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RESEARCH ARTICLE

A STUDY ON ANTI-MICROBIAL RESISTANT ENVIRONMENTAL BACTERIA FROM AURANGABAD CITY MUNICIPAL AREA MAHARASHTRA, INDIA

Abhijit S. Thorat and Sanjay B. Attarde

School of Environmental and Earth Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon (M.S.) India.

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Abstract

With rapid industrial development and progress of civilization, the problem of increase in waste generation becomes more complex in urban areas like Aurangabad fastest growing city in Asia in Marathwada, Maharashtra in India. In this study, we explore the diversity of bacteria in sewage wastewater with the assessment of the antibiotic resistance of the isolated bacteria. The water samples: sewage wastewater was collected from around the Aurangabad City of Maharashtra, India, and was processed for isolation, which was characterized for identification following phenotypic techniques. A total of 09 bacteria were isolated from the sewage samples studied, among which 3 were gram-negative and 06 were gram-positive. Our present study evidently revealed that these wastes as confirmed in the current study, the growth of gram-positive rods shape cocci, and even gram-negative rods too; in which the diversity of gram-positive bacteria was found greater, as we compared to the gram-negative bacteria. These bacteria isolates have the potential to cause infections and possess varied antibiotic resistance. Thus, this is imperative for the regular vigilance of such wastewater bacteria to combat bacterial multiple antibiotic resistance. We can conclude that the useful bacteria might be isolated from the Aurangabad city area which will be helpful for bioconversion of sewage/solid waste management.

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Introduction:-

The current environmental concerns have stimulated the interest of institutions, industries, and the public in two critical concepts: sustainability and circular economy (Ingrassia et al., 2020, 2019). Solid Waste is defined as waste type that includes principally household waste/domestic waste with sometimes the addition of commercial waste collected by a municipality within a given area. Whichever solid or semisolid form and usually eliminate industrial hazardous wastes. Microorganisms that reside in solid wastes are clustered under Solid Waste Microflora (SWM). The most common microorganisms that normally originate in solid waste are bacteria and fungi. In the Niger Delta region of Nigeria, it has been reported that gram-positive and gram-negative pathogenic bacteria were isolated from fishpond water (Njoku, 2015). From samples of residential water, Mukhopadhyay (2012) recovered a large number of antibiotic-resistant bacteria, including coliforms, *Pseudomonas aeruginosa*, and *Enterococcus* spp. Mulamattathil (2014) provided examples of how to distinguish and identify bacteria from sewage water samples and established the profiles of bacteria's antibiotic resistance. These microorganisms use the components of the waste as the

Corresponding Author:- Abhijit S. Thorat

Address:- School of Environmental and Earth Sciences, Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon (M.S.) India.

