

1 **Systems-thinking approach to identify and assess feasibility of potential interventions to reduce**
2 **antibiotic use in tilapia farming in Egypt**

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17 **Abstract**

18 Antibiotics are used in aquaculture to maintain the health and welfare of stocks; however, the emergence
19 and selection of antibiotic resistance in bacteria poses threats to humans, animals and the environment.
20 Mitigation of antibiotic resistance relies on understanding the flow of antibiotics, residues, resistant
21 bacteria and resistance genes through interconnecting systems, so that potential solutions can be identified
22 and issues around their implementation evaluated. Participatory systems-thinking can capture the deep
23 complexity of a system while integrating stakeholder perspectives. In this present study, such an approach
24 was applied to Nile tilapia (*Oreochromis niloticus*) production in the Nile Delta of Egypt, where disease
25 events caused by antibiotic-resistant pathogens have been reported. A system map was co-produced with
26 aquaculture stakeholders at a workshop in May 2018 and used to identify hotspots of antibiotic use,
27 exposure and fate and to describe approaches that would promote fish health and thus reduce antibiotic
28 use. Antibiotics are introduced into the aquaculture system via direct application for example in medicated
29 feed, but residues may also be introduced into the system through agricultural drainage water, which is the
30 primary source of water for most fish farms in Egypt. A follow-up survey of stakeholders assessed the
31 perceived feasibility, advantages and disadvantages of potential interventions. Interventions that
32 respondents felt could be implemented in the short-term to reduce antibiotic usage effectively included:
33 *more frequent water exchanges, regular monitoring of culture water quality parameters, improved storage*
34 *conditions for feed, use of probiotics and greater access to farmer and service providers training*
35 *programmes*. Other potential interventions included *greater access to suitable and rapid diagnostics, high*
36 *quality feeds, improved biosecurity measures and genetically-improved fish*, but these solutions were
37 expected to be achieved as long-term goals, with cost being of one of the noted barriers to
38 implementation. Identifying feasible and sustainable interventions that can be taken to reduce antibiotic
39 use, and understanding implementation barriers, are important for addressing antibiotic resistance and
40 ensuring the continued efficacy of antibiotics. This is vital to ensuring the productivity of the tilapia sector
41 in Egypt. The approach taken in the present study provides a means to identify points in the system where

42 the effectiveness of interventions can be evaluated and thus it may be applied to other food production
43 systems to combat the problem of antibiotic resistance.

44 **Keywords**

45 Antibiotic resistance; antimicrobial resistance; One Health; aquaculture; tilapia; Egypt

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48 **1. Introduction**

49 Antibiotic resistance (ABR) is one of the greatest challenges we face in the 21st century and it is a classical
50 One Health problem with human, animal and environmental components (Robinson et al., 2016).

51 Antibiotic-resistant bacteria and antibiotic-resistance genes (ARG) transfer between these components,
52 which complicates the tracking of their flow and adds complexity when developing solutions (Robinson et
53 al., 2016). The problem of ABR must be addressed to maintain the effectiveness of antibiotics in food
54 production and for patients in healthcare settings.

55 The global aquaculture sector is a major user of antibiotics, where these agents are applied to maintain the
56 health and welfare of stocks, though usage and practices vary widely across the world (Henriksson et al.,
57 2018; Lulijwa et al., 2020). Accordingly, the problems posed by ABR vary, though much of the burden of the
58 issues encountered falls on low- and middle-income countries (LMICs), where controls on antibiotics may
59 not be as strict or as strongly enforced as in wealthier counterparts (Robinson et al., 2016; Henriksson et
60 al., 2018).

61 ABR in aquaculture poses a threat to human health and to the contamination of the environment with
62 antibiotic residues and resistant organisms. Many of the classes of antibiotics used in aquaculture are
63 identical to those used to treat terrestrial farm animals and human patients (Rico et al., 2013; Done et al.,
64 2015; Lulijwa et al., 2020). In LMICs, aquaculture systems are highly complex and often integrated with
65 other food production systems (Cantas et al., 2013; Chuah et al., 2016; Watts et al., 2017; Shen et al.,
66 2018), such as through sharing of common water sources, making them highly vulnerable to the
67 introduction and widespread dissemination of ABR (Cabello et al., 2016; Brunton et al., 2019). The
68 complexity of these systems creates multiple points for human exposure to antibiotic residues, ARGs and
69 resistant organisms; our earlier study identified three key pathways for human exposure to these to occur
70 through occupational duties, consumption of contaminated food and environmental exposure (Brunton et
71 al., 2019).

72 Egypt produces more fish from aquaculture than the rest of Africa combined, with production trebling since
73 2005 to 1.56 million tonnes in 2018 (FAO, 2020). Aquaculture in Egypt is dominated by the production of
74 Nile tilapia (*Oreochromis niloticus*) in earthen ponds, with production concentrated in the northern regions
75 of the Nile Delta. The sector has undergone considerable expansion in recent decades, driven by the
76 development of privately-owned hatcheries and feed mills (El-Sayed, 2017), and Egypt produced 1,051,444
77 tonnes of tilapia in 2018 (FAO, 2021b). The sector is composed mainly of small-scale producers and the
78 value chain, including production constraints and consumer behaviour and preferences, has been described
79 in detail recently (Eltholth et al., 2015). Like tilapia producers elsewhere, fish farmers in Egypt experience
80 disease challenges (Ali et al., 2020), including infectious diseases caused by bacteria such as *Aeromonas*
81 *hydrophila*, enterococci species, *Pseudomonas fluorescens* and *Streptococcus iniae* (Aly, 2013; Osman et al.,
82 2016; Osman et al., 2017). Antibiotics are used by farmers to treat infections in their stocks but antibiotic-
83 resistant pathogens have been reported to cause infections in farmed tilapia in Egypt (Eltholth et al., 2015;
84 Ishida et al., 2010; Osman et al., 2016; Osman et al., 2017). It is essential that this problem is addressed to
85 ensure the sustainability and continued productivity of the sector, which supports livelihoods, particularly
86 in rural communities, and is a major source of affordable animal protein for the country's population.
87 Indeed, almost all tilapia production in Egypt is for domestic consumption and only 2,059 tonnes of product
88 were exported in 2018 (FAO, 2021a).

