previously studies on antibiotic resistance mainly focused on clinical microorganisms to address
59 the direct threat of emerging antibiotic resistant bacteria on public health. With the emergence of
60 superbugs (multidrug resistant human pathogens) in medical treatment, both medical and public
61 concern has increased. This has led to recognition that this is a problem that requires an
62 interdisciplinary vision since it clearly involves not only human medicine but also agriculture and
63 veterinary medicine as well as a range of environmental domains. The result has been formulation of
64 the “One-Health” concept to engage scientists and practitioners across these disciplines to study the

65 problem and its remedies in a cross-disciplinary manner. Antibiotic resistance has been described as
66 the “quintessential” One–Health issue (Robinson *et al.*, 2006). It involves antimicrobial resistance
67 (AMR) in three main domains (Figure 1): Human health, animal agriculture and the environment.
68 The United Nations through its agencies: the UNEP, the World Health Organization (WHO), the Food
69 and Agriculture Organization (FAO), and the World Organisation for Animal Health (OIE) work
70 jointly to promote collective action to minimize the emergence and spread of AMR. As illustrated in
71 Figure 1, soil is a central component in One-Health since it harbors a large natural resistome but also
72 receives ARB and ARGs from both human and animal wastes which can be returned to humans
73 through vegetable and animal products, through surface, ground and reclaimed water, and via aerosols.
74 This is particularly important for soil science research because of: i) its central role as a reservoir,
75 thus potential source for resistance traits, ii) the many routes of dissemination including to humans
76 and iii) the large natural background of resistance which makes it more difficult to assess human-
77 enhanced resistance and high risk AMR.

78

79 **2 Detection of antibiotic resistance genes in the environment**

80 One of the major needs to address the antibiotic resistance issue is improved AMR surveillance
81 incorporating clinical, agricultural and environmental data at local, national and global levels (Hughes
82 *et al.*, 2016). This has been a challenge for the environmental domain since a legacy of methods tuned
83 to the environment has not been established. In the last decade, molecular methods to quantify ARGs
84 in the environment and their distribution and propagation in wastewater and sludge, aquaculture, soil
85 and atmosphere have been developed or improved. These are primarily of three types: PCR based
86 amplification of ARGs, hybridization of DNA to ARG gene fragments and searching metagenomic
87 sequence data for ARGs. The former includes the polymerase chain reaction (PCR), quantitative real-
88 time PCR (qPCR), DNA microarray and DNA hybridization. The traditional target sequence
89 amplification-based methods, PCR and qPCR, have been widely applied to detect and quantify ARGs
90 in diverse environmental samples because of their high sensitivity for ARGs detection under low

91 abundance in natural environments. However, due to the limitations of primers available for ARGs
92 amplification, PCR and qPCR approaches could only effectively detect well-studied ARGs. Besides,
93 ARGs primers may cause biases and false negative/positive results, and only several ARGs could be
94 detected in one set using traditional methods (Laht *et al.*, 2014; Titilawo *et al.*, 2015; Xiong *et al.*,
95 2015). In recent years, improvements have provided high-throughput (highly parallel) qPCR for
96 detection of hundreds of ARGs in one run (Looft *et al.*, 2012). To further improve its efficiency and
97 coverage, 294 primer sets for ARGs and some mobile genetic elements (MGEs) were developed (Zhu
98 *et al.*, 2013) and validated by sequencing its amplicons (Johnson *et al.*, 2016). This was subsequently
99 expanded to 384 primers (Wang *et al.*, 2016). Recently, the results from over 500 diverse samples
100 were evaluated, and together with updated reference data and a new primers design tool,
101 EcoFunPrimer, Primer set 2.0 for highly parallel qPCR was tested and reported (Stedtfield *et al.*, 2018).

