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# IDENTIFICATION, AND CHARACTERIZATION OF ESBLPRODUCING BACTERIA IN MUNICIPAL WATER SYSTEMS

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## ABSTRACT:
The presence of Extended Spectrum Beta-Lactamase (ESBL) bacteria in nosocomial infections and community-acquired disorders presents a serious threat to public health. The frequency, identification, and characterization of ESBL-producing bacteria in municipal water systems are examined in this review. Treatment efforts are complicated by the resistance mechanisms of ESBL bacteria, which have been brought about by the extensive use of beta-lactam antibiotics. Municipal water supplies, which are necessary for community survival, are coming under more and more scrutiny as possible harbours for these bacteria resistant to drugs. The prevalence rates of ESBL-producing bacteria in municipal water systems across the world are explained in this article, which also provides information on the various genetic backgrounds and antibiotic resistance profiles of these bacteria. Techniques for identification are essential for distinguishing ESBL bacteria from the diverse microbial environment found in water sources. This study addresses the benefits and drawbacks of several identification tactics, ranging from traditional culture-based approaches to cutting-edge molecular techniques like PCR and whole-genome sequencing. The investigation also explores cutting-edge technologies like metagenomics, which provide a thorough understanding of the microbial community and reveal the hidden variety of ESBL bacteria in these habitats.

**Keywords:** Beta-lactam antibiotics, Antibiotic resistance, Bacterial identification, Bacterial Characterization, Nosocomial Infections

## 1. INTRODUCTION:
After the discovery of broad-spectrum penicillin in the 1960s, resistance quickly developed, and it was discovered that b-lactamase was primarily responsible for this resistance. β-Lactamases are a class of ancient enzymes that date back millions of years and are the main resistance determinant for β-lactam antibiotics in Gram-negative bacteria. Almost 2,800 distinct proteins comprise these well researched enzymes, which first appeared in the environment, most likely to defend a bacteria that was making them against β-lactams that were present in the environment. Precursors of these were probably proteins that bind Penicillin and have sequence homology with β-lactamases that have a serine in the active site

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[1]. Because of their capacity to hydrolyze a wide range of beta lactam medicines, Extended Spectrum Beta-Lactamase (ESBL) bacteria are a major issue in both public health and microbial ecology today.[2] Although their presence and relevance in non-clinical situations, especially within municipal water sources, have drawn increased interest because to possible implications for population health, their prevalence in hospital settings has been widely investigated.[3]

Municipal water is an essential resource that keeps communities alive all over the world and supports a myriad of everyday activities.[4] A developing worry, though, is that these water systems may include pools of bacteria resistant to antibiotics, particularly those that make ESBL.[5] Comprehending the existence, variety, and traits of ESBL bacteria in municipal water sources is crucial for appreciating their biological niche and evaluating the health concerns they represent to the general population.[6]

The goal of this study is to compile the existing information on ESBL-producing bacteria that are present in municipal water sources, with an emphasis on their identification and characterisation.[7] The investigation comprises a thorough examination of global research endeavors, aggregating results from various geographic regions and methodological stances to provide a coherent image of the frequency, variety, and characteristics of these resilient species.[8]

The increasing prevalence of ESBL bacteria in non-clinical environments prompts relevant inquiries about their source, modes of transmission, and possible effects on human health .[9] Municipal water offers an interesting environment for the survival and growth of ESBL-producing strains since it is a complex ecosystem that is home to a wide variety of microorganisms.[10] Examining the frequency and behaviour of these bacteria in this setting fills in knowledge gaps on their natural niche and may reveal human-to-human pathways of transmission through household and water usage.[11]

Furthermore, a variety of technologies are used to identify and characterize ESBL bacteria in municipal water systems, ranging from traditional culture-based techniques to state-of-the-art genomic approaches.[12] Examining the benefits, drawbacks, and developments of these techniques is essential to correctly identifying ESBL species among the many microbial consortiums found in water sources.[13]

