Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

Estimation of Bacterial contamination in different Fresh Water fishes and implementation of time-dependent UV-radiation to control the existence of Fish Microflora

1. Dr. PYV Satyanarayana
Head, Dept. of Zoology & Fisheries
Sri YN College(A)
Narsapur.

2. Sub Lt. G.Sunitha
Lecturer in Zoology & Fisheries
Sri YN College(A)
Narsapur.

3. P.Vazra.Suneela
Head, Dept. of Microbiology
Sri YN College(A)
Narsapur.

ABSTRACT

The present study attempted to evaluate the existence and survival of microorganisms in common sea fishes available in Narsapur; West Godavari District and also evaluated the effects of UV_radiation to reduce the proliferation of bacterial pathogens. A total of 4 categories of fresh water fishes were collected from local markets in Narsapur. Each sample was subjected to UV-irradiation at different time intervals such as 5 min, 10 min & 15 min. The bacterial existence pattern of the isolates were determined through biochemical identification, respectively. Most of the raw fish samples were found to be contaminated with huge number of microorganisms including
the coliforms (E.coli and Klebsiella spp.), Salmonella spp., Shigella spp., Staphylococcus spp., Pseudomonas spp.The incidence of coli forms and other harmful bacteria is considered a serious threat to the public health upon consumption of such fishes. When subjected to UV-irradiation, the propagation rate of pathogens was significantly reduced, in many cases 100% reduction was observed, especially after 15 min of UV radiation.

KEYWORDS

Bacteria, Fresh Water fish, Fish pathogen, UV-radiation

1. INTRODUCTION

Fish is one of the major food sources globally, serving as a source of micronutrients of significance. It is also the basis of many livelihoods. In Narsapur; West Godavari District, fishes contribute to around 60% of the total national demand for animal proteins and contribute to economic growth as well as poverty alleviation. The fisheries sector in Narsapur supports the livelihood of more than 84,000 people, directly and indirectly. Thus, ensuring the production of high-quality and safe fish products has great global and national importance.
A major challenge to maintain the quality of fish products is their susceptibility to various pathogenic microorganisms. Additionally, antibiotic-resistant bacteria are also prevalent in fish products as a result of extensive use of antibiotics in agriculture sectors as well as in aquaculture systems .Microorganismslike *E.coli,* Klebsiella *spp*., Pseudomonas *spp*., Listeria spp., Aeromonas *spp*., *Salmonella spp*., and Staphylococcus aureus, identified in various fishes produced in Narsapur were found to be resistant against commercially available antibiotics . Consuming contaminated fish could cause the spread of major food-borne diseases globally, where the typical antibiotics might not be useful for treatment. Therefore, it is crucial to explore strategies to eliminate microorganisms from fish products before exporting and selling them to consumers.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

The strategies to minimize microbial contamination of fish products are not widely investigated. Over the last decade, gamma irradiation , chemical treatment with formic acid , acidic electrolyzed oxidizing water , ultrasound, and UV radiation have been found to be successful in decontaminating fishes. To minimize the health impacts of gamma irradiation in our previous works, this study utilized UV radiation. UV radiation is low-cost and non-ionizing radiation with germicidal properties at wavelengths in the range of 200–280 nm. The UV ray can damage microbial DNA and inactivate their cellular functions .The downside of using the UV method is that accidental exposure to UV rays can cause skin burns and eye injuries. Nonetheless, if proper precaution is taken, it is a relatively safe and effective method. The primary goal of this study was to detect the presence of drug-resistant bacteria in various fishes, available in the local markets of Narsapur. After identifying the bacteria, the efficacy of the UV radiation was tested for eliminating the bacteria from the fish samples. This work is a contribution toward the global effort of ensuring food safety for consumers and reducing the outbreak of diseases caused by drug-resistant bacteria.

2. MATERIALS AND METHODS

2.1. STUDY AREA AND SAMPLING

A total of 04 fish samples of 04 categories were analyzed: *Arius jella,Mugil cephalus,Cluponella cultriventris and Lutijanus gibbus* . The samples were collected from different local shops of Narsapur within a time frame of April 2025 to June, 2025. All the samples were collected aseptically and transported immediately to the laboratory using sterile polyethylene bags with ice.

