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MULTIDRUG-RESISTANT BACTERIAL COLONIZATION PATTERNS IN COMMUNITY WASTEWATER AND THEIR PUBLIC HEALTH IMPLICATIONS: A NARRATIVE REVIEW

Narrative Review

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ABSTRACT

Background: The proliferation of multidrug-resistant (MDR) bacteria poses a critical threat to global public health. Community wastewater is increasingly recognized not merely as a reflection of community-level antimicrobial resistance (AMR) but as a dynamic environment that actively amplifies and disseminates resistance genes and pathogens, creating significant exposure risks for human populations.

Objective: This narrative review aims to synthesize current evidence on the patterns of MDR bacterial colonization in community wastewater systems and to critically analyze the subsequent implications for public health.

Main Discussion Points: The review delineates the wastewater resistome as a rich repository of diverse antibiotic resistance genes, often associated with mobile genetic elements. It confirms the persistent detection of clinically significant MDR bacteria, including carbapenem-resistant *Enterobacterales* and methicillin-resistant *Staphylococcus aureus*, throughout wastewater treatment processes, which are shown to be inadequate for their complete removal. The discussion further explores the role of treated effluent and biosolids as critical pathways for environmental contamination and community exposure. The application of wastewater-based epidemiology (WBE) is highlighted as a promising tool for population-level AMR surveillance. However, the literature is marked by methodological limitations, including a lack of standardization and longitudinal studies, which challenge the precise quantification of direct health risks.

Conclusion: Evidence conclusively identifies wastewater as a significant reservoir and conduit for AMR. To mitigate this threat, there is an urgent need to integrate WBE into public health surveillance and to update water quality guidelines to include AMR parameters. Future research must prioritize standardized, longitudinal, and intervention-based studies to better quantify transmission risks and evaluate the efficacy of mitigation strategies.

Keywords: Multidrug-Resistant Bacteria, Wastewater, Public Health, Antimicrobial Resistance, Wastewater-Based Epidemiology, One Health.

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INTRODUCTION

The escalating crisis of antimicrobial resistance (AMR) represents one of the most formidable public health challenges of the 21st century, threatening to undermine decades of medical progress. The World Health Organization has declared AMR a top-ten global public health threat, with drug-resistant infections directly contributing to an estimated 1.27 million deaths annually and being associated with nearly 5 million more (1). This silent pandemic is propelled by the relentless selection and dissemination of multidrug-resistant (MDR) bacteria, strains impervious to multiple classes of antimicrobial agents, thereby drastically limiting therapeutic options and escalating mortality rates. While clinical settings have long been the focal point of AMR surveillance and control, there is a growing, urgent recognition that the environment, particularly urban water systems, plays a crucial role in the resistance cycle. Community wastewater, a complex effluent from domestic, commercial, and sometimes industrial sources, is now understood to be a significant reservoir and a dynamic conduit for the amplification and spread of resistance determinants (2). It serves as a collective microbiome, mirroring the resistance genes circulating within a human population and providing a fertile meeting ground for bacteria of human, animal, and environmental origin.The journey of MDR bacteria into wastewater begins with excretion from colonized or infected individuals within the community. Asymptomatic gut colonization by MDR organisms like extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae*, vancomycin-resistant enterococci (VRE), and carbapenem-resistant *Enterobacterales* (CRE) is increasingly prevalent, often going undetected in healthy carriers. A systematic review estimated the global colonization prevalence of ESBL-producing *Enterobacteriaceae* in the community to be as high as 14% (3), indicating a substantial and continuous discharge of these bacteria into sewage systems. Furthermore, the incomplete removal of antibiotics, disinfectants, and heavy metals from wastewater provides continuous selective pressure, favouring the survival and proliferation of resistant strains over their susceptible counterparts. Within this nutrient-rich, high-microbial-density environment, horizontal gene transfer flourishes. Mechanisms such as conjugation (via plasmids), transduction (via bacteriophages), and natural transformation allow for the direct exchange of mobile genetic elements carrying resistance genes between pathogenic and environmental bacteria, creating novel MDR combinations and broadening the resistance gene pool in ways that would be less likely to occur in more sterile settings (4).