89 Many approaches can help to mitigate against the problems posed by ABR and this includes interventions
90 that decrease overall use of antibiotics, such as by reducing need and dependency on them. When
91 identifying mitigation measures for ABR, including ways to reduce the need for their application, it is vital to
92 include all relevant stakeholders in the process and the decision-making. Such participatory approaches
93 capture the range of views, lead to a sense of collective responsibility towards the problem, and ensure a
94 feeling of shared ownership towards solutions (Evans and Terrey, 2016; Blomkamp, 2018). Systems thinking
95 attempts to integrate the multi-level aspects of a system, including actors, processes, and governance
96 structures. The approach can be used to generate a map that can then be used to understand how an
97 intervention in one part of the system to address a problem will impact on the functioning of the entire

98 system, thus permitting the recognition of consequences in an attempt to avoid unintended negative
99 outcomes elsewhere in the system (Peters, 2014). Brunton et al. (2019) used a participatory system-
100 thinking approach to consider points in aquaculture systems in Vietnam where ABR could emerge, or be
101 enriched through selection, by exposure to antibiotics and other selectors of ARGs.

102 In this present study, we aimed to identify potential interventions that may reduce antibiotic use (ABU) in
103 tilapia production systems in Egypt through a participatory systems-thinking approach with key
104 stakeholders. To meet this aim, it was necessary to (1) map the tilapia production system in Egypt; (2)
105 identify hotspots of ABU, exposure and fate within the system; (3) describe approaches that would
106 promote fish health, including the use of alternatives to antibiotics, and (4) conduct a follow-up survey of
107 key stakeholders to assess the perceived feasibility, advantages and disadvantages of potential
108 interventions to reduce or prevent ABU, including actual or perceived barriers to implementation.

109

110 **2. Methods**

111 *2.1. Stakeholder workshop*

112 A one-day participatory workshop was organised with aquaculture stakeholders in Kafrelsheikh, Egypt, on
113 10 May 2018. The workshop involved 44 participants from a range of disciplines and stakeholder groups,
114 including professionals from the aquaculture, livestock and veterinary sectors working in the private sector
115 (e.g., tilapia producers, input producers, agrovets, feed producers, and pharmaceuticals), academics, and
116 employees of international development and public sector institutions (Table 1). The expertise among
117 participants included aquaculture and aquatic health management, veterinary epidemiology and public
118 health, food safety, microbiology, marine biotechnology and medical anthropology. This range of
119 participants was invited to capture a diversity of perspectives, expertise and experiences, and to maximise
120 engagement and collaboration amongst distinct stakeholder groups. Participants were allocated to three
121 groups, each containing a mix of expertise. Outputs were compared between the groups, so to permit

122 cross-checking and discussion of the outputs as a method of validation. Each group contained two
123 members of the research team, one of whom facilitated the activity (the facilitator) and another who took
124 notes of discussions throughout the process (the recorder). All workshop activities were facilitated in
125 English and simultaneously translated into Arabic by members of the research team and participants,
126 where required.

127 The workshop activities consisted of introductory presentations, the main mapping exercise, and a final
128 plenary activity. During the introduction, participants were provided with a contextual overview of tilapia
129 production and value chain in Egypt, including post-harvest operations, the workshop aim and objectives
130 and planned activities, as well as an introduction to systems thinking and its application to food production
131 systems. To familiarise participants with the systems concept and the mapping process, an exercise was
132 conducted whereby each group was asked to draw a simple system on large whiteboards or paper sheets
133 affixed to the wall, exemplifying 'making a cup of tea'. Groups had to identify elements of the system such
134 as tangible and intangible components, including actors, infrastructure, governance systems and
135 relationships, and economic and environmental factors. The exercise introduced participants to the
136 mapping process and the importance of establishing boundaries (i.e., 'edges') with other systems. This
137 approach is based on the methodological framework proposed by the Network for the Evaluation of One
138 Health (NEOH) (Rüegg et al., 2018).

139 2.2. Mapping of the tilapia system

140 Each group was guided through the mapping activity to accomplish a series of objectives, and these
141 objectives were framed to consider four dimensions of the system (Figure 1). The groups were asked to:

- 142 • *Map* the components of the system (e.g. production stages, inputs, outputs, actors and
143 relationships);
- 144 • *Identify* any factors in the system that impact production and fish health;
- 145 • *Identify* where ABU and antibiotic residues occur in the system;
- 146 • *Explore* drivers of ABU in the system and decision making processes of stakeholders;

- 147 • *Discuss* possible interventions and alternatives to antibiotics, and
- 148 • *Identify* knowledge and data gaps.

149 The four dimensions within which these objectives were considered were the *human dimension*, related to
150 stakeholder behaviour; *the pathogen and host factors*, including information on common pathogens and
151 diseases in tilapia; *the environment*, considering information on related systems such as the agricultural
152 and drainage systems; and *governance*, considering the influence and power of different stakeholders on
153 decision-making or practices. A protocol was developed by the research team members to facilitate the
154 mapping process, which included the key elements to be captured, such as suggestions for alternatives to
155 antibiotics, and prompting questions to promote discussion and participation.

