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RESEARCH ARTICLE

Assessment of antibiotic resistance genes in dialysis water treatment processes

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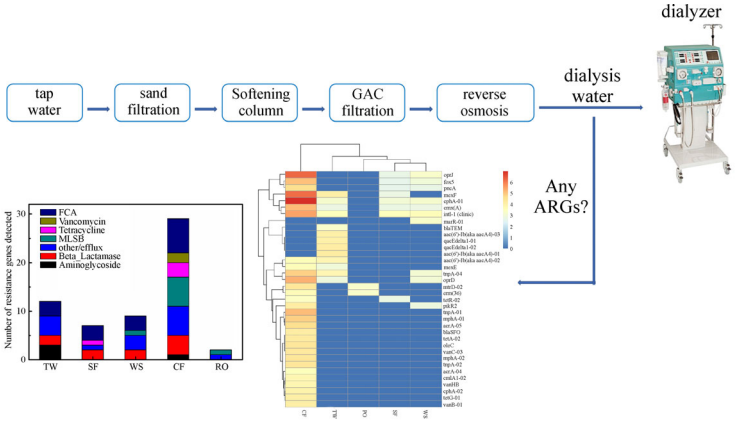
HIGHLIGHTS

- Quantitative global ARGs profile in dialysis water was investigated.
- Totally 35 ARGs were found in the dialysis treatment train.
- 29 ARGs (highest) were found in carbon filtration effluent.
- *erm* and *mtr*D-02 occurred in the final effluent.
- The effluent was associated with health risks even after RO treatment.

GRAPHIC ABSTRACT



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ABSTRACT

Dialysis water is directly related to the safety of hemodialysis patients, thus its quality is generally ensured by a stepwise water purification cascade. To study the effect of water treatment on the presence of antibiotic resistance genes (ARGs) in dialysis water, this study used propidium monoazide (PMA) in conjunction with high throughput quantitative PCR to analyze the diversity and abundance of ARGs found in viable bacteria from water having undergone various water treatment processes. The results indicated the presence of 35 ARGs in the effluents from the different water treatment steps. Twenty-nine ARGs were found in viable bacteria from the effluent following carbon filtration, the highest among all of the treatment processes, and at 6.96 Log (copies/L) the absolute abundance of the *cphA* gene was the highest. Two resistance genes, *erm* (36) and *mtr*D-02, which belong to the resistance categories macrolides-lincosamides-streptogramin B (MLSB) and other/efflux pump, respectively, were detected in the effluent following reverse osmosis treatment. Both of these genes have demonstrated the potential for horizontal gene transfer. These results indicated that the treated effluent from reverse osmosis, the final treatment step in dialysis-water production, was associated with potential health risks.

1 Introduction

Antibiotic resistance is an emerging public health problem because of the clinical abuse of antibiotics in recent years. Antibiotic resistance genes (ARGs), as the physical carrier, are thus regarded as a group of emerging contaminants (Rysz and Alvarez, 2004; Pruden et al., 2006). ARGs could be detected not only in specimen from patients treated with

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antibiotics but also in related environmental samples, such as tap water, in hospitals. In fact, tap water (drinking water) was found to be a rather stable reservoir for bacteria carrying ARGs in many cases though it present extremely oligotrophic conditions (Bouki et al., 2013; Xiong et al, 2015; Xu et al., 2016). The emergence and proliferation of ARGs have greatly influenced the therapeutic efficacy of antibiotics, and intraspecific and interspecific horizontal gene transfer among bacterial colonies can further promote the inheritance and proliferation of these genes (Allen et al., 2010). Furthermore, the emergence of drug-resistant "super bacteria" has exacerbated the biosafety risks present in tap water under the combined actions of antibiotics, heavy metals, and other environmental stresses. For example, in the drinking water system of New Delhi, India, a super bacterium carrying a multi-resistant NDM-1 plasmid was detected (Walsh et al., 2011). Once antibiotic resistant bacteria enter the human body, the horizontal gene transfer between endogenous and exogenous bacteria inside the body may occur, raising the prospect that endogenous bacteria become antibiotic resistant (van den Braak et al., 1998) and potentially lead to the failure of resistance management therapies.