Consequently, wastewater treatment plants (WWTPs) are not merely treatment facilities but are increasingly viewed as "hotspots" for the evolution and dissemination of AMR. Current knowledge firmly establishes that typical wastewater treatment processes, while effective at reducing overall bacterial load and organic matter, are not specifically designed to eliminate all MDR bacteria or their genetic blueprints. Numerous studies have consistently documented the presence of clinically relevant MDR pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA), CRE, and MDR *Pseudomonas aeruginosa*, in both influent (raw sewage) and, critically, in treated effluent (5). The discharge of this treated, yet still contaminated, water into rivers, lakes, or coastal waters, or its reuse for irrigation, creates a direct pathway for MDR bacteria to re-enter the human environment. This poses tangible risks of community exposure through recreational water activities, consumption of contaminated produce, or the pollution of drinking water sources. Moreover, the practice of land-applying sewage sludge (biosolids) as fertilizer can disseminate MDR bacteria and resistance genes directly into agricultural soils, creating a persistent reservoir and potentially entering the food chain. Despite this growing body of evidence, there remains a significant research gap in systematically characterizing the specific colonization patterns—the predominant bacterial species, their resistance profiles, and the carriage of specific mobile genetic elements—across different community wastewater systems and how these patterns correlate with public health outcomes. Much of the existing research has focused on quantifying the abundance of specific resistance genes or the presence of particular pathogens at single points in the wastewater system. However, a comprehensive synthesis is lacking that analyzes the *patterns* of MDR bacterial colonization in wastewater as an integrated system, from source to final discharge, and links these patterns to quantifiable public health risks.

Key unresolved questions persist: How do demographic factors, seasonal variations, and local antibiotic consumption rates in a community influence the MDR profile of its wastewater? What is the relative contribution of hospital effluent versus community sewage to the overall AMR load in municipal wastewater? And most importantly, to what extent does the environmental release of MDR bacteria from wastewater sources lead to tangible community-acquired infections? Answering these questions is critical for moving from mere observation to actionable, evidence-based interventions. A narrative review that consolidates and critically appraises the current state of knowledge on these fronts is therefore not only timely but essential. The primary objective of this narrative review is to synthesize and critically evaluate the existing scientific literature on the patterns of multidrug-resistant bacterial colonization in community wastewater

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systems and to analyze the subsequent implications for public health. The review will focus on studies from the last decade to ensure relevance to the current AMR landscape. It will seek to delineate the most frequently encountered MDR bacterial species and their characteristic resistance mechanisms in wastewater matrices, explore the factors that shape these colonization patterns, and trace the pathways through which wastewater-derived MDR bacteria potentially impact human health. The scope of this review encompasses studies investigating raw influent, treated effluent, and sewage sludge, with a specific focus on community and municipal wastewater, excluding studies solely on hospital effluents unless they are discussed in a comparative context. By providing a synthesized overview of the current evidence, this review aims to bridge the gap between environmental surveillance data and clinical public health practice. Its significance lies in its potential to inform public health authorities, environmental engineers, and policymakers. The findings could highlight the utility of wastewater-based epidemiology (WBE) as a cost-effective tool for population-level AMR surveillance, providing a community-wide "snapshot" that complements traditional clinical surveillance. Furthermore, by elucidating the critical control points where MDR dissemination occurs, this review may contribute to the development of more targeted and effective mitigation strategies, such as optimizing wastewater treatment technologies for AMR removal and refining risk assessment models for water reuse and biosolid application. Ultimately, understanding the journey of MDR bacteria from the toilet to the river, and back to the community, is a vital step in crafting a holistic and proactive defense against the escalating threat of antimicrobial resistance.