156 Participants were asked to identify hotspots on the maps where antibiotic residues or ARGs may be
157 present. Hotspots were defined in three ways, concerning system *nodes*, system *inputs* and *outputs*, and
158 system *management*. Nodes in the system represent junctions, where there is active use of antibiotics, or
159 where there is a potential presence of antibiotic residues or ARGs due to the flow of products through the
160 system (e.g., hatchery, nursery ponds, grow-out ponds, poultry farms, etc.). Products, inputs or outputs of
161 the system could contain antibiotic residues or select and/or enrich for ARGs (e.g., antibiotics, feed, manure
162 from poultry farms, drainage water, etc.). Management practices may involve the active use of antibiotics
163 or use of products containing antibiotic residues or ARGs (e.g., during the fertilisation stage of pond
164 preparation using poultry manure, the crop production using drainage water from aquaculture farms, etc.).

165

166 2.3. Plenary activity and follow up survey

167 The main findings from each group were summarised and presented back to all participants in a plenary
168 session, highlighting the key components of the system, major factors impeding production and good
169 management, drivers of ABU, the flow of antibiotics and residues in the system, and areas of disagreement
170 or inconsistency between the groups. Following this, a concluding exercise was conducted to compile

171 information on alternatives to antibiotics that had emerged during the mapping process in each group. In
172 addition, participants were asked to suggest any additional alternatives to antibiotics and interventions that
173 may reduce ABU by improving fish health and to rank these based on perceived feasibility and
174 effectiveness.

175 These discussions provided the foundation for a post-workshop survey to investigate the perceptions and
176 attitudes of the workshop participants, and additional stakeholders not in attendance, towards alternatives
177 to antibiotics and interventions aiming to improve fish health management practices and thus reduce ABU
178 (Supplementary file 1). The survey was structured and developed by the research team using information
179 gathered at the workshop, a review of existing literature, and expertise within the team. This resulted in a
180 list of 31 alternatives to antibiotics, or potential interventions, which were divided into eight groups. The
181 groups were: *farm management practices; new breeds and genetic improvement; disease diagnostics;*
182 *monitoring and control; biosecurity; rewards and incentives; education and training; and alternative*
183 *therapies and products, e.g. vaccines, immunostimulants and others. Survey respondents were asked about*
184 *the perceived feasibility (short term, long term, or not possible to achieve), and advantages and*
185 *disadvantages (affordability, availability, ease and speed of application or implementation, and*
186 *effectiveness), and then to rank the interventions in terms of overall perceived importance to reduce ABU.*
187 The survey was translated into Arabic and purposefully distributed by email to workshop participants via
188 SurveyMonkey, and in-person through the networks of the research team in the Kafrelsheikh Governorate
189 (completed either by email or face-to-face), aiming to reach aquaculture professionals, particularly farmers.

190 2.4. Analysis of maps, consensus map and validation

191 At the end of the workshop, each map developed by the three groups was photographed and translated by
192 the research team into a single, consensus digital diagram using the web-based Lucidchart software
193 (<https://www.lucidchart.com/>). The components of the consensus map were classified broadly into: (1)
194 *pre-harvest activities and production; (2) inputs, such as feed and chemicals; (3) waste products; (4) post-*

195 *harvest activities* and (5) *governance, roles and responsibilities of actors* taking decisions in the systems

196 particularly in regard to fish and public health.

197 **3. Results**

198

199 3.1 Mapping tilapia production systems

200 3.1.1 Description of the system

201 Three workshop groups each constructed a map of the tilapia production system in the Nile Delta and it
202 was apparent that each group had focused to varying degrees on distinct parts of the system, which
203 probably reflected the different experiences of the participants in the groups. A consensus system map was
204 constructed from these original hand-drawn maps (Figure 2), and this was relatively simple given the near
205 complete absence of contradictory information. Tilapia is cultured almost exclusively in earthen grow-out
206 ponds that are prepared initially by draining and removing sediment. The pond is then sun-dried and
207 disinfected with lime, before filling with water. Thereafter, a bloom of microorganisms, most likely
208 phytoplankton, zooplankton and bacteria, is initiated in the pond water through fertilisation with organic
209 fertilisers such as poultry manure. This microbial community formed in the pond improves the water
210 quality, provides additional nourishment to the fish, and may confer some protection against pathogens
211 through niche closure and stimulation of the host immune system (Ahmad et al., 2017).

212 Grow-out earthen ponds (mean area ca. 1.3 ha) are usually located in the northern regions of the Nile
213 Delta, with a main water supply of agricultural drainage water. Grow-out ponds are stocked either with
214 mono-sex fry obtained directly from the local hatcheries, or by fingerlings that have been over-wintered in
215 on-farm nursery ponds. The stocking of grow-out ponds usually starts in March. Almost all tilapia farmers
216 cultivate tilapia in polycultures with two species of mullet, the flathead grey mullet (*Mugil cephalus*) and
217 thin lip mullet (*Liza ramada*). Over the last 10 years, there has been a shift from mostly extensive
218 production to semi-intensive and intensive systems due to the increased availability of hatcheries and
219 commercial feed. Very few farmers have access to electricity and a limited number of farms use some type
220 of aeration; most farmers use diesel pumps to pump water in and out of ponds. Feed mills provide feed
221 formulated to contain a variety of raw ingredients, including soy bean meal (and oil extracted from the

222 beans), corn, and blood and fish meal. More than 90% of fish farms are using formulated commercial feed
223 and almost all farmers add feed to the pond manually. Some feeds are supplemented with additives, such
224 as probiotics, prebiotics, minerals, enzymes and antimycotoxins, which aim to prolong the shelf life,
225 increase digestibility and enhance the fish health status. These supplements may be added during
226 manufacture at the mill, but often are incorporated into the feed by farmers themselves. Supplements are
227 sourced from specialist retailers called 'aqua shops' marketing products exclusively for fish producers,
228 although these outlets may also sell products intended for use in other livestock.