Dialysis water is in direct contact with the human body and its quality is closely related to the safety of patients receiving dialysis. At present, the inlet water for dialysis is generally obtained directly from municipal tap water. Subsequently, an in-depth tap water treatment, such as sand filtration, water softening, carbon filtration, and reverse osmosis, is applied to meet the water standards for dialysis (Damasiewicz et al., 2012; Diao and Sang, 2015). The sand filtration is mainly used to remove residual particles from tap water, whereas the water-softening device is mainly used to reduce the hardness. The carbon filtration device is installed after the water-softening device to adsorb residual chlorine. This step is important because residual chlorine in the water may corrode the reverse osmosis membrane and reduce its service life; also, an excess amount of chlorine entering the body of patients receiving dialysis may lead to hemolytic anemia (Junglee et al., 2010). The high retention membrane used in reverse osmosis can retain 95%–98% of the salt and almost all of the microorganisms present in water as well as materials with a molecular weight of more than 200 Da (Pontoriero et al., 2004). However, after treatment by reverse osmosis, the treated water may still pose a health risk in regard to antibiotic resistance bacteria.

Prior studies have assessed the contamination of drinking water based on the presence of ARGs. For example, Xi et al. (2009) surveyed resistance genes in water and found that the abundance of most resistance genes in tap water was higher than at the original sources. When tap water is used as the inlet water for dialysis, its safety should be further verified in this regard. At present, the primary concerns associated with the effluent of dialysis water are related to conventional water quality parameters (Damasiewicz et al., 2012; Shahryari et al., 2016), and few reports have addressed the impact on ARGs introduced by the treatment processes. Therefore, it is necessary to gain an in-depth understanding regarding the presence of antibiotic resistance bacteria following the treatment processes in order to reduce the health risks faced by patients receiving dialysis.

In this paper, the treatment processes of dialysis water at a hospital (No. 2 Hospital, Xiamen, China) were assessed. Using propidium monoazide (PMA) in combination with high throughput quantitative PCR, the absolute abundance and diversity of ARGs in the effluent from the various processing units used for dialysis water treatment were analyzed. It is expected that this investigation will provide a more comprehensive understanding about how these individual processes impact antibiotic resistance bacteria and ARGs. These observations will also provide the theoretical support for preventing the contamination by antibiotic resistance bacteria during water treatment and to further reduce the health risks brought by this contamination in these processes.

2 Materials and methods

2.1 Sample collection and treatment

The water samples were collected from five effluent outlets located along the dialysis water treatment processes at the hospital and were numbered 1–5 (Fig. 1). Aseptic

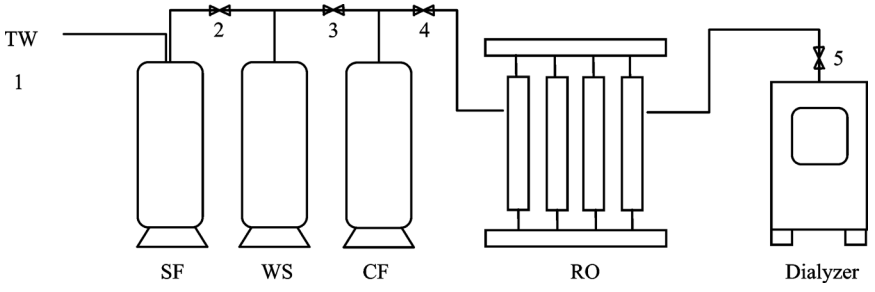

Fig. 1 Process flow diagram. (TW-tap water, SF-sand filtration, WS-water softening, CF-carbon filtration, RO-reverse osmosis; 1-5 means the sampling port).

techniques used during the sampling process were ensured. The values of Dissolved Oxygen (DO), pH, and temperature were measured on-site using a multi-parameter water quality analyzer (Multi 3420, WTW, Germany). After collection, the water samples were immediately sent to the laboratory for analysis. The volume of each water sample was about 20 L, except for the reverse osmosis sample (50 L). The physical and chemical parameters of about 100 mL from each sample were measured in accordance with the methods listed in China's national standards. In addition, another 100 mL of each sample was used for the determination of microbiological parameters. The remaining water samples were filtered through 0.22 μm mixed cellulose ester membranes (GPWP04700, Millipore, Ireland) and used for subsequent experiments.