substrate for their growth. They produce and increase these wastes by utilizing the various mechanisms that make up solid waste. Further, a wide variety of pathogenic microorganisms have been reported to be up to date in these organic wastes. No significant study was carried out in the field of solid waste microflora and pathogenic bacteria in the study area of Aurangabad City Municipal Corporation area (AMC). The occurrence of resistant to multiple antibiotics among bacteria, having the capacity to cause many health threats to humans, is not uncommon, in our times, in the environment, including aquatic bodies observed by various researchers such as Poonia, 2014; Zang, 2009 and Baquero, 2008. So, we are interested to investigate the Bacteria present in sewage wastes and to find out its applications since, the beginning of humankind has been generating waste in the form of bones and other parts of animals they slaughter for their food or the wood they cut to make their carts, with the progress of civilization, the waste generation become more complex in nature. Microbial antibiotic resistance has been stated as one of the significant threats to both human and environmental health at the moment, despite the fact that antibiotics have successfully treated numerous life-or-death situations in the past in clinical settings (Van Boeckel, 2014). Taneja, 2019 provided a microbiological examination of drinking water from various institutions in North India to demonstrate the existence of coliform bacteria, including *Escherichia coli*. According to Ajayi, gram-positive bacteria like *Streptococcus* species, *Staphylococcus aureus*, and *Bacillus* species, as well as gram-negative bacteria like *Escherichia coli*, *Pseudomonas* species, *Klebsiella* species, *Enterobacter* species, *Proteus* species, *Cyrobacter* species, and *Shigella* species, were found in the pond water in 2014. Multiple antibiotic-resistant gram-negative bacteria have been identified as organic pollutants of natural water sources Krumperman, 1983 and Adefisoye, 2017. In our earlier research, Nandi, 2016, showed that gram-negative and gram-positive bacteria from environmental sources exhibit a wide variety of MAR indices. Determining the existence of potentially dangerous bacteria in sewage and the forms of the isolated microorganisms' antibiotic resistance are the two goals of the currently underway investigation. Furthermore, a wide variability of moribund microorganisms has been stated to be existing in these organic wastes. (Sonawane et. al., 2010). No significant study was made in the field of solid waste microflora in the Aurangabad City (AMC) study area. So, we were interested to investigate the microorganisms present in sewage wastes and to find out their applications. (Mohapatra, 2006). The sewage waste generated in urban usually said that municipal sewage waste (MSW). 27.6% of India's population resides in urban areas (census 2011). It is predicted 45% reside in cities by 2021. In India, waste quantity increased from 45 million tons in 2011 to 70 million tons in 2021. (Kumar and Gaekwad, 2004). Aurangabad city generates large amounts of solid waste and sewage which is poorly disposed and untreated so, there is an instantaneous requirement for intended scientific integrated solid waste and sewage management for the city. Earlier studies showed that on average, each person in urban areas produces half a kilogram of garbage each day Haq et. al., 1999. According to EPA in 1989, only 20 % of this Municipal Solid Waste (MSW) is being reprocessed, while 70% goes into landfills and 10% is incinerated. 1982; Blight and Mbande, 1996. Every single day huge amounts of sewage waste materials are produced in all municipalities of India. In urban areas, solid and the extreme organic content of sewage effluent is between 65 and 80%. Along with population increase and rising per capita GNP, India's metropolitan areas are producing more solid garbage. Only (AMC) Aurangabad City Municipal Corporation (AMC). 10.05 million inhabitants produce about 3000 tonnes of rubbish every day. In the Aurangabad city region, daily rubbish generation per person ranges from 0.35 kg to 0.4 kilogramme. The recommended structure effectively collects the entire amount of garbage produced. About 10-15% of waste is recycled formally, while 35-50% is self-disposed of or illegally dumped rubbish. As a result, given the right circumstances, this degradable fraction may be combined with another substance to create soil conditioners and biofertilizers that would be beneficial. as per Parr and Hornick, 1992. The five main categories that make up the soil's microbial population include actinomycetes, fungus, algae, and protozoa. Among these groups, bacteria are the most prevalent and crucial for digesting trash, according to Sultana (1997). Bacteria consume waste products for their own metabolism, and as a result, they create some straightforward and practical mixes that are crucial for the health of the soil, plant growth, and the overall maintenance of the natural ecosystem's delicate balance. Composting is the controlled biodegradation or conversion of organic material, typically under aerobic circumstances, through which a substance is changed into a product that is stable and soil-like material known as manure. The number of microorganisms along with rodents and creatures play a vigorous character for the sewage waste deprivation. The most significant part is played by bacteria; hence it is possible to use efficient microorganisms for the deliberate decomposition of solid organic waste. Given the crucial role that garbage decomposition plays, this investigation was conducted to create a repeatable process and search for more active decomposer bacteria that could effectively and efficiently break down organic wastes and produce components that are beneficial for plant sustenance. The isolation and characteristics of solid waste vary from place to place; factors that influence the quantity and composition are the average income level, sources, population, social behaviour, climate, and the market for waste materials. Similar studies have been carried out by

various worker i.e. Haq, et. al., 1999; Joerger, et. al., 2009; Ramesh and Mathivanan, 2009; Sultana, 1997; Zaved, et. al., 2008 and Chetan, et. al., 2017.

