8(4): 324-330 (2014) DOI: 10.3153/jfscom.201439

Journal of FisheriesSciences.com

E-ISSN 1307-234X

ORIGINAL ARTICLE/ORİJİNAL ÇALIŞMA

FULL PAPER TAM MAKALE

AEROMONAS HYDROPHILA SENSITIVITY TO DISINFECTANTS

Deyan Stratev*, Ivan Vashin

Department of Hygiene and Control of Food Products from Animal Origin, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, Bulgaria

Received: 07.01.2014 / Accepted: 28.04.2014 / Published online: 04.09.2014

Abstract: The aim of the present study was to determine the sensitivity of *Aeromonas hydrophila* to disinfectants and to propose an efficient method for control of fish diseases caused by this pathogen The tested *A. hydrophila* strains were inactivated by the combinations of glutaraldehyde, formaldehyde and quaternary ammonium salts (HMI® Glicofin) and glutaraldehyde, (ethylenedioxy) dimethanol and benzalkonium chloride (Aldesept®). Iodine (Desinfekt B®), *n-alkyl-dimethyl-benzyl-ammonium chloride* (HMI® Roda Super), ethanol (40%, 50% and 70%) and potassium permanganate (1000 mg/l) were also found effective against *A. hydrophila*. At a concentration of 5 mg/L, potassium permanganate reduced *A. hydrophila* (ATCC 7965) counts by 3% ($\log_{10}$ 0.25), while the strain was completely inactivated by 100 mg/L. Under the influence of 5 mg/L and 100 mg/L potassium permanganate, the clinical *A. hydrophila* isolate counts were reduced by 0.7% ($\log_{10}$ 0.06), and 2.3% ($\log_{10}$ 0.19), respectively. Most tested disinfectants were effective against *A. hydrophila*, with the exception of potassium permanganate at 5 mg/L.

Keywords: *Aeromonas hydrophila*, Disinfectants, Sensitivity

* **Correspondence to:** **Deyan STRATEV**, Department of Hygiene and Control of Food Products from Animal Origin, Faculty of Veterinary Medicine, Trakia University, 6000 Stara Zagora, BULGARIA

E-mail: deyan.stratev@trakia-uni.bg

Journal abbreviation: **J FisheriesSciences.com**

Öz: ***Aeromonas hydrophila'*nın Dezenfektanlara Duyarlılığı**

Bu çalışmanın amacı *Aeromonas hydrophila*' nın dezenfektanlara karşı duyarlılığının belirlenmesi ve söz konusu patojenden kaynaklanan balık hastalıklarının kontrolüne etkin bir öneri getirmektir. Deneylerde kullanılan *A. hydrophila* suşları glutaraldehit, formaldehit ve dörtlü amonyum tuzları (HMI® Glicofin) kombinasyonu ile glutaraldehit, (etilendioksi) dimetanol ve benzalkonyum klorit (Aldesept®) kombinasyonu ile inaktif hale gelmiştir. İyodin (Desinfekt B®), *n-alkali-dimetil-benzil-amonyum klorit* (HMI® Roda Super), etanol (%40, %50 ve %70) ve potasyum permanganat (1000 mg/L) de *A. hydrophila*'ya karşılı etkili olmuştur. 5 mg/L konsantrasyonunda kullanıldığında, potasyum permanganat *A. hydrophila* (ATCC 7965) koloni sayısını %3 ($\log_{10}$ 0.25) azaltırken, 100 mg/L konsantrasyonunda suşlar tamamen inaktive olmuşlardır. 5 mg/L ve 100 mg/L potasyum permanganat konsantrasyonlarının etkisi altında klinik *A. hydrophila* suşlarının koloni sayısı sırasıyla %0.7 ($\log_{10}$ 0.06) ve %2.3 ($\log_{10}$ 0.19) azalmıştır. 5 mg/L konsantrasyonundaki potasyum permanganat dışında deneylerde kullanılan dezenfektanların birçoğunun *A. hydrophila*'ya karşı etkili olduğu tespit edilmiştir.

