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**Research Article**

# ISOLATION OF PATHOGENIC BACTERIA FROM STAGNANT WASTE WATER AROUND RESIDENTIAL BUILDINGS

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## ABSTRACT

Worldwide water born pathogen contamination and disease arise major issues between the communities. Due to heavy rain most of the places were sink in to the rain water around. In addition, residing peoples were discharge their septic tank and sewage water in to stagnant water. After 2-3 months duration stagnant rain water contaminated by pathogenic microorganism. Such pathogenic organism causes many diseases to the people who are living near such contaminated area. The aim of the investigation was to isolate and identify pathogenic bacteria from stagnant water around residential area. For this study two locations were selected, and contaminated water samples were collected from Mettu theru (L1), and Srinvasapuram( L2). Isolated bacteria from L1 were *Chryseobacterium sp., Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Bacillus megaterium, Enterococcus sp, Streptococcus sp, Serratia marcescens* and fro L2 were *Staphylococcus aureus, Proteus vulgaris, Bacillus cereus, Providencia rettgeri, Escherichia coli, Serratia marcescens, Chryseobacterium, Pseudomonas aeruginosa,* Enterococcus. Pathogenesity of the isolates measured by antibiotic sensitivity test. The sensitivity and resistance profile were unique in each bacterium. Based on the research Ecoli has the highest antibiotic resistance profile against majority of the antibiotics similarly Streptococcus and staphylococcus had maximum resistance. This research concludes that we should avoid stagnant water very near to the residential area, also need to create awareness to the people about the effects contaminated water.

**Keywords:** Water born, Resistance profile, Contaminated, Pathogenesity.

## INTRODUCTION

Water is a necessary for every living organism without water no life existing in the world. However, heavy rain and flood due to climatic change could affect human. Recently heavy rain and flood in India extremely spoiled the human's life in addition it spread many infectious disease within the community. Many of the residential area building were partially sink in stagnant water for few months. The stagnant water around buildings is the source of biodiversity, which contain complex microbial community. Lack of awareness within the people they are discarding their septic tank water and sewage water to empty plot due to absence of proper drainage system. Sewage is wastewater, which comprise solid and liquid layer, it contains organic, inorganic and some toxic substances (Zhang *et al*., 2016). In developing area no regular garbage collection, people dump their household waste in to empty land. Such activity highly contaminates water, soil and ground water too. Water quality is vital for human health; many parameters influence the quality of water, such as microbial indicators and chemical composition. A study reported, The environmental impact of sewage disposal are numerous and constitutes major problems faced inour ecology resulting from human activities such as waste from households, industries and agriculture leading to pollution (Schwarzenbach *et al*., 2010). Sewage is wastewater which consists of solid and liquid layer collected and conveyed through sewers, treated or untreated and disposed of into large bodies of water (Zhang *et al*., 2016)

United Nations (UN) reports, there would be raise of 1.1 billion population in next 15 years(United Nations,2013; Massoud *et al*., 2010) therefore large number of people will migrate from urban to cities to access basic

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facilities and better standard of living(Tabraiz *et al*., 2016). Water is the main transmission medium not only of algae but also of protists. Protists, particularly species from the genera Acanthamoeba, Naegleria, Saccamoeba, Hartmannella and Vexillifera, control the number of bacteria and to a lesser extent also of fungi (Defra 2011; Percival *et al*. 2014). Likealgae, some represent hosts for pathogenic bacteria of the generaLegionella, Burkholderia and Mycobacterium.Additionally, species from the genera Cryptosporidium,Microsporidia and Giardia can act as human pathogens, causingdiarrhoea, nausea, keratitis and encephalitis. Species that can form Oocysts represent particular problems, since they areless sensitive to water chlorination (Valster 2011; Novak Babic, *et al*., 2020).

