

Antibiotic Resistance in *Micrococcus sp* Isolated from Freshwater Fish Farms *Catla Catla*

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Abstract:

Freshwater and marine fish are affected by very few pathogens, in specific flexibacteria, aeromonads and vibrios, several pathogens may present as only skin infections. Ulcer, hemorrhage, scale reduction, tail and fin rot, dropsy involves many bacterial diseases, is present in fishes. Ten bacterial genera were isolated, where these fish pathogens belonging to *Micrococcus sp.*, *Bacillus sp.*, *Aeromonas sp.*, *Flavobacterium sp.*, *Staphylococcus sp.*, *Enterobacteria sp.*, *E. coli*, *Pseudomonas sp.*, *Salmonella sp.*, *Shigella sp.*, associated with *Catla catla*. These pathogen isolated from freshwater fish *Catla catla* were characterized the pathogen as being susceptible to antibiotics such as Amoxycillin, Azithromycin, Cefixime, Ciprofloxacin, Chloramphenicol, Furazolidin, Gentamicin, Levofloxacin, Tetracycline and Vancomycin. The present study reports the results of inhibition of pathogen by Amoxycillin and Azithromycin.

Introduction:

Antibiotics are microorganism-generated compounds and have many applications, such as infection control, prophylaxis, food preservation, and as growth promoters (Refsdal and Forsberg, 2000). The emergence of resistance is an incredibly complicated process and its environmental importance is not well understood (Alanis, 2005). Resistance to bacteria from various environments has risen at an unprecedented pace and is becoming a major concern worldwide. In the environment, the prevalence of resistant bacteria depends on the exposure of these bacteria to various antibiotics, resulting in the production of new strains of bacteria resistant to specific antibiotics.

Since no antibiotics are used for animals in the wild, antibiotics are susceptible to bacteria isolated from those sources. However the emergence of antibiotic resistant strains from wildlife sources has been triggered by many human activities and resistant bacteria have been detected in the aquatic environment (Afser and Ali, 1981). The discharge of sewage effluent

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into water is known to be one of the routes of transition to aquaculture of antibiotic resistant bacteria. The isolation from fish and sea water of antibiotic resistant bacteria in which urban waste is discharged suggests a risk to health.

In normal enteric bacteria and pathogenic bacteria, Oomycetes fungi (Barde et al. 2020, 2021) the overuse of antibiotics has undoubtedly led to multiple antibiotic resistance. Antibiotic abuse not only increases the tolerance of pathogenic bacteria, but also of indigenous animal flora, which in turn affects the normal human microbial flora (Al-Zahrani, 1988). In both pathogenic and natural flora bacteria that inhabit our intestinal tract, multiple resistance to antibiotics is commonly found.

Several antibiotic resistant bacteria were isolated at different locations in Muscat, Oman, from various sewage effluents (Al-Bahry et al., 2009). Moreover, the presence of multiple resistance to antibiotics in green turtle eggs in Oman was controlled (Al-Bahry et al., 2009, 2011). The value of these findings is because the green turtles in Oman are the world's second largest species. Thus, in their migratory and feeding environments, green turtles may be exposed to various contaminants, such as sewage. It is still under investigation whether sewage effluent can be a factor in the spread of multiply resistant bacteria to marine environments in the Gulf of Oman.

Antibiotic in aquaculture

The discovery of antibiotics, fish farmers and livestock producers' growth-promoting and disease-fighting capabilities started with the use of such drugs in animal feed. For animals, antibiotics routinely used for the treatment of human infections are often used, either for medication, for prophylactic reasons or for promotion of growth. Subtherapeutic doses of antibiotics have generally been used for the last reason, and this has led to the promotion of resistance.

Accumulated scientific evidence suggests that some uses of antibiotics in animals that manufacture food can lead to resistance to antibiotics in intestinal bacteria, and that this resistance can then be spread to the general population, causing diseases that are immune to medication. These antibiotic uses can also give rise to antibiotic resistance in non-pathogenic bacteria, whose resistance genes can be transmitted to disease-causing bacteria, resulting in human infections that are immune to antibiotics. The study from the Invitation Conference of the European Union on the Microbial Threat (EU, 1998) acknowledged that the food chain is the main route of transmission of resistant microorganisms from animals to humans. Other authors confirm this pattern (Nawaz et al., 2001).