2.2. SAMPLE PROCESSING WITH AND WITHOUT UV RADIATION

The appropriate lengths and weights of each fish sample were measured and all the samples were divided into 4 parts. The samples were labeled as raw sample, UV for 5 min, UV for 10 min & UV for 15 min. All the samples were homogenized with peptone buffer water (PBW) (Hi-Media Laboratories Pvt. Limited, The UV radiation (5 min, 10 min & 15 min) was performed on the homogenized samples in a laminar flow hood with a wavelength of 260 nm. Then the raw and the UV treated fish samples were serially diluted up to 10 min^n for microbiological analysis.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

Fish samples

Sample mixing on Cyclo Mixer

2.3. ESTIMATION OF TOTAL VIABLE BACTERIA (TVB), TOTAL FECAL COLIFORM (TFC), *E. coli, Klebsiella* spp., and *Staphylococcus* spp

In order to determine the Total Viable Bacteria (TVB), Total Fecal Coliform (TFC), and presence of Staphylococcus aureus, 0.1 ml of sample from each dilution of the suspension was spread out onto Nutrient agar, Sabouraud's Dextrose Agar (SDA) and Mannitol Salt Agar (MSA) plates consecutively . For TVB and Staphylococcal assay, plates were incubated at 37 °C for 24 h, while for estimating the fecal coliforms, plates were incubated at 44.5 °C for 24 h. For the detection of *E. coli* and Klebsiella spp., 0.1 ml of samples from each dilution of the suspension was spread onto the Eosin Methylene Blue (EMB) agar and MacConkey agar plate, respectively, which were incubated at 37 °C for 24 h.

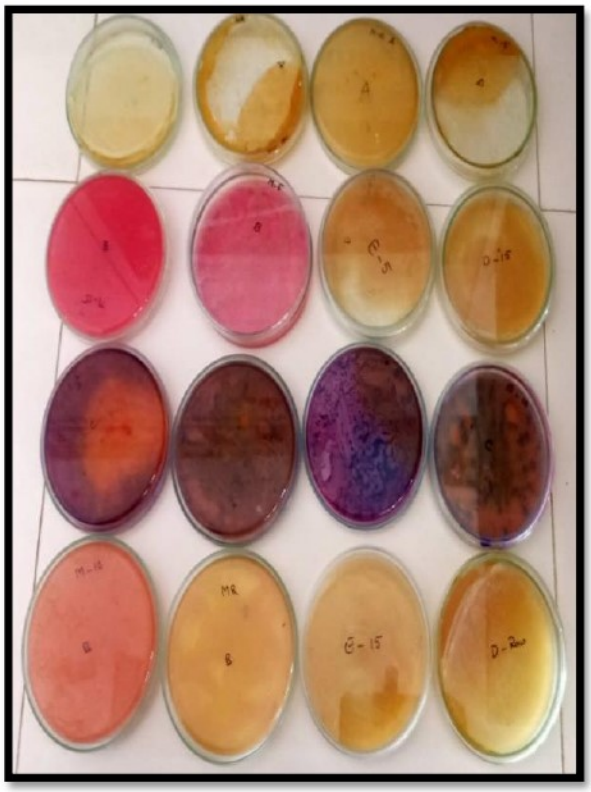
Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

Culture Media

Cultural Plates after incubation.

Culture Plates at Incubator



Cultural Plates after incubation.

