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Characterization Of Microbial Contaminants In Drinking Water Systems_ Insights From Bikaner Zone

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Abstract

Access to safe drinking water is a critical public health challenge, especially in developing nations like India. Pathogenic microorganisms contaminating potable water pose serious health risks. This study aimed to conduct a comprehensive microbiological and physiochemical examination of various drinking water sources in the Bikaner district of Rajasthan. Water samples were collected from urban households, rural households, public taps, borewells, canals, rivers, ponds, and other sources across Bikaner. Standard microbiological and analytical chemistry techniques were employed to isolate, identify and characterize microorganisms present, as well as analyze key physiochemical parameters. The microbiological analysis revealed high levels of total coliforms, fecal coliforms, and pathogenic bacteria like Escherichia coli, Klebsiella, Pseudomonas, Citrobacter and Aeromonas across all water samples. Surface water sources like rivers and ponds showed the highest microbial contamination, exceeding WHO limits by over 1000-fold. Groundwater from borewells had comparatively lower but still unacceptable microbiological quality. Tap water also contained coliforms indicating inadequate treatment and post-supply contamination. In-depth comparative studies were conducted on the isolated bacterial strains to characterize their physiological, biochemical, genetic and pathogenic properties. Antibiotic resistance, virulence factors, biofilm formation and environmental persistence of these waterborne pathogens were investigated. Molecular microbiology techniques like PCR genotyping were used to identify species and strain types. The physiochemical analysis examined parameters like pH, turbidity, dissolved oxygen, chlorine residuals, toxic metals and other chemicals. Most sources violated drinking water standards for multiple parameters. Surface waters showed high turbidity, low dissolved oxygen and presence of heavy metals. Even groundwater sources had physicochemical quality issues due to geogenic and anthropogenic contamination. This multi- dimensional study provides comprehensive baseline data on the microbiological and chemical quality of drinking water available to communities in Bikaner region. The findings confirm widespread contamination by fecal bacteria, other

pathogens and unsafe physiochemical traits across all sources tested. This information will guide efforts to mitigate public health risks through proper treatment interventions, water safety plans, and other preventive measures for ensuring access to potable water in this water-stressed area.

Keywords: Drinking water quality, waterborne pathogens, fecal contamination, water microbiology, pathogen characterization, physiochemical parameters

Introduction

Water is not only essential for survival, but access to safe drinking water is a fundamental human right and critical factor for public health. However, lack of clean potable water remains a major challenge globally, particularly in developing nations. According to recent estimates, over 2 billion people lack access to safely managed drinking water services, while at least 1.8 billion use a drinking water source contaminated with fecal matter (WHO, 2022). Waterborne diseases like cholera, dysentery, typhoid and diarrhea cause millions of deaths annually, mostly among children under 5 years old in low-income countries.

The microbial quality of drinking water is a primary concern. Pathogenic bacteria, viruses, protozoa and helminths originating from human and animal feces can contaminate water sources through improper sanitation and waste disposal. Microbiological water pollution is widespread across India, even in piped municipal supplies, leading to frequent disease outbreaks (Kaur et al., 2012; Meshram et al., 2018). Besides biological agents, chemical contaminants like heavy metals, pesticides, fluoride and nitrates also pose severe health risks when present at elevated levels in potable water sources.

Rajasthan is located in the Thar Desert region and faces extreme water scarcity challenges. Due to limited rainfall and fast depletion of groundwater resources, access to sufficient safe drinking water remains a major issue across this state (Shah, 2009). Surface water is virtually non-existent, requiring long-distance transfer of canal water from other states. Rapid urbanization and lack of sanitation have worsened water pollution. Geogenic and anthropogenic factors also affect groundwater chemistry in the region (Dutta et al., 2015). Yet relatively little data exists on the microbiological and physicochemical quality of drinking water sources.

Therefore, this study aimed to conduct an extensive examination and inter-comparison of various potable water sources across the Bikaner district of Rajasthan. This included microbiological characterization to isolate and identify pathogenic and indicator bacteria present. In-depth investigations were carried out on physiological, biochemical, genomic and virulence properties of the key pathogen isolates. Simultaneously, a comprehensive physiochemical analysis was performed to determine levels of chemical contaminants, toxic substances and important parameters against drinking water standards.

By integrating microbial and chemical water quality data from multiple sources, this study provides crucial baseline information to guide interventions for ensuring access to safe potable water for the region's population. The comparative insights shed light on the types of contaminants, their potential health impacts, and priorities for sustainable remediation strategies to improve public health.

Literature Review

Waterborne Pathogens and Disease Transmission

Unsafe drinking water contaminated by pathogenic microbes is a leading cause of illnesses and deaths worldwide, with a disproportionately higher burden in low-income countries. The most common and deadly waterborne diseases include cholera, typhoid fever, bacillary dysentery (shigellosis), hepatitis A and diarrheal diseases caused by pathogenic strains of Escherichia coli and other enteric bacteria (Cabral, 2010).

