

1 **Current disease treatments for the ornamental pet fish**  
2 **trade and their associated problems**

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26

27 Abstract

28 The trade in live ornamental fishes to be held as companion animals or displayed in public  
29 aquaria has an estimated global annual value of US\$15-20 billion. Supply chains for  
30 ornamental pet fishes often involve many more parties than for fish farmed as food fishes,  
31 and at each stage fishes are exposed to stressors including handling, confinement, crowding,  
32 mechanical disturbance, and poor water quality. If chronic, these stressors can compromise  
33 their immune system, making fishes more susceptible to pathogens. Mortality and morbidity  
34 from infectious disease can result in considerable welfare impacts and massive economic  
35 losses for the industry, and the range of infective agents seen in ornamental species is well  
36 documented. However, treating these diseases is not straightforward with practices varying  
37 greatly across the trade and with several approaches having unintended consequences, such  
38 as the emergence of resistant strains of pathogens. While disease treatments for a handful of  
39 fish species (e.g. koi, goldfish) have received focused research attention, for the home  
40 aquarium owner, there is an increasing reliance on products based on natural compounds  
41 which have received far less scientific attention. This review aims to highlight the gaps in our  
42 knowledge surrounding the range of disease treatments used across the ornamental pet fish  
43 trade, with a particular focus on freshwater tropical species destined for home aquaria.  
44 Consideration is given to the potential problems arising from these treatments, including  
45 microbial resistance and effects of treatments themselves on fish health and welfare.

46

47 Key words: home aquaria; bacteria; freshwater tropical fish; antimicrobial resistance;  
48 welfare; medicines.

49

## 50 **Introduction**

51 The trade in live ornamental fishes to be held as companion animals, and their associated  
52 products, represents an economically important global industry. In the UK alone, the industry  
53 is valued at ~£400 million (King, 2019) with the vast majority of this trade in tropical  
54 freshwater species with > 4500 species comprising 90% of the market (Iqbal and Shalij, 2019;  
55 King, 2019; OATA, 2020). The global ornamental industry relies on transnational supply  
56 chains to provide fish keepers in countries such as the UK, USA and Germany, with fish  
57 reared/collected from nations such as Singapore, Israel, and Japan (OATA, 2018; Olivier,  
58 2003). These supply chains involve many parties including importers, exporters, distributors,  
59 wholesalers, and retailers. At each stage in the supply chain, fishes can be exposed to a variety  
60 of stressors which can result in mortality (Jones et al., 2021). These stressors include handling,  
61 confinement and crowding (Barton and Iwama, 1991), mechanical disturbance during transit  
62 (Masud et al., 2019), poor water quality, accumulation of toxic waste products, and reduction  
63 in dissolved oxygen (Sampaio and Freire, 2016). Exposure to stressors over a period of time  
64 can compromise the immune system of the fish making them more susceptible to bacterial  
65 diseases (Eslamloo et al., 2014). In addition to the effects of long-term stress on the immune  
66 system, fishes are routinely netted for grading or preparing for transport which can result in  
67 loss or damage to the epidermal mucus layer that protects fishes against pathogens (Fouz et al.,  
68 1990; Raj et al., 2011).

69 Susceptibility to disease is therefore a major challenge for the ornamental pet fish trade and is  
70 the second most frequent cause of ornamental fish mortality and morbidity, after collective  
71 water quality issues (Lewbart, 2001). These mortalities result in economic losses for the  
72 ornamental fish industry (Cardoso et al., 2021) and an overreliance on antibiotics has fostered  
73 resistant strains of pathogens (Weir et al., 2012). However, treating these diseases is not  
74 straightforward, and research has focused primarily on one or two species. Although the  
75 majority (95%) of freshwater pet fishes are tropical, research into ornamental fish diseases is  
76 most advanced for cyprinid species (e.g. goldfish, koi) where development of a vaccine for koi  
77 herpesvirus disease looks promising for the industry (Klafack et al., 2022). However, for small  
78 freshwater ornamentals, destined for lives in home aquaria, far less scientific consideration has  
79 been given and more effective treatments are needed to prevent disease. In many importing  
80 countries, there is an increasing reliance by home aquarists on products based on natural  
81 compounds, potentially due to their perceived environmental sustainability (Arapi and Cable,

82 unpublished data). However, these disease treatments have generally been overlooked within  
83 the scientific literature and their efficacy and effects on general fish health and welfare are  
84 under-researched.

85 Here we review disease management strategies within the freshwater ornamental fish trade  
86 with a particular focus on tropical species destined for home aquaria. We look first at more  
87 traditional approaches such as the use of antibiotics and the emergence of antimicrobial  
88 resistance. In the second part of this review, we present a systematic review of information  
89 available on treatments available to home aquarists highlighting the gaps in our knowledge  
90 surrounding the range of disease treatments used across the ornamental fish trade and areas for  
91 future research.

92

## 93 **Diseases in ornamental fishes**

94

95 Infectious diseases cause significant mortalities to stocks of ornamental fishes, including in  
96 aquaculture and research facilities, sometimes resulting in cumulative mortalities >50%  
97 (Hawke et al., 2013; Kumar et al., 2015). Cyprinid herpesviruses 2 and 3, are particularly  
98 damaging to koi and goldfish farms respectively, with outbreaks across Asia causing  
99 mortalities as high as 95% (Dharmaratnam et al., 2018; Jiang et al., 2020; Piewbang et al.,  
100 2024). While these outbreaks are undoubtedly costly to the industry, data on the direct  
101 economic impact of infectious diseases within the ornamental trade is sparse, not least as there  
102 are few incentives for fish farmers to report disease outbreaks in countries without a legal  
103 obligation to do so, and where reputational damage could occur.

104

105 Ornamental fishes are susceptible to infection by a plethora of microorganisms including  
106 metazoa, protozoa, bacteria, algae, fungi, and viruses. Many diseases are caused by Gram  
107 negative bacteria which are ubiquitous within ornamental fish holding water and can  
108 opportunistically infect immunocompromised individuals (Roberts et al., 2009; Smith et al.,  
109 2012). There is considerable overlap in the environment favoured by many ornamental fish  
110 species and that required for bacterial growth (e.g. warm temperatures, high concentrations of  
111 nutrients, and good oxygenation) (Gomez et al., 2013; Smith et al., 2012). There are also some  
112 notable protozoan and fungal-like diseases that commonly occur when the immune system of

113 ornamental fishes is compromised. While viral diseases are an issue for ornamental fishes,  
114 prevention of viral diseases generally relies on maintaining virus-free breeding stocks,  
115 vaccination where available, and good biosecurity including disinfecting setups between  
116 production cycles, UV sterilization of water, and implementation of sufficient quarantine  
117 periods (Cardoso et al., 2019). There is an extensive literature on koi herpesvirus disease  
118 (KHVD) and a commercial vaccine available in some countries (Klafack et al., 2022).  
119 However, for the majority of tropical freshwater fish that are traded to the home aquarium  
120 owner, there are no commercial chemical treatments for viruses, therefore viruses are not  
121 explored in great detail here.

122

### 123 *Bacterial diseases*

124 Gram-negative bacteria known to cause disease issues in ornamental fishes include  
125 *Flavobacterium columnare* (columnaris disease), *Edwardsiella* spp., *Aeromonas* spp. (koi  
126 ulcerative disease and motile aeromonad disease), *Pseudomonas* spp. (pseudomoniasis), and  
127 *Vibrio* spp. (vibriosis). While there are other examples, these five bacteria are particularly  
128 significant for the ornamental trade; the symptoms of the diseases they cause are summarised  
129 in Table 1. There are comparatively fewer Gram-positive bacterial diseases reported within  
130 ornamental fishes; the two main Gram-positive bacteria infecting ornamental fishes held in  
131 home aquaria are *Streptococcus* spp. (streptococcosis) and *Nocardia* spp. (nocardiosis).  
132 *Mycobacterium* spp. (mycobacteriosis) and *Nocardia* spp. can appear weakly Gram-positive  
133 but also presents as an acid-fast bacterium.

134

135 Many of the bacteria summarised in Table 1 can cause tail/fin rot in fishes (Emany et al., 1995;  
136 Sasmal et al., 2004). This occurs when bacteria colonise the fin tissue and cause necrosis and  
137 degeneration, often unevenly, giving the fins a ragged appearance (Lewbart, 2001; Roberts et  
138 al., 2009). Poor water quality and fin damage due to aggression or inappropriate handling can  
139 predispose fishes to fin rot (Rao et al., 2013). In severe cases, the fin can be significantly  
140 reduced, and the infection can progress to the body surface (Chatterjee et al., 2020; Rao et al.,  
141 2013). Fin rot can compromise the commercial value of ornamental fishes destined for home  
142 aquaria, particularly in species prized for their long flowy fins e.g., fighting fish, *Betta*  
143 *splendens*.

144

145 *Parasitic diseases*

146 There are numerous parasites, which present considerable economic and welfare-related  
147 concerns to the ornamental industry from a diverse range of taxa including protists,  
148 monogeneans and crustaceans (Evans and Lester, 2001; Florindo et al., 2017; Iqbal and  
149 Haroon, 2014). Some common parasites for pet fishes are *Piscinoodinium* spp. (freshwater  
150 velvet disease), *Ichthyophthirius multifiliis* (white spot disease), skin and gill flukes  
151 (*Gyrodactylus* and *Dactylogyrus* spp.), and roundworms (e.g. *Camallanus* and *Capillaria* spp.).  
152 Freshwater velvet disease is caused by the dinoflagellates *Piscinoodinium* sp. which have a  
153 direct life cycle with three stages lasting between 4 and 15 days (Lieke et al., 2020; Noga,  
154 2010). Stage one is a free-swimming dinospore which attaches to the epithelium of the host's  
155 skin or gills with a specialised extension known as a rhizoid. Once attached, the dinospores  
156 become trophonts (feeding stage) which actively parasitise upon the host's cells. Finally, the  
157 trophonts detach, encapsulate themselves, and turn into tomons (reproductive stage) (Lieke et  
158 al., 2020). White spot disease, caused by the ciliate *Ichthyophthirius multifiliis*, is arguably one  
159 of the most common parasitic diseases of ornamental fishes (Iqbal and Haroon, 2014;  
160 Matthews, 2005) and if not treated promptly, can result in high mortalities (Ekanem et al.,  
161 2004; Ezz EI Dien et al., 1998; Francis-floyd et al., 2016). Motile theronts (infective stages) of  
162 this parasite burrow into the epithelial tissues of the skin, fins, or gills of the host fish where  
163 they transform into spherical trophonts (feeding stage) and feed on the surrounding tissue  
164 (Dickerson, 2011). Two genus of fluke (monogenean) which are particularly prevalent in the  
165 ornamental fish industry are *Gyrodactylus* and *Dactylogyrus*. The former reproduces  
166 viviparously while the latter are oviparous (Scott, 1982; Zhang et al., 2022). Regardless of  
167 reproductive method, they have direct life cycles and relatively short generation times leading  
168 to rapid transmission between host fish (Zhang et al., 2022). Using specialised hooks they  
169 attach to the external body surfaces including skin, fins, and gills (favoured by *Dactylogyrus*  
170 spp.) feeding off the mucus and epithelial cells of the host (Cable et al., 2002; Said, 2008). In  
171 terms of endoparasites of ornamental fishes, perhaps the most significant are nematodes,  
172 specifically *Camallanus* and *Capillaria* spp. Both species are similar in clinical presentation,  
173 both targeting the gastrointestinal tract of the host fish (Palmeiro and Roberts, 2012). However,  
174 *Capillaria* spp. have a direct life cycle whereas *Camallanus* spp. typically uses an intermediate  
175 copepod host, although they have exhibited flexibility in this regard directly infecting fish (De,  
176 1999; Levsen, 2001; Palmeiro and Roberts, 2012).

177

178

179 *'Fungal-like' diseases*

180 Aside from microsporidian parasites, diseases caused by 'true' fungal pathogens of fishes are  
181 relatively rare (Noga, 2010); although rare *Aspergillus*, *Rizopus*, and *Mucor* have been reported  
182 to cause disease within ornamental fishes (Haroon et al., 2014; Iqbal and Sajjad, 2013). Of the  
183 microsporidia, two species commonly infect pet fishes - *Pseudoloma neurophilia* and  
184 *Pleistophora hyphessobryconis*, the latter being the causative agent of "neon tetra disease",  
185 despite being nonspecific to neon tetras and infecting a broad range of fish species (Kent and  
186 Sanders, 2020; Sanders et al., 2010). These diseases present a particular problem for zebrafish  
187 used as model organisms, as they often have subclinical effects on behaviour which can  
188 confound interpretation of behavioural endpoints (Estes et al., 2021; Kent and Sanders, 2020;  
189 Midttun et al., 2020). For fish destined for ornamental purposes, these diseases represent less  
190 concern, typically resulting in subclinical effects with mortality only in some chronic cases  
191 (Kent and Sanders, 2020; Sanders et al., 2020). Additionally, there is no proven treatment for  
192 either pathogen with evaluation of treatment options in its infancy and current guidance to  
193 euthanise severely infected fishes (Lavin et al., 2023). Therefore, these diseases are not  
194 considered further here.