The incidence of resistance in non-typhoidal *Salmonella enterica* and *Campylobacter* spp., and to a lesser degree in *Vero cytotoxin-producing Escherichia coli* 0157 coli, has been substantially increased in developed countries (VTEC 0157). In non-typhoidal S, there is also a rise in the occurrence of resistance. In developing countries, however these rises have been almost entirely associated with the use of antimicrobials in human medicine, unlike the situation found in developed countries (Theobald et al., 2000).




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According to a survey by the European Federation of Animal Health (FEDESA), 4700 tons (35 percent) of all antibiotics administered in the European Union were consumed by farm animals in 1999, while 8500 tons were consumed by humans (8500 tons) (65 percent). Of the antibiotics administered to livestock, 3900 tons (29% of total use were administered to help sick animals recover from disease, while 786 tons (6% of total use were fed as growth promoters to farm animals. The survey reported that since 1997, when animals consumed approximately 1600 tons as feed additives, the amount of antibiotics used as growth promoters had dropped by half (EU, 2002).

Material and Methodology

Specimen collection

Fish specimens are collected from local water reservoirs of marathwada region, Maharashtra, India and transported to laboratory in crushed ice container. All the chemicals and media used in this work were obtained from Himedia Pvt. Ltd, Mumbai.

Isolation of Microorganisms

Fish specimens are proceeded for normalized at room temperature by placing in distilled water for 1Hr, followed by addition of 1gm sample to nutrient broth enrichment of microorganisms in Nutrient Broth. Microorganisms were isolated by spread plate and streak plate method on nutrient agar. Identification were carried out with the help of physiological and biochemical tests and Bergey's manual of systematic Bacteriology.

Antibiotic sensitivity test

All biochemically characterized isolates were proceeded for antibiotic sensitivity test by disc diffusion method. 24hr old active culture were spread on nutrient agar plate and antibiotic soaked discs were placed aseptically over it. After 24hr incubation at optimum conditions, plates were observed for zone of inhibition.

Results and Discussion:

All the biochemically characterized isolates were also subjected to antibiotic sensitivity test. The presence of clear area (zone of inhibition) surrounding the antibiotic disk represents the sensitivity of bacterium and absence of clear area represents the resistance of bacterium. Results were interpreted as per standard of CLSI (Clinical and Laboratory Standards Institute).

Table 1: Antibiotic sensitivity pattern of bacterial isolates from *Catla catla*.

Sr. No	Bacterial genus	Total Nos of isolated identified	Nos of isolated resistance antibiotics	
1	<i>Micrococcus sp.</i>	17	11	64.7
2	<i>Bacillus sp.</i>	16	10	62.5
3	<i>Corynebacteria sp.</i>	13	2	15.4
4	<i>Alcaligenes sp.</i>	14	2	14.3

5	<i>Aeromonas sp.</i>	18	10	55.6
6	<i>Streptococcus sp.</i>	11	1	9.1
7	<i>Flavobacterium sp.</i>	15	9	60.0
8	<i>Clostridium sp.</i>	12	1	8.3
9	<i>Proteus sp.</i>	11	1	9.1
10	<i>Staphylococcus sp.</i>	16	6	37.5
11	<i>Enterobacteria sp.</i>	17	6	35.3
12	<i>E. coli</i>	18	5	27.8
13	<i>Pseudomonas sp.</i>	18	6	33.3
14	<i>Chromobacterium sp.</i>	10	1	10.0
15	<i>Citrobacter sp.</i>	9	2	22.2
16	<i>Klebsiella sp.</i>	10	2	20.0
17	<i>Salmonella sp.</i>	16	7	43.8
18	<i>Serratia sp.</i>	11	1	9.1
19	<i>Shigella sp.</i>	17	4	23.5

Of the seventeen different genera of bacteria isolated from *Catla catla* from all the district of Marathwada, the most dominant ten genus which were found to be dominant in appearance and showing more resistance to test antibiotics were selected. The genus belonging to *Micrococcus sp.*, *Bacillus sp.*, *Aeromonas sp.*, *Flavobacterium sp.*, *Staphylococcus sp.*, *Enterobacteria sp.*, *E. coli*, *Pseudomonas sp.*, *Salmonella sp.*, *Shigella sp.*, were selected as these genera are known to be associated with fishes and human diseases.

