

Microbial Population Characterization Associated with Effluents obtained from Seafood Processing Industries

S. Gini¹, S. Illanjiam², S. Priya Grace², C. Shanmugasundaram², T. Srinivasan²,
P. Nirmala², R.V. Prabu², K.N. Gayathri², S.R. Sriram Kumar² and A. Ganesh Kumar^{2*}

¹Department of Microbiology, New Prince Shri Bhavani Arts and Science College,
Affiliated to University of Madras, Chennai (Tamilnadu), India.

²Center for Research & Development, Department of Microbiology,
Hindustan College of Arts & Science, Affiliated to University of Madras, Padur, OMR, Chennai (Tamilnadu), India.

(Corresponding author: A. Ganesh Kumar*)

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ABSTRACT: In this study, microorganisms were isolated and identified from seafood processing effluent from Chennai. Microorganisms were isolated after the samples were serially diluted, inoculated on Nutrient agar media and Sabouraud Dextrose agar, respectively. The spread plate technique was used for the isolation process. The bacterial isolates were described by Gram staining and identification by biochemical assays. The colony and morphology of the isolates were also noted. Wet mount technique with lacto phenol method was used to examine the fungal isolates. According to the results, nine bacterial and four fungal isolates from the effluent were found, with *Pseudomonas* species being the most prevalent. *Staphylococcus aureus*, *Bacillus* species, *Micrococcus* species, *Salmonella* species, *Vibrio* species, *Escherichia coli*, *Klebsiella* species, and *Shigella* species. The isolated and identified fungi are *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Curvularia*, *Alternaria*. The isolates also showed resistant to some antibiotics. These bacteria causes ill effects to humans, so this effluent should be treated properly and treated seafood processing effluent can be used for agricultural purposes. So the study will be helpful to identify and isolate the pathogenic microorganism and also used for the future generation.

Keywords: Seafood processing Effluents, Bacteria, Fungi, Morphological, Biochemical, Antibiotic Sensitivity.

INTRODUCTION

The world is abundant with resources that are beneficial to all life (Sarala Thambavani *et al.*, 2009). Because of its harmful effects on living things, pollution is a serious environmental problem globally. Unchecked growth has led to a significant pollution issue in recent decades as a consequence of the accumulation of trash and industrial effluents in water bodies (Tamilselvi *et al.*, 2012). The majority of organizations are reliant on water, and a sizeable amount of their effluent is often dumped into waterways either untreated or insufficiently treated, which results in water pollution (Pandey and Carney 1998). Water-borne illness epidemics are mostly caused by the viruses, bacteria, fungus, protozoa and helminthes that are often found in wastewater influents. High strength effluent from seafood processing activities contains organic contaminants in soluble, colloidal, and particle forms (Shahidi *et al.*, 1999). The viability of the seafood itself, as well as the conservation of the environment, depend on the microbial composition of the effluent from the processing of seafood. Pathogenic bacteria pose the greatest risk to people. It can have an impact on the environment and induce a number of ailments in people. Industrial Effluents entering the water bodies is one of major sources of environmental toxicity. It not only affects the quality of water but also has deleterious impact on the aquatic ecosystems (Kaur

et al., 2010).

Microbial contaminants can also be used to gauge the quality of water. The numerous forms of microbiological contaminants in wastewater are usually challenging, expensive, and time-consuming to detect, isolate, and identify. In order to prevent this, indicator organisms are always employed to assess the relative risk of a certain pathogen's potential existence in wastewater (Paillard *et al.*, 2005). Since they have the metabolic ability to utilize the environment's resources and can find a suitable niche, bacteria may thrive in polluted habitats (Ilyina *et al.*, 2003). *Thiobacillus*, *Acinetobacter*, *Nitrosomonas*, *Nitrobacter*, *Achromobacter*, *Alcaligenes*, *Bacillus*, *Flavobacterium*, *Micrococcus*, and *Pseudomonas* are the most common bacteria found in various industrial effluents.

Fungi are abundant in nature and can take the form of filamentous and multicellular mold as well as unicellular yeast. Despite their widespread prevalence, their existence and importance in aquatic ecosystems have received little attention (Kirk *et al.*, 2001).

A severe and expanding global hazard to human health is antibiotic resistance. Local action is required to stop the development of antibiotic resistance through the environment. Antibiotic resistance is undoubtedly a global problem. Wastewater treatment facilities are important repositories of antibiotic resistance genes and

sources of their propagation in the environment (Amy Pruden *et al.*, 2013). The ecosystem is harmed by human and animal excretion of antibiotics due to uncontrolled intake (Pazda *et al.*, 2020).