2.2 PMA treatment

PMA is a nucleic-acid dye that is able to penetrate through the damaged cell membrane and, after photo-crosslinking with the intracellular DNA, inhibit DNA amplification during PCR (Zhang et al., 2015). This approach prevents amplification of DNA from dead cells and makes it possible to quantify the total number of viable bacteria in a sample. A total of 25 μL of PMA (40013, Biotium, USA) stock solution at a concentration of 1 mg/mL was added into 475 μL of sterile water to prepare a PMA working solution with a final concentration of 100 μmol/L (Quan, 2010). Subsequently, the filters corresponding to each water treatment process were placed in six-well plates and covered with the PMA working solution. After incubating the plates in the dark for 5 min, the plates were placed on ice at 20 cm away from a 650-W halogen lamp (220 V, 3400K, OSRAM, Munich, Germany). After irradiating the plates for 4 min to induce the photo-crosslinking reaction, the filters were cut into pieces and the bacterial DNA was extracted using a FastDNA SPIN Kit (MP Biomedicals, USA).

2.3 High-throughput fluorescence quantitative PCR

DNA amplification was performed using a SmartChip Real-Time PCR System (WaferGen Inc., USA). A total of 296 pairs of primers were selected, which included 285 pairs of primers for genes with resistance against conventional antibiotics, 8 pairs of primers for transposon genes, 2 pairs of primers for integron genes (*intI-1(clinic)* and *CintI-1(class1)*), and primers for the 16S rRNA gene. The volume of the PCR reaction system was 100 nL. The final concentration of each reagent in the system was: 1 × LightCycler 480 SYBR® Green I Master Mix (Roche, USA), 1 ng/μL BSA, 2 ng/μL DNA templates, and 1 μmol/L forward and reverse primers. The reaction conditions were: 95°C for 10 min; 40 cycles of 95°C for 30 s and 60°C for 30 s. The melting curve was automatically set. According to its sensitivity, a CT value of 30 was used as the detection limit of the instrument.

2.4 Absolute fluorescence quantitative PCR

A QuantStudio® 6 Flex Real-Time PCR System (Applied Biosystems, USA) was used to perform SYBR Green absolute fluorescence quantification of the 16S rRNA gene in all samples (Barbau-Piednoir et al., 2013). The qPCR reaction volume was 20 μL: 10 μL of 2 × LightCycler 480 SYBR® Green I Master Mix, 0.25 μL each of forward and reverse primers (at a concentration of 10 μmol/L), 7.5 μL of ddH_2O, and 2 μL of templates. The PCR reaction conditions were: 95°C for 5 min; 40 cycles of 95°C for 15 s, 60°C for 60 s, and 75°C for 15 s. The melting curve was plotted and a standard curve was drawn based on 10-fold serial dilutions of plasmids with known concentrations. The results were analyzed according to the standard curve.

2.5 Data analysis

The relative copy number in the high-throughput quantitative PCR experiments was calculated using formula (1), which was based on the method developed by Looft et al. (2012). The relative copy number of the 16S rRNA gene obtained from the high-throughput quantitative PCR and the absolute copy number of the 16S rRNA gene obtained from the absolute fluorescence quantitative PCR were fitted via Pearson correlation, and it was found the two values had a significant correlation. Therefore, the absolute abundance of ARGs could be calculated using formula (2).

$$\text{The relative number of a gene} = 10^{[(30-\mathrm{CT})-(10/3)]}. \quad (1)$$

The absolute abundance of an antibiotic resistance gene = the relative copy number of the antibiotic resistance gene/the relative copy number of the 16S rRNA gene × the absolute copy number of the 16S rRNA gene. (2)

3 Results and discussion

3.1 Operational efficacies of the dialysis water treatment processes

The results from measuring the physical, chemical, and microbiological parameters of the effluents from the various units of the dialysis water treatment processes are shown in Table 1. It can be seen that the water quality parameters of the effluent from the reverse osmosis process met the YY 0527-2005 standard for dialysis water (State Food & Drug Administration of China, 2005). Because this water treatment process is conducted in an enclosed environment, the temperature, DO, and pH values were minimally impacted. With the progression of the process