195 Diseases caused by the fungal-like oomycetes or 'water moulds' (e.g. *Saprolegnia*, *Achyla*, and  
196 *Dictyuchus* spp. etc.) are relatively common in freshwater fishes in part due to their low host  
197 specificity (Gozlan et al., 2014). They are ubiquitous within aquatic environments and typically  
198 cause secondary infections, opportunistically infecting lesions caused by parasitic or bacterial  
199 diseases (Noga, 2010). Alternatively, they can infect hosts by colonising physical trauma  
200 caused by aggression or inappropriate handling (Khoo, 2000). Poor water quality and stressors  
201 such as crowding and unsuitably low temperatures seem to increase the likelihood of infection  
202 (Howe and Stehly, 1998; Noga, 2010; Udomkusonsri and Noga, 2005). Fish eggs are also at  
203 risk of infection from oomycetes. By initially colonising dead eggs, water moulds can spread  
204 to healthy eggs, which they could not otherwise colonise, and can cause extensive egg mortality  
205 (Eissa et al., 2013; Thoen et al., 2011).

206

## 207 **Disease management**

208

### 209 **Transboundary spreading and zoonotic potential**

210 Within the ornamental fish industry, live animals are transported on a global scale in substantial  
211 quantities and whenever live animals are transported from one location to another there is  
212 opportunity for co-transport of pathogens (Ariel, 2005). Therefore, a key aspect of disease  
213 management is risk consideration for the spread of pathogenic organisms. Additionally, while  
214 ornamental fishes are destined to spend their lives in captivity, escapes or intentional releases  
215 can pass on pathogens to naïve natural populations through a process known as transboundary  
216 spreading (Chan et al., 2019; Munson et al., 2024). The trade in ornamental fishes has been  
217 implicated in spreading viruses, bacteria, and parasites to native fishes outside their natural  
218 ranges (Dikkeboom et al., 2004; Taylor et al., 2013; Whittington and Chong, 2007). This is  
219 typically an issue with cold water ornamental species (e.g. koi and goldfish), as many countries  
220 importing ornamental fishes are located in temperate regions where tropical species are less  
221 likely to survive on release. However, some typically tropical species have been found in  
222 temperate regions (Munson et al., 2024), and rising global water temperatures may increasingly  
223 exasperate the problem.

224 Humans are also susceptible to transboundary spreading via fish-human zoonoses. In 2012, a  
225 systematic review of ornamental fish-human zoonoses highlighted *Mycobacterium marinum*  
226 as the greatest cause for concern, with at least 32 recorded case reports (single occurrence of  
227 human illness) and 16 case series (two or more occurrences of human illness) of human *M.*  
228 *marinum* infection linked to ornamental fish exposure, including potential links to three deaths  
229 in immunocompromised individuals (Weir et al., 2012). Other cases have been reported since  
230 (Bouceiro-Mendes et al., 2019; Huang et al., 2012), and a more recent review found strong  
231 molecular and epidemiological evidence for zoonotic *Mycobacterium* transmission (Gauthier,  
232 2015). Gauthier (2015) also highlighted three other zoonotic pathogens (*Clostridium*  
233 *botulinum*, *Streptococcus iniae*, and *Vibrio vulnificus*). However, these were more related to  
234 consumption or handling of food fishes, with little relevance to fish kept as pets. The true extent  
235 of human-fish zoonoses is likely underestimated due to limited awareness and  
236 monitoring/surveillance (Haenen et al., 2020a; Ziarati et al., 2022). For example, infection by  
237 *Mycobacteria marinum*, is not notifiable in most countries (Ahmed et al., 2020; Haenen et al.,  
238 2020a). Due to the relative frequency of exposure, professionals within the ornamental industry



239 are most at risk from zoonotic diseases, but there can also be a risk to the home aquarist  
240 (Haenen et al., 2020a).

241 Addressing the zoonotic issues described above will require a ‘One Health’ approach (CDC,  
242 2020) which has been adopted successfully in combating a number of zoonoses from terrestrial  
243 animals such as zika, rabies, and hendra virus (reviewed in Horefti, 2023). A framework has  
244 already been proposed for a ‘One Health’ approach to sustainable food-producing aquaculture  
245 (Stentiford et al., 2020) and while animals destined for human consumption naturally pose a  
246 greater zoonosis risk, consideration of a similar framework for ornamental fishes is warranted.  
247 An additional complexity associated with the risk of zoonotic diseases, is the release of  
248 antibiotics and antimicrobial resistance genes to the environment where they can pass to human  
249 pathogens (Santos and Ramos, 2018). Effluent from an ornamental fish market in China  
250 identified numerous antibiotics, antimicrobial resistance genes, and potential opportunistic  
251 human pathogens (Liu et al., 2021) and effluent from ornamental fish farms in Sri Lanka  
252 contained multidrug-resistant *Aeromonas* spp. (Dhanapala et al., 2021). Recommendations  
253 already exist which could be implemented into a ‘One Health’ approach including monitoring  
254 ornamental fish stocks and consignments for zoonotic and AMR pathogens, raising awareness  
255 of zoonotic diseases, promoting proper hygiene and biosecurity practices, monitoring and  
256 regulating antibiotic use (Dhanapala et al., 2021; Haenen et al., 2020b, 2020a; Kušar et al.,  
257 2017; Phillips Savage et al., 2022; Weir et al., 2012; Ziarati et al., 2022).

258

259

## 260 **Disease Treatments**

261 The prevalence and severity of diseases within ornamental fishes, along with their zoonotic  
262 potential, highlights the need for effective antimicrobial treatments. ‘Antimicrobial’ is an  
263 umbrella term which describes any chemical (synthetic or naturally derived) that kills or  
264 inhibits growth of microorganisms. As such, this spans a wide range of chemicals including  
265 disinfectants, antiseptics, antibiotics, antifungals, antivirals, antiparasitics and antiprotozoals.  
266 In the literature, the terms ‘antimicrobial’ and ‘antimicrobial resistance’ are usually used  
267 synonymously with ‘antibiotic’ and ‘antibiotic resistance’ respectively. Throughout this  
268 review, where possible the class of chemical discussed (e.g. antibiotic, antifungals,  
269 disinfectants, antiseptics, etc.) will be used, accepting that some compounds may have more

270 than one specific action. When referring to a combination of these compounds, or where it is  
271 not clearly stated in previous literature, the general term ‘medications’ will be used.

272 Ornamental fish may be administered medications in different ways. Bath treatments are where  
273 the fish is added to water containing relatively low concentrations of the medication, typically  
274 for a period of 2-60 minutes (Loh, 2015). For dip treatments fish are exposed to the chemical  
275 in a contained volume of water for shorter durations with typically higher concentrations of  
276 medications (Mashima and Lewbart, 2000). Prolonged immersion can also be used, where a  
277 very low concentration of medication is introduced to the tank water for a longer exposure  
278 duration, typically many days (Loh, 2015). While injections are an option for treating fishes,  
279 particularly in the case of vaccination, they are usually impractical for small ornamental fishes  
280 particularly in commercial settings. This is due to their small body sizes, frequency and size of  
281 consignments, individual fish value, cost, and labour intensity involved (Yanong, 2003). The  
282 final major route of administration is through medicated feeds. Here, fish food is coated with  
283 medications before feeding or they are added to the feed during production. This method of  
284 administration requires less medication than dip or bath treatments, but relies on the fish having  
285 an appetite, which can be non-existent in severe cases of infection (Yanong, 2003).

286 There are few data on the comparative efficacy of different routes of administration, most of  
287 which come from an aquaculture setting. When silver perch (*Bidyanus bidyanus*) were treated  
288 with antiparasitics to control monogenean gill parasites, oral treatments were comparable to bath  
289 treatments for one medication but performed worse for another (Forwood et al., 2013). Some  
290 fish medications can reduce palatability leading to feed and hence medication being rejected,  
291 hampering effectiveness (Forwood et al., 2013; Marking et al., 1988). In red pacu (*Colossoma*  
292 *brachypomum*) and koi carp (*Cyprinus carpio koi*) oral, intramuscular injections (and  
293 intraperitoneal in koi), and bath treatments of the antibiotic enrofloxacin all provided  
294 therapeutic blood concentrations (Lewbart et al., 1997; Udomkusonsri et al., 2007). However,  
295 in the oral treatments gastric lavages were used rather than medicated feed which eliminates  
296 the limitation of palatability but increases labour intensity significantly. In Nile tilapia  
297 (*Oreochromis niloticus*) the antibiotic oxytetracycline was more effective against an  
298 *Aeromonas hydrophila* infection when provided as medicated feed rather than as a bath  
299 treatment (Julinta et al., 2017). Similarly, in common carp (*C. carpio*), the antibiotic florfenicol  
300 was more readily absorbed when provided as a medicated feed rather than a bath treatment  
301 (Jangaran Nejad et al., 2017).

302 Ornamental fish supply chains often involve many parties in both the exporting and importing  
303 country. Most freshwater ornamentals originate from fish farms in Asia Pacific countries such  
304 as Singapore, Indonesia, and the Philippines (Jones et al., 2021). In these farms, fishes may be  
305 treated with medications prophylactically (preventatively; in absence of disease),  
306 therapeutically (to cure active disease), and possibly in some cases for growth promotion,  
307 despite the latter having no scientific basis and some evidence it could decrease gut health  
308 (Trushenski et al., 2018; Weir et al., 2012; Zhou et al., 2018). Obtaining data on treatments at  
309 this stage is difficult, as ornamental fish farms are typically small family operated businesses  
310 and are reluctant to share husbandry practices established through many years of trial and error  
311 (Chapman, 2000). As fishes are traded between different parties, they could experience  
312 different medications at each stage in the supply chain, particularly during long transports  
313 between importing and exporting countries where medications are often added prophylactically  
314 to increase survival (Cole et al., 1999; Dobiasova et al., 2014). Fishes may even be treated with  
315 chemicals banned in the destination country, as different countries have different rules and  
316 regulations on fish medication use (Haenen et al., 2020). For example, in the UK, all antibiotics  
317 used to treat animals fall into class POM-V (prescription only medicine – Veterinarian) and  
318 must be prescribed by a veterinarian (Veterinary Medicines Directorate, 2016). However, in  
319 the US many antibiotics used to treat fish are available to purchase online or over the counter  
320 (Zhang et al., 2020). In addition, in countries where antibiotics are available, there are  
321 generally strict regulations in place for antibiotic use in finfish aquaculture destined for human  
322 consumption, but not for ornamental fish aquaculture. Regulations do not typically exist for  
323 ornamental fishes, or are unenforced if in place (Weir et al., 2012). For example, the US Food  
324 and Drug Administration (FDA) regards ornamental fishes as a low regulatory priority  
325 (Yanong, 2003). Once the fish have reached the end user in the supply chain, the home aquarist,  
326 the choice of treatments will likely be very different to those routinely used in commercial  
327 settings, particularly in exporting countries. It is worth noting, fish are typically given  
328 quarantine periods before being added to aquaria both in a commercial setting and when kept  
329 by the more dedicated hobbyist. For example, public aquaria generally quarantine new  
330 individuals for a minimum of 30 days before introducing to established aquaria (Hadfield and  
331 Clayton, 2011). During this time, typically at least two weeks, fish can undergo health  
332 monitoring to look for early warning signs of disease in addition to the application of a variety  
333 of prophylactic fish medications. However, while health monitoring as a preventative tool has  
334 been used successfully for tropical ornamentals within research facilities (Collymore et al.,  
335 2016; Mocho, 2016), it is not well developed for the home aquarist.

336

### 337 **Treatments within commercial settings**

338 The scarcity of data on antibiotic use within the ornamental trade was recognised by Weir and  
339 colleagues (2012). They sought to rectify this by surveying aquaculture-allied professionals  
340 with expertise in ornamentals and antibiotic use. The surveys covered all aspects of antibiotic  
341 use in the ornamental sector including purpose, production phase, and class of antibiotic used.  
342 Most participants stated antibiotics were commonly used, mostly therapeutically rather than  
343 prophylactically. Quinolones, tetracyclines, and nitrofurans were among the most used classes  
344 of antibiotic, although occasional to frequent use was reported for all classes by at least some  
345 respondents. However, the majority of survey participants were from North America (92 out  
346 of 113; 81.4%). This becomes problematic for interpretation considering that Asia and Europe  
347 contribute ~57% and ~28% of global ornamental exports respectively (Dey, 2016). Therefore,  
348 the surveys likely do not capture the true use of antibiotics within the trade.

349 Another way to obtain data on fish medication use within the trade could be the screening of  
350 carriage water for medication residues. When 50 consignments of ornamental fishes imported  
351 into the Netherlands from 13 countries were screened for medication residues, 49 of them  
352 contained one or more antibiotics at detectable levels, including antibiotics which are banned  
353 in the EU such as chloramphenicol and nitrofurans (36 and 68% of consignments respectively;  
354 Haenen et al., 2020). By examining carriage water samples, data can be collected on fish  
355 medication use from exporting countries independently of reporting bias. However, if  
356 medications are used early in the production cycle prior to export then there is a possibility  
357 they would not be represented. Additionally, different classes of antibiotics vary in their  
358 persistence within aquatic environments (Kümmerer, 2009). Therefore, there is the potential  
359 for less stable compounds to be underrepresented. Another way to estimate use of fish  
360 medications is through sales data, however, these data are not readily available on a global  
361 scale. It is important to monitor antibiotic use within the trade as inappropriate use can result  
362 in antimicrobial resistance (AMR).

