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Microbial Biofilm Diversity and Prevalence of Antibiotic Resistance Genes in Drinking Water Distribution System of Peshawar, Pakistan

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Abstract: The occurrence of microbial communities harboring antibiotic resistance bacteria and antibiotic resistance genes in the drinking water distribution system pose a significant threat to the aquatic ecosystem and to public health, especially in developing countries. In this study, we have used next-generation sequencing technology to explore bacterial community diversity and the abundance of antibiotic resistance genes in biofilms collected from the drinking water distribution system of Peshawar, the capital city of the Khyber Pakhtunkhwa province of Pakistan. The results showed that *Proteobacteria* were the most abundant phyla (89.79%) in all biofilm samples, followed by *Bacteroidetes* (3.48%) and *Actinobacteria* (2.79%). At genus level, *Pseudomonas* was the most common (22.45%) in all biofilm samples. Overall, bacterial diversity and richness was higher in biofilm samples collected from the consumer end than the source site. Bacterial diversity was also dependent on the piping material (GI vs. PVC) and water supply (direct vs. indirect). Functional annotation reveals a differential abundance of common metabolic pathways at source and consumer end. Resistome analysis revealed a prevalence of resistance genes against 12 classes of antibiotics in all samples with macrolides resistance being the commonest at the consumer end (42.1%) and fluoroquinolone resistance at the source end (24%). To our knowledge, this is the first study that provides new insight and evidence into the microbial community diversity and antibiotic resistance in the drinking water supply system of Peshawar. These findings may ultimately help the authorities to design and implement effective strategies for controlling biofilms and ensuring a continuous supply of safe drinking water to the community.

Keywords: shotgun metagenomic sequencing; bacterial diversity; core species; functional potential; resistome

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1. Introduction

Water is one of the main components of life on planet Earth and critically important for all human, animal and plant survival. Only second to oxygen, water is essential for the smooth functioning of the human body. Therefore, provision, access and an adequate supply of safe drinking water has been recognized as a fundamental human right, a top priority issue and one of the eight components of primary health care identified by the International Conference on Primary Health Care in Alma-Ata in 1978 [1]. In modern times, the distribution of drinking water to communities is generally achieved by an engineered,

drinking water distribution system (DWDS), consisting of pipes that distribute water from the source (reservoirs or aqueducts) to the end users. A DWDS, although ensuring a continuous supply of safe and aesthetically pleasing drinking water, always has the risk of contamination from toxic elements and microbes [2] rendering the water unsafe for drinking. Recent estimates suggest more than two billion people across the world drink from water sources contaminated with fecal microbes and as a result, they are at high risk of contracting waterborne diseases such as diarrhea, cholera, dysentery, typhoid and polio [3].

Microbiological contamination of drinking water occurs either due to planktonic (free floating) bacteria in flowing water or bacterial biofilms grown on the inner surface DWDS pipes. Bacterial biofilms are aggregates of microorganisms attached to a surface (biotic or abiotic) and/or each other and surrounded by a self-produced biofilm matrix (slime) that consists mainly of lipids, proteins, polysaccharides and nucleic acids [4]. Biofilm formation is so far the most successful form of life that not only protect microbes against the action of antibiotics [5] but also helps in metabolic collaboration and communication with other species to maintain hemostasis [6]. Bacterial biofilms growing in DWDS is the main source of microbiological contamination of drinking water that can pose several problems to human health. They can affect the esthetic quality of water, act as pathogen reservoirs, play a role in corrosion of the water distribution pipes [7] and can harbor antibiotic resistance bacteria (ARB) and antibiotic resistance genes (ARGs) [8]. There is growing evidence that ARBs and ARGs have polluted not only untreated drinking water sources, such as surface [9] and ground waters [10], but even tap and bottled water [11]. These ARGs have the ability to pass from one bacteria to another in DWDS biofilms and human pathogenic bacteria through horizontal or vertical gene transfer, making them resistant to antibiotics. The water flow may then dislodge antibiotic resistant bacteria and ultimately affect human health directly thorough the consumption of contaminated drinking water [12]. Human beings are also exposed to ARB and ARGs indirectly when they use contaminated water for activities like bathing, swimming and consumption of irrigated food produce, thus posing a risk to human health. Antibiotic resistance has been recognized as an emerging and eminent threat to public health across the globe. ARB related infections in humans are extremely difficult and expensive to treat, require longer stays in hospitals and are associated with significantly high mortality and morbidity [13]. Recent estimates suggests that approximately 700,000 people die each year because of resistant infections and if the problem is not controlled by a coordinated action plan, it would further result in 10 million deaths per year and a cumulative USD 100 trillion loss by the year 2050 [14].

In order to ensure a supply of safe and esthetically pleasing drinking water, minimize health risks and protect humans against waterborne diseases; in many developed countries, water treatment and disinfection is mandatory by law. For this purpose, different strategies such chlorination and drinking water treatment plants are being employed across the world. Although recent evidence suggests that drinking water treatment plants are not capable of completely removing ARB and ARG [15], the issue may be more serious in developing countries like Pakistan where there is no prior disinfection or physical and chemical treatment of drinking water supplies to the communities. Faulty and poorly managed distribution systems with leaking pipes and cross connection with sewerage lines may further worsen the situation. As a result, the water quality is greatly impaired. A number of studies have reported bacteriological contamination of drinking water from different cities and waterborne diseases continue to be a significant threat to public health in Pakistan [16]. Successful tackling of the issue requires a thorough characterization of the bacterial communities and the occurrence of ARGs in DWDS, using advanced technologies.