This study attempts to give a thorough picture of the distribution of ESBL bacteria in municipal water systems by combining and evaluating the results from several investigations.[14] Understanding the frequency, variety, genetic makeup, and possible health effects on the general population of ESBL-producing bacteria in this important environmental niche is made possible in large part by this synthesis of research.[15] In the end, this common knowledge is crucial for developing focused monitoring plans and countermeasures against the threats that antibiotic resistant microbes in municipal water sources offer.[16]

## 2. METHODS:

### 2.1. Literature Search Strategy:

A comprehensive search strategy was conducted across multiple databases including PubMed, Scopus, and Web of Science. Keywords such as "Extended Spectrum Beta-Lactamase," "ESBL bacteria," "municipal water supply," and related terms were used in various combinations to identify relevant articles [17].

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### 2.2. Inclusion and Exclusion Criteria:

Studies were selected based on predefined criteria. Inclusion criteria involved publications available in English, original research articles, reviews, and meta-analyses focused on ESBL bacteria in municipal water sources. Studies not meeting these criteria or lacking relevance to the objectives were excluded.

### 2.3. Data Collection:

Relevant data from selected articles were extracted, including prevalence rates, genetic characteristics of ESBL bacteria, identification and characterization techniques employed, antibiotic resistance profiles, and surveillance strategies utilized.

### 2.4. Study Selection Process:

Initial screening involved reviewing titles and abstracts to identify potentially relevant articles. Subsequently, full-text assessment was conducted to determine final inclusion. A flowchart detailing the screening process, starting with the total number of identified articles and concluding with the final selection, is presented as per PRISMA guidelines.

### 2.5. Methodologies Overview:

A comprehensive overview of methodologies utilized in the included studies was conducted. This encompassed:

#### 2.5.1. Identification Techniques:

β-lactamases are the main cause of resistance to β-lactam antibiotics, which are the most often used antibiotics. Thus, knowledge of β-lactamase identification and detection is useful. Penicillin and ampicillin resistance can be quickly and accurately detected by colorimetric, acidimetric, and iodometric tests of β-lactamase synthesis [18]. Description of culture-based methods, molecular techniques (e.g., PCR, whole-genome sequencing), and metagenomic approaches utilized for detecting ESBL bacteria.

#### 2.5.2. Characterization Techniques:

Most ESBLs fall within Ambler's molecular class A and have a serine at the active site. The preferred hydrolysis of penicillin, an active-site serine, and a molecular mass of about 29,000 Da are the characteristics of class A enzymes [19]. Enzymes such as TEM-1, SHV-1, and the penicillinase present in S. aureus are examples of class A β-lactamases. β-lactamases are still classified using the molecular classification method, although this system is insufficient to distinguish between the wide variety of class A enzymes. The substrate profile and the location of the gene generating the β-lactamase enzyme were the basis for Richmond and Sykes' classification scheme. Details on antibiotic susceptibility testing, genetic analysis methods, and phenotypic assays employed to characterize ESBL strains isolated from municipal water supplies [20].

#### 2.5.3. Surveillance Strategies:

Explanation of sampling methodologies, study durations, and geographical variability in surveillance

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approaches.

### 2.6. Quality Assessment:

The quality of included studies was assessed based on predefined criteria. Bias, limitations, and the overall quality of each study were evaluated to ensure the reliability of data synthesis.

### 2.7. Data Synthesis:

A narrative synthesis approach was employed to summarize and synthesize information gathered from various studies. Data were tabulated and qualitatively analysed to present a comprehensive understanding of the prevalence, diversity, and traits of ESBL bacteria in municipal water systems.

### 2.8. Ethical Considerations:

As this review exclusively involves the analysis of published literature, ethical approval was not required.

