ELSEVIER

International Journal of Food Microbiology 76 (2002) 165–168

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Short communication

Antibiotic resistance of *Aeromonas hydrophila* isolated from marketed fish and prawn of South India

G. Vivekanandhan [a], K. Savithamani [a], A.A.M. Hatha [b], P. Lakshmanaperumalsamy [a,*]

[a] *Department of Environmental Sciences, Bharathiar University, Coimbatore-641 046, Tamil Nadu, India*
[b] *Department of Aquaculture and Fishery Microbiology, M.E.S. Ponnani College, Ponnani, Kerala, India*

Received 18 February 2001; received in revised form 16 August 2001; accepted 27 November 2001

Abstract

A total of 319 strains of *Aeromonas hydrophila* were isolated from 536 fish and 278 prawns for a 2-year period. All the strains were tested for resistance to 15 antibiotics and 100% of the strains was resistant to methicillin and rifampicin followed by bacitracin and novobiocin (99%). Only 3% of the strains exhibited resistance against chloramphenicol. The multiple antibiotic resistance (MAR) indexing of *A. hydrophila* strains showed that all of them originated from high-risk sources.

Keywords: *Aeromonas hydrophila*; Fish; Prawn; Antibiotic resistance

1. Introduction

Wide use of antibiotics to treat bacterial infections and incorporation of subtherapeutic dose of antibiotics into feeds for cultured organism resulted in a global increase in antibiotic resistance among pathogenic bacteria. The problem is more serious in developing countries, where antibiotics are used widely. In India, antibiotics are extensively applied in animal husbandry and aquaculture.

The use of antibiotics is the most important factor in amplifying the level of resistance in a given reservoir (Wegener and Frimodt-Moller, 2000). Multiple antibiotic resistance (MAR) among *Aeromonas hydrophila* strains has been reported from many parts of the world (Pettibone et al., 1996; Son et al., 1997; Ko et al., 1998; Rajeswari Shome and Shome, 1999). Under these circumstances, it will be worthwhile to find out the prevalence of antibiotic resistance of the *Aeromonas* strains that may be considered as an emerging pathogen and to identify the high-risk source.

2. Materials and methods

The fish and prawn samples were collected from a major fish market of Coimbatore, Tamil Nadu, South India. The samples were collected in sterile polyethylene bags and brought to the laboratory in an ice chest. The samples were processed within 2 h of collection. Body surface, gill and intestinal content of fish were aseptically swabbed using sterile cotton buds, inoculated into alkaline peptone water (peptone—10.0 g; sodium chloride—10.0 g; distilled water—1000 ml;

* Corresponding author. Tel.: +91-422-422222; fax: +91-422-422387.
E-mail address: lps@mailcity.com (P. Lakshmanaperumalsamy).

0168-1605/02/$ - see front matter
PII: S0168-1605(02)00009-0

pH—8.4, Shread et al., 1981) for pre-enrichment at 37 °C for 18 h. The enriched cultures were streaked on starch ampicillin agar (beef extract—1.0 g; peptone—10.0 g; sodium chloride—15.0 g; phenol red—0.025 g; soluble starch—10.0 g; ampicillin—10 μg/ml; agar—15.0 g; distilled water—1000 ml, pH—7.4, Palumbo et al., 1985) plates and incubated at 37 °C for 24 h. Yellow to honey coloured, amylase and oxidase positive colonies were isolated and presumptively considered as *Aeromonas* species (Conn, 1957). Further, the isolates were identified as *A. hydrophila* using Kaper's multitest media (Kaper et al., 1979) and confirmed on the basis of biochemical characteristics (Esteve, 1995). The reference strain *A. hydrophila* MTCC 646 (Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh, India) was used for comparison.