In order to characterize microbial communities growing in DWDS, different methods are being used. Of these, conventional culturing techniques relying on isolation and detection of culturable microbes is the most commonly used method throughout the world [17]. However, these methods have limited applicability for the of study microbial biofilms growing in DWDS, the majority (in fact >99%) of which contain microorganisms that

cannot be cultured/grown in a laboratory but are still viable [18]. Nowadays, nucleic acid based, culture-independent methods are increasingly being used to study environmental biofilms including those growing in DWDS. Of these, next generation sequencing (NGS) technologies are one of the most accurate and high throughput methods to characterize microbial diversity in DWDS biofilms [18]. The NGS-based approach not only provides broad-spectrum identification of different microbes, along with taxonomic classification and pathogen detection, it also provides a deep insight into the functional potential of the metagenome and ARGs profiling. In the recent past, NGS technology has been increasingly used in studying DWDS biofilms and drinking water quality monitoring in different countries of the world. However, it has never been used to characterize microbial biofilm diversity and the prevalence of antibiotic resistance genes in the absence of drinking water treatment and poorly managed DWDS in Peshawar, the capital of the Khyber Pakhtunkhwa province of Pakistan. The city is famous for its geo-strategic importance and is currently hosting the highest number of refugees and displaced persons in the country. It is a rapidly growing metropolitan city of Pakistan with an estimated population of 4.2 million and a growth rate of 3.7% [19]. The Government of Khyber Pakhtunkhwa is struggling hard to maintain an uninterrupted supply of clean drinking water to the ever growing population of the city. A number of studies have reported microbiological contamination of drinking water in the city [20,21]. However, the scope of these studies to identify potential health risks to the population is limited, primarily due to using species-specific culturing techniques that are inherently biased as the total bacterial populations have not been taken into account. Furthermore, till now, there has been no study of the occurrence of antibiotic resistance and bacterial biofilms in the oldest and poorly managed DWDS of the city. In the current study, for the first time, we have used advanced, shotgun metagenomic sequencing technology to assess microbial biofilm diversity and the prevalence of antibiotic resistance in the DWDS of Peshawar, Pakistan.

2. Materials and Methods

2.1. Study Area and Sampling

Public water in Peshawar city is mainly drawn from ground sources and supplied to the community directly without any filtration or disinfection. In terms of water supply, Peshawar city is divided into urban (Zone A, B, C and D), rural and cantonment zones. Drinking water supply to these areas falls under the jurisdiction of the Water and Sanitation Services Peshawar (WSSP), Public Health Engineering Department and Cantonment Board Peshawar, respectively. Based on WHO sample size for drinking water quality assessment, a total of nineteen DWDS (n = 19) sites were randomly selected. Of these, 12 sites were selected from urban areas, 5 from rural and 2 from cantonment area (Figure 1). From each DWDS, two biofilm samples were collected; one from the source site and the other from the consumer end. In total, 38 biofilm samples were collected. Sample collection at consumer end was done at minimum of 100 m distance from the source site.

All the sampling procedures were carried out following American Public Health Association (APHA) approved guidelines. Biofilm samples were collected by opening off the pipes, maintaining sterile conditions by using sterile disposable gloves and instruments. Biofilms were removed from the inside of the pipes by swabbing and scrapping off the biofilms using sterile spatula and swab in 360 degree manner to ensure representation of whole biofilm samples growing on the whole pipe rather than only a subsection. The samples were immediately transferred aseptically into sterile, 50 mL Falcon tubes. The samples were kept on ice during collection, transported to the lab, maintaining cold chain and processed within 24 h after collection.

