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ANTIMICROBIAL SUSCEPTIBILITY OF *EDWARDSIELLA ICTALURI*

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ABSTRACT: *Edwardsiella ictaluri* isolates collected over the past 7 yr from outbreaks of disease in fish occurring in different geographic areas were screened against 37 antimicrobial agents. *Edwardsiella ictaluri* were found to be susceptible to most agents active against gram negative bacteria such as aminoglycosides, cephalosporins, the "newer" penicillins, quinolones, tetracyclines, chloramphenicol, nitrofurantoin, and potentiated sulfonamides. Resistance was observed against colistin, sulfonamides, and several agents regarded as effective for gram positive bacteria. There was no evidence of unusual antimicrobial resistance associated with *E. ictaluri* or of developing resistance.

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 in less than 10 yr has become a leading cause of bacterial mortality in channel catfish.

The disease is seasonal, occurring in the early summer and autumn months when water temperatures are in the range of 25–30 C. The prevention and treatment of ESC has become of major importance. Antibiotics are currently the common means of control and are applied usually to the feed mixture either prophylactically or to treat an existing infection. The only data available on antimicrobial susceptibility for *E. ictaluri* are notes in the original description of ten isolates (Hawke, 1979). Knowledge of susceptibility is important (1) to determine whether the antimicrobials currently in use are effective, (2) to determine which agents are effective and could be used in the future, and (3) to determine whether there is a trend toward resistance. Therefore a study was begun to obtain antimicrobial susceptibility data on a large number of isolates of *E. ictaluri*. Since these isolates represented different geographic areas and years of isolation, comparisons could be made as to developing differences in antimicrobial pattern or resistance.

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MATERIALS AND METHODS

Isolates

A total of 118 isolates of *E. ictaluri* was collected and studied. Their origins, distributions, and year of isolation are given in Table 1. Isolates were maintained in culture and transferred annually.

Antimicrobial susceptibilities

Thirty-seven compounds were screened for antimicrobial activity against *E. ictaluri*. The susceptibility of this microorganism to penicillin G (PG), methicillin (METH), cloxacillin (CLOX), ticarcillin (TIC), moxalactam (MOX), cefoperazone (CEF), sulfadiazine (SD), triple sulfa (SSS), sulfamethoxazole/trimethoprim (SXT), ormetoprim/sulfadimethoxine (Romet, Hoffman-LaRoche, RO5), trimethoprim (TRIM), streptomycin (STR), neomycin (NEO), kanamycin (KAN), polymyxin B (PB), colistin (COL), nalidixic acid (NA), oxolinic acid (OA), cinoxacin (CIN), lincomycin (LIN), clindamycin (CLD), novobiocin (NVB), spectinomycin (SPT), bacitracin (BAC), nitrofurantoin (NF), and erythromycin (ERY) was determined by disk sensitivity tests using the method described by Bauer et al. (1966). Results were interpreted as susceptible, intermediate, or resistant, based on zone diameters of inhibition (National Committee for Clinical Laboratory Standards, 771 East Lancaster Avenue, Villanova, Pennsylvania 19085, USA).

Minimum inhibitory concentrations (MIC) were determined for ampicillin (AMP), cephalothin (CEPH), gentamicin (GENT), tetracycline (TET), carbenicillin (CARB), chloramphenicol (CHLR), tobramycin (TOBR),

TABLE 1. Distribution of isolates of *Edwardsiella ictaluri* used in this study by geographic area and year of isolation.

Year	ALA[a]	GA	MS	Other	Total
1977	0	1	0	0	1
1978	2	1	0	0	3
1979	0	2	0	0	2
1980	0	1	1	0	2
1981	0	3	3	0	6
1982	0	4	20	1[b]	25
1983	3	1	19	2[c]	25
1984	7	0	45	2[d]	54
Total	12	13	88	5	118

[a] ALA = Alabama; GA = Georgia; MS = Mississippi.
[b] One isolate from Wyoming.
[c] One isolate from Tennessee; one from California.
[d] Two isolates from Arkansas.

amikacin (AMIK), cefoxitin (CEFX), cefamandole (CEFM), and cefotaxime (CEFT) using commercially prepared MIC panels (Micro Media Systems, Potomac, Maryland 28054, USA) following the manufacturer's protocol.

For analysis by year of isolation, isolates from 1977 through 1981 were grouped together. The disc sensitivity data were analyzed for differences between year and locality of isolation using the Tukey's test for multiple comparisons among proportions. The mean MIC values of each group were evaluated for variations using the Scheffe test for multiple comparisons. These tests were selected because of the high disparity in numbers between groupings.

RESULTS

A collection of *E. ictaluri* isolated from various geographic areas over a 7-yr period was evaluated against 37 antimicrobial agents. Due to the large number of isolates, some were not tested against each agent. The antimicrobial disc susceptibility results are listed in Table 2. *Edwardsiella ictaluri* was uniformly susceptible to NA, OA, CIN, SXT, RO5, TRIM, NEO, STR, KAN, NF, TIC, MOX, and CFP. Resistance was noted for COL, SD, SSS, LIN, CLD, METH, CLOX, and BAC. Intermediate results were obtained with PB, NVB, ERY, PG, and SPT.

