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Minimal Bactericidal Concentration of Sanitizers against *Edwardsiella tarda* Strains Isolated from African Catfish *Clarias gariepinus*

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Abstract

This study evaluated the minimum bactericidal concentration (MBC) of six commercial sanitizers such as hydrogen peroxide, glutaraldehyde, benzalkonium chloride, sodium hypochlorite, iodophor and pursue in distilled water, physiological saline, pond water and tryptic soy broth, as suspending media, against *Edwardsiella tarda* strains isolated from African catfish *Clarias gariepinus*. The MBC results suggest that benzalkonium chloride and hydrogen peroxide are effective to kill *E. tarda* at lower concentrations ranging from 0.78-3.13 µg/ml in comparison to other sanitizers. These results would help the catfish farmers to determine the actual dose required to sanitize the hatchery or nursery water and to avoid *E. tarda* infection.

Keywords: *Clarias gariepinus*, *Edwardsiella tarda*, Sanitizers, Minimum bactericidal concentration.

Introduction

Edwardsiella tarda, one of the zoonotic pathogens, is a Gram-negative, oxidase negative, motile, short, rod-shaped bacterium of 2–3 µm long and 1 µm in diameter. Gastrointestinal disease caused by *E. tarda* has been frequently reported by spencer *et al.*[16], which is characterized by watery to bloody diarrhea that could be prolonged or intermittent, anorexia and vomiting. Engel and Martin[8] documented few instances of ulcerative colitis, which is a progressive form of gastroenteritis. *Edwardsiella tarda* is intracellular by nature and can successfully colonize a wide variety of human tissues causing

extraintestinal infections. This also causes peritonitis, multiple liver abscesses, cholangitis, meningitis, cholecystitis, salpingitis, bronchopneumonia, empyema, skin and genitourinary tract infections. These infections may further lead to septic shock particularly in immunocompromised patients. Disease caused by *E. tarda* is of high concern in tropical and sub-tropical region where dietary habits include consuming raw fish and seafoods[16, 17].

Catfish farming sector is a relatively new development in India. With intensification in culture, the incidence of catfish diseases is increasing in India. Bacterial diseases continue to be the major economic factor for commercial catfish farming and the development of antimicrobial resistance in pathogens particularly of zoonotic species has also become a global public health problem. *Edwardsiella tarda* primarily causes edwardsiellosis in catfish and also infects reptiles, amphibians and mammals[15]. This disease has a considerable economic effect on aquaculture industry worldwide[3]. The incidence, identification and pathology of edwardsiellosis in various fish have been reviewed[15]. According to them, this disease is usually associated with poor maintenance of aquaculture ponds. Infected fishes exhibit abnormal swimming behavior, loss of pigmentation, exophthalmia, eye opacity, abdominal swelling, petechial hemorrhage in fin and skin, rectal hernia and internally, watery and bloody ascites in the abdominal space and congested liver, spleen and kidney.

In order to eliminate human pathogens including *E. tarda* from aquaculture environment, the use of chemical sanitizers are more frequent[11]. Chemicals such as chlorine, chlorine dioxide, iodine, quaternary ammonium compounds (QACs), and peroxyacetic acid are commonly used as decontaminating agents in aquaculture industries[6]. A wide range of disinfectants are used by aquaculturists, but the concentrations to be used are mostly based on laboratory suspension tests and the range of bacteria tested may be too limited[7]. Increased knowledge and better understanding of the bactericidal capacity of disinfectants are essential to optimize sanitation procedures depending on the needs and also to reduce costs and environment waste. Therefore, the objective of this study was to evaluate the minimum bactericidal concentration (MBC) of commercially used sanitizers against *E. tarda* strains isolated from African catfish, *Clarias gariepinus*.

Materials and Methods

Determination of minimum bactericidal concentration (MBC) of sanitizers

Two strains of *Edwardsiella tarda* (CI-1 and NI-1) isolated and identified from African catfish, *Clarias gariepinus* (Burchell 1822) and from the collections of Department of Aquatic Animal Health, Faculty of Fishery Sciences, Kolkata were used. The commercial sanitizers used were hydrogen peroxide, glutaraldehyde, benzalkonium chloride, sodium hypochlorite, iodophor and pursue (Table 1). Available iodine in iodophor and chlorine in hypochlorite was estimated by the iodometric method[2]. After ascertaining the level of available compounds, they were suitably diluted to get the desired concentration.

The minimum bactericidal concentration (MBC) of six sanitizers (Table 1) to the test strains was determined by broth dilution method[14] in four suspending media such as distilled water (DW), physiological saline (PS), pond water (PW) and tryptic soy broth (TSB). The test strains were grown in TSB separately for 18 h at 30±2°C and centrifuged to remove the cells. The cells were suspended in 5 ml sterile physiological saline, vortexed thoroughly and used immediately. A graded concentration of the sanitizers, viz., 0.1, 0.2, 0.39, 0.78, 1.56, 3.13, 6.25, 12.5, 25, 50 and 100 ppm were prepared separately along with control in 10 ml volumes of respective suspending media. After careful mixing, approximately 10^5 CFU/ml of respective *E. tarda* strains were inoculated aseptically in to the respective tubes, in duplicate, and incubated at 30±2°C for 24 h. A loopful of inoculum from each tube was then streaked on to tryptic soy agar, and incubated for 72 h at 30±2°C. The plates were observed for growth or no growth. The MBC was determined as the minimal concentration showing no growth at 72 h.

