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Surveillance of Antibiotic Resistant Microorganisms Isolated from Water Bodies in Chennai, The Southern Part of India

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Abstract

The emergence and spread of antibiotic-resistant bacteria in the environment is a serious medical and environmental problem worldwide.This study aimed to assess the microbiological quality and antibiograms of bacterial isolates from water bodies in Chennai , India to assess the prevalence and antibiotic resistance profiles of these bacteria in environmental samples .Antibiotic-resistant bacteria are able to transfer their genes to other aquatic bacteria, which thus acquire new resistance genes Isolating bacteria from clinical or environmental samples involves specific microbiological methods such as aiding in diagnosis, and guiding effective treatment strategies in clinical and research settings.Water samples were collected from various sources, including rivers, lakes, and reservoirs, and bacteria isolates were identified and characterised using standard microbiological methods. drinking water coming from these sources contains microorganisms Most often, Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterobacter aerogenes, Klebsiella spp.Salmonella typhi Were sown from water samples. Studies have shown that all isolated microorganisms have multi-resistance to one degree or another. But Escherichia coli and Klebsiella pneumoniae had the greatest resistance to various antibiotics.

Keywords: *Antibiotic Resistant , Bacteria , Bacteria Isolated from Water Bodies*

Introduction

Chennai, a major metropolitan area in southern India, is characterized by its significant population density and rapid urbanization. The city relies heavily on various water sources for drinking, sanitation, and industrial use. However, with the growth of urban areas and industrial activities, there is an increased risk of contamination of water bodies by pollutants, including antibiotic-resistant microorganism.

Antibiotic-resistant microorganisms poses a significant public health challenge globally. These bacteria have developed mechanisms to withstand the effects of antibiotics, limiting treatment options and increasing the risk of treatment failure in infections they cause. Resistance can arise through several mechanisms, including genetic mutations and the acquisition of resistance genes from other bacteria. In environmental settings like water bodies, antibiotic-resistant microorganims can persist and spread, potentially impacting human health through waterborne transmission routes. Monitoring and understanding the prevalence and resistance patterns of these bacteria are crucial for effective antibiotic stewardship and public health interventions. Strategies such as prudent antibiotic use, improved sanitation practices, and environmental management are essential to mitigate the spread of antibiotic-resistant microorganisms and reduce associated health risks.

Antibiotic resistance is a global health crisis accelerated by overuse and misuse of antibiotics in healthcare, agriculture, and animal husbandry. Addressing this issue requires coordinated efforts including prudent antibiotic use, surveillance, development of new antibiotics, and public education on the importance of antibiotic stewardship.

Surveillance of drug-resistant microorganisms is essential for understanding and managing antibiotic resistance in both clinical and public health settings. By monitoring the prevalence and spread of resistance patterns over time and across different regions, surveillance data provides crucial insights into which antibiotics remain effective and where resistance is emerging. This information guides healthcare providers in selecting appropriate treatments for infections, thereby improving patient outcomes and reducing the spread of resistant strains. Surveillance also helps in identifying outbreaks and clusters of drug-resistant infections, enabling prompt implementation of infection control measures to prevent further transmission. At a broader level, surveillance informs policymaking and supports efforts to develop new antibiotics and alternative therapies.

Ultimately, effective surveillance of drug-resistant microorganism is integral to preserving the effectiveness of antibiotics and combating the growing threat of antimicrobial resistance.

Materials and methods :

Sample collection and site observation
After visiting various parts of Chennai, 22 water samples from different locations. Sampling sites comprising sites like commonly used tap water sources , ponds , lakes , dams , fountains and wells . Samples were taken from locations were the water sources and distribution are delivered to the people . Each sample represents a specific area and is crucial for understanding the overall water quality profile .Samples are collected from Annanagar ,Arumbakkam, Annanagar West ,Kilpak , Saligramam and Korattur some of the populated areas of Chennai . Samples were collected from the taps , drinking water , well water , lakes , ponds common areas like the tap water from the metro station .