#### 363 *Antimicrobial resistance (AMR) within ornamental fish pathogens*

364 One of the main problems associated with the disease treatments used during the commercial  
365 phases of the ornamental trade is the emergence of antimicrobial resistance (AMR), a concept  
366 typically associated with antibiotic use (Narendrakumar et al., 2023). AMR develops rapidly  
367 in bacterial populations (Baym et al., 2016) and the last systematic review on the extent of

368 AMR within the ornamental industry was published more than a decade ago (Weir et al., 2012).  
369 Therefore, an update on the subject is prudent. While recent reviews have considered AMR in  
370 aquaculture (Preena et al., 2020b), the focus is on food fishes with little emphasis given to fish  
371 kept as companion animals. Some bacteria have intrinsic resistance to different classes of  
372 antibiotics. For example, all Gram-negative bacteria possess intrinsic resistance to  
373 glycopeptides (e.g. vancomycin) and lipopeptides (e.g. daptomycin) due to reduced  
374 permeability of their outer membrane (Reygaert, 2018). Other mechanisms can confer intrinsic  
375 resistance such as the chromosomally encoded efflux pumps in *Pseudomonas aeruginosa*.  
376 These pumps confer resistance to a wide range of antibiotics (e.g. tetracycline,  
377 chloramphenicol, and norfloxacin) by actively transporting them out of the cell (Li et al., 1994).  
378 This intrinsic resistance emphasises the need to establish which bacterial pathogen is being  
379 treated and target with suitable antibiotics rather than treating prophylactically. When  
380 antibiotics are used inappropriately acquired resistance can also occur.

381 Acquired resistance can arise through natural mutations resulting in AMR genes which confer  
382 resistance by four main routes: reducing antibiotic uptake (Quinn et al., 1986), modification of  
383 antibiotic target (Jaktaji and Mohiti, 2010), inactivating the antibiotic compound (Murray and  
384 Shaw, 1997), or its active expulsion via efflux pumps (Li and Nikaido, 2009). AMR genes can  
385 exist in variable abundances within bacterial populations but can become more common when  
386 inappropriate antibiotic use acts as a selection pressure causing a genetic bottleneck increasing  
387 their relative abundance in the new population (Mahrt et al., 2021). Additionally, AMR genes  
388 are often present on plasmids, which can be transferred between bacteria through any method  
389 of horizontal gene transfer (HGT) such as transformation, transposition, and conjugation  
390 (Reygaert, 2018; Strahilevitz et al., 2009). Acquiring AMR genes is not always a benefit fitness  
391 for the organism, for example the genes *Staphylococcus aureus* acquires to become resistant to  
392 methicillin and other  $\beta$ -lactam antibiotics also significantly decreases its growth rate (Rolinson,  
393 1998). Regardless, the acquisition of AMR genes in pathogens make the diseases they cause  
394 considerably harder to treat.

395 The rise in AMR has implications for treating ornamental fishes, and also for human health as  
396 reservoirs of AMR genes can be passed by HGT to human pathogens (Lupo et al., 2012; Preena  
397 et al., 2020b). This becomes particularly problematic as many antibiotics useful for treating  
398 fish diseases are also used for treating human diseases (Cabello, 2006). To test for the extent  
399 of AMR in the ornamental trade, pathogens have been obtained from ornamental fishes by  
400 culturing swabs taken from the skills and gills, or the homogenized internal organs, or even

401 filtered from the water. Once cultured, bacteria are exposed to antibiotics either using minimum  
402 inhibitory concentration (MIC) or disk diffusion assays to determine if the pathogens are  
403 resistant or susceptible to the tested concentrations of antibiotics (CLSI, 2022). The prevalence  
404 of AMR within the ornamental trade is wide ranging and within most classes of antibiotic (Weir  
405 et al., 2012). Nevertheless, high resistance to penicillin, tetracycline, sulphonamide, and  
406 quinolone classes were commonly reported.

407 The PRISMA method was used to undertake a systematic review to update our understanding  
408 of the prevalence AMR within the ornamental industry with a focus on fish held as companion  
409 animals (Page et al., 2021). In May 2022 and repeated in October 2023, the following search  
410 term: ‘Antimicrobial resistan\* AND Ornamental fish\*’ was searched on Web of Science and  
411 Scopus retrieving articles published from 2010 onwards. The search returned 61 and 46 results  
412 from Web of Science and Scopus respectively. After duplicates were removed 71 unique  
413 articles remained which underwent initial screening of relevance by reading the title and  
414 abstract, removing irrelevant articles (removed n=18). The full texts of the remaining 53  
415 articles were then assessed against the following inclusion criteria: papers presenting data from  
416 < 20 isolates were not included for brevity (removed n = 11), must be original research not a  
417 review paper (removed n = 6), data must be provided on pathogens isolated from ornamental  
418 fishes or their water, data on pathogens from food fish species or a mixture of the two where  
419 pathogens from ornamentals could not be distinguished were not included (removed n = 10),  
420 article must be in English (removed n = 1), and must provide data on the percent of isolates  
421 resistant to the antibiotics (removed n = 5). An additional study was removed as it was not  
422 accessible. The remaining 19 studies were accepted, and the following data were extracted:  
423 bacterial species studied (species and number of isolates), geographical origin of samples  
424 (country and type of facility), biological origin of samples (fish species, organ sampled, water  
425 samples), antibiotics tested and percent of isolates resistant, and multiple antibiotic resistance  
426 (MAR) index (number of antibiotics an isolate is resistant to divided by the total number of  
427 antibiotics tested). Where data were only presented graphically, numerical values were  
428 obtained using GetData Graph Digitizer ver. 2.26.0.20.

429 In the analysis of the results from this systematic approach and similarly to the findings of  
430 Weir, there was considerable variation in resistance to most of the antibiotics tested (Table 2).  
431 Generally, bacterial isolates showed relatively low resistance (mean % of isolates resistant: 2.8  
432 – 22.9) to the following antibiotic classes; 3<sup>rd</sup> generation cephalosporins (e.g. cefotaxime,  
433 ceftazidime, and ceftriaxone), carbapenems (e.g. meropenem, doripenem, imipenem), and

434 aminoglycosides (e.g. tobramycin, amikacin, and gentamycin). High resistance was generally  
435 shown (mean % of isolates resistant: 21.2 – 87%) to penicillins (e.g. ampicillin, amoxicillin)  
436 and tetracyclines (e.g. tetracycline, oxytetracycline), indicating heavy use of these antibiotics  
437 within ornamental fish culture.

438 The results of this review are generally in accordance with Weir et al. (2012) as penicillins and  
439 tetracyclines were largely ineffective in their findings. However, some classes of antibiotics  
440 performed better in recent years than in the previous findings. Weir found high resistance to  
441 cepheems (the subclass of antibiotics that includes the cephalosporins and cephamycins) in some  
442 cases. In the present review most cepheems showed relatively low resistances aside from the 1<sup>st</sup>  
443 generation cephalosporins. As Weir does not distinguish between the generations of  
444 cephalosporins it is difficult to make direct comparisons. Conversely, some classes of drugs  
445 performed worse. While carbapenems generally showed low resistance, some studies found  
446 levels of resistance higher than those found by Weir, possibly indicating an increase in the use  
447 of these antibiotics.

448 Less than half of the studies reported the MAR index of the isolates they were testing (Table  
449 2). A MAR index > 0.2 indicates the isolate originates from an environment with a high  
450 antibiotic presence (Davis and Brown, 2016). Of the studies which did report MAR (n = 7), all  
451 reported isolates with MAR > 0.2. Additionally, in four of the studies, all isolates tested  
452 exceeded the 0.2 threshold. Whilst this is a relatively small sample size, it does hint at the  
453 prevalence of unsuitable antibiotic treatments within the ornamental fish trade.

454 *In vitro* assays such as MIC and disk diffusion are considered the gold standard to determine  
455 an antibiotic's effectiveness against pathogens (Khan et al., 2019). However, effectiveness  
456 shown in these tests is not guaranteed to translate to successful treatment of diseases *in vivo*.  
457 Comparatively few *in vivo* efficacy trials have been undertaken for antibiotics used with  
458 ornamental fishes, relative to *in vitro* trials and there is a clear paucity in data on *in vivo* efficacy  
459 of many antibiotics used. Zebrafish with skin scrapes experimentally infected with *Aeromonas*  
460 *hydrophila* showed significantly higher survival when treated with the antibiotic gentamicin in  
461 ultrapure water compared to gentamicin in slightly saline water (0.9% NaCl) and the untreated  
462 infected fish (Gao et al., 2021). Clearly more *in vivo* treatment trials are needed on a range of  
463 fish species and pathogens to guarantee antibiotic effectiveness, allow targeted commercial  
464 treatments based on scientific evidence, and reduce the risk of AMR. It is worth noting that  
465 antibiotics are not the only class of treatment where resistance can be developed. For example,

466 bacteria can also become resistant to disinfectants (Tong et al., 2021). Equally other taxa of  
467 pathogen can become resistant to their respective treatments such as anthelmintic resistance in  
468 monogeneans (Waller and Buchmann, 2001), or antifungal resistance in fungal pathogens (Lee  
469 et al., 2023). However, given the scale of antibiotic use, the prevalence of bacterial disease  
470 within the ornamental fish industry, and the threat resistance poses to human health, we have  
471 focused on antibiotic resistance in this review.

472

## 473 **Treatments within home aquaria**

474 The unsuitability of antibiotic treatments for home aquaria, coupled with growing concern in  
475 the rise of AMR presents the need for alternative treatments. There are a range of alternative  
476 treatments available to the home aquarist, some of which may also be used at a commercial  
477 level. Most of these treatments contain therapeutic dyes or plant-derived essential oils as their  
478 active ingredients. In a recent survey of over 350 participants, it was found that hobbyists  
479 purchase proportionally more natural products compared to retailers but also that there is a  
480 tendency by hobbyists not to adhere to manufacturer instructions for exact dosing protocols  
481 (Arapi and Cable, unpublished data). In addition to this, in a home aquaria setting treatments  
482 may be administered without proper diagnosis leading to treatment failure and implications for  
483 animal welfare. To determine the quantity of scientific information available on these  
484 alternative treatments, a systematic search of ornamental fish treatments from the BSAVA  
485 (2020) Small Animal Formulary Part B: Exotic Pets Appendix III; Proprietary fish medicine  
486 vendors, was carried out using Web of Science and Scopus in October 2022, October 2023 and  
487 June 2024. The search term was mainly the name of the product, however, where products had  
488 generic names which generated excessive hits the brand name was specified prior to the product  
489 using the Boolean operators “”. Table 3 summarises the number of articles returned and  
490 whether or not the article related to the treatment of ornamental fishes and/or their pathogens  
491 *in-vitro*. It is immediately clear that there is a substantial lack of knowledge on the efficacy of  
492 most of these treatments even though most are marketed on a global scale. Given the lack of  
493 knowledge on specific treatments, the following sections are focused around the main active  
494 ingredients in order to highlight gaps in our knowledge and stimulate further research.

495

496



497 *Plant-derived essential oils*

498 A number of plant-derived essential oils have been marketed to treat diseases within home  
499 aquaria. One such oil is cajuput oil, a mixture of essential oils derived from the cajuput tree  
500 (*Melaleuca cajuputi*). Cajuput oil mostly consists of terpenes, such as 1,8-cineole and limonene  
501 (Schelkle et al., 2015b) although the exact composition of essential oils can vary seasonally  
502 and regionally and can exhibit distinct chemotypes (Homer et al., 2000; Idrus et al., 2020).  
503 MELAFIX (API) is a product marketed globally to control home aquarium bacterial diseases  
504 where the active ingredient is 1% cajuput oil. The minimum inhibitory concentration (MIC)  
505 and minimum bactericidal concentration (MBC) of MELAFIX on an assortment of known fish  
506 pathogens (*Aeromonas salmonicida* subsp. *salmonicida*, *Listonella anguillarum*, *Pasteurella*  
507 *piscicida*, *Photobacterium damsela* subsp. *piscicida*, and *Streptococcus iniae*) was determined  
508 using the microbroth dilution technique (Shivappa et al., 2015). MELAFIX was unsuccessful  
509 in inhibiting the growth of pathogens and all but one isolate had MBCs and MICs greater than  
510 the maximum concentration. The concentration tested (up to 83.2  $\mu\text{l ml}^{-1}$ ) was two orders of  
511 magnitude greater than the recommended daily dose of MELAFIX (i.e. dose for 7 days at 5 ml  
512 per 10 US gallon = 0.13  $\mu\text{l/ml}$ ). However, these *in vitro* tests were done in Mueller-Hinton  
513 broth, which is likely to be more nutrient rich than home aquarium water and it is possible that  
514 MELAFIX would be more successful in inhibiting bacterial growth at more representative  
515 nutrient concentrations. Additionally, testing was conducted at 37°C which would increase the  
516 volatility and hence reduce efficacy of the active components of this botanical oil. Temperature  
517 also has the potential to influence MIC testing directly (Smith et al., 2018). Furthermore, *in*  
518 *vitro* MIC assays performed using emulsified cajuput oil against the pathogens *A. hydrophilia*,  
519 and *F. columnare*, significantly reduced optical density when compared to a no-treatment  
520 control (O’Brine, et al., submitted for publication 2023), suggesting some anti-bacterial  
521 activity.

522 While it is unclear the extent of the anti-bacterial activity of MELAFIX, there is speculation  
523 that beneficial effects of the product may be due to immunostimulant rather than antimicrobial  
524 properties as there is evidence within the literature that essential oils can have antioxidant  
525 potential. In silver catfish (*Rhamdia quelen*) bathing in 50  $\mu\text{l l}^{-1}$  of nano-encapsulated tea tree  
526 oil (derived from the congener *Melaleuca alternifolia*) for 1 h daily, reduced hepatic oxidative  
527 damage caused by experimental infection with *Pseudomonas aeruginosa* (Souza et al., 2017).  
528 Similarly, free tea tree oil protected *R. quelen* from hepatic oxidative damage caused by  
529 *Aeromonas hydrophilia* infection (Baldissera et al., 2017), where hepatic oxidative damage

530 may be a key factor in the progression of bacterial disease (Baldissera et al., 2018; Biazus et  
531 al., 2017; Castro et al., 2017).