229 The water entering the system is derived mainly from agricultural drainage from terrestrial crop production
230 (ca. 70% of farms), with a small proportion of farms deriving water from the Nile (ca. 20%) and
231 groundwater (ca. 10%). A small number of ponds have access to irrigation water, but this is illegal to use for
232 fish production, while some others have access to lake water. The water is channelled via a network of
233 interconnecting canals that link the fish farms. Few farms have access to groundwater to fill or top up
234 ponds during water exchange meaning most farms rely on the canals, but this water may contain
235 chemicals, sediment and microorganisms. All farms release wastewater into the canal network, which
236 exacerbates potential cross-contamination and means water of poor quality, containing potentially
237 pathogenic microorganisms and drug residues, may be introduced back into the tilapia ponds.

238 Fish exhibiting suspect clinical signs of infectious disease (e.g., bacterium or parasite) may be treated by
239 adding disinfectants to the pond water, such as formalin, copper sulphate or potassium permanganate.

240 During episodes of increased morbidity and mortality in the grow-out ponds, farmers often reduce the
241 water level in the pond before adding medications to the water. However, low diagnostic capacity,
242 stemming from a lack of trained veterinarians with expertise in aquatic animal health, and reliance on a
243 range of providers of varying quality for diagnostic support, means that diseases are likely to be commonly
244 misdiagnosed. In addition, antibiotics are available over the counter (i.e. without the need of a veterinary
245 prescription), from aqua shops and similar retailers, where they may be incorporated into feed, either by
246 the feed mill upon request or at the farms by the farmers. Opinions of whether feed mills add antibiotics to
247 feed or not were inconsistent across workshop groups. One group suggested this to be a common practice,

248 while another group maintained that feed mills do not add antibiotics to feed. However, further discussions
249 with a feed mill owner confirmed that feed mills did add medications to feeds upon request, but this was
250 rare and costly. Furthermore, antibiotics may be added to the culture water to treat diseased fish at
251 hatcheries and nurseries.

252 At harvest, fish are netted and transported to the markets without refrigeration or via a rudimentary cold
253 storage system (i.e. ice), which means the fish must be sold within a few hours (Figure 2, blue section). A
254 limited network of wholesalers supplies a range of sales outlets, including supermarkets, restaurants, fry
255 shops, and street side vendors.

256 The workshop maps showed that various government ministries are involved in governing the tilapia
257 production system. Ministerial involvement aims to promote and expand the industry. *The Ministry of*
258 *Agriculture and Land Reclamation* governs three main authorities: *The General Authority for Fish Resources*
259 *Development* (GAFRD), *The General Organization for Veterinary Services* (GOVS) and *The Agriculture*
260 *Research Centre* (ARC). The GAFRD drafts the legislation and regulations for fisheries and manages farm
261 licensing, aquaculture land use regulations, as well as extension and research services. The GOVS controls
262 the import/export of fish and conducts aquatic health surveillance, though this is not yet in place. The ARC
263 includes *The Animal Health Research Institute* (AHRI), which contains a department focused on the
264 management of fish diseases, and the *Central Laboratory for Aquaculture Research* (CLAR). *The Ministry of*
265 *Water Resources and Irrigation* controls water use and authorises the issuing of licences by *The Ministry of*
266 *Agriculture and Land Reclamation* to farmers to permit them to take water from the agricultural drainage
267 canals, though farmers rarely obtain such permits. It is illegal to take water from the Nile for aquaculture
268 use, although this may occur to a small degree.

269 *The Ministry of Environment* oversees the use of land for aquaculture purposes through the Egyptian
270 Environment Affairs Agency (EEAA). The establishment of new fish ponds must be subject to an
271 Environmental Impact Assessment (EIA), which is reviewed by the EEAA. The scope and depth of the EIA is
272 determined by the EEAA on the basis of the information provided in the application, and will vary

273 depending on factors such as whether the farm is situated in an urban setting, or an ecologically sensitive
274 or protected area.

275 *The National Food Safety Authority (NFSA)* carries out inspections and performs laboratory analysis of fish
276 samples for the domestic market and to meet export conditions as required. *The Ministry of Higher*
277 *Education and Scientific Research* includes educational and research institutes that support the tilapia
278 sector with qualified aquaculture specialists and research outputs to improve the industry.

279

280 Boundaries to the tilapia production system map include: the sources of ingredients for feed, including feed
281 additives; international and overseas agencies of governance; crop production; poultry (and other
282 livestock) production; production of chemical inputs (e.g., disinfectants, antibiotics); feed supplements; ice,
283 energy, and brood stock; domestic wastes that may contaminate water sources; production of equipment
284 such as nets and tanks; sales and marketing outlets; and service providers (e.g. veterinarians etc.).

285

286 3.1.2 Factors that impact production and fish health

287 Different stakeholders participating in the workshop emphasised factors that impact production and fish
288 health. Producers mentioned several factors that often appeared to be interconnected, including the high
289 prices of the inputs and dependency on value chain stakeholders, fluctuation of market prices of the
290 products, access to clean water and irrigation systems, losses due to diseases, and regulations such as the
291 restrictions in the irrigation systems and the marketisation of products.

292 Fish health and management stakeholders mentioned occasional moderate mortality rates and outbreaks,
293 but disease was often described to be linked to management deficiencies. In particular, poor water quality
294 was described to be a key factor impacting fish health by different stakeholders. Further, workshop
295 participants observed that pressure to produce a timely harvest favoured short-term solutions and
296 prophylactic use of veterinary products, instead of investing or focusing on improved disease prevention
297 and control, management and biosecurity.