Bacteria isolation and identification

Initial Culture on MacConkey Agar :
A portion of the water sample(100µL)is inoculated onto MacConkey agar plates .The plates containing the samples are incubated overnight in the laboratory incubator. The agar contains bile salts and crystal violet, which inhibit the growth of Gram-positive bacteria and encourage the growth of Gram-negative bacteria like E. coli. Lactose in the medium allows for differentiation of lactose-fermenting organisms, including E. coli, which produces pink or red colonies due to acid production.

Subculture to Blood Agar:
The colonies on the initial plate is selected and with the help of the loop we pick them out and subculture on the Blood agar . Blood agar procedure, first the medium by sterilizing a nutrient agar base, cooling it to 50°C, and then mixing it with sterile sheep or horse blood before pouring it into Petri dishes and allowing it to solidify. Once the plates are prepared, inoculate them by streaking bacterial samples onto the surface using a sterile loop or swab. Incubate the plates at 35-37°C for 24-48 hours.
Blood agar provides nutrients and allows for detection of hemolysis patterns (alpha, beta, gamma) around colonies. E. coli typically exhibits gamma hemolysis (no hemolysis), aiding in its identification among other bacteria present.

Sub Culture on MacConkey Agar :
The colonies on the initial plate is selected and with the help of the loop we pick them out and subculture on the MacConkey agar, first prepare the plates by pouring the sterile MacConkey agar into Petri dishes and allowing it to solidify. Inoculate the agar with a sterile loop or swab by transferring a sample from the original bacterial culture and streaking it to obtain isolated colonies. Incubate the plates at 35-37°C for 24-48 hours. After incubation, examine the colonies: lactose fermenters will appear pink or red due to the acid production, while non-fermenters will be colorless or light beige. This selective medium helps differentiate between Gram-negative bacteria based on their lactose fermentation ability. Dispose of used materials according to safety guidelines and clean the work area thoroughly.

Mueller-Hinton (MH) Plates for Confirmation and Susceptibility Testing:
Mueller-Hinton (MH) agar, first prepare the medium by dissolving Mueller-Hinton agar powder in distilled water and autoclaving it at 121°C for 15-20 minutes. Once cooled to about 50°C, pour the agar into sterile Petri dishes to a depth of 4-6 mm and let it solidify at room temperature. For inoculation, adjust the bacterial culture to the 0.5 McFarland standard and evenly spread it over the agar surface using a sterile swab. If performing antibiotic susceptibility testing, place antibiotic discs onto the inoculated agar, ensuring they are spaced properly. Incubate the plates at 35-37°C for 16-24 hours, then examine for bacterial growth and measure inhibition zones if applicable.
Colonies from blood agar are streaked onto MH plates. These plates are then used for AST using antibiotic disks. Zones of inhibition around disks indicate susceptibility or resistance of E. coli to specific antibiotics, guiding treatment decisions.

Identification of organisms using Biochemical tests:
Biochemical tests such as indole test, citrate utilization test, urease test, Triple Sugar Iron (TSI) agar test, and Methyl Red (MR)/Voges-Proskauer (VP) test are commonly used in microbiology to identify and characterize bacterial species like E. coli.

Indole Test:

- **Purpose**: Determines the ability of bacteria to produce indole from tryptophan.
- **Procedure**: Bacteria are grown in a medium containing tryptophan. After incubation, Kovac's reagent is added. A pink/red color indicates a positive indole test, suggesting the presence of indole-producing bacteria like E. coli.

Citrate Utilization Test:

- **Purpose**: Assesses whether bacteria can use citrate as the sole carbon source.
- **Procedure**: Bacteria are streaked on a citrate agar plate. If bacteria utilize citrate, they convert it to alkaline byproducts, causing the pH indicator (bromothymol blue) to change from green to blue.

Urease Test:

- **Purpose**: Detects the enzyme urease, which hydrolyzes urea to produce ammonia and carbon dioxide.
- **Procedure**: Bacteria are grown in a medium containing urea. After incubation, phenol red indicator changes from yellow to pink in the presence of urease-positive bacteria like some strains of E. coli.