Material and Methods:-

Study Area:

Aurangabad is an A grade municipal corporation and capital of Marathwada located at N19°53'47"–E75°23'54" of Maharashtra state. The city is bounded by mountains on all directions, and which is titled "The City of Gates" and the strong presence of these can be felt as one drives through the city. Aurangabad is the tourism capital of Maharashtra and is the fifth largest city in Maharashtra. It has an average rainfall of 756.6 mm and maximum and minimum temperatures of the city are 42.6°C and 8.1°C respectively.

Sample Collection:

An aggregate of 09 waste samples were collected from waste transfer site of Aurangabad Municipal Corporation (AMC) area and rest 5 from Industrial zone, landfill site of Naregaon of Aurangabad. Soil blended with waste was tested and gathered in sterile zip-lock plastic keeping up aseptic conditions, put at 4°C and stamped as requirement be to their source and area. All the microorganism cultures were maintained at 4°C in nutrient agar wholly the cultures were sub-cultured at 15 to 20 days interval time. Nine samples sewage waste were collected during January 2021 to February 2023 from nine different areas of Urban and industrial regions of Aurangabad city area stored at 4°C and marked according to their source and location as shown in table 1.

Table 1:- Showing source and location of Sewage Sample collected in AMC area.

Sr. No.	Strain Code	Location
1	S1	Solid Waste Collection from Housing Colonies in AMC Area
2	S2	Solid Waste in Aurangabad City Area Dump (Garbage, behind Ghati Hospital
3	S3	Soil surface, Salim Ali lake side of Delhi Gate, Aurangabad
4	S4	Transportation of Solid Waste in AMC Area Wet soil, drain side of Naregaon Area Aurangabad
5	S5	Solid Waste Dump Sites in AMC Area drain side garbage, Aurangabad
6	S6	Soil surface, Segregation of Waste and Recycled items in AMC Area, Aurangabad
7	S7	Waste Encroached Road in Chikalthana Industrial Area wet soil of drain
8	S8	Housing Colony Waste Dumping Sites in Waluj Industrial Area wet soil of drain.
9	S9	Refuse, slaughterhouse, Padegaon Area, Aurangabad

Three bacterial samples such S1 to S3 showed better growth on their favorable medium. According to Holt, 1984 and Forbes, 2007, and as previously mentioned by Nandi, 2016, the isolated bacteria were characterised using phenotypic approaches, such as colony morphology study, gram-staining and biochemical assays, including sugar fermentation for identification. Five antibiotics were tested on isolated bacteria by Hi-Media in India: gentamicin GEN, 30 g/disc, 30 g/disc of chloramphenicol C, and 10 g/disc of ampicillin AMP., methicillin MET; 5 µg/disc and piperacillin PI: 100 µg/disc, following disc diffusion Bauer, 1966. The results, in terms of zone diameter of the inhibition (ZDI) values of the test antibiotics, were interpreted following the guidelines of the Clinical and Laboratory Standards Institute Chatterjee, 2010. Previous investigation groups have stated on this unattractive trend in the literature (Ahsan 1999; Agarwal 2005; Bandela et. al. 2010; Jha et. al. 2011; Ezeah and Roberts 2012; Kadafa et. al. 2013; Butu and Mshelia 2014; Uwadiogu and Iyi 2014; Thorat and Chavan 2021). Sewage waste sites are not supposed to be located very near to residential areas. In AMC area, waste dump sites which are at random distributed very close to residences and public buildings. Site S₄ and S₈ gives a typical picture of such waste dump sites in Naregaon site in one of the Government reserved areas within the Aurangabad city municipal corporation area (AMC).