102 The rapid development of high-throughput sequencing (HTS) technology in the past decade has
103 enabled sequence-based metagenomic analysis to be widely applied in diverse ecological, medical,
104 engineering and physical studies (Kircher and Kelso, 2010), including in assaying ARB and ARGs in
105 environmental samples (Zeng *et al.*, 2015; Ma *et al.*, 2016). HTS technique can rapidly generate large
106 amounts of sequence data at a relatively low cost (Kircher and Kelso, 2010). Metagenomic analysis
107 is a culture-independent molecular approach, which uses the generated metagenomic sequences to
108 search, annotate and predict targeted genes. For annotation and detection of ARGs in environmental
109 samples, the generated metagenomic sequences are filtered for quality control and then compared
110 against a reference ARG database that contains sequences of the known ARGs. With the increasing
111 concerns of ARB and ARGs in both medical treatment and environmental monitoring, several ARG
112 databases have been constructed to facilitate the ARGs annotation, including ARDB (Liu and Pop,
113 2009), CARD (Jia *et al.*, 2017), ARG-ANNOT (Gupta *et al.*, 2014) and ResFinder (Zankari *et al.*,
114 2012). One structured database derived from combining ARBD and CARD was designed to search
115 metagenomic data and classify the annotation results into 24 ARG types and 1208 subtypes (Yin *et*
116 *al.*, 2018).

117 A variation of the above approach of searching for ARGs in metagenomic data is to first
118 assemble the short-read ARG fragments to gain more reliability in gene identification. This can be
119 done by gene-targeted assembly tools such as Xander (Wang *et al.*, 2015) or Mega GTA (Li *et al.*,
120 2017). One study reports this result in soils for 35 ARGs (Dunivin and Shade, 2018).

121 Challenges remain on improving the detection limit and identifying untargeted ARGs, which can
122 be potentially-emerging, problematic ARGs. All of the above molecular methods are based on
123 sequence information from known ARGs. None of these methods can detect cryptic ARGs. Further,
124 many ARG sequences may be part of the natural resistome, code for functions that by themselves do
125 not confer resistance, or may not be expressed.

126 The comparison of antimicrobial resistance among different environmental niches with culture-
127 based clinical (pathogen) resistance is necessary for improving our knowledge on the evolution,
128 spread and risk of ARGs in the environment. However, standard methods and criteria specifically
129 designed for meeting this need in environmental samples is lacking. One challenge for use of culture-
130 based methods for environmental samples is that > 99% of environmental microorganisms are not
131 cultivable, and another is that pathogen recovery can be inefficient due to their injury or stress from
132 being in the environment. Therefore, improvement of methods through combining molecular methods,
133 perhaps informed by DNA sequence or biochemical markers from clinical or culture-based studies,
134 with high-throughput sequencing will provide a comprehensive overview of antimicrobial resistance
135 as well as specific areas of higher risk in diverse environmental samples. One example of that kind
136 of method is epicPCR which can link the host and ARG without culturing in high throughput method
137 (Hultman *et al.*, 2018). Future attention should also be paid to tracking antimicrobial resistance from
138 pollution sources versus natural environments to human-related environments, as important guidance
139 for management of antimicrobial resistance risk.

140

141 **3 Antibiotic resistance genes in wastewater and sludge**

142 The major environmental sources of AMR, and especially those that are clinically relevant and

143 have the potential to reach humans are from i) urban wastewater treatment plants, ii) use in
144 aquaculture, iii) use in animal agricultural for growth promotion and therapeutic use. Other sources,
145 not discussed here, but should not be neglected, are antibiotic production and formulation factories
146 and used as a ‘pesticide’ on some food crops. Furthermore, the use of antibiotics for treatment of
147 companion animals, and transmission of ARB by wildlife, including birds, and the irrigation of crops
148 with reclaimed wastewater, are sources to also be recognized (Fig. 1).