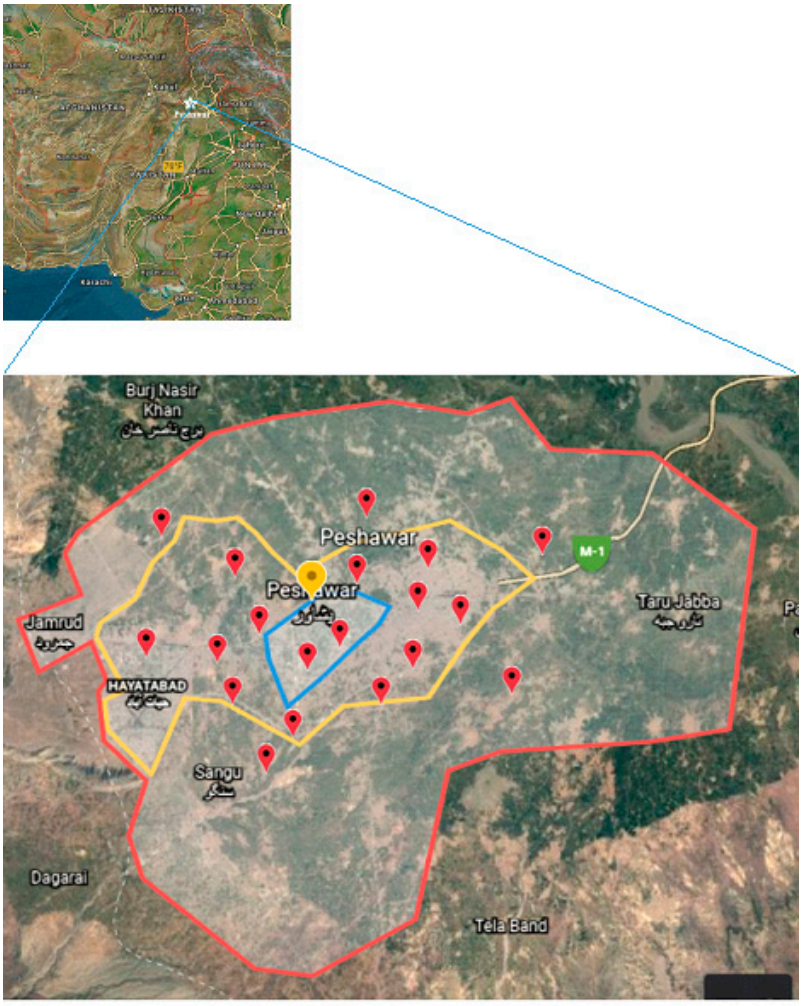

Figure 1. Map of Peshawar city showing geographical locations of sample collection area.

2.2. DNA Extraction

Biofilm samples stored at −80 °C were allowed to thaw at room temperature. After thawing, 5 mL of PBS (Phosphate Buffer saline) was added to the Falcon tubes containing biofilm, followed by vortex and centrifugation at 10,000 rpm to allow settling down of biofilm contents in the form of a pellet. Genomic DNA was extracted from the pellet using ZymoBIOMICS DNA Miniprep Kit (Zymo Research, Irvine, CA, USA) following manufacturer's instructions. A 1% agarose gel electrophoresis was used to assess DNA quality. DNA quantity was measured by a NanoDrop Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA). On average, each biofilm sample yielded 14 ng/mL of genomic DNA. The final extracted DNA samples were stored at −20 °C till further analysis.

2.3. Shotgun Metagenomic Sequencing and Quality Filtering

Extracted DNA samples were sent to Rehman Medical Institute (Center for Genomic Sciences) for library preparation and high throughput sequencing on Illumina Miseq sequencing platform (Illumina Inc., San Diego, CA, USA). Library was constructed using Illumina Nextera XT DNA Library Preparation Kit (FC-131-1096, Illumina Inc., CA, USA) and Nextera XT Index Kit v2 Set A (FC-131-2001, Illumina Inc., CA, USA) following manufacturer's instructions, followed by paired-end sequencing (2 × 150 bp) on MiSeq platform. Primary quality analysis of the reads was done by FastQC quality assessment tool [22]. Human associated sequences were removed through KneadData (v. 0.6.1) by mapping the reads against the GRCh37.p13 reference genome (GCF_000001405.25 assembly). Sequencing reads with quality score < 30 and length < 60 were removed by

using Trimmomatic version 0.33 [23]. The quality filtered reads were used for downstream analysis.

2.4. Bioinformatics Analysis

In order to assess bacterial diversity and, the quality filtered sequencing reads were separately aligned against NCBI's nonredundant protein reference database [24] (NCBI-NR) using DIAMOND aligner v.0.9.25 [25]. DIAMOND alignment files (.daa) for each sample were imported into MEtaGenome Analyzer (MEGAN 6) software for the taxonomic and functional annotation of aligned quality filtered sequencing reads [26]. For comparison, sequencing reads from all the samples were normalized to the sample with lowest read count followed by removal of unassigned reads. Antimicrobial resistance was assessed by using the ARIBA (Antimicrobial Resistance Identification by Assembly) [27].

2.5. Statistical Analysis

For statistical analysis of taxonomic and functional profiles, STAMP (Statistical Analysis of Metagenomic Profiles) statistical tool was used [28]. The analysis used Fischer's exact *t*-test along with Storey's FDR correction at 95% confidence interval. Corrected *p*-values (q-values) of $\leq$0.05 were considered significant.

3. Results

3.1. Taxonomic Diversity

3.1.1. Alpha Diversity

The Alpha diversity indices (i.e., Shannon−Weaver and Simpson's reciprocal index), based on bacterial richness and evenness in samples, were calculated by resolving the next generation sequencing reads on the phylum and genus levels. Table 1 demonstrates the alpha diversity indices at both taxonomic levels for DWDS biofilms at the source and consumer end. At genus level, consumer-end biofilms possessed higher alpha diversity (Simpson's reciprocal index = 18.09; Shannon−Weaver index = 5.14) than the source-end biofilms (Simpson's reciprocal index = 14.168; Shannon−Weaver index = 4.716). A similar trend was also observed at phylum level. However, such a trend of increment for the Shannon−Weaver index was more prominent at phylum level, while on genus level, Simpson's reciprocal index showed a clear distinction between the sources and consumer-end biofilm samples.

Table 1. Diversity indices of source and consumer-end biofilm samples at phylum and genus levels. The indices are calculated as mean ± standard deviation.