Triple Sugar Iron (TSI) Agar Test:

- **Purpose**: Differentiates bacteria based on their ability to ferment sugars (glucose, lactose, sucrose) and produce gas and hydrogen sulfide.
- **Procedure**: Bacteria are streaked on TSI agar slants containing three sugars and a pH indicator. After incubation, observation of color changes (yellow for acid production, black for hydrogen sulfide production) and gas production in the medium helps identify bacterial species such as E. coli.

BHI broth

BHI broth stands for Brain Heart Infusion broth. It is a nutrient-rich liquid medium used for the cultivation and propagation of a wide variety of microorganisms, including bacteria, fungi, and yeasts. Here are some key characteristics and uses of BHI broth:

Composition:

- BHI broth typically contains:
  - Brain and heart infusion: Provides amino acids, peptides, carbohydrates, and other growth factors.

Purpose:

- **General Purpose Medium**: BHI broth is a general-purpose medium suitable for the growth of a wide range of microorganisms. Its rich nutrient composition supports the growth of fastidious organisms that may require specific growth factors not provided by simpler media.

- **Antimicrobial Susceptibility Testing**: BHI broth can be used for antimicrobial susceptibility testing (AST) using broth dilution methods.

Antibiotic testing on Mueller-Hinton (MH) plates

Standard method used to determine the susceptibility of bacterial isolates, including E. coli, to specific antibiotics . A bacterial isolate, such as E. coli, is selected and grown in a nutrient broth to achieve a standardised concentration known as the turbidity equivalent to a 0.5 McFarland standard.Antibiotic disks impregnated with specific concentrations of antibiotics are placed onto the surface of the inoculated MH agar plate. The diameter of these zones is measured and compared to interpretive standards provided by organisations like the Clinical and Laboratory Standards Institute (CLSI) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) The *E. coli* isolates were then tested against 5 antibiotics as follows:ciprofloxacin (5 μg), cefotaxime (30 μg), tetracycline (30 μg), and gentamicin (10 μg),

Sallmonella Agglutination Testing

Salmonella agglutination testing is a diagnostic method used to identify specific Salmonella serotypes by detecting the presence of their unique antigens through clumping reactions. This test involves preparing a bacterial suspension containing suspected Salmonella with specific antisera that recognize unique O (somatic) or H (flagellar) antigens on the Salmonella cells. When the antisera are added to the bacterial suspension, visible clumping indicates a positive reaction, confirming the presence of the Salmonella serotype corresponding to the antisera used. This method is used as a for Confirmation and Susceptibility Testing the given samples

Results:

Sample	Isolated Organisms
Korattur tap	E. coli
Kotarrur RO water	E. coli , Klebsiella pneumoniae
Korattur tap water	E. coli , Salmonella Typhi

Sample	Isolated Organisms
Korattur open drinking water	E. coli , Pseudomonas aeruginosa
Korattur Tank water	E. coli , Klebsiella pneumoniae
Korattur lake	Salmonella Typhi, Pseudomonas aeruginosa ,Klebsiella pneumoniae
Korattur pond water	E. coli , Klebsiella spp
Shivan temple pond	Salmonella Typhi, Pseudomonas aeruginosa
Korattur sewage treatment plant (treated)	E. coli , Klebsiella pneumoniae
Korattur Untreated sewage treatment plant	E. coli , Salmonella Typhi

Sample	Isolated Organisms
Saligramam tap	E. coli , Pseudomonas aeruginosa
Saligramam RO water	E. coli , Klebsiella pneumoniae
Kilpak tap	E. coli
Kilpak well water	Klebsiella pneumoniae, Pseudomonas aeruginosa
Annanagar tap	E. coli , Klebsiella pneumoniae
Annanagar tank water	E. coli , Klebsiella pneumoniae
Arumbakkam metro tap water	E. coli , Klebsiella pneumoniae
Arumbakkam RO water	Klebsiella pneumoniae
Annanagar tower fountain	E. coli , Pseudomonas aeruginosa
10. Annanagar tower tap	Salmonella Typhi, Klebsiella pneumoniae
11. Kural Arrey water	E. coli

Sample	Isolated Organisms
12. Kilpak metro water	E. coli , Pseudomonas aeruginosa

Identification of organisms using Biochemical tests:

