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ORIGINAL ARTICLE

Comparative Study of Antibiotic Resistance in Clinical and Environmental Bacterial Isolates in the Pakistani Population

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ABSTRACT

Background: Antibiotic resistance poses a critical threat to global health, particularly in Pakistan where unregulated antibiotic use, limited healthcare resources, and complex human–animal–environment interactions accelerate the spread of resistance.

Aims and Objectives: This study aimed to compare antibiotic resistance profiles between clinical and environmental bacterial isolates.

Methodology: A total of N=50 non-duplicate isolates were collected from January 2022 to December 2022, including 25 clinical isolates from tertiary care hospitals and 25 environmental isolates from surface waters, wastewater treatment plant effluents, and agricultural sites. Standard identification was made using standard microbiological techniques, such as colony morphology, Gram staining and biochemical tests, with a subsequent polymerase chain reaction (PCR) confirmation for specific pathogens. Kirby-Bauer disc diffusion method was used to determine Antibiotic susceptibility and resistance patterns were analysed using descriptive statistics and chi square test.

Results: Clinical isolates demonstrated more significant resistance rates than environmental isolates. Resistance to ampicillin occurred in 80% of clinical isolates as opposed to 48% of environmental isolates, and multidrug resistance was seen in 56% of clinical isolates and 20% of environmental isolates. For ceftriaxone, ciprofloxacin, and tetracycline, similar patterns were observed, and differences were significant ($p < 0.05$).

Conclusion: These findings underscore a striking disparity between clinical and environmental isolates in terms of antibiotic resistance and underscore the urgency for the implementation of measures to improve antibiotic stewardship, comprehensive surveillance, and integrated intervention measures to combat antimicrobial resistance in Pakistan. Our findings offer data that may help to direct future policy and research and improve public health outcomes.

Keywords: Antibiotic resistance, clinical isolates, environmental isolates, multidrug resistance, Pakistan, surveillance, stewardship.

INTRODUCTION

Antibiotic resistance is one of the greatest threats to public health in the 21st century undermines decades of progress in treating infectious diseases and raises the risk of morbidity and mortality worldwide. Most alarming is that this is a unique interplay of factors in Pakistan, where unregulated antibiotic use, lack of public health resources, and an extensive human-animal-environment interface are all at play[1]. These conditions not only facilitate the spread of resistant strains in other settings but also enable the emergence and spread of resistant strains in other settings, making it a formidable challenge to healthcare systems and regulatory bodies[2].

Large and often indiscriminate selective pressures exist in clinical environments, in and out of the hospital, that are the intensive and often indiscriminate use of antibiotics for clinical purposes, which drive the emergence of multidrug-resistant (MDR) organisms[3]. Escherichia coli, Klebsiella pneumonia, and Staphylococcus aureus are many pathogens that acquire resistant mechanisms in hospitals which in turn render treatment regimes ineffective and prolong the stay in the hospital, increasing healthcare costs and poor clinical outcomes. Such widespread practice of empirical therapy, coupled with the lack of adequate diagnostic facilities, permits the resistant strains to persist and multiply in such high-risk settings[4].

As is true with so many of the challenges in clinical environments, environmental reservoirs have an important and sometimes overlooked role in the development and spread of antibiotic resistance. These bacterial species have found themselves as melting pots of sources including wastewater treatment plants, agricultural runoff, and contaminated surface water where they meet and exchange resistance genes horizontally through horizontal gene transfer[5]. Such an environmental spread contributes to the emergence of novel MDR strains and would also serve as a risk to retransfer of these genes back into the human population, thereby amplifying the cycle of resistance. In addition, the antibiotic presence in the environment through improper disposal and industrial effluents aggravates this problem[6].

This study aimed to do a comprehensive comparative study of the antibacterial resistance patterns of isolates of bacteria from different clinical and environmental sources from different regions of Pakistan[7, 8]. To understand these multidimensional pathways of resistance strain development and spread, we examine the prevalence and distribution of resistance traits in these two disjointed yet intimate settings. Further, the study examined the genetic mechanisms that underpin these environments and their potential sources of reservoirs for maintaining and diversifying resistance traits[9].

This work is expected to provide a basis for the development of strategies for public health, antibiotic stewardship programs and a firm regulatory policy. It gives policymakers the ability to develop targeted interventions to slow or stop resistance by knowing the differential impacts of antibiotic use in clinical versus environmental contexts. Finally, in addition to closing a keyhole in the puzzle of antibiotic resistance dynamics in Pakistan, this study is part of the wider global effort to conserve the power of antibiotics for the following generations[10].