Anahtar Kelimeler: *Aeromonas hydrophila*, Dezenfektanlar, Duyarlılık

Introduction

Bacteria from the genus *Aeromonas* belong to the *Aeromonadaceae* family. They are Gram-negative facultatively anaerobic rods, oxidase and catalase positive (Parker and Shaw, 2011). Janda and Abbott (2010) classify aeromonads into two large groups. The one includes psychrophilic, immotile species as *A. salmonicida*, and the other – motile mesophils as *A. hydrophila*. Aeromonads naturally inhabit aquatic environments and cause disease in cold-blooded, warm-blooded animals and humans (Igbinosa et al., 2012). *A. hydrophila*-caused fish diseases include dermal ulceration, haemorrhagic septicaemia, red sore disease, red rot disease, and scale protrusion disease (Cipriano er al., 1984). *A. hydrophila* causes substantial economic losses due to high mortality and poor quality of produce (Ibrahem et al., 2008). According to Aoki (1999) most cultured and wild freshwater fish species as brown trout (*Salmo trutta*), rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*), ayu (*Plecoglossus altivelis*), carp (*Cyprinus carpio*), channel catfish (*Ictalurus punctatus*), clariid catfish (*Clarias* batrachus), Japanese eel (*Anguilla japonica*), American eel (*Anguilla rostrata*), gizzard shad (*Dorosoma cepedianum*), goldfish (*Carassius auratus*), golden shiner (*Notemigonus crysoleucas*), snakehead fish (*Ophicephalus striatus*) and tilapia (*Tilapia nilotica*) are sensitive to this pathogen. The adequate hygiene, periodic drying and disinfection of pools are important for prevention of *A. hydrophila*-induced fish diseases.

Disinfectants are products destroying or inhibiting microbial growth, used for treatment of surfaces or objects (McDonnell and Russell, 1999). Aeromonads are sensitive to sodium hypochlorite, quaternary ammonium salts, iodoform, 2-chlorophenol and glutaraldehyde (ICMSF, 1996). Alcohols are effective antimicrobial agents. They exhibit a rapid broad-spectrum antimicrobial activity against vegetative forms of bacteria, viruses and fungi but do not possess sporocidic effect (McDonnell and Russell, 1999). The investigations of the efficiency of potassium permanganate for control of *A. hydrophila* infections are few. *In vitro* investigations on the sensitivity of *A. hydrophila* to potassium permanganate are necessary prior to evaluate its efficacy in fish (Schrader et al., 2013). According to McDonnell and Russell (1999) glutaraldehyde is an important dialdehyde used for disinfection. It possesses a broad spectrum of activity against bacteria and their spores, fungi and viruses. Formaldehyde is a highly reactive chemical interacting with proteins, DNA and RNA (McDonnell and Russell, 1999), and benzalkonium chloride is a broad-spectrum disinfectant used in aquaculture (Tonguthai, 2000). Gottardi (1991) affirms that iodine exhibits a rapid bactericidal, fungicidal, tuberculocidal, virucidal and sporicidal effect. It penetrates rapidly in microbes and attacks key groups of proteins. Quaternary ammonium compounds are irreversibly bound to phospholipids and proteins of cell membranes, thus reducing their permeability (Maris, 1995).

The aim of the present study was to determine the sensitivity of *Aeromonas hydrophila* to disinfectants and to propose an efficient method for control of fish diseases caused by this pathogen. To this end, the sensitivity of two *Aeromonas hydrophila* strains to six disinfectants at various concentrations was tested.

Materials and Methods

Disinfectants

Disinfectants, tested concentrations and exposure times used in this research are presented in Table 1. Working solutions are prepared *ex tempore* in sterile distilled water.

Bacterial cultures

One reference (ATCC 7965) and one clinical *A. hydrophila* strain were used. The reference strain was purchased by the National Bank for Industrial Microorganisms and Cell Cultures, Sofia, Bulgaria. The clinical isolate originated from a silver carp with haemorrhagic septicaemia and was kindly provided by National Reference Laboratory on Fish, Mulluscs and Crustacean Diseases, Sofia, Bulgaria. They were stored in a freezer and inoculated on soybean casein digest broth (Merck, Germany) prior to the experiment at 28°C for 24 h. Then the strains were inoculated on soybean casein digest agar (Merck, Germany) and incubated at 28°C overnight. Bacterial suspensions with density $\log_{10}$ 8.32 cfu/mL for both the reference and the clinical strains were then prepared.