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

IDENTIFICATION OF CULTURAL CHARACTERS

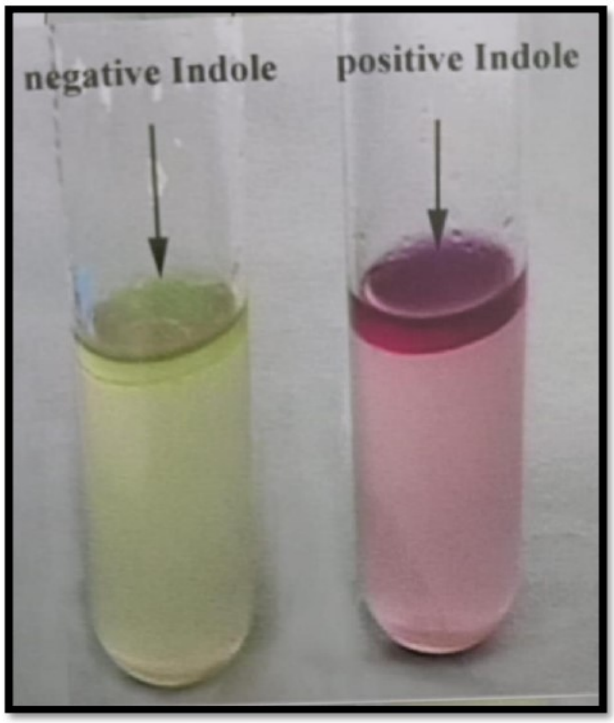
S.NO		E.Coli	Salmonella	Klebsiella	Staphylococcus
1.	Nutrient Agar	Colonies are large, thick, grayish white, moist, smooth, opaque or translucent discs at 37° C. After 48 hrs colonies are light pink.	Medium sized strains, 2-3 min in diameter, off-white, moist, with smooth convex surfaces and complete margins.	Circular, dome – shaped, mucoid, translucent.	It forms fairly large yellow & white colonies.
2.	Sabouraud's Dextrose Agar (SDA)	Not found	Not found	Not found	Not found
3.	Mannitol Salt Agar (MSA)	Not found specific growth of E,coli	Pinkish Zone colonies are often capable of growing on this media, but not present.	No change	Circular,2-3 nm in diameter, with a smooth shiny surface. Pigmented golden yellow colour.
4.	MacConkey Agar	Colonies are circular, moist, smooth, appear flat & pink lactose fermenting colonies.	Colourless colonies between 2 to 3 mm in diameter after 24 hrs of incubation.	Large, mucoid & red with diffusing red pigment, 2 – 3 nm in diameter.	Light pink or red colonies, not perfect growth.
5.	Eosin Methylene Blue (EMB)	Metallic Strain, large, blue-black colonies often with a green metallic green.	No specific growth	Pink or purple dark centered mucoid colonies.	Small colonies, smooth, round, small white colonies after 48 hrs incubation.

2.5. BIOCHEMICAL TESTS FOR THE CONFIRMATIVE IDENTIFICATION

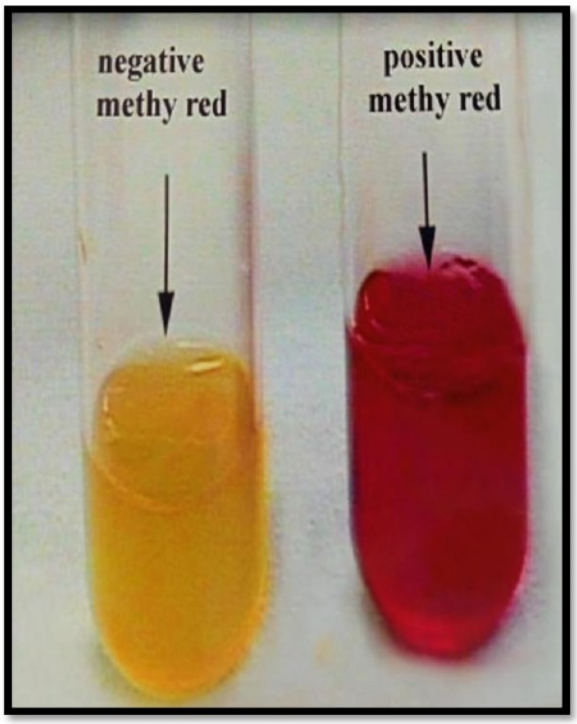
For the final identification of all the bacterial isolates, several biochemical tests were performed by following the standard protocol. Triple Sugar Iron (TSI) test determines bacterial ability to utilize sugar and produce H_2S, Methyl-Red (MR) and Voges-Proskauer (VP) tests identify metabolic pathway, Motility Iodole and Urease (MIU) test determine bacterial motility, Urease utilization, and Indole production ability, Citrate utilization test checks ability to utilize citrate as carbon and energy source, and Catalase and Oxidase tests check the presence of catalytic enzymes.

