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Review article

Risks of pharmaceutical chemicals used in aquaculture: alternatives and current perspective

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ABSTRACT

Aquaculture is the fastest worldwide growing activity with respect to food production. However, the emergence of infection diseases is the principal risk factor in this industry. To control them, it has been used diverse veterinary drugs, antibiotics, solutions, colorants, which have toxic effects for associated biota, with ecological level impact, bacterial resistance promoters, and water bodies' contamination. The present review show some negative aspects of their use and some alternatives for their substitution like: the use of probiotics implementation, terrestrial plants extraction and aquatic microalgae (marine and freshwater) with therapeutic properties, biodegradable and also molecular biotechnology implementation, genomic and proteomic terrestrial for development of resistant strains diseases and make the aquaculture a sustainable activity.

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1. Introduction

Aquaculture is the fastest worldwide growing activity and plays an important role on food assurance (Wang et al., 2007). However, in this sector it expands in different directions, the importance of aquaculture health increase and is important to find new strategies to avoid diseases introduction and dispersion of infection diseases (Caceres and Vazquez, 2013). So that aquaculture has become a key component for animal health industry and this has caused a great demand for biological and pharmaceutical products, to eliminated bacterial and viral infections. In aquaculture historically it has been used different biochemical products like formalin, copper sulphate, malachite green and especially antibiotics such as ox tetracycline, chloramphenicol and kanamycin (Alderman and Michel, 1992). All these chemical compounds were used indiscriminately, without testing, to determined time and dose of administration to cultured species. Also, they have not carried out appropriate analysis to established etiological agent causing infectious process and right antibiotic supply (Millanao et al., 2012). All this has generated a serious pollution problem not only in water bodies surrounding aquaculture production systems, but also to terrestrial environment because many wastes are released to environment without any treatment, so chemicals compounds that were used can reach fresh and marine water and terrestrial habitat which impact on biodiversity. Otherwise, it can pass through trophic chains into final consumer that can produce a public health problem caused by bioaccumulation process (Anónimo, 2010). Because of this, the strategies of disease control in aquaculture should focused on prevention through good management practices rather than use of chemicals and antibiotics to control them (Subasinghe et al., 2009; Millanao et al., 2011). This review aims to give an overview of risks caused by chemical drugs used for disease control in aquaculture and development strategies to reduce their use in this industry.

2. Aquaculture diseases and chemical pharmaceutical applications

At worldwide regions where aquaculture activity was made, the presence of diseases were inherent, for what they are conditioning factor for productive sector and severe impact in social and economic in many countries (FAO, 2016). Such diseases are not nature infection, caused by environmental pollution conditions surrounding these production systems like: pesticides, heavy metals, detergent, among others and the infections caused by bacteria, virus, fungus and parasites which caused disease when variation of aquatic conditions was present in surrounding culture fishes like temperature, oxygen, pH, salinity, water hardness, ammonium toxic compounds, carbon dioxide, among others; causing stress and decrease of immune response in aquatic organisms who made it sensitive to infection process (Jiménez, 2006). On other hand, it is important to stand out that most aquaculture diseases due to poor implementation of practices like: general cleaning facilities, production beakers or ponds, as well as aquatic organisms transportation between regions, states or countries the mobilization of a large number of organisms across regions, states or countries without complying quarantines periods and consequently spread of pathogens (Negrete et al., 2004).

Therefore, associated with aquaculture production, a chemical and veterinarian drugs were development to prevent and counteract infectious outbreaks. However, this has allowed the indiscriminate use of different solutions, chemicals, industrial dyes like: malachite green, crystal violet, methylene blue, formalin, hydrogen peroxide and iodophor solution, which are low cost and easy acquisition (Zhang et al., 2012; Chambel et al., 2014). To this problem, in recent years were made several assessments to establish the use of chemical and antibiotic effects in aquaculture, since aquatic organisms can accumulate in their tissues these compounds and violate the food security breach (Meador, 2006; Tacon and Barg, 2001). Likewise water production systems discharges may contain compounds impact negatively water bodies, soil and biodiversity habitat surrounding producing farms, creating environmental and public health problems (Meador, 2006; Cabello, 2006). Then, principal drugs used in treatments of several biological infections were described considering their aquatic activity importance.