Table 1. Sanitizers used for the determination of minimal bactericidal concentration against *Edwardsiella tarda*

Name of the sanitizer	Source
Benzalkonium chloride (BKC) - *A mixture of alkyl benzyl dimethyl ammonium chlorides of various even-numbered alkyl chain lengths,* $C_{22}H_{40}CIN$	LOBA Chemie, Mumbai
Glutaraldehyde - Pentane-1,5-dial, $C_5H_8O_2$	LOBA Chemie, Mumbai
Sodium hypochlorite, NaClO	Merck Ltd., Mumbai
Hydrogen peroxide, H_2O_2	RFCL Ltd., New Delhi
Iodophor - *Complexes of iodine and non-ionic surface active agents, usually polyvinyl pyrrolidone,* *I_2	CDH Laboratory Reagent, New Delhi
Pursue - Didecyl dimethyl ammonium chloride, $C_{22}H_{48}ClN$	Amway, India

* Active ingredient

Results

The MBC results of sanitizers such as benzalkonium chloride, glutaraldehyde, sodium hypochlorite, hydrogen peroxide, iodophor and pursue against two *E. tarda* strains in distilled water, physiological saline, pond water and TSB are presented in Table 2. The MBC values for both *E. tarda* strains varied markedly in different suspending media, with highest being in TSB, wherein the MBCs ranged from 25 to

100 µg/ml. The next highest MBC values were recorded in pond water, with MBC values ranging from 1.56 to 25 µg/ml for both strains. The MBCs of sanitizers recorded in distilled water and physiological saline were almost the same, ranging from 0.78 to 12.50 µg/ml. Significant differences existed in the MBCs among the suspending media ($P<0.05$), *E. tarda* strains ($P<0.05$), sanitizers ($P<0.05$) and combination of factors ($P<0.05$).

Table 2. Minimal bactericidal concentration (MBC) of sanitizers against *Edwardsiella tarda* strains in distilled water (DW), physiological saline (PS), pond water (PW) and tryptic soy broth (TSB)

Sanitizers	MBC in (µg/ml), *E. tarda* strains and suspending media							
	E. tarda CI-1				*E. tarda* NI-3			
	DW	PS	PW	TSB	DW	PS	PW	TSB
Benzalkonium chloride	0.78	1.56	1.56	50.00	3.13	1.56	6.25	100.00
Glutaraldehyde	3.13	1.56	12.50	50.00	1.56	3.13	6.25	100.00
Sodium hypochlorite	12.5	3.13	25.00	50.00	6.25	1.56	12.5	100.00
Hydrogen peroxide	3.13	1.56	6.25	50.00	0.78	3.13	12.5	50.00
Iodophor	1.56	3.13	12.50	25.00	3.13	3.13	12.5	100.00
Pursue	3.13	6.25	12.50	50.00	1.56	0.78	3.13	50.00

Discussion

The use of sanitizers such as benzalkonium chloride, glutaraldehyde, hydrogen peroxide, sodium hypochlorite and iodophor is a common practice in aquaculture and few of them in food processing industries. Often, sanitizers are added to the water as a prophylactic measure to combat the diseases and eliminate the bacterial pathogens in water used for food processing and aquaculture. As shown in Table 2, the MBCs for two *E. tarda* strains varied markedly with different suspending media possibly due to the intrinsic susceptibility of the strains to a biocide. Because, the susceptibility phenotype expressed by the cell can vary significantly according to the prevailing physicochemical environments[5, 12]. The highest MBC value was recorded in TSB (25-100 µg/ml) followed by pond water (0.78-25 µg/ml) for both the strains. The results of MBC as observed in TSB corroborate the observations of Kollanoor *et al.*[10] for *E. tarda* using Mueller Hinton broth. The high MBC value in TSB and pond water is obvious as both of them contained organic matter, which interferes with the effect of sanitizers to inhibit growth of bacteria. The impact of

environmental factors and organic load of pond water on the efficacy of sanitizers was also reported in earlier studies[4, 13]. Naik *et al.*[13] reported that variation in the organic load in water and sediment is expected between different ponds and within ponds in a day and this is bound to have an impact on the efficacy.

Although the MBC values of sanitizers recorded in distilled water and physiological saline were insignificantly different (P>0.05), BKC and hydrogen peroxide were more effective against both strains with low MBC values ranging from 0.78 to 3.13 µg/ml than other sanitizers tested. This was followed by glutaraldehyde and iodophor with values ranging from 1.56 to 3.13 µg/ml and finally pursue and sodium hypochlorite with MBC ranging from 0.78 to 12.5 µg/ml. Similar observations were made by Kumar and Abraham[11] on *E. tarda* isolated from stinging catfish *Heteropneustes fossilis*. Aarestrup and Hasman[1] reported that hydrogen peroxide was effective against Enterobacteriaceae with MBCs ranging from 0.002% (20 ppm) to 0.016% (160 ppm). In an earlier study, Kollanoor *et al.*[10] reported the MBC values for *E. tarda* strains with caprylic acid (10 mM), monocaprylin (5 mM) and sodium caprylate (100 mM) using Mueller Hinton broth. Goswami and Prasad[9] recorded a minimal lethal concentration of 1.5 ppm BKC against *E. tarda* in sterile distilled water and unchlorinated water as suspending media. The results of the present study suggest that sanitizers like BKC and hydrogen peroxide are effective at lower concentrations in comparison to other sanitizers such as glutaraldehyde, iodophor, pursue and sodium hypochlorite. It further revealed that the susceptibility of *E. tarda* vary with suspending media, strains, sanitizers and combination of factors.

The results presented here would be beneficial to the aquaculturists and food processors as they can work out the effective concentration of sanitizers to be used in the respective industries to contain *E. tarda* infection, especially in catfish aquaculture systems. Rotational use of different disinfectants is recommended to avoid development of resistance or selection of resistant strains in an environment, which is frequently disinfected.

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