Isolation of microorganism:

The samples had been taken from metropolitan sewage and community sludge. Isolation of these microorganisms until get single colony had been completed by using serial dilution method and streaking method 26 agar plate were prepared aseptically. Afterward agar plate was completed, the fast growing of bacteria plate was taken. The diverse shape of microbes on the plate was selected by using aseptic technique. The selective bacteria on the agar plate were streaked. Plate was divided into 4 parts. The streak agar plate is sealed and kept in 30°C incubator for growing. After 24 hours, purification of bacteria was done using by another spread plate technique and incubation. Further that, from the earlier plate, gram staining was done. Samples was observed under microscope and characterized based on

Chetan, et. al., 2017. Identification of microorganism, biochemical test was performed by standard procedure based on Bergey's manual. For the identification of these microorganisms from municipal sewage, several biochemical identification methods such as gram stain, spore forming, strict anaerobes, starch hydrolysis, Voges-Proskauer and swollen cell test had been used. There are numerous biochemical identification systems completed of municipal sludge with colour difference of the test bacterium was detected.

Results and discussion:-

The development of bacteria is influenced by several physiochemical factors, including the media, pH, temperature, incubation time, carbon source, etc. Microorganisms can flourish in a variety of humidity conditions. The range of moisture content of the samples that were obtained for the current investigation initially varied from 20.10 to 70.20%. The Aurangabad city municipal area and industrial area samples revealed the determined moisture content (70.20%) whereas the BCSS (2) sample revealed the minimal humidity content (25.09%). Different soils' bacterial populations are closely correlated with their relative humidity. The highest bacterial densities are found in areas with high moisture content, and aerobic bacterial activity is typically best when soil moisture retention capacity is between 45 and 80% of the soil moisture holding capacity. The study suggests that in order to protect human health and the environment from waste concerns, beneficial microorganisms should be segregated from the surrounding environment for safe bioconversion of organic waste. The established process of waste degradation as revealed in this investigation is believed to be both appropriate and effective. The goal of the study was to determine how sewage, decomposed waste, and bacterial suspension affected biomass output.

There has been an acceleration in the emergence and dissemination of multiple antibiotic resistant (MAR) bacteria due to the increasing and careless use of antibiotics in clinical practices, veterinary medicine, and agricultural practices, which is limiting the efficacy of the currently available antibiotics in treating human diseases caused by bacterial infections globally. Li, 2006. Obviously, the antibiotic resistances are not narrow in clinical settings, rather reported in agricultural as well as various environmental settings, and foods (Penki, et. al., 2022). Previously, we have reported the isolation of potentially pathogenic bacteria from the riverine as well as municipal sewage wastewater receiving polluted effluents in Aurangabad City Municipal Corporation area, Maharashtra, India Sonawane and Thorat, 2010. A total of 09 sewage i.e. bacteria isolated are: S1, S2, S3, S4, S5, S6, S7, S8 and S9 show different morphological attributes of their colonies developed on nutrient agar plates as shown in Figure 2. Three of the isolated bacteria in Figure 2 (S4, S5, and S6) were gram-negative rods, while the remaining six isolates were either gram-positive rods (n = 10; S1, S2, S3, S7, S8 and S9) or gram-positive cocci (n = 1; S7). Tables 2 and 3 show the results of biochemical and sugar fermentation tests conducted on the isolated bacteria. Table 4 shows the microorganisms that have been identified after being characterised phenotypically. The occurrence of bacterial contamination of natural water resources with various microorganisms has been recorded. Table 5 shows the MAR indices and antibiotic resistance phenotypes of the isolated bacteria. According to Pasalari et al.'s 2019 research, *Pseudomonas aeruginosa*, *Escherichia coli*, and other gram-negative bacteria were successfully isolated from the fishpond. It showed resistance phenotypes for tetracycline erythromycin, cefuroxime tetracycline erythromycin, and cefuroxime tetracycline erythromycin ciprofloxacin in *Pseudomonas aeruginosa*, *Escherichia coli*, and *Salmonella typhi*, respectively. The bacterial isolates in the current study displayed resistance to at least one of the tested antibiotics; S2 strain had the lowest level of resistance to a single antibiotic (MET; MAR index: 0.2), while S1 strain had the highest level of resistance to four antibiotics (AMP-GEN-MET-PI; MAR index: 0.8). Six of the nine isolated bacteria displayed MAR phenotypes with resistance to two or more antibiotics, with MAR indices ranging from 0.5 to 0.7; in contrast, five isolates with resistance to two antibiotics had MAR indices of 0.4, as shown in Table 5. The S1 strain to chloramphenicol and the S2 strain to gentamicin both showed intermediate susceptibility to these two antibiotics. And for S4 and S9 strains to piperacillin. Penki and Rout (2021) highlighted worry about how the majority of bacterial isolates have MAR indices greater than 0.2 due to excessive antibiotic loads in natural water supplies. The immediate study, where the MAR indices for the microbial isolates ranged from 0.2 to 0.8, recommended their isolated microbe's suggestion with niches having high levels of antibiotic contamination as well as the "human fecal source of origin" for most of the isolates in accordance with the measures stated previously (Tembekar, 2005 and Kaneene, 2007). in the AMC area.

