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ANTIMICROBIAL SUSCEPTIBILITY OF *EDWARDSIELLA ICTALURI* ISOLATED FROM DIFFERENT FISH SPECIES

MAHMOUD MOSTAFA MAHMOUD
Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Assiut 71526, Egypt

ABSTRACT

Edwardsiella ictaluri strains isolated from different fish species in different geographic areas (Japan, Indonesia, Vietnam and USA) were screened against 15 antimicrobial agents. The antimicrobial susceptibility was examined using the Minimum Inhibitory Concentration (MIC) test. Except for only one of the tested strains (DTHN01), the others were found to be susceptible, with high percentages, to most agents active against gram negative bacteria such as aminoglycosides, β lactams, quinolones, tetracyclines, and chloramphenicol. Resistance was observed against colistin, sulfisozole, Polymixin B sulfate and Vancomycin.

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***Key words*:** Antimicrobial susceptibility, *Edwardsiella ictaluri*, MIC

INTRODUCTION

Edwardsiella ictaluri is the etiologic agent of an acute septicemic disease of catfish which has been termed “enteric septicemia of catfish” (ESC). It was first isolated in 1976 (Hawke, 1979) and causes economic losses in channel catfish, *Ictalurus punctatus*, aquaculture. Subsequently, *E. ictaluri* infection was recorded in many fish species worldwide including catfishes and non-catfishes (Evance *et al.*, 2011; Hassan *et al*., 2012; Plumb and Hanson, 2011). The prevention and treatment of this disease has become of major importance.

Antibiotics are still the common means of control and are applied usually to the feed mixture either prophylactically or to treat an existing infection. Hawke (1979) was the first to test the antimicrobial susceptibility of 10 *E. ictaluri* isolates. Later, Waltman and Shotts (1986) screened 118 *E. ictaluri* isolates retrieved in the United States for susceptibility to 37 antimicrobials using the disc sensitivity test. They found that the majority of isolates were susceptible to most agents active against Gram-negative bacteria, but resistance was observed against colistin and sulfonamides in more than 90% of isolates.

Reger *et al*. (1993) likewise tested the antimicrobial susceptibility of American *E. ictaluri* isolates and found full susceptibility to gentamicin, and doxycycline. Thereafter, Stock and Wiedemann (2001) studied the antimicrobial susceptibility of 41 *E. ictaluri* strains to 71 antibiotics. All these isolates originated from American channel catfish, and hardly any acquired resistance was detected. Recently, Dung *et al*. (2008) discussed the in vitro susceptibility of 64 Vietnamese isolates and described the acquired resistance developed against some antimicrobial agents. Evaluation of the susceptibility to commonly used antimicrobials will assist in determination of their efficacy and emphasize which agents are effective and can be used in the future.

In the last few years, *E. ictaluri* isolates were recovered from different fish species including striped catfish, *Panagasius hypophthalmus*, in Indonesia and Vietnam (Yuasa *et al*., 2003 and Hassan *et al*., 2010) and ayu, *Plecoglossus altivelis*, in Japan (Nagai *et al*., 2008; Sakai *et al*., 2008; Hassan *et al*., 2012). Those isolates were characterized biophysically, biochemically, antigenically and genetically (Hassan *et al*., 2012; Mahmoud and Nakai, 2011). Yet, they have not been tested for their susceptibility to antimicrobial agents.

The current study was designed to assess the *in vitro* susceptibility of the used strains to different antimicrobial agents. This is the first report on the antimicrobial susceptibility of the current strains.

MATERIALS and METHODS

Bacterial strains:
A total of 12 isolates of *E. ictaluri* of different origin was collected and studied. Their sources, distributions, and year of isolation are given in Table 1. Isolates were maintained in glycerol broth and kept at -80°C.

Antimicrobial agents:
Fifteen antimicrobial agents were screened for their activity against *E. ictaluri.* These agents are ampicillin, amoxicillin (β lactams); tetracycline,

oxytetracycline (tetracyclines); kanamycin, gentamycin, neomycin, streptomycin (aminoglycosides); chloramphenicol; nalidixic acid and oxolinic acid (quinolones); sulfisozole; colistin; polymixin B sulfate and vancomycin. They were dissolved in appropriate solvents to make stock solutions and then further diluted in sterile distilled water according to the methods recommended by the Clinical and Laboratory Standards Institute (CLSI M7-A7, 2006).