Experimental design

The sensitivity of experimental *A. hydrophila* strains to disinfectants was determined as per Mainous et al. (2011). One hundred µl of bacterial suspension were pipetted in sterile Eppendorf tubes. To each tube were added either 0.9 mL sterile phosphate-buffered saline with pH 7.2 (control) or working solutions of tested disinfectants. After exposure time was over, 100 µL of each bacterial suspension + disinfectant mixture were transferred in tubes containing 9.9 mL sterile phosphate-buffered saline (pH 7.2) and inverted for inactivation of the respective disinfectant. Tenfold dilutions and inoculations on GSP agar (Merck, Germany) were then performed. Petri dishes were incubated in a thermostat at 28°C for 24 hours and colonies were counted. The control sample was run after 6 hours, corresponding to the longest time of exposure to one of tested disinfectants. The experiment was conducted at room temperature (22°C). The log reduction measure of efficacy was calculated as per Eryilmaz and Akin (2007) as followed: $\log_{10}$ reduction = $\log_{10}$ pre-disinfection count – $\log_{10}$ disinfection count. A log reduction value of 5 or higher was accepted as indicating a satisfactory bactericidal activity.

Results and Discussion

The results for the sensitivity of experimental *A. hydrophila* strains to tested disinfectants are presented in Table 2 and Table 3. Table 2 shows that the combination glutaraldehyde, formaldehyde and quaternary ammonium salts (HMI® Glicofin) and glutaraldehyde, (ethylenedioxy) dimethanol and benzalkonium chloride (Aldesept®) inactivated completely (100%, $\log_{10}$ 8.32) *A. hydrophila* (ATCC 7965). Iodine (Desinfekt B®), *n-alkyl-dimethyl-benzyl ammonium chloride* (HMI® Roda Super), ethanol (40%, 50% and 70%) and potassium permanganate (1000 mg/L) were also effective against *A. hydrophila* (ATCC 7965) achieving a complete inactivation (100%, $\log_{10}$ 8.32). At a concentration of 5 mg/L, potassium permanganate reduced *A. hydrophila* (ATCC 7965) bacterial counts by 3% ($\log_{10}$ 0.25), whereas at 100 mg/L, inactivated completely the reference strain (100%, $\log_{10}$ 8.32).

The clinical *A. hydrophila* strain was also sensitive to tested disinfectants (Table 3). HMI® Glicofin, Aldesept®, Desinfekt B®, HMI® Roda Super, ethanol (40%, 50% and 70%) and potassium permanganate (1000 mg/L) were 100% effective against the strain and inactivated it completely (100%, $\log_{10}$ 8.32). Five mg/L potassium permanganate reduced the counts of the clinical *A. hydrophila* isolate by 0.7% ($\log_{10}$ 0.06), and at concentration of 100 mg/L – by 2.3% ($\log_{10}$ 0.19).

The control sample exhibited a reduction by 0.8% ($\log_{10}$ 0.07). Aquaculture species are extensively developing and thus, diseases in them are among the most significant challenges. Motile aeromonads are considered as important etiological agents of fish diseases (John and Hatha, 2013). *A. hydrophila* caused diseases are among the most devastating in freshwater fish species, manifested with acute or chronic signs and high death rates. Medicated feeds and water treatment are necessary for control of diseases caused by motile aeromonads (Schrader et al., 2013).

Table 1. Disinfectants tested in the study

Trade name	Active ingredient(s)	Concentration	Exposure time
HMI® Glicofin[1]	Glutaraldehyde Formaldehyde Quaternary ammonium salts	1.5 %	6 hours
HMI® Roda Super[1]	*n-alkyl-dimethyl-benzyl-ammonium chloride*	0.5 %	10 min
Desinfekt B®[1]	Iodine	1.25 %	6 min
Aldesept®[1]	Glutaraldehyde (Ethylenedioxy) dimethanol Benzalkonium chloride	1 %	30 min
Ethanol	Ethanol	40 %	1 min
		50 %	
		70 %	
Potassium permanganate[2]	Potassium permanganate	5 mg/L	5 min
		100 mg/L	
		1000 mg/L	

[1] The working solution and exposure time are according the manufacturer's recommendations.

[2] The concentrations are recommended by Noga (1996).