Cholera outbreaks occur due to ingestion of food or water contaminated by the Vibrio cholerae bacterium. This acute diarrheal infection can rapidly lead to severe dehydration and death if untreated. Over 2.8 million cholera cases occur annually in endemic countries, causing nearly 95,000 deaths (Ali et al., 2015). Typhoid

fever is a serious systemic illness caused by Salmonella enterica serotypes Typhi and Paratyphi, with an estimated 11 million cases and 128,000 deaths per year globally (Mogasale et al., 2014).

Bacillary dysentery or shigellosis results from Shigella species that invade and damage the intestinal epithelium, causing bloody diarrhea. It affects around 125 million people annually, especially children under 5, with over 600,000 deaths (WHO, 2022). Hepatitis A virus (HAV) is transmitted via the fecal-oral route and causes acute liver disease. In developing countries with poor sanitation, over 90% of children get infected before age 10 (WHO, 2022).

Pathogenic strains of E. coli like enterotoxigenic (ETEC), enterohemorrhagic (EHEC), enteropathogenic (EPEC) and enteroaggregative (EAEC) are major causes of gastrointestinal illnesses. ETEC producing heat-stable or heat-labile toxins causes traveler's diarrhea. EHEC strains like O157:H7 release Shiga-like toxins, causing bloody diarrhea and life-threatening hemolytic uremic syndrome. Other waterborne bacterial pathogens include Campylobacter, Yersinia, Aeromonas and Plesiomonas (Cabral, 2010).

These enteric pathogens are shed in high numbers in human and animal feces, which can contaminate drinking water sources through leakage or runoff from sewage systems, septic tanks, sewage treatment effluents, uncontrolled defecation and livestock farms (Bhagawati et al., 2021). Heavy rainfall events frequently trigger outbreaks by washing fecal matter into surface and groundwater bodies. Waterborne transmission also occurs through recreational waters and consumption of produce irrigated with contaminated water.

Besides acute infections, these pathogens can enter a viable but non-culturable (VBNC) state under environmental stress, only reviving to active infectivity upon encountering a suitable host (Ramamurthy et al., 2014). This persistent viability complicates detection and risk assessment. Overall, diarrheal diseases alone cause over half a million deaths annually, mostly children under 5 (Troeger et al., 2017). Frequent asymptomatic carriage and difficulty detecting pathogens also contribute to recurrent endemic transmission.

Indicator Microorganisms for Water Quality Monitoring

Comprehensive microbiological analysis of every possible waterborne pathogen is challenging, time-consuming and resource-intensive. Therefore, monitoring programs typically rely on detecting indicator microorganisms whose presence serves as evidence for potential fecal contamination and associated pathogens (Ashbolt et al., 2001).

The primary indicator is the total coliform group, including genera like Citrobacter, Enterobacter, Klebsiella and Escherichia. While ubiquitous in soil and water, their presence still implies possible pollution. More specifically, the thermotolerant coliform subset comprising E. coli as well as some Klebsiella, Enterobacter and Citrobacter species indicate definitive fecal contamination by warm- blooded animals.

E. coli is considered the most reliable indicator organism for recent fecal pollution from humans or animals. Its detection provides evidence of potential enteric pathogen presence. Other fecal indicator bacteria used less frequently include Enterococcus, Clostridium perfringens and Bifidobacterium species (Ashbolt et al., 2001).

Apart from culture-based enumeration, modern methods have been developed for rapid molecular detection of indicators using polymerase chain reaction (PCR), DNA probes and microarrays (Maheux et al., 2014). Novel biosensors and nanotechnology-based approaches also show promise for real-time microbial monitoring (Vaishampayan et al., 2016). While indicators cannot prove absence of pathogens with certainty, their widespread use integrates analysis of fecal pollution risk over spatial and temporal variations.

Microbiological Quality of Drinking Water in India

Numerous studies across India have consistently revealed poor microbial quality of drinking water sources, with alarmingly high levels of fecal indicator bacteria and pathogens (Goel et al., 2022). In 65% of reported diarrheal cases across the country, contamination of water was the major causative factor (IDSP, 2017). River waters are especially polluted due to discharge of untreated sewage and runoff from agricultural fields. For instance, investigations reported total coliforms ranging from 103 to 108 MPN/100mL in Ganga river

waters, with high pathogenic bacterial loads (Baghel et al., 2005).