Taxonomical Levels	Shannon−Weaver Index		Simpsons's Reciprocal Index	
	Source End	Consumer End	Source End	Consumer End
Phylum level	0.728 ± 0.326	1.186 ± 0.197	0.933 ± 0.376	1.353 ± 0.296
Genus level	4.716 ± 0.753	5.147 ± 0.574	14.168 ± 7.131	18.09 ± 7.773

3.1.2. Beta Diversity

Beta diversity (the relative abundance of bacterial taxa in each sample) analysis was also performed by principal coordinate analysis (PCoA), employing the Bray–Curtis dissimilarity matrix [29] at genus level. An increase in distance between the samples is a representative of higher dissimilarity at observed taxonomic level. Figure 2 shows the PCoA plot for the source and consumer-end biofilm samples. As shown in the figure, samples from both the consumer and source ends assembled into four distinct clusters. The majority of samples clearly clustered together into a large core group comprising of 28 samples that demonstrated nearly similar taxonomic profiles, showing a similar microbiome composition at genus level. However, some consumer samples and five source samples clustered separately, thus having a different microbiome composition than the core DWDS samples.

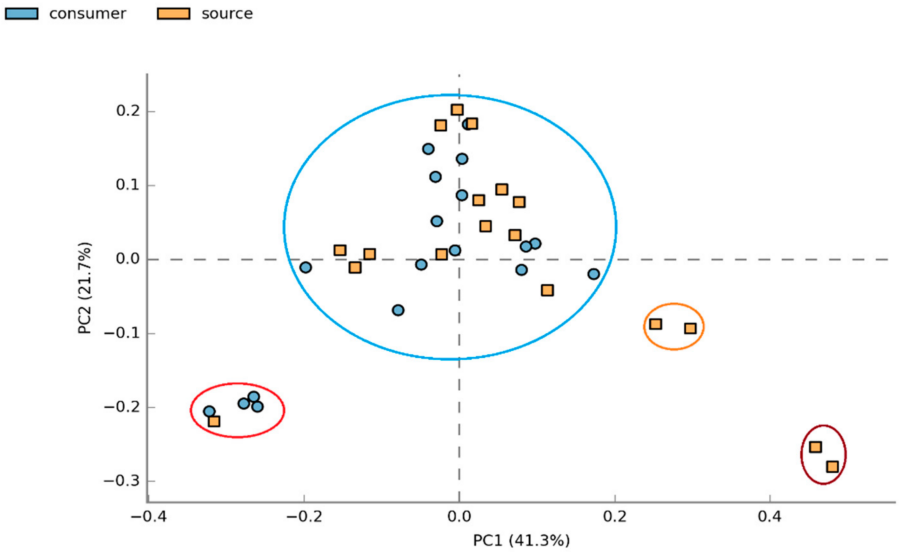

Figure 2. Principal coordinate analysis (PCoA) plot of bacterial communities in DWDS based on Bray–Curtis dissimilarity.

3.2. Bacteria Community Composition

Taxonomic and Differential Abundance Profiles

The taxonomic profile for both source and consumer-end biofilms was generated by the alignment of quality filtered reads against the NCBI nonredundant protein database followed by annotation using Metagenome Analyzer (MEGAN). The top ten phyla were selected. Of these, *Proteobacteria* (90–92%) was found to be the most abundant phyla in both the source and consumer-end biofilm samples, followed by *Bacteroidetes* and *Actinobacteria*. Differential abundance profiling by Fischer's exact t test revealed *Proteobacteria* (p-value = 0.011) to be in significantly higher abundance in the source-end biofilm samples (Figure 3). Although *Bacteroidetes* (p-value = 0.071) and *Actinobacteria* (0.116) were more abundant in consumer than source-end biofilm samples, the differences were statistically insignificant ($p > 0.05$).

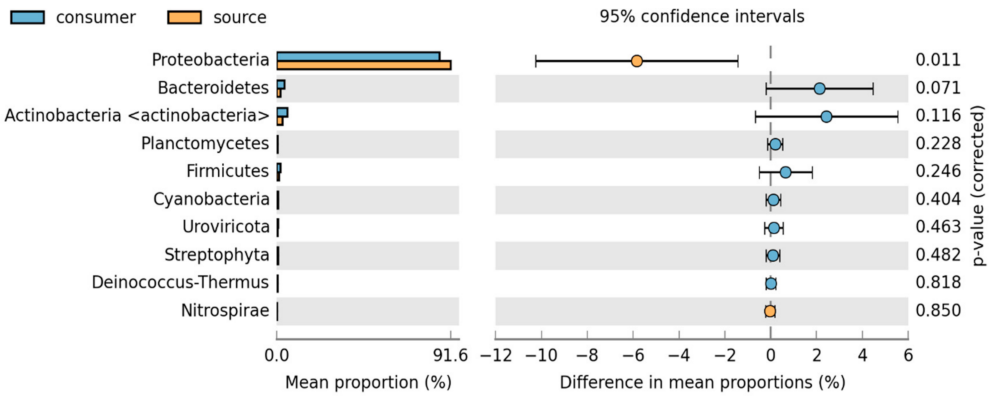

Figure 3. Differential abundance profile (phylum level) between source and consumer-end biofilm samples on the basis of Fischer's exact t-test with Storey's FDR correction at 95% confidence interval.

For the genus level abundance analysis, the top 30 genera were selected as shown in Figure 4. *Pseudomonas* and *Xanthomonas* were the most abundant genera found in both source and consumer-end biofilm samples. Of all the 30 genera, only *Fibrella* genus appeared to be in significantly high (p-value = 0.039) abundance in the consumer-end rather than the source-end samples.

