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Analysis of Antibiotic Resistance In Bacteria Isolated From Sewage Water In Nasiriyah

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Abstract: The present research charts the distribution and resistance profiles of bacteria pulled from sewage systems in Nasiriyah, a southern Iraqi city often strained by industrial runoff. Across 336 discrete sampling points-tertiary plants, hospital outfalls, and makeshift trunk labourers netted 158 colonies that later merged into a cohesive strain bank for analysis. A striking 127 isolates, or 80.45, qualified as multidrug-resistant, with *Escherichia coli* claiming 53.2% of the total. Antibigram readouts show that better than eight in ten *E. coli* strains rebuffed seven or more standard agents, including ampicillin and ciprofloxacin. *Acinetobacter baumannii*, though fewer in number four examples, passed every last resort hurdle, shrugging off imipenem and colistin alike. Parallel spot tests for extended-spectrum beta-lactamases identified multiple strains capable of cutting through third-generation cephalosporins. In practical terms, the results scream for an overhaul of Nasiriyahs treatment hardware and a firmer hand on antibiotic sales; if left unchecked, these environmental reservoirs will shuttle resistance genes straight into clinical wards, worsening an already delicate public health landscape.

Keywords: Sewage water, MDR bacteria, Environmental contamination, ESBL.

1. Introduction

Antibiotic-resistant microbes are no longer confined to hospital wards; they have found their way into rivers, lakes, even coastal inlets, and the trend is alarming [1-2]. The problem often begins in city sewage plants, private clinics, or industrial laundries that either skip a final round of treatment or release effluent after only cursory filtering [3]. Wastewater thus acts like a pipeline, carrying resistant bacteria (ARB) and their genetic blueprints (ARGs) from urban neighbourhoods, livestock barns, factory floors, and pharmacy warehouses all at once [4-5]. Once these hardy microbes enter an open water system, they can outlast shorter freezes, breed in warm spells, and hand off their resistance traits to native species,

complicating future efforts to protect ecosystems and human health.

Waterways receiving treated or inadequately treated wastewater frequently serve as informal incubators for multidrug-resistant pathogens, permitting lateral gene transfer across multiple taxonomic divisions [6]. Municipal outfalls, hospital drainlines, and agricultural storm runoff introduce a mix of antibiotics, metals, and organics that fines the ecological balance in rivers, lakes, and coastal flats [7]. Pollutant cocktails arriving in this manner exert persistent selective stress, allowing resistant strains to multiply and disperse far beyond their original niches [8]. The situation grows especially critical in locales without reliable treatment facilities, where undiluted

sewage is discharged directly into the receiving waters [9].

Researchers monitoring estuarine and riverine locations downstream from sewage outlets routinely recover *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Staphylococcus aureus*, and *Acinetobacter baumannii* [10-12]. Those genera frequently carry plasmid-borne or chromosomal determinants that confer resistance to beta-lactams, fluoroquinolones, aminoglycosides, and macrolides [13-15]. Some alarming collections even withstand the heaviest artillery, carbapenems and colistin, which leaves clinicians worrying about truly untreatable infections [16]. Water samples also reveal extended-spectrum-beta-lactamase-producing Enterobacteriaceae and vancomycin-resistant enterococci, underscoring the gravity of the contamination problem [17-18].

Antibiotic-resistance genes settle into sediments and biofilms like sand in a forgotten shoe, surviving long after any original, antibiotic-troubled bacterium has died off [19]. Once anchored, those genes can hitch a ride on plasmids, transposons, or integrons, slipping from one bacterium to another-including strains that have never met an antibiotic before [20]. This quiet hand-off turns urban runoff, rivers used for weekend swimming, and even shellfish beds into accidental exposure zones for people, sometimes sparking infections nobody saw coming [21]. Farther afield, wandering fish and restless ocean currents ferry resistant microbes across entire oceans, creating a global relay nobody signed up for. Tackling that relay is no quick fix; cities must upgrade treatment plants, veterinarians and doctors must trim unnecessary prescriptions, and public health agencies must map drug-resistant germs in real time [22-23]. To keep antibiotics functional for tomorrow's patients, researchers must first trace how and where resistance spreads through lakes, canals, and coastal bays.