Cover Page

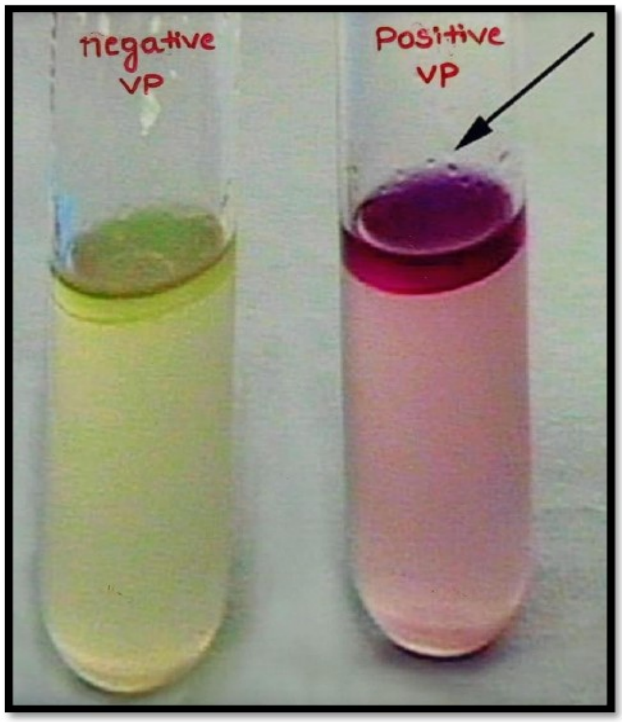
INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in



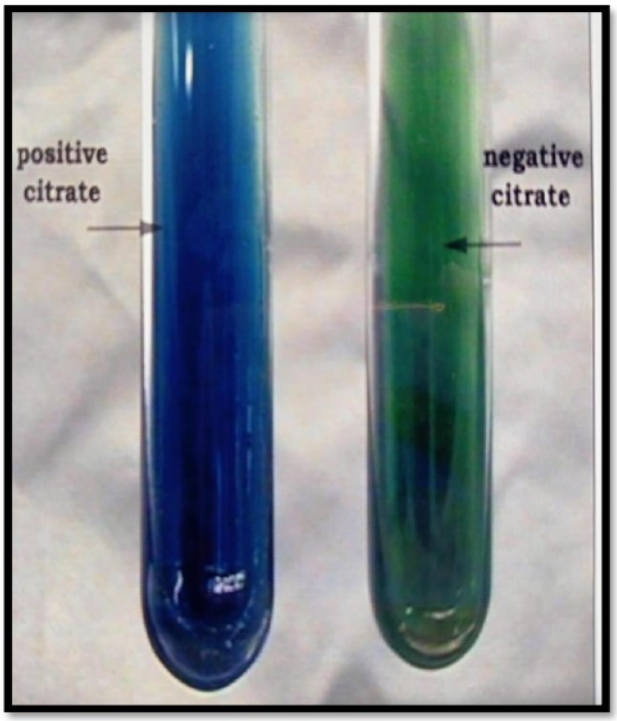
INDOLE TEST



METHYL RED TEST



VOGES-PROSKAUER (VP) TEST



CITRATE UTILISATION TEST

Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

Name of the Micro-organism	Indole Test	Methle Red	VP Test	Urease	Citrate	Cas Production	Motility	Catalase test	Oxidase
E. Colo	+ve	+ve	-ve	-ve	-ve	+ve	+/- ve	-ve	-ve
Salmonella	-ve	+ve	-ve	-ve	+/- ve	+ve	+ve	-ve	-ve
Shigella	+ve	+ve	-ve	-ve	+/- ve	-ve	-ve	-ve	-ve
Klebsicella	-ve	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve
Staphylococcus	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve

Methyl-Red (MR) and Voges-Proskauer (VP) tests identify microorganisms based on their ability to ferment carbohydrates via different metabolic pathways. Depending on whether the MR and VP tests are positive or negative, different bacteria can be detected. The positive MR and negative VP successfully confirmed the presence of *E.coli* and *Salmonella* spp. in our case. Positive motility and positive indole production for *E.coli* and positive motility and negative indole production for *Salmonella* spp. supported the previous results. Among all the bacteria, only *Klebsiella* spp. showed a positive result for the citrate utilization test only *Shigella* spp. showed a negative result for the catalase test , and *Vibrio* spp. and *Pseudomonas spp*. showed a positive result for the oxidase test , which aligned with general observations. Positive results in the MR and the catalase test and negative results in the indole and oxidase tests confirmed the presence of *Staphylococcus* spp.