Table 2:- Showing Biochemical test results for bacteria isolated from sewage samples.

Strain code	Biochemical tests							
	TSI	Ci-trate	In-dole	Cata-lase	Oxi-dase	MR	VP	DN-ase

S1	Y/Y	-	-	+	-	-	+	+
S2	Y/Y	+	-	+	+	-	+	-
S3	Y/Y	+	-	+	+	-	+	-
S4	Y/Y	+	-	+	-	+	-	-
S5	P/Y	-	-	+	+	-	-	+
S6	Y/Y	-	-	+	+	-	-	+
S7	Y/Y	-	-	+	+	+	-	-
S8	P/P	+	-	+	+	-	-	-
S9	Y/Y	-	-	+	+	-	-	-

Note: MR: Methylred; VP: Voges-Proskaur; P: Pink; Y: Yellow; -: Negative; +: Positive; TSI: Triplesugariron.

Table3:- Showing Sugarfermentationtestresultsforbacteriaisolatedfromsewagesamples.

Straincode	Sugars				
	Sorbitol	Sucrose	Lactose	Maltose	Mannitol
S1	+	+	+	+	+
S2	+	+	W	-	+
S3	-	W	-	-	-
S4	+	+	-	-	W
S5	-	-	-	-	-
S6	+	+	+	+	+
S7	+	+	W	+	+
S8	-	-	-	-	-
S9	-	-	-	-	-

Note: W: Weaklypositive; -: Negative; +: Positive.

Table4:- Showing Identityofbacteriaisolatedfromsewagesamples.

Straincode	Bacterialidentity
S1	Corynebacteriumxerosis
S2	Bacilluscereus
S3	Bacillusubtilis
S4	Bacillusmegaterium
S5	Chryseobacteriumindologenes
S6	Micrococcusluteus
S7	Bacilluscirculans
S8	Pseudomonasaeruginosa
S9	Bacillusindicus

Table5:- Showing AntibioticresistanceprofilesandMARindicesfortheisolatedsewagebacteria.

