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Antibiotic Resistance of *Aeromonas* SP. Isolated from Marketed Fish Samples

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ABSTRACT

Aeromonas sp. are opportunistic pathogens causing a wide range of diseases including urinary tract infection, peritonitis, gastroenteritis. Special attention was paid to *Aeromonas* sp. due to its association with a wide range of human illnesses. In the present studya total of 200 *Aeromonas* sp. were isolated from 350 fish samples. All the isolates were tested for resistance to 10 antibiotics and all the isolates were resistant to methicillin and erythromicin, 97% to rifampicin, 91% to vancomycin, 85% towards trimethoprim, 82% towards cefaperazone, 81% to cefamendole, 62% towards tetracycline, 58% to chloramphenicol and 59% to gentamicin. The least percentage of the resistance was shown towards chloramphenicol. The multiple antibiotic resistance indexing of *Aeromons* sp showed that all of them originated from high risk sources.

Keywords: *Aeromonas*, Fish, Antibiotic resistance.

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INTRODUCTION

The emergence of resistance of bacteria to antibacterial drugs is a common phenomenon. Emergence of resistance reflects evolutionary processes that take place during antibacterial drug therapy. The antibacterial treatment may select for bacterial strains with physiologically or genetically enhanced capacity to survive high doses of antibacterials[1]. Antibiotic-susceptibility pattern is also important for selective isolation of microorganisms.[2] Environmental contamination with antibiotics and other pollutants contributes to the maintenance and spread of antibiotic resistance genes.[3]Resistance to diverse groups of antibiotics is another concerning characteristic of *Aeromonas* sp.[4]High resistance rates against penicillin's and first generation cephalosporins have been described and are associated with the production of chromosomally encoded beta-lactamases.[5] Due to these reasons, it will be worthwhile to find out the prevalence of antibiotic resistance of the *Aeromonas* sp. that may be considered as an emerging pathogen and to identify the high risk source.

MATERIALS AND METHOD

The fish gut samples were collected in pre-sterilized polyethylene bags from Champakkara market of Ernakulam district and transported to the laboratory in a portable ice chest. Sample processing was done aseptically within 2 - 5 h of collection. Intestinal tissue weighing 5 g was homogenized with 10 ml of sterile water and centrifuged at 3500 rpm for 5 min at 4 °C. The supernatant was serially diluted until $1x10^{-8}$ dilution. Pipetting up and down in the liquid for few times will ensure that the sample in the tube is mixed and uniform. About 100 µL from each dilution was spread evenly over the entire surface of the starch ampicillin agar plate and the plates were incubated at 37 °C for 24 hours. A characteristic yellow to honey coloured colonies were selected and used for further testing. After enrichment and streaking onto SAA, honey coloured colonies were subjected to Gram staining as well as enzymatic tests such as oxidase and catalase were also performed. The oxidase and catalase positive colonies were then purified by repeated streaking on the nutrient agar and were maintained in the nutrient agar slants. Antibiotic sensitivity test was carried out by disc diffusion method.[6] The *Aeromonas* sp. were tested against the following antibiotic discs: gentamycin (10 mcg); chloramphenicol (30mcg); cefamendole (30mcg)); rifampicin (5mcg); erythromycin(15 mcg); trimethoprim (5mcg); tetracycline (30mcg); vancomycin (30mcg); cefoperazone (75mcg); methicillin (5mcg).Mueller-Hinton agar (HiMedia, India) were prepared, sterilized and poured onto sterile petriplates. The medium was allowed to solidify. Pure cultures grown in nutrient broth for 6–8 hours were swabbed over MHA using sterile cotton swabs. Using antibiotic disc dispenser, discs were placed on the agar surface with sufficient space so as to avoid overlapping of inhibition zones. After thirty minutes of pre-diffusion time, the plates were incubated at 37 ^{o}C for 18-24 hours. After incubation, the diameter of the inhibition zone was measured and compared with the interpretative chart provided by the manufacturer and classified as resistant, intermediate and sensitive. Multiple antibiotic resistance (MAR) index of an isolate is the number of antibiotics to which the test isolate displayed resistance divided by the total number of antibiotics to which the test organism has been evaluated for sensitivity. It is denoted by a/b, where 'a' represents the total number of antibiotics to which the test isolate displayed resistance and 'b' represents the total number of antibiotics to which the test organisms has been evaluated for sensitivity. MAR index value higher than 0.2 is considered that the isolate have originated from high risk source of contamination. MAR index value less than or equal to 0.2 is considered that the isolate have originated from animals in which antibiotics are used very rarely or never used. [7]

The percentage of *Aeromonas* sp. showing resistance against each antibiotic is given in Table1.