3. RESULTS AND DISCUSSION

In Narsapur; West Godavari District, fisheries play a big role in providing food security to the population along with contributing to the development of the national economy and employment opportunities. Fisheries also significantly contribute to the export earnings of Narsapur.
The biggest challenge to maintain the quality of the fish products is their contamination with drug-resistant bacteria. This poses a serious threat to global public health as well as the economy. Various life-threatening diseases may be triggered by the propagation of drug-resistant bacteria through fish. Additionally, the drug resistance can be transferred to clinically important strains of the natural environment through horizontal gene transfer, affecting the whole ecosystem. The current study attempted to address this dire issue by implementing the non-ionizing UV radiation technique to decontaminate fish products commonly produced in Narsapur; West Godavari District.

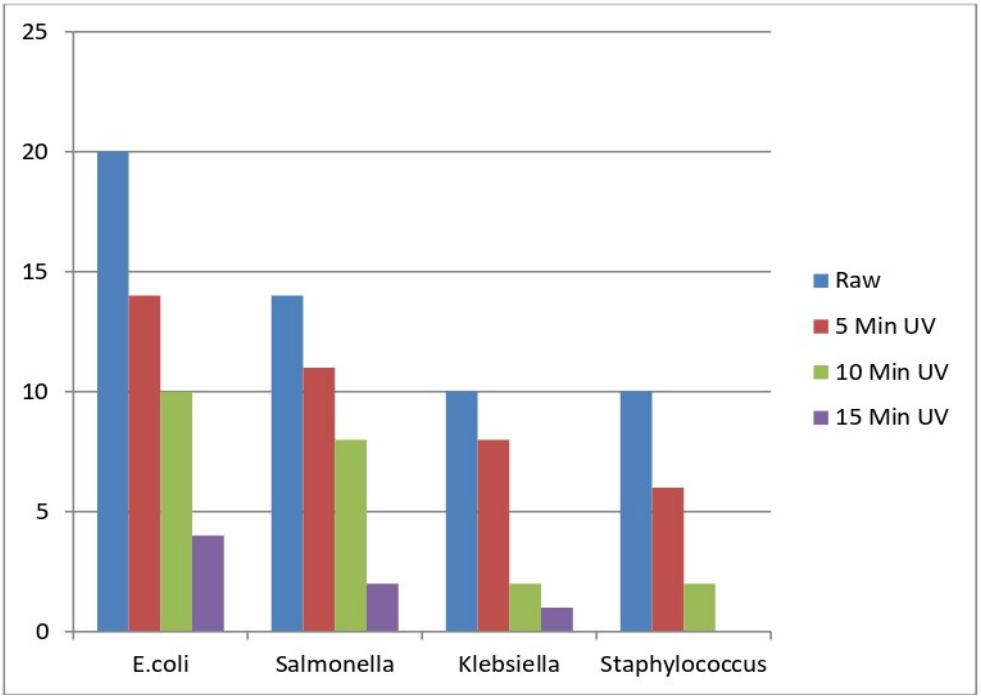
Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

The first step was to identify the microorganisms present in the tested fish samples. A variety of bacterial pathogens were detected including *E. coli, Klebsiella* spp, *Pseudomonas* sp., Shigella spp., *Salmonella* spp., Vibrio spp., and *Staphylococcus* spp. in all of the 04 categories of fish samples based on the colonies formed in agar plates The isolated microorganisms were also biochemically identified .The TSI test confirmed the presence of all the microorganisms. The colors of the slanted part (aerobic environment) and the bottom part (anaerobic environment) of the TSI medium in a test tube differentiate among microorganisms, based on the differences in various carbohydrate fermentation patterns, gas, and H_2S production . The color patterns that were noted aligned with the patterns generally noticed for *E. coli, Klebsiella* spp, *Pseudomonas* spp., *Shigella* spp., *Salmonella* spp., *Vibrio* spp. and *Staphylococcus* spp. . The rest of the tests provided supportive information.

