ORIGINAL ARTICLE

A preliminary test and analysis to identify the presence of cefadroxil-resistant microbes in drinking water of prominent areas of Anantapur Urban

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Abstract

Introduction: With the increase in the usage of OTC antibiotics, there are growing chances of antibiotic misuse, resulting in the emergence of antibiotic insensitivity in humans and antibiotic resistance in microorganisms. The increasing trend of antibiotic-resistant organisms necessitates a preliminary survey to identify the presence of antibiotic-resistant organisms in the populated areas of Anantapur urban. **Methods:** Highly populated areas of Anantapur were selected based on the population density data collected from the officials of village secretariats ("Grama Sachivalayam," an official term given by the State Government of Andhra Pradesh in Telugu Language). Random sampling of household drinking water from each area was carried out, and these samples were subjected to antibiotic resistance testing against cefadroxil in the laboratory. **Results:** The results indicate the presence of cefadroxil-resistant bacteria in almost 11 areas out of 15 tested. It also shows the presence of cefadroxil-resistant bacterial and fungal species in the water samples collected and tested. **Conclusion:** This study intensifies the importance of extending this to different areas of Anantapur to ascertain the complete picture of the antibiotic resistance state of the microbes by including other antibiotics also.

Key words: Anantapur, cefadroxil, drinking water, microbial resistance, population

INTRODUCTION

Nowadays, it has become common for people to take antibiotics to treat bacterial infections. Whenever someone encounters a bacterial infection, they tend to resort to antibiotics for treatment. However, in the context of the contemporary push for recycling and reusing resources, concerns about antibiotic resistance have been mounting. The continuous and sometimes improper use of antibiotics has contributed to the emergence of antibiotic resistance. As bacteria evolve and develop resistance mechanisms, antibiotics become less effective in treating infections, posing a significant challenge for health-care professionals.

A study conducted by Kraupner *et al.* tested effluent water from a wastewater treatment plant in Sweden and identified 149 antibiotic-resistant species of *Escherichia coli.* In addition, other antibiotic-resistant bacteria have been observed, such as methicillin-resistant *Staphylococcus aureus*, vancomycin-resistant enterococcus, multidrug-resistant mycobacterium tuberculosis, and carbapenem-resistant *Enterobacteriaceae* gut bacteria.[1] These bacteria exhibit resistance against antibiotics. Moreover, in some countries, waterborne diseases have become increasingly common, and the bacteria causing these diseases are developing resistance against antibiotics.[2]

This growing concern led us to investigate the presence or absence of cefadroxil-resistant species in the drinking water samples of Anantapur Urban. The importance of cefadroxil

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in the treatment of infectious diseases cannot be understated. It belongs to the class of beta-lactam antibiotics, which are widely used for treating various bacterial infections. In the year 2020, cefadroxil was prescribed 2 million times in the United States alone and held a market share of 30 million USD, making it one of the most sold drugs in the beta-lactam antibiotics category. The city of Anantapur Urban covers an area of over 15.98 square kilometers and accommodates a population of 340,613. The city faces around 50,000 reported infectious disease cases per year, ranging from acute diarrhea to hepatitis A and E.[3]

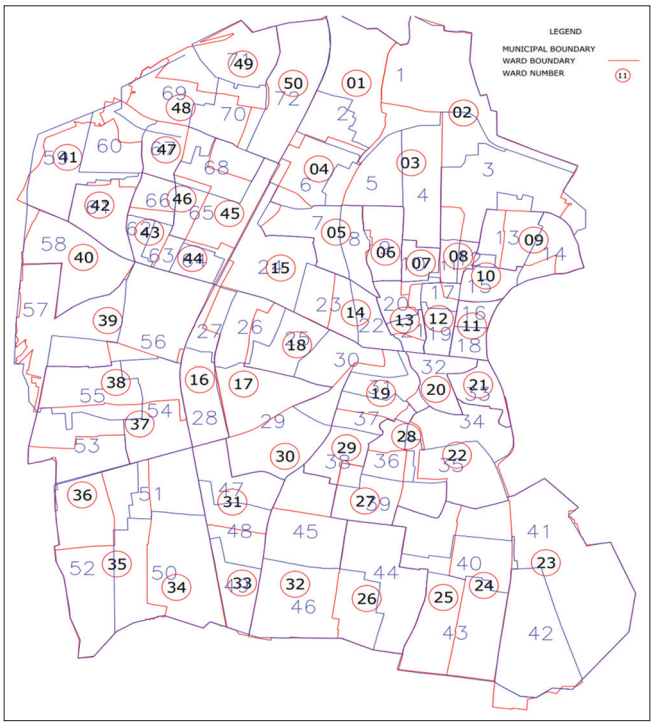

Figure 1: The map of Anantapur clearly divided by areas

To carry out our study, we have selected highly populated areas within Anantapur Urban, and from each area, we collected two water samples. These samples were subjected to laboratory testing to identify the presence or absence of cefadroxil-resistant species.