3. Drugs obtained from triphenylmethane

3.1. Green malachite (GM) and crystal violet (CV)

Both dyes are obtained from triphenylmethane and were used like biocides and fungicides for decades in aquaculture. Particularly, GM (4-[(4-dimethylaminophenyl)-phenyl-methyl]-N, N-dimethyl-aniline) is a basic dye

used since 1933 in aquatic industry for treatments of protozoan and fungus which affect fishes and crustaceans (Bergwerff and Scherpenisse, 2003; Srivastava et al., 2004; Sudova et al., 2007; Gopinathan et al., 2015). Even when there are reports that shown hurtful effects like mentioned in Srivastava et al., (2004), which prove that GM increase their toxicity with respect exposition time, temperature and concentration; likewise reported variations in hematologic parameters in different organisms. Other studies like Lee et al. (2006), shown that GM is a potential teratogenic and carcinogenic compound. Culp et al. (1999) reported that GM is a mutagen which produce chromosomic fractures and reduce fertility in salmon rainbow trout. Srivastav and Dayalanand (2015), showed that GM produce respiratory toxicity and cellular stress with affectations in processes of cellular division and multiplication.

Atmid-eighties, efforts were redoubled to eradicate use of both dyes compounds, since there is great variety of literature that has confirmed risk and harmful effects of its application. Moreover, studies suggest that after application of this dyes, it is necessary to maintain long periods without their application in water and organisms because of the production of wastes and persistent residues (GESAMP, 1997). Likewise, it was documented the GM impact on soil biota like fungus: *Penicillium roqueforti*; Gram positive and Gram negative bacteria included like: *Bacillus subtilis*, *Azotobacter chroococcum*; yeast like: *Saccharomyces cerevisiae*; earth worms (*Eisenia foétida*) and cultured plant seed germination from different families, because it produced cytotoxicity, genotoxicity, and also the detection of DNA polymorphism and fragmentation (Gopinathan et al., 2015). It also produced affectations on water bodies' biota since wastes affect planktonic crustacean larvae like cladocerans by GM toxicology and sensibility. Also it exist *P. stylirostris* nauplii reports which mentioned that when exposed to GM in 80 µg L⁻¹ concentration inhibit metamorphosis process (Buschmann, 2001; Lannacone and Alvaríño, 2007).

For these reasons, the use of GM was restricted and forbidden for Food and Drug Administration FDA in United States of America and countries like Canada, European Union, Denmark, China, Chile, Japan, Thailand and among others southeast Asiatic countries (Zhang et al., 2012; Eissa et al., 2013). In Mexico, it does not exist restriction or normativity about GM use in aquaculture. It can be found and sell in any aquarist or veterinary store without any additional information about used risks in bacterial treatment of fish infections the risks to use it, as well as their low cost (SAGARPA-ASTILAPIA-CONAPESCA, 2009). However, in countries with better GM regulations, was illegal used causing negatively effects in environmental and human public health (Zhang et al., 2012).

On other hand, CV dye, whose formula was 4-[bis (4-dimethylaminophenyl)methylene cyclohexa-2,5-dien-1-ylidene-dimethylazanium chloride) was used in human and veterinary medicine as necessary input in Gram staining and textile dye, besides being used like therapeutic in aquaculture sector. CV was classified as recalcitrant dye and it is known to maintain in environment for long period time (Parshetti et al., 2011) and toxic for terrestrial and aquatic biota (Au et al., 1978). CV *in vitro* studies concluded that it is a mitotic poisoned agent, also it was considered like biological risk substance being responsible to promote tumor growing and powerful carcinogen in some fish species (Au et al., 1978; Fan et al., 2009). Although GM and CV substances were forbidden in United States, European Union, China and in others countries, it was being applied routinely affecting quality and safety of diverse aquatic origin products, generating export and detection problems of products for being risky for human health. For example, the database Rapid Alert System for Food and Feed from European Union reported in 2003-2007, 131 total cases products derived from catfish, trout, tilapia, salmon, shrimp and caviar (Veterinary Residues Committee, 2009).