MACCKONKEY AGAR

	E.coli	Salmonella	Klebsiella	Staphylococcus
Raw	20	14	10	10
5 Min UV	14	11	8	6
10 Min UV	10	8	2	2
15 Min UV	4	2	1	0

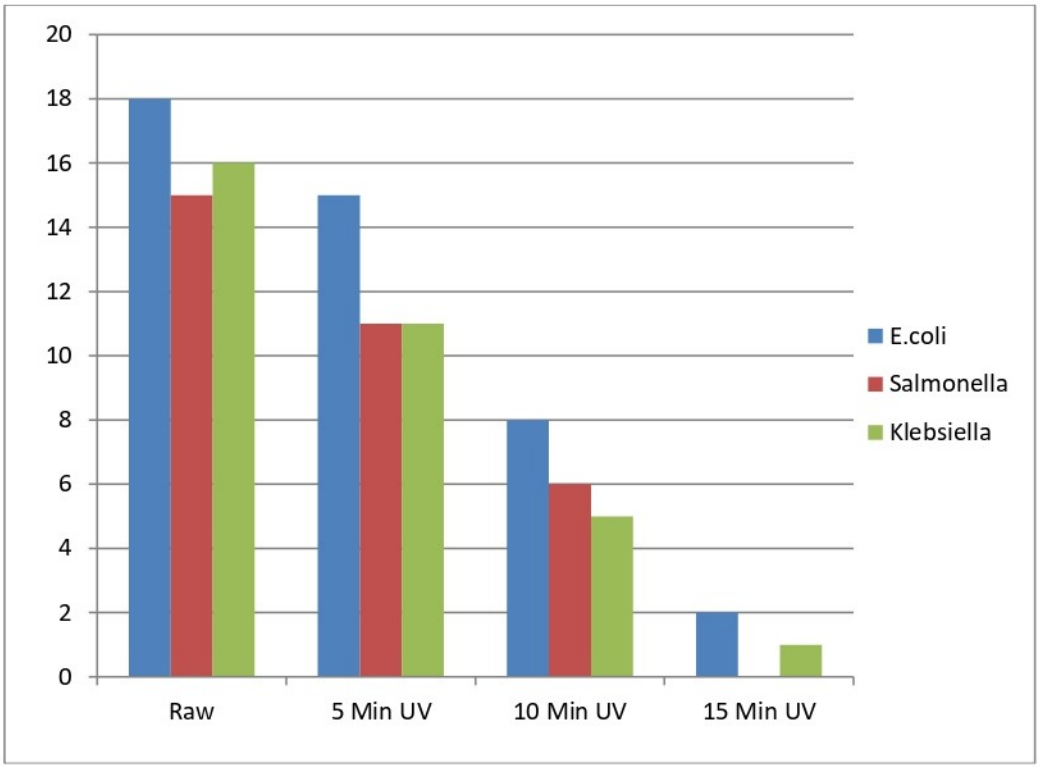


Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

MANNITOL SALT

	E.coli	Salmonella	Klebsiella	Staphylococcus
Raw	18	15	16	10
5 Min UV	15	11	11	8
10 Min UV	8	6	5	6
15 Min UV	2	0	1	0