Table 1 The physical and chemical parameters of different processes

AVE	TW	SF	WS	CF	RO
Temperature (°C)	21.24	20.28	20.42	20.78	25.60
DO (mg/L)	8.66	8.64	8.52	8.65	8.06
Residual chlorine (mg/L)	0.73	0.63	0.40	0.02	0.01
Conductivity (μs/cm)	91	106	112	92	–
pH	6.46	6.56	6.67	6.73	6.86
Turbidity (NTU)	0.17	0.20	0.18	0.23	0.08
NO_3^--N (mg/L)	1.73	1.95	2.13	1.73	–
NO_2^--N (mg/L)	–	–	–	–	–
NH_4^+-N (mg/L)	0.04	0.03	0.03	0.04	0.04
PO_4^{3-}-P (mg/L)	0.01	0.01	–	0.01	–
TOC (mg/L)	1.12	1.08	1.19	1.05	0.11
Ca^{2+} (mg/L)	8.62	8.00	–	–	–
Mg^{2+} (mg/L)	1.52	1.40	–	–	0.01
Total bacteria /100mL	3	60	130	1.15E + 04	45

Notes: 1) "–" indicates not detected; 2) TW-tap water, SF-sand filtration, WS-water softening, CF-carbon filtration, RO-reverse osmosis.

operation, the physical and chemical parameters of the effluent from the reverse osmosis process, including residual chlorine, Ca^{2+}/Mg^{2+}, Total Organic Carbon (TOC), ammonia nitrogen, nitrite nitrogen, nitrate nitrogen, and PO_4^{3-}-P, had reached their lowest levels, with some of these substances registering below detection limits (Table 1). The total number of bacteria in the effluent from the reverse osmosis process was 45 CFU/100 mL, i.e. 0.45 CFU/mL, which complied with the YY 0527-2005 standard (< 100 CFU/mL). In summary, this process worked well and was up to standards.

3.2 Absolute abundance of ARGs in the treatment processes of dialysis water

As described in the method section, PMA staining was applied in this study. With this method, only the nucleic acid fragments from the viable cells could be amplified and quantified. As shown in Fig. 2, the absolute abundance of ARGs in the effluent at each step of the water treatment process ranged from 10^2–10^7 copies/L. In the tap water, the absolute abundance of ARGs was 10^3–10^5 copies/L, with a maximum abundance of 3.2×10^4 copies/L. Except for that of tetracycline, the absolute concentrations of ARGs for all other antibiotics decreased by 0.03–1.90 orders of magnitude after the sand filtration treatment, indicating that the sand filtration device played a clear role in the reduction of antibiotic resistance bacteria. After the water softening treatment, the absolute abundance of ARGs was 10^1–10^4 copies/L, which was higher than the absolute abundance of ARGs in the effluent of the sand filtration device. After the treatment with the carbon filtration device, the absolute abundance of ARGs reached its highest level of 10^4–10^7 copies/L. In particular, the absolute abundance of genes with resistance against β-lactam antibiotics was highest to about 1.0×10^7 copies/L. Although the genes with resistance to only two kinds of antibiotics were detected in the effluent of the reverse osmosis treatment, their absolute concentration was 1.76×10^3 copies/L, which was the same as the number of live bacteria in this water, indicating that the live bacteria detected in the effluent of the reverse osmosis treatment may have been multi-resistant. In addition, one of the resistance genes belonged to the category of efflux pump resistance genes, which has often been reported to be