532 The one *in vitro* efficacy study mentioned above also established the effect of MELAFIX on  
533 marine and freshwater general fish health *in vivo* (Shivappa et al., 2015). Aquaria (75 l, n =3)  
534 were stocked with 10 clownfish (marine) or goldfish (freshwater) and underwent daily dosing  
535 with MELAFIX for 2 weeks with water changes performed on day 7 and daily for 2 weeks post  
536 treatment. No distress behaviours or histopathological changes (compared to a control tank  
537 without MELAFIX) due to MELAFIX treatment were noted. A study evaluating the  
538 antiparasitic properties of cajuput oil (at comparable concentrations to MELAFIX) against  
539 *Gyrodactylus turnbulli* infection in guppies (*P. reticulata*) also observed no signs of  
540 behavioural distress during the first hour of treatment (Schelkle et al., 2015a) where fish were  
541 treated in isolation. While these studies suggest no negative effects of MELAFIX, behavioural  
542 observations following MELAFIX treatment have been limited to temporary distress  
543 behaviours.

544 West Indian bay tree (*Pimenta racemose*) oil is another essential oil mixture promoted as an  
545 antimicrobial treatment for ornamental fish pathogens. PIMAFIX (API) is a product marketed  
546 globally to combat fungal infections in home aquaria where its active ingredient is 1% *Pimenta*  
547 *racemose* essential oils. The oils consist mostly of eugenol, chavicol (phenols), and the  
548 monoterpene myrcene (McHale et al., 1977; Schelkle et al., 2015b). No published studies were  
549 identified on the efficacy of PIMAFIX for treating fungal infections (or fungal-like oomycete  
550 infections) in fishes. However, preliminary data supporting a significant reduction in fungal  
551 hyphae growth on ricefish eggs (*Oryzias woworae*) using PIMAFIX as a stand-alone treatment  
552 compared to a methylene blue control has been established (Snellgrove, D and O'Brine, T,  
553 unpublished data). West Indian bay oil has antifungal properties when its fumes ( $28 \times 10^{-3}$  mg  
554 ml<sup>-1</sup> air) were used to inhibit growth of two phytopathogenic fungi *Phytophthora cactorum*  
555 ( $69.1 \pm 11.6\%$  inhibition) and *Cryponectria parasitica* ( $75.2 \pm 2.4\%$  inhibition) (Kim et al.,  
556 2008). Many plant-based essential oils, particularly thyme, *Thymus vulgaris*, and oregano,  
557 *Origanum vulgare*, have strong antifungal properties against *Saprolegnia parasitica*, perhaps  
558 the most common fungal-like pathogen of fishes (Gormez and Diler, 2014; Nardoni et al., 2019;  
559 Tampieri et al., 2003). However, these essential oils contain different volatile constituents to  
560 West Indian bay oil (Nardoni et al., 2019; Schelkle et al., 2015b). Therefore, it is unclear if  
561 West Indian bay oil would be as effective and warrants further investigation. Regardless,

562 PIMAFIX, in combination with MELAFIX was found to be 95% effective as an antiparasitic  
563 agent against *Gyrodactylus turnbulli* infection in guppies (Schelkle et al., 2015b).

564 Another plant-derived treatment used within fish-keeping communities is garlic (*Allium*  
565 *sativum*). Allicin, the major active compound of garlic has broad antimicrobial capabilities with  
566 demonstrated antibacterial, antiparasitic, antifungal, and antiviral activity (reviewed in: Ankri  
567 and Mirelman, 1999). While there are garlic-based fish keeping products on the market in the  
568 UK, most are marketed as dietary supplements or appetite stimulants, whilst occasionally  
569 listing disease prevention as a secondary function. Despite this, 'home-made' garlic treatments  
570 are often used and can be prepared in various ways including dried (Schelkle et al., 2013),  
571 crushed (Fridman et al., 2014), and minced/pureed (Sasmal et al., 2005; Schelkle et al., 2013).  
572 However, the most scientific attention has been given to garlic extracts created with a variety  
573 of solvents including water, methanol, and ethanol, with the latter extracting the greatest variety  
574 of active chemical compounds (Ahmadniaye Motlagh et al., 2020; Fridman et al., 2014;  
575 Gholipour-Kanani et al., 2012; Saha and Bandyopadhyay, 2017; Sahandi et al., 2023; Sasmal  
576 et al., 2005).

577 Fluke infections (*G. turnbulli*) were controlled in guppy (*P. reticulata*) by using Chinese freeze  
578 dried garlic powder (0.03 mg ml<sup>-1</sup>), freeze dried garlic flakes (1 mg ml<sup>-1</sup>) and allyl disulphide  
579 (an allicin derivative, 0.5 mg ml<sup>-1</sup>) as successfully as a levamisole control group (Schelkle et  
580 al., 2013). This is in agreement with Fridman et al. (2014) who found 1 hour baths of 7.5 and  
581 12.5 ml l<sup>-1</sup> aqueous garlic extract significantly reduced *G. turnbulli* prevalence and severity in  
582 *P. reticulata*. Additionally, when their food was supplemented with 10-20% dried garlic  
583 powder for 14 days, intensity and prevalence of *G. turnbulli* and *Dactylogyrus* sp. (another  
584 fluke) infection was significantly reduced (Fridman et al., 2014). Ethanolic extract of garlic  
585 was found to treat goldfish (*C. auratus*) infected with tichonodinid ciliates (protozoan  
586 parasites) in a dose dependent manner with 15 mg l<sup>-1</sup> completely curing the infection after 8  
587 days of treatment (Saha and Bandyopadhyay, 2017). White spot disease in sailfin molly  
588 (*Poecilia latipinna*) and guppy (*P. reticulata*) was cured after 5 and 4 days of bath exposure to  
589 0.1 g l<sup>-1</sup> garlic extract respectively (Gholipour-Kanani et al., 2012).

590 The effectiveness of garlic is not limited to treating parasites, when tested *in-vitro*, crude  
591 aqueous garlic extract inhibited 80% of the growth of *Aeromonas* sp. and *Psuedomonas* sp.  
592 strains at concentrations of 0.40-0.41% and 0.99-1.43% respectively (Sasmal et al., 2005).  
593 Supplementing fish diets with garlic seems to confer protective benefits. When goldfish (*C.*

594 *auratus*) were a fed garlic paste-supplemented diet at a rate of 1 g per 100 g feed, they did not  
595 exhibit fin/tail rot up to 30 days post *Pseudomonas fluorescens* challenge whereas 20% of fish  
596 fed the control diet did (Sasmal et al., 2005). Additionally, when aqueous garlic extract was  
597 incorporated into the diet of female guppy juveniles (*P. reticulata*) for 80 days at levels of 0.1  
598 - 0.2 ml kg<sup>-1</sup> diet, non-specific skin mucus immune parameters were elevated, with optimum  
599 levels found at 0.15 ml kg<sup>-1</sup> (Ahmadniaye Motlagh et al., 2020).

600 Despite this promise there is some concern over the effects of garlic treatments on fish health.  
601 When white spot infected guppy (*P. reticulata*) were given long term (14 day) baths of a  
602 therapeutic dose of garlic extract (0.1 g l<sup>-1</sup>) histopathological changes were seen in key organs.  
603 A multitude of changes occurred in the gill tissue including epithelial hyperplasia, interstitial  
604 edema resulting in severe epithelial lifting in secondary lamellae, degeneration of secondary  
605 lamellae, reduced length of primary lamellae, severe lamellar fusion, increased space between  
606 filaments, vasodilatation, and blood congestion. Whereas in the liver, nuclear pyknosis,  
607 cytoplasm and vacuolar degeneration and hepatic necrosis were seen (Sahandi et al., 2023).  
608 Furthermore, fluke infected (*G. turnbulli* and *Dactylogyrus* sp.) guppies (*P. reticulata*) fed 10-  
609 20% garlic powder-supplemented feed showed elevated muscular dystrophy relative to  
610 untreated infected fish when histologically examined (Fridman et al., 2014). Garlic treatments  
611 have been shown to cause slight fin damage in a dose-dependent manner in fluke infected  
612 guppies, although this was not quantified. However, the authors noted the damage healed  
613 relatively quickly (within weeks of the treatment ceasing; Schelkle et al., 2013). It is unclear  
614 whether the gill or liver tissue changes in the previously mentioned studies reverted as longer-  
615 term monitoring was not in place and hence should be investigated.

616 Care should be taken when dosing with garlic-based treatments. The LC50 (the concentration  
617 which killed 50% of the treated animal) of ethanolic extract of garlic in goldfish was found to  
618 be 28.67 mg l<sup>-1</sup> in 4 day bioassays (Saha and Bandyopadhyay, 2017). Mortality was seen from  
619 concentrations of 20 mg l<sup>-1</sup> upwards, coupled with erratic swimming and irregular opercular  
620 movements which is close to the apparent effective dose of this extract at 15 mg l<sup>-1</sup>. In healthy  
621 guppies, aqueous garlic extract (15 ml l<sup>-1</sup>) started to cause mortality after 1 h of exposure with  
622 100% mortality shown by 6 h of exposure (Fridman et al., 2014). Again, this was close to the  
623 effective dose of 12.5 ml l<sup>-1</sup> for 1 h. The possibility of overdosing when treating with garlic  
624 perhaps limits its practicality as a treatment for the inexperienced home aquarist.

625 *Salt (Sodium Chloride)*

626 There is evidence that salt may be an effective treatment against a range of fish parasitic  
627 diseases including *Ichthyophthirius multifiliis* (white spot disease). *In vitro* studies of short  
628 exposures (24 h) to varying levels of salt (2.5-20 g l<sup>-1</sup>) show the infective theronts of *I.*  
629 *multifiliis* are susceptible to salt concentrations greater than 2.5 g l<sup>-1</sup> (50% mortality) with 5-10  
630 g l<sup>-1</sup> being most effective resulting in ≥ 95% mortality (Shinn et al., 2006). Feeding stage  
631 trophonts are less susceptible and can survive 10 h of exposure to 15 g l<sup>-1</sup> with 0% mortality,  
632 but not 10 h at 20 g l<sup>-1</sup> (Lahnsteiner and Weismann, 2007). Salt baths of variable concentrations  
633 and lengths have been tested *in vivo* in a variety of food fish species such as catfish and trout  
634 with varying success. Generally, baths of 5 g l<sup>-1</sup> or more for extended periods > 7 days are  
635 effective in treating white spot in these species (reviewed in Picón-Camacho et al., 2012).  
636 However, there are comparatively fewer studies in ornamental fishes.

637 When an outbreak of white spot occurred in an aquarium retailer's stock of black mollies,  
638 *Poecilia sphenops*, a sea salt bath (10 g l<sup>-1</sup>) reduced parasite burden to zero in 3 days at 27 °C  
639 without fish mortality (Maceda-Veiga and Cable, 2014). Similarly, when fingerlings of  
640 iridescent shark catfish (*Pangasianodon hypophthalmus*) were treated for white spot with 1%  
641 salt and elevated temperatures (24 to 30 °C) the infection cleared up after 15 days (Mamun et  
642 al., 2020), although mortality rates were high. It is possible the infection was too advanced as  
643 salt baths are reportedly more effective in the early stages of white spot (Maceda-Veiga and  
644 Cable, 2014); when the same treatment was applied to angelfish, *Pterophyllum scalare*, and  
645 gold gourami, *Trichopodus trichopterus*, with a lighter parasite burden, survival was improved  
646 (Mamun et al., 2021). The use of elevated temperatures was likely important as an increased  
647 temperature accelerates the *I. multifiliis* lifecycle and hence emergence of the salt-susceptible  
648 theront stage (Dickerson, 2011; Lahnsteiner and Weismann, 2007).

649 Another parasitic disease of ornamental fishes that seems to be effectively controlled with salt  
650 is external infection with flukes. The *in vitro* and *in vivo* survival of the guppy-parasitising  
651 fluke congeners *Gyrodactylus turnbulli* and *G. bullatarudis* were tested under varying salt  
652 concentrations (Schelkle et al., 2011). Survival of both fluke species decreased with increasing  
653 salt concentrations, surviving < 1 h at 33 g l<sup>-1</sup>. However, when tested *in vivo* on guppies,  
654 *Poecilia reticulata*, the two parasites differed in their tolerances. To establish the preventative  
655 effects of salt, the guppies were gradually habituated to 3 or 7 g l<sup>-1</sup> salinity over 7 days and then  
656 experimentally infected by close proximity to a donor fish while anaesthetised. *G. turnbulli*  
657 failed to establish on 100% of guppies acclimated to 7 g l<sup>-1</sup> whereas *G. bullatarudis*  
658 successfully established on ~72% guppies at the same salinity. Furthermore, a similar pattern

659 emerged when using higher concentration salt baths as a treatment for guppies already infected  
660 with *Gyrodactylus*. When exposed to 15 min baths (five baths for juveniles) of 15 and 25 g l<sup>-1</sup>,  
661 survival of both parasites decreased with increasing salinity. However, *G. bullatarudis* was less  
662 affected by the salt baths with 73.3% efficacy at 25 g l<sup>-1</sup> compared to 100% efficacy in *G.*  
663 *turnbulli*. These treatments should be used with caution, particularly in juveniles. Routine  
664 monitoring post-experiment showed increased mortalities in the juvenile fish exposed to the  
665 shorter salt baths. This mortality was not fully quantified and thus remains anecdotal in nature.  
666 More studies should be done to investigate the efficacy of salt baths for treating ornamental  
667 fishes with parasites.