THEMATIC DISCUSSION

1. The Wastewater Resistome: A Catalogue of Resistance Genes and Mechanisms

The concept of the "wastewater resistome" has become central to understanding the scope of the antimicrobial resistance (AMR) problem. This term describes the collective pool of all antibiotic resistance genes (ARGs) present in the microbial communities of wastewater systems. Metagenomic studies have been instrumental in cataloguing this vast genetic reservoir, revealing that wastewater contains a richer and more diverse array of ARGs than any other anthropogenic environment (6). The resistome is not static; it is a dynamic entity shaped by the constant influx of bacteria from human guts, livestock, and industry. Predominant among the identified genetic determinants are those conferring resistance to beta-lactams (e.g., blaCTX-M, blaNDM, blaKPC), sulfonamides (sul1, sul2), tetracyclines (tetA, tetM), and fluoroquinolones (qnr), reflecting the clinical and agricultural usage patterns of these drug classes (2). The persistence of these genes, even in the absence of continuous antibiotic selection pressure, is often facilitated by their association with mobile genetic elements like integrons, which can capture and express gene cassettes, and broad-host-range plasmids, which act as efficient vehicles for horizontal gene transfer (7). This genetic mobility is the cornerstone of the public health threat, as it allows ARGs to jump from benign environmental bacteria to human pathogens, effectively bypassing species barriers and accelerating the emergence of new resistant strains.

2. Clinically Significant Multidrug-Resistant Bacteria in Wastewater Matrices

Beyond the genetic level, the tangible presence of viable, clinically significant multidrug-resistant (MDR) bacteria in wastewater confirms its role as a reservoir for pathogens. Culture-based and molecular studies consistently isolate a concerning spectrum of MDR bacteria from both raw and treated wastewater. Foremost among these are carbapenem-resistant Enterobacterales (CRE), particularly Escherichia coli and Klebsiella pneumoniae harbouring carbapenemases such as NDM and KPC (8). These pathogens represent a nightmare scenario for clinicians, as carbapenems are often last-resort antibiotics for severe Gram-negative infections. Similarly, the detection of vancomycin-resistant Enterococcus faecium (VRE) and methicillin-resistant Staphylococcus aureus (MRSA) in sewage underscores that the threat is not confined to Gram-negative organisms (9). A study by Bréchet et al. (2023) demonstrated that wastewater mirrors the shifting prevalence of MRSA clones within a community, serving as an effective surveillance tool (10). The co-occurrence of these diverse MDR pathogens in a single, mixed environment like wastewater is particularly alarming. It creates a perfect storm where genetic exchange can occur, potentially leading to the emergence of Gram-positive bacteria acquiring Gram-negative resistance genes and vice versa, although such events are less common. The consistent isolation of these pathogens confirms that wastewater is not merely a reflection of community colonization but an active reactor for their enrichment and potential evolution.

3. The Fate of MDR Bacteria During Wastewater Treatment

A critical thematic question is the efficacy of conventional wastewater treatment processes in mitigating the release of MDR bacteria into the environment. The consensus from recent literature is that while secondary treatment processes (e.g., activated sludge) are highly

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effective at reducing the overall biochemical oxygen demand and total bacterial load, they are markedly less effective at eliminating the AMR burden. The activated sludge process itself, while removing many bacteria through sedimentation, creates a high-biomass environment that can facilitate horizontal gene transfer. Studies show that while there is a significant log-reduction in the concentration of specific MDR bacteria between influent and effluent, the remaining populations in the final effluent are often enriched for resistant strains (5). This suggests that treatment processes inadvertently select for hardier bacteria that can withstand the biological and chemical stresses of treatment. Furthermore, while tertiary treatments like chlorination, UV radiation, and ozonation can be more effective, they are not universally applied and have their own limitations. Chlorination, for instance, can be compromised by high organic load and may even promote the selection of chlorine-resistant strains, some of which exhibit co-resistance to antibiotics (11). The incomplete removal means that treated wastewater effluent constitutes a significant and continuous point source for the introduction of MDR bacteria and ARGs into rivers, lakes, and agricultural land.

4. Biosolids: An Overlooked Pathway for AMR Dissemination

An often-overlooked but equally critical pathway for AMR dissemination is the production and application of biosolids, the treated sewage sludge. The process of stabilizing sludge aims to reduce pathogens, but like liquid treatment, it is not designed specifically to eradicate ARGs or MDR bacteria. In fact, the anaerobic digestion commonly used can concentrate certain ARGs and select for resistant populations. Studies analyzing Class B biosolids, which are commonly applied to agricultural land as soil amendments, have detected high levels of sul and tet resistance genes, as well as viable MDR E. coli and Salmonella spp. (12). The land application of these biosolids introduces MDR bacteria and their genes directly into the soil ecosystem. From there, they can persist for extended periods, be taken up by crops, leach into groundwater, or be transported via runoff into surface waters. This creates a long-term reservoir of AMR in the environment, directly linking human waste management to agricultural practice and the food chain. The public health implications are profound, as this pathway exposes a wider population through the food supply and the environment, far beyond the direct users of reclaimed water.