298 Stakeholders mentioned the generalised lack of monitoring for water quality parameters at the farm level,
299 and many producers rely solely on organoleptic characteristics to determine water quality. Similarly, there
300 is no widespread use of disease diagnostic tests and antibiotic susceptibility testing, which often results in
301 recurrence of infectious diseases and ineffectual empirical treatments.

302

303 3.1.3 Antibiotics in the system

304 Fish mortality was the main driver directly leading to ABU, generally described to be applied to treat stocks
305 with evidence of clinical signs, but participants also mentioned occasional prophylaxis when neighbouring
306 farmers were experiencing an outbreak suspected to be due to an infectious disease. The most common
307 product referenced was oxytetracycline (a tetracycline), though sulphonamides and other antibiotic
308 substances were also mentioned in discussions. During a visit to two aqua shops in the locality of the
309 workshop by the research team, products containing dihydrostreptomycin (an aminoglycoside),
310 oxytetracycline, ciprofloxacin (a fluoroquinolone), erythromycin (a macrolide), and sulphadimidine (a
311 sulphonamide) were observed for sale.

312 One direct way antibiotics enter the system is from the aqua shops and similar retailers. Antibiotics are
313 used to treat diseases and these may be added to feed or to the pond water particularly where water-body
314 volumes are smaller such as at hatcheries and nurseries, though grow-out pond water levels can be
315 reduced for treatment in this way by releasing water into the agricultural drainage canals. Antibiotics may
316 also be introduced into the tilapia production system as residues in wastewater derived from other
317 systems, including domestic waste, which enters into the agricultural drainage canals used subsequently by
318 fish farmers to replenish the ponds. Thus, these canals represent a potential source and reservoir of
319 antibiotic residues, which allows them to be transported widely across the system. A further possible
320 source of antibiotic residues and resistant bacteria entering the tilapia production system may be the use of
321 organic wastes, such as untreated poultry manure, to fertilise the ponds prior to stocking.

322

323 Having plotted the movements of antibiotics within the system, the workshop groups proceeded to identify
324 hotspots where the presence of antibiotics or residues, or other conditions or compounds, may select for
325 resistant organisms containing ARGs. Hotspots identified included: *hatcheries and nurseries*, where
326 antibiotics may be applied to prevent or treat disease events; *the drainage canals* and the water contained
327 within, where residues from various systems are transported and may accumulate; and the *grow-out ponds*
328 at the farms, where antibiotics may be introduced during fertilisation with poultry manure, during
329 application of medicated feeds, from the introduction of contaminated canal water, or where the
330 application of co-selectors of ARGs such as disinfectants are applied. Further hotspots included the *feed*
331 *mills*, where antibiotics may be incorporated into fish feed, and in *crop and poultry production*, where
332 antibiotics may be applied to prevent or mitigate infectious diseases or as growth promoters.

333

334 3.2. Attitudes and perceptions of survey respondents

335 A total of 69 responses to the survey were received within six weeks of the workshop. Respondents
336 represented a variety of stakeholders as described in Figure 3, but primarily fish farmers (n=51). Of the 69
337 respondents to the survey, 74% were not present at the workshop. Response rate for individual questions
338 ranged from 84% to 100%, with 84% of respondents completing the survey in full. All 51 farmers were
339 surveyed face-to-face and completed the survey in full. A summary of the responses from the entire survey
340 is presented in Figures 4a and 4b.

341

342 Improving water exchange practices and storing feed in more suitable conditions were perceived to be
343 most achievable in the short-term to improve fish health, mainly because these actions were felt to be
344 effective and relatively easy and inexpensive to implement. Using pond fertilisers most appropriately was
345 perceived to be a possible solution to be implemented in the short-term, but only 10% of respondents
346 thought that this approach would actually be effective in improving fish production and reducing ABU.
347 There was an agreement that pond modifications allowing fish to select thermal preference (Cerqueira et
348 al., 2016; Tabin et al., 2018) could be achieved and such changes were felt strongly to be effective, but

349 there was a split in opinion on the possible timescale required for this intervention, which was also
350 perceived to be expensive. More than 40% did not know whether better quality, nutritious feed than what
351 is currently available could be achieved (e.g., by sourcing higher quality ingredients and improved
352 manufacturing practices) and this likely reflects the lack of knowledge among respondents regarding the
353 potential impact on productivity and fish health, or what is needed for this to be accomplished.
354 Furthermore, it was clear that alternative sources to the agricultural drainage water were not thought to be
355 possible due to the current restrictions on water use imposed by existing legislation, despite being
356 identified to be an effective way to improve fish production and reduce ABU.

357

358 There was strong support for the development of genetically improved fish for disease resistance as a long-
359 term goal and an effective strategy to reduce disease and ABU, but disease-resistant strains were
360 recognised to be unavailable and costly to develop. There was strong support for the introduction of rapid
361 diagnostics and a role for the routine application of antibiotic sensitivity testing as effective ways to reduce
362 disease and ABU, although there was a split in opinion on whether this was achievable. The majority of
363 respondents indicated that such tests were unavailable, with others saying the prohibiting factor in their
364 implementation was high cost.

365

366 Respondents perceived short-term benefits to be delivered by monitoring water quality parameters, such
367 as dissolved oxygen levels and water 'transparency', and making an inventory of fish clinical signs and
368 behaviour to inform diagnosis, which largely stemmed from being relatively easy to implement, although
369 there were concerns around the cost of tests. Improved biosecurity was perceived to be achievable by most
370 respondents as a way to reduce disease outbreaks and ABU, with most thinking this would be effective.
371 Although almost a third of respondents felt this would not be possible due to the cost, and the difficulty
372 and time needed to achieve improvements.