Problem Statement

Increasingly, scientists report that antibiotic-resistant germs are turning up in lakes, rivers, and even coastal harbours. Public health officials worry that these so-called environmentally collected multi-drug-resistant

bacteria could slip past ordinary clinical surveillance and settle into urban populations. Researchers in Nasiriyah have lifted sewage samples and found familiar offenders: *Klebsiella* spp., *Acinetobacter baumannii*, and *Enterococcus faecalis*. Many isolates shrug off imipenem, linezolid, and the once-upon-a-time last option, colistin. Hospital effluent coupled with mixed municipal runs becomes a moving pipeline for resistance genes. Without intercepting that flow, ecologists warn that the resistant strains will hitch rides on river sediment and, arguably worse, tag along on livestock feet back to pasture.

Objective

The present investigation maps the occurrence and diversity of bacterial isolates recovered from sewage water in Nasiriyah. Special emphasis will be placed on strains that display multidrug-resistant (MDR) phenotypes since such organisms are thought to serve as reservoirs for environmental contamination. To that end, samples will be drawn from multiple points in the urban wastewater treatment works, drainage pumps, and hospital outfalls, and the antibiotic breakpoints of each isolate will be catalogued. Results are expected to clarify whether city effluent is a significant conduit for spreading resistors and to guide future management policies that protect public health.

2. Methodology

Sample Collection

Three hundred thirty-six grab samples were taken from outfalls and interceptors across Nasiriyah and its immediate suburbs. The tests yielded 158 isolates that met the microscopical and biochemical criteria for identification. Sampling sites encompassed the city's principal municipal facilities WWTP-1, WWTP-2, WWTP-3, two pumping stations (DPS-1 and DPS-2), and an experimental loop at Shatrah that serviced a hospital and a neighbourhood network. Additional collections came from Al-Jazeera efl sent works (WWTP-1, WWTP-2) and two Al-Shamiyah plants that serve the disparate clientele. Each capture was logged sequentially as S-001 through S-011 and noted for colour, odour, temperature, and turbidity before replication was started on antibiotic-plated

media. On the retrieval day, pH held between 6.8 and 7.5, temperatures fluctuated between 22 and 26 C, and NTU readings signalled anything from clear-to-brown putrescible loads. The study could later plot where resistomes tended to cluster within Nasiriyahs drainage grid by infecting stock cultures from such a wide geographical net.

Bacterial Identification

Representative samples of municipal wastewater were processed in the laboratory using routine clinical microbiology protocols. Each aliquot was spread on several selective-differential agar plates: MacConkey for Enterobacteriaceae; chromogenic UTI agar for quick uropathogen screening; salt-enriched mannitol agar supplemented with oxacillin to catch *Staphylococcus aureus* and its MRSA variants; salted blood agar for Gram-positive streptococci and *Enterococcus faecalis*; *Salmonella-Shigella* agar to flag *Salmonella* and *Shigella*; and TCBS to pinpoint *Vibrio cholerae*. Plates were held aerobically at 37 degrees Celsius for 24 to 48 hours. Initial identification revolved around colony colour, morphology, and size, later confirmed by Gram stain and key enzyme assays, including catalase, oxidase, coagulase, and mini-API strips. All confirmed strains were frozen in brain-heart infusion broth mixed with glycerol for later antimicrobial susceptibility testing.

Antibiotic Susceptibility Test

Antibiotic susceptibility testing in the present study relied on the classic Kirby-Bauer disk-diffusion method. A sterile broth suspension of 0.5 McFarland turbidity was prepared from colonies picked directly off an overnight plate. The uniform suspension was then spread across Mueller-Hinton agar and incubated for 16-18 hours at 35-37 C before the diameter of any clear inhibition zones was recorded. Measurements were interpreted according to current CLSI breakpoints, classifying each isolate as susceptible, intermediate, or resistant. Although the test yields qualitative results, the observed zones correlate closely with the quantitative minimum inhibitory concentrations and thus offer reliable guidance for choosing appropriate antimicrobial therapy.