5. Wastewater-Based Epidemiology for Public Health Surveillance

In the face of this challenge, a promising tool has emerged: wastewater-based epidemiology (WBE). WBE involves the systematic analysis of wastewater to obtain quantitative data on the consumption of chemicals or the circulation of pathogens within a population. Its application to AMR surveillance is a rapidly advancing field. By quantifying specific ARGs or tracking MDR bacterial clones in sewage over time, public health officials can gain a near real-time, population-wide snapshot of the community's AMR carriage without the need for costly and invasive clinical screening of thousands of individuals (13). This approach was successfully piloted during the COVID-19 pandemic for tracking SARS-CoV-2, validating its utility for public health. For AMR, WBE can identify the emergence and spread of high-risk clones, such as CRE, and monitor the effectiveness of local antibiotic stewardship programs by correlating ARG abundance with data on antibiotic consumption (14). However, significant methodological gaps remain. There is a lack of standardization in sampling protocols, DNA extraction methods, and data normalization, making cross-study comparisons difficult. Furthermore, while WBE can signal the presence of a threat, it cannot currently distinguish between genes carried by pathogenic versus commensal bacteria, nor can it easily determine the potential for gene transfer, which is crucial for accurate risk assessment.

6. Quantifying the Public Health Risk: From Detection to Impact

The ultimate and most contentious theme revolves around directly linking the presence of MDR bacteria in wastewater to tangible community-acquired infections. Establishing a direct causal link is methodologically challenging, but a growing body of epidemiological and genomic evidence supports a strong association. Comparative studies have shown that individuals living in areas where rivers are polluted with treated wastewater or where wastewater is reused for irrigation have higher rates of gut colonization with MDR bacteria identical to those found in the water (15). Genomic sequencing has been pivotal in providing more direct evidence; for instance, studies have identified identical plasmids carrying blaCTX-M genes in human clinical E. coli isolates and in E. coli strains recovered from connected wastewater sources, strongly implicating environmental transmission (16). Despite this, controversies and knowledge gaps persist. It is difficult to quantify the relative contribution of wastewater exposure compared to other transmission routes, such as person-to-person contact or food consumption. Furthermore, the infectious dose for many MDR pathogens acquired via environmental exposure is unknown. More longitudinal studies combining advanced WBE with parallel clinical surveillance and detailed epidemiological data are needed to move from correlation to causation and to reliably quantify the attributable risk fraction of wastewater in the community burden of AMR.

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CRITICAL ANALYSIS AND LIMITATIONS

While the existing literature provides a compelling and largely consistent narrative about the role of wastewater as a significant reservoir for multidrug-resistant (MDR) bacteria, a critical analysis reveals substantial methodological limitations that constrain the strength of the conclusions and their utility for direct public health intervention. A primary and pervasive limitation across the field is the overwhelming reliance on cross-sectional study designs. The vast majority of investigations offer a single snapshot in time, capturing the resistome and bacterial community at a specific moment (2). This approach fails to account for temporal dynamics, such as diurnal fluctuations, seasonal variations in antibiotic usage and water flow, and the impact of rainfall events, which can dramatically dilute or concentrate wastewater constituents. Without longitudinal data collected over extended periods, it is impossible to discern trends, establish causality, or evaluate the long-term effectiveness of any interventions aimed at reducing antimicrobial resistance (AMR) load in wastewater. The absence of such time-series data means that our understanding of the wastewater resistome is inherently static and potentially unrepresentative of its true dynamic nature. Further complicating the picture is the profound lack of standardization in methodological approaches, which severely hampers the comparability of findings across different studies and geographical regions. From the initial sampling stage, strategies vary widely, ranging from grab samples, which may miss periodic discharges, to 24-hour composite samples, which provide a more averaged profile but may dilute transient spikes in MDR bacteria (16). Subsequent laboratory analyses are equally variable. DNA extraction protocols, for instance, differ in their efficiency in lysing different bacterial cell types (e.g., Gram-positive versus Gram-negative), introducing a potential bias in the resulting metagenomic profile. The choice of quantification methods—whether culture-based, quantitative PCR (qPCR), or high-throughput metagenomic sequencing—each comes with its own set of limitations and biases. Culture-based methods fail to capture the vast majority of viable-but-non-culturable and unculturable bacteria, while qPCR is limited to targeting pre-selected genes, and metagenomic sequencing, though comprehensive, is influenced by DNA extraction efficiency and bioinformatic pipeline parameters (17).