Table 2. Sensitivity of *A. hydrophila* (ATCC 7965) to tested disinfectants

Disinfectant	Concentration	Bacterial counts after the exposure log_{10} cfu/mL	Reduction, %	Log reduction
HMI® Glicofin	1.5 %	0	100	8.32
HMI® Roda Super	0.5 %	0	100	8.32
Desinfekt B®	1.25 %	0	100	8.32
Aldesept®	1 %	0	100	8.32
Ethanol	40 %	0	100	8.32
	50 %	0	100	8.32
	70 %	0	100	8.32
Potassium permanganate	5 mg/L	8.07	3	0.25
	100 mg/L	0	100	8.32
	1000 mg/L	0	100	8.32
Control	–	8.25	**0.8**	**0.07**

Table 3. Sensitivity of clinical *A. hydrophila* strain to tested disinfectants

Disinfectant	Concentration	Bacterial counts after the exposure log_{10} cfu/mL	Reduction, %	Log reduction
HMI® Glicofin	1.5 %	0	100	8.32
HMI® Roda Super	0.5 %	0	100	8.32
Desinfekt B®	1.25 %	0	100	8.32
Aldesept®	1 %	0	100	8.32
Ethanol	40 %	0	100	8.32
	50 %	0	100	8.32
	70 %	0	100	8.32
Potassium permanganate	5 mg/L	8.26	0.7	0.06
	100 mg/L	8.13	2.3	0.19
	1000 mg/L	0	100	8.32
Control	–	8.25	**0.8**	**0.07**

We have studies the efficacy of several disinfectants available as commercial products at concentrations and exposure times recommended by the manufacturers. They contained one or several active ingredients as glutaraldehyde, formaldehyde and quaternary ammonium salts (HMI® Glicofin), *n-alkyl-dimethyl-benzyl-ammonium chloride* (HMI® Roda Super), iodine (Desinfekt B®), glutaraldehyde, (ethylenedioxy) dimethanol and benzalkonium chloride (Aldesept®). Goni-Urriza et al. (2000) have tested the sensitivity of 12 *A. hydrophila*, 22 *A. sobria* and 104 *A. caviae* strains to glutaraldehyde. Most strains were destroyed at 0.02%. For 6 of tested strains, including 4 *A. hydrophila*, the minimum bactericidal concentrations range was from 0.04% to 0.16% glutaraldehyde. Esteban et al. (1999) demonstrated that 2% glutaraldehyde was effective against *A. hydrophila*. Similar results were reported by Mainous et al. (2011). In their study, *A. hydrophila* was found to be sensitive to 2% glutaraldehyde solution after 1 min exposure. Palumbo and Buchanan (1988) observed that *A. hydrophila* was sensitive to 1-min exposure to 0.125% glutaraldehyde at 25°C. According to Tonguthai (2000) formaldehyde could be used for disinfection at concentrations of 0.001-0.0015%, and for disinfection of hatcheries –0.02% are recommended. Benzalkonium chloride exhibits bactericidal and fungicide effects at concentrations between 0.0001% and 0.000125% or as baths at concentration of 0.02% for 30 min. The combinations of glutaraldehyde, formaldehyde and quaternary ammonium salts (1.5% solution of HMI® Glicofin) and glutaraldehyde, (ethylenedioxy) dimethanol and benzalkonium chloride (1% solution of Aldesept®) destroyed completely the experimental *A. hydrophila* strains.

Potassium permanganate is a strong oxidiser with a marked oxidation effect through the MnO_4^- ions. During organic matter oxidation (bacteria or fish tissues), MnO_4^- ions are reduced to MnO_2, which is relatively no-toxic, insoluble and biologically unavailable (Lasier et al., 2000). In our study, 5 mg/L potassium permanganate was found ineffective against the tested *A. hydrophila* strains. Schraderet et al. (2013) established a minimum inhibitory concentration of potassium permanganate of 158 mg/l. According to our results, the reference *A. hydrophila* (ATCC 7965) strain was destroyed by 100 mg/L potassium permanganate. On the opposite, for the clinical *A. hydrophila* isolate, potassium permanganate at 100 mg/L was not effective and only reduced the bacterial counts by 2.3%. Both tested strains were destroyed by potassium permanganate at a concentration of 1000 mg/L. Other researchers having investigated the antibacterial effect of potassium permanganate against *Aeromonas* spp. Alam et al. (2011) have reported that concentrations of 15, 20 and 30 mg/L had no effect against the microbial agent.