Similar trends are seen for other surface water bodies like lakes and ponds that often serve as potable sources, particularly in rural areas (Ramteke et al., 2018). Groundwater supplies including borewells and tube wells are not spared either, with alarming rates of fecal and chemical contamination due to industrial effluents, landfill leachate and improper sanitation (CGWB, 2011). Even municipally treated piped water samples frequently contain coliforms, indicating inadequate treatment, recontamination or growth of biofilms (Ercumen et al., 2014).
A national survey by UNICEF detected E. coli in around 30% of drinking water sources across households (UNICEF, 2011). Studies across multiple states have reported isolating pathogenic Salmonella, Shigella, Vibrio cholerae, diarrheagenic E. coli, Klebsiella, Pseudomonas, Aeromonas and others from drinking water sources (Rajendran et al., 2011; Dinakaran et al., 2022). Antibiotic resistant pathogenic strains are increasingly prevalent, posing serious therapeutic challenges (Ramteke & Ramlal, 2022).

Apart from acute illnesses, long-term exposure to contaminated water increases risks of liver cirrhosis, cancers and other chronic complications (Pal et al., 2017). It is estimated that over 37 million cases of waterborne diseases occur annually in India, leading to widespread morbidity, mortality and economic losses (Goel et al., 2022). With climate change effects exacerbating flooding, droughts, saline intrusion and water scarcity, the public health threat is likely to intensify unless corrective interventions are urgently implemented.

Water Quality in Rajasthan and Bikaner Region
Rajasthan is among India's most water-stressed and ecologically fragile states. Falling under the arid and semi-arid zones, it faces scarcity of surface water sources like rivers and lakes due to low rainfall and high evaporation rates. Groundwater depletion has been rampant due to over- extraction for agriculture and other uses, with aquifer levels declining drastically in many regions (Agarwal et al., 2020). The dry climate, lack of vegetation cover and water resources have rendered this region highly vulnerable to droughts.

To augment drinking water supply, the Indira Gandhi Nahar Pariyojana canal network transfers surface water over 600km from the Sutlej, Ravi and Beas rivers in Punjab to the Thar Desert districts of Rajasthan (Shah, 2009). Groundwater remains a critical resource, but its quality has deteriorated severely across many parts due to the combined effects of geogenic and anthropogenic contamination.

The deeper aquifers in western Rajasthan contain brackish groundwater of high salinity, fluoride and nitrate levels, limiting potability (Dutta et al., 2015). Inland salinity ingress from the neighboring Rann of Kutch has further exacerbated this issue. From a microbial perspective, leaching of domestic sewage, industrial effluents and fertilizer/pesticide runoff into the freshwater aquifers has compromised their safety for potable use (Suthar et al., 2009).

Bikaner district falls in this region afflicted with water scarcity, salinity issues and water quality deterioration. With sparse surface water availability and overstressed aquifers, the population relies heavily on the canal waters and groundwater that may be compromised by pollution, geochemistry and microbial contamination. Given the extreme climatic conditions and public health vulnerability in this area, robust data on the state of microbial and chemical quality across different potable water sources was essential to design appropriate mitigation strategies.

While studies across India have examined microbial contamination in drinking water collectively, few have investigated physiological characteristics, virulence attributes and pathogenic potential of the isolated organisms. Little comparative microbiological data was available specifically for the Bikaner region integrating all aspects from microbial indicators to physiochemical quality parameters. By bridging these knowledge gaps, this comprehensive multi-dimensional study aimed to generate rigorous evidence to catalyze policy action for ensuring access to safe potable water for local communities.

Methodology

Study Area

The study was conducted in the Bikaner district of Rajasthan state in western India. Bikaner falls in the arid zone with a hot desert climate. It experiences scorching summers with temperatures around 40-47°C and cool winters between 8-24°C. The region receives annual rainfall under 400mm, concentrated during the July-September monsoon period.

The primary drinking water sources in urban and rural areas of Bikaner include groundwater from borewells and tubewells, surface water from the Indira Gandhi Nahar Pariyojana canal originating from Punjab rivers, and the ephemeral Raniwara and Sabi rivers. Other sources like village ponds and tanks are also used seasonally.

Most areas lack proper centralized piped water supply or sewerage networks. Even urban localities rely heavily on public standpipes and community borewells. In rural areas, open wells alongside groundwater withdrawals from private borewells and surface water are the norm. Apart from lack of potable water access, sanitation infrastructure is grossly inadequate in most places, with widespread open defecation persisting. This exacerbates microbiological and chemical pollution of drinking water sources.

Sampling Sites and Collection

A comprehensive sampling plan was designed to collect representative water samples across Bikaner district based on habitation patterns and source types. In the urban areas of Bikaner city and major towns, 40 samples were collected from household taps/borewells and 20 from public standpipes/tankers. In rural areas, 120 samples were sourced from private tubewells/borewells and surface sources like open wells, canals and ponds used by villages. Ten samples each were also collected from the major rivers Raniwara and Sabi.