149 Domestic wastewater treatment plants, also known as urban wastewater treatment plants
150 (UWTPs), also receive ARGs and ARBs from domestic and clinical sources but cannot remove them
151 nor most antibiotics by the most commonly used technologies applied in the treatment of domestic
152 effluents. Therefore, in spite of reducing the abundance of bacteria in the effluents, wastewater
153 treatment, fulfilling the legal recommendations (e.g. Directive 91/271/EEC on Urban Waste Water
154 Treatment and following amendments in the European Union), leads to the continuous release of ARB
155 and ARGs into the environment (Vaz-Moreira *et al.*, 2014; Manaia *et al.*, 2016). Unfortunately, the
156 risks posed by these high loads of ARBs and ARGs are still poorly understood (Czekalski *et al.*, 2014;
157 Li *et al.*, 2016; Osinska *et al.*, 2016; Proia *et al.*, 2016; Li *et al.*, 2018).

158 The recognition of antibiotic resistance in waste water effluents has stimulated the study of
159 occurrence and potential control of antibiotic resistance in UWTPs. Most abundant classes of ARGs
160 were found in raw influents and final effluents, accumulated in activated sludge during
161 secondary/biological treatment or reaching the UWTP surrounding environment (Karkman *et al.*,
162 2018). Not surprisingly, these genes are frequently detected in or statistically associated to bacterial
163 genera and species known to be hosted transiently or permanently by humans (Narciso-da-Rocha *et*
164 *al.*, 2014; Narciso-da-Rocha *et al.*, 2018). In addition, the presence of mobile genetic elements
165 (MGEs), involved in horizontal gene transfer (HGT), particularly, plasmids and phages, as well as,
166 genetic recombination elements in the wastewater metagenome, highlights the potential for the
167 propagation of ARGs, within and across different environmental compartments (Dolejska and
168 Papagiannitsis, 2018). Of note, is the fact that this core wastewater resistome (totality of resistance

169 genes) and mobilome (the totality of elements involved in HGT and genetic recombination) is
170 continuously evolving by the rapid incorporation of genes emerging in clinical settings (Karkman *et*
171 *al.*, 2018).

172 Advanced treatment options, such as chlorination, ozonation or UV, or combined disinfection
173 approaches may reduce the environmental emission of ARGs from UWTPs (Dodd, 2012; Giannakis
174 *et al.*, 2018). The reactivation and regrowth of bacteria after disinfection, particularly of fast growing
175 bacteria, also may need attention in order to minimize the proliferation of ARB or ARGs after
176 disinfection and during water storage (Becerra-Castro *et al.*, 2016; Sousa *et al.*, 2017).

177

178 **4 Antibiotic resistance genes in aquaculture**

179 As a global source of fish and shellfish, the significance of aquaculture has increased remarkably
180 in the last 50 years (Watts *et al.*, 2017), with the aquaculture production already surpasses the catch
181 of the wild growing aquatic species. The term aquaculture can refer to a large spectrum of activities.
182 The extensive aquaculture relies on native species which are helped by removing predators and
183 competing species whereas in the intensive aquaculture the cultured species are more isolated from
184 the environment and the food is supplied externally. On the other hand, the term covers everything
185 between farming salmon offshore in the cold Atlantic water to tropical integrated aquaculture where
186 excess animal feed and livestock manure are nutrients for the aquaculture. Therefore, it is not too
187 surprising that there is no single picture of the antibiotic use in aquaculture.

188 Use of antibiotics in aquaculture is strictly regulated in Europe, Japan and North America. In
189 those regions, the use of antibiotics is limited to therapeutic applications where only a limited number
190 of antibiotics are approved. As an example, only five different antibiotics are authorized for use in
191 aquaculture in the UK, compared to 13 different ones in China (Liu *et al.*, 2017). Some forms of
192 aquaculture use antibiotics in a sustainable way, with the salmon production in Norway as probably
193 the best example. Between 1987 and 2013 the antibiotic use in that production system was reduced
194 by 99% concurrent with a 20-fold increase in the tonnage produced (Norwegian Ministry of Health