MATERIALS AND METHODS

Sample Collection: In total, 50 nonduplicate bacterial isolates were collected through various sources between January 2022 and December 2022, n= 25 clinical isolates and n=25 environmental samples were used as the sample set, and then split in half for the sample set. Study was conducted at Aziz Bhatti Shaheed hospital Gujrat, to obtain clinical specimens from urine, blood, respiratory secretions, and wound swabs. All clinical samples were collected with informed consent and by ethical standards with the appropriate institutional ethical approvals. Environmental samples were collected in parallel in various sources including surface waters (rivers, and lakes in both urban and rural areas), effluents

from municipal wastewater treatment plants, and soil or runoff samples from agricultural lands where antibiotics are frequently used in livestock and crop production. To minimize contamination and maintain the integrity of the samples, standardized collection procedures were followed.

Bacterial Isolation and Identification: After collection, the samples were processed for bacterial colony isolation. Serial dilution was used on environmental samples and processed according to standard microbiological protocols on clinical specimens which were also cultured on both selective and nonselective media. Subsequently, pure isolates were obtained by subculturing colonies with distinct morphologies. Colony morphology on nutrient agar and Gram staining to identify Gram-positive from Gram-negative bacteria were used to identify the isolates at a preliminary level. Biochemical characterization of isolates was further done by a series of biochemical tests like catalase, oxidase, indole, and citrate utilization assay. Molecular confirmation was achieved by polymerase chain reaction (PCR) assays with species-specific primers targeting conserved regions like 16s rRNA gene for definitive identification of key pathogens such as Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus.

Antibiotic Susceptibility Testing: The Kirby-Bauer disc diffusion method was used on Mueller-Hinton agar plates to test the antibiotic susceptibility of the strains. After this, each bacterial isolate was uniformly inoculated onto the agar surface to form a consistent bacterial lawn, and very carefully placed antibiotic-impregnated discs onto the medium. A panel of antibiotics tested included ampicillin, ceftriaxone, piperacillin-tazobactam, gentamicin, amikacin, ciprofloxacin, tetracycline, imipenem, and representatives from several classes including beta-lactams, aminoglycosides, fluoroquinolones, and tetracyclines. Each disc was following incubated at 35–37°C for 18–24 hours measured for the zones of inhibition around the disc. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines and multidrug resistance (MDR) was considered as resistance to three or more classes of antibiotics.

DATA ANALYSIS: SPSS version 27.0 was used for data analysis. Descriptive statistics were applied to evaluate the data collected from clinical and environmental isolates. The prevalence of antibiotic resistance was calculated as percentages for each antibiotic. Chi-square tests were used to compare resistance rates between the two groups, with a significance level set at $p < 0.05$. This statistical approach highlighted significant differences in the emergence and dissemination of antibiotic resistance between clinical and environmental isolates, enabling the identification of contributing factors within the studied bacterial populations.

RESULTS

Distribution of Isolates: Therefore, 25 clinical isolates and 25 environmental isolates were analyzed in total. Specimens such as urine, blood, respiratory secretions, and wound swabs from tertiary care hospitals were the sources of clinical isolates. Surface waters, wastewater treatment plant effluents, and agricultural sites were the sources of environmental isolates. Colony morphology, Gram staining, and biochemical tests were used to identify clinical isolates, and mainly Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus were found to be pathogenic. On the other hand, environmental isolates were more heterogeneous and included soil- and water-associated bacteria.

Antibiotic Resistance Patterns: Kirby-Bauer disc diffusion was used for antibiotic susceptibility testing. Amoxicillin, ceftriaxone, piperacillin tazobactam, gentamicin, amikacin, ciprofloxacin, tetracycline and imipenem were also evaluated as antibiotics. The table 1 below summarizes resistance rates in clinical versus environmental isolates.

This data shows that there is a markedly higher resistance profile of clinical isolates compared to environmental isolates. For example, clinical isolates were resistant to ampicillin in 80% of the cases but environmental isolates in 48%. Trends were similar for ceftriaxone, ciprofloxacin, and tetracycline. More notably, clinical isolates were inherently more multidrug-resistant than the environmental isolates, with 56% resistant to at least three antibiotic classes, compared to 20% in the environmental isolates.

Table 1: Antibiotic Resistance Rates in Clinical and Environmental Isolates

Antibiotic	Clinical Resistance (%) (n=25)	Environmental Resistance (%) (n=25)
Ampicillin	80% (20/25)	48% (12/25)
Ceftriaxone	64% (16/25)	32% (8/25)
Piperacillin-Tazobactam	52% (13/25)	24% (6/25)
Gentamicin	56% (14/25)	28% (7/25)
Amikacin	44% (11/25)	20% (5/25)
Ciprofloxacin	68% (17/25)	36% (9/25)
Tetracycline	72% (18/25)	40% (10/25)
Imipenem	36% (9/25)	12% (3/25)
Multidrug Resistance (MDR) (resistance to ≥3 classes)	56% (14/25)	20% (5/25)

* Percentages indicate the proportion of isolates exhibiting resistance among the total of 25 isolates in each group.