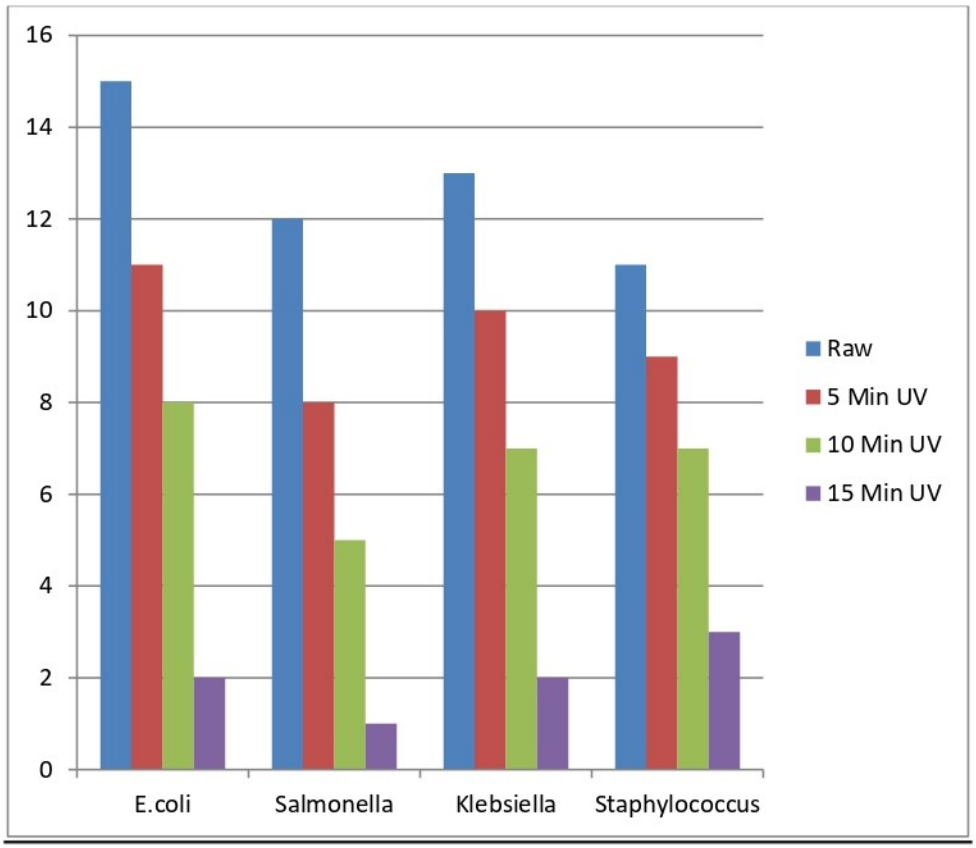


Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

NUTRIENT AGAR

	E.coli	Salmonella	Klebsiella	Staphylococcus
Raw	15	12	13	11
5 Min UV	11	8	10	9
10 Min UV	8	5	7	7
15 Min UV	2	1	2	3

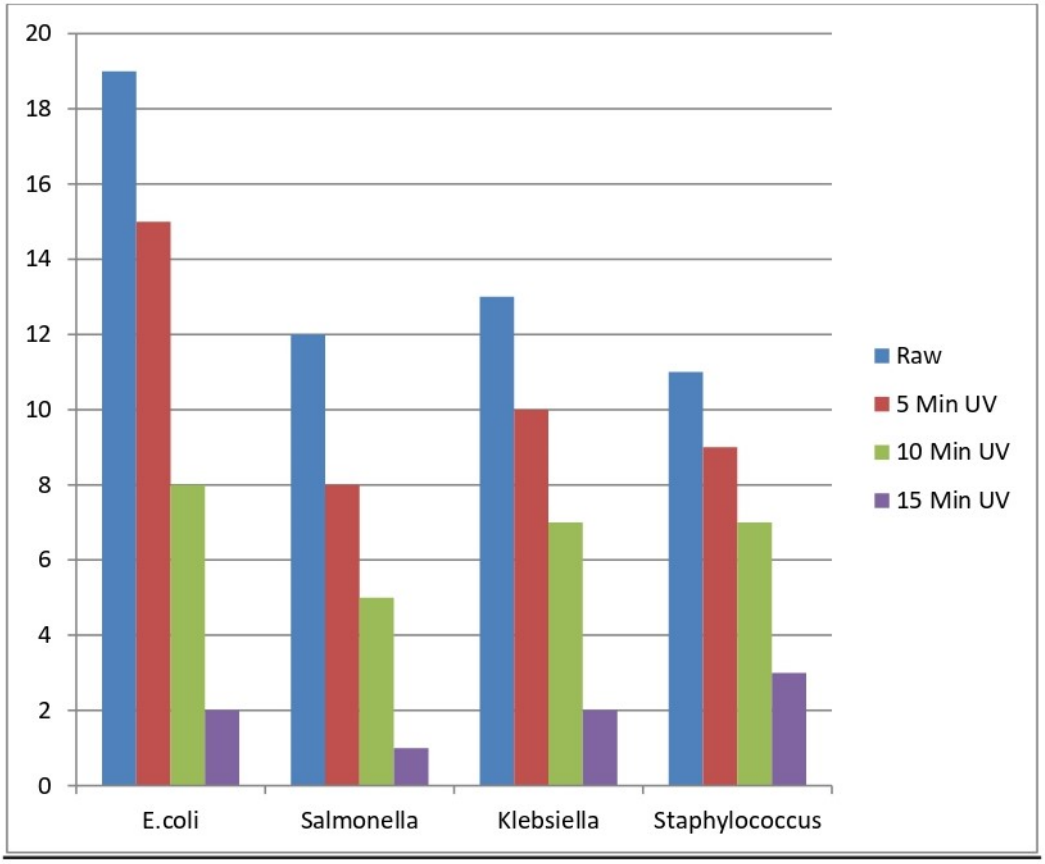


Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

EOSIN METHYLENE BLUE

	E.coli	Salmonella	Klebsiella	Staphylococcus
Raw	19	12	13	11
5 Min UV	15	8	10	9
10 Min UV	8	5	7	7
15 Min UV	2	1	2	3