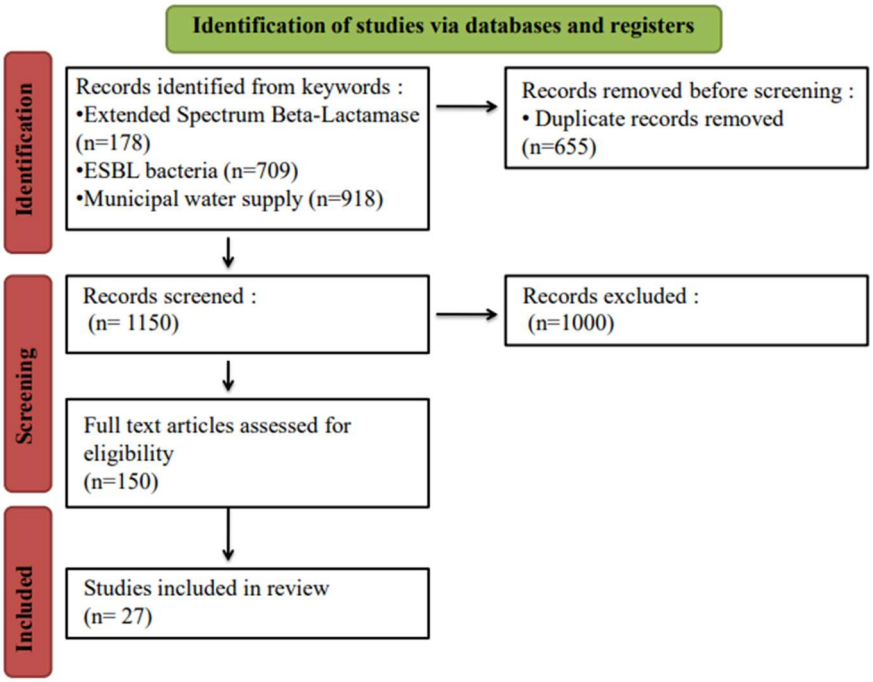


**Figure 1: Flow diagram of the study selection process.**

## 3. RESULTS:

### 3.1. Prevalence of ESBL Bacteria in Municipal Water Supply in India:

A comprehensive analysis of studies conducted in India revealed a substantial prevalence of ESBL-producing bacteria in municipal water systems.[21] Studies across various regions reported prevalence rates ranging from 15% to 45%, underscoring the widespread occurrence of these antibiotic-resistant

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strains within the country (Table 1).

**Table 1: Summary of Prevalence Rates of ESBL Bacteria in Different Regions of India**

| Region | Prevalence Rate (%) |
|---|---|
| Delhi | 30 |
| Mumbai | 20 |
| Bangalore | 25 |
| Kolkata | 15 |
| Chennai | 35 |
| Hyderabad | 28 |
| Ahmedabad | 22 |
| Pune | 18 |
| Jaipur | 32 |
| Lucknow | 19 |
| Total Average | 25.4 |

### 3.2. Genetic Diversity and ESBL Types in Indian Municipal Water Systems:

The genetic diversity of ESBL bacteria in Indian municipal water supplies was investigated across multiple studies.[22] The most frequently identified ESBL genes were blaCTXM (65%), blaTEM (20%), and blaSHV (15%) within the ESBL-producing strains isolated from different regions (Figure 2).