MATERIALS AND METHODS

A. Sample collection

Effluent was collected in screw capped sterilized bottles from seafood processing industries in Chennai and it is stored at 4°C.

B. Isolation of Bacteria from seafood processing effluent

Using the serial dilution approach, 1 ml of effluent was transferred into 9 ml of sterile water in several test tubes (10^{-1} , 10^{-2} , 10^{-3} , 10^{-4} and 10^{-5}). To ensure a uniform dispersion of the microorganism, each sample was shaken for two minutes after being set aside. Next, using a sterile pipette, 0.1 ml of each solution was put into several sterile Petri plates, where the samples were then spread evenly. The plates were kept for incubation at 37°C. The plates were examined for colonies and other morphological characteristics after incubation.

C. Identification of Bacterial Isolates

The bacterial isolates were characterized and identified based on their morphology and biochemical reactions. The isolates were identified up to species based on comparative analysis of the observed characteristics with the standard description of bacterial strains in Bergey's Manual of Determinative Bacteriology.

Colony morphology. The isolates were characterized based on their shape, colour, elevation, margin, and texture.

Gram Staining. Prepare the smear on the clean slide with a loopful of sample then air dry. Crystal Violet was added and kept for about one minutes and rinse the slide with water. Flood the slide with gram's iodine for one minute and wash with water. Then wash with 95% alcohol or acetone for about 10-20 seconds and rinse with water. Add safranin for about one minute and then wash with water.

Motility. Take a clean, scratch free glass slide and add culture, then place a cover slip. This preparation was viewed under microscope.

Biochemical reactions. Various biochemical tests were being carried out for the identification of bacterial isolates that were isolated in the seafood processing effluent.

Indole Test. This experiment was done to determine whether bacteria produce the tryptophanase enzyme, which can hydrolyze tryptophan into Indole pyruvic acid and ammonia. A colony of the test organism was introduced into test tubes with peptone water with a sterile wire loop. For 24 hours, the tube was incubated at 37°C. Kovac's reagent was added to the medium following incubation. Positive results are shown by the formation of red colour.

Methyl red test. This test is to find bacteria that could make and keep stable acid end products from the fermentation of glucose. After combining Peptone Bacteriological, Dipotassium Dihydrogen Phosphate, and glucose, the mixture was autoclaved and organisms was inoculated and kept at 37°C. Finally, a few drops of methyl red solution were added to the mixture. A

development of red colour indicate a positive result.

Voges Proskauer test. This test was performed to show if the bacterium has butanediol fermentation and can split glucose to acetoin. In this test, Peptone, Dipotassium Dihydrogen phosphate and glucose were mixed together, then sterilized and the test organisms were inoculated and incubated at 37°C. Then Alphanaphtol was then added followed by potassium hydroxide. The formation of pink coloration shows a positive result.

Citrate test. This test was done to identify bacteria that can utilize citrate as their only carbon source. In Simmons's citrate agar organisms were inoculated and incubated at 37°C during 24 - 48 hours. A colour change from green to blue shows a positive result.

Urease test. This test was performed to identify *Enterobacteriaceae* that produce Urease enzyme, which hydrolyze urea to give ammonia and carbon dioxide. The test organism was inoculated into the media and incubated at 35°C – 37°C for 18-24 hours. A pink coloration in the medium showed a positive result.

Sugar fermentation test. This test is used to determine the ability of bacteria to utilize different sugars, like glucose, fructose, lactose and sucrose. The sugar solutions were prepared and poured into test tubes with Durham's tube. The sugar was autoclaved, after which a loopful of test organism was introduced into the sugar solution. A yellow coloration shows acid production, while the collection of gas bubbles in the Durham's tube shows gas production.

D. Antimicrobial susceptibility testing

The Clinical and Laboratory Standards Institute recommended using the Kirby-Bauer disc diffusion technique on Mueller-Hinton agar to determine the antimicrobial resistance pattern of the isolated strain from effluent water samples. Colonies from an overnight pure culture were mixed in sterile normal saline (0.85%) in test tubes with the turbidity adjusted to 0.5 McFarland standard to provide an inoculum for each isolate. Using sterile swabs, the bacterial solution was uniformly spread over Mueller Hinton agar plates and allowed to settle for three minutes before the antibiotics were added. Chloramphenicol, Ampicillin, Gentamycin, Amoxycillin Tetracycline and Ciprofloxacin are popular antibiotics used in this test. For 24 hours, plates were incubated at 35°C, and the diameters of zone of inhibition were measured and results interpreted according to Clinical Laboratory Standards institute.