668 The effectiveness of salt in treating parasitic diseases has led to speculation on its potential to  
669 treat external bacterial infections, particularly those caused by species sensitive to salt, such as  
670 *Flavobacterium columnare* (Columnaris disease). *In vitro* data suggest short baths (15 mins)  
671 of 4% NaCl were 95-100% effective in killing *F. columnare* strains (Suomalainen et al., 2005).  
672 However, *in vivo* in experimentally infected rainbow trout, *Oncorhynchus mykiss*, all fish  
673 succumbed to *F. columnare* infection by 6 days post infection despite being treated with 4%  
674 NaCl baths on days 1 (15 min bath), 3, and 5 (both 5 min baths; Suomalainen et al., 2005).  
675 While the salt baths were ineffective at reducing fish mortality, a delay in mortality caused by  
676 columnaris disease was seen, potentially through salt killing bacteria shed from the fish  
677 epidermis, reducing the transmission rate to conspecifics.

678 Despite little evidence to suggest salt dips can be used therapeutically for bacterial diseases,  
679 prolonged exposure to salt may be effective as a preventative measure. When four species of  
680 fish, including goldfish (*C. auratus*) were acclimated to higher salinities for a period of 4-10  
681 weeks, mortality was significantly reduced following experimental *F. columnare* infection  
682 (Altinok and Grizzle, 2001). In untreated goldfish, mortality was 66.5% at 5 days post exposure  
683 to *F. columnare*, compared to 40.8% in fish acclimated to 1 ‰, and 0 % in fish acclimated to  
684 3 and 9 ‰. These results could be explained by reduced adherence and biofilm formation  
685 capabilities of *F. columnare* at salinities  $\geq 3\text{‰}$  (Altinok and Grizzle, 2001; My et al., 2020).  
686 Adherence and biofilm formation are both important factors in the initial colonisation of fish  
687 tissue and thus disrupting them may have been a key factor in reducing mortalities (Declercq  
688 et al., 2021).

689 While 3-9 ‰ salinity seems to be an effective preventative measure for columnaris disease in  
690 goldfish, the long-term effects of salinity on the health of freshwater ornamental fishes are

691 unclear. Goldfish kept for 21 days at 8-10‰ had significantly reduced growth, whereas koi  
692 kept at 12‰ for 4 months did not (Luz et al., 2008; Sharma et al., 2017). Goldfish kept at 5‰  
693 for 21 days showed decreased blood pH, blood ionic imbalance and alteration of gill structure  
694 with increased mucus secretion, swollen blood vessels and lesions (Da Silva et al., 2021).  
695 There are clear gaps in our understanding of the effects of long-term salt exposure on  
696 freshwater ornamental fish health. Additionally, salt treatments are not suitable for aquaria  
697 containing live plants as many freshwater plants are intolerant of even low levels of salt  
698 (Tootoonchi and Gettys, 2019).

699

#### 700 *Therapeutic dyes*

701 There are a range of therapeutic dyes with antiparasitic, antifungal and antibacterial properties  
702 that have been used to treat ornamental fishes. Malachite green has a long history of use in fish  
703 treatment. It was first reported as a treatment for fungal infection of trout and as a disinfection  
704 treatment for their eggs in 1936 (Foster and Woodbury, 1936). In the 1960s it was demonstrated  
705 to be a useful antiparasitic agent, particularly against white spot (Johnson, 1961) and for the  
706 rest of the 20<sup>th</sup> century was used routinely in aquaculture as an antiparasitic, antifungal and egg  
707 disinfectant. However, in 2000 malachite green was banned in the EU for use in food fishes  
708 due to its persistence in fish tissues and evidence of toxic and carcinogenic properties (Sudova  
709 et al., 2007). Despite this, malachite green is still used in many products commonly sold to  
710 control parasitic and fungal diseases in ornamental fishes.

711 Malachite green is known to be highly toxic to fishes and the recommended therapeutic dose  
712 can often come close to the lethal dose, which can vary greatly between species (Intorre et al.,  
713 2007; Souza et al., 2020; Sudova et al., 2007). For example, in jewel cichlid, *Hemichromis*  
714 *bimaculatus*, no harmful effects were observed at 0.25 - 0.5 mg l<sup>-1</sup> malachite green for 96 h,  
715 which is greater than maximum recommended prolonged dose for ornamental fishes: 0.2 mg l<sup>-1</sup>  
716 (Noga, 2010; Souza et al., 2020). However, in goldfish, *Carassius auratus*, and zebrafish,  
717 *Danio rerio*, the recommended short-bath therapeutic dose (2 ppm for 0.5 h) caused 10%  
718 cumulative mortality in both species by 14 days post treatment (Intorre et al., 2007). In addition,  
719 fish treated with the therapeutic dose showed sub-lethal responses to the malachite green  
720 including reduced activity and loss of equilibrium. When the same concentration was applied  
721 over an extended period (2.5 h) the mortality was increased in goldfish (90% cumulative  
722 mortality at 14 days post treatment) but not zebrafish (Intorre et al., 2007). The effectiveness

723 of malachite green may also vary between species. When iridescent shark catfish fingerlings,  
724 *P. hypophthalmus*, received a combination treatment of formalin (25 ppm) and malachite green  
725 (0.1 mg l<sup>-1</sup>) a heavy infection of white spot was not successfully treated (Mamun et al., 2020),  
726 but the same treatment successfully resolved an infection in mollies, *Poecilia sphenops*, in 6  
727 days without any mortality (Maceda-Veiga and Cable, 2014). Treatment of scaleless fishes  
728 with malachite green is not recommended due to its toxic effects (Mamun et al., 2020) and is  
729 likely to be a contributing factor in the difference between these two studies.

730 Another therapeutic dye used in the trade is methylene blue which is used as an antiparasitic  
731 for fishes and an antifungal bath treatment for eggs. It increases hatch rate in ornamental fish  
732 eggs at 3 mg l<sup>-1</sup>, although this is species-specific occurring in angelfish, *Pterophyllum scalare*,  
733 but not in zebrafish, *Danio rerio* (Chambel et al., 2014). Methylene blue is considered to have  
734 a lower toxicity than malachite green (Alam et al., 2011; Bolivar et al., 2012), but is used in  
735 higher doses to achieve the same effects (2 mg l<sup>-1</sup> compared to 0.1 mg l<sup>-1</sup>) and can result in  
736 mortality in some species (Tieman and Goodwin, 2001). Repeated exposure to 2 mg l<sup>-1</sup> reduced  
737 growth performance and some immunological markers in goldfish (Soltanian et al., 2021).  
738 However, despite this, when challenged with *Aeromonas hydrophilia*, treated goldfish had  
739 better survival than untreated challenged goldfish. The use of methylene blue (1 ppm) in  
740 combination with 2% salt as a bath treatment was partially successful at resolving a heavy  
741 white spot infection in iridescent shark catfish, *P. hypophthalmus* (Mamun et al., 2020), where  
742 the salt possibly contributed negatively to treatment performance as treated fish showed  
743 extensive epithelial damage and had sloughed off epidermis (Mamun et al., 2020).

744 Acriflavine is another dye used for the control of parasitic infections and egg disinfection in  
745 ornamental fishes (Plakas et al., 1999), although there is little scientific evidence for its  
746 effectiveness. It is not suitable for use in ornamental fish larvae where it causes high mortalities  
747 (up to 100% in zebrafish larvae) using lower than recommended therapeutic doses (Meinelt et  
748 al., 2002; Plakas et al., 1999). Furthermore, toxicity is increased in harder water which makes  
749 it unsuitable for treatment of fishes which thrive in harder waters such as East African lake  
750 cichlids (Meinelt et al., 2002; Santos et al., 2023).

751

#### 752 *Other common active ingredients*

753 Lice-Solve (Vet Ark) is a treatment marketed at combating ectoparasitic crustaceans where the  
754 active ingredient is emamectin benzoate. While no studies have been performed on the



755 product's efficacy against crustaceans, one study has looked at its potential as an anthelmintic  
756 treating the gastrointestinal nematode *Pseudocapillaria tomentosa* in zebrafish (Kent et al.,  
757 2019). Lice-Solve was tested at 10 and 3 x the manufacturers recommended dose in four 24 h  
758 bath treatments. Both concentrations proved 100% effective although the weaker dose was  
759 tested on populations with less severe infections (80 vs 30% prevalence). It is unclear if the  
760 lower dose would have been as effective with greater parasite prevalence. The safety of the  
761 product was also assessed in concentrations of 10 and 5 x the manufacturers dose. No mortality  
762 was seen. However, behaviours indicative of stress (rapid respiration, staying near tank bottom)  
763 were seen in the higher concentration although fish recovered on cessation of treatment.  
764 Additionally, histological analysis of gill, liver, intestine, and kidney tissue revealed no  
765 toxicological effects.

766 Ethacridine lactate is a topical antiseptic, generally effective against Gram-positive bacteria  
767 and used to prevent wound infection in humans (Reinhardt et al., 2005). However, despite  
768 being used in therapeutic products (Table 3) nothing is known about its effectiveness as  
769 treatments for bacterial infections in fishes.

770 Copper sulphate is an inorganic salt with various antimicrobial properties, widely considered  
771 to be an effective antiparasitic and antifungal agent. It has been used in aquaculture for many  
772 years to combat pathogens such as *Piscinoodinium* spp., *Ichthyophthirius multifiliis*, and  
773 *Saprolegnia parasitica* (reviewed in Tavares-Dias, 2021). Comparatively little is known about  
774 its effectiveness as an antibacterial treatment for ornamentals but there is some evidence to  
775 suggest it could be used as a preventative treatment. In channel catfish, *Ictalurus punctatus*, 24  
776 h exposure to copper sulphate (25 mg l<sup>-1</sup>) prior to infection with *Edwardsiella ictaluri* reduced  
777 mortalities relative to untreated fish (Griffin and Mitchell, 2007). MICs of copper sulphate for  
778 a variety of pathogenic bacteria range from 100-1600 µg ml<sup>-1</sup> (Benhalima et al., 2019), much  
779 higher than the 25 mg l<sup>-1</sup> used by Griffin and Mitchell (2007), suggesting the effect was due to  
780 prior exposure rather than direct antibacterial effects.

781 Praziquantal is an active ingredient often found in anthelmintic treatments for ornamental  
782 fishes (Table 3). A recent review has shown it has good efficacy against a number of cestode  
783 and monogenean fish pathogens in both food fish and ornamental species (Norbury et al.,  
784 2022). It is relatively safe to use, with toxic concentrations rarely reached during conventional  
785 therapy. However, there is limited evidence on its environmental impacts and there is potential  
786 for resistance to develop within targeted pathogens (Norbury et al., 2022).

787 *Euthanasia*

788 From a welfare perspective, in severe cases of disease, euthanasia may be the most ethical  
789 solution and should be carried out humanely. Overdose with a suitable waterborne anaesthetic  
790 (e.g. buffered tricaine methosulphate - MS 222) followed by destruction of the brain is  
791 frequently promoted as a humane method of euthanizing fishes (AVMA, 2020; Metcalfe and  
792 Craig, 2011; Sloman et al., 2019). However, options for the home aquarist can be limited due  
793 to the unavailability of anaesthetics to the general public in some countries. Clove oil appears  
794 to be most typically used by the home aquarist (Fernandes et al., 2017). Care should be taken  
795 to ensure the anaesthetic used is suitable for the fish species, as anaesthetics vary in efficacy,  
796 dosage, and perception by fishes in a species-specific manner and a ‘one size fits all’ approach  
797 should not be taken (Davis et al., 2015; Perret-Thiry et al., 2022; Readman et al., 2017).

798

## 799 **Conclusion**

800 The ornamental fish trade is a multi-billion dollar global industry with a responsibility for the  
801 welfare of a large number of fishes that are routinely traded internationally. With mortality and  
802 morbidity from disease resulting in significant detriment to fish welfare and economic losses  
803 for the industry, our review clearly highlights some large scientific gaps in our knowledge that  
804 require urgent attention. While the types of pathogens occurring in ornamental fishes are well-  
805 established, within commercial practices AMR is certainly a growing problem. Building on the  
806 previous systematic review in this field by Weir et al. (2012) we have shown that antimicrobial  
807 resistance is still an issue with many classes of antibiotics such as tetracyclines and penicillins  
808 proving ineffective treatments and some resistance being shown to previously effective  
809 antibiotics such as carbapenems. Inappropriate treatments of infections within the ornamental  
810 trade, particularly the use of antibiotics, will continue to fuel antimicrobial resistance with  
811 potential risks to human health. The paucity of *in vivo* studies on the efficacy of antibiotics in  
812 treating ornamental fish diseases makes it very difficult to make recommendations for  
813 commercial practice. Further research into efficacy to allow targeted treatment of fishes is  
814 clearly needed. We recommend collaboration between policy makers, stakeholders within the  
815 industry (e.g. ornamental fish producers and wholesalers) and environmental scientists to  
816 develop and implement international ‘One Health’ frameworks to curb the rise in AMR and  
817 reduce risks to human health.