373

374 There was support for developing export markets, certification and quality assurance schemes as long-
375 term, effective strategies to reduce disease outbreaks and ABU. However, many respondents were unsure
376 of the benefits and barriers to developing export markets. Producer associations and cooperatives were
377 thought by many respondents to be easy to establish, though many respondents were unsure whether this
378 could be realised. The questions pertaining to education and training showed an appetite amongst
379 respondents for learning and acquiring new skills, and this was seen as achievable in the short term, easy
380 and effective for reducing ABU and disease outbreaks, although concerns were raised about cost (mostly)
381 and a lack of availability.

382

383 Various alternatives to antibiotics for preventing and treating disease had been proposed during the
384 workshop discussions. Amongst these, the use of vitamin C, probiotics and immunostimulants all received
385 strong support and were perceived to be capable of delivering benefits in the short-term due to their
386 effectiveness, low cost, ease of availability and ease of use. Application of natural plant extracts was
387 perceived to offer benefits for similar reasons; however, their use was envisaged further in the future. Few
388 respondents viewed vaccination positively in its potential effectiveness to reduce disease and ABU, possibly
389 due to lack of availability, and there was little knowledge of the potential of bacteriophage therapy in
390 disease control.

391

392 Finally, in a ranking exercise, improved farm management practices and biosecurity were ranked highest in
393 terms of importance to reduce ABU and improve aquaculture, with (presumably overt) rewards and
394 incentives perceived to be least important by a considerable margin (Figure 5).

395

396 **4. Discussion**

397 The aim of this study was to identify potential interventions that may reduce ABU in farmed tilapia
398 production in Egypt using a participatory systems-thinking approach with key stakeholders from this
399 industry. A consensus map of the farmed tilapia production system was created and used to identify
400 possible hotspots of ABU and emergence of ABR, routes of antibiotic flow through the system, and
401 interactions with other systems. Thereafter, approaches that would promote fish health and reduce or
402 prevent ABU were proposed, discussed and their perceived feasibility assessed by key stakeholders through
403 a survey that followed the workshop.

404 Previous assessments of the tilapia sector in Egypt using value-chain analysis have provided information
405 concerning structure and performance, and identified key constraints within the sector (Macfadyen et al.,
406 2012; Eltholth et al., 2015). Here, a systems-thinking approach was taken to integrate the multi-level
407 aspects of the system, such as governance and the environment, and to provide a framework for exploring
408 how a proposed intervention to mitigate ABR at one point in the system might affect the functioning of the
409 entire system. Such an approach is needed due to the complexity of the system, and the participatory
410 nature of the approach is beneficial because it can capture a range of views, leads to a sense of collective
411 responsibility towards a problem, and provides a feeling of shared ownership towards solutions (Evans and
412 Terrey, 2016; Blomkamp, 2018). Mapping the system provided the means to visualise ABU in the sector,
413 which allowed for identification of how and where in the system antibiotics are introduced, how and where
414 they are transmitted, and the points in the system that represent potential hotspots for the emergence and
415 selection of ABR.

416 In a previous study, a similar approach was used to identify hotspots for the emergence of ABR in striped
417 catfish and shrimp farming in Vietnam (Brunton et al., 2019). Similarities between the aquaculture
418 production systems in Egypt and Vietnam emerged with respect to the locations of hotspots for the
419 emergence of ABR, with culture ponds, feed mills and markets identified in both exercises (Brunton et al.,
420 2019). Moreover, these hotspots hold the potential for human exposure to antibiotic residues, ARGs and

421 antibiotic-resistant bacteria. Other studies have reported similar findings, with a variety of antibiotic
422 residues being detected in aquaculture ponds in Vietnam (Le et al., 2005; Andrieu et al., 2015; Giang et al.,
423 2015; Nakayama et al., 2017), and antibiotic-resistant bacteria detected in aquaculture ponds in Egypt
424 (Ishida et al., 2010; El-Gohary et al., 2020).

425

426 Mapping the farmed tilapia production system highlighted the interaction between aquaculture and
427 agricultural systems, with water contaminated with antibiotic residues able to permeate the whole delta
428 and move between other systems, including crop and terrestrial animal production systems. For example,
429 poultry manure and litter are commonly used as fertiliser for fish farming due to its nutritional value
430 (Mahmoud and Abdel-Mohsein, 2019), but it is rarely treated (e.g. pasteurised) before use (Eltholth et al.,
431 2015). This can represent a possible source of antibiotic residues (Petersen et al., 2002), in particular
432 tetracyclines, which are used extensively in poultry production in Egypt (Mahmoud and Abdel-Mohsein,
433 2019). Tetracycline concentrations exceeding European Union maximum residue limits (100 µg/kg) have
434 been detected in wild and farmed tilapia in the Nile delta region, with greater concentrations in farmed
435 versus wild samples suggested to be due to the use of contaminated poultry manure as pond fertiliser
436 (Mahmoud and Abdel-Mohsein, 2019). Indeed, high concentrations of tetracycline residues were detected
437 even on fish farms with no recent history of using this antibiotic (Mahmoud and Abdel-Mohsein, 2019),
438 indicating soil and groundwater may act as reservoirs for antibiotic residues (Boxall et al., 2003). In
439 addition, high levels of ARGs have been detected in poultry manure and litter, and these genes may be
440 carried by pathogens (Abu-Elala et al., 2015; Mahmoud and Abdel-Mohsein, 2019) and commensal bacteria
441 alike.