195 and Care Services 2015). The main factors behind such development are implementation of strict use
196 of vaccines and hygienic requirements, which partly resulted from active scientific research in this
197 field. There are obviously other than scientific and technological challenges involved since the uses
198 of antibiotics in aquaculture varies tremendously even with the same fish species. The reason behind
199 that difference is still not clear but it probably includes not only lack of vaccination, but also high fish
200 density and sub-optimal fishing practices which includes at least underdeveloped hygiene and feeding
201 with unknown components that might contain antibiotics or other agents causing the selection
202 pressure for antibiotic resistance. Additionally, aquaculture systems integrating the wastes from
203 livestock production are effective for nutrient cycling, but may have potential problems of
204 disseminating antibiotic resistance (Cabello *et al.*, 2016).

205

206 **5 Antibiotic resistance genes in soil**

207 Of all the environments impacted by humans, soil undoubtedly contains the richest and most
208 diverse populations of microorganisms (Thompson *et al.*, 2017) and DNA sequence (Rodriguez-R *et*
209 *al.*, 2018). Soil bacteria carry antibiotic resistance genes that are also found in human clinical
210 pathogens, and novel ones that are for the first time being discovered (Lau *et al.*, 2017). A key human
211 activity of concern with respect to antimicrobial resistance that impacts the soil environment is the
212 land application of fecal material, largely through agricultural practice (O'Connor *et al.*, 2005; Larney,
213 2011; Wang *et al.*, 2018). Organic amendments of animal (manures) or human (i.e. biosolids) origin
214 are widely used as a valued source of nutrients and for crop production and organic matter for soil
215 improvement. The use of antibiotics for prophylaxis or therapy in commercial food animal production
216 will select for and enrich ARB in the digestive tract, and these bacteria will end up in the manure
217 (Zhu *et al.*, 2013). Manure will also contain residues of antibiotics that have been excreted intact in
218 the urine or feces (Pope *et al.*, 2009). The microbial and chemical composition of raw manures will
219 vary with the commodity (i.e. poultry, swine, beef, dairy) and the medications used. Likewise,
220 biosolids contain residues of antibiotics and other potential co-selective agents such as biocides

221 (Sabourin *et al.*, 2012). Treatment practices that reduce the abundance of ARB and destroy antibiotic
222 residues will reduce soil loading rates of these contaminants and are therefore desirable (Lau *et al.*,
223 2017; Tien *et al.*, 2017).

224 Another increasingly important practice of concern with respect to potentiating antibiotic
225 resistance in soils is irrigation with reclaimed wastewater (Christou *et al.*, 2017). Irrigation with
226 nutrient-rich raw or poorly treated sewage is sometimes practiced in lower income countries (Thebo
227 *et al.*, 2017). Turfgrass in green spaces in urban or peri-urban settings may also be irrigated with
228 reclaimed wastewater (Wang *et al.*, 2014). Depending on the degree of treatment, reclaimed
229 wastewater will contain ARB and residues of pharmaceuticals including antibiotics (Pan and Chu,
230 2018).

231 Soil contamination with metals and organic chemicals other than antibiotics that co-select for
232 antibiotic resistance are of concern. Contamination of soil with copper and zinc from mining activities
233 or the application of manure from animals that consume feed supplemented with the metals can co-
234 select for genetic elements that confer resistance to both metals and antibiotics (Poole, 2017). Copper
235 is also commonly spread on fruits and vegetables as a pesticide against bacterial and fungal plant
236 pathogens. Interestingly, some herbicides decrease the sensitivity of Gram negative bacteria to some
237 antibiotics, possibly through upregulating efflux (Kurenbach *et al.*, 2015). Likewise, there is a
238 concern that the widespread use of fungicides in crop production will select for resistance that will
239 end up eroding the efficacy of fungicides used to treat human fungal or yeast infections (Jensen, 2016).
240 Fungicides are also entrained into soil through the application of biosolids (Chen *et al.*, 2013).