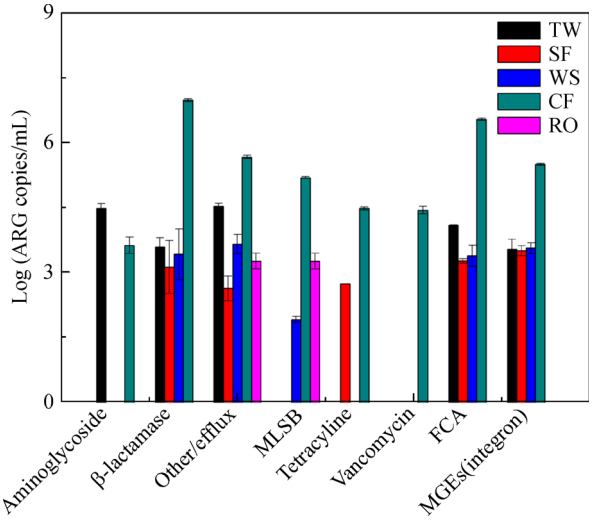

Fig. 2 Absolute abundance of ARGs. (TW-tap water, SF-sand filtration, WS-water softening, CF-carbon filtration, RO-reverse osmosis).

carried as a plasmid and is associated with horizontal gene transfer (Friehs, 2004); therefore, this gene may demonstrate an increased risk of transmission.

In general, mobile genetic elements (MGEs) were detected in the effluent from all of the processes, except that of the reverse osmosis, and their absolute abundance reached up to 10^3–10^6 copies/L. This indicated that these steps in the water treatment process were also associated with a certain risk of horizontal gene transfer prior to beginning the reverse osmosis treatment. Through these processes, the resistance genes against various antibiotics and the integron-associated MGEs demonstrate a similar trend, as their absolute abundance in the carbon-filtered water was the highest, indicating that the carbon filtration device had an enrichment effect on ARGs and/or was a repository of such genes. This result may be due to the fact that inside the carbon filter chamber the residual chlorine adsorbed by the activated carbon and the natural porous structure of the activated carbon can provide a good environment for microorganisms to grow, leading to the active proliferation of microorganisms inside the filter and formation of a biofilm. It has been shown that the generation of resistance genes and horizontal gene transfer tend to occur in biofilm (Schwartz et al., 2003), thus leading to an increase in the types of ARGs and their intraspecific and interspecific inheritance. Although a large number of ARGs and antibiotic resistant bacteria were effectively removed during the reverse osmosis, the applied pressure during reverse osmosis is relatively high and may easily cause contamination of the osmosis membrane and leakage of microorganisms. The leakage of viable antibiotic resistant bacteria may further increase the risk to patients, and if the resistance genes carried by these bacteria enter into a pathogen, it could increase the risk of resistant infections.

3.3 The diversity of resistance genes in the treatment processes of dialysis water

According to the type of antibiotics, the corresponding resistance genes can be divided into eight main categories: aminoglycosides, β-lactams, tetracyclines, sulfonamides, fluoroquinolones/chloramphenicol/amide alcohols (FCA), macrolides-lincosamides-streptogramin B (MLSB), other/efflux pump, and vancomycin. As shown in Fig. 3, a total of 35 ARGs and one MGE were detected in the various effluents. Among them, 12, 7, and 9 ARGs were detected in the tap water, sand filtration effluent, and water softening effluent, respectively. This indicated an overall reduction of certain ARGs throughout these steps in the treatment process. However, the number of ARGs in the effluent of the carbon filtration treatment was the largest at 29, which was nearly 3 times that detected in the other processes. ARGs from all eight categories were detected in the effluent from the carbon filtration process, indicating that the carbon filtration device had a certain enrichment effect on antibiotic resistance bacteria and denoting a potential risk of health problems present in the water before it reaches the reverse osmosis membrane. However, after treatment by reverse osmosis, only two ARGs were detected.

Notably, ARGs in the other/efflux pump category were detected in the effluent samples from all five processing units. As mentioned earlier, the resistance genes acting through an efflux pump mechanism are often carried by plasmids (Schwartz et al., 2003), which can have an increased risk of horizontal gene transfer and promote the proliferation of resistance genes.