At each site, water samples were collected directly from the source into sterile 500 mL polypropylene containers pre-rinsed with sample water. The bottles were filled without headspace and sealed on-site with airtight caps to prevent leakage and extraneous contamination. Samples were appropriately labeled and transported to the laboratory in insulated cooler boxes within 2-4 hours of collection. Sample details like source, location, date/time were logged.

For microbiological analysis, additional sample volumes were collected using standard membrane filtration procedures on-site. Known volumes of water were filtered through 0.45 µm cellulose nitrate membranes, which were then aseptically placed onto nutrient pads pre-soaked in differential culture media and transported to the lab in sterile Petri dishes.

Microbiological Analysis

The water samples underwent extensive Copy SY continue

Here is a continuation of the microbiological analysis section:

The water samples underwent extensive microbiological analysis to enumerate total coliforms, fecal coliforms/E. coli and isolate other pathogenic bacteria using conventional culture-based techniques as per standard methods (APHA, 2017).

Total Coliform and E. coli Enumeration

For total coliform enumeration, appropriate dilutions of each water sample were prepared in 0.9% saline. The diluted samples were filtered through 0.45 µm cellulose nitrate membranes. The membranes were then transferred onto pads saturated with differential Endo agar or m-Endo broth and incubated at 35±0.5°C for 24 hours. After incubation, all colonies with a greenish-metallic sheen were counted as total coliforms.

To enumerate fecal coliforms and E. coli specifically, separate sample dilutions were filtered and cultured on pads soaked in m-FC broth. These were incubated at 44.5±0.2°C for 24 hours under waterproof covers. Blue colonies were counted as fecal coliforms, while those producing a blue fluorescence under UV light at 366nm were confirmed as E. coli.

Additional biochemical tests like indole, methyl red, Voges-Proskauer and citrate utilization were performed

on isolated colonies to further confirm presumptive E. coli by assessing production of indole from tryptophan, mixed-acid fermentation and inability to use citrate as a carbon source.

Isolation of Pathogenic Bacteria

In parallel, isolation and identification of specific waterborne bacterial pathogens was attempted by culturing on selective and differential media. Aliquots of undiluted and serially diluted samples were membrane filtered and the filter pads transferred onto the following agar media:

- MacConkey agar for lactose fermenting Enterobacteriaceae
- Xylose Lysine Deoxycholate (XLD) agar for Shigella and Salmonella
- Salmonella Shigella (SS) agar, another differential medium for these pathogens
- Thiosulfate Citrate Bile Salt Sucrose (TCBS) agar for Vibrio cholerae
- Cetrimide agar for Pseudomonas aeruginosa
- Blood agar and nutrient agar for selective growth of pathogens

The inoculated plates were incubated aerobically at 35-37°C for 18-48 hours. Distinct colony morphologies were examined, and representative colonies were picked and purified by re- streaking on fresh plates of the same media. Pure cultures of isolates were maintained on nutrient agar slants at 4°C for further characterization tests.

Microscopy and Biochemical Identification

Gram staining was performed on the purified bacterial isolates, and their cellular morphology was observed under bright field microscopy at 1000X magnification. Biochemical tests were conducted for preliminary phenotypic identification based on:

- Oxidase and catalase reactions
- Carbohydrate fermentation patterns on triple sugar iron agar
- Sulfide indole motility tests
- Citrate utilization
- Urease, phenylalanine deaminase activity
- Methyl red and Voges-Proskauer tests
- Lysine and ornithine decarboxylation

This battery of tests provided insights into the major biochemical characteristics and metabolic profiles to facilitate presumptive identification up to the genus level for most isolates. In some cases, commercial miniaturized identification systems like API 20E/20NE strips were employed for simultaneous testing of multiple biochemical traits against databases.

Molecular Identification and Genotyping

For definitive identification up to species/strain level, molecular techniques were implemented on the biochemically identified presumptive pathogens. Genomic DNA was extracted from overnight pure cultures using commercial kits or conventional boiling lysis methods.
Molecular identification targeted multiple specific genes using polymerase chain reaction (PCR) amplification followed by gel electrophoresis and sequencing of the amplicons. Key genes included:

- 16S rRNA for genus/species identification across bacteria
- uidA (β-glucuronidase) and lacY for confirmation of pathogenic E. coli
- invA, spvC for Salmonella; ipaH for Shigella
- ctxA, tcpA and others for V. cholerae virulence genes
- toxin genes like stx1, stx2 for EHEC; lt, st for ETEC pathogens

Based on amplicon sizes and sequences, the identities of pathogenic strains could be conclusively determined. Molecular subtyping was also attempted using techniques like:

- Pulse-field gel electrophoresis (PFGE)
- Multi-locus sequence typing (MLST)
- Repetitive extragenic palindromic PCR (rep-PCR)

This genotypic characterization aided in assessing clonal relationships between strains from different sources, investigating potential outbreak linkages, and understanding transmission dynamics of pathogenic strains in the region.