E. Isolation of Fungi from seafood processing effluent

Samples were spread on Sabouraud Dextrose Agar medium and incubate at 25°C for 5 days. After 5 days of incubation, the mycoflora was identified on basis of morphology and characterization. Moreover, for subculture, fungal spores were spot in Sabouraud Dextrose Agar medium with needle and grow at 25°C.

F. Morphological Identification of fungi - Lacto Phenol Cotton Blue Staining

The colony of the fungal culture was taken and deposited on a few drops of lacto phenol cotton blue using a sterile wire loop. The mixture was then well mixed. Then a cover slip was kept above it and the slide was now ready

to be examined.

RESULTS

This study was based on the microbial community identified in the industrial effluent from seafood processing. The outcome revealed a significant microbial population in this wastewater. Gram positive and gram negative bacteria were identified in the wastewater produced during the processing of seafood. Table 1 displays the quantitative estimation and the microbial population's colony forming unit. The majority of the strains generate yellow and green pigment on Nutrient agar plates. The results were compared to those from the Bergey Manual, which revealed six gram-negative bacilli and the isolates identified as *Pseudomonas aeruginosa*, *Vibrio cholerae* sp, *Klebsiella* sp, *Salmonella* sp., *Shigella* sp and *Escherichia coli*. And three gram positive organisms such as *Staphylococcus aureus*, *Bacillus* sp and *Micrococcus* sp. The identified genus are shown in Table 2 and 3 displays the antibiotic sensitivity pattern of isolated strains. Organisms exhibited resistance and

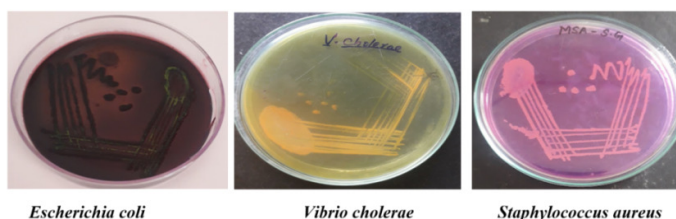
susceptibility towards various antibiotics, this shows that even antibiotic resistance bacteria are present in effluent. The fungi isolated and identified are *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Curvularia*, *Alternaria*. and tabulated in Table 4.

The Bacterial Count for Wastewater Samples. The bacterial count of the wastewater samples and the colony forming unit were calculated and were carried out as shown in Table 1.

Table 1: Bacterial count for Sea food Processing Effluent samples.

Samples	Number of Colonies	CFU/ML
S1	157	1.57×10^7
S2	112	1.12×10^7
S3	87	8.7×10^6
S4	62	6.2×10^6
S5	76	7.6×10^6
S6	83	8.3×10^6
S7	134	1.34×10^7
S8	64	6.4×10^6
S9	91	91×10^6

Keys: CFU = Coliform Unit; ml = Millitres



Biochemical Reactions of the Bacterial Isolates from Sea food Processing Effluent. The biochemical reactions were carried out to identify the bacterial isolates into the genus level.

Antibiotic Sensitivity Pattern. The antibiotic sensitivity pattern of various organism are shown in Table 3.

Identification of the Fungal Isolates from Sea food Processing Effluent The fungal isolates from both samples were identified using a wet mount method procedure, as shown in Table 4.

DISCUSSION

Water is needed in enormous quantities by the companies that prepare seafood for cleaning and for washing the machinery and also used to preserve the seafood. As a

result, the wastewater that is released as effluents from seafood processing factories into the environment is rather significant. Many members of the Enterobacteriaceae family were included among the isolates from seafood processing effluents used in this investigation, along with a small number of gram positive bacteria, fungus, and other microbes. Water is utilized for home and industrial reasons and is the medium in which the majority of organisms live, according to Gwana *et al.* (2017). They include a wide range of microbial pathogens known to pollute water, such as viruses, parasites, fungus, and bacteria, all of which may be linked to waterborne illness, thereby it contaminates the environment making it unfriendly and health risks.

Table 2: Biochemical Reactions Tests of the Bacterial Isolates for Sea food Processing Effluent Samples.