Cover Page

INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY EDUCATIONAL RESEARCH
ISSN:2277-7881(Print); IMPACT FACTOR :9.014(2025); IC VALUE:5.16; ISI VALUE:2.286
PEER REVIEWED AND REFEREED INTERNATIONAL JOURNAL
(Fulfilled Suggests Parametres of UGC by IJMER)
Volume:14, Issue:8(3), August, 2025
Scopus Review ID: A2B96D3ACF3FEA2A
Article Received: Reviewed : Accepted
Publisher: Sucharitha Publication, India
Online Copy of Article Publication Available : www.ijmer.in

Multiple factors could be responsible for the variable bacterial contamination noticed in the raw fish samples. Contamination could occur in any of the stages of preharvest, processing, packaging, storage, and distribution. It is difficult to specify the exact reason behind the presence of some microorganisms in certain fish categories. Factors like the condition of the freshwater where fish were grown, storage temperature, the water source used to make the ice for fish storage, and hygiene conditions during handling and selling could all control the growth of some microorganisms over the other .

CONCLUSION:-

Ensuring good quality food is currently a major priority globally. Our microbial analysis of different fresh water fishes *Arius jella,Mugil cephalus,Clupemella ciltriventris and Lutijanus gibbus* available in Narsapur, West Godavari District showed that they can be easily contaminated with various drug-resistant pathogens. This could ultimately lead to severe food-borne disease outbreaks and endanger many lives. The study addressed this issue by demonstrating the efficacy of non-ionizing UV radiation (260 nm) in decontaminating fish products. 15 min of radiation was sufficient in most cases to completely eliminate the bacteria.

References

1.R. Noor, M. Acharjee, T. Ahmed, K.K. Das, L. Paul, S.K. Munshi, N.J. Urmi, F. Rahman, M. Alam
Microbiological study of major sea fish available in local markets of Dhaka city, Bangladesh
J. Microbiol. Biotechnol. Food Sci., 2 (4) (2013), pp. 2420-2430
2. M.M. Shamsuzzaman, M.M. Mozumder, S.J. Mitu, A.F. Ahamad, M.S. Bhyuian
The economic contribution of fish and fish trade in Bangladesh
Aquac. Fish., 5 (4) (2020), pp. 174-181
3.M.H. Rokibul, A. Mrityunjoy, D. Eshita, K.D. Kamal, A. Tasnia, A.A. Muhammad, K.F. Kazi, N. Rashed
Microbiological study of sea fish samples collected from local markets in Dhaka city
Int. Food Res. J., 20 (3) (2013), p. 1491
4. R. Noor, M.F. Hasan, M.M. Rahman
Molecular characterization of the virulent microorganisms along with their drug-resistance traits associated with the export quality frozen shrimps in Bangladesh
SpringerPlus, 3 (1) (2014), pp. 1-7
5. R. Noor, F. Hasan, S. Munna, M. Majibur Rahman
Demonstration of virulent genes within Listeria and Klebsiella isolates contaminating the export quality frozen shrimps
Int. Aquat. Res., 7 (2) (2015), pp. 157-161
6. M.G. Rasul, B.C. Majumdar
Abuse of antibiotics in aquaculture and it's effects on human, aquatic animal and environment
Saudi J. Life Sci., 2 (3) (2017), pp. 81-88
7. F.E. Hossain, S. Chakraborty, N.C. Bhowmick, M.A. Rahman, F. Ahmed
Comparative analysis of antibiotic resistance pattern of bacteria isolated from fish of cultured and natural ponds: a study based on noakhali region of Bangladesh
Biores. Commun. (BRC), 4 (2) (2018), pp. 586-591