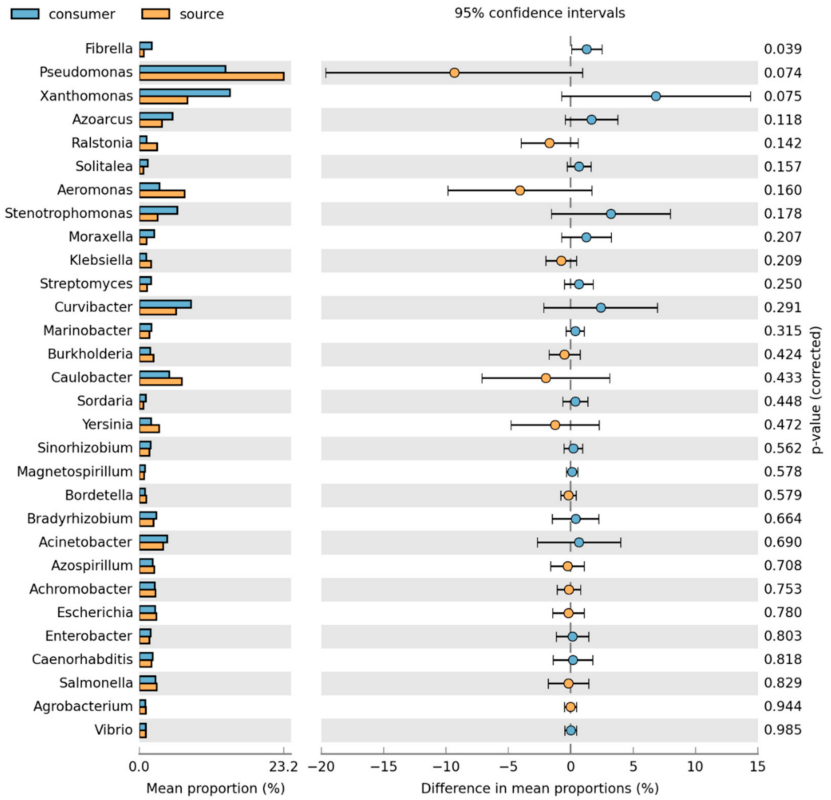

Figure 4. Differential abundance profile (genus level) between source and consumer-end biofilm samples on the basis of Fischer's exact *t*-test with Storey's FDR correction at 95% confidence interval.

3.3. DWDS Features and Their Relationship with Bacterial Biofilm Community

We have further assessed whether the water supply or piping material in DWDS have any impact on community composition using abundance profiles at genus level. In Pakistan, DWDS receive water either directly (from the tube well and supply it to the community) or indirectly from an overhead storage tank. Figure 5 demonstrates the differential abundance profile between direct and indirect source biofilms. Genus *Caulobacter* was found to be of significantly (p-value = 0.00077) high abundance in direct rather than indirect source biofilms. Furthermore, pathogenic bacterial genera namely *Pseudomonas* (p-value = 0.034) and *Burkholderia* (p-value = 0.015) were also found to be significantly more ($p < 0.05$) abundant in direct source biofilms. In indirect source biofilms, *Xanthomonas* and *Stenotrophomonas* were more abundant, though the difference from the direct source biofilms was not significant.

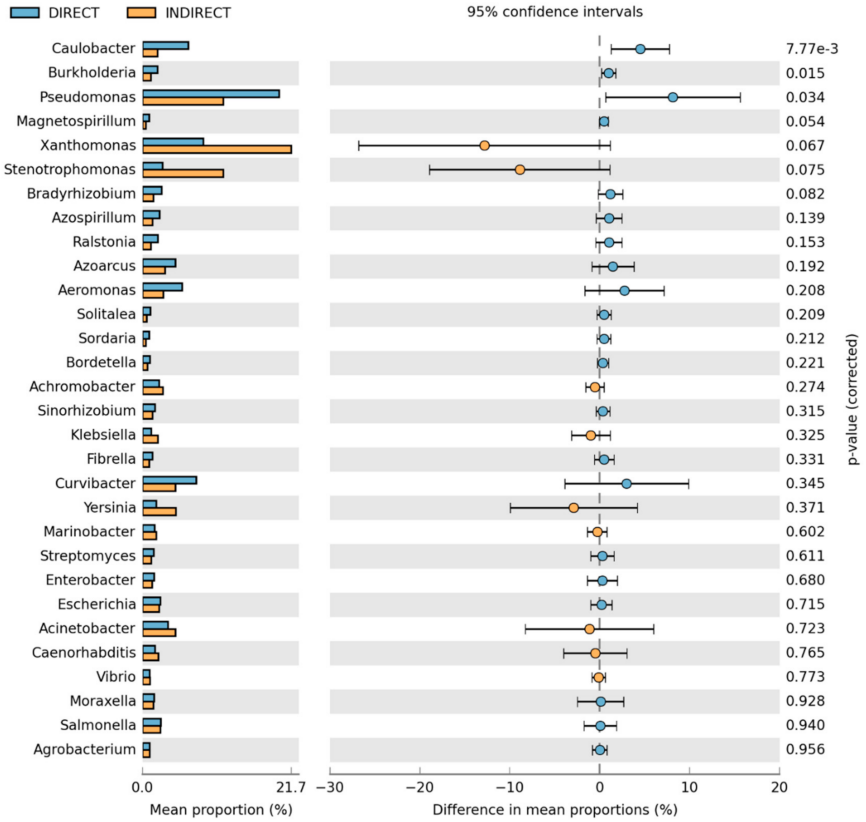

Figure 5. Differential abundance profile (genus level) between direct and indirect source biofilm samples on the basis of Fischer's exact *t*-test with Storey's FDR correction at 95% confidence interval.