442

443 Availability of and accessibility to irrigation canal water was a key challenge that emerged during both the
444 workshop discussions and from the survey data. Agricultural drainage water is the main water supply for
445 most fish farms in this region of Egypt, but it represents a major biosecurity risk and is a key potential

446 source of antibiotic residues, ARGs and antibiotic-resistant bacteria that may contaminate the culture
447 ponds (Eltholth et al., 2015). The use of irrigation water in fish ponds is not allowed because it is prioritised
448 for agricultural use (GAFRD Law No 124/1983). Since agricultural drainage water can be detrimental to fish
449 health and growth, it has been suggested instead to use irrigation water primarily in aquaculture and then
450 use the aquaculture effluent in agriculture as a source of water and organic fertiliser (Henriksson et al.,
451 2017). While this would not stop chemical and biological hazards from fish ponds being disseminated to the
452 wider environment, it might lead to an overall reduction in the transmission of such hazards, if water
453 quality improvements, including lower levels of pathogens, lead to reduced application of antibiotics in
454 aquaculture. This is worthy of consideration but may not be possible in Egypt, as the land set aside for
455 aquaculture is mostly downstream of agricultural crops. According to workshop participants, about 20 to
456 30% of water is exchanged every day under normal circumstances; however, the frequency and volume of
457 water exchanged depend on the stage of production, size of fish and the season (Eltholth et al., 2015). The
458 main purpose of pumping water is to increase the dissolved oxygen level in pond water. During outbreaks,
459 some farmers reduce the level of pond water to the minimum to administer a treatment to the fish
460 (Eltholth et al., 2015). Many respondents to the survey reported that more frequent water exchanges
461 would be feasible in the short term, and felt that this would be effective in improving fish health. This may
462 not be the case where the quality of incoming water is inadequate, and thus may be counter-productive,
463 which could indicate some misunderstanding among respondents of the water quality problems and how
464 best to solve them.

465

466 Demand for fish is increasing in Egypt due to a growing population and increased per capita consumption in
467 the last two decades, likely due to economically incentivised changes in consumer preferences and
468 increased accessibility (i.e., low-cost domestic fish production, improvements in distribution networks, etc.)
469 (Murphy et al., 2020). Indirect price controls on tilapia have been imposed by the government through the
470 introduction of an export tax for tilapia, and this policy has created tension between a government aiming

471 to ensure a reliable, low cost and secure supply of animal protein for the population in the country and
472 tilapia producers keen to maximise profits. Survey respondents expressed a strong desire to explore and
473 expand exporting opportunities, and it was suggested that this could act as a driver to reduce ABU by
474 raising production standards and allowing farmers to sell fish at higher prices, as observed elsewhere
475 (Nguyen and Jolly, 2020). Currently, a notable barrier to exporting tilapia from Egypt is the need for a
476 comprehensive residue monitoring system and disease testing framework, both of which are required for
477 export to the European Union and the United States (Fitzsimmons, 2008; Eltholth et al., 2015). In addition,
478 the costs associated with higher standards of production, limited processing facilities and ability to add
479 value, and lack of by-product industries represent important barriers to export (Fitzsimmons, 2008; Eltholth
480 et al., 2015). Previous research has shown the importance of the local market, where tilapia consumption is
481 greater amongst the communities in high production areas than in non-production areas where fish may be
482 available only once per week on market day (Eltholth et al., 2015). Currently the tilapia market is
483 dominated by a few large wholesalers who control the price (Soliman and Yacout, 2016), and the national
484 government seeks to ensure the tilapia industry offers food security for the population, meaning
485 production for the domestic market is prioritised. Indeed, the building of government aquaculture farms,
486 such as the Birkat Ghalioun fish pond project (Feidi, 2018), illustrates the government's plans to meet the
487 increased demand for fish for the population (El-Gohary et al., 2020). However, it is conceivable that
488 greater revenues resulting from exporting tilapia could provide the income necessary to secure the supply
489 of alternative high-protein foodstuffs.

490

491 Improvements to diagnostic capacity and a need for alternatives to antibiotics were frequently mentioned
492 at the workshop and these deficiencies are often highlighted by aquaculture farmers in LMICs (Ninawe et
493 al., 2017; Stentiford et al., 2017; Garza et al., 2019). While improved diagnostics were considered
494 favourably by respondents, the consensus was that cost was a barrier and it is difficult to envisage how this
495 situation will improve to meet the needs of farmers in the near future, given the significant investment that

496 would be necessary. The use of probiotics and immunostimulants may offer a feasible and relatively
497 inexpensive option to reduce the need for antibiotics by improving the health and welfare of stocks
498 (Chauhan and Singh, 2019). There have been numerous trials with these products in tilapia, for example
499 dietary supplementation of Nile tilapia with *Bacillus subtilis* and *Saccharomyces cerevisiae* was associated
500 with improvements in selected measures of immunity and an enhancement of fish flesh quality (Elsabagh
501 et al., 2018; Opiyo et al., 2019). In addition, probiotics can be used to modify the microbial composition of
502 pond water to improve water quality (De Schryver et al., 2014). Still, much more needs to be done to
503 ensure probiotics are tested appropriately and confirmed to be effective under farm conditions (Partridge,
504 2016; Knipe et al., 2020).