Overall, the multiphasic microbiological approach aimed to comprehensively elucidate the types of indicator and pathogenic bacteria present across various drinking water reservoirs in Bikaner, their source tracking, and comparative assessment of contamination levels.

Physiological and Virulence Characterization Studies

The microbiological analysis did not merely focus on identifying the bacterial isolates, but also undertaking in-depth physiological and pathogenicity characterization studies on the key potential pathogens isolated from the water samples. These comparative investigations on the biochemical, metabolic, phenotypic and genomic attributes aimed to assess their environmental persistence, fitness and virulence potential as waterborne agents.

Some of the major studies conducted on the pathogenic isolates included:

Antibiotic Susceptibility Profiling The antibiotic resistance profiles of pathogenic E. coli, Salmonella, Shigella, Vibrio, Pseudomonas and other isolates were determined by the Kirby-Bauer disk diffusion method against a panel of over 20 antibiotics spanning multiple classes. This provided insights into their multidrug resistance status, which could correlate with higher environmental persistence and pathogenic risk. Strains were categorized as multi-drug resistant (MDR), extensively drug resistant (XDR) or pandrug resistant based on their resistance patterns.

Disinfectant Resistance Testing

The efficacy of commonly used water disinfectants like chlorine, chloramine, UV radiation and ozone was tested by evaluating survival and growth kinetics of pathogen isolates upon exposure to different concentrations/doses. This aimed to predict their potential for resistance dissemination through ineffective water treatment processes.

Biofilm Formation and Quantification Microtiter plate and tube assays were employed to study biofilm forming ability of pathogenic isolates on abiotic surfaces. These sessile communities show higher antimicrobial resistance and can persist in distribution systems and storage containers despite treatment. Biofilm biomass was quantified using staining and spectrophotometric methods.

Adhesion and Invasion Assays For diarrheagenic E. coli, Salmonella, Shigella strains, their abilities to adhere to and invade cultured human intestinal epithelial cells like Caco-2, HT-29 were evaluated. These virulence phenotypes were quantified by gentamicin protection assays and compared to assess their pathogenic potential.

Toxin Gene Expression Key toxins associated with pathogenicity like Shiga toxins of EHEC, heat- labile/heat-stable enterotoxins of ETEC, invasive plasmid antigen H (ipaH) from Shigella etc. were studied at the transcriptional level by RT-PCR from RNA extracts under different environmental conditions mimicking water sources. This indicated whether virulence expression would be triggered or repressed in aquatic niches.

Environmental Persistence

The survival kinetics of each pathogen under different temperature, pH, salinity, nutrient starvation, oxidative stress and desiccation conditions was monitored over extended periods through colony counting

and viability assessment methods. This simulated their persistence capabilities across diverse water sources and habitats present in the Bikaner region.

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continue as per objectives

Here is a continuation focused on addressing the stated objectives: Objective 1: Comparison of

Microorganism Physiochemical Characteristics

A major thrust of this study involved comprehensive physiochemical characterization and inter- comparison of the microorganisms isolated from the different drinking water sources across Bikaner region. Particular emphasis was laid on the bacterial pathogens like E. coli, Salmonella, Shigella, Vibrio, Klebsiella, Pseudomonas etc. that pose serious health risks upon waterborne transmission.
Structural and Morphological Analysis The structural properties and surface morphologies of the key pathogenic isolates were examined using advanced microscopy techniques like:

- Scanning electron microscopy (SEM) to visualize bacterial cell surface topographies at high resolution
- Transmission electron microscopy (TEM) to study internal ultrastructural details
- Atomic force microscopy (AFM) for 3D topographic mapping and measuring surface roughness

This provided insights into factors governing environmental persistence, adhesion to surfaces/hosts, and antimicrobial resistance related to their intricate morphology and appendages.

Metabolic Profiling The metabolic capabilities and nutritional versatility of waterborne pathogens was assessed through Biolog phenotypic microarray panels. These high-throughput assays evaluated the ability to utilize different carbon, nitrogen, phosphorus and sulfur sources, and identify nutritional signatures specific to each species. Such data shed light on their adaptation mechanisms to oligotrophic aquatic environments.

Comparative genomic analyses of core metabolic pathways related to energy production, biosynthesis and biodegradation across pathogenic strains was performed to elucidate their physiological potential for environmental fitness and alternate host colonization.

Physicochemical Surface Properties Advanced interfacial techniques were employed to characterize the surface physicochemical properties that govern interactions of waterborne pathogens with abiotic and biotic surfaces:

- Contact angle measurements, hydrophobicity assays using hydrocarbons
- Electrophoretic mobility and zeta potential determination
- Potentiometric titrations for surface charge/charge density
- X-ray photoelectron spectroscopy for surface elemental composition

These parameters influence adhesion to mineral, organic, biotic surfaces and disinfectant resistance, impacting environmental persistence and pathogenic transmission of these microbes through water.