According to US EPA, 2012, pathogenic microorganism is danger for human when it contaminates all types of water bodies. In developed and developing countries water supply networks in hospital, school and homes were identified as a hotspot for waterborne pathogen (Ling *et al*., 2018). Every year 3.4 million people die from water related diseases (WHO, 2014). Similarly, UNICEF (United Nations Children's Fund) reported every day 4000 children die due to contaminated water (UNICEF, 2014). Fayomi *et al*., 2019, discussed in their study, the sewage disposal practice followed in Nigeria are release of septic tank in to soil will pollute soil and water environment (Nowak and Imperowicz, 2016), sewage water discarding into storm water can cause air pollution (Palamuleni,2002), sewage in to pond water is the source for vector diseases (Petrie *et al*., 2015) and direct dumping of sewage waste in river increase toxic substances in food chain through plants, animals and finally human (Ajibush and Terdoo, 2013). Similarly, a study said, potable water supply network harbours multifaceted microbial ecosystems, affecting the lives of 3.6 billion people living in urbanized areas (Fangqiong Ling *et al*., 2018). Diversity of microbes and germs are responsible for gastrointestinal infection which causes symptoms like fever, vomiting, diarrhoea and abdominal pain was recorded in 29.53% cases. Similarly, 27% of cases were caused by Shigella sp., *Cryptosporidium parvum,*(10.99%), Naegleria fowleri (16.84%) Adenovirus 3(10.08%), *E. coli* 0157:H7 (12.63%), Leptospira (6.59%) and Schistosoma sp (29.53%) (Pandey *et al*., 2014). According to Rajasulochana and Rebba, 2015 water contaminated with faecal coliform harshly affects the performance of human; some of the main pathogen responsible for water contamination is *Camphylobacter, Salmonella, Staphylococcus aureus, Clostridium botulinum, Pseudomonas eruginosa, Escherichia coli* and *Vibrio cholera.*

Antibiotic and drug resistance is growing concern globally due to waste water pollution in environment. A study examined, discharge of septic tank water in to empty land in the residential area could make antibiotic as well as drug resistance bacteria due to extensive use of antibiotics through human therapy animal husbandry and agricultural practice as a result increase number of antibiotic resistant bacteria (Vaseeharan *et al*., 2005; Martinez, 2009). Antibiotic resistance is major public health concern (Moore *et al*., 2010) such resistant bacteria present in waste water and sewage contaminated surface water, ground water as well as drinking water(Luczkiewiz *et al*.,2010;Lobova *et al*., 2008). Water quality used for chemical industry and health is one of the most important segments in health management. Numerous human diseases having bath in rivers, lakes, ponds and coastal sea waters in the area of river and sewage inflow, swimming pools are associated with the presence of opportunistic pathogens from Pseudomonas Sps, aeromonas, staphylococcus and other microorganisms groups, being able to generate infections by contact with skin, mucous membrane, nosopharyngeal cavity, respiratory ducts, eyes, ears and urogenital passages. Pyogenic infection of injuries, meningitis, urinary system, respiratory system, inflammation of the middle ear and eyes are typical diseases caused by contaminated water. Wound infections, peritonitis, meningitis, endocarditis, septicemia, corneal ulcers, nosocomial infections, urinary tract infections, gastroenteritis of people who bathe or use water in other ways are caused *by Aeromona shydrophila*. Infections of skin, nosopharyngeal cavity, eyes, outer ear among bathing people could be caused by recreational waters polluted by *S.aureus.* All the above mentioned species of bacteria survive in water longer than classical indicators of sanitary state and they are not connected with faecal contamination present in water (Rajasulochana and Rebba, 2015). This study isolates and identifies the pathogenic bacteria from stagnant waste water around residential area it is the causative agent for number of disease within the community.

## MATERIALS AND METHODS

### Description of the study area

Stagnant waste water samples were collected from residential area at Thanjavur district. Thanjavur is the seventh biggest city in Tamil Nadu. Thanjavur is famous for art, and architecture, Most of the Great Living Chola Temples, are approved UNESCO World Heritage Monuments. Recent climate change and heavy rain after heavy rain, water were stagnant around the residential building more than three months, in addition public were discharge their septic tank water together with stagnant rain water. Which promote the growth of various microorganisms. Contaminated water sample (100 ml) were collected from two locations around Thanjavur city, Tamil Nadu, India. such as Location 1 (L 1) Mettu Theru ,10°47'60.0"N 79°07'46.1"E and (L2) Srinivasapuram 10°47'21.4"N 79°07'31.9"E. Figure 1 and 2 Shows map showing sampling location (Figure 1 and 2).