818 Also concerning is the lack of scientific information surrounding the range of treatments  
819 available to most home aquarists to combat infections in their ornamental fishes. Our  
820 systematic review found minimal research into many of the commercial products available,  
821 and within that a predominance of *in vitro* rather than *in vivo* studies. Also lacking is  
822 consideration of the impacts of these treatments on general ornamental fish health and  
823 behaviour in order to fully evaluate the risks and benefits associated with these treatments.  
824 Many of the more traditional approaches such as salt and therapeutic dyes have significant side-  
825 effects. The emergence of AMR, coupled with a consumer demand for sustainable products  
826 highlights the need to develop further treatments that can be used throughout the trade to reduce  
827 disease and improve welfare. Ideal products for disease treatment in pet fishes should have  
828 high efficacy with minimal risk of resistance developing, be non-toxic to the fish with a  
829 significant margin between the effective dose and sub-lethal effects in the fish, such as adverse  
830 behavioural responses. More research is needed to understand fully these characteristics within  
831 existing treatments, but also into the potential for new treatments to reduce disease and improve  
832 ornamental fish welfare.

833

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**Table 1.** Summary of the main diseases reported for freshwater fishes held as companion animals in home aquaria. For more detailed reviews which include gross signs of common diseases, see Cardoso et al. (2019, 2022).

Microorganism Category	Disease	Symptoms	Examples of Susceptible Species	References
Gram-negative bacteria	Columnaris disease ( <i>Flavobacterium columnare</i> )	Yellowy white filamentous lesions predominantly affecting skin, gills and fins.	Mollies, platies, goldfish, koi, guppies.	Yamazaki et al., 1990; Decostere et al., 1998, 2002; Altinok, 2004; Roberts et al., 2009; Declercq et al., 2013.
	<i>Edwardsiella piscida</i>	Discoloured skin patches, external haemorrhages, indication of septicaemia, erratic swimming, exophthalmia.	Koi carp, goldfish, zebrafish.	Buján et al., 2018; Choe et al., 2017; McDermott & Palmeiro, 2020; Reichley et al., 2017; Zhang et al., 2021.
	<i>Edwardsiella ictauluri</i>	Enteric septicaemia.	Catfishes, considered emerging disease in ornamentals.	Hawke et al., 1981; Hawke et al., 2013; Kumar et al., 2019; McDermott & Palmeiro, 2020.
	Koi ulcerative disease ( <i>Aeromonas salmonicida</i> )	Cutaneous lesions which progressively erode the skin, exposing underlying musculature.	Koi, goldfish.	Elliott and Shotts, 1980; Gan et al., 2015.
	Motile aeromonad disease ( <i>A. hydrophilia</i> , <i>A. veronii</i> biovar <i>veronii</i> , <i>A. caviae</i> ).	Sloughing of scales, skin, fins, abdominal dropsy, exophthalmia, haemorrhage; in systemic cases haemorrhagic septicaemia.	Wide range of ornamentals.	Hossain et al., 2019c; Hossain & Heo, 2021; Jagoda et al., 2014; Sreedharan et al., 2012.

	<i>Pseudomonas</i> spp. infection.	Tail and fin rot, haemorrhage, and ulceration.	Wide range of ornamentals.	El-Hady and Samy, 2011; Fayed et al., 1997.
	Vibriosis ( <i>Vibrio</i> spp.).	Ulceration, haemorrhage and haemorrhagic septicaemia.	Wide range of ornamentals.	Roberts et al., 2009.
Gram positive bacteria	Streptococcosis	Haemorrhages (particularly around the operculum, eye, and base of fins), exophthalmia, cloudy eyes, ulcerations, and erratic spiral swimming behaviour.	Cyprinids and cichlids such as red-tail black shark, rainbow shark, oscar, blue ram.	Lazado et al., 2018; Raissy et al., 2012; Tukmechi et al., 2009; Russo et al., 2006; Tukmechi et al., 2009; Velappan and Munusamy, 2018; Lazado et al., 2018.
Acid fast bacteria	Nocardiosis	Presents similarly to mycobacteriosis; distinguished by the branching, filamentous morphology of the bacteria.	Neon tetras, oscar, goldfish, and Chinese high-fin banded shark.	Conroy, 1964; Sheikhzadeh et al., 2020; Wang et al., 2007.
	Mycobacteriosis	Chronic (survival 4-8 weeks) and acute infections (survival 5- 16 days). Ascites, lethargic behaviour, spinal defects, loss of appetite, scale loss, exophthalmia, emaciation, ulcerations and depigmentation.	Reported in over 150 species, including ornamentals.	Decostere et al., 2004; Puk and Guz, 2020; van der Sar et al., 2004; Gauthier and Rhodes, 2009; Puk and Guz, 2020; Wang et al., 2007.

Protozoan Parasites	Velvet disease ( <i>Piscinoodinium</i> spp.)	In heavily infected fishes, a rusty-coloured velvet-like appearance. Mortality can occur due to extensive epithelial damage compromising osmoregulatory functions.	Wide range of ornamentals, typically tropical species.	Ferraz and Sommerville, 1998; Lieke et al., 2020.
	White spot disease ( <i>Ichthyophthirius multifiliis</i> )	White raised spots covering most of body. Flashing, and general lethargic behaviour often seen. High mortality if not treated promptly.	Wide range of ornamentals.	Dickerson, 2011; Ekanem et al., 2004; Ezz EI Dien et al., 1998; Francis-floyd et al., 2016; Iqbal and Haroon, 2014; Matthews, 2005.
Metazoan Parasites	<i>Gyrodactylus</i> spp. (Skin fluke)	Affects the skin, gills and fins of the fish. Symptoms depend on infection site. Thickening of the fins/fin erosion, skin erosion, gill histopathological changes, excess mucus production all common.	Wide range of ornamentals.	Dewi et al., 2018; Mohamed et al., 2010; Mousavi et al., 2013; Thilakaratne et al., 2003; Trujillo-González et al., 2018; Yandi et al., 2017
	<i>Dactylogyrus</i> spp. (Gill fluke)	Chiefly affects the gills but also infects skin of the fish. Causes histopathological changes to gill	Largely cyprinid and poeciliid species such as barbs, koi, guppies, goldfish, and tetras.	Dewi et al., 2018; Mohammadi et al., 2012; Molokomme et al., 2023; Thilakaratne et al.,

		tissue and impaired respiratory function. Reduced growth, lethargy and gill flashing behaviours often seen.		2003; Trujillo-González et al., 2018; Yandi et al., 2017
	<i>Camallanus</i> spp. (nematode)	Subclinical in many cases. In heavily infected fish symptoms include poor body condition, abdominal swelling, lethargy, red worms protruding from vent, abnormal faeces and mortality.	Various ornamental species, particularly livebearers.	Ferreira et al., 2019; Kim et al., 2002; Levsen, 2001; Martins et al., 2007; Palmeiro and Roberts, 2012
	<i>Capillaria</i> spp. (nematode)	Similar symptoms to <i>Camallanus</i> however worms protruding from vent will be white.	Various ornamental species, particularly cichlids.	Adel et al., 2013; Dewi et al., 2018; Palmeiro and Roberts, 2012; Rahmati-Holasoo et al., 2023; Thilakaratne et al., 2003
Fungal-like diseases	Oomycetes ('water moulds' e.g. <i>Saprolegnia</i> , <i>Achyla</i> , and <i>Dictyuchus</i> spp.)	Cottony thread-like lesions on body surface. Often secondary infection of pre-existing lesions.	Wide range of ornamentals.	Gozlan et al., 2014; Howe and Stehly, 1998; Khoo, 2000; Noga, 2010; Udomkunsri and Noga, 2005.

**Table 2.** Studies reporting antimicrobial resistance in bacteria isolated from ornamental fishes since 2010. Only studies containing data on  $n \geq 20$  isolates are reported. Antibiotics listed from least to most effective. Antibiotics with the same % resistant isolates are grouped together. MAR = Multiple antimicrobial resistance index = number of antibiotics resistant to/ total number of antibiotics tested. Where data were only represented graphically, numerical values were obtained using GetData Graph Digitizer ver. 2.26.0.20.

Study	Bacterial spp. (n of isolates)	Geographical origin	Biological sample	Antibiotics tested and % of isolates resistant	MAR (min-max, mean)
(Čížek et al., 2010)	<i>Aeromonas</i> spp. (n = 72)	Czech Republic, Koi farms	Koi carp <i>Cyprinus carpio koi</i> , gills, and skin swabs	Oxytetracycline (50%); Ciprofloxacin (25%); Trimethoprim (15%); Chloramphenicol, Florfenicol (7%)	-
(Dias et al., 2012)	<i>Aeromonas</i> spp., <i>A. aquariorum</i> (n = 43), <i>A. hydrophila</i> (n = 67), <i>A. veronii</i> (n = 94), <i>A. culicicola</i> (n = 16)	Portugal, ornamental fish importer	Various ornamental fish species, skin, and water samples	Carbenicillin (96%); Ampicillin (94%); Amoxicillin (95%); Erythromycin (88%); Tetracycline (80%); Ticarcillin (73%); Trimethoprim/sulfamethoxazole (36%); Cephalothin (32%); Kanamycin (31%); Gentamicin (28%); Cefoxitin (24%); Ticarcillin/clavulanic acid (22%); Tobramycin (19%); Ciprofloxacin (18%); Norfloxacin (16%); Amoxicillin/clavulanic acid (15%); Chloramphenicol (13%); Netilmicin (11%); Piperacillin (7%); Amikacin (6%); Ceftriaxone (5%); Ceftazidime, Cefoperazone (4%); Piperacillin/ clavulanic acid (3%); Imipenem (1%); Cefotaxime, Cefepime, Aztreonam (0%)	-



(Sreedharan et al., 2012)	<i>Aeromonas caviae</i> (n = 20)	India, goldfish farms	Water samples from goldfish <i>Crassius auratus</i> , culture systems	Norfloxacin, Ofloxacin, Pefloxacin, Sparfloxacin, Pipemidic acid, Nalidixic acid, Amoxicillin, Doxycycline HCl, Ampicillin, Colistin, Bacitracin, Vancomycin, Erythromycin, Oxytetracycline, Tetracycline Methicillin, Penicillin G, Cloxacillin, Ticarcillin, Oxacillin, Cephaloridine, Clindamycin, Lincomycin, Fusidic acid, Polymixin B (100%); Novobiocin (60%); Cephalothin, Oleandomycin, Spiramycin (40%); Piperacillin, Carbenicillin, Cephalexin, Cefazolin, Cephadrine Cephadroxil, Chlortetracycline, Minocycline, Chloramphenicol, Rifampicin, Cefaclor, Cephoxitin, Cefamandole, Cefriaxone, Ceftazidime, Cefoperazone, Ceftizoxime, Imipenem, Amikacin, Gentamycin, Kanamycin, Neomycin, Netillin, Streptomycin, Tobramycin, Azithromycin, Tylosine, Clarithromycin, Nitrofurazone, Furazolidone, Furaxone, Trimethoprim, Sulfadiazine, Sulfafurazole, Sulfaphenzole, Ciprofloxacin, Nitrofurantoin, Enrofloxacin, Floxidine, Nitroxoline, Fosfomycin (0%)	0.243-0.457, -
	<i>Aeromonas jandaei</i> (n = 23)	India, goldfish farms	Water samples from goldfish <i>C. auratus</i> , culture systems	Norfloxacin, Ofloxacin, Pefloxacin, Sparfloxacin, Amoxicillin, Doxycycline HCl, Ampicillin, Bacitracin, Erythromycin, Oxytetracycline, Tetracycline Methicillin, Penicillin G, Cloxacillin, Ticarcillin, Cephaloridine, Clindamycin, Lincomycin, Fusidic acid, Polymixin B (100%); Colistin (74%); Oleandomycin (56.5%); Cephalexin, Cefazolin, Cephadrine, Cephalothin, Cephoxitin (30.4%); Oxacillin (26.1%); Pipemidic acid, Nalidixic acid, Vancomycin (26%); Spiramycin, Piperacillin, Carbenicillin, Cephadroxil, Chlortetracycline, Minocycline, Chloramphenicol, Rifampicin, Cefaclor, Cefamandole, Cefriaxone, Ceftazidime, Cefoperazone, Ceftizoxime, Imipenem, Amikacin, Gentamycin, Kanamycin, Neomycin, Netillin, Streptomycin, Tobramycin, Azithromycin, Tylosine, Clarithromycin, Nitrofurazone, Furazolidone, Furaxone, Trimethoprim, Sulfadiazine, Sulfafurazole, Sulfaphenzole, Ciprofloxacin, Novobiocin, Nitrofurantoin, Enrofloxacin, Floxidine, Nitroxoline, Fosfomycin (0%)	0.243-0.457, -
(Rose et al., 2013)	Various spp. (n = 64)	USA, ornamental fish importer	Various ornamental fishes, kidneys	Tetracycline (77%); Trimethoprim/sulphamethoxazole (73%); Azithromycin (68%); Cefadroxil (65%); Ampicillin, Ciprofloxacin (58%); Gentamycin (50%); Kanamycin (35%); Cefotaxime (16%)	