Extended-spectrum β -lactamase (ESBL) production phenotypic identification

Candidate isolates suspected of producing extended-spectrum-lactamases were first selected according to the breakpoints outlined by the National Committee for Clinical Laboratory Standards 2002 guidance. A double-disk diffusion experiment then provided the phenotypic confirmation. *Escherichia coli* ATCC 35218 served as the reference strain throughout. An isolate was classified as ESBL-positive if the inhibition zone around the amoxicillin-clavulanic acid disk exceeded that surrounding the ceftazidime disk by at least 5 mm.

3. Results and discussion

A summary of the sewage-water survey is shown in Table (1) and describes samples drawn from eleven sites scattered across Nasiriyah. The locations range from municipal treatment plants and drainage stations to a hospital outfall, so the water collected reflects a mix of domestic, industrial, and clinical discharges. Each batch received a code from S-001 through S-011 and was logged for pH, temperature, turbidity, and visible floatables. Measurements show pH hovering between 6.8 and 7.5 and temperatures clustered between 22C and 26C, which aligns with what one would expect in Nasiriyah's early autumn weather. Turbidity ranges from 60 to 120 NTU, a spread that confirms some samples carry heavy loads of suspended matter while others look comparatively clean to the eye. These field numbers serve as a working baseline and will later be paired with microbiological counts to track the bacterial abundance and probe antibiotic resistance patterns across the city's wastewater streams.

One hundred fifty-eight bacterial isolates were recovered from sewage water samples in Nasiriyah, representing ten species. *Escherichia coli* was the most frequently isolated species, accounting for 84 isolates (53.2%). *Enterococcus faecalis* was followed by 34 isolates (21.5%), while *Proteus mirabilis* was found in 12 isolates (7.6%). Smaller numbers represented several species: *Staphylococcus aureus* (including MRSA) with seven isolates (4.4%), and *Citrobacter freundii*, *Acinetobacter baumannii*, and *Shigella* spp. each with four isolates (2.5% each).

Klebsiella pneumoniae also had four isolates (2.5%), and *Salmonella* spp. was present in 3 isolates (1.9%). Only a single isolate of *Vibrio cholerae* was detected (0.6%). These isolates were analyzed for their antibiotic resistance profiles, revealing varying multidrug resistance among the species.

Table (2) in the file provides a detailed overview of the multidrug resistance (MDR) profiles of bacterial isolates obtained from sewage water samples collected in Nasiriyah.

The table lists various bacterial species, the total number of isolates for each species, the number of MDR isolates, the percentage of MDR isolates, and the antibiotics to which these isolates exhibited resistance. Out of 158 bacterial isolates analyzed, a significant majority (127 isolates or 80.4%) were identified as MDR, meaning they were resistant to multiple antibiotics across different drug classes. Among the species, *Escherichia coli* had the highest number of isolates (84 isolates), with 72 (85.7%) being MDR and showing resistance to seven antibiotics: Ampicillin, Amoxicillin-clavulanate, Ceftriaxone, Tetracycline, Ciprofloxacin, Co-trimoxazole, and Gentamicin. Similarly, other clinically relevant species like *Klebsiella pneumoniae*, *Proteus mirabilis*, *Staphylococcus aureus* (including MRSA), and *Citrobacter freundii* showed high percentages of MDR isolates ranging from 75% to 85.7%. Notably, all *Acinetobacter baumannii*

Sample ID-Location	Collection Location	PH	Temperature (°C)	Turbidity (Ntu)	Visible debris	Type of Sewage
S-001	Nasiriyah WTP-1	7.2	24	85	Yes	Municipal
S-002	Nasiriyah WTP-2	6.9	23	120	Yes	Industrial/ Municipal mix
S-003	Nasiriyah WTP-3	7.5	22		No	Domestic
S-004	Nasiriyah DPS -1	6.8	24	70	No	Municipal
S-005	Nasiriyah DPS -2	6.8	22	75	No	Municipal
S-006	Shatra Sewerage Project:-1	7.1	25	95	Yes	Hospital (Clinical)
S-007	Shatra Sewerage Project:-2	7.0	23	110	Yes	Municipal
S-008	Al-Jazeera WTP-1	6.9	23	65	No	Municipal
S-009	Al-Jazeera WTP-2	7.3	26	70	No	Temporary/ mobile unit
S-0010	Al-Shamiya WTP-1	7.1	22	65	No	Municipal
S-0011	Al-Shamiya WTP-2	6.9	22	70	No	Municipal