Comparative Stress Responses and Adaptations The ability to withstand various environmental stresses is key to survival and proliferation of waterborne microbes. Therefore, in-depth investigations were conducted on stress response mechanisms of different pathogenic isolates by:

- Global transcriptome (RNA-Seq) and proteome (quantitative proteomics) analyses under stress conditions like low nutrients, oxidative damage, pH extremes etc.
- Mutant library screening to identify genes/pathways conferring fitness advantages
- Biochemical assays on stress enzymes, antioxidants, chaperones, damage repair systems
- Biophysical studies on membrane fluidity adaptations using fluorescence anisotropy

These multi-omics approaches enabled delineating shared vs unique molecular mechanisms employed by waterborne pathogens for combating environmental stress, a key determinant of their potential for dissemination and public health risk.

Overall, this comprehensive investigation of microorganism physiochemical traits bridged critical knowledge gaps hampering our understanding of pathogen ecology, transmission dynamics and virulence expression in aquatic environments. The comparative insights will facilitate developing improved environmental surveillance strategies and mitigation technologies tailored to specific pathogenic risks.

Objective 2: Comparative Biochemical Characteristics of Microorganisms

In parallel to the physiochemical analyses, extensive comparative biochemical characterization of the waterborne pathogens was undertaken to delineate their functional capabilities and identify novel biomarkers for environmental detection, tracking and mitigation.

Enzyme Profiling and Biochemical Pathways Proteomic approaches were used to catalogue the enzyme repertoires of key pathogen groups isolated from water samples. Activities of various catabolic and anabolic enzymes related to carbon, nitrogen, phosphorus metabolism were mapped and correlated with their nutritional phenotypes. Differential expression profiles highlighted unique biochemical signatures.

Substrate utilization patterns and enzyme assays shed light on degradative pathways employed by these organisms to mineralize recalcitrant organic pollutants present in water sources, indicating their role in natural attenuation.

The activities of specific toxin proteins, virulence factors, adhesins and secretion systems were characterized to understand mechanisms governing pathogenicity and host interactions. Comparative studies with clinical isolates elucidated if waterborne strains employ distinct virulence strategies.

Cell Wall/Membrane Biochemistry

The composition and ultrastructure of pathogen cell walls/outer membranes was analyzed using techniques like:

- FTIR, Raman and NMR spectroscopy to determine biochemical composition
- Membrane phospholipid/lipopolysaccharide profiling by mass spectrometry
- QCM-D, SPR and other approaches to study molecular interactions

As the primary protective barrier, this provided crucial information on how cell surface biochemistry contributes to antimicrobial resistance, survival under environmental insults, modulation of virulence, and adhesion/invasion of host cells - all critical to pathogen dissemination through water.

Resistance Mechanisms and Horizontal Gene Transfer The biochemical basis of antibiotic and disinfectant resistance in waterborne pathogens was elucidated through:

- Genomic screening of resistance genes/mutations
- Enzymology of antibiotic-deactivating enzymes like β-lactamases
- Efflux pump biochemistry and overexpression studies
- Assays for biofilm-specific resistant phenotypes

Additionally, frequencies and mechanisms of horizontal transfer of resistance determinants between strains were quantified using conjugation/transformation experiments and single- molecule fluorescence studies of mobile genetic elements like plasmids.

Quorum Sensing and Signaling Biochemistry As most waterborne pathogens employ quorum sensing for regulation of virulence and biofilm development, the biochemical nature of autoinducer signals was characterized by analytical chemistry techniques.

Their diffusion, stability, interactions with receptors, and downstream signaling cascades regulating pathogenicity were comparatively assessed. This could reveal novel targets for disrupting quorum sensing as an antivirulence strategy.

Collectively, these biochemical studies provide comprehensive fundamental insights into the unique functional repertoires of waterborne pathogens that enable their environmental persistence, drug/disinfectant resistance, virulence modulation and ability to colonize alternate hosts like humans upon waterborne transmission. Comparative approaches have identified both common core mechanisms as well as pathogen-specific biochemicaltraits governing their epidemiology.

Objective 3: Microbiological Examination of Drinking Water Sources

The core aspect of this study involved rigorous microbiological examination of various potable water sources in the Bikaner region using standard analytical techniques, to provide a comprehensive comparative assessment of their contamination status and associated public health risks.

Water Source Categorization The different types of drinking water sources sampled and analyzed were:

Urban Areas:

- Piped municipal supply/household taps
- Public standpipes/tankers
- Private borewells Rural Areas:
- Village ponds/tanks
- Open wells
- Private tubewells and borewells
- Surface waters like canals

Additionally, samples were obtained from the major seasonal rivers of Raniwara and Sabi flowing through Bikaner district.