ARGs can be divided into four main categories according to the mechanisms of resistance: antibiotic inactivation, ribosome protection, efflux pump mechanism, and unknown mechanisms of resistance. As shown in Fig. 4, the proportion of ARGs acting via the efflux pump mechanism was the highest (50%) in the effluents from all five processing units, further indicating that such water treatment processes are associated with a risk of horizontal

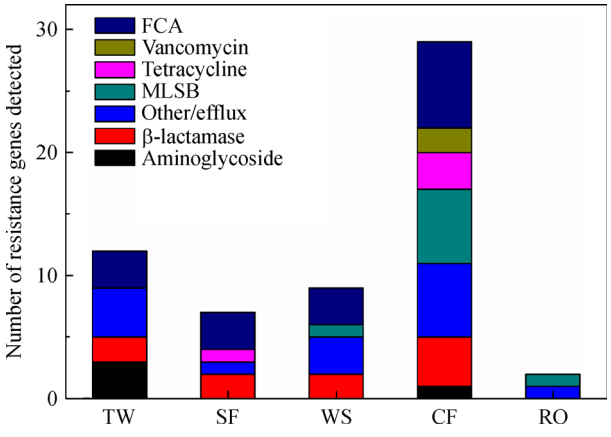

Fig. 3 Distribution of ARGs from the different processes. (TW-tap water, SF-sand filtration, WS-water softening, CF-carbon filtration, RO-reverse osmosis).

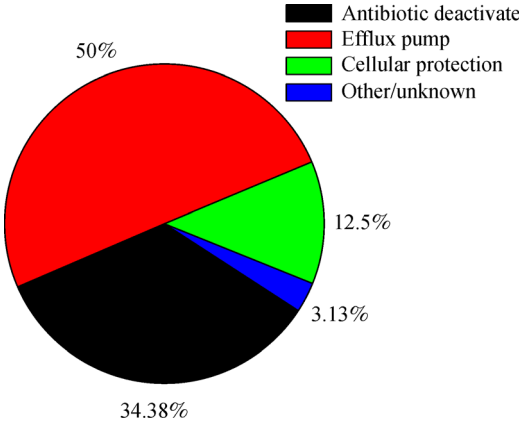

Fig. 4 Classification of antibiotic resistance gene based on the mechanism of resistance.

gene transfer, and this may accelerate the proliferation of resistance genes.

3.4 Profile of ARGs

The thermal image shown in Fig. 5 indicates that the abundance and diversity of ARGs in the effluent from carbon filtration were significantly higher than those in the effluents from the other processing units. The genes *cmx (A), cphA-01*,and *intI-1(clinic)* were all detected in tap water as well as the effluents of the sand filtration, water softening, and carbon filtration processes. Among these genes, *intI-1(clinic)* is an MGE and *cmx(A)* acts via the efflux pump mechanism of resistance. Both of these genes are associated with the possibility of horizontal gene transfer. In comparison, an increased number of ARGs (a total of 14) were detected from the effluent from carbon filtration, and most of these resistance genes, such as *cmlA-02*, act via an efflux pump mechanism or as an MGE, indicating that resistance behaviors associated with horizontal gene transfer are accommodated inside the carbon filter. This suggested that the carbon filtration device had a certain enrichment effect on ARGs, and likely be the place for the occurrence of horizontal gene transfer and inducing the bacterial ARGs. Among the genes with MLSB antibiotic resistance, genes such as *erm* that are resistant to macrolide antibiotics were detected in both the effluents from the carbon filtration and the reverse osmosis processes. Roberts (2003) have shown that the *erm* gene can be easily captured by MGEs, such as plasmids or transposons, and can be transferred between different host bacteria. Therefore, although only two ARGs, *erm* and *mtr*D-02 (a resistance gene that belongs to the other/efflux pump category), were detected in the effluent of the reverse osmosis treatment, both genes possess the possibility of horizontal gene transfer, which may result in the proliferation of these resistance genes. Thus, the effluent of the reverse osmosis process is associated with a relatively substantial health risk.