The disc susceptibility pattern was fairly consistent regardless of geographic areas or year of isolation. Those antimicrobials with intermediate results are shown in Table 3 and are separated as to geographic area and year of isolation. Some statistically significant antibiotic resistant differences existed by geographic area, but no trend was noted.

The mean MIC values of 85 isolates of *E. ictaluri* against 11 antimicrobial agents are listed in Table 4. The MIC values were low (i.e., susceptible) to all the agents evaluated. In addition, no single isolate gave a resistant MIC value for any antimicrobial used. No significant variation in

TABLE 2. Patterns of antimicrobial disc susceptibility of isolates of *Edwardsiella ictaluri* to 26 antimicrobial agents.

Antimicrobial agent	No. isolates tested	% Susceptible	% Intermediate	% Resistant
Polymyxin B[a]	85	24.7	30.6	44.7
Colistin[a]	106	2.8	1.9	95.3
Nalidixic acid[b]	106	94.3	5.7	0.0
Oxolinic acid[a]	106	100.0	0.0	0.0
Cinoxacin[b]	106	100.0	0.0	0.0
Sulfadiazine[b]	97	3.1	2.1	94.8
Triple sulfa[b]	81	4.9	3.7	91.4
SXT[b]	106	100.0	0.0	0.0
Romet	81	100.0	0.0	0.0
Trimethoprim[b]	51	100.0	0.0	0.0
Neomycin[a]	106	100.0	0.0	0.0
Streptomycin[b]	94	95.7	4.3	0.0
Kanamycin[b]	106	100.0	0.0	0.0
Novobiocin[a]	106	29.2	35.8	35.0
Lincomycin[a]	106	0.0	0.0	100.0
Clindamycin[a]	93	0.0	0.0	100.0
Nitrofurantoin[b]	106	99.1	0.0	0.9
Erythromycin[b]	105	2.9	27.6	69.5
Penicillin G[b]	99	27.3	23.2	49.5
Methicillin[b]	96	0.0	0.0	100.0
Cloxacillin[a]	72	0.0	0.0	100.0
Ticarcillin[b]	50	100.0	0.0	0.0
Spectinomycin[a]	59	3.4	10.2	86.4
Bacitracin[a]	60	0.0	0.0	100.0
Moxalactam[b]	106	91.9	8.9	0.0
Cefaperazone[b]	71	96.1	3.9	0.0

[a] Interpretation based on zone diameter as recommended by BBL Sensi-Disc Antimicrobial Susceptibility Test Discs package insert, July 1984.
[b] Interpretation based on zone diameter as recommended by the National Committee for Clinical Laboratory Standards, 771 East Lancaster Avenue, Villanova, Pennsylvania 19085, USA.

TABLE 3. Differences between the resistance of isolates of *Edwardsiella ictaluri* to five antimicrobial agents based on geographic area and year of isolation.

Group	% Resistant				
	PB[i]	NVB	ERY	PG	SPT
1981	40.0[b]	16.7[d]	91.7[b,c]	25.0[c,d]	80.0
1982	66.7[a,c,d]	17.4[d]	21.7[a,c,d]	17.4[c,d]	54.6[d]
1983	22.7[b,d]	13.6[d]	57.1[a,b,d]	57.1[a,b]	83.3[a,b]
1984	46.9[b,c]	57.1[a,b,c]	91.8[b,c]	69.0[a,b]	97.3[b]
AL[j]	54.6	0.0[f,g]	75.0	91.7[f,g]	83.3
GA[j]	40.0	16.7[e,g]	58.3	25.0[e]	75.0
MS[j]	45.9	35.4[e,f]	44.9	44.4[e]	89.1
Other	0.0	0.0	33.9	66.7	66.7

[a] Differs significantly ($P < 0.05$) from 1981.
[b] Differs significantly ($P < 0.05$) from 1982.
[c] Differs significantly ($P < 0.05$) from 1983.
[d] Differs significantly ($P < 0.05$) from 1984.
[e] Differs significantly ($P < 0.05$) from AL.
[f] Differs significantly ($P < 0.05$) from GA.
[g] Differs significantly ($P < 0.05$) from MS.
[h] Differs significantly ($P < 0.05$) from Other.
[i] PB = polymyxin B; NVB = novobiocin; ERY = erythromycin; PG = penicillin G; SPT = spectinomycin.
[j] AL = Alabama; GA = Georgia; MS = Mississippi.

MIC values between groups based on geographic area or year of isolation was found.