### Sample collection

Samples were collected from the Mettu theru L1, and Srinvasapuram L2. Each location 100 ml of samples was collected in sterile containers. All the samples were transported immediately and stored in laboratory at Kunthavai Nacchiyaar Govt. Arts College for women, Thanjavur.


**Figure 1**. Shows the location of Thanjavur district on India map.

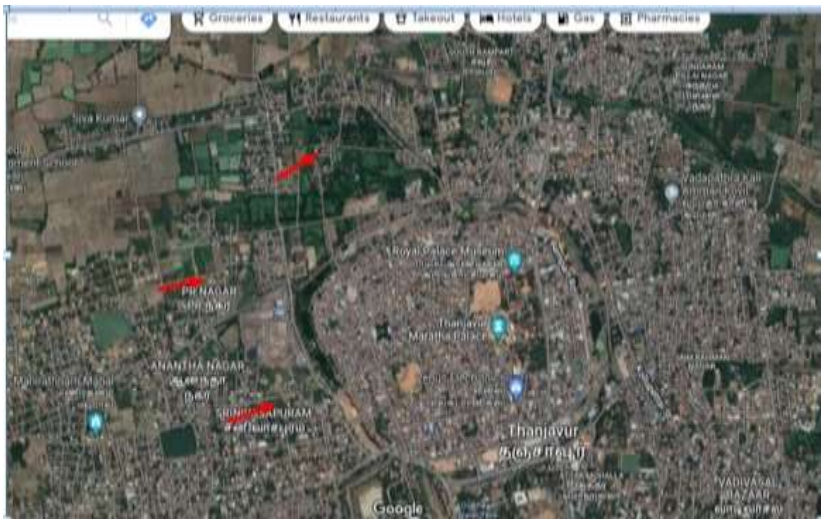


**Figure 2**. Map showing satellite view of sampling location.

**Isolation of pathogenic bacteria**

One ml of sample was suspended in 10 ml saline solution for three hours under shaken (180 rpm). Serial dilution techniques were used for the isolation of pure culture. Colonies were purified by twice subculturing using streaking plate method. Young culture was performed Gram staining, All isolates were subjected to biochemical identification test. From the primary isolation plate, Individual colonies were picked up separately and purified by quadrant streaking in nutrient agar plates (Cuppuccino and Sherman, 1996). After successful growth of bacteria the pure culture was sub cultured in nutrient agar slats incubated at $37^0$ C to achieve vigorous growth. Then the bacterial isolates were incubated at $37^o$ C for 24 hours (Kannan, 2002).

**Microbial and Biochemical characteristics of isolates**

The pure culture of metal tolerant strain was characterized by morphologically and biochemically using standard procedure from Bergey's Manual of Systematic Bacteriology 1994(Holt, 1994; Cuppuccino and Sherman, 1996).

**Susceptibility testing**

Antibiotic susceptibility of the bacterial isolates from stagnant waste water sample was assessed with Kirby-Bauer disc diffusion method, on Muller Hinton agar plates.

**Kirby-Bauer disc diffusion method**

The disk diffusion method is performed using Mueller-Hinton Agar (MHA), The inoculum for the disk diffusion method is prepared using a suitable broth such as tryptic soy broth and MHA agar. Sterilize by autoclaving at 121°C for 15 min. Cool the agar medium to 40-50°C. Pour the agar into sterile glass or plastic petri dish on a flat surface to a uniform depth of 4 mm, allow solidifying. From a pure bacterial culture (not more than 48 hours, old except for slow growing organisms), take four or five colonies with a

wire loop. Transfer colonies to 5 ml of Trypticase soy broth or 0.9% saline. Dip a sterile cotton swab into the standardized bacterial suspension. Inoculate the colonies on agar plate. Place antibiotic disk on the surface of the inoculated and dried plate. Incubate plates in an inverted position at 30°C or at an optimum growth temperature. Observe for the zone of inhibition after 16 to 18 hours. Slow growing organisms may require longer incubation period. The antibiotic disc used(mg) were Rifampicin (R) (50), Neomycin (N) (30), Gentamicin (G)(50), Vancomycin (V) (10), Chlramphenical (C)(30), Tetracycline (T) (30mg), Streptomycin (S) (10), Penicillin-G (P)(10 units), Ampicillin (A) (25) and Erythromycin E (10 mg).