To combat antibiotic resistance, it is crucial to conduct antibiotic resistance surveys and studies like ours to monitor the prevalence of antibiotic-resistant bacteria in various environments, including water sources. Understanding the extent of antibiotic resistance can help in devising better strategies for the appropriate use of antibiotics and implementing measures to preserve their effectiveness and continued efficacy in combating infectious diseases.

METHODOLOGY

This research paper outlines the methodological approach employed to acquire the map of Anantapur urban for investigating antibiotic resistance. Collaboration with the Anantapur Municipal Corporation was initiated, and our research team explained the project's objectives to them, formally requesting the Anantapur Urban Map. The municipal office processed the request and sent the map through electronic mail [Figure 1], facilitating the identification of highly populated areas [Figure 2] for water sample collection. This map proved essential in our efforts to assess antibiotic resistance by targeting specific locations. The cooperative approach exemplified the significance of data sharing and partnerships in scientific research, fostering mutual trust between researchers and governing bodies and contributing to a comprehensive understanding of antibiotic resistance patterns in Anantapur urban.

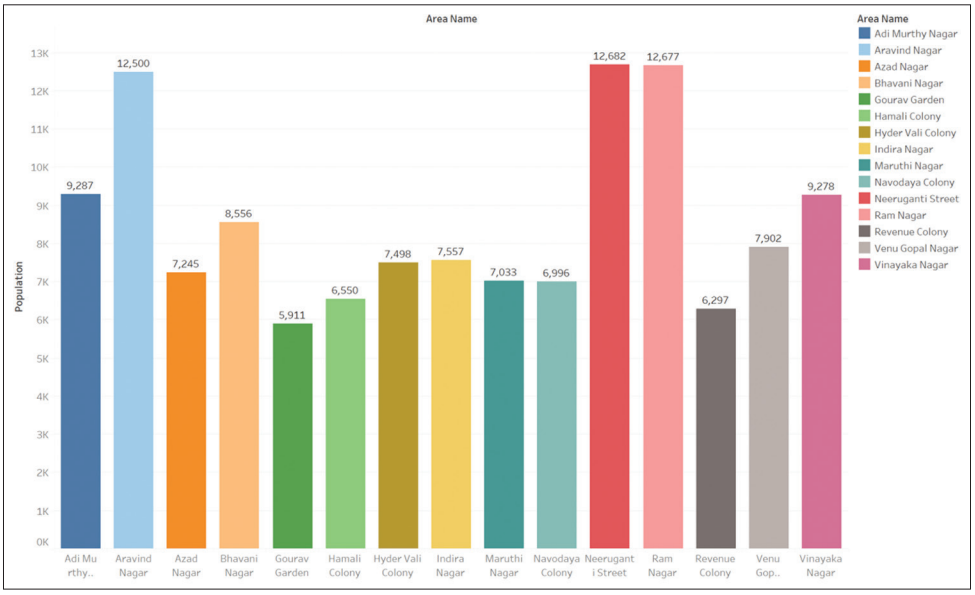

Figure 2: A graph indicating the selected areas with population

The areas of Anantapur were carefully selected based on the number of households and grouped according to their proximity. The geographical locations of the classified areas (Aadi Murthy Nagar, Aravind Nagar, Azad Nagar, Bhavani Nagar, Gourav Garden, Hamali Colony, Hydervali Nagar, Indira Nagar, Maruthi Nagar, Navodaya Cology Neeruganti Nagar Street, Ram Nagar, Revenue Colony, Venu Gopal Nagar, and Vinayak Nagar) were accurately marked on maps using the Google Maps Add Stop tool [Table 1]. This approach aimed to enhance the efficiency and organization of the data collection process, ensuring systematic investigation and yielding valuable insights into the prevalence of cefadroxil resistance in the sampled regions. Each area was assigned a unique customized code for easy identification. Subsequently, two water samples were collected from each selected area [Figure 3] to test for the presence of cefadroxil-resistant species. To optimize collection efficiency, areas located in close proximity were tested on the same day.