Isolates	Gram Staining	Motility	Catalase	Oxidase	Indole	Methyl Red	Voges Praskauer	Citrate	Urease	TSI	Organism
S1	-	+	+	+	-	-	-	+	-	-/-	<i>Pseudomonas aeruginosa</i>
S2	+	-	+	-	-	+	+	-	+	+/-	<i>Staphylococcus aureus</i>
S3	+	-	+	+	-	-	+	-	-	-/+	<i>Micrococcus</i> sp
S4	-	+	+	-	+	+	-	-	+	+/+	<i>Escherichia coli</i>
S5	-	-	+	-	-	-	+	+	+	-/+	<i>Klebsiella</i> sp
S6	+	+	-	-	-	-	+	+	-	+/-	<i>Bacillus</i> sp
S7	-	+	+	+	+	+	-	+	-	-/-	<i>Vibrio cholerae</i>
S8	-	+	+	-	-	+	-	+	-	-/+	<i>Salmonella</i> sp
S9	-	-	+	-	+	+	-	-	-	-/+	<i>Shigella</i> sp

Table 3: Antibiotic sensitivity pattern Bacterial Isolates from Sea food Processing Effluent.

Microorganisms	Total number of isolates	Chloramphenicol	Amoxycillin	Ampicillin	Ciprofloxacin	Gentamycin	Tetracycline
<i>Pseudomonas aeruginosa</i>	27	37%	33%	89%	30%	22%	44%
<i>Staphylococcus aureus</i>	14	43%	64%	79%	43%	29%	64%
<i>Micrococcus</i> sp	8	25%	38%	88%	75%	62%	25%
<i>Escherichia coli</i>	14	29%	36%	71%	33%	43%	50%
<i>Klebsiella</i> sp	11	28%	18%	72%	19%	46%	46%
<i>Bacillus</i> sp	8	37%	75%	88%	37%	25%	25%
<i>Vibrio cholerae</i>	17	47%	18%	18%	18%	47%	47%
<i>Salmonella</i> sp	7	29%	29%	86%	29%	43%	57%
<i>Shigella</i> sp	6	33%	33%	33%	33%	33%	33%

Table 4: Identification of the Fungal Isolates from Sea food Processing Effluent Samples.

ORGANISM	TEXTURE	SIZE
<i>Aspergillus niger</i>	Velvety to coarse, rough woolly and cottony.	The conidiophores ranging from 900 – 1600 µm in length. Vesicles are globose.
<i>Aspergillus flavus</i>	Flat or rugose, mat like colony, surface pigment is yellow to yellow green.	The conidiophores ranging from 850 µm in length and 5-8 µm in width. Vesicles are large and globose.
<i>Aspergillus fumigatus</i>	Velvety to floccose, rugose, bluish green to gray colonies.	The conidiophores ranging from 150 µm – 300 µm in length. Flask shaped vesicles.
<i>Curvularia</i>	Velvety to woolly, dark brown to black olive green pigment	Slender and geniculate conidiophore.
<i>Alternaria</i>	Flat colonies, dark brown to dark olive green colony.	Conidiophores are septate and darkly pigmented.
<i>Rhizopus</i> sp	Cottony to wolly, Gray colonies, salt and pepper colony surface.	Sporangiophores are long and straight. Sporangia are round and hyaline. Well developed rhizoids .

The majority of the bacteria that are common in many sectors come from waterways. It is bacteria that break down both organic and inorganic substances. Several studies revealed that numerous bacterial species were identified from various industrial effluents (Yazdi *et al.*, 2001). According to the current study, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* sp, *Staphylococcus aureus*, *Micrococcus* sp, *Bacillus* sp, *Shigella* sp, *Vibrio cholerae*, and *Salmonella* sp were among the bacteria and fungi such as *Aspergillus* sp isolated from the effluent of seafood processing, along with *Curvularia*, *Alternaria* (Olugasa *et al.*, 2000).

According to Lin *et al.* (2008); Ali and Naseem (2012), *Pseudomonas* species are among the most prevalent species of bacteria that break down phenolic compounds when they are isolated from sites that have been contaminated by various industries. This finding is consistent with our study of effluent from seafood processing. The findings of the current study also show that the majority of the species in the industrial effluents were gram-negative bacilli. *Pseudomonas* species cause serious eye infections in immune weakened individuals. Excessive concentrations of this bacterium in swimming pool and canal water might result in rashes and superficial infections of the outer ear canal (Calderon and Mood 1982).

Several employees have said that gram-positive bacteria are present in effluent. The genus *Micrococcus* the common gram-positive bacteria that were isolated from effluent for this investigation. These bacterial strains also present in an industrial effluent by Ali and Naseem (2012). This bacteria can cause serious illnesses and is highly transmissible since it is resistant to antibiotics like penicillin and ampicillin (Smith *et al.*, 1999).