## RESULTS AND DISCUSSION

The recent heavy rain rapidly damages the people in Tamil Nadu. Due to the heavy rain the residential building were partially sank and many empty lands also filled with rain water. This situation extent to three to four months for complete drain of water meanwhile the peoples who are living in this area were discarding of their septic tank and used water to this empty land. This leads to contaminate stagnant rain water due to high organic and inorganic waste in septic tank water and used water could pollute the nature of water and also it enhances the growth of pathogenic and parasitic microorganism. In most developing countries, it is estimated that 90% of sewage is disposed of into watercourses like rivers, lakes, streams and ponds without being treated thereby resulting in pollution of the environment (Ahmed *et al*., 2017). Similarly, large amount of these wastewaters seeps into the soil, polluting aquifers and groundwater which are as a result of leakage in on-site sanitation facilities and lack of sewerage pipes for disposing wastewater in low- and middle-income countries (Ajibola, 2019). Disposal of untreated waste into the environment can cause oxygen depletion as a result of microbial decomposition of organic matter, destructing the marine ecosystem, and also, affect the quality of water, making it unsafe for drinking and can lead to diseases such as dysentery, cholera and typhoid (Ajibuah and Terdoo, 2013; Alghobar and Suresha, 2015). In Tamil Nadu Chennai was one of the places heavily affected by rain on November 2021, The News Minut reported that the whole Chennai sank due to rain and also sewage water mixed with rainwater.

In this investigation, totally 136 isolates were obtained from Stagnant water sample at Mettu theru L1 and Srinvasapuram L2. Isolated bacterial pathogens were Chryseobacterium *sp., Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aeruginosa, Escherichia coli, Bacillus megaterium, Enterococcus sp, Streptococcus sp, Serratia marcescens* and *Staphylococcus aureus, Proteus vulgaris, Bacillus cereus, Providencia rettgeri, Escherichia coli, Serratia marcescens, Chryseobacterium, Pseudomonas aeruginosa, Enterococcus* respectively. Among this highest number of E coli, Streptococcus and Staphylococcus aureus were observed in both sites.

Antibiotic profile of isolates (Table 1) was unique in each strain, based on the results Ecoli is highly susceptible in both sites next Staphylococcus aureus from L1 and Proteus vulgaris from L2 more susceptible than other isolates. E. coli from L1 sensitive to Neomycin, Gentamicin, Chlramphenical, Tetracycline, Streptomycin and Erythromycin similarly from L2 Ecoli has same pattrn of sensitivy. Next *Serratia marcescens* from L1sensitive to Gentamicin, Chlramphenical, Tetracycline, Streptomycin, Penicillin, Amoxicilin and Erythromycin and likewise from L2 Gentamicin, Chlramphenical, Tetracycline, Streptomycin and Penicillin. Least level of sensitivity observed in Bacillus megaterium, Baceillus cereus and Enterococcus sp., Also resistant level of Chryseobacterium, Staphylococcus were observed in two antibiotics of Gentamicin, Amoxicillin.