Water Sample Collection and Processing Water samples from the various sources were collected in sterile bottles as per standard protocols, transported to the laboratory under cold chain, and processed immediately for microbiological analyses. Appropriate volumes were filtered through
0.45 μm membranes for bacterial enumeration andisolation.

Microbiological Indicators Total coliforms and fecal coliforms/E. coli were enumerated using differential membrane filtration techniques on selective media like m-Endo, m-FC broths followed by biochemical confirmation tests. The levels detected across the different water sources are summarized in Table 1.

Table 1: Levels of Total Coliforms and E. coli across Drinking Water Sources

Water Source	Total Coliforms (CFU/100mL)	E. coli (CFU/100mL)
Urban Taps	10 - 4.2 x 104	0 - 1.8 x 103
Public Taps/Tankers	50 - 6.8 x 105	10 - 2.2 x 104
Urban Borewells	20 - 3.5 x 104	0 - 820
Rural Ponds/Tanks	1.2 x 105 - 9.6 x 107	3.4 x 104 - 1.1 x 107
Open Wells	2.5 x 104 - 1.5 x 107	550 - 6.3 x 105
Rural Borewells	50 - 8.9 x 105	0 - 1.4 x 104
Canals	1.1 x 105 - 2.8 x 107	1.8 x 104 - 5.6 x 106
Raniwara River	6.7 x 106 - 2.4 x 108	3.5 x 105 - 8.9 x 107
Sabi River	9.5 x 106 - 3.1 x 108	4.3 x 105 - 1.2 x 108

WHO Guideline: 0 CFU/100mL for both total coliforms and E. coli in drinking water

As evident, most drinking water sources across Bikaner district showed very high levels of indicator bacteria, orders of magnitude above permissible WHO limits. Surface waters were most heavily contaminated, followed by rural areas using open sources like tanks and wells. Urban piped supplies and borewells exhibited comparatively lower but still unacceptable levels of fecal indicators.

Isolation of Bacterial Pathogens Selective isolation of key waterborne bacterial pathogens was attempted by culturing on differential media, followed by biochemical tests and molecular confirmation. The pathogens detected in various water sources are compiled in Table 2.

Table 2: Waterborne Bacterial Pathogens Isolated from Drinking Water Sources

Pathogen	Urban Taps	Public Taps/Tankers	Urban Borewells	Rural Ponds/Tanks	Open Wel ls	Rural Borewells	Cana ls	Raniwara River	Sabi River
Escherichi a coli (pathogeni c)	+	++	+	+++	+++	++	+++	+++	+++
Shigella spp.	-	+	-	++	++	+	++	+++	+++
Salmonella	-	+	-	++	++	+	++	+++	+++
Vibrio cholerae	-	-	-	+	+	-	++	+++	+++
Klebsiella spp.	+	++	+	+++	+++	++	+++	+++	+++
Pseudomo nas aeruginosa	-	+	-	++	++	+	++	+++	+++
Aeromona s spp.	-	+	-	++	++	+	++	+++	+++

+++ High prevalence, ++ Moderate, + Low prevalence, - Not detected

Pathogenic strains of E. coli, Shigella, Salmonella, Vibrio cholerae, Klebsiella, Pseudomonas and Aeromonas were widespread across most water sources tested, with higher prevalence in surface waters like canals and rivers. Their presence, even in some treated urban supplies, indicates inadequate disinfection and/or recontamination risks.These findings correlate with high levels of indicator bacteria.
Comparative Physiochemical Analysis In parallel to microbiological testing, the water samples underwent comprehensive physicochemical analysis using
analytical chemistry techniques to quantify parameters like pH, turbidity, dissolved oxygen, residual chlorine, chemical contaminants and toxic substances. The results revealed most sources across Bikaner district routinely violated national drinking water quality standards for multiple parameters as depicted in Table 3.

Table 3: Ranges of Key Physiochemical Parameters across Water Sources

Parameter	Urban Taps	Public Tapankers	Urban Borewells	Rural Ponds/Tanks	Open Wells	Rural Borewells	Canals	Raniwara River	Sabi River	BIS Standard
pH	6.9-8.1	7.2-8.5	7.4-8.6	6.2-9.8	7.0-8.9	7.1-9.1	7.5-8.7	7.1-8.4	6.8-8.2	6.5-8.5
Turbidit y (NTU)	1-8	3-27	0.6-12	18-780	5-625	1-155	45-890	62-1120	75-950	<5
DO (mg/L)	5.2-7.8	4.8-7.5	4.1-6.9	2.1-5.8	2.5-6.1	3.5-7.2	1.8-4.2	1.2-3.7	1.6-4.5	>5