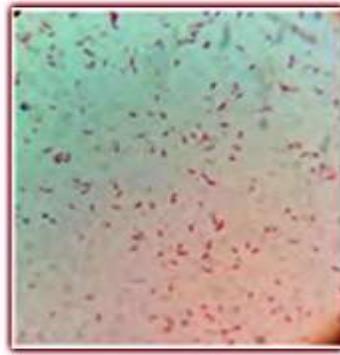
IsolatedBacteria	Antibioticresistance Profile					MAR index
	AMP	C	GEN	MET	PI	
S1:C.xerosis	R	S	S	R	S	0.4
S2:B.cereus	R	S	S	R	R	0.6
S3:B.subtilis	R	S	S	R	R	0.6
S4:B.megaterium	R	S	S	R	S	0.4
S5: C.indologenes	R	S	S	R	R	0.6
S6:M.luteus	R	S	S	R	S	0.4
S7:B.circulans	R	S	S	R	S	0.4
S8:P.aeruginosa	R	R	S	R	S	0.6
S9:B.indicus	S	S	S	R	S	0.2

Note: (n=9) AMP:ampicillin;C:chloramphenicol;GEN:gentamicin;MET:methicillin;PI: piperacillin.

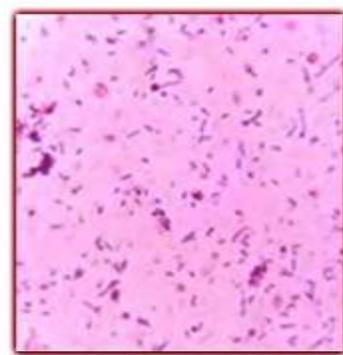




S1: *Corynebacterium xerosis*



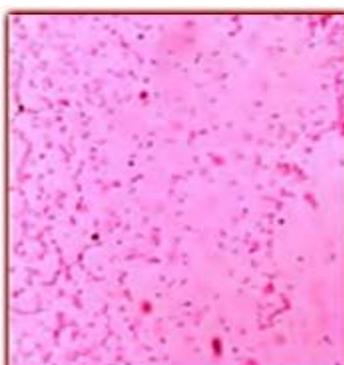
S2: *Bacillus cereus*



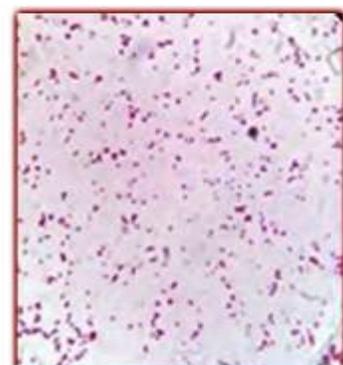
S3: *Bacillus subtilis*



S4: *Bacillus megaterium*



S5: *Chryseobacterium indologenes*



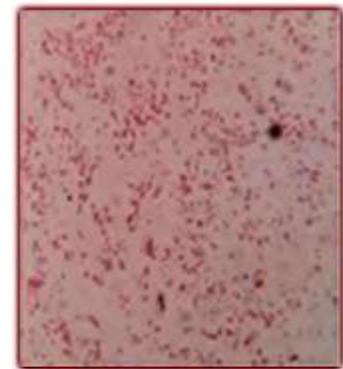
S6: *Micrococcus luteus*



S7: *Bacillus circulans*



S8: *Pseudomonas aeruginosa*



S9: *Bacillus indicus*

Figure 2: Showing gram staining property of bacteria isolated from sewage samples.

Conclusions:-

Municipal sewage is a combination of many substrates, making it the perfect enrichment medium for the development of a wide adaptation of microorganisms. The metabolic activity of the microbes in this environment concerns in the making of various enzymes and bioactive mixtures allied to other conservational conditions. It is vital to realize the waste-derived microorganisms in ecological terms and as a biotechnology source. Our current research has clearly revealed that sewage, as shown in the study, supports the growth of gram-positive rods, cocci, and gram-negative rods as well. The diversity of gram-positive bacteria was higher than that of gram-negative bacteria. The isolated bacteria were capable of infecting humans and had a range of MAR indices and antibiotic resistances. To combat the bacterial multiple antibiotic resistances, there is an urgent and imperative need to maintain regular vigilance of such environmental bacteria of clinical relevance and to prepare effective Indian guidelines for the prudent use of antibiotics that can also serve as a good substitute in industries.

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