4. Drugs obtained from thionine

4.1. Methylene blue (MB)

The MB also named methylthionium chloride is a cationic dye whose molecular formula is C₁₆H₁₈N₃SCl₃H₂O. This compound was used at paper dye industry, leather, cotton, pasta, wool, among others (Gupta et al., 2011). MB was used in aquaculture to prevent and control saprolegniasis, red mouth disease, ichthyophthiriasis among other fish diseases (Xu et al., 2012). However, skin contact through water caused adverse effects in human health like skin discoloration, reddening or dryness. Eyes exposition to MB cause irritation, tearing and burning sensation in eyelids. MB ingestion caused gastrointestinal tract irritation, along with headache, dizziness, nausea and vomiting. Also has been recorded that inhaled can lead to convulsions, cyanosis, confusion and sweating. Another consequences must be heart rate increase, shock, jaundice, quadriplegia,

methemoglobinemia and necrosis tissue causing permanent damage to humans and animals (Hameed and Ahmad, 2009; Muthuraman and Teng, 2009; Razmara et al., 2011; Manoj et al., 2016).

The inadequate use of MB represent a danger to aquatic biodiversity because at high concentrations in water bodies, it decrease light penetration, retard photosynthetic activity, inhibited the biota grow, can be toxic to invertebrates like *Daphnia magna* and *Artemia franciscana* (Lannacone and Alvaríño, 2007; Cortés and Bartolome, 2012), affect the trophic web in water bodies and a tendency to chelate metal ions which produce micotoxose for fishes and other organisms (Mahony et al., 2002; Garg et al., 2004). Before discharge waters with MB content must be treated adequate to avoid serious environmental damages (Xinge et al., 2015). That is why MB was forbidden for their use in aquaculture in many countries like United States and Japan, but it requires a better worldwide clear regulation (Li et al., 2016).

5. Other solutions

The copper sulphate (CS) whose molecular formula is CuSO_4 , was used since 1904 to decrease proliferation of phytoplankton concentration in water bodies (Haughey et al., 2000; Mazon et al., 2002). In aquaculture CS was used for ectoparasites, bacteria, fungi and harmful microalgae control (Mazon et al., 2002; Straus et al., 2003; Reddy et al., 2006). Although CS use is not approved by FDA like therapeutic agent, it was used in massive way for aquaculture activity (Straus et al., 2003). In water bodies, the increase of this heavy metal concentration was causing toxic problems in several species (Ferreira et al., 2014).

The inappropriate use of CS must cause damage like other heavy metals because is toxic in high concentrations (Reddy et al., 2006) and although organisms requires an specific quantity of metal ions to make efficiently their physiological process, excess must cause stress in aquatic organisms manifesting in a sub lethal or dead form (Scelzo, 1997). The sub lethal dose of CS involve organism problems like alterations or increase in respiratory rates (Handy, 2003; Yeh et al., 2004), since cooper ions interrupted the gills osmoregulation process (Massaut, 1999). In some species showed behavior problems (Burba, 1999), metamorphosis inhibition molt, malformations (Scelzo, 1997) and grow decrease (Méndez and Green, 2005). However, it was documented that copper accumulation and toxic level depends from each specie and water quality (Perkins et al., 1997), since that therapeutic effectivity of CS was decrease with total water hardness and alkalinity increase. The copper toxicity increase when water pH decrease (Straus, 2003; Çogun y Kargin, 2004).

Discharging wastewaters from aquaculture who contain copper affects surrounding vegetation with cytotoxic responses like structural and functional membrane disturbances, cellular cycle perturbations, genotoxicity, grow and photosynthesis inhibition, and chlorophyll content decrease (He et al., 2005) and in mankind, the copper consume (10-20 g copper salt) can produce acute toxicity, blood, leaver, kidneys and brain diseases. The acryflavine was used like disinfectant solution since 1913 and in aquaculture was recommended for bacterial and fungi diseases treatment. Also was used to disinfect the fish roe to obtain viable larvae with lowest potential pathogen microorganisms' load and increase their hatching percentage (Rodríguez et al., 2011). However, it was reported that their use as egg disinfectant has an effect in *Danio rerio* fish embryo development, with mortality rate and not egg viability of 36%, with respect 10% observed with disinfectants made with E3 medium and salinated water (IMTA-SEMARNAT, 2011). Table 1 show obtained results from mentioned compounds and mean lethal doses (LD_{50}) obtained from different experimental organisms.