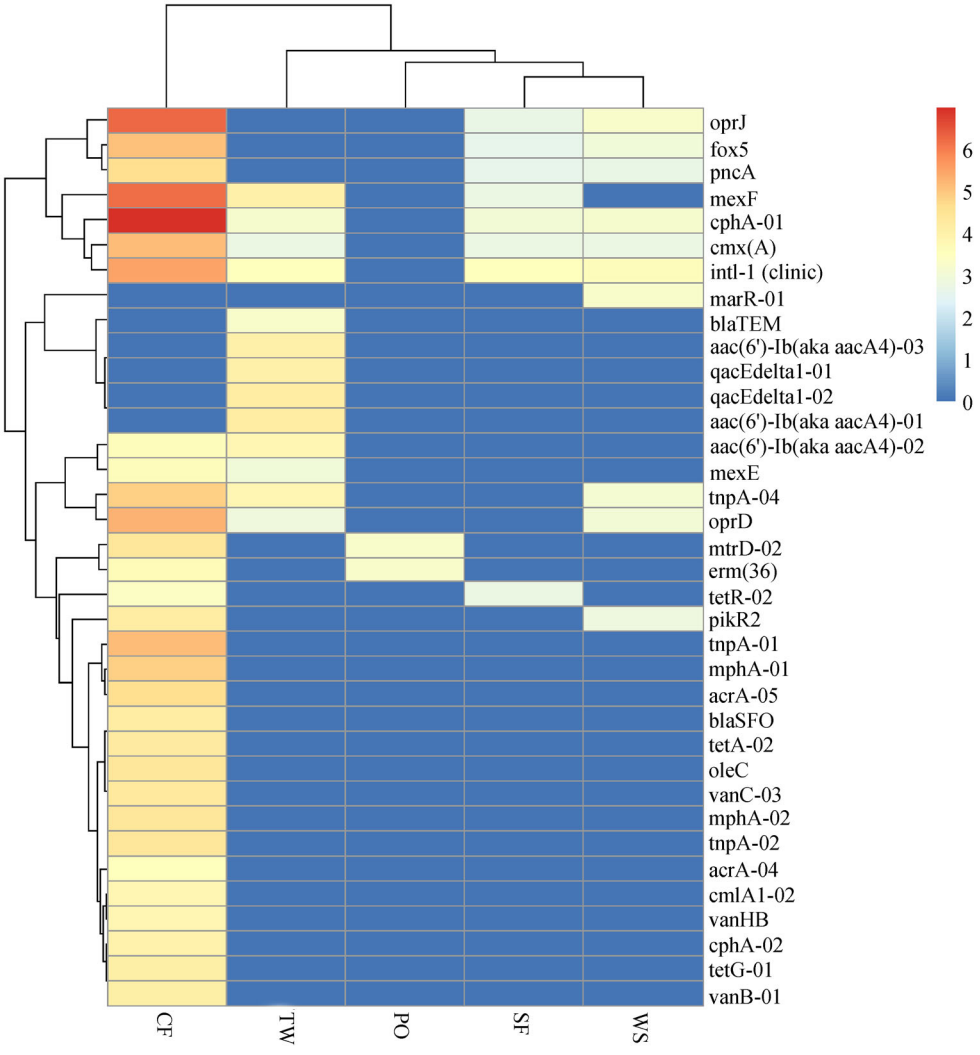

Fig. 5 Profile of resistance genes. (TW-tap water, SF-sand filtration, WS-water softening, CF-carbon filtration, RO-reverse osmosis).

4 Conclusions

1) For physical and chemical parameters, the dialysis water treatment processes achieved relatively high removal efficiency. In particular, the efficacy of the reverse osmosis device was significantly high.

2) The distribution of ARGs gradually changed throughout the water treatment processes, and the number and abundance of ARGs in the effluent of the carbon filtration process were the highest. After treatment with the reverse osmosis device, the number and abundance of ARGs were significantly reduced and only two resistance genes were detected, indicating that the reverse osmosis device demonstrated a good efficacy in reducing the presence of ARGs. However, the two ARGs present in this effluent had the potential of horizontal gene transfer.

3) The identification of many resistance genes before entering the reverse osmosis device supported the possibility of horizontal gene transfer during the treatment processes, and this may in turn promote the proliferation of resistance genes and pose potential health risks to hemodialysis patients.