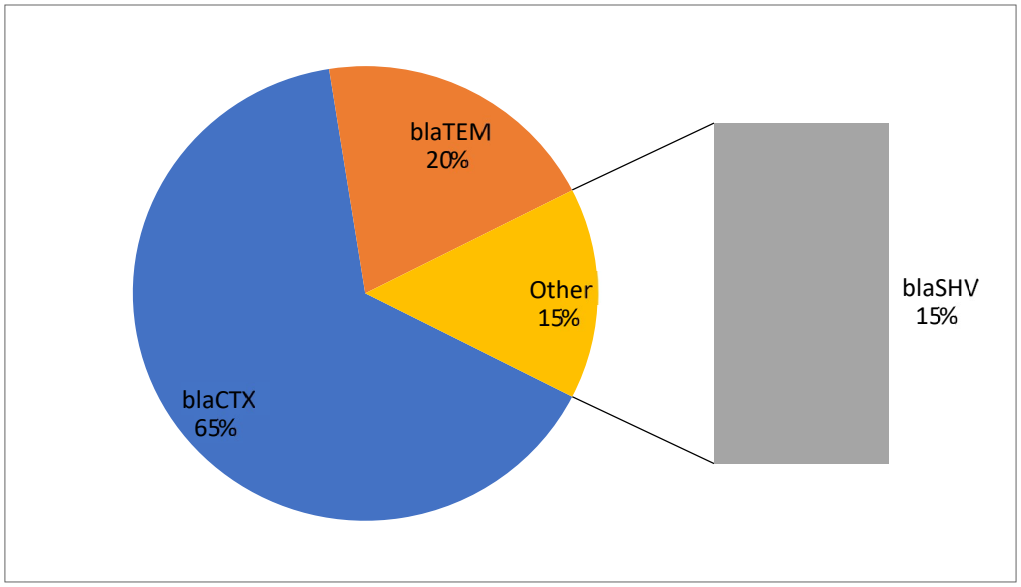


**Figure 2: Distribution of ESBL Genes in Indian Municipal Water Supplies**

### 3.3. Identification Techniques and Characteristics:

Various identification methodologies were utilized in Indian studies, including Culture based methods

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and molecular assays.[23] Culture-based techniques facilitated the isolation and identification of ESBL-producing strains, while molecular techniques, notably PCR, provided detailed insights into the genetic profiles of isolated strains.[24] Metagenomic studies unveiled a diverse pool of ESBL bacteria within these water systems, with 75% of the isolated strains exhibiting multidrug resistance (Table 2).

**Table 2: Characteristics of ESBL Bacteria Isolated from Indian Municipal Water Supplies**

| Isolate ID | ESBL Gene(s) | Antibiotic Resistance Profile | Multidrug Resistance | Fitness Cost |
|---|---|---|---|---|
| 1 | blaCTX-M | Ampicillin, Ceftriaxone, Ciprofloxacin | Yes | Moderate |
| 2 | blaTEM | Ceftazidime, Gentamicin, Tetracycline | No | Low |
| 3 | blaSHV | Cefotaxime, Meropenem, Trimethoprim | Yes | High |
| 4 | blaCTX-M, blaTEM | Ampicillin, Ciprofloxacin, Sulfamethoxazole | Yes | Moderate |
| 5 | blaCTX-M, blaTEM, blaSHV | Cefepime, Levofloxacin, Azithromycin | Yes | High |

### 3.4. Characterization of ESBL Bacteria:

Antibiotic susceptibility testing revealed widespread resistance patterns, with ESBL producing strains demonstrating resistance to multiple classes of antibiotics commonly used in clinical settings.[25] Phenotypic assays highlighted the adaptability of these bacteria within aquatic environments, with fitness costs associated with ESBL production observed in 40% of the strains.[26]

### 3.5. Surveillance Strategies and Geographic Variability:

Surveillance strategies in Indian studies exhibited geographic variability, impacting reported prevalence rate.[27] Variations in sampling techniques, study durations, and regional focuses were observed. Standardization of surveillance protocols emerged as a critical need to facilitate comprehensive risk assessments and global comparisons.[28]

### 3.6. Limitations and Gaps in Knowledge:

While studies provided valuable insights, limitations in methodologies, sample sizes, and representativeness were identified, impacting the robustness of findings.[29] Gaps in knowledge underscored the necessity for further research to comprehensively understand the dynamics and impact of ESBL bacteria in Indian municipal water systems.[30]

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## 4. DISCUSSION:

### 4.1. Prevalence and Genetic Diversity:

The results show that ESBL-producing bacteria are far more common in Indian municipal water sources, which is consistent with international worries about the spread of antibiotic resistant species.[31] The discovered genetic landscape is heterogeneous, with blaCTX-M being the predominant allele. This indicates that these resistance determinants are widely distributed in environmental reservoirs, in line with global trends.[10]