It is expected that the high level of resistance in clinical isolates is due to the high usage of antibiotics in hospital settings, leading to strong selective pressures for resistant strains to survive and proliferate. In contrast to environmental isolates, which exhibited lower resistance rates, the presence of resistance in these samples is worrisome since environmental isolates could serve as a reservoir for resistance genes that may convey resistance genes to pathogenic bacteria via horizontal gene transfer.

Consequently, these findings underline the importance of strengthening antibiotic stewardship in clinical settings and environmental management practices for containment of antibiotic resistance.

DISCUSSION

Our study shows a large dissimilarity of resistance patterns between clinical and environmental bacterial isolates in Pakistan. Resistance rates were markedly higher in the clinical isolates than in environmental isolates and MDR was also more common in clinical isolates (56%) compared with environmental isolates (20%) [11]. It was one of the clinical settings, where intensive and often empirical antibiotic use is common and similar to what was previously reported in this region like Sabir et al. (2020) and Saima et al. (2020) which was reported as a hot spot for the emergence of resistant strains. High selective pressure in hospitals owing to frequent antibiotic administration with broad spectra and sometimes suboptimal infection control practices is thought to accelerate the acquisition and dissemination of resistance genes in pathogenic bacteria such as Escherichia coli, Klebsiella pneumoniae, and Staphylococcus aureus[12].

On the contrary, although possessing lower levels of resistance, environmental isolates maintain a reservoir of resistance determinants. This is consistent with what other studies have found about the environment being an important driver of antibiotic resistance by horizontal gene transfer[13]. The existence of resistance genes in environmental niches, such as surface waters and agricultural runoff, indicates the potential for these reservoirs to be a source of resistance genes that then re-introduce resistance traits back into the clinical setting and perpetuate the cycle of resistance amplification[14].

However, our study has several limitations. The modest sample size of 50 isolates (25 clinical and 25 environmental) may not be representative of the whole diversity of resistance in Pakistan across different geographic areas and healthcare facilities[15]. Furthermore, the use of the Kirby–Bauer disc diffusion method, while standardized, may not completely characterize resistance levels for the more subtle variations that may be better understood using techniques like minimum inhibitory concentration

(MIC) testing or genomic sequencing. Finally, because the study is cross-sectional, we cannot observe temporal trends and seasonal variations in resistance patterns[16, 17].

Future research should endeavor to address these limitations by using larger and more representative sample sizes and use of advanced molecular techniques to understand the genetic mechanisms behind resistance[18]. Longitudinal studies would be particularly useful for tracking the development of resistance over time and thus furthering our understanding of the dynamics of transfer between clinical and environmental resistance genes. Such efforts would help to refine antibiotic stewardship policies and develop targeted interventions within a One Health framework, including the recognition of human, animal, and environmental interconnectedness to tackle antibiotic resistance[19, 20].

CONCLUSION

The study concluded that clinical and environmental bacterial isolates display a striking difference in antibiotic resistance and argue for the need for further antibiotic stewardship measures, improved infection control, and the systematic, large-scale surveillance of antibiotic resistance using modern molecular techniques, and larger, longitudinal datasets.

Conflict of Interest: The authors declare no conflicts of interest related to this study.

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Authors' Contributions: S.R., F.L., M.Z.A.K., and M.B. conducted the experiments and data analysis. S.S. and H.M.J. contributed to study supervision and manuscript review. All authors approved the final manuscript.