In aquaculture, antimicrobial agents are used as growth promoter for maximum food production and for clinical treatment of infectious diseases in farm animals, so as to control further transmission of infection in animals as well as in humans. Inadequate selection or indiscriminate use of antimicrobial agents may develop resistance in bacteria. Thus, the clinical treatment of these infections become more complicated because of the development of bacterial resistance to most first-line antibiotics (i.e. amoxicillin, ciprofloxacin, levofloxacin). These resistant bacteria could pass to human via food chain (Papadopolou *et al.*, 1997), contact via industrial exposure, waste runoff from animal production facilities (Schroeder *et al.*, 2002) and are responsible for the cause of foodborne diseases. Several studies have been reported on antibiotic susceptibility of bacteria and many of the resistant bacteria isolated from fishes-related samples and which particularly include *Escherichia spp.*, *Enterobacter spp.*, *Salmonella spp.*, other *Enterobacteriaceae* members (Sugita *et al.*, 1996; Spanggaard *et al.*, 1993; Novita 2020; Kanchan *et al.*, 2020).

Antibiotic Sensitivity in *Micrococcus sp.*

Out of Seventeen samples, 8.76 ± 2.44 % of the isolates were resistant to Amoxycillin, none of isolates were resistant to Azithromycin, 22.36 ± 1.31 % of the isolates were resistant to Cefixime, 60.08 ± 1.57 % of the isolates were resistant to Ciprofloxacin, 90.34 ± 2.19 % of the isolates were resistant to Chloramphenicol, 67.14 ± 1.01 % of the isolates were resistant to

Furadantin, $65.78 \pm 1.31\%$ of the isolates were resistant to Gentamicin, $85.96 \pm 0.87\%$ of the isolates were resistant to Levofloxacin, $6.13 \pm 1.16\%$ of the isolates were resistant to Tetracycline, and none % of the isolates were resistant to Vancomycin

With *Micrococcus sp.* $82.89 \pm 0.76\%$ of the isolates were susceptible to Amoxycillin, 100 % of isolates were resistant to Azithromycin, $70.17 \pm 1.58\%$ of the isolates were susceptible to Cefixime, $32.01 \pm 1.16\%$ of the isolates were susceptible to Ciprofloxacin, $4.38 \pm 0.87\%$ of the isolates were susceptible to Chloramphenicol, $24.99 \pm 2.00\%$ of the isolates were susceptible to Furadantin, $27.62 \pm 2.00\%$ of the isolates were susceptible to Gentamicin, $10.52 \pm 0.75\%$ of the isolates were susceptible to Levofloxacin, $90.78 \pm 1.31\%$ of the isolates were susceptible to Tetracycline, and $96.48 \pm 0.43\%$ of the isolates were susceptible to Vancomycin

So, the data suggests that, Vancomycin, Tetracycline and Amoxycillin are the most suitable antibiotic for inhibition the growth of bacteria at the optimum level. The resistance of the bacterium indicates the exposure to antibiotics.

Table 2: Antibiotic sensitivity pattern of *Micrococcus sp.* Isolates from *Catla catla*.

Antibiotic	Sensitive(%)	Intermediate(%)	Resistance(%)
Amoxycillin	82.89 ± 0.76	8.33 ± 1.91	8.76 ± 2.44
Azithromycin	100	0	0
Cefixime	70.17 ± 1.58	6.13 ± 0.43	22.36 ± 1.31
Ciprofloxacin	32.01 ± 1.16	7.89 ± 1.51	60.08 ± 1.57
Chloramphenicol	4.38 ± 0.87	5.69 ± 2.32	90.34 ± 2.19
Furadantin	24.99 ± 2.00	7.45 ± 1.16	67.1 ± 2.01
Gentamicin	27.62 ± 2.00	6.57 ± 0.75	65.78 ± 1.31
Levofloxacin	10.52 ± 0.75	4.38 ± 0.87	85.96 ± 0.87
Tetracycline	90.78 ± 1.31	3.06 ± 0.43	6.13 ± 1.16
Vancomycin	96.48 ± 0.43	3.50 ± 0.43	0

Resistance is expressed in two types. First, latent or intrinsic tolerance, that is to say, the species is not usually susceptible to the drug in question. This may be due to the failure of the antibacterial agent to penetrate and meet the target site of the bacterial cell, or to the absence of affinity between the antibacterial agent and its target (site of action), or to the absence of a target in the cell. Second, resistance gained, where the species is usually susceptible to a specific drug, but certain strains demonstrate resistance to the drug, which may be mediated by a variety of mechanisms (Yurima *et al.*, 2014).

The antibiotic is no longer capable of healing or treating the illness caused by the infective agent as resistance arises. A small rise in the minimum inhibitory concentration (MIC) for the antibiotic from the normal value, which is not usually of clinical importance, may detect a low level of resistance (Armando *et al.*, 2016; Barde, 2021).

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