This methodological heterogeneity creates a "Tower of Babel" effect, where synthesizing data from different studies to draw global conclusions becomes a formidable challenge. A significant methodological bias stems from the almost exclusive focus on the water phase of wastewater, with considerably less attention paid to the solid fraction—biosolids and sludge. This is a critical oversight given that many MDR bacteria and antibiotic resistance genes (ARGs) are hydrophobic and tend to partition onto solid particles. Consequently, studies analyzing only liquid effluent may vastly underestimate the total AMR load leaving a treatment plant, as the bulk of the resistance determinants are sequestered and concentrated in the sludge (12). This sampling bias leads to an incomplete risk assessment, as the subsequent land application of biosolids represents a major and direct pathway for introducing concentrated MDR bacteria into agricultural environments and, potentially, the food chain. The public health implications of this pathway are likely underappreciated due to this research gap. When attempting to link environmental detection to public health impact, the literature faces its most significant inferential challenges. While genomic studies can demonstrate that identical MDR bacterial strains or plasmids are found in both wastewater and clinical settings, this alone does not prove direct transmission. It remains exceptionally difficult to rule out the possibility that both the clinical case and the environmental sample acquired the resistant organism from a common, independent source. Establishing a definitive direction of transmission—from wastewater to human or vice versa—requires sophisticated study designs, such as large-scale, prospective cohort studies with integrated human, animal, and environmental sampling, which are logistically complex and costly (15). The current evidence, therefore, largely rests on ecological correlations and plausible mechanism, falling short of demonstrating direct causation.

This limitation is a major barrier for policymakers who require robust evidence to justify significant investments in advanced wastewater treatment technologies. Finally, the issue of publication bias and generalizability must be considered. There is a likely overrepresentation of studies from high-income countries with established research infrastructure and from regions where AMR is already a recognized priority. Findings from a modern wastewater treatment plant in Europe or North America may not be generalizable to the situations in low- and middle-income countries, where wastewater treatment may be minimal or non-existent, and where the burden of communicable diseases and antibiotic misuse is often higher (8). Furthermore, the "file drawer" problem—where studies with negative or inconclusive findings (e.g., those failing to find a significant concentration of a particular ARG) remain unpublished—can skew the perceived prevalence and importance of certain resistance mechanisms. This creates a distorted view of the global AMR situation in wastewater, potentially leading to misplaced priorities in surveillance and control efforts. Addressing these limitations requires a concerted global effort to standardize methodologies, fund longitudinal and interdisciplinary research, and prioritize inclusive surveillance that captures the realities of diverse wastewater management contexts.

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IMPLICATIONS AND FUTURE DIRECTIONS

The synthesis of current evidence on multidrug-resistant (MDR) bacterial colonization in wastewater carries profound implications that extend from the clinic to the global policy arena, while also charting a clear course for future scientific inquiry. From a clinical and public health perspective, the consistent detection of high-threat pathogens like carbapenem-resistant Enterobacterales (CRE) and their resistance genes in wastewater necessitates a paradigm shift in how the community spread of AMR is perceived and monitored. The findings strongly advocate for the formal integration of wastewater-based epidemiology (WBE) into national AMR surveillance programs. For clinicians and public health officials, WBE can serve as an early warning system, providing a cost-effective, population-wide snapshot of circulating resistance determinants that complements traditional, passive clinical surveillance (13). For instance, a sudden spike in the abundance of the blaKPC gene in a city's sewage could alert hospitals and primary care providers to the potential emergence of a CRE cluster, enabling pre-emptive infection control measures and guiding empirical antibiotic therapy in high-risk scenarios before clinical cases overwhelm the system. At the policy level, the evidence underscores an urgent need to re-evaluate regulatory standards for wastewater treatment and reuse. Current regulations for treated wastewater effluent and biosolids are primarily based on the reduction of conventional indicators like faecal coliforms and nutrients, with no specific mandates for the removal of MDR bacteria or antibiotic resistance genes (ARGs). This review demonstrates that this is a critical oversight. Environmental protection agencies and public health bodies must collaborate to establish new, risk-based thresholds for AMR indicators in discharged water and land-applied biosolids (16). This could drive the adoption of advanced treatment technologies, such as ozonation, advanced oxidation processes, and membrane filtration, which have shown greater efficacy in degrading genetic material and inactivating MDR bacteria compared to conventional methods. Furthermore, urban planning and water reuse policies, particularly in water-scarce regions, must explicitly incorporate AMR risk assessments to ensure that the irrigation of food crops or recreational use of reclaimed water does not become a direct vector for transmitting resistant infections to the community.