Micrococcus is resistant to ampicillin and ciprofloxacin in this investigation as well.

Staphylococcus aureus is one of the isolates found in the wastewater samples, and it is one of the most serious threats to public health since it has been linked to several episodes of food poisoning (Wadhwa *et al.*, 2002). In the study by Ulfata *et al.* (2022), *Staphylococcus* had the greatest resistance to Penicillin while displaying the greatest sensitivity to Teicoplanin. *Staphylococcus aureus* exhibits resistance to the antibiotics ampicillin and amoxicillin as well as chloramphenicol. This is comparable to our work, which demonstrates antibiotic resistance to drugs like ampicillin and amoxycillin.

Escherichia coli has been found to be one of the contaminants of wastewater, this organism has high rate prevalence in water samples also responsible for Gastroenteritis and produce large quantities of toxins. They are most specific indicator for faecal contamination occur only in the feces of warm blooded mammals. The presence of *Escherichia coli* in the wastewater samples is an indication of fecal contamination. In this study also *Escherichia coli* was identified and resistant to Ampicillin and susceptibility to other antibiotics. *Escherichia coli* are part of the normal flora of the human intestines. Some strains of *Escherichia coli* have been linked to diarrhea, gastroenteritis and urinary tract infections (Olugasa *et al.*, 2000).

Salmonella sp are extensively dispersed in the environment and get into aquatic bodies through faeces, drainage flows, and improperly handled waste discharges. *Salmonella* sp. causes a variety of human illnesses, including enteric fever, gastroenteritis, and bacteremia, which have a major impact on health in underdeveloped nations (Zaki *et al.*, 2009). *Salmonella*

isolated during this investigation shown ampicillin resistance.

The primary source of *Bacillus* species and other Gram positive bacilli are soil, water, dust, air, feces, vegetation, wounds and abscesses. Incidence of *Bacillus* sp in Canal water is due to fecal contamination and domestic waste. If the water is used without proper treatment, it can cause life threatening diseases. It was resistant to antibiotic ampicillin and shows sensitivity to other antibiotics.

Klebsiella sp is inherently environmental organisms that survive and sometimes multiply in suitable waters. Presence of *Klebsiella* sp in the effluent indicates that domestic waste is its source and it may become a risk factor if this water is used for irrigation purpose as the bacteria will contaminate the crops especially vegetables and will cause diseases in consumers.

Vibrio sp is commonly present in water and considered to be pathogenic and causes many harmful diseases in humans. It can cause cholera, which is an acute, diarrheal illness that can result in severe dehydration and even death. *Shigella* sp. were resistance to Streptomycin (Pazhani *et al.*, 2008). In this present study *Shigella* was susceptible to various tested antibiotics.

The isolated fungal species agrees with the other research work *Aspergillus* spp. in wastewater. *Aspergillus niger*, *Rhizopus* sp., *Aspergillus flavus*, *Aspergillus fumigatus*, *Alternaria*, and *Curvularia* are among the isolates of fungus from seafood processing effluent, which is consistent with research by Aakash and Mahesh (2021). The microbial pathogens that are known to pollute wastewater and sludge, consequently damaging the environment and posing a health concern (Bassey *et al.*, 2017).

According to our research, all of these species of bacteria and fungus may be found in sea food processing effluents. As medications are widely accessible in underdeveloped nations, people may self-administer antibiotics, which would further raise the incidence of drug-resistant strains. A significant factor in the emergence of antibiotic-resistant bacteria in the environment is the long-standing practice of administering low dosages of antibiotics over an extended length of time to promote animal growth (Pareek *et al.*, 2015).

CONCLUSIONS

In conclusion, the extensive use of antibiotics in the production and processing of seafood is clearly supported by the large microbial load in the effluent and their resistance to several common antibiotics. If discharged without being properly treated, effluents harboring resistant bacteria may pollute the receiving river and other natural water bodies. As it enters the food chain, seafood taken from contaminated waterways may act as passive carriers of resistance that can harm people. Hence, identifying the risk factors involved in the transmission and spread of drug-resistant bacteria may be aided by early detection, the identification of antibiotic resistance patterns, and the characterization of the isolates from a seafood processing industries. Hence, good waste management procedures in processing facilities would not only preserve seafood cleanliness but

also safeguard the environment.

FUTURE SCOPE

The effluent were studied for various other parameters and toxicity study can be done for the reuse of effluent in agriculture. This in future can be used for the environment and save water for the future generation.

Conflict of Interest. None.

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