Mainous et al. (2011) reported that 30%, 50% and 70% ethanol destroyed *A. hydrophila* for 1 min. Our results were similar, as both tested *A. hydrophila* strains were killed by ethanol at 40%, 50% and 70% after a minute.

Tonguthai (2000) outlined that iodine was commonly used for disinfection of hatcheries and pools at a dose of 0.0001-0.0005%. Palumbo and

Buchanan (1988) observed that 0.001% iodoform inactivated *A. hydrophila* for 10 min at 25ºC. In our experiments, we have also confirmed the bactericidal effect of iodine against *A. hydrophila*, by establishing that 1.25% Desinfekt B® inactivated the tested strains for 6 minutes.

Quaternary ammonium salts had a bactericidal effect against *A. hydrophila* at a concentration of 0.008% after 1 min exposure at 25ºC (Palumbo and Buchanan, 1988). In the present study, n-alkyl-dimethyl-benzyl-ammonium chloride (0.5% solution of HMI® Roda Super) was also found to have a bactericidal effect against the tested *A. hydrophila* strains after 10-min exposure.

In aquaculture, disinfection is used for control of common diseases. It could be a part of routine practice in programmes for biocontrol and prevention of specific diseases. On the other hand, it is used as a routine sanitary measure for reduction of morbidity rates or stamping out at farms. The main principles of disinfection in aquaculture farming include treatment at concentrations and exposure times, sufficient to kill all pathogens. The toxicity of disinfectants is the cause for the ban on their use in open water farming; disnifection is eligible only in hatcheries and storage tanks (OIE, 2010).

Conclusion

The combinations of glutaraldehyde, formaldehyde and quaternary ammonium salts (HMI® Glicofin) and glutaraldehyde, (ethylenedioxy) dimethanol and benzalkonium chloride (Aldesept®) were effective against *A. hydrophila*. Iodine (Desinfekt B®), *n-alkyl-dimethyl-benzyl-ammonium chloride* (HMI® Roda Super), ethanol (40%, 50% and 70%) and potassium permanganate (1000 mg/L) were also found effective against *A. hydrophila*. At concentration of 5 mg/L, potassium permanganate was not effective in destroying A. *hydrophila* (ATCC 7965), whereas at 100 mg/L, inactivated completely the reference strain. At concentrations of 5 mg/L and 100 mg/L, potassium permanganate was not effective against the tested *A. hydrophila* isolate. The results from the present study could be useful for aquaculture farmers with respect to control *A. hydrophila* diseases in fish.

References

Alam, M., Rahman, M.M., Foysal, M.J., Hossain, M.N., (2011). Determination of lethal concentration and antibacterial activity of commonly used disinfectants, *International Journal of Natural Sciences*, **1**(4): 102-105.

Aoki, T., (1999). Motile Aeromonads (*Aeromonas hydrophila*). In: Woo, P.T.K., Bruno, D.W. (Eds.), Fish diseases and disorders, Volume 3: Viral, Bacterial and Fungal Infections, CABI Publishing, London, 427-453.

Cipriano, R.C., Bullock, G.L., Pyle, S.W., (1984). *Aeromonas hydrophila* and motile aeromonad septicemias of fish. US Fish and Wildlife Publications, Paper 134.

Eryilmaz, M., Akin, A., (2007). Comparison of efficacy of some hand antiseptics against meticilline-resistant Staphylococcus aureus, *Türk Mikrobiyoloji Cemiyeti Dergisi*, **37**(3), 161-163.

Esteban, J., Gadea, I., Fernandez-Roblas, R., Molleja, A., Calvo, R., Acebron, V., Soriano, F., (1999). Pseudo-outbreak of Aeromonas hydrophila isolates related to endoscopy, *Journal of Hospital Infection*, **41**(4): 313-316.

doi: 10.1053/jhin.1998.0531

Goni-Urriza, M., Pineau, L., Capdepuy, M., Roques, C., Caumette, P., Quentin, C., (2000). Antimicrobial resistance of mesophilic *Aeromonas* spp. isolated from two European rivers, *Journal of Antimicrobial Chemotherapy*, **46**(2): 297-301.

doi: 10.1093/jac/46.2.297

Gottardi, W., (1991). Iodine and iodine compounds. In: Block, S. (Ed.), Disinfection, sterilization, and preservation, 4th edn, Philadelphia, 152-166.