This research paper details a methodological investigation into the susceptibility of commercially available cefadroxil formulations, specifically Cefadrox-500 mg, under acidic pH conditions. Pure drug samples were not used to reflect real-world consumption patterns. Instead, cefadroxil from a pharmacy store was utilized for the study. Test solutions were prepared at a concentration of 2 g/100 ml and adjusted to acidic pH levels using appropriate buffers to mimic acidic environments encountered in certain physiological settings. The research findings shed light on the behavior of cefadroxil in acidic conditions and its potential efficacy against bacterial infections within acidic bodily regions. This study provides valuable insights for optimizing cefadroxil therapy in patients facing infections in acidic environments, thereby contributing to a comprehensive understanding of the antibiotic's performance in relevant conditions.[4]

Table 1: Google maps add stops for sample collection from respective area

Area	Location Coordinates
Aadi Murthy Nagar	14° 40' 48.1" N 77° 35' 46.5" E
Aravind Nagar	14° 40' 05.9" N 77° 35' 41.9" E
Azad Nagar	14° 41' 02.9" N 77° 35' 11.3" E
Bhavani Nagar	14° 41' 12.0" N 77° 36' 11.2" E
Gourav Garden	14° 41' 47.7" N 77° 35' 30.1" E
Hamali Colony	14° 39' 48.1" N 77° 36' 09.1" E
Hyder Vali Nagar	14° 41' 10.7" N 77° 35' 12.7" E
Indira Nagar	14° 41' 46.5" N 77° 35' 18.0" E
Maruthi Nagar	14° 40' 39.5" N 77° 35' 11.5" E
Navodaya Colony	14° 39' 27.7" N 77° 36' 38.4"E
Neeruganti Nagar Street	14° 40' 58.6" N 77° 36' 24.5"E
Ram Nagar	14° 40' 23.9" N 77° 35' 04.8" E
Revenue Colony	14.690928° N 77.598194° E
Venu Gopal Nagar	14° 41' 12.0" N 77° 36' 11.2" E
Vinayaka Nagar	14° 41' 09.8" N 77° 36' 48.7" E

Spread Plate Method to Identify the Resistant Species

The samples collected were subjected to testing for the presence of antibiotic-resistant organisms using two types of agar media: Nutrient agar[5] and Sabouraud dextrose agar[6]. The Petri plates were arranged under the laminar air flow to maintain a sterile environment. Sterile antibiotic-containing media were poured into the Petri plates and allowed to solidify. Following this, 200 µL of the water samples was inoculated onto the media using a micropipette, and the sample was evenly spread using an Sterile L-bend rod. The Petri plates were then sealed with parafilm and incubated in the incubator for 24 h. On the following day, the Petri plates were analyzed under a colony counter to determine the presence or absence of cefadroxil-resistant species. This carefully designed experimental procedure allowed for the systematic assessment of antibiotic resistance in the water samples, providing valuable insights into the prevalence of cefadroxil resistance in the studied regions.[7,8]

RESULTS AND DISCUSSION

The objective of this research was to assess the presence of cefadroxil-resistant microbes in selected areas of Anantapur. The water samples collected from each area were tested using both Nutrient agar and Sabouraud dextrose agar media. Nutrient agar revealed the presence of cefadroxil-resistant bacteria, while Sabouraud dextrose agar indicated the presence of cefadroxil-resistant fungal species in the respective areas.

Table 2 presents the results of testing water samples from selected areas in Anantapur for cefadroxil-resistant bacteria and fungi using Nutrient agar and Sabouraud dextrose agar media, respectively. Vinayaka Nagar (VN) exhibited the highest number of cefadroxil-resistant microbes, with 31 bacterial colonies and 16 fungal species colonies. Following closely, Ram Nagar (RM) showed significant resistance, with 27 bacterial colonies and 27 fungal species colonies. Maruthi Nagar (MR) also displayed notable resistance, with 19 bacterial

Figure 3: A representation of how water samples was collected form each household

Table 2: Area-wise number of resistant colonies obtained in the samples tested

Area	Sample 1		Sample 2	
	Nutrient Agar	Sabouraud dextrose agar	Nutrient Agar	Sabouraud dextrose agar
Adi Murthy Nagar (AM)	-	-	-	-
Aravind Nagar (AD)	11	21	21	17
Azad Nagar (AZ)	19	11	-	5
Bhavani Nagar (BN)	-	1	-	-
Gourav Garden (GG)	-	-	-	-
Hamali Colony (HC)	-	-	-	2
Hyder Vali Nagar (HVN)	-	-	-	-
Indira Nagar (IN)	-	-	-	-
Maruthi Nagar (MR)	19	23	26	31
Navodaya Colony (NC)	2	-	-	-
Neeruganti Nagar (NS)	-	4	-	-
Ram Nagar (RM)	27	29	15	27
Revenue Colony (RV)	-	-	1	1
Venu Gopal Nagar (VGN)	5	19	-	-
Vinayaka Nagar (VN)	31	27	16	27