(Jagoda et al., 2014)	<i>Aeromonas</i> spp., <i>A. veronii</i> (n = 34), <i>A. veronii</i> atypical (n = 7), <i>A. hydrophila</i> (n = 4), <i>A. caviae</i> (n = 3), <i>A. dhakensis</i> atypical (n = 2), <i>A. jandaei</i> (n = 1), <i>A. enteropelogenes</i> (n = 1)	Sri Lanka, commercial aquaria and breeding farms	Various septicaemic ornamental fishes, kidneys and liver	Amoxicillin (98.1%); Tetracycline (59.5%); Erythromycin (54.7%); Trimethoprim/sulphamethoxazole (26.4%); Nitrofurantoin (22.6%); Neomycin (9.4%); Chloramphenicol, Enrofloxacin (7.5%)	-
(Chung et al., 2017)	<i>Aeromonas veronii</i> (n = 30)	Korea, ornamental fish importer	Various ornamental fishes, organs sampled unknown	Ampicillin (96.7%); Tetracycline (80%); Piperacillin (73.3%); Sulfamethoxazole/trimethoprim (56.7%); Enrofloxacin (46.7%); Marbofloxacin (40%); Gentamicin (30%); Tobramycin (26.7%); Ceftiofur (20%); Imipenem (10%); Chloramphenicol (6.7%); Amikacin (3.3%); Amoxicillin/clavulanic acid, Cefpodoxime, Nitrofurantoin (0%)	-
(Hossain et al., 2018)	<i>Aeromonas</i> spp., <i>A. hydrophila</i> (n = 30), <i>A. veronii</i> (n = 32), and <i>A. punctata</i> (n = 3)	Korea, ornamental fish stores	Healthy goldfish <i>C. auratus</i> , whole body	Amoxicillin, Nalidixic acid, Levofloxacin, Amikacin (100%); Ampicillin (98.46%); Tetracycline (92.31%); Rifampicin (86.15%); Cephalothin (61.45%); Trimethoprim/sulfamethoxazole (44.62%); Doxycycline (26.15%); Gentamicin, Kanamycin (6.15%); Cefoxitin, Imipenem, Chloramphenicol (4.61%); Ciprofloxacin, Ofloxacin (1.54%); Ceftriaxone (0%)	-
(Hossain et al., 2019a)	<i>Aeromonas</i> spp., <i>A. veronii</i> biovar <i>veronii</i> (n = 26), <i>A. veronii</i> biovar <i>sobria</i> (n = 3), <i>A. hydrophila</i> (n = 8), <i>A. caviae</i> (n = 3), <i>A. enteropelogenes</i> (n = 2), and <i>A. dhakensis</i> (n = 1)	Korea, ornamental fish stores	Zebrafish <i>Danio rerio</i> , whole body	Amoxicillin, Nalidixic acid, Oxytetracycline (100%); Ampicillin (93.2%); Tetracycline (74.42%); Rifampicin (67.44%); Imipenem (65.15%); Trimethoprim/sulfamethoxazole (27.91%); Cephalothin (25.58%); Ciprofloxacin (6.98%); Chloramphenicol (4.65%); Nitrofurantoin (2.33%); Amikacin, Kanamycin, Cefotaxime (0%)	0.19-0.44, -

(Hossain et al., 2019b)	<i>Aeromonas</i> spp., <i>A. veronii</i> (n = 34), <i>A. dhakensis</i> (n = 10), <i>A. caviae</i> (n = 3) and <i>A. enteropelogenes</i> (n = 2)	Korea, ornamental fish stores	Healthy guppy <i>Poecilia reticulata</i> , whole body	Amoxicillin, Nalidixic acid, Oxytetracycline (100%); Ampicillin (92.30%); Imipenem (71.15%); Cephalothin, Tetracycline (51.92%); Trimethoprim/sulfamethoxazole (50%); Gentamicin (21.15%); Kanamycin (13.46%); Nitrofurantoin (7.69%); Chloramphenicol (5.77%); Ciprofloxacin (5.76%); Cefotaxime (3.85%); Ceftriaxone (1.92%); Amikacin (0%)	0.28-0.67, -
(Hossain et al., 2019c)	<i>Aeromonas</i> spp., <i>A. hydrophila</i> (n = 30), <i>A. veronii</i> biovar <i>veronii</i> (n = 32), and <i>A. caviae</i> (n = 3).	Korea, ornamental fish stores	Healthy goldfish <i>C. auratus</i> , whole body	Amoxicillin, Nalidixic acid (100%); Ampicillin (98.46%); Tetracycline (92.31%); Rifampicin (90.77%); Cephalothin (66.15%); Trimethoprim/sulfamethoxazole (49.23%); Doxycycline (26.15%); Gentamicin, Kanamycin (6.15%); Cefoxitin, Imipenem, Chloramphenicol (4.62%); Ciprofloxacin (1.54%); Cefotaxime, Levofloxacin, Ofloxacin, Amikacin (0%)	0.22-0.56, 0.36
(Delalay et al., 2020)	Various spp. (Exact isolate number not reported, 1448 isolates used in entire study, n > 20 per antimicrobial)	Switzerland, various sources (private owners, fish stores etc.)	Various species of clinically ill ornamental fishes, liver spleen and kidney	Sulphonamide (67%); Oxolinic acid (44.6%); Erythromycin (44.1%); Oxytetracycline (41.5%); Tetracycline (36.9%); Cefepime (32.7%); Florfenicol (31.5%); Sulphonamide/trimethoprim (27.2%); Chloramphenicol (18.3%); Gentamicin (14.1%); Amikacin (12.2%); Norfloxacin (10.8%)	-
			Clinically ill koi <i>C. carpio koi</i> , liver spleen and kidney	Sulphonamide (74.7%); Oxolinic acid (73.7%); Oxytetracycline (63.8%); Tetracycline (60.5%); Erythromycin (50.5%); Florfenicol (42.2%); Chloramphenicol (41.3%); Norfloxacin (36.1%); Sulphonamide/trimethoprim (34.6%); Amikacin (34.3%); Cefepime (24.7%); Gentamicin (24%)	-
			Clinically ill goldfish <i>C. auratus</i> , liver spleen and kidney	Sulphonamide (64%); Oxolinic acid (48%); Oxytetracycline (45.5%); Tetracycline (39.1%); Erythromycin (32%); Sulphonamide/trimethoprim (29.2%); Norfloxacin (19%); Chloramphenicol (16%); Gentamicin (8%)	-

(Haenen et al., 2020)	<i>Aeromonas</i> spp. (n = 59)	Global ornamental fish consignments imported into the Netherlands	Various tropical ornamental fishes, skin, and internal organs	Tetracycline (85%); Flumequine (53%); Trimethoprim/sulfamethoxazole (30%); Neomycin (34%); Nitrofurantoin (17%); Florfenicol (9%)	-
(Preena et al., 2020a)	Various spp. (n = 25)	India, ornamental fish farm	Clinically infected koi, gills, liver, and spleen. Data also provided on goldfish but isolates n < 20	Furazolidone (72.72%); Bacitracin (45.5%); Trimethoprim, Cephalothin, Cefalexin (45.45%); Vancomycin, Rifampicin, Azithromycin, Streptomycin, Ampicillin (36.36%); Nalidixic acid (30%); Oxytetracycline, Perfloxacin (27.3%); Nitrofurantoin, Sulphadiazine, Erythromycin, Tobramycin, Gentamycin, Cefoxitin, Cefazolin, Cefoperazone (18.2%); Aztreonam (18%); Amikacin, Colistin, Enrofloxacin, Chloramphenicol, Co-trimoxazole, Imipenem, Amoxyclav (9.1%); Piperacillin, Ceftazidime (9%) Cefepime, Cefixime/clavulanic acid, Cefotaxime, Cefotaxime/clavulanic acid, Ceftriaxone, Cefuroxime, Amoxicillin, Doripenem, Meropenem, Piperacillin/tazobactam, Kanamycin, Ciprofloxacin, Levofloxacin, Norfloxacin, Polymyxin-B (0%)	0-0.34, -
(Saengsitthisak et al., 2020)	<i>Aeromonas</i> spp., <i>A. sorbria</i> (n = 41), <i>A. hydrophilia</i> (n = 18), and <i>A. caviae</i> (n = 5)	Thailand, ornamental fish shops	Clinically diseased goldfish <i>C. auratus</i> , koi <i>C. carpio koi</i> and red swordtail <i>Xiphophorus hellerii</i> , liver and spleen	Amoxicillin (93.75%); Oxytetracycline (79.69%); Erythromycin (75%); Sulfamethoxazole/trimethoprim (46.88%); Ciprofloxacin (40.63%); Enrofloxacin, Norfloxacin (25%); Gentamicin (17.19%); Amikacin (12.5%); Nitrofurantoin (7.81%); Chloramphenicol (6.25%); Ceftazidime (0%)	-
(Sicuro et al., 2020)	Various spp. (exact isolate number not reported, estimated n ≥ 91)	Italy, ornamental fish importer	Various ornamental fishes, kidneys	Lincomycin (97.1%); Erythromycin (86.9%); Ampicillin (86.5%); Oxytetracycline (80.7%); Tetracycline (78.9%); Oxolinic acid (76.1%); Amoxicillin (74.3%); Amoxicillin/clavulanic acid (60%); Enrofloxacin (57.8%); Neomycin (54.9%); Furazolidone (53.2%); Sulfamethoxazole/trimethoprim (48.4%); Spectinomycin (47.8%); Flumequine (42.7%); Gentamicin (41.2%); Chloramphenicol (34.7%); Florfenicol (21.9%)	-

(Dhanapala et al., 2021)	<i>Aeromonas</i> spp., <i>A. veronii</i> (n = 122), <i>A. hydrophilia</i> (n = 15), <i>A. caviae</i> (n = 8), <i>A. jandaei</i> (n = 7), <i>A. dhakensis</i> (n = 6), <i>A. sobria</i> (n = 1), <i>A. media</i> (n = 1), and <i>A. popoffii</i> (n = 1)	Sri Lanka, commercial aquaria and breeding farms	Water samples, skin and mucous samples from various healthy ornamental fishes, kidney samples from various moribund ornamental fishes	Amoxicillin (92.5%); Enrofloxacin (67.1%); Nalidixic acid (63.4%); Erythromycin (26.1%); Tetracycline (23.6%); Imipenem (18%); Gentamicin, Trimethoprim/sulfamethoxazole (16.8%); Nitrofurantoin (8.1%); Doxycycline (5%); Chloramphenicol (3.7%); Rifampicin (2.5%); Ceftazidime (1.2%)	0.08-0.86, -
(Preena et al., 2021)	Various spp. from Enterobacteriaceae group (n = 84)	India, ornamental fish farms	Moribund goldfish <i>C. auratus</i> , gills, liver, kidney, and spleen	Ampicillin, Vancomycin (100%); Amoxicillin, Bacitracin (84.6%); Cephalothin, Oxytetracycline (76.9%); Cefazolin, Azithromycin, Erythromycin, Nitrofurantoin, Rifampicin (69.2%); Aztreonam, Nalidixic acid, Colistin (61.5%); Sulphadiazine (53.8%); Cefalexin, Cefuroxime, Cefoperazone, Gentamycin, Streptomycin, Polymyxin-B (46.1%); Amikacin, Amoxyclav, Piperacillin, Piperacillin/tazobactam, Cefixime/clavulanic acid, Cefotaxime, Cefotaxime/clavulanic acid, Cefepime, Carbapenem, Tobramycin, Furazolidone (38.4%); Cefoxitin (30.7%); Meropenem, Levofloxacin, Norfloxacin, Co-trimoxazole, Chloramphenicol (23%); Doripenem, Imipenem, Enrofloxacin, Trimethoprim (15.3%); Ceftriaxone (7%); Ciprofloxacin, Perfloxacin (0%)	0.21-0.85, 0.45
(Guz and Puk, 2022)	Rapidly growing <i>Mycobacterium</i> spp. (n = 50)	Poland	Diseased ornamental fishes	Rifampicin, Isoniazid (100%); Doxycycline (78%); Ciprofloxacin (44%); Sulfamethoxazole, Tobramycin (40%); Clarithromycin (16%); Amikacin (6%); Kanamycin (2%);	-
	Slowly growing <i>Mycobacterium</i> spp. (n = 49)			Isoniazid (97.96%); Rifampicin (93.88%); Ciprofloxacin (16.33%); Tobramycin (10.2%); Doxycycline (6.12%); Clarithromycin (2.04%); Amikacin, Kanamycin, Sulfamethoxazole (0%)	-

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(Au-Yeung et al., 2022)	<i>Aeromonas</i> spp. (n = 22-38) Data also presented on <i>Pseudomonas</i> spp. however resistance prevalence only assessed for <i>Aeromonas</i> spp.	Hong Kong, ornamental fish shops	Carriage water containing either zebrafish ( <i>D. rerio</i> ), southern platys ( <i>Xiphophorus maculatus</i> ), or koi ( <i>Cyprinus rubrofuscus</i> )	Tetracycline (97.4%); Oxalinic acid (95%); Oxytetracycline (94.9%); Enrofloxacin (70%); Doxycycline (55%)	-
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**Table 3.** Systematic search of common “over the counter” products for treating bacterial, fungal, and parasitic diseases in ornamental fishes. The list of products was taken from ‘BSAVA (2020) Small Animal Formulary Part B: Exotic Pets Appendix III; Proprietary fish medicine vendors’ with additional products added if identified, although will not be exhaustive on a global scale. Products were included if they were applicable to treating pet fishes destined for freshwater aquaria. Searches were performed in Web of Science (WoS) and Scopus databases in October 2022, October 2023 and June 2024. Where products had generic names which generated excessive hits the brand name was specified prior to the product using the Boolean operators “”.