Table 1.Percentage of *Aeromonas* isolates showing resistance to various antibiotics

Antibiotics	Percentage of resistance (%)
Cefamandole	81
Cefaperazone	82
Chloramphenicol	58
Erythromycin	100
Gentamycin	59
Methicillin	100
Rifampicin	97
Tetracyclin	62
Trimethoprim	85
Vancomycin	91

RESULTS AND DISCUSSION

All the 200 isolates were subjected to antibiotic susceptibility test against 10 commercially available antibiotics for screening of multidrug resistance. In the present study, all the isolates were resistant to methicillin and erythromicin, 97% to rifampicin, 91% to vancomycin, 85% towards trimethoprim, 82% towards cefaperazone, 81% to cefamendole, 62% towards tetracycline, 58% to chloramphenicol and 59% to gentamicin. The least percentage of the resistance was shown towards chloramphenicol. The results are presented in Figure 1.

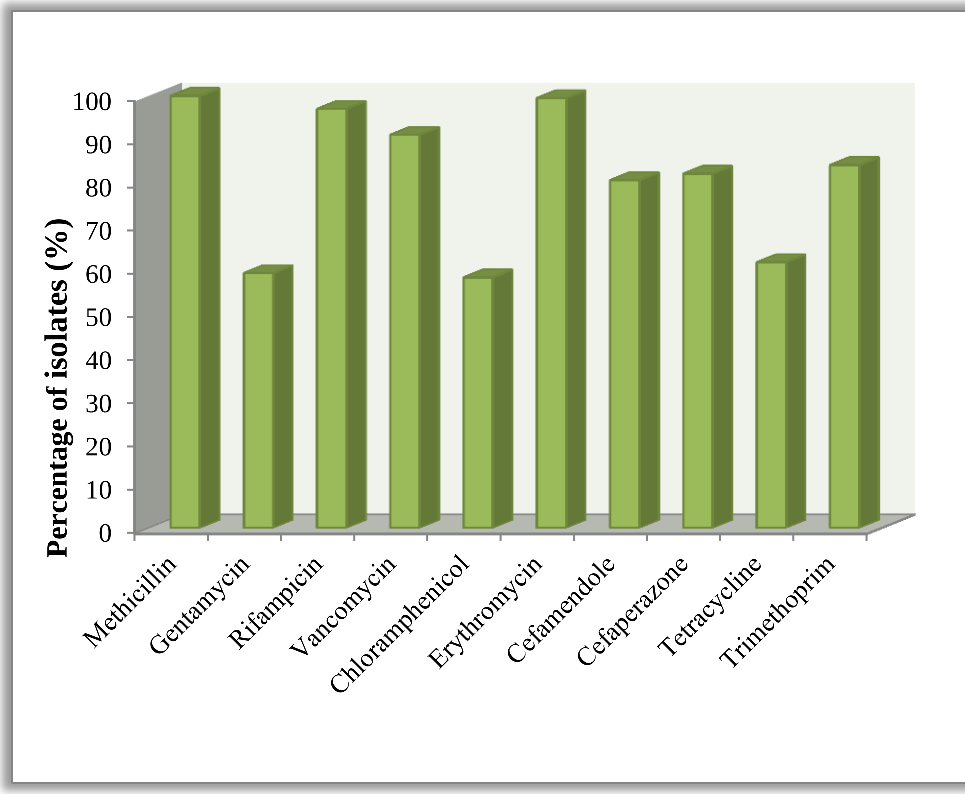

Figure 1: Percentage of *Aeromonas* isolates showing resistance to various antibiotics

All *A. hydrophila* strain of human origin were resistant to methicillin[8] and also in another study all strains of *A. hydrophila* from marketed fish and prawn were resistant to methicillin and more than 95% isolates were resistant to erythromycin.[9]This shows a consistency in the resistance pattern as the repeated exposure to the same antibiotics therefore provides selective pressure, which makes the surviving bacteria more likely to be resistant.

The results of *Aeromonas* sp. showing resistance against each antibiotic is given in figure 4.3. Multidrug resistance was exhibited by most of the isolates. The isolates showed resistance to a minimum of atleast 3 antibiotics. A maximum of 35 (18%) isolates showed resistance to all the 10 antibiotics and 27% of isolates to 9 antibiotics. Further, 26.5% isolates showed resistance to 8 antibiotics, 17% to 7 antibiotics, 6% to 6 antibiotics, 4% to 5 antibiotics, 1.5% to 4 antibiotics and 0.5% to 3 antibiotics (figure 2).