The bacterial abundance profile at genus level also depends on the piping material (galvanized iron vs. polyvinyl chloride) (Figure 6). *Pseudomonas* was the most common bacterial genus in biofilms grown in PVC pipes while *Xanthomonas* was most abundant in GI pipes. However, significant differences were only found in Vibrio (p-value = 0.017), *Azospirillum* (p-value = 0.021), *Marinobacter* (p-value = 0.024), *Moraxella* (p-value = 0.027) and *Klebsiella* (p-value = 0.045) with all these genera being significantly abundant in GI rather than PVC piper biofilms.

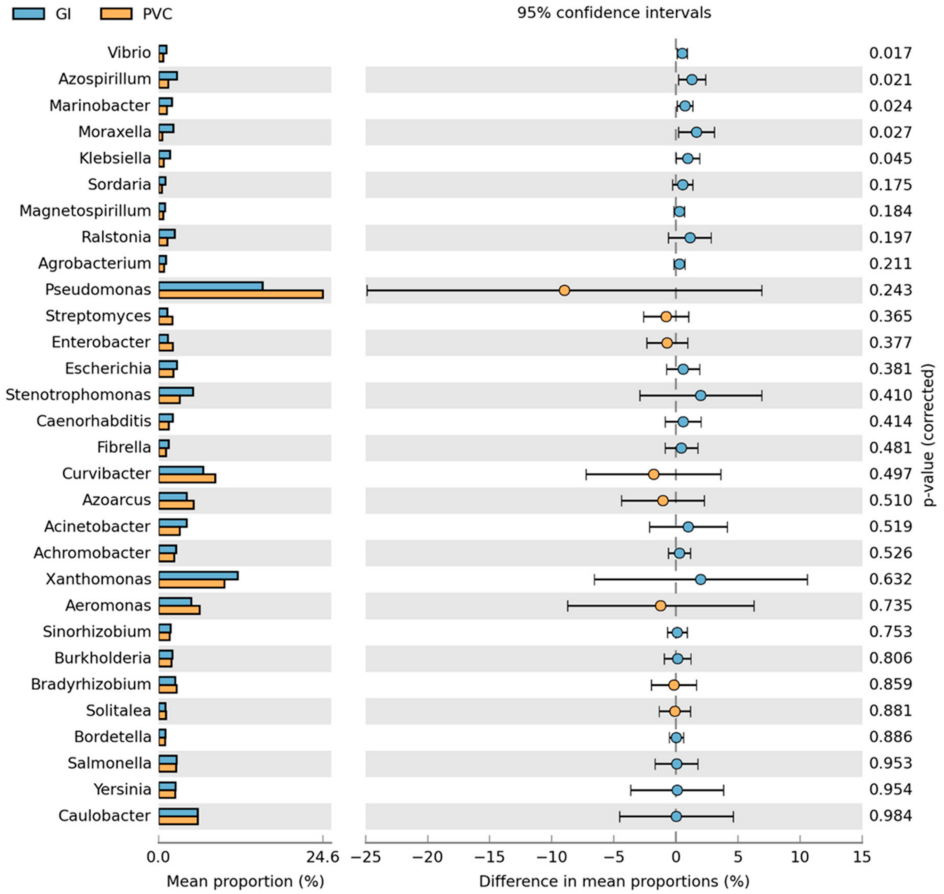

Figure 6. Differential abundance profile (genus level) between GI and PVC pipe biofilm samples on the basis of Fischer's exact *t*-test with Storey's FDR correction at 95% confidence interval.

3.4. Functional Annotation of DWDS Biofilm Metagenome

The metagenome predicted functional profiling of the biofilm samples revealed that most of the contigs were involved in common metabolic pathways such as replication, recombination and repair (10–13%), protein metabolism and amino acid transportation (10–11%) and energy production and conversion (Figure 7). Genes associated with replication, amino acid metabolism, energy production, lipid metabolism, secondary metabolite biosynthesis and defense mechanisms were comparatively more abundant in consumer than source biofilm samples.