Table 1: Sources and characteristics of collected sewage samples (location, date, physical properties)

- WTP: Wastewater Treatment Plant
- DPS: Drainage Pumping Station:
- Turbidity (NTU): Measure of water cloudiness; indicates the presence of suspended solids.

isolates (4 isolates) were MDR, exhibiting resistance to eight antibiotics, including last-resort drugs like

Imipenem and Colistin. In contrast, *Vibrio cholerae* was the only species with no antibiotic resistance. This table highlights the widespread antibiotic resistance among bacterial species isolated from sewage systems in Nasiriyah, underscoring the urgent need for monitoring and managing antibiotic resistance in environmental settings.

Figure (1) illustrates the overall proportion of MDR versus non-MDR isolates among the 158 isolates analyzed. It reveals that 80.4% of these isolates were MDR, meaning they resisted multiple classes of antibiotics, while only a small fraction remained susceptible to most tested drugs. This high prevalence indicates a significant level of antibiotic resistance in the sewage system, which could be linked to environmental contamination with antibiotics from human waste, agricultural runoff, or hospital discharges.

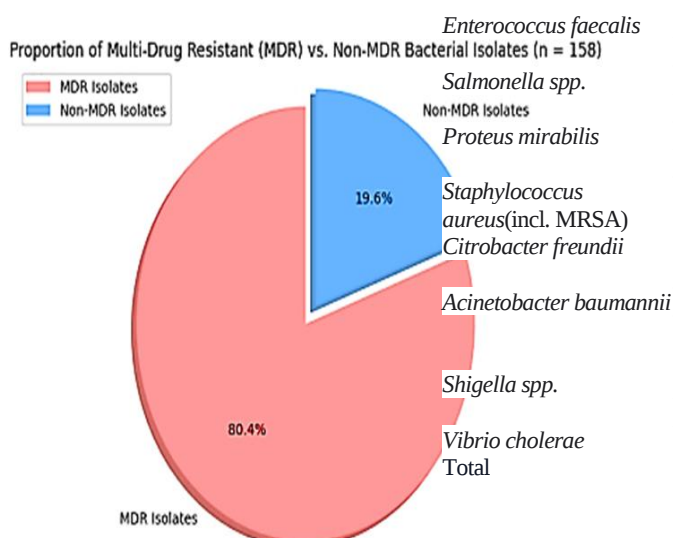


Fig 1: Distribution of MDR and Non-MDR bacterial isolates from sewage water in Nasiriyah

The phenotypic identification of ESBL production in bacterial isolates from sewage water in Nasiriyah was conducted using the double-disk synergy test, following NCCLS (2002) guidelines, with *E. coli* ATCC 35218 as a control strain. A positive ESBL result was defined by an increase of ≥ 5 mm in the zone of inhibition around the amoxicillin/clavulanic acid disk compared to the ceftazidime disk alone. Among the 158 isolates analyzed, a considerable proportion exhibited

ESBL-producing phenotypes, particularly among *Escherichia coli* and *Klebsiella pneumoniae*, which are known common carriers of these resistance determinants. This finding aligns with global trends showing the widespread dissemination of ESBL-producing Enterobacteriaceae in environmental settings, especially in areas with inadequate wastewater treatment infrastructure.