Table 3: Categorization of tested area based on Presence of Cefadroxil Resistance Microbes

Area	Sample 1		Sample 2		Degree of concern	Degree of risk
	Nutrient agar	Sabouraud dextrose agar	Nutrient Agar	Sabouraud dextrose agar		
Aravind Nagar (AD)	+	+	+	+		High risk
Maruthi Nagar (MR)	+	+	+	+		High risk
Vinayaka Nagar (VN)	+	+	+	+		High risk
Ram Nagar (RM)	+	+	+	+		High risk
Azad Nagar (AZ)	+	+	-	+		High risk
Revenue Colony (RV)	-	-	+	+		Medium risk
Venu Gopal Nagar (VGN)	+	+	-	-		Medium risk
Hamali Colony (HC)	-	-	-	+		Emerging
Navodaya Colony (NC)	+	-	-	-		Emerging
Bhavani Nagar (BN)	-	+	-	-		Emerging
Neeruganti Nagar (NS)	-	+	-	-		Emerging
Adi Murthy Nagar (AM)	-	-	-	-		Safe
Gourav Garden (GG)	-	-	-	-		Safe
Hyder Vali Nagar (HVN)	-	-	-	-		Safe
Indira Nagar (IN)	-	-	-	-		Safe

colonies and 23 fungal species colonies. In Aravind Nagar (AD), 11 bacterial colonies and 21 fungal species colonies were observed, while Azad Nagar (AZ) showed 19 bacterial colonies and 5 fungal species colonies. Venu Gopal Nagar (VGN) displayed 5 bacterial colonies and 19 fungal species colonies. Neeruganti Nagar (NS) exhibited 4 fungal species colonies, while Navodaya Colony (NC) showed 2 bacterial colonies. Revenue Colony (RV) displayed 1 bacterial colony and 1 fungal species colony. Bhavani Nagar (BN) exhibited 1 bacterial colony. On the other hand, areas such as Hamali Colony (HC), Gourav Garden (GG), Hyder Vali Nagar (HVN), Indira Nagar (IN), and Adi Murthy Nagar (AM) did not show any presence of cefadroxil-resistant microbes in either sample. These findings provide valuable insights into the distribution and prevalence of cefadroxil-resistant bacteria and fungi in the tested areas, contributing to a comprehensive understanding of antibiotic resistance patterns in Anantapur.

The tested areas in Anantapur were categorized based on the degree of risk [Table 3] in descending order. In the High Risk

Zone, Aravind Nagar, Maruthi Nagar, Vinayaka Nagar, Ram Nagar, and Azad Nagar showed a significant concern with growth observed in both Nutrient Agar and Sabouraud dextrose agar media. In the Medium Risk Zone, Revenue Colony and Venu Gopal Nagar displayed growth in either of the media, indicating a moderate level of antibiotic resistance concern. The Emerging Risk Zone included Hamali Colony, Navodaya Colony, Bhavani Nagar, and Neeruganti Nagar, where growth was observed in only one of the media, suggesting the potential development of antibiotic resistance. In contrast, the Safe Zone comprised Adi Murthy Nagar, Gourav Garden, Hyder Vali Nagar, and Indira Nagar, where no growth was observed in either media, signifying a lower risk of antibiotic resistance. This ordering provides valuable insights for health-care authorities to prioritize monitoring and intervention strategies to address the escalating concern of antibiotic resistance in Anantapur.

CONCLUSION

The study was carried out to test the status of antibiotic-resistant species (cefadroxil was taken as drug to analyze the resistant species) in Anantapur urban through testing the water samples collected from selected areas of Anantapur. The work concludes with the presence of cefadroxil-resistant bacterial and fungal organisms in 11 areas out of 15 tested. In contrast, the Safe Zone, encompassing Adi Murthy Nagar, Gourav Garden, Hyder Vali Nagar, and Indira Nagar, demonstrates a lower risk of antibiotic resistance, with no growth observed in either media. However, it is important to note that this study had limitations, as it tested only selected areas and collected only two samples through random sampling. A comprehensive analysis covering all areas and a larger sample size would provide a more comprehensive understanding of the prevalence and distribution of antibiotic resistance in Anantapur. Despite these limitations, the findings offer valuable insights, to conduct a complete survey to ascertain the overall extent of antibiotic resistance.

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