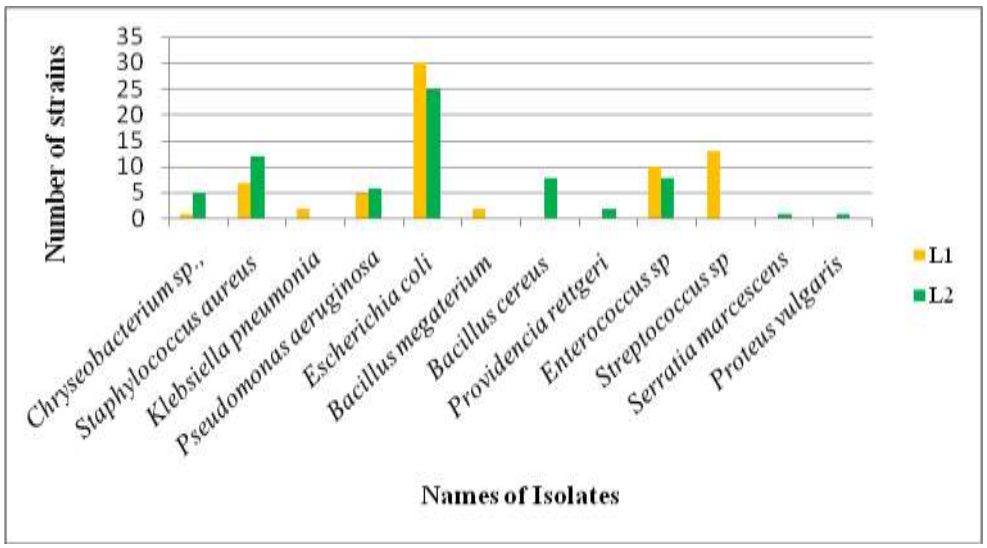


**Figure 3**. Shows isolation of bacteria sp., on Location 1(L1) and Location (L2).

**Table No 1**. Antibiotic profile of isolates in different pathogens.

| | No of coloies | Sensitive | | | | | | | | | | Resistant | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | R | N | G | V | C | T | S | P | A | E | R | N | G | V | C | T | S | P | A | E |
| *Chryseobacterium* | 1 | * | | * | | | | | | * | | | | * | | | | | | * | |
| *S. aureus* | *7* | | | | * | * | * | | * | * | * | | * | * | | * | | | * | * | * |
| *Klebsiella pneumonia* | *2* | | | * | | | * | * | | * | * | | * | | | | * | * | | * | |
| *P. aeruginosa* | *5* | | | * | | * | | * | | | | | * | * | | | * | * | | * | |
| *Escherichia coli* | *30* | | * | * | | * | * | * | | * | * | * | * | * | | * | * | * | | * | |
| *Bacillus megaterium* | *2* | | | * | | | | * | | | | | | | * | * | | | * | * | * |
| Enterococcus sp | *10* | | | | | | * | | * | | | | | * | * | | * | * | * | | |
| Streptococcus sp | *15* | | | | * | | | | | | | * | | | | * | * | | * | | |
| *Serratia marcescens* | 1 | | | * | | * | * | * | * | * | | | * | * | | | | * | | * | * |
| **Isolates on L2 site** | | | | | | | | | | | | | | | | | | | | | |
| *S, aureus* | 12 | * | | * | * | | | | | * | * | | | * | | | | | | * | |
| *Proteus vulgaris* | 1 | | | * | * | | | | * | * | | | | * | * | * | * | * | * | | * |
| *Bacillus cereus* | 8 | | | * | | | | * | | | | | | | * | * | | | * | * | * |
| *Providencia rettgeri* | 2 | | | | | | | | | | | | | * | | | * | | * | | |
| *Escherichia coli* | 25 | | * | * | | * | * | * | | * | * | * | * | * | * | * | | | * | * | * |
| *Serratia marcescens* | 1 | | | * | | * | * | * | * | | | | * | * | | | | * | | | * |
| *Chryseobacterium* | 5 | * | | * | | | | | | * | | | | * | | | | | | * | |
| *P. aeruginosa* | 6 | | | * | | * | | * | | | | | * | * | | | * | * | | * | |
| *Enterococcus* | 8 | * | | | | | * | | * | | | | | * | * | | * | * | * | | |

## CONCLUSION

This study concludes that stagnant rain water mixed with sewage water is the source for the growth of pathogenic microbial population; this will lead communicative disease in the society. We need to aware the people about the impacts of waste discharge to environment and waste disposal. Insist the corporation to channelize the root of rain water harvesting too. This study isolate number of pathogenic bacteria from stagnant waste water from location 1 and 2 and also analysed their antibiotic profile of the isolates.

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