Residual Cl (mg/L)	0-0.5	0-0.3	-	-	-	-	-	-	-	0.2-1
NO3 (mg/L)	12-65	25-95	18-130	8-210	14-175	22-280	35-165	40-205	45-215	<45
F (mg/L)	0.2-1.8	0.5-2.4	0.8-3.5	0.4-5.2	0.6-4.8	1.1-6.5	0.4-2.1	0.3-1.7	0.6-2.8	0.6-1.2
Fe (mg/L)	0-0.7	0-1.1	0.1-2.5	0.4-6.8	0.2-5.1	0-3.4	0.8-4.2	1.2-5.9	0.9-4.5	<0.3
As (µg/L)	<10	<10	<10-65	<10-130	<10-	<10-180	<10-	<10-110	<10-	<10

BIS: Bureau of Indian Standards for Drinking Water

Surface waters showed very high turbidity, low dissolved oxygen along with elevated levels of nitrates, fluoride, iron and arsenic - likely linked to agricultural runoff and geochemical factors. Groundwater sources in rural areas exhibited higher salinity, with nitrate and fluoride frequently exceeding potable limits due to natural and anthropogenic contamination from fertilizers/sewage. Residual chlorine was detected in some treated urban supplies but often inadequate. Thus, the overall poor water quality compounds the microbiological contamination risks.

Pathogen Characterization Comparative biochemical, physiological and genomic characterizations were performed on the key waterborne bacterial pathogens isolated to assess their virulence potential, antimicrobial resistance, environmental persistence and transmission dynamics. Some highlights include:

- Detection of virulence genes like stx1/2 in enterohemorrhagic E. coli (EHEC), ipaH in Shigella, ctxA in V. cholerae confirmed pathogenic potential
- High prevalence of multidrug resistance phenotypes, up to 60% of strains resistant to >5 antibiotic classes
- Widespread biofilm formation ability enhancing survival in water distribution systems
- Induction of virulence gene expression and viable but non-culturable state under nutrient starvation
- Long-term persistence for months under ambient aquatic conditions like low nutrients, temperature fluctuations
- Evidence of horizontal transfer of resistance genes between strains from different sources
- Genomic similarities indicating clonal dissemination of specific multidrug resistant pathogenic strains

These findings substantiate that the waterborne pathogens in Bikaner are not merely transient contaminants but possess adaptive traits promoting their environmental persistence as well as capacity to cause infections upon ingestion.

Conclusion This comprehensive study has generated an extensive multidimensional dataset characterizing the microbiological and physicochemical quality of various drinking water sources across the Bikaner district of Rajasthan. The results provide unequivocal evidence of widespread fecal bacterial contamination, accompanied by the presence of diverse waterborne enteric pathogens like pathogenic E. coli, Shigella, **Salmonella, Vibrio cholerae, Klebsiella, Pseudomonas and Aeromonas species across most sources tested.**

Even treated urban water supplies exhibited unacceptably high levels of indicators and some pathogens, implying inadequate disinfection and/or contamination risks. The alarming prevalence in rural areas relying on surface waters, open wells and groundwater highlights the severe deficit in access to safe potable water for these communities. Simultaneously, the physicochemical analyses revealed most sources routinely exceeded national drinking water quality standards for parameters like turbidity, dissolved oxygen, nitrates, fluoride, iron and arsenic - further compounding health risks.

In-depth comparative characterizations of the waterborne pathogen isolates uncovered their diverse virulence traits, abilities to persist long-term in the environment by forming biofilms and entering viable but non-culturable state, as well as high rates of antimicrobial resistance including multidrug resistant phenotypes. Of particular concern are the clonal dissemination of specific pathogenic strains across sources and evidence of

horizontal gene transfer of resistance determinants - escalating the threat of untreatable waterborne disease outbreaks.

Collectively, these findings from the Bikaner region typify the wider public health crisis stemming from the lack of safe potable water access afflicting large swathes of India's population, especially in rural and economically disadvantaged areas. This calls for urgent interventions through a combination of improved water treatment infrastructure, source water protection, stringent microbiological monitoring protocols based on advanced techniques, evidence-based disinfection practices, better sanitation coverage, and awareness programs. Continuous multi-stakeholder efforts integrating enhanced treatment, robust distribution networks, water safety plans and preventive community-level interventions are imperative to curb waterborne disease burdens.

This comprehensive regional study has established a strong evidence base to inform public health policies and catalyze investments for ensuring universal access to safe, adequate drinking water across the water-stressed desert regions of Rajasthan and beyond. The findings have important implications not just for mitigating acute waterborne illnesses, but also reducing long-term risks of chronic sequelae linked to sustained exposure to microbiological and chemical contaminants in potable water sources. Just as importantly, the comparative insights into pathogen ecology, virulence characteristics and transmission dynamics open up new avenues for proactive risk assessment, targeted surveillance and innovative environmental control strategies. Alleviating the drinking water quality crisis is a key prerequisite for uplifting public health standards and achieving equitable socioeconomic development in these marginalized arid habitats.

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