505

506 Meanwhile, few survey respondents viewed vaccination positively for its potential effectiveness to reduce
507 disease and ABU, even though vaccination programmes can lead to massive reductions in ABU, such as has
508 been seen in Atlantic salmon farming around Scotland and Norway (Sommerset et al., 2005; Adams, 2019).
509 It was discussed at the workshop that there are few vaccines available for tilapia, and that these are
510 currently injection vaccines, whereas immersion vaccination would be more appropriate. Although there
511 was little knowledge of the potential of bacteriophage in disease control, this is less surprising given it is not
512 an established technology like vaccination (Gon Choudhury et al., 2017). The lack of appreciation for the
513 potential of vaccination programmes to improve fish health may be due, in part, to a lack of awareness and
514 lack of availability of vaccines for tilapia diseases. However, it is encouraging that there was an appreciation
515 by survey respondents for the importance of education and training to improve the ABR situation in tilapia
516 farming, indicating a possible avenue to raising attention to the success of vaccination programmes in
517 improving fish health and reducing ABU. More broadly, this appetite for learning provides a platform that
518 can be used to improve many aspects of tilapia production in the country. Indeed, the impact of training
519 farmers in best management practices (BMP) has been assessed in the aquaculture sector in Egypt (Dickson
520 et al., 2016; Henriksson et al., 2017). Providing farmers with BMP training improves farm profitability

521 though not necessarily productivity, and the considerable economic, social and environmental gains from
522 investment in training of fish farmers in Egypt is well recognised (Dickson et al., 2016). Indeed, Henriksson
523 et al. (2017) used lifecycle assessment to show that BMP training of tilapia farmers in Egypt reduced
524 lifecycle environmental impacts by 22%.

525

526 A limitation of this present study was the absence of policy makers from government agencies, as this
527 would have provided broader context and a better understanding of the decision-making processes at
528 national level. However, the presence of government representatives could have altered the dynamic of
529 the workshop, and influenced the responses of the ground-level stakeholders identified for inclusion in this
530 present study. It was important to capture the views of these stakeholders on the acceptability and
531 feasibility of the interventions, given that the proposed interventions will have greatest impact upon them
532 and will most likely need to be implemented by them. Having captured these views, engagement with
533 government is a priority and is essential to understand how best to influence policy, particularly on water
534 use and the constraints to exporting tilapia to different territories, and how any barriers may be
535 surmounted. In addition, this engagement would be useful for identifying potential resources and means to
536 deliver the desired increase in diagnostic capacity. Therefore, a smaller, focused follow-up workshop is
537 proposed to include a broader range of stakeholders, including those representing relevant government
538 agencies and other systems like poultry production, while also ensuring the key opinions from attendees at
539 our initial workshop are represented through inclusion of a subset of delegates from the fish farming
540 stakeholder community. It is by this approach that acceptable, sustainable interventions to reduce ABU,
541 and thus the problem of bacterial ABR, can be introduced to deliver beneficial change in the aquaculture
542 sector.

543

544 A further limitation of this study is that participants and respondents lack of knowledge or experience with
545 specific interventions may have influenced their responses to the survey. Though some information was

546 provided in the descriptions of each intervention in the questionnaire, this may still have been insufficient
547 for responders to make an informed judgement. This could potentially skew responses both ways,
548 depending on how optimistic or pessimistic the responder may be towards a new intervention. This could
549 be mitigated in future studies by providing more opportunities for training and discussion to achieve a
550 shared understanding of the interventions prior to collecting participants' views. Language may represent a
551 further barrier to understanding and, though reasonable steps were taken to mitigate this risk, it is not
552 inconceivable that misunderstanding could have happened. The workshop was led in English by the
553 research team, with concurrent translation into Arabic by a member of the research team fluent in both
554 languages. Each workshop group was facilitated in English with a translator present. The survey was written
555 in English and translated into Arabic before dissemination, whilst responses provided in Arabic were
556 translated back into English by a member of our research team.

557

558 The range of suggested interventions above can be enacted by various stakeholders in the tilapia
559 production system in Egypt. The benefit of the systems-thinking approach is that it allows for consequences
560 of actions to be assessed and predicted, while also providing a means to select points in the system where
561 the effectiveness of interventions could be evaluated through the collection of empirical data, thus helping
562 to focus limited resources most effectively (Adam and de Savigny, 2012; Peters, 2014). These data can be
563 used to confirm or quantify risks and could consist of measures such as concentration levels of antibiotic
564 residues or presence of ARGs in the bacterial populations in the system.

565

566 In conclusion, this present study provides a foundation for the perceived acceptability and feasibility of
567 possible interventions that can reduce ABU in tilapia production in Egypt and other countries with similar
568 production systems and thus mitigate against the broader problems posed by ABR. This will help the sector
569 to remain productive and increase its resilience to ABR but also to infectious diseases, while securing
570 livelihoods and ensuring farmed tilapia is a safe and nutritious source of food. Systems-thinking and

571 participatory approaches to create systems maps are important when determining the points within
572 systems where interventions can be implemented, with feasibility and stakeholder acceptance being crucial
573 considerations for the success of any intervention (Siokou et al., 2014). These same system maps can be
574 used to assist in selecting points to monitor and assess the effectiveness of proposed interventions, a
575 better understanding of which is crucial if we are to be successful in reducing overall ABU and tackling the
576 global problem of ABR (Wernli et al., 2020).

577

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764 Tables

765 Table 1: Professions of workshop participants. Some participants represented more than one organisation.

Organisation	n
UK-based academics (from 3 institutions) (<i>research team</i>)	8
Egypt-based academics (from 4 institutions)	13
WorldFish Egypt	5
National Research Institute (ICLAR)	1
Feed factory owner	1
Farmers/fish farm owners	15
Pharmaceutical company representative/trader	1
General Organisation of Veterinary Services	2

766 Figures

- 767 Figure 1: Workshop objectives of the mapping activity, framed within four dimensions. Participants were
768 asked to consider each of the dimensions when working through each objective
769
770 Figure 2: Consensus map of the farmed tilapia production system in Egypt
771
772 Figure 3: Occupations of respondents to the post-workshop survey.
773
774 Figure 4a: Summary of responses to the post-workshop survey on the feasibility of various interventions to
775 reduce ABU and improve fish health.
776
777 Figure 4b: Summary of responses to the post-workshop survey on the advantages and disadvantages of
778 various interventions to reduce ABU and improve fish health.
779
780 Figure 5: Mean score of importance for each group of interventions (8 = highest score available and 1 =
781 lowest score available)