Susceptibility testing:
Minimum Inhibitory Concentration (MIC) tests were carried out using microdilution procedure in Mueller-Hinton broth. Each antimicrobial agent was two-fold serially diluted using 96-well sterile plastic microdilution plates. Final concentrations of 0.1-625 μg/ml were tested for all antimicrobial agents. Inocula were prepared by suspending bacterial cultures (25°C, 48 hours) in Mueller-Hinton broth. Approximately 1×10^5 colony forming units (cfu) of the strains were then inoculated on the antibiotic-containing plates and on antibiotic-free control plates, after which plates were incubated at 25°C for 48 hours. The MIC was detected as the lowest concentration of the antimicrobial agent with no visible bacterial growth. Each antimicrobial agent was examined trice and the mean MIC values were evaluated for variations using the student *t* test for multiple comparisons. The interpretation of the MIC results as susceptible, intermediate and resistant was done following the interpretive standards (in μg/ml) of Clinical and Laboratory Standards Institute (2007).

RESULTS

The patterns of antimicrobial susceptibility of the tested *E. ictaluri* strains (n=12) showed that all strains were susceptible to the used β lactam antibiotics (ampicillin and amoxicillin). Also, two of the aminoglycoside antibiotics (kanamycin and gentamycin) exhibited the same pattern with 100% susceptible strains, while the other two antibiotics displayed less effectiveness where 75% and 25% only of the strains were susceptible to neomycin and streptomycin respectively. The susceptibility to tetracyclines (tetracycline and oxytetracycline), quinolones (nalidixic and oxolinic acids), and chloramphinicol were almost same (91.7 % susceptible strains). On the other hand, sulfisozole, colistin, polymixin B, and vancomycin proved ineffectiveness against *E. ictaluri* with 100% resistance. The detailed data are shown in Table 2.

An overview of the MIC values for the different *E. ictaluri* isolates is shown in Table 3. For the pathogen isolated from different fish species, MIC values did not display big difference in between except for DTHN01 strain that was isolated from striped catfish in Vietnam. All strains had high MIC values which were equal to or above 58.6 μg/ml in case of sulfisozole, colistin, polymixin B, and vancomycin.

Table 1: Sources of *Edwardsiella ictaluri* strains

Bacterial strains	Bacterial sources			
	Host fish	Locality	Year	Reference
JCM1680 (ATCC33202)	Channel catfish [a]	USA	1970s	Hawke *et al*. (1981)
JF0384	Striped catfish [b]	Indonesia	2003	Yuasa *et al*. (2003)
DTHN01	Striped catfish	Vietnam	2009	Hassan *et al*. (2010)
PH0744	Ayu	Japan	2007	Nagai *et al*. (2008)
FPC1095	Ayu [c]	Japan	2008	Sakai *et al*. (2009a)
FPC1096	Ayu	Japan	2008	Sakai *et al*. (2009a)
FPC1100	Ayu	Japan	2008	Sakai *et al*. (2009a)
PT0801	Ayu	Japan	2008	Hassan *et al*. (2012)
AH0816	Ayu	Japan	2008	Hassan *et al*. (2012)
AH0901	Ayu	Japan	2009	Hassan *et al*. (2012)
D4	Ayu	Japan	2008	Hassan *et al*. (2012)
Oth29	Forktail bullhead [d]	Japan	2008	Hassan *et al*. (2012)

[a] *Ictalurus punctatus* [b] *Panagasius hypophthalmus*
[c] *Plecoglossus altivelis* [d] *Pleteobagrus nudiceps*

Table 2: Patterns of antimicrobial susceptibility of *Edwardsiella ictaluri* strains

Antimicrobial agent	% Susceptible	% Intermediate	% Resistant
Ampicillin	100	0	0
Amoxicillin	100	0	0
Tetracycline	91.7	8.3	0
Oxytetracycline	91.7	0	8.3
Kanamycin	100	0	0
Gentamycin	100	0	0
Neomycin	75	16.7	8.3
Streptomycin	25	66.7	8.3
Chloramphenicol	91.7	0	8.3
Nalidixic acid	91.7	0	8.3
Oxolinic acid	91.7	0	8.3
Sulfisozole	0	0	100
Colistin	0	0	100
Polymixin B sulfate	0	0	100
Vancomycin	0	0	100

Table 3: Minimum inhibitory concentration (MIC) values (μg/ml ± SD) of *Edwardsiella ictaluri* strains to 15 antimicrobial agents