Pure cultures were grown in brain heart infusion broth (BHIB) (Hi-Media, Mumbai, India) for sensitivity testing. Mueller Hinton agar (Hi-Media) was used for all solid media. Disc diffusion method for antibiotic susceptibility was conducted as described by Bauer et al. (1966). The *A. hydrophila* strains were tested against the following antibiotic discs (Hi-Media): bacitracin, 10 units; chloramphenicol, 30 μg; erythromycin, 15 μg; gentamycin, 10 μg; kanamycin, 30 μg; methicillin, 5 μg; nalidixic acid, 30 μg; neomycin, 30 μg; novobiocin, 30 μg; polymyxin-B, 300 μg; rifampicin, 5 μg; streptomycin, 10 μg; tetracycline, 30 μg; trimethoprim, 5 μg; vancomycin, 30 μg. After enrichment in BHIB for 6–8 h at 37 °C, the cultures were streaked on Mueller Hinton agar plates using a cotton swab. With an antibiotic disc dispenser, the discs were placed on the agar surface sufficiently separated so as to avoid overlapping of the inhibition zones. After 30 min of prediffusion time, the plates were incubated at 37 °C for 18–24 h. After the incubation period, the diameter of the inhibition zones was measured and compared with the interpretive chart of Performance Standards for Antimicrobial Disk Susceptibility Tests, Dec. 1993 (Hi-Media) and classified as resistant, intermediate and sensitive.

The MAR index when applied to a single isolate is defined as *a*/*b*, where '*a*' represents the number of antibiotics to which the isolate was resistant and '*b*' represents the number of antibiotics to which the isolate was exposed. MAR index value higher than 0.2 is considered to have originated from high-risk sources of contamination like human, commercial poultry farms, swine and dairy cattle where antibiotics are very often used. MAR index value of less than or equal to 0.2 considered as the origination of strain from animals in which antibiotics are seldom or never used (Krumperman, 1985).

3. Results and discussion

The percentage of *A. hydrophila* strains showing resistance against each antibiotic is given in Table 1. All the strains were resistant to methicillin, which was similar to the findings of Motyl et al. (1985) who reported that all *A. hydrophila* strains of human origin were resistant to methicillin. In contrast, Pettibone et al. (1996) observed that only 54% of the strains was resistant to this antibiotic. However, Kampfer et al. (1999) reported that no significant differences could be observed between clinical and non-clinical *Aeromonas* isolates, although the clinical isolates showed a few more positive results with respect to antibiotic resistance.

More than 95% of the strains was resistant to bacitracin, erythromycin, neomycin, novobiocin, polymyxin-B and rifampicin. The least resistance was noted for chloramphenicol (3.7%), gentamycin (7.5%), streptomycin (8.7%) and nalidixic acid (16.9%). About

Table 1
Percentage frequency of antibiotic resistant *A. hydrophila* strains from fish and prawns

Antibiotics	Source	
	Fish ($n=268$)	Prawn ($n=51$)
Bacitracin	99.0	100.0
Chloramphenicol	4.4	0.0
Erythromycin	97.3	98.0
Gentamycin	8.2	3.9
Kanamycin	89.9	100.0
Methicillin	100.0	100.0
Nalidixic acid	16.7	17.01
Neomycin	94.4	98.0
Novobiocin	98.8	100.0
Polymyxin-B	95.8	98.0
Rifampicin	99.6	100.0
Streptomycin	9.3	5.8
Tetracycline	53.3	41.1
Trimethoprim	64.9	80.3
Vancomycin	83.2	94.1

51.4% of the strains was resistant to tetracycline. The antibiotic resistance among the strains of both fish and prawns differed at minimum (Table 1).

Local selective pressures can influence the antibiotic resistance. Chang and Bolton (1987) found that more percentage of Asian isolates of *A. hydrophila* were resistant to tetracycline and rifampicin than Australian isolates. More than 50% of the *A. hydrophila* strains was resistant to tetracycline and occurrence of tetracycline resistant strains of *A. hydrophila* from different sources was reported (Ansary et al., 1992; Ramteke et al., 1993; Pettibone et al., 1996; Son et al., 1997; Kampfer et al., 1999).

Kanamycin, neomycin and polymyxin-B were the other antibiotics, to which a high frequency of resistance was observed (Table 1). In contrast, Ramteke et al. (1993) have not recorded any polymyxin-B resistant strains. Ramteke et al. (1993) and Pettibone et al. (1996) have not noticed any Kanamycin resistant strain, whereas the investigation of Ansary et al. (1992) supported the existence of kanamycin resistant strains, with a frequency of about 38.2%. About 67% of the *A. hydrophila* strains obtained from fish and prawn exhibited resistance against trimethoprim (Table 1). This is in contrast to the findings of Ansary et al. (1992) who have reported only 8% of the trimethoprim resistant strains.