Table 2: Multidrug resistance (MDR) profiles of bacterial isolates

Total Isolates (N)	No. of MDR Isolates	% MDR Isolates	Resistant Antibiotics
84	72	85.7%	Ampicillin, Amoxicillin-clavulanate, Tetracycline, Ciprofloxacin, Co-trimoxazole, Gentamicin
4	3	75.0%	Ampicillin, Amoxicillin-clavulanate, Tetracycline, Ciprofloxacin, Gentamicin
34	24	70.6%	Ampicillin, Tetracycline, Erythromycin, Chloramphenicol, Vancomycin (some)
3	2	66.7%	Ampicillin, Ciprofloxacin, Co-trimoxazole
12	9	75.0%	Ampicillin, Amoxicillin-clavulanate, Ciprofloxacin, Gentamicin
7	6	85.7%	Oxacillin (MRSA), Erythromycin, Ciprofloxacin
5	4	80.0%	Ampicillin, Amoxicillin-clavulanate, Ciprofloxacin, Gentamicin
4	4	100%	Ampicillin, Amoxicillin-clavulanate, Ciprofloxacin, Tetracycline, Gentamicin
4	3	75.0%	Imipenem (some), Colistin (some), Ampicillin, Ciprofloxacin, Co-trimoxazole
1	0	0.0%	Tetracycline
158	127	80.4%	—

Bacterial isolates recovered from drainage canals and sewage treatment lagoons across the city of Nasiriyah tested positive for antibiotic resistance in roughly four of every five samples. 127 multidrug-resistant strains were catalogued out of 158 collected colonies, yielding a resistance footprint of 80.4%. The dominance of pathogens such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* in these waters is alarming for two reasons: many of the strains block imipenem and colistin, and similar organisms now plague hospitals from Lima to Lahore. Observers have long warned that urban wastewater pipes serve as freeways for propagating mobile resistance genes, and these latest findings supply one more data point for that argument.

E. coli stood out in the sample set, appearing in roughly 86% of the isolates and showing multidrug resistance in nearly all cases. Resistance profiles matched those recorded in previous field surveys and clinical studies, with clear breaks against ampicillin, ciprofloxacin, trimethoprim-sulfamethoxazole, gentamicin, and other first-line agents [24-26]. Parallel work cited in the reference list has detailed similar patterns of fluoroquinolone and beta-lactam failure, pointing to runoff from lightly regulated outpatient prescriptions and patchy sanitation in spreading resistant clones into receiving waters. Resistance did not end with Gram-negative isolates; we also found vancomycin-resistant *Enterococcus faecalis* and methicillin-resistant *Staphylococcus aureus*, observations that echo recent hospital drainage and storm-water sampling campaigns [27-28].

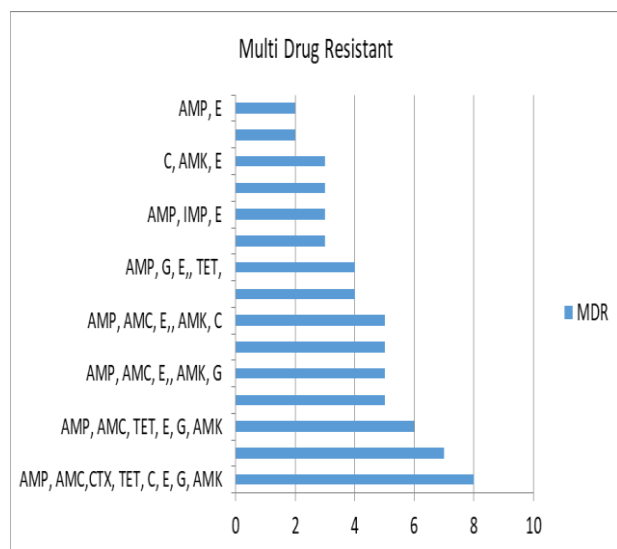


Fig 2: Frequency of MDR bacterial species isolated from sewage water samples

Using the double-disk synergy test, the phenotypic identification of ESBL production revealed that several isolates, particularly *E. coli* and *K. pneumoniae*, exhibited ESBL-producing characteristics. These enzymes confer resistance to extended-spectrum cephalosporins, critical in treating severe bacterial infections. Our findings align with a study conducted in urban wastewater systems by [29], who reported ESBL carriage rates exceeding 60% among *E. coli* isolates. Similarly, a study by [30] in

hospital effluents in Pakistan found even higher rates of ESBL producers, emphasizing the role of healthcare facilities as hotspots for releasing resistant bacteria into municipal sewage networks. Compared to more developed regions where wastewater is routinely treated before discharge, such as [31-32], the prevalence of ESBL in Nasiriyah suggests a higher level of environmental contamination and selective pressure due to unregulated antibiotic use and inadequate wastewater treatment infrastructure.