241 Given the chemical, biological and physical complexity of manures and biosolids it is extremely
242 difficult to determine which specific agent or classes of agents interact with soil microorganisms to
243 promote antibiotic resistance. Laboratory evidence suggests that in the presence of antibiotics, viruses
244 (bacteriophage) disseminate antibiotic resistance genes more rapidly in manured soil than in the
245 absence of antibiotics (Ross and Topp, 2015).

246 Soil can also affect the growth inhibition and selection pressure of antibiotics on the microbes

247 by sorption of the antibiotic reducing their bioavailability, which is determined by the chemical
248 properties of the antibiotic and the soil matrix. In some cases soil reduces the growth inhibition of
249 pathogens by certain antibiotics and for other antibiotics it does not (Chander *et al.*, 2005; Subbiah *et*
250 *al.*, 2011). More recent mechanistic work shows that soils and their associated mineral and organic
251 constituents affects the bioavailability of tetracycline as measured by a bioreporter linked to ARG
252 expression (Chen *et al.*, 2017). Antibiotic residues that are sorbed on clay mineral surfaces turned on
253 the bioreporter suggesting that they would be able to provide selective pressure for antimicrobial
254 resistance (Zhang *et al.*, 2018).

255

256 **6 Antibiotic resistance genes in the atmosphere**

257 Due to its emerging threat to public health, the existence of ARGs in the atmosphere, especially
258 urban air, has given rise to more attention. Comprehensive knowledge of the types and abundance of
259 ARGs in the air and whether they are associated with potential pathogens can provide new guidance
260 for assessment of air quality.

261 The relative abundance profiles of ARGs are spatially and temporally distributed in ambient air
262 of various cities. On heavy air pollution days, the increase of airborne particles is conducive to
263 suspending of microbes by providing more adhesion sites (Hu *et al.*, 2018). There was a higher
264 richness with 64 ARG types in bacterial biota in Beijing smog than in other environments, such as
265 pharmaceutically polluted environments, wastewater/sludge, animals and other terrestrial sources
266 (Pal *et al.*, 2016). These results indicate that airborne transmission plays a crucial role in the global
267 environmental distribution and exposure of antimicrobial resistance compared with other routes.
268 Evidence support that such a risk is attributed to diverse antibiotic usage in different cities. In rural
269 settings, airborne particulate matter promoted the transmission of microbial biota harboring
270 antimicrobial resistance genes downwind of cattle feedlots (McEachran *et al.*, 2015). The dispersal
271 patterns of particulate matter-borne ARGs were also impacted by physicochemical factors,
272 meteorological parameters and bacterial communities (Hu *et al.*, 2018). Pathogens in clinical ambient

273 air may be resistant to multiple drugs, threatening inhabitants as well as people outside hospitals via
274 airborne spread (Huang *et al.*, 2012).

275 Unfortunately, knowledge on airborne ARGs in geographically, culturally, and economically
276 different areas on a global scale is lacking. Their health impact is still unknown. On the other hand,
277 considerable attention is paid on particulate matter mass concentration, e.g. PM 2.5, while biological
278 parameters such as ARGs or ARB are yet to be adequately evaluated (Li *et al.*, 2018).

279 280 **7 Transmission of antibiotic resistance genes in the environment**

281 Two major factors determine the fate of ARGs in the environment: dispersal mechanisms and
282 selection. The dissemination of resistance genes is attributed to physical and biological forces
283 throughout various environments. Physical forces drive the dispersal of ARGs, such as wind and
284 waters, including erosion and leaching, but so do wild animals, e.g. birds, deer, raccoons. Major routes
285 of human exposures are from contaminated foods, e.g. vegetables with exposure to manures or
286 reclaimed wastewater, or meats contaminated with fecal waste from slaughter (Fig. 1). Less likely
287 sources are from drinking water, which is highly source dependent, and air. Pets can also be a source
288 and a recipient from their owner, and thereby cycle the AMR (Fig. 1). Proximity to human activities
289 provides an enriched source of ARGs for potential dispersal. For example, human proximity is closely
290 related to the antibiotic resistance profiles of the gut bacteria of wild mammals, whose habitats can
291 be affected by antibiotics (Allen *et al.*, 2010).