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References

Allen H K, Donato J, Wang H H, Cloud-Hansen K A, Davies J, Handelsman J (2010). Call of the wild: Antibiotic resistance genes in natural environments. Nature Reviews. Microbiology, 8(4): 251–259

Barbau-Piednoir E, Bertrand S, Mahillon J, Roosens N H, Botteldoorn N (2013). SYBR®Green qPCR *Salmonella* detection system allowing discrimination at the genus, species and subspecies levels. Applied Microbiology and Biotechnology, 97(22): 9811–9824

Bouki C, Venieri D, Diamadopoulos E (2013). Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: a review. Ecotoxicology and Environmental Safety, 91(4): 1–9

Damasiewicz M J, Polkinghorne K R, Kerr P G (2012). Water quality in conventional and home haemodialysis. Nature Reviews. Nephrology, 8(12): 725–734

Diao Z, Sang H (2015). Hemodialysis water treatment system and water quality monitoring. Medical Equipment, 28(1): 31–34

Friehs K (2004). Plasmid copy number and plasmid stability. Advances in Biochemical Engineering/Biotechnology, 86(1): 47–82

Junglee N A, Rahman S U, Wild M, Wilms A, Hirst S, Jibani M, Seale J R (2010). When pure is not so pure: Chloramine-related hemolytic anemia in home hemodialysis patients. Hemodialysis International. International Symposium on Home Hemodialysis, 14(3): 327–332

Looft T, Johnson T A, Allen H K, Bayles D O, Alt D P, Stedtfeld R D, Sul W J, Stedtfeld T M, Chai B, Cole J R, Hashsham S A, Tiedje J M, Stanton T B (2012). In-feed antibiotic effects on the swine intestinal microbiome. Proceedings of the National Academy of Sciences of the United States of America, 109(5): 1691–1696

Pontoriero G, Pozzoni P, Andrulli S, Locatelli F (2004). The quality of dialysis water. Journal of Italian Nephrology, 21(Supplementary 30): S42–S45

Pruden A, Pei R, Storteboom H, Carlson K H (2006). Antibiotic resistance genes as emerging contaminants: studies in northern Colorado. Environmental Science & Technology, 40(23): 7445–7450

Quan T Z (2010). Selective detection of viable pathogens in water using propidium monoazide combined with qPCR and its application. Dissertation for the Doctoral Degree. Beijing: Tsinghua University (in Chinese)

Roberts M C (2003). Acquired tetracycline and/or macrolide-lincosamides-streptogramin resistance in anaerobes. Anaerobe, 9(2): 63–69

Rysz M, Alvarez P J J (2004). Amplification and attenuation of tetracycline resistance in soil bacteria: Aquifer column experiments. Water Research, 38(17): 3705–3712

Schwartz T, Kohnen W, Jansen B, Obst U (2003). Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. FEMS Microbiology Ecology, 43(3): 325–335

Shahryari A, Nikaeen M, Hatamzadeh M, Vahid Dastjerdi M, Hassanzadeh A (2016). Evaluation of bacteriological and chemical quality of dialysis water and fluid in Isfahan, central Iran. Iranian Journal of Public Health, 45(5): 650–656

State Food & Drug Administration of China (2005). Standard for Water for Haemodialysis and Related Therapies. Beijing: State Food & Drug Administration of China

van den Braak N, van Belkum A, van Keulen M, Vliegenthart J, Verbrugh H A, Endtz H P (1998). Molecular characterization of vancomycin-resistant *Enterococci* from hospitalized patients and poultry products in The Netherlands. Journal of Clinical Microbiology, 36(7): 1927–1932

Walsh T R, Weeks J, Livermore D M, Toleman M A (2011). Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: An environmental point prevalence study. Lancet. Infectious Diseases, 11(5): 355–362

Xi C, Zhang Y, Marrs C F, Ye W, Simon C, Foxman B, Nriagu J (2009). Prevalence of antibiotic resistance in drinking water treatment and distribution systems. Applied and Environmental Microbiology, 75 (17): 5714–5718

Xiong W, Sun Y, Zhang T, Ding X, Li Y, Wang M, Zeng Z (2015). Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. Microbial Ecology, 70(2): 425–432

Xu L, Qian Y, Su C, Cheng W, Li J, Wahlqvist M L, Chen H (2016). Prevalence of bacterial resistance within an eco-agricultural system in Hangzhou, China. Environmental Science and Pollution Research International, 23(21): 21369–21376

Zhang S, Ye C, Lin H, Lv L, Yu X (2015). UV disinfection induces a VBNC state in *Escherichia coli* and *Pseudomonas aeruginosa.* Environmental Science & Technology, 49(3): 1721–1728