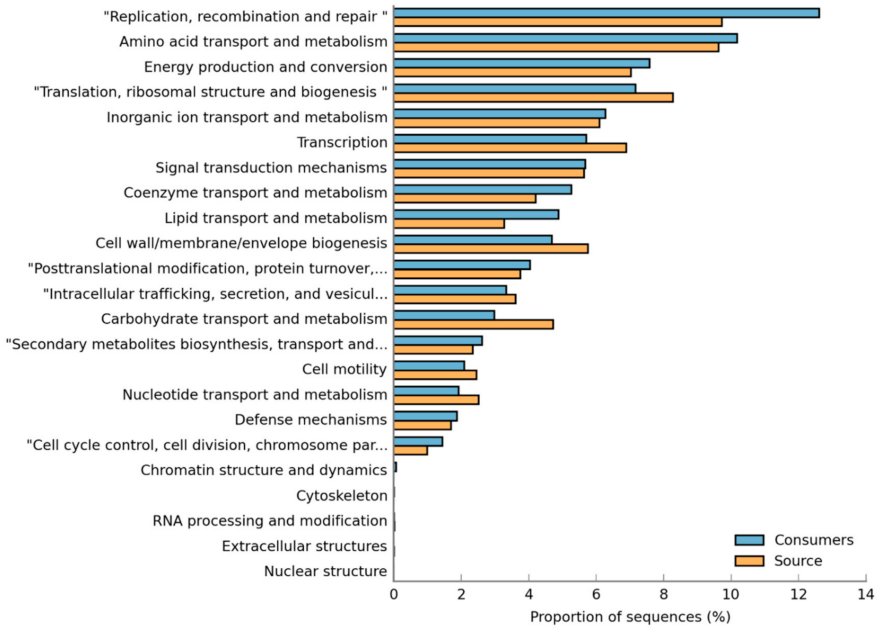

Figure 7. Relative percentage bar plot of metabolic pathways associated with genes identified in consumer and source biofilm samples.

3.5. Resistance Genes Prevalence in DWDS Biofilms

In total, antibiotic resistance genes against 12 different antibiotic classes were characterized in both consumer source end biofilm samples (Figure 8). Of these, the genes associated with resistance against macrolides (45%), aminoglycosides (14%), glycopeptides (12%), cephalosporin (6%), elfamycin (5%) and lincosamide (4%) were comparatively more abundant in consumer-end biofilms while fluoroquinolones (25%), rifamycin (5%), aminocoumarin (5%) and carbapenem (3%) resistance genes were more abundant in source-end biofilm samples.

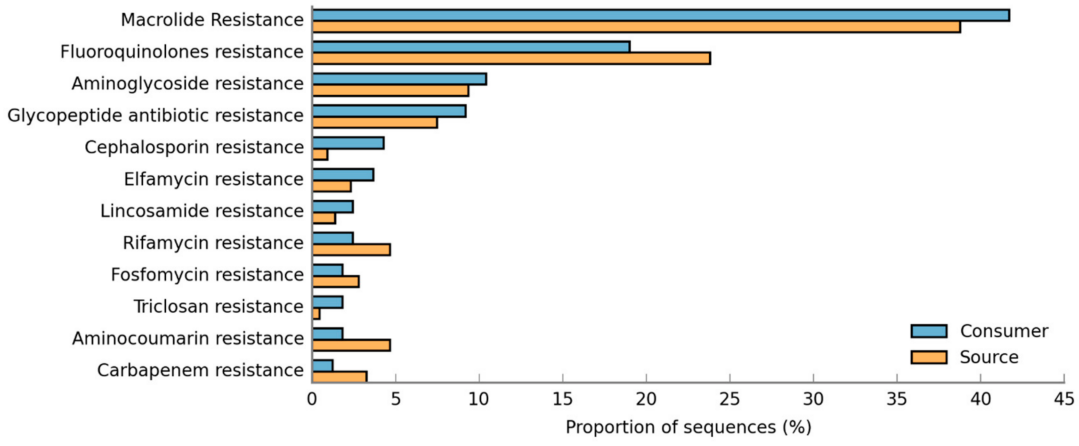

Figure 8. Relative percentage bar plot of antibiotic resistance gene classes identified in consumer and source samples.

4. Discussion

4.1. Genral Discussion

The regular assessment of water quality is an important component of public health monitoring programs throughout the world. Currently, monitoring programs utilize conventional culturing techniques involving isolation and enumeration of microbes to assess the microbiological contamination of drinking water [17,30]. However, the requirement for

a unique isolation and culturing procedure for each microorganism, the long incubation hours to grow and the ability to characterize only a few bacterial species, in contrast to a full scale bacterial load greatly limits the applicability of the conventional techniques for drinking water quality assessment and monitoring [31]. Moreover, the emergence of ARBs and ARGs as public health risks have also compelled the monitoring authorities to pay more attention to overall microbial load and antibiotic resistance in drinking water rather than focusing only on disease-causing pathogens, such as cholera and typhoid fever. The detection and characterization of these emergent pathogens and resistance and virulence genes require advanced, culture-independent molecular biology techniques. Recently, nucleic acid-based identification techniques, such as next-generation sequencing (NGS), are gaining popularity as they are culture independent, allow direct and simultaneous identification of multiple microorganisms without incubation, characterize ARGs, assess functional potential and have a turnaround time of only a few hours [32]. In the last two decades, NGS-based techniques have increasingly been used in water quality assessment and monitoring throughout the world. In the current study, we have also used advanced next-generation sequencing technology to provide a deeper insight into the bacterial composition and presence of antibiotic resistance genes in the DWDS of Peshawar, Pakistan.