Antimicrobial agent	*Edwardsiella ictaluri* strains											
	JCM1680	JF0384	DTHN01	PH0744	FPC1095	FPC1096	FPC1100	PT0801	AH0816	AH0901	D4	Oth29
Ampicillin	1.2±0.0	1.2±0.0	1.2±0.0	0.9±0.4	1.2±0.0	1.2±0.0	0.9±0.6	1.2±0.0	0.6±0.0	1.2±0.0	0.6±0.0	0.6±0.0
Amoxicillin	0.1±0.0	0.2±0.1	0.1±0.0	0.6±0.5	0.1±0.0	0.4±0.2	0.1±0.0	0.1±0.0	0.5±0.0	0.1±0.0	0.2±0.1	0.2±0.1
Tetracycline	0.3±0.0	0.3±0.0	14.6±6.8*	0.5±0.2	0.6±0.0	0.5±0.2	0.5±0.2	0.6±0.0	0.6±0.0	0.3±0.0	0.6±0.0	0.5±0.0
Oxytetracycline	0.2±0.1	0.6±0.0	19.5±0.0*	0.9±0.4	1.2±0.0	1.2±0.0	0.5±0.2	0.6±0.0	0.6±0.0	0.6±0.0	0.6±0.0	1.2±0.0
Kanamycin	1.8±0.8	1.2±0.0	0.9±0.4	3.6±1.7	0.9±0.4	0.9±0.6	0.9±0.6	1.2±0.0	1.6±0.6	1.2±0.0	1.2±0.0	1.2±0.0
Gentamycin	1.2±0.0	1.2±0.0	1.2±0.0	0.5±0.2	1.2±0.0	1.2±0.0	0.5±0.2	1.2±0.0	1.2±0.0	1.2±0.0	0.6±0.0	1.2±0.0
Neomycin	5.9±4.9	1.8±0.8	625±0.0*	3.6±1.7	2.4±0.0	3.6±1.7	2.4±0.0	1.5±0.3	4.8±0.0	1.5±1.3	3.6±1.7	3.6±1.7
Streptomycin	4.8±0.0	1.5±1.3	625±0.0*	4.8±0.0	4.8±0.0	4.8±0.0	4.8±0.0	1.5±1.3	4.8±0.0	1.5±1.3	4.8±0.0	4.8±0.0
Chloramphenicol	0.3±0.0	0.3±0.0	19.5±0.0*	0.2±0.1	0.3±0.0	0.3±0.0	0.2±0.1	0.3±0.0	0.5±0.2	0.3±0.0	0.3±0.0	0.3±0.0
Nalidixic acid	0.3±0.0	0.2±0.0	234.4±110.4*	0.2±0.0	0.2±0.2	0.2±0.2	0.5±0.2	0.5±0.2	0.1±0.0	0.6±0.0	0.2±0.0	0.2±0.0
Oxolinic acid	0.1±0.0	0.04±0.0	625±0.0*	2.4±0.0	1.2±0.0	3.6±1.7	0.2±0.0	0.1±0.0	2.4±0.0	0.1±0.0	2.4±0.0	2.4±0.0
Sulfisozole	78.1±0.0	78.1±0.0	312.5±0.0	156.3±81.9	156.3±0.0	156.3±0.0	156.3±0.0	78.1±0.0	156.3±0.0	29.3±13.7	156.3±0.0	156.3±0.0
Colistin	78.1±0.0	156.3±0.0	117.2±55.2	156.3±0.0	312.5±0.0	234.4±110.4	78.1±0.0	156.3±0.0	156.3±0.0	156.3±0.0	78.1±0.0	78.1±0.0
Polymixin B sulfate	78.1±0.0	28.5±55.2	78.1±0.0	58.6±27.6	117.2±55.2	78.1±0.0	78.1±0.0	78.1±0.0	117.2±55.2	117.2±55.2	156.3±0.0	58.6±27.6
Vancomycin	156.3±0.0	197.8±162.1	156.3±0.0	156.3±0.0	234.4±110.4	234.4±110.4	312.5±0.0	156.3±0.0	156.3±0.0	312.5±0.0	312.5±0.0	312.5±0.0

* Significant difference among strains tested against the same antibiotic (*$p<0.05$*)

DISCUSSION

E. ictaluri is currently isolated from many fish species everywhere. It was first recorded in channel catfish in USA (Hawke, 1979), however, later on the infection was reported in different localities and the pathogen was isolated from different fish species including cultured Japanese eel, *Anguilla japonica*; European sea bass, *Dicentrarchus labrax*; rainbow trout, *Oncorhynchus mykiss*; wild rudd, *Scardinius erythrophthalmus*, and ornamental fishes such as green knife fish, *Eigemannia virscens*; danio, *Danio devario*, and rosy barb, *Puntius conchonus* (reviewed in Evance *et al*., 2011; Plumb and Hanson, 2011) and recently isolated from in Japan (Nagai *et al*., 2008; Sakai *et al*., 2008).