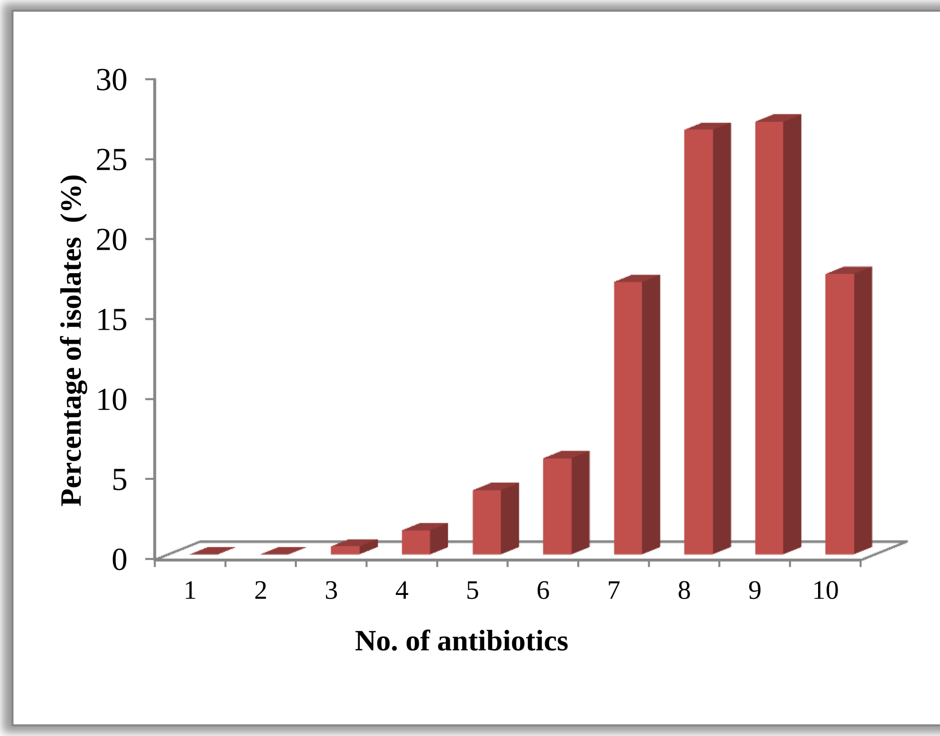

Figure 2: Percentage of isolates resistance to number of antibiotics

Aeromonas aquariorum was susceptible to chloramphenicol and tetracycline and 13% of the isolates were resistant to gentamicin[10]but in the current study more than 50% of the isolates were resistant towards these antibiotics, this discrepancy might be due to the difference in the source of the isolates, which is of fish origin in the current study whereas theirs were from stool samples.

In a study, 23.5% of the *A. hydrophila* strains of fish origin were resistant to gentamicin[11] and also the *Aeromonas* isolates from fish samples showed 93.1% resistance towards gentamicin[12]. In this study about 59% of the isolates from fish samples were resistant to gentamicin. This difference might be due to the geographical location and local selective pressure which influences the level of the antibiotic resistance. Chloramphenicol resistance is an extremely rare trait in *Aeromonas* sp[13]. Sensitivity of *Aeromonas* towards chloramphenicol is reported earlier [14]. Similarly low-level of resistance towards chloramphenicol was observed among the isolates of this study compared to the other antibiotics used. Low-level of resistance to chloramphenicol in *Aeromonas* sp. may be due to decreased permeability. From the present study, chloramphenicol was found to be more effective in inhibiting growth of the *Aeromonas* sp. isolates than other drugs. Therefore, it would be the first drug of choice in application against *Aeromonas*. Antibiotic resistance study in frozen fish isolates resulted in 44% of resistance to tetracycline, 58.4% to gentamycin, 54.5% to erythromycin, 9% to chloramphenicol and 57.1% to rifampicin[15].