Using shotgun metagenomic sequencing, we have found differentially abundant bacterial taxa and antibiotic resistance genes in both the source and the consumer-end biofilm samples. Overall, bacterial diversity indices as assessed by Shannon−Weaver (species richness) and Simpson's reciprocal index (species evenness) were higher in consumer-end biofilm samples. Although, the exact mechanism of why such disparities in microbial community composition exist at source and consumer end, there are several possible explanations for this phenomena. First, it may be due to a convergence of fluvial networks at the consumer end which is further dependent on the corrosion of the DWDS pipeline system resulting in altered microbial distribution at community level [33,34]. Second, an increase in alpha diversity downstream of the distribution system is also correlated with the dispersion of microbial communities due to continuous water flow [33]. Third, the water from the source to the consumer end has to travel a lot of distance thus providing sufficient time for microorganism to grow, form biofilms, dislodge and regrow at a distant point from the source. The comparative analyses of the source and the consumer-end biofilm samples showed significant bacterial community diversity at both phylum and genus level. *Proteobacteria* were found to be the most abundant phyla in both the source and consumer-end biofilm samples. These results are in concordance with the previous studies, demonstrating a higher abundance of *Proteobacteria* in DWDS [35–37]. *Proteobacteria* phylum is comprised of bacterial species that possess an extensive ability to generate biofilms in distribution systems [38,39]. These species can produce cellulose that acts as a molecular glue to strengthen the biofilm integrity and allows the protection of *Proteobacteria* communities from common drinking water disinfection procedures [40]. Therefore, an abundance of *Proteobacteria* in drinking water is related to their biofilm production potential. At genus level, the source-end biofilms showed a significantly higher abundance of *Caulobacter* and pathogenic bacterial genera, i.e., *Pseudomonas* and *Burkholderia*. *Caulobacter* species produce a bioadhesive that allows higher enumeration and dispersion in freshwater ecosystems [41]. Moreover, *Pseudomonas* and *Burkholderia* species have adherence potential using extracellular polymeric substances in water distribution systems and this property is further facilitated by the surface roughness of the pipeline distribution systems [42]. Hence, persistence of these bacterial communities is due to increased adhesion in response to factors promoting biofilm formation.

Microbial diversity, survival and regrowth within the DWDS also depends on complex and multidimensional interactions between microorganisms and the physiochemical characteristics of the DWDS, such as hydraulic conditions, nutrient availability and piping material. Of these, the piping material is the most common and important factor affecting microbial diversity within DWDS. The piping infrastructure in DWDS is in constant contact

with drinking water and provides a large surface area for microbial biofilm growth. These pipes are made of different materials, including cast iron, steel, plastic and copper. Previous studies have reported that DWDS made of iron and steel harbor a high density of microbial biofilms/biomass compared to plastic pipes, such polyvinylchloride [43,44]. In our study, we have also found a significantly high differential abundance of bacterial genera growing in GI pipes. Previous studies have also shown GI pipes to be the most favorable material for bacterial adhesion and hence biofilm formation [43,45]. The exact mechanism of enhanced biofilm formation in these piping systems is not known. However, the release of organic matter and phosphorus (biogenic compounds) have been observed in these piping systems which may stimulate biofilm formation [45]. These findings are specifically important in the context of Peshawar city where 65% of the pipes in DWDS are made of GI [46]. The piping system may harbor diverse bacterial biofilm communities, including pathogenic bacteria that are released into the drinking water and pose significant threat to public health.

Metagenomic analysis further demonstrated that the relative abundance of antibiotic resistance genes was higher in the consumer than the source biofilm samples. Similar finding have also been reported in other studies [47], indicating the regrowth of antibiotic resistance bacteria when the water is transported through the distribution system. It is not clear how the bacteria acquire resistance during transportation although Lv et al. suggested that horizontal genes transfer, cross/co resistance to antimicrobial agents or heavy metals and chromosomal mutations might play a role [48]. The DWDS may be an important reservoir of antibiotic resistance genes and thus require enhanced surveillance for risk assessment and preventive strategies in order to protect public health.

4.2. Limitations of the Study

Although our study is the first of its kind to report on bacterial biofilm diversity and antibiotic resistance in the untreated DWDS of a mega city from Pakistan, using shotgun metagenomic sequencing technology, it has some limitations. First, the relatively small sample size of the study makes it difficult to generalize the findings for the whole city. Second, due to time and budget constraints, we could not assess how seasonal variations, antibiotics and heavy metal concentrations affect biofilm diversity and its impact on drinking water quality. Third, we were also not able to assess the exposure, risk and potential health impacts in the local population.

4.3. Practical Implications and Future Directions

The findings of the study suggest that the DWDS in Peshawar is an important source of microbiological and antibiotic resistance gene pollution of drinking water and may pose a significant threat to community health. Public health authorities of the city are required to pay special attention to DWDS biofilms and adopt biofilm limiting strategies, such as the installation of a biofilm-resistant piping system, together with the removal of organic and inorganic pollutants and the use of disinfectants. Future studies should focus on (1) monitoring the occurrence, fat and transport of ARBs, ARGs, mobile genetic elements and virulence genes from source to tap water and humans, via drinking water; (2) epidemiological studies to assess the human health risk due to waterborne contaminants, using risk modelling; (3) the evaluation and monitoring of antibiotics in drinking water and associated factors; and (4) propose and evaluate the effectiveness of low-cost water-treatment methods.

5. Conclusions

In summary, the current study provides a deeper insight into bacterial biofilm diversity and antibiotic resistance in DWDS of Peshawar city. The results showed that the bacterial diversity and abundance of antibiotic resistance genes varies between source and consumer-end biofilms. The type of water source and piping material also affect bacterial diversity. All these findings suggest further studies to assess human health risks and effective strategies

to minimize the harmful health impact on the community, using low cost, community-based, mitigation and monitoring approaches.

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