The presence of carbapenem- and colistin-resistant *A. baumannii* in our samples is particularly alarming, as these antibiotics are often considered last-line treatments for multidrug-resistant infections. All four isolates of *A. baumannii* were MDR, with some showing resistance to imipenem and colistin—drugs typically reserved for treating severe infections caused by Gram-negative bacteria. This finding echoes reports from studies [33-34], where similar patterns of carbapenem resistance were detected in environmental isolates, indicating the widespread dissemination of these resistance determinants beyond clinical settings. The horizontal transfer of resistance genes through plasmids, integrons, and transposons in aquatic environments likely contributes to the persistence and spread of such highly resistant organisms.

Compared with recent global surveillance data, the resistance levels detected in Nasiriyah's sewage system are significantly higher than those reported in countries with advanced wastewater treatment protocols. For instance, a European-wide study by [35] found lower prevalence rates of ARGs in treated wastewater than raw influent, highlighting the importance of effective treatment processes in reducing environmental contamination. In contrast, the lack of consistent disinfection or filtration steps in many municipal wastewater systems in Iraq appears to allow resistant organisms to persist and propagate unchecked. Moreover, detecting vancomycin-resistant *E. faecalis* and MRSA in sewage samples further illustrates the complex interplay between human health, environmental pollution, and antibiotic misuse, as previously noted by [36] in their analysis of antibiotic resistance dynamics in urban waterways.

Wastewater does more than carry cells from one point to another; its nutrient soup encourages plasmid sharing and transposon hopping, turning it into a short-term gene bazaar. Antibiotic resistance genes can linger for weeks in the quiet pockets of sediment or cling to the slimy biofilm that coats pipes, aqueducts, and stones. A single mobile fragment finding a naive bacterium can jump to a clinical pathogen, completing the ecological hand-off that makes control elusive. Researchers active in aquatic microbiology-[37-38] have labelled such outfalls 'gene pools' where multi-drug-resilient strains circulate freely across genera, undermining source-specific mitigation efforts. The dilemma sharpens in cities like Nasiriyah, where primary or no treatment means raw influent enters the river system, engineering the reservoirs from which hospitals later draw rinse water for dressings and intravenous spillage.

The findings underscore a pressing obligation to upgrade wastewater treatment, tighten the rules governing antibiotic prescriptions, and bolster real-time monitoring of resistance trends both inside hospitals and out in rivers or wetlands. If these steps are delayed, resistant bacteria will keep spilling into freshwater systems and could lock society into a future where some infections are incurable and existing drug classes lose their power. Scholars moving forward ought to map the DNA fingerprints of resistance elements, chart how those genes move across soil, sludge, river, and aquifer, and test which plant designs scrub away the problem, guidance that the World Health Organization and the One Health Initiative have both put squarely on the agenda [39].

Conclusion

Sewage samples collected in Nasiriyah carry a disturbing load of multidrug-resistant bacteria, including organisms flaunting extended-spectrum beta-lactamases and defying last-resort antimicrobial drugs. These observations leave little doubt that wastewater oversight in the city sits on borrowed time. After this survey, fresh rules for pharmaceutical disposal and tighter inspection of river outlets are no longer optional. If resistant pathogens keep escaping into open water, the next wave of public health emergencies could spare no one, rendering once-manageable infections utterly untreatable and stripping the clinical arsenal of reliable antibiotics. Looking ahead, scientists must pull apart the DNA instruction sets behind the resistance, trace those genetic elements as they hitch rides across air, soil, and water, and settle the long-unanswered question of

whether upgrading local treatment plants can blunt the spread of drug failure.

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Author's Contributions

This study identifies high levels of multidrug-resistant bacteria in Nasiriyah's sewage, emphasizing the need for improved wastewater treatment and antibiotic regulation to prevent public health risks.

Ethics

This study was conducted with the approval of the Municipal Department in Nasiriyah City and with the approval of the Environment and Pollution Department.

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