Bacitracin resistant strains were found to be 99% and to our knowledge, such a level of resistance against this antibiotic among the strains of *A. hydrophila* has not been reported so far. Among the strains tested, most of the strains were resistant to erythromycin. This is partially supported by Ansary et al. (1992) and Son et al. (1997). However, Pettibone et al. (1996) have not reported any erythromycin resistant *A. hydrophila* strains. The variation in the drug resistance may well be related to the source of the *A. hydrophila* isolates and the frequency and type of antimicrobial agents prescribed for treating *Aeromonas* infections, e.g. in cultured fish in different geographical areas (Son et al., 1997).

The chloramphenicol resistant strains were few among *A. hydrophila* from fish. None of the strains isolated from prawns was chloramphenicol resistant. Similar findings have been recorded from Malaysian and American fish isolates (Ansary et al., 1992; Pettibone et al., 1996). Resistance towards chloramphenicol, erythormycin, kanamycin, nalidixic acid, streptomycin, sulphamethoxazole-trimethoprim and tetracycline has been observed among *A. hydrophila* isolates from *Tilapia mossambica* (Son et al., 1997).

About 8.2% of *A. hydrophila* strains from fish and 3.9% strains from prawns were found resistant to gentamycin. Ansary et al. (1992) reported that about 23.5% of the *A. hydrophila* strains isolated from healthy and diseased fish expressed resistance to this antibiotic, which was considerably higher than the resistance encountered in our findings. However, Ramteke et al. (1993) reported that none of the *A. hydrophila* strains from fish and environmental samples was resistant to gentamycin.

In the antibiotic era, increase in the levels of resistance of clinical strains of *A. hydrophila* to commonly used antibacterial agents has been observed (Ko et al., 1996). Like enteric Gram-negative bacteria, the emergence of resistance among aeromonads will be accelerated by the clinical use of antibiotics (Chaudhury et al., 1996).

The results of MAR index of *A. hydrophila* strains and the percentage of occurrence are given in Table 2. The strains from fish showed resistance to minimum of at least three antibiotics. About 31.3% of strains was resistant to 10 antibiotics followed by 26.1%, 17.5% and 10.4% of 11, 9 and 12 antibiotics, respectively. Interestingly, 1.9% of the strains was resistant to 14 antibiotics. *A. hydrophila* strains from prawns exhibited the resistance between 8 and 13 antibiotics. About 45.1% of the strains was resistant to 10 anti-

Table 2
The percentage occurrence of multiple antibiotic resistance (MAR) index of *A. hydrophila* strains from fish and prawns

MAR index	Source	
	Fish ($n=268$)	Prawns ($n=51$)
0.1	0	0
0.2	0.37	0
0.33	0.74	0
0.40	0.74	0
0.46	2.98	0
0.53	5.22	3.92
0.60	17.53	5.88
0.66	31.34	45.09
0.73	26.49	37.25
0.80	10.44	5.88
0.86	2.23	1.96
0.93	1.86	0

biotics followed by 37.3% to 11 antibiotics. Six percent of the strains was resistant to 9 and 12 antibiotics, while 3.9% and 2% were resistant to 13 and 8 antibiotics.

The results revealed that the strains might have originated from high-risk source of contamination. MAR *A. hydrophila* were reported from environmental sources as well as freshwater fish (Pathak et al., 1993; Pettibone et al., 1996). The release of MAR organisms through faeces may ultimately pave way for the contamination of fish and shellfish in the aquatic environment (Grabow et al., 1973, 1976).

Acknowledgements

The authors are thankful to the Head, Department of Environmental Sciences, Bharathiar University, Coimbatore for providing necessary laboratory facilities and also to Indian Council of Agricultural Research (4 (49)/97-ASR-1 dt 22.09.2001) for the financial support.

References

Ansary, A., Haneef, R.M., Torres, J.L., Yadav, M., 1992. Plasmids and antibiotic resistance in *Aeromonas hydrophila*. J. Fish Biol. 15, 191–196.

Bauer, A.W., Kirby, W.M.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standard single disk method. Am. J. Clin. Pathol. 36, 493–496.

Chang, B.J., Bolton, S.M., 1987. Plasmids and resistance to antimicrobial agents in *Aeromonas hydrophila* clinical isolates. Antimicrob. Agents Chemother. 31, 1281–1282.

Chaudhury, A., Nath, G., Shukla, B.N., Sanyal, S.C., 1996. Biochemical characterization, enteropathogenicity and antimicrobial resistance plasmids of clinical and environmental *Aeromonas isolates*. J. Med. Microbiol. 44, 434–437.