Brand	Product	Active ingredients	Target pathogen	Search term	Hits WoS	Hits Scopus	Unique Hits	# Relevant	Notes on Relevance
API (USA)	Melafix	Cajeput oil	Bacteria	Melafix	3	3	3	2	Related to product
	Pimafix	West Indian Bay oil	Fungi	Pimafix	2	2	2	1	Related to product
	White Spot Cure	Malachite green, Polyvinylpyrrolidone	Protozoa	"API" White Spot Cure	0	0	0	0	
Aquarium Münster (Germany)	Argumor	Diflubenzuron	Flukes and crustaceans	Argumor	0	0	0	0	
	Dactymor forte	Cyromazine	Flukes	Dactymor forte	0	0	0	0	
	Dessamor	Copper sulphate, Ethacridine lactate, Methylthioninium chloride, Acriflavine	Fungi	Dessamor	0	0	0	0	
	Ektomor	Sodium perborate, Magnesium peroxide	Fungi, bacteria, and protozoa	Ektomor	0	0	0	0	
	Faunamor	Methylthioninium chloride, Malachite green, Methylrosaniline, Acriflavine	Protozoa	Faunamor	0	0	0	0	
	Medimor	Ethacridine lactate, Methylthioninium chloride, Methyl orange, Acriflavine	Fungi, bacteria, and protozoa	Medimor	0	0	0	0	
	Odimor	Copper sulphate, Malachite green	Fungi, bacteria, and protozoa	Odimor	0	0	0	0	
	Protomor	Malachite green, Ethacridine lactate, Methylthioninium chloride, Gentian violet	Protozoa	Protomor	0	1	1	0	Unrelated to product
Virumor	Potassium peroxymonosulphate	Fungi and bacteria	Virumor	0	0	0	0		

<b>Blue planet (Australia)</b>	Aquari cycline	Tetracycline hydrochloride	Bacteria	Aquari cycline	0	0	0	0	
	Fluke & tapeworm tablet	Praziquantel	Flukes and cestodes	Fluke and tapeworm tablet	1	1	1	0	Unrelated to product
	Multi cure	Malachite green, Methylene blue, Acriflavine	Protozoa and Fungi	"Blue planet" Multi cure	0	0	0	0	
	Para cide	Trichlorfon	Crustaceans and Flukes	"Blue Planet" Para cide	0	0	0	0	
	Tri sulfa	Sulfadizine, Sulfadimidine, Sulfamerazine	Bacteria, Fungi, and protozoa	Tri sulfa	9	9	14	0	Unrelated to product
<b>eSHa Labs (Netherlands)</b>	eSHA 2000	Ethacridine lactate, Copper sulphate, Proflavine hemisulphate	Fungi, bacteria, and protozoa	eSHA 2000	10	0	10	0	Unrelated to product
	eSHA alx	Lufenuron	Crustaceans	eSHA alx	0	0	0	0	
	eSHA EXIT	Ethacridine lactate, Malachite green, Methylene blue	Protozoa	eSHA EXIT	1	0	1	0	Unrelated to product
	eSHA gdex	Praziquantel	Flukes and cestodes	eSHA gdex	0	0	0	0	
	eSHA Hexamita	Copper sulphate, Ethacridine lactate, Acriflavine, Methylene blue	Fungus, bacteria and protozoa	eSHA Hexamita	0	0	0	0	
	eSHA-ndx	Levamisole hydrochloride, Sodium metabisulphite, Methyl parahydroxybenzoate	Nematodes	eSHA-ndx	0	0	0	0	
<b>Interpet (UK)</b>	Anti Crustacean Parasite Plus	Sodium chlorite	Crustaceans	Anti Crustacean Parasite Plus	0	0	0	0	
	Anti Fungus & Finrot Plus	2-phenoxyethanol	Fungi and bacteria	Anti fungus and Finrot Plus	0	0	0	0	
	Anti Internal Bacteria Plus	Formaldehyde, Bronopol, Benzalkonium chloride	Bacteria	"Interpet" Anti Internal Bacteria	0	0	0	0	
	Anti Parasite, Slime & Velvet Plus	Formaldehyde, Benzalkonium chloride, Copper EDTA, Quinine bisulphate	Protozoa and Flukes	Anti Parasite, Slime & Velvet Plus	0	0	0	0	



	Anti White Spot Plus	Formaldehyde, Malachite green	Protozoa	"Interpet" Anti White Spot Plus	0	0	0	0	
	Swimbladder Treatment Plus	Formaldehyde, Bronopol, Benzalkonium chloride	Bacteria	Swimbladder Treatment Plus	0	0	0	0	
<b>JBL (Germany)</b>	Aradol Plus	Diflubenzuron	Crustaceans	Aradol Plus	0	0	0	0	
	Ektol bac Plus	Benzalkonium chloride, Methylene blue, Polyvinylpyrrolidone iodine	Bacteria	Ektol bac Plus	0	0	0	0	
	Ektol fluid Plus	Methylene blue, Benzalkonium chloride	Bacteria	Ektol fluid Plus	0	0	0	0	
	Fungol Plus	Malachite green oxalate, Ethacridine lactate, Povidone iodine	Fungi	Fungol Plus	0	0	0	0	
	Furanol Plus	Nifurpirinol	Bacteria	Furanol Plus	1	1	2	0	Unrelated to product
	Gyrodol Plus	Praziquantel	Flukes and cestodes	Gyrodol Plus	0	0	0	0	
	Nedol Plus	Benzimidazole	Worms	Neodol Plus	1	1	1	0	Unrelated to product
	Oodinol Plus	Copper sulphate, Methylthionimium chloride	Protozoa	Oodinol Plus	0	0	0	0	
	Punktol Plus	Malachite green oxalate, Methylthionimium chloride	Protozoa	Punktol Plus	0	0	0	0	
	Spirohexol Plus	2-amino-5-nitrothiazole	Protozoa	Spirohexol Plus	0	0	0	0	
<b>King British (UK)</b>	Bacteria control	Formaldehyde, Magnesium sulphate, Sodium chloride, Allantoin	Bacteria	"King British" Bacterial Control	0	0	0	0	
	Disease Clear	Silver proteinate	Fungi and bacteria	"King British" Disease Clear	0	0	0	0	
	Fin Rot & Fungus Control	2-phenoxyethanol	Fungi and bacteria	"King British" Fin rot and fungus control	0	0	0	0	

	Original Formula WS3	Malachite green, Acriflavin, Quinine sulphate	Protozoa	Original Formula WS3	1	1	1	0	Unrelated to product
	White Spot Control	Malachite green, Acriflavin, Quinine sulphate	Protozoa	"King British" White Spot Control	0	0	0	0	
<b>NT Labs (UK)</b>	Aquarium Anti White-spot & Fungus	Formaldehyde, Malachite green	Fungi and protozoa	Aquarium Anti White-spot and Fungus	0	0	0	0	
	Aquarium Anti-Fluke & Wormer	Flubendazole	Flukes and worms	Aquarium Anti-Fluke and Wormer	0	0	0	0	
	Aquarium Anti-Internal Bacteria	Chloramine T	Bacteria	Aquarium Anti-Internal Bacteria	0	0	0	0	
	Aquarium Anti-Parasite	Copper sulphate, Formaldehyde	Protozoa	Aquarium Anti-Parasite	1	1	1	0	Unrelated to product
	Aquarium Anti-Ulcer & Finrot	Acriflavin, Aminoacridine hydrochloride, Formaldehyde	Bacteria	Aquarium Anti-Ulcer and Finrot	0	0	0	0	
	Aquarium Disease Solve	Acriflavine, Methylene blue	Pathogens	Aquarium Disease Solve	3	1	3	0	Unrelated to product
	Aquarium Swimbladder Treatment	Acriflavine, Aminoacridine hydrochloride	Bacteria	Aquarium Swimbladder Treatment	4	1	4	0	Unrelated to product
<b>Oase (Germany)</b>	AntiArgulus	Diflubenzuron	Crustaceans	AntiArgulus	0	0	0	0	
	AntiFungus	Ethic lactate mono, Methyl chloride, Acrid monocular	Fungi	"Oase" AntiFungus	0	0	0	0	
	AntiParasite	Methythionium chloride, Malachite green oxalate, Methylrosabilinium chloride, Acriflavine monochloride	Protozoa	"Oase" AntiParasite	0	0	0	0	
<b>Seachem (USA)</b>	Cupramine	Copper sulphate	Protozoa	Cupramine	3	9	11	1	*Only relevant result is for marine ornamentals
	Focus	Nitrofurantoin	Fungi and bacteria	"Seachem" Focus	0	0	0	0	

	Kanaplex	Kanamycin sulphate	Fungi and bacteria	Kanaplex	0	0	0	0	
	Metroplex	Metronidazole	Bacteria and protozoa	"Seachem" Metroplex	0	0	0	0	
	Neoplex	Neomycin sulphate	Bacteria	Neoplex	5	18	19	0	Unrelated to product
	Paraguard	Aldehyde, Malachite green, Polymers	Fungi, bacteria and protozoa	Paraguard	4	13	13	0	Unrelated to product
	Polyguard	Sulfathiazole, Malachite green, Nitrofurantoin, Nitrofurural, Quinacrine dihydrochloride	Fungi, bacteria and protozoa	Polyguard	10	19	29	0	Unrelated to product
	Sulfaplex	Sulfathiazole	Fungi, bacteria and protozoa	Sulfaplex	0	0	0	0	
<b>Sera (Germany)</b>	Argulol	Denatonium benzoate, Emamectin benzoate	Crustaceans	Argulol	0	0	0	0	
	Bakto Tabs	Nifurpirinol	Bacteria	Bakto Tabs	0	0	0	0	
	baktopur	Acriflavine, Methylene blue, Phenyl glycol	Bacteria	baktopur	0	0	0	0	
	costapurF	Malachite green, Formaldehyde	Fungi and protozoa	costapurF	0	0	0	0	
	ectopur	Sodium perborate, Sodium borate, Sodium chloride	Fungi, bacteria and protozoa	ectopur	0	0	0	0	
	Flagellol	Nitro thiazolylazane, Ascorbic acid, Menadione sodium bisulphite	Protozoa	Flagellol	0	0	0	0	
	mycopur	Acriflavine chloride, Copper sulphate/chloride	Fungi and Flukes	mycopur	0	0	0	0	
	Nematol	Emamectin benzoate	Nematodes	"Sera" Nematol	17	0	17	0	Unrelated to product
	Phyto med Baktazid	Thyme oil	Bacteria	Phyto med Baktazid	0	0	0	0	
	Phyto med Catappa	Catappa extract	Fungi, bacteria and external parasites	Phyto med Catappa	1	0	1	0	Unrelated to product
	Phyto med Mycozid	Thyme oil	Fungi	Phyto med Mycozid	0	0	0	0	
	Phyto med Protazid	Quinine hydrochloride	Protozoa	Phyto med Protozid	0	0	0	0	
	Phyto med Tremazid	Peppermint oil	Flukes	Phyto med Tremazid	0	0	0	0	

	Protazol	Phenyl methylum hydroxide	Fungi and protozoa	Protazol	0	0	0	0	
	Tremazol	Praziquantel	Flukes and cestodes	Tremazol	0	0	0	0	
<b>Tetra (UK)</b>	ContraIck Plus	Methylthioninium chloride, Malachite green oxalate, Methylrosanilinium chloride, Acriflavine chloride	Protozoa	ContraIck Plus	0	0	0	0	
	Fungistop Plus	Ethacridine lactate, Methylthionium chloride, Acriflavine chloride		Fungistop Plus	0	0	0	0	
	Fungus Guard Tablets	Nitrofurazone, Potassium dichromate	Fungi	Fungus Guard Tablets	0	0	0	0	
	GeneralTonic Plus	Ethacridine lactate, Acriflavine, Methylene blue, 9-Aminoacridine hydrochloride	Bacteria and protozoa	GeneralTonic Plus	0	0	0	0	
	Goldmed	Formaldehyde, Malachite green oxalate	Fungi, bacteria, and protozoa	Goldmed	2	2	3	0	Unrelated to product
	Ick Guard Tablets	Victoria green, Acriflavine	Protozoa	Ick Guard Tablets	0	0	0	0	
	Lifeguard Tablets	1-chloro-2,2,5,5-tetramethyl-4-imidazolidinone	Fungi, bacteria, and protozoa	Lifeguard Tablets	2	2	2	0	Unrelated to product
<b>VetArk (UK)</b>	Chlormaine T	Sodium N-chloro-para-toluenesulfonamide	Bacteria, protozoa, and flukes	Chloramine T	1	0	1	0	Unrelated to product
	Fluke-Solve	Praziquantel	Flukes	Fluke-Solve	0	0	0	0	
	Fluke-Solve Plus	Emamectin benzoate, Nitroscanate, Praziquantel	Flukes	Fluke-Solve Plus	0	0	0	0	
	Lice-Solve	Emamectin benzoate	Crustaceans	Lice-Solve	1	1	1	1	Related to product
<b>Waterlife (UK)</b>	Medizin	Formaldehyde, Malachite green, Methylene blue, Alkyl-dimethyl-benzyl-ammonium chloride	Fungi and protozoa	"Waterlife" Medizin	0	0	0	0	
	Myxazin	Benzalkonium chloride, Formaldehyde, Malachite green, Acriflavine	Bacteria	Myxazin	0	0	0	0	
	Nova+	Tea tree extract	Fungi, bacteria and protozoa	"Waterlife" Nova+	0	0	0	0	
	Octozin	Dimetridazole	Protozoa	Octozin	0	0	0	0	

Protozin	Malachite green, Formaldehyde, Copper sulphate	Fungi and protozoa	Protozin	1	2	2	0	Unrelated to product
Sterazin	Formaldehyde, Malachite green	Flukes	Sterazin	0	0	0	0	