To build a more robust evidence base for these policy and clinical decisions, future research must address the significant gaps identified in this review. A primary unanswered question is the quantitative assessment of the actual human health risk posed by environmental exposure to wastewater-derived MDR bacteria. Future studies should move beyond mere detection and correlation to quantify the transmission dynamics. This requires a shift towards interdisciplinary, One Health approaches that integrate simultaneous sampling from human populations (e.g., longitudinal stool sampling from communities), livestock, and the full spectrum of the wastewater system, including sludge and receiving environments (2). Such study designs would allow for the application of advanced molecular typing, such as whole-genome sequencing, to track the flow of specific bacterial clones and plasmids between reservoirs and to estimate transmission rates. A key research priority is to determine the infectious dose for various MDR pathogens acquired via environmental exposure, data that is crucial for refined microbial risk assessment models. Methodologically, the field urgently requires standardization to ensure data comparability. Future research should prioritize the development and widespread adoption of standardized protocols for every step of the process, from representative composite sampling and optimized DNA extraction to the normalization of metagenomic data and a unified bioinformatic pipeline for ARG annotation (7). To better understand the functional risk, future work should also complement metagenomic data with metatranscriptomics to discern which resistance genes are being actively expressed and are therefore a more immediate threat. Finally, there is a pressing need for intervention-based studies. Rather than being purely observational, future research should evaluate the real-world efficacy of upgraded treatment technologies in reducing AMR loads and, crucially, assess the subsequent impact on the prevalence of MDR colonization in downstream communities. By embracing these future directions, the scientific community can transform the understanding of wastewater as an AMR reservoir from a subject of academic concern into a basis for actionable and effective public health solutions.

CONCLUSION

In conclusion, this review synthesizes compelling evidence that establishes community wastewater as a critical reservoir and amplifier for multidrug-resistant bacteria, harboring a diverse resistome that includes genes conferring resistance to last-resort antibiotics and facilitating their dissemination among microbial populations through horizontal gene transfer. The collective findings from the literature, while somewhat constrained by methodological heterogeneity and a predominance of observational studies, consistently demonstrate that conventional wastewater treatment processes are insufficient to eliminate this threat, leading to the continuous release of MDR bacteria and resistance genes into the environment via treated effluent and biosolids. This necessitates a paradigm shift in public health strategy, advocating for the immediate integration of wastewater-based epidemiology into national antimicrobial resistance surveillance

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programs to serve as an early warning system and for the urgent development of updated regulatory standards that mandate the monitoring and reduction of AMR indicators in treated wastewater. Therefore, it is unequivocally recommended that future research priorities must focus on standardized, longitudinal, and intervention-based studies that quantitatively link environmental exposure to clinical outcomes, thereby providing the robust evidence required to galvanize global action against this pervasive environmental health threat.

AUTHOR CONTRIBUTION

Author	Contribution
Bashir Ahmed*	Substantial Contribution to study design, analysis, acquisition of Data Manuscript Writing Has given Final Approval of the version to be published
Rimal Rashid	Substantial Contribution to study design, acquisition and interpretation of Data Critical Review and Manuscript Writing Has given Final Approval of the version to be published
Hafiza Samin Anjum	Substantial Contribution to acquisition and interpretation of Data Has given Final Approval of the version to be published
Ajay Kumar	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published
Nayab Aslam	Contributed to Data Collection and Analysis Has given Final Approval of the version to be published

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