Mesophilic aeromonads from three riverine freshwaters showed 37% resistance to cefamandole, 17% to trimethoprim and 15% to erythromycin [16]. Resistance to gentamycin, chloramphenicol, tetracycline and rifampicin was found to be <5%. The results of the above mentioned study were contrasting to the results of our study in which the isolates showed higher resistance to all the above antibiotics. Such a high level of multiple resistance may arise from selective pressure due to the indiscriminate use of antibiotics. In the present study resistance towards rifampicin was found to be 97%, which is similar to the study of Pettibone and colleagues[17]where 97% of *A. hydrophila* strains from fish were resistant to rifampicin and 85% to vancomycin. This study is in contrast to Alzainy who reported that 15.4% resistance was observed towards rifampicin. Also the present study shows that 50% increase in the resistance towards tetracycline (62%) and chloramphenicol (58%) compared to the isolates of lived fish and frozen fish in which 33.4% showed resistance to tetracycline and 30.8% towards chloramphenicol[18]. Almost all the 57 isolates of *Aeromonas* from food samples such as fresh and frozen chicken, game birds, pasteurized milk, baby food, bakery products, fruit, vegetables, fish and water were resistant to vancomycin one exception [19]and is similar to the isolates of this study in which 91% were resistant to vancomycin. These results show that the strains might have been previously exposed to antimicrobial drugs and developed resistance. This means that antimicrobial drugs are used inappropriately and a further development of the resistance may be expected, so the number of effective antimicrobial drugs is diminishing. Since *Aeromonas* is a microorganism that may threaten human health, transmission of the reduced susceptibility may have negative consequences for humans. All the strains of *Aeromonas* were resistant to tetracycline and furthermore the highest resistance encountered was trimethoprim (67.9%)[20]. The resistance level to trimethoprim in our study was found to be 85% which was comparable to the findings of Yucel and Citak [21], who has reported 63%-100% of trimethoprim resistant strains, and these results are supported by Vivekanandhan and colleagues [9]who have reported that 67% of resistant isolates from marked fish and prawn but this is in contrast to the findings of another study which has reported that only 8% were resistant to trimethoprim[11]. These differences may be related to the source and species of *Aeromonas* recovered, the method of isolation, the frequency of use of certain antimicrobial agents in a specific geographic area, or to other unknown factors. In a previous study it has been reported that the third-generation cephalosporins moxalactam, cefotaxime, and cefoperazone were uniformly active against the 60 isolates of three different species of *Aeromonas* and second-generation cephalosporins (cefoxitin and cefamandole) inhibited approximately 50% of the 60 strains tested (8) but the results of this study was contradicting to the above report, by isolates showing 81% resistance to second-generation cephalosporins (cefamandole) and 82% resistance to third-generation cephalosporins (cefoperazone). Out of 8 strains of *Aeromonas* from skin infections of common freshwater fish only two strains were resistant to cefamandole[22] which is also contradicting to the current study. 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 [23]. The results obtained in this study indicate that multiple antibiotic resistance among the isolates is often seen in *Aeromonas* sp. isolated from fish, since geographic locations and local selective pressure influence the antibiotic resistance levels. Multidrug resistant *A. hydrophila* were reported from environmental sources as well as freshwater fish.[17]The results of MA Rindex of *Aeromonas* sp. and the percentage of occurrence are given in Table 2.

Table 2 Percentage of isolates showing MAR frequency

MAR Index	Percentage of isolates (%)
0.2-0.4	0.5
0.4-0.6	5.5
0.6-0.8	23
0.8-1	71

In the present study about 71% of the isolates showed MAR index value between 0.8-1. This shows that the multidrug resistant *Aeromonas* sp. was predominant in environmental samples. The MAR index results revealed that the strains might have originated from high-risk source of contamination. In this study, high incidence of multiple antibiotic resistance amongst *Aeromonas* species isolated from marine fishes was observed suggesting that the seawater is extremely polluted. A high incidence of antimicrobial resistance, has been found among *Aeromonas* sp. isolated from marine fishes in developing countries like India has to be considered as serious public health concern. Emergence of drug resistant microbe is a global concern. Wide spread use of antibiotics for treating bacterial diseases, sub-therapeutic use of antibiotics in animal husbandry and aquaculture are held responsible for emergence of antibiotic resistance [24]. In developing countries including India, the situation is more precarious due to less stringent regulatory control of antibiotics with extensive use of antibiotics in animal husbandry and aquaculture [25, 9].

CONCLUSION

The increasing rates of antimicrobial resistance observed in *Aeromonas* sp. is perceived as a potential danger for human health. The present study points out that environmental contamination with antibiotics and other pollutants contributes to the maintenance and spread of antibiotic resistance genes. Special attention should be given to the indiscriminate use of antibiotics, such as erythromycin, methicillin rifampicin and vancomycin, since the resistance shown is already high. The trend of consuming ready to eat semi-raw foods is getting popular. Fishes and raw meat may be an important reservoir for *Aeromonas* sp. and of public health significance.

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