292 The selection pressure exerted on environmental bacteria can be of two types: selection for the
293 microbes that are carrying ARGs that is independent of the resistance trait, e.g. for particular carbon
294 sources, faster growth rate, resistances to stressors like pH; and selection for ARG trait which can be
295 the antibiotic itself or co-selection for resistances that are genetically linked to another (or multiple)
296 ARGs. The type of selective agent (chemical speciation and concentrations), co-exposure to other
297 selective agents (exposure period) and under what environmental conditions are essential for
298 differential bacterial growth (Baquero *et al.*, 2009; Larsson *et al.*, 2018). An overview of the

299 characteristics, variability and abundance of ARGs and mobile genetic elements, and their
300 transmission efficiencies in various circumstances is necessary to better differentiate ambient from
301 anthropogenic sources of antibiotics and ARGs in the environment (Hunter *et al.*, 2008; Zhu *et al.*,
302 2017; Larsson *et al.*, 2018). Such transfer and selection patterns of genes and bacteria are important
303 guides to distinguish the most urgent exposure and risk reduction practices.

304

305 **8 Conclusions and One Health perspective**

306 Humans profoundly impact the environment, in particular through agricultural practice. There
307 is a need to better understand what specific practices represent what risk with respect to AMR in soil,
308 water, air, and human health. This needs to be done considering the realities of how agriculture and
309 aquaculture are undertaken in high, middle and lower-income settings. There is also a need to better
310 understand how the fate and impacts of microbial and chemical contaminants that reach soil and water
311 vary with climate, particularly extremes of temperature and rainfall.

312 Importantly, changes in food animal production practices that reduce antimicrobial use will
313 reduce selection for and transmission of antibiotic-resistant bacteria. One practice that has long been
314 questioned is the use of sub-therapeutic doses for growth promotion in animal production. It has been
315 widely practiced because it does speed time to market and hence lower cost of food, but it has been
316 targeted as a non-essential practice that promotes AMR. Progress is being made though as this practice
317 has been banned in the EU since January 2006 and in the U.S. since January 2017. This could only
318 be accomplished in concert with changes in production systems that minimize disease and maintain
319 farmer profitability. Currently vaccination is an economically viable practice especially with high
320 value species (e.g. salmon) in some developed countries. Development of alternatives to antibiotics,
321 like vaccines, is needed but they must be cost competitive to have an impact on antibiotic use. This
322 is an important area for research.

323 The relevance of ARB and ARGs as environmental contaminants is now accepted but there still
324 exist some key priority issues. There is currently limited evidence that permit an objective and

325 quantitative assessment of the human health risks posed by the occurrence of ARB in the environment.
326 For this reason, it is difficult to define threshold values for the maximum admissible levels of ARB
327 and ARGs in treated wastewater, in sludge and manure to be used in agriculture. Increasing water
328 scarcity and soil degradation will drive increased need for water reuse, and beneficial reuse of
329 municipal sludges and manures, likely increasing inputs of ARGs and ARBs into soil. The movement
330 of people and food between higher and lower income countries will contribute to the global
331 transmission of resistance from regions with poor sanitary conditions. Improving water sanitation and
332 hygiene in lower income countries should be a global priority. Overall, adequate risk assessment
333 frameworks, policy development and implementation, definition of circular economy good practices
334 and ensuring adequate sanitation are priorities to reduce antibiotic resistance burden and global
335 transmission (Pruden *et al.*, 2013).