Table 1
Mean lethal doses (LD_{50}), of some therapeutics used in aquaculture.

Therapeutic	Specie	LD_{50} (mgL^{-1})	Exposition time (hour)	Author
Methylene blue	<i>Daphnia magna</i>	2.37	24	Lannacone and Alvaríño, 2007
		1.20	48	
		0.54	72	
		0.38	96	
		1.16	24	
	<i>Artemia franciscana</i>			Cortés and Bartolome, 2012

Green malachite	Daphnia magna	9.27	24	Lannacone and Alvaríño, 2007	
		4.65	48		
		3.23	72		
		2.97	96		
	Wistar rats (170-200g)	275 mgkg ⁻¹	24	24 Perineal application	Clemmensen et al., 1984
	Wistar rats (170-200g)	>2000 mgkg ⁻¹	24	24 Dermic exposition	Clemmensen et al., 1984
	Mices NMRI	50 mgkg ⁻¹	24		Clemmensen et al., 1984
Heteropneustes fossilis	0.24	95		Srivastav y Dayalanand, 2015	
Oncorhynchus mykiss		1.40	3	Bills et al., 1977	
		2.35	3		
		6.80	6		
Chloridate acriflavine	Artemia franciscana	0.2217	24	Cortés and Bartolome, 2012	
Cooper sulphate	Phallocerus caudimaculatus	0.05		Ferreira et al., 2014	
	Hyphessobrycon eques	0.16	96		
	Brachydanio rerio	0.13			
	Piaractus mesopotamicus	10.36			
	Cryphiops caementarius		3.875	24	Mendoza, 2009
			3.399	48	
			2.55	72	
			2.054	96	
			0.94 ppm	48	
	Piaractus brachypomus			Velasco et al., 2006	
	Echinogammarus olivii	0.25		Bat et al., 1999	
	Sphaeroma serratum	1.98	96		
	Palaemon elegans	2.52			
	Astacus astacus		192.2	24	Burba, 1999
		173.5	48		
		153.9	72		
		148.4	96		
		660.9 ppb	24		
Artemesia longinaris		378.5 ppb	48	Scelzo, 1997	
		212.3 ppb	75		
Homarus americanus	100	96	96	Mc Leese, 1974	

5.1. Antibiotics

The antibiotics are active biological molecules with an increasing use in human and veterinary health as well as in livestock, agricultural and aquaculture industry. In aquaculture, antibiotics were used during the production process to avoid (prophylactic use) and treat (therapeutic use) bacterial diseases (FAO/SEAFDEC/CIDA, 2000). But they were also exploited like growth promoters and it is recommended for fish handling. This practice has proved to be ineffective and generally, it has not been probed for Fish Inspection Services (FAO, 2002).

By common practice in production units, fish producers apply directly in culture water the antibiotics or incorporate them in the food source (Defoirdt et al., 2011), generating problems to human health, due to the presence of fish tissue and derivatives waste (Cabello, 2006). Similarly, there are reports that show some secondary effects occurred by the use and application of high quantities of antibiotics like allergies and toxicity problems in people who have direct contact with water and organisms (Grave et al., 1996; Lillehaug et al., 2003). However, the indiscriminate antibiotic use resulted in an increase of bacterial resistance. Epidemiological and molecular evidence

indicate that genes involved in this resistance can be transmitted from aquatic bacteria to bacteria capable of infecting humans and terrestrial animals (Cabello, 2004; Sallum and Chen, 2008). An example of these are R-plasmids which contains determinants for tetracycline resistance encoded in Tn1721 gene, which have been spread between *Aeromonas salmonicida*, *Aeromonas hydrophila*, *Aeromonas caviae* and *E. coli* in fish and humans obtained from different geographic locations in Europe (Rhodes et al., 2000). Similarly, enrofloxacin waste can lead bacterial resistance development, without forgetting that those classified antibiotics were potential carcinogenic and mutagenic potential as furazolidone (He et al., 2009; Hu et al., 2007). More than 10 different antibiotics are used in prophylactic way in some aquatic species, principal of economic and commercial importance like shrimps and salmon whose aquatic cultures were made in different water bodies like: rivers, lakes, oceans and adequate facilities (Defoirdt et al., 2011). However, the lack of good practices for handling and disposal of antibiotics results in environmental effects ranging from beneficial microbes removal who were attendant to aquatic systems balance to waste that can reach aquatic environment wastewater via when organic matter (feces, dead bodies and food) are used as agricultural land fertilizers (Bayd and Massaut, 1999; Lalumera et al., 2004; Woodward, 2006). An example of development of microorganism resistance is *Aeromonas hydrophila*; opportunistic pathogen, which is commonly isolated from infectious processes of aquatic organisms and has high resistance to various antibiotics as shown in Table 2. The highest challenge in aquaculture health was to find other strategies to control diseases.