Antimicrobial treatment is still the major control method. Plumb and Schwedler (1982) recommended the use of terramycin mixed with feed at the rate of

2.5 g/100 lb of fish per day for 10-14 days. Oxytetracycline and ormetoprim–sulfadimethoxine have both been used with varying success, but some *E. ictaluri* isolates are resistant. Also, not all isolates from the same case exhibited the same resistance pattern (Taylor and Johnson, 1991). The importance of this finding to choice of medication is not yet known. Some isolates have also been found to be susceptible to kanamycin, streptomycin, neomycin, nitrofurantoin, and/or oxolinic acid in vitro (Waltman and Shotts 1986), but none of these are approved for treating the infection in food fish. In addition, more judicious use of oxytetracycline and ormetoprim-sulfadimethoxine has resulted in a significant decline in resistant isolates in Mississippi (Noga, 2010), making them more available for use.

The present study confirmed that tetracycline and oxytetracycline are still effective against *E. ictaluri* with 91.7 % of the tested isolates being susceptible. Only bacteria isolated from the striped catfish in Vietnam were resistant to both drugs which can be attributed to the acquired resistance from their previous use in prophylaxis or treatment of aquaculture in Vietnam (Dung *et al*., 2008). The β lactam antibiotics (ampicillin and amoxicillin), used in the current work, showed 100% effectiveness against the pathogen. That correlates well with the results of Waltman and Shotts (1986) who described a low MIC for ampicillin. Also, Dung *et al*. (2008) stated that none of the isolates used in their study (n=64) displayed acquired resistance against amoxicillin. Of the aminoglycosides tested in the present study, two (kanamycin and gentamycin) proved their efficacy in killing the pathogen completely (100% susceptibility). Similar results have been mentioned previously (Waltman and Shotts, 1986) who found 0% resistance and low MIC value (0.5- 4 µg/ml) for kanamycin. Reger *et al.* (1993) reported that all examined strains were susceptible to gentamicin. Also, Dung *et al*. (2008) described no resistance against kanamycin or gentamycin. However, the present work described the developing of resistant or intermediate resistant strains against either neomycin or streptomycin, and the strain DTHN01 represented the resistant one with high MIC values (625 µg/ml) for both antibiotics. These results come in accordance with data shown by Dung *et al*. (2008) who found acquired resistance to streptomycin (83 % resistance). Chloramphenicol elicited 91.7% effectiveness against the used strains, with only one strain being resistant (DTNH01). More or less same results have been recorded in different *E. ictaluri* isolates elsewhere (Stock and Wiedemann, 2001). Oxolinic acid has been used to treat fish diseases and has been shown to be effective with little toxicity (Endo *et al*., 1973; Rogers and Austin, 1983; Austin *et al*., 1983). The strains used here exhibited slight resistance against the tested Quinolones (nalidixic and oxolinic acids) with only one resistant strain (DTHN01), i.e. 8.3% resistance with high MIC values of 234.4 and 625 µg/ml for both agents respectively. In their study, Stock and Wiedemann (2001) found that *E. ictaluri* isolates were naturally sensitive to quinolones.

On the other hand, all the used strains were resistant to sulfisozole, colistin, polymyxin B, and vancomycin (100% resistance for all of them) and MICs not less than 58 µg/ml. Several literatures described the resistance of *E. ictaluri* isolates against these antimicrobial agents (Dung *et al*., 2008; Reger *et al*., 1993; Stock and Wiedemann, 2001; Waltman and Shotts, 1986).

The results of antimicrobial susceptibility of *E. ictaluri* studied here revealed that the strains exhibited a homogeneous antimicrobial susceptibility pattern either in their susceptibility to many antimicrobial agents or resistance to others. Only the strain DTHN01, isolated from the striped catfish in Vietnam, exceptionally showed resistance to many of the used antimicrobial agents. The reason for its resistance to almost all used antimicrobial agents may be attributed to the use of these agents in the routine aquaculture practice that enabled *E. ictaluri* to acquire such resistance as mentioned above (Dung *et al*., 2008).

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قابلية بكتيريا الإدواردسيللا إكتالورى المعزولة من أنواع مختلفة من الأسماك لمضادات الميكروبات

محمود مصطفى محمود

تم فحص القابلية لخمس عشرة من مضادات الميكروبات فى سلالات من بكتيريا الإدواردسيللا إكتالورى المعزولة من أنواع مختلفة من الأسماك من مناطق جغرافية متعددة. استخدمت طريقة تعيين التركيز الأدنى المثبط فى تحديد قابلية البكتيريا للمضادات الميكروبية المستعملة. وقد وجد أنه باستثناء سلالة واحدة من البكتيريا (DTHN01) كانت السلالات الأخرى المختبرة قابلة للتثبيط بنسب عالية بواسطة معظم المضادات الميكروبية المستخدمة ضد البكتيريا سالبة الجرام مثل الأمينوجليكوزيد، البيتالاكتام، الكينولون، التتراسيكلين والكلورامفنيكول. بينما لوحظ أن هذه السلالات البكتيرية كانت مقاومة للكوليستين، السلفاسوزول، البوليمكسين ب والفانكوميسين.