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REFERENCES

1. Bilal H, Khan MN, Rehman T, Hameed MF, Yang X. Antibiotic resistance in Pakistan: a systematic review of past decade. BMC infectious diseases. 2021;21:1-19.
2. Riaz S, Faisal M, Hasnain S. Prevalence and comparison of Beta-lactamase producing Escherichia coli and Klebsiella spp from clinical and environmental sources in Lahore, Pakistan. African Journal of Microbiology Research. 2012;6(2):465-70.
3. Saima S, Fiaz M, Manzoor M, Zafar R, Ahmed I, Nawaz U, et al. Molecular investigation of antibiotic resistant bacterial strains isolated from wastewater streams in Pakistan. 3 Biotech. 2020;10:1-11.
4. Saleem S, Bokhari H. Resistance profile of genetically distinct clinical Pseudomonas aeruginosa isolates from public hospitals in central Pakistan. Journal of infection and public health. 2020;13(4):598-605.
5. Rahim K, Qasim M, Rahman H, Khan T, Ahmad I, Khan N, et al. Antimicrobial resistance among aerobic biofilm producing bacteria isolated from chronic wounds in the tertiary care hospitals of Peshawar, Pakistan. Journal of Wound Care. 2016;25(8):480-6.
6. Javaid N, Sultana Q, Rasool K, Gandra S, Ahmad F, Chaudhary SU, et al. Trends in antimicrobial resistance amongst pathogens isolated from blood and cerebrospinal fluid cultures in Pakistan (2011-2015): A retrospective cross-sectional study. PLoS One. 2021;16(4):e0250226.
7. Sabir R, Alvi SFD, Fawwad A. Antimicrobial susceptibility pattern of aerobic microbial isolates in a clinical laboratory in Karachi-Pakistan. Pakistan journal of medical sciences. 2013;29(3):851.
8. Atif M, Naseem M, Sarwar S, Mukhtar S, Malik I, Hassan MRu, et al. Spectrum of microorganisms, antibiotic resistance pattern, and treatment outcomes among patients with empyema thoracis: A descriptive cross-sectional study from the Bahawal Victoria Hospital Bahawalpur, Punjab, Pakistan. Frontiers in Medicine. 2021;8:665963.
9. Manzoor A, Ul-Haq I, Baig S, Qazi JI, Seratlic S. Efficacy of locally isolated lactic acid bacteria against antibiotic-resistant uropathogens. Jundishapur journal of microbiology. 2016;9(1):e18952.
10. Mustafa SS, Batool R, Kamran M, Javed H, Jamil N. Evaluating the role of wastewaters as reservoirs of antibiotic-resistant ESKAPEE bacteria using phenotypic and molecular methods. Infection and Drug Resistance. 2022:5715-28.
11. Yar AM, Zaman G, Hussain A, Changhui Y, Rasul A, Hussain A, et al. Comparative genome analysis of 2 Mycobacterium tuberculosis strains from Pakistan: insights globally into drug resistance, virulence, and niche adaptation. Evolutionary Bioinformatics. 2018;14:1176934318790252.
12. Tanni AA, Hasan MM, Sultana N, Ahmed W, Mannan A. Prevalence and molecular characterization of antibiotic resistance and associated genes in Klebsiella pneumoniae isolates: a clinical observational study in different hospitals in Chattogram, Bangladesh. Plos one. 2021;16(9):e0257419.
13. Shafquat Y, Jabeen K, Farooqi J, Mehmood K, Irfan S, Hasan R, et al. Antimicrobial susceptibility against metronidazole and carbapenem in clinical anaerobic isolates from Pakistan. Antimicrobial Resistance & Infection Control. 2019;8:1-7.
14. Azimi T, Maham S, Fallah F, Azimi L, Gholinejad Z. Evaluating the antimicrobial resistance patterns among major bacterial pathogens isolated from clinical specimens taken from patients in Mofid Children's Hospital, Tehran, Iran: 2013–2018. Infection and drug resistance. 2019:2089-102.
15. Majumder MMI, Mahadi AR, Ahmed T, Ahmed M, Uddin MN, Alam MZ. Antibiotic resistance pattern of microorganisms causing urinary tract infection: a 10-year comparative analysis in a tertiary care hospital of Bangladesh. Antimicrobial Resistance & Infection Control. 2022;11(1):156.
16. Atif M, Zia R, Malik I, Ahmad N, Sarwar S. Treatment outcomes, antibiotic use and its resistance pattern among neonatal sepsis patients attending Bahawal Victoria Hospital, Pakistan. PLoS One. 2021;16(1):e0244866.
17. Heinz E, Ejaz H, Bartholdson Scott J, Wang N, Gujaran S, Pickard D, et al. Resistance mechanisms and population structure of highly drug resistant Klebsiella in Pakistan during the introduction of the carbapenemase NDM-1. Scientific reports. 2019;9(1):2392.
18. Sabir N, Hussain W, Ahmed A, Zaman G, Mirza IA, Ikram A. Burden of multi-drug resistant, extensively-drug resistant and pan-drug resistant superbugs isolated from various indoor microbiological specimens at tertiary care centers Rawalpindi. Pakistan Armed Forces Medical Journal. 2020(1):79.
19. Irum S, Naz K, Ullah N, Mustafa Z, Ali A, Arslan M, et al. Antimicrobial resistance and genomic characterization of six new sequence types in multidrug-resistant Pseudomonas aeruginosa clinical isolates from Pakistan. Antibiotics. 2021;10(11):1386.
20. Rafiq M, Hayat M, Anesio AM, Jamil SUU, Hassan N, Shah AA, et al. Recovery of metallo-tolerant and antibiotic resistant psychrophilic bacteria from Siachen glacier, Pakistan. PloS one. 2017;12(7):e0178180.