Table 2.A
hydrophila antibiotic resistance.

Antibiotics	<i>Aeromonas hydrophila</i> (resistance percentage)
Ciprofloxacin	25
Nalidixic acid	25
Amikacin	50
Gentamicin	25
Streptomycin	100
Chloranphenicol	50
Ceftriaxone	25
Erythromycin	100
Carbenicillin	100
Ampicillin	100
Penicillin	100
Methicillin	100

For all above, the regulation use of antibiotics is primordial in local region and their specific variations between countries development. Since 1996, some countries like Norway (Beveridge, 1996), maintained a register about antibiotics use, other countries do not have a law for this. In Mexico it exists the rule: in which it contemplate the use of oxytetracycline, sarafloxacin, enrofloxacin and florphenicol for bacterial diseases control who affect cultured organisms, but it takes updating the international agreements generated from antibiotic use reported harmful effects and also fulfill with legal frameworks and established relevant sanctions, that is why it needs a lot of studies and work to do.

5.2. Alternatives development and implementation

As aquaculture sector is growing, alternatives are being developed to antibiotic and therapeutic substitution compounds, because excessive use caused bacterial resistance problems, environmental ecotoxicity and bioaccumulation. With respect dyes, the MG was replaced gradually with salt baths (sodium chloride) for fungi infection treatments and those caused by protozoa, except *Ichthyophthirius multifiliis* and chloramine used for protozoa infection diseases and gills flavobacteriosis in salmonids (Sudova et al., 2007). The biological control in other way, using competitive exclusion has taken great interest in infection process control, like using bacteriophages like therapeutic agents in aquaculture. As well as using purified phages preparations, as renewed therapeutic alternative, since they are capable to infect and kill bacterial host, even antibiotic resilient, showing

high specificity, auto replicable, reliability, and easily to select new phages (Nakai et al., 1999; Romero, 2015). However, the research advances are few.

Another alternative that has positive results was the use and implementation of probiotics in this prophylactic and prevention diseases process in aquaculture (Zizhong et al., 2009)(Table 3). The probiotics was defined like live microorganisms which supplied in adequate quantities have benefic effect in host. The first commercial probiotic used was registered in 1992 with a no pathogen strain of *Vibrio alginolyticus* which improved the shrimp yield crops of Ecuador and Mexico (Verschuere et al., 2000). The principal bacterial groups proved like probiotics in aquatic culture organisms were strains of genus *Vibrio*, *Pseudomonas*, *Bacilos*, yeasts and *Lactobacilos* which finality is the reinforcement of gastrointestinal barrier, better nutritional assimilation, immunity stimulation and growth promoters (Monroy et al., 2012). However, the probiotics aquaculture research is at first early development phases, since it is necessary to characterize fish, mollusk and crustacean microbiota, in order to select specific strains and obtained highest benefits that already obtained.

Table 3

Probiotic studies which show diseases control.

Benefit	Aquatic specie	Author
Lactococcus garvieae and Streptococcus iniae control	Oncorhynchus mykiss	Brunt and Austin, 2005
Vibrio inhibition	Penaeus sp., Oncorhynchus mykiss	Moriarty, 1998; Sakai et al., 1995; Parada et al., 2007
Vibrio control	Penaeus monodon, Paralichthys olivaceus, Oncorhynchus mykiss.	Dalmin et al., 2001; Taoka et al., 2006; Reyes-Becerril et al., 2008;
Forunculosis caused by Aeromonas salmonicida control	Oncorhynchus mykiss	Nikoskelainen et al., 2001
Three strains of genus Bacillus sp. to Aeromonas hydrophila control	Pterophyllum scalare	Monroy et al., 2010

The Worldwide Health Organization expressed their position to renew the use of herbology and another alternatives to be used in health sector with therapeutic purposes, specifically to aquaculture apply (Morales et al., 2013). The herbology, is an alternative using extracts and essences of medical plants, like nature or innocuous compound (Kirubakaran et al., 2010). Because phytochemicals can be chemical therapeutic molecules, their interest use was increased because their low cost and easily handling (Sivaram et al., 2004; Van Hai, 2015).