Conn, H.J., 1957. Staining methods. In: Society of American Bacteriologists Committee on Bacteriological Technic (Eds.), Manual of Microbiological Methods. McGraw-Hill Book, New York, p. 16.

Esteve, C., 1995. Numerical taxonomy of Aeromonadaceae and Vibrionaceae associated with reared fish and surrounding fresh and brackish water. Syst. Appl. Microbiol. 18, 391–402.

Grabow, W.O.K., Middendorff, I.G., Prozesky, O.W., 1973. Survival in maturation ponds of coliform bacteria with transferable drug resistance. Water Res. 7, 589–1597.

Grabow, W.O.K., Van Zyl, M., Prozesky, O.W., 1976. Behaviour in conventional purification process of coliform bacteria with transferable and non-transferable drug resistance. Water Res. 10, 717–723.

Kampfer, P., Christmann, C., Swings, J., Huys, G., 1999. In vitro susceptibilities of *Aeromonas* genomic species to 69 antimicrobial agents. Syst. Appl. Microbiol. 22, 662–669.

Kaper, J.B., Lockman, H., Colwell, R.R., 1979. Medium for the presumptive identification of *A. hydrophila* and enterobacteriaceae. Appl. Environ. Microbiol. 38, 1023–1026.

Ko, W.C., Yu, K.K., Liu, C.Y., Huang, C.T., Leu, H.H., Chuang, Y.C., 1996. Increasing antibiotic resistance in clinical isolates of *Aeromonas* strains in Taiwan. Antimicrob. Agents Chemother. 40, 1260–1262.

Ko, W.C., Wu, H.-M., Tsung, C.C., Yan, J.-J., Wu, J.-J., 1998. Inducible β-lactam resistance in *A. hydrophila*: therapeutic challenge for antimicrobial therapy. J. Clin. Microbiol. 36, 3188–3192.

Krumperman, P.H., 1985. Multiple antibiotic indexing of *E. coli* to identify high-risk sources of fecal contamination of foods. Appl. Environ. Microbiol. 46, 165–170.

Motyl, M.R., McKeinely, G., Janda, J.M., 1985. In vitro susceptibilities of *Aeromonas hydrophila*, *Aeromonas sobria* and *Aeromonas caviae* to 22 antimicrobial agents. Antimicrob. Agents Chemother. 28, 151–153.

Palumbo, S.A., Maxino, F., Williams, A.C., Buchanan, R.L., Thayer, D.W., 1985. Starch–ampicillin agar for the quantitative detection of *Aeromonas hydrophila*. Appl. Environ. Microbiol. 50, 1027–1030.

Pathak, S.P., Gaur, A., Gopal, K., 1993. Distribution and resistance pattern in *Aeromonas hydrophila* from some organs of catfish, *Clarias batrachus*. Indian J. Microbiol. 33, 195–200.

Performance Standards for Antimicrobial Disk Susceptibility Tests, Dec. 1993, 4th edn., NCCLS. 10 (7).

Pettibone, G.W., Mear, J.P., Sampsell, B.M., 1996. Incidence of antibiotic and metal resistance and plasmid carriage in *Aeromonas* isolated from brown bullhead (*Ictalurus nebulosus*). Lett. Appl. Microbiol. 23, 234–240.

Rajeswari Shome, Shome, B.R., 1999. Antibiotic resistance pattern of fish bacteria from freshwater and marine sources in Andamans. Indian J. Fish. 46, 49–56.

Ramteke, P.W., Pathak, S.P., Gautam, A.R., Bhattacherjee, J.W., 1993. Antibiotic susceptibility pattern of *Aeromonas* isolated from drinking water sources. Int. J. Toxicol., Occup. Environ. Health 2, 32–34.

Shread, P., Donovan, T.J., Lee, J.V., 1981. A survey of the incidence of *Aeromonas* in human faeces. Soc. Gen. Microbiol. Q. 8, 184.

Son, R., Rusul, G., Sahilah, A.M., Zainuri, A., Raha, A.R., Salmah, I., 1997. Antibiotic resistance and plasmid profile of *Aeromonas hydrophila* isolates from cultured fish, Tilapia (*Tilapia mossambica*). Lett. Appl. Microbiol. 24, 479–482.

Wegener, H.C., Frimodt-Moller, N., 2000. Reducing the use of antimicrobial agents in animals and man. J. Med. Microbiol. 49, 111–113.