Ibrahem, M.D., Mostafa, M.M., Arab, R.M.H., Rezk, M.A., (2008). Prevalence of *Aeromonas hydrophila* infection in wild and cultured tilapia nilotica (*O. niloticus*) in Egypt. In: Eighth International Symposium on Tilapia in Aquaculture. Proceedings. Cairo, Egypt, 1257-1271.

ICMSF (International Commission on Microbiological Specifications for Foods), (1996). Microorganisms in foods 5. Microbiological specifications in food pathogens, Blackie

Academic and Professional, London, 524 pages.

Igbinosa, I., Igumbor, E., Aghdasi, F., Tom, M., Okoh, A., (2012). Emerging aeromonas species infections and their significance in public health, *The Scientific World Journal*, (in press).

doi: 10.1100/2012/625023

Janda, J.M., Abbott S.L., (2010). The genus Aeromonas: taxonomy, pathogenicity, and infection, *Clinical Microbiology Reviews*, **23**(1): 35-73.

doi: 10.1128/CMR.00039-09

John, N., Hatha, A.A., (2013). Distribution, Extracellular Virulence Factors and Drug Resistance of Motile Aeromonads in Fresh Water Ornamental Fishes and Associated Carriage Water, *International Journal of Aquaculture*, **3**(17): 92-100.

doi: 10.5376/ija.2013. 03.0017

Lasier, P.J., Winger, P.V., Bogenrieder, K.J., (2000). Toxicity of manganese to Ceriodaphnia dubia and Hyalella azteca, *Archives of Environmental Contamination and Toxicology*, **38**(3): 289-304.

doi: 10.1007/s002449910039

Mainous, M.E., Kuhn D.D., Stephen S.A., (2011). Efficacy of Common Aquaculture Compounds for Disinfection of Aeromonas hydrophila, A. salmonicida subsp. salmonicida, and A. salmonicida subsp. achromogenes at Various Temperatures, *North American Journal of Aquaculture*, **73**(4): 456-461.

doi: 10.1080/15222055.2011.630265

Maris, P., (1995). Modes of action of disinfectants, *Scientific and Technical Review*, **14**(1): 47-55.

McDonnell, G., Russell, A., (1999). Antiseptics and Disinfectants: Activity, Action, and Resistance, *Clinical Microbiology Reviews*, **12**(1): 147-179.

Noga, E., (1996). Fish disease: Diagnosis and Treatment. Blackwell Publishing Company, Iowa. ISBN 978-0-8138-2558-8, 367 pages.

OIE, (2010). Methods for disinfection of aquaculture establishments. In: Manual of Diagnostic Tests for Aquatic Animals. **http://web.oie.int/eng/normes/fmanual/1.1.3_DISINFECTION.pdf.**

Palumbo, S., Buchanan, R., (1988). Factors affecting growth or survival of Aeromonas hydrophila in foods, *Journal of Food Safety*, **9**(1): 37-51.

doi: 10.1111/j.1745-4565.1988.tb00506.x

Parker, J., Shaw, J., (2011). Aeromonas spp. clinical microbiology and disease, *Journal of Infection*, **62**(2): 109-118.

doi: 10.1016/j.jinf.2010.12.003

Schrader, K., Marcuslene, D.H., Darwish, A.M., (2013). In vitro comparisons of the inhibitory activity of florfenicol, copper sulphate and potassium permanganate towards Aeromonas hydrophila and Flavobacterium columnare, *Aquaculture Research*, **44**(2): 212-219.

doi: 10.1111/j.1365-2109.2011.03024.x

Tonguthai, K., (2000). The use of chemicals in aquaculture in Thailand. In: Arthur, J.R., Lavilla-Pitogo, C.R., Subasinghe, R.P. (Eds.), Use of Chemicals in Aquaculture in Asia. Proceedings of the Meeting on the Use of Chemicals in Aquaculture in Asia, Philippines, ISBN 9718511490. p. 207-220.