At this point of view, nature antivirals were poor studied and only patent who described therapeutic agent from natural origin was U.S. Pat. N°6.440.466 which provide a prophylactic or therapeutic composition with effective plant extracts of *Lantena camera*, *Aegle marmelos*, *Ocimum sanctum*, *Mimosa púdica*, *Cynodon dactylon*, *Curcuma longa* and *Allium sativum*, optionally combined with diluents or excipients pharmacological accepted (Modak, 2011). Although, medicinal plants and herbs, are widely known, documented and used in human health and as potential biocides, are few therapy studies for aquaculture species, allowing opening in phytochemistry area field research.

At same context, the marine algae also have therapeutic value than can be the clue to fight diseases from different origins, since obtained marine macroalgae have a wide spectrum at biological systems activity. Some sulphate polysaccharides, synthetized from red algae species, possess antiviral activity, the halogen terpenoids has antiparasitic effect and fucoidan like stimulants of immunologic system and anti-inflammatory effect, among others (Ondanza and Rincones, 2008) (Table 4). Nevertheless, still few or null information of their supply in aquaculture with therapeutic purposes.

Table 4

Benefits used plants and algae to control aquaculture diseases.

Plant	Studied specie	Benefits	Author
Psidium guajava	Trout	Necrosis hematopoietic infectious control	Wei y Chen, 2000
Ocimum sanctum	Shrimp	Yellowhead disease control	Direksaburokom et al.,1996
Allium sativum	Trout	Control of Saprolegnia	Alcántara, 1990
Helenium quadridentatum	Trout	Saprolegniasis	Marqués, 1990
Calophyllum antillanum	Shrimp	Yellowhead control disease	Silveira, 2000
Allium sativum	Freshwater fishes	Aeromonas, Pseudomonas and Vibrio control	Silveira et al., 2002
Protium copal	Freshwater fishes	Gram negative control	Silveira, 2000
Odogonium capilare	Carassius auratus	Vibrio fluvialiscontrol	Negrete, 2010
Ulva faciata	Shrimp	V. fischeri, V. alginolyticus, V. harveyi and Aeromonas sp. control	Selvin et al., 2012
Azadirachta indica, Ocimum sanctum, Curcuma longa	Carassius auratus	Aeromonas hydrophilacontrol	Harikrishnan et al., 2009
Azadirachta indica, Lippia verlanderi	Litopenaeus vannamei	Vibrio parahaemolyticuscontrol	Morales et al., 2013

Through molecular biotechnology, genomic and proteomic it is possible to impact genetic modification and improvement to develop resistant diseases strains, identification and control of pathogens and vaccines for infectious process treatments affecting or impact to aquaculture (Melamed et al., 2002). However, some researchers have implemented techniques like bioremediation with purpose to remedy environmental damage by therapeutic or antibiotics compounds used autochthonous organisms from contaminated site or from other sites (exogenous) as aquatic plants like *Spirodela polyrrhiza* (L.) (Waranusantigui et al., 2003) and *Posidonia oceanica*(L.) (Ncibi et al., 2007), *Salvinia minima* (Sanchez and Ramirez, in press) as removal agents MB and *Pistia stratiotes* (L.); *Myriophyllum aquaticum* (L.) heavy metals removals such copper (Bustamante and Gonzalez, 2014). As well as bacterial use like *Agrobacterium radiobacter* (Parshetti et al., 2011), *Pseudomonas aeruginosa* (El-Naggar et al., 2004) biodegrading capable and minimizing dye toxicity such as CV.

5.3. Conclusions and perspectives

The present review has shown the relevance of generating new research lines focused on control and detection of waste, strengthening legal frameworks for regulation in aquaculture and promoting convergence between government policies with progress and achievements researchers to risks and impact compounds to animals, human and environmental health.

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