DISCUSSION

Enteric septicemia of catfish caused by *E. ictaluri* results in severe economic losses to catfish culture. Antimicrobial treatment is currently 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. Because catfish are cultured for human consumption, federal restrictions dictate which antimicrobial agents may be used for controlling disease. Currently only sulfamerazine and oxytetracycline are cleared for use in catfish culture. The isolates of *E. ictaluri* studied here were uniformly resistant to sulfonamides, confirming the earlier findings of Hawke (1979). This presently leaves oxytetracycline as the sole agent which may be used to control ESC in food fish. Fortunately, isolates of *E. ictaluri* were susceptible to tetracyclines, and no evidence of resistance was noted. At least two disadvantages may be associated with the use of tetracyclines. First, they are bacteriostatic agents and thus the killing of *E. ictaluri* rests with the fish's immune system. Second, the use of tetracyclines has led to the development of plasmid-mediated antimicrobial resistance in other bacteria pathogenic to fish such as *Aeromonas hydrophila* (Shotts et al., 1976), *E. tarda* (Aoki et al., 1977), *A. salmonicida* (Aoki et al., 1971) and *Vibrio anguillar-*

TABLE 4. Minimum inhibitory concentration (MIC) values of 85 isolates of *Edwardsiella ictaluri* to 11 antimicrobial agents.

Antimicrobial agent	MIC (μg/ml)			
	Range	MIC50	MIC90	Mean
Ampicillin	0.25–4.00	0.25	1.00	0.48
Cephalothin	1.00–4.00	1.00	1.00	1.03
Gentamicin	0.50–4.00	2.00	2.00	1.44
Tetracycline	0.25–1.00	0.25	0.50	0.35
Carbenicillin	8.00	8.00	8.00	8.00
Chloramphenicol	0.50–2.00	1.00	2.00	1.08
Tobramycin	0.50–4.00	2.00	4.00	1.64
Amikacin	1.00–8.00	4.00	8.00	3.84
Cefamandole	1.00–2.00	1.00	1.00	1.01
Cefoxitin	1.00–4.00	1.00	1.00	1.10
Cefataxime	2.00	2.00	2.00	2.00

um (Aoki et al., 1974). Therefore indiscriminant use of tetracyclines should be avoided and isolates should be screened routinely for the development of resistance.

Recently, a potentiated sulfonamide, ormetoprim/sulfadimethoxine was cleared for use in salmonid furunculosis (Maestrone, 1984). *Edwardsiella ictaluri* was susceptible to this agent even though it was resistant to sulfonamides. These bacteria were susceptible to TRIM, and when TRIM was potentiated with a sulfonamide, even though the bacteria were resistant to the latter agent, the effectiveness of TRIM was enhanced (Bushby, 1973). We suggest that efforts should be made to have a potentiated sulfa cleared for use in catfish culture on the basis of its effectiveness and the necessity of having a second antimicrobial available.

Other antimicrobial agents which were highly effective against *E. ictaluri* were the aminoglycosides, the quinolones, cephalosporins, the "newer" penicillins, the furans and chloramphenicol. Certain aminoglycosides have been evaluated for use in controlling fish diseases (kanamycin: Conroy, 1962, 1963; Gilmartin et al., 1976; gentamicin: Kingsford, 1975; Gilmartin et al., 1976). However, the possible toxic effects and the expense involved have prevented their widespread use. The quinolones are very active against *E. ictaluri* and would be a possible choice for further study. 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, 1982; Austin et al., 1983). For economic reasons, the cephalosporins and the "newer" penicillins, although effective, are not likely candidates for use in controlling fish diseases. The furans are effective and have been used in the treatment of bacterial diseases of non-food fish (Kubota and Hagita, 1963; Hayashi et al., 1964; Snieszko and Axelrod, 1971). A similar compound, Furanace (nifurpirinol), has been found to be very effective in treating several bacterial diseases of fish in non-food fish (Dainippon Pharmaceutical Co., 1975). Chloramphenicol also is well known as an effective agent in the treatment of bacterial fish diseases (Kingsford, 1975; Snieszko, 1975). However, like tetracycline, it is bacteriostatic. Chloramphenicol is not absorbed from the water (Nusbaum and Shotts, 1981) and therefore must be injected or fed for use in systemic diseases.

Edwardsiella ictaluri is resistant to LIN, CLD, METH, CLOX, and BAC as expected since these agents are primarily active against gram positive bacteria. *Edwardsiella ictaluri* is resistant to colistin, an agent effective against many gram negative bacteria (except *Proteus* spp., some *Serratia* and *Aeromonas*, *E. tarda*, and certain non-fermentative gram negative bacteria).

Edwardsiella ictaluri exhibits a very homogeneous antimicrobial susceptibility pattern. Very little variation in resistance was found except with PB, NVB, ERY, PG, and SPT. Analysis of the susceptibility of *E. ictaluri* to these agents reveals some significant differences, but no general trends.

Although *E. ictaluri* shows no present trend toward the development of antimicrobial resistance, investigators should monitor the susceptibility pattern of isolates routinely as antimicrobial treatment continues to be used.

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