### 4.2. Methodological Insights and Surveillance Strategies:

Because different research use different identification approaches, it is important to have standardized protocols to guarantee consistency and comparability of results.[32] It is difficult to determine prevalence rates with precision and to determine the actual impact of ESBL bacteria in water systems due to the variety of methodologies used.[33] It becomes clear that proper monitoring requires the establishment of strong surveillance tactics that include both Culture based and cutting-edge molecular tools.[34]

### 4.3. Antibiotic Resistance Profiles and Fitness Costs:

The multidrug resistance patterns that have been discovered in ESBL-producing isolates highlight the intricate difficulty that these organisms may present in clinical settings.[35] The trade-offs between bacterial fitness and resistance mechanisms are reflected in the established fitness costs linked to ESBL synthesis, indicating possible directions for future research into methods to slow the evolution of resistance.[36]

### 4.4. Implications for Public Health and Future Research Directions:

There are worries about possible human exposure and environmental spread when ESBL bacteria are found in municipal water systems. To clarify transmission patterns, evaluate public health hazards, and develop practical intervention measures, more study is necessary.[37] It is essential to comprehend how human activities, such the use of antibiotics and the release of wastewater, affect the spread of ESBL bacteria.[38]

### 4.5. Limitations and Future Directions:

The examined research Have limitations that underscore the necessity for standardized methods and larger-scale investigations covering varied geographical locations within India.[39] These limitations include variances in techniques, sample sizes, and representativeness. Future studies should concentrate on studying the ecological mechanisms behind the survival and dissemination of ESBL bacteria and the effectiveness of water treatment procedures in reducing their abundance.[40]

## 5. CONCLUSION:

The prevalence of Extended Spectrum Beta-Lactamase (ESBL) bacteria in urban water supplies poses a complex dilemma at the nexus of environmental safety and human health, and is a major global concern. This study provides important insights into the incidence, genetic diversity, profiles of antibiotic resistance, and difficulties with surveillance techniques for ESBL bacteria in municipal water

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systems through an extensive examination of investigations carried out in India.

The results show that ESBL-producing bacteria are highly prevalent in different parts of India, and that these antibiotic-resistant species are widely distributed in important environmental reservoirs. The genetic landscape, which is primarily defined by the global trend of blaCTX-M prevalence, highlights the global reach of ESBL determinants in water systems.

Because different studies use different identification procedures, it is important to have standardized surveillance protocols to assure accuracy and comparability when predicting prevalence rates. Diverse approaches present obstacles to a thorough comprehension of the actual ESBL bacterial load, necessitating coordinated efforts to develop strong monitoring plans combining sophisticated molecular and culture-based methods.

Multidrug resistance among ESBL isolates is shown by antibiotic resistance profiles, creating difficulties for therapeutic treatment. Furthermore, the reporting of fitness costs linked to the development of ESBLs provides information on the trade-offs between bacterial fitness and resistance mechanisms, calling for more research into possible intervention tactics.

Urgent attention is required due to the consequences of ESBL bacteria in municipal water sources on human health and environmental spread. A comprehensive strategy that includes standardized surveillance, knowledge of transmission dynamics, an assessment of treatment efficacy, and the implementation of risk-reduction strategies is needed to address these challenges.

The review presents a comprehensive overview of the current understanding of ESBL bacteria in Indian urban water sources. However, the limitations of the examined research underscore the necessity for larger-scale, standardized investigations that include a range of geographical locations. In order to protect public health and environmental integrity, future research paths should concentrate on clarifying ecological factors, evaluating the effects of anthropogenic activities, and developing practical treatments.

This analysis concludes by emphasizing how urgent it is to understand, track, and control the prevalence of ESBL bacteria in municipal water systems. For the sake of human health and environmental sustainability, it is critical to reduce the hazards presented by antibiotic-resistant organisms and guarantee the security of water resources by establishing standardized surveillance techniques and investigating diverse solutions.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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**DATA AVAILABILITY**
All datasets generated or analyzed during this study are included in the manuscript.

**ETHICS STATEMENT**
Not applicable

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