336 The One Health framework was developed in recognition of the inter-domain system in which
337 antibiotic resistance resides and circulates (Fig. 1). Among the three domains, human health is the
338 spotlight, due to multidrug-resistance genes have been prevalent in several important pathogens. To
339 achieve the goal of One Health, veterinarians, physicians, food safety professionals, wastewater
340 treatment plant operators, environmental experts and regulators are obliged to work together. At a
341 policy level, all countries were asked to formulate a “National Action Plan” to combat antimicrobial
342 resistant appropriate for their country (WHO, 2017). Many have done so, but the challenge is now to
343 effectively implement and monitor these plans, and to validate the efficacy of practices intended to
344 reduce the development and spread of AMR. Thus the overriding importance of undertaking
345 surveillance is across the One Health Framework.

346

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357

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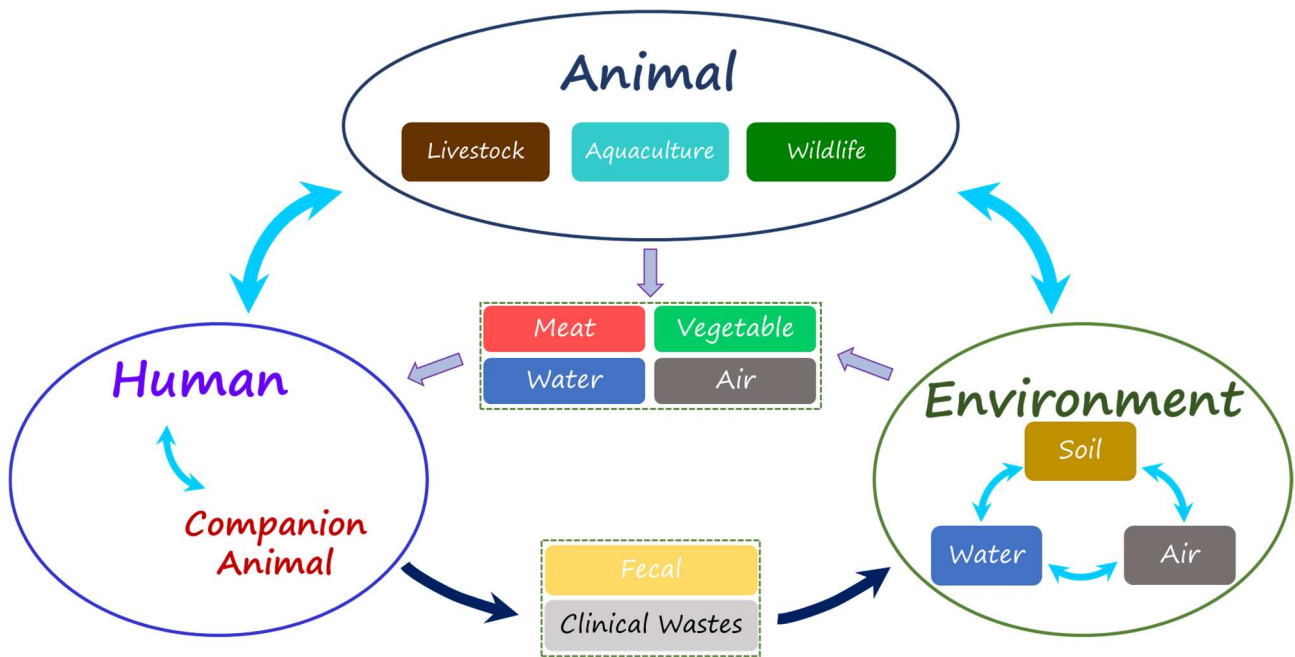
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605

606 Fig. 1 The transmission of antibiotic resistance in the One Health framework with Human Health,
 607 Animals and Environment as the major domains through which antibiotic resistance genes and
 608 microbes reside, amplify and spread. Most arrows are in both directions reflecting these pathways,
 609 and the cyclic nature of the problem. Human exposure can be directly from animals and from food,
 610 water and air, but humans can also spread resistance to those domains, and especially via fecal wastes.
 611 Companion animals are grouped with humans because of higher potential for ARG exposure and
 612 bidirectional transfer.