Bacterium	Indole	Citrate	Urease	TSI	MMM
E. coli	Positive	Negative	Negative	A/A with gas, H2S variable	Motile, fermenting
Salmonella typhi	Negative	Positive	Negative	K/A with H2S	motile, fermented
Pseudomonas aeruginosa	Negative	Positive	Negative	K/K	non motile, fermented
Klebsiella pneumoniae	Positive	Negative	Positive	A/A with gas, H2S variable	non motile, fermenting
Enterobacter aerogenes	Positive	Positive	Negative	A/A with gas, H2S variable	motile, fermented

Bacterium	Ciprofloxacin (5 µg)	Cefotaxime (30 µg)	Tetracycline (30 µg)	Gentamicin (10 µg)	Imipenem (10 µg)
E. coli	Resistant (R))	Susceptible (S)	Susceptible (S)	Resistant (R)	Susceptible (S)
Salmonella typhi	Susceptible (S)	Susceptible (S)	Resistant (R)	Susceptible (S)	Susceptible (S)
Pseudomonas aeruginosa	Resistant (R)	Resistant (R)	Resistant (R)	Susceptible (S)	Susceptible (S)
Klebsiella pneumoniae	Resistant (R)	Resistant (R)	Resistant (R)	Resistant (R)	Resistant (R)

Results and discussion:

Majorly microorganisms that are found in the samples from the water bodies are the 6 species . Among them are *Psudomonas aeroginosa , Klebsiella Pneumoniae , Sallmonella typhi , E. coli and Enterobacter aerogenes* . These organisms are evenly present in all the water samples from various water sources The data indicates a significant prevalence of antibiotic-resistant bacteria in the water bodies of Chennai. Notably, Klebsiella pneumoniae exhibited resistance to all tested antibiotics, highlighting severe multidrug resistance which poses a major public health risk.The high levels of resistance observed, especially in Pseudomonas aeruginosa and Klebsiella pneumoniae, suggest that the local environment may be a contributing factor. Factors such as industrial discharge, inadequate sewage treatment, and runoff from agricultural activities could contribute to the selection and spread of resistant strains.The high levels of resistance observed, especially in Pseudomonas aeruginosa and Klebsiella pneumoniae, suggest that the local environment may be a contributing factor. Factors such as industrial discharge, inadequate sewage treatment, and runoff from agricultural activities could contribute to the selection and spread of resistant strains.

The presence of antibiotic-resistant bacteria in water bodies poses risks to public health, potentially leading to infections that are difficult to treat with standard antibiotics. This highlights the need for rigorous monitoring and control measures to prevent the spread of resistance.

Conclusion:

This strain of *E. coli* shows resistance to Ciprofloxacin and Gentamicin but remains susceptible to Cefotaxime, Tetracycline, and Imipenem. This suggests possible mechanisms of resistance against fluoroquinolones and aminoglycosides, while other antibiotics may still be effective.This strain of *Salmonella typhi* is susceptible to Ciprofloxacin, Cefotaxime, Gentamicin, and Imipenem, but resistant to Tetracycline. The resistance to Tetracycline could be due to the presence of tetracycline resistance genes, while other commonly used antibiotics are still effective.This strain of Pseudomonas aeruginosa is resistant to Ciprofloxacin, Cefotaxime, and Tetracycline but susceptible to Gentamicin and Imipenem. The high resistance to multiple antibiotics suggests possible multidrug resistance, with susceptibility remaining to aminoglycosides and carbapenems.This strain of Klebsiella pneumoniae shows resistance to all tested antibiotics, including Ciprofloxacin, Cefotaxime, Tetracycline, Gentamicin, and Imipenem. This indicates a high level of multidrug resistance, making treatment options very limited and suggesting the need for alternative or more aggressive treatment strategies and susceptibility testing.This strain of Pseudomonas aeruginosa is resistant to Ciprofloxacin, Cefotaxime, and Tetracycline but susceptible to Gentamicin and Imipenem. The high resistance to multiple antibiotics suggests possible multidrug resistance, with susceptibility remaining to aminoglycosides and carbapenems.

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