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Identification and assessment of antimicrobial resistance bacteria in a hemodialysis water treatment system

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ABSTRACT

The water treatment process is a vital factor for hemodialysis (HD) patients. This study aimed to assess the degree of contamination of HD water by bacteria at the HD center of Mohammedia, Morocco, in addition to evaluating the antimicrobial resistance of isolated bacteria. Fifty-four water samples were taken, the appropriate cultures were used to isolate the pathogenic bacteria, which were identified biochemically and molecularly by 16S RNA sequencing. Their susceptibility to antimicrobial drugs was determined by the disk diffusion method. Approximately 5.5% of water samples were above the norm. The isolated bacteria that colonized the HD systems were mostly Gram-negative bacilli, such as *Stenotrophomonas maltophilia*, *Pseudomonas* spp., and *Burkholderia cepacian*. Results of the antibiotics test showed remarkable resistance levels. Among *Pseudomonas* spp. and *S. maltophilia*, 10 strains were classified as multidrug-resistant (MDR), and 4 as extensively drug-resistant (XDR). The diversity of bacterial strains isolated in the water used for HD treatments, and their worrying resistance levels pose a significant risk to patients. For these reasons, an urgent need for periodic microbiological monitoring of water after each treatment step must be applied, and the treatment process should also be optimized.

Key words: bacterial contamination, dialysis water, hemodialysis, MDR bacteria, Morocco

HIGHLIGHTS

- Overall, the water quality in the Mohammedia HD center does comply with the minimum requirement of hemodialysis water.
- Bacteria with pathogenic potential in hemodialysis water.
- Bacteria with high antimicrobial resistance.

BACKGROUND

Recently, the number of people with chronic kidney disease who undergo hemodialysis (HD) has increased significantly. The quality of water used for dialysis is an essential factor to consider since the blood of dialyzed patients encounters 300–600 liters of water per week through a non-selective membrane at each stage of the purification process (e.g., dialysis water, generator, and vascular access) (Ward 2011), which causes the risk of microorganism transmission to be high. Bacterial infections are a major cause of morbidity and mortality in patients with chronic HD (i.e., the 2nd leading cause after cardiovascular disease). On the one hand, kidney failure directly or indirectly alters neutrophil and lymphocyte functions, and, on the other hand, extrarenal purification induces cytokine release. In addition, dialysis patients often have comorbidities that make them more vulnerable to side effects (e.g., hypertension, diabetes, and cardiovascular disease) (Coresh *et al.* 2007).

Generally, the input water used for dialysis is usually obtained from municipal tap water. Subsequently, tap water is treated extensively by sand filtration and softening methods. Carbon filtration and reverse osmosis (RO) are applied to meet the water standards for dialysis (Tong *et al.* 2001; Damasiewicz *et al.* 2012). Sand filtration is mainly used to remove residual particles, while water softening is primarily used to reduce hardness. A carbon filtration device is installed to adsorb residual chlorine. This step is essential because residual chlorine can corrode the RO membrane and reduce its service life. In addition,

excessive transfer of chlorine into the bodies of dialysis patients can lead to hemolytic anemia (Junglee *et al.* 2010). The membrane used in RO can retain 95–98% of the salt and nearly all microorganisms present in the water (Pontoriero *et al.* 2004). However, after RO, treated water may still present a health risk concerning antibiotic resistance.

Prior studies, in different countries, have shown the presence of several microorganisms in water and have found that dialysis water is an important means of spreading antibiotic-resistant bacteria (Montanari *et al.* 2009; Damasiewicz *et al.* 2012; Heidarieh *et al.* 2016; Shahryari *et al.* 2016). Hence, in order to reduce the health risks incurred by patients in HD centers, management and microbiological monitoring of the quality of the dialysis water becomes imperative. This study has been conducted to determine, for the first time, the bacteriological quality of the water used in a public HD center at the Provincial Hospital Center (PHC) of Mohammedia, Morocco, based on phenotypic and molecular identification of the predominant bacteria, as well as the study of their profile and their resistance to antibiotics.

MATERIALS AND METHODS

This study was conducted in a public HD center with 15 dialysis machines, which included a device explicitly used for the HD treatment of patients infected with the hepatitis B virus. Each year, the center conducts more than 5,000. A water treatment system is an integrated unit that includes a water tap that is pretreated with a filtration system, water softener, and activated carbon filter, followed by the final RO purification step. The treated water is stored in a tank, distributed to the dialysis machines. After each session, the devices are rinsed and disinfected according to the protocols in force.

Sample collection

This study was conducted over 5 months, from February 2019 to June 2019. A total of 54 water samples were collected, and the water samples (250 mL) were taken under aseptic conditions according to the established water sampling guidelines (DES 2018). The sampling ports are shown in Figure 1. After sampling, the bottles were clearly labeled and transported immediately to the epidemiology and hygiene laboratory in the facility to be analyzed within a maximum period of 1–2 h after collection.

Bacterial analysis

A filtration technique was used to count the suspended bacteria in the water samples. A 100 mL fraction of each diluted sample was filtered through a membrane with 0.45 μm diameter pores, and the membrane was then placed on plate count agar (PCA, Oxoid®) and incubated at 21 ± 2 °C for 7 days. The number of colonies found was expressed as the average number of colony-forming units (CFU/mL), according to international standards for microbiological cultures of dialysis water and dialysis liquids (ANSI/AAMI/ISO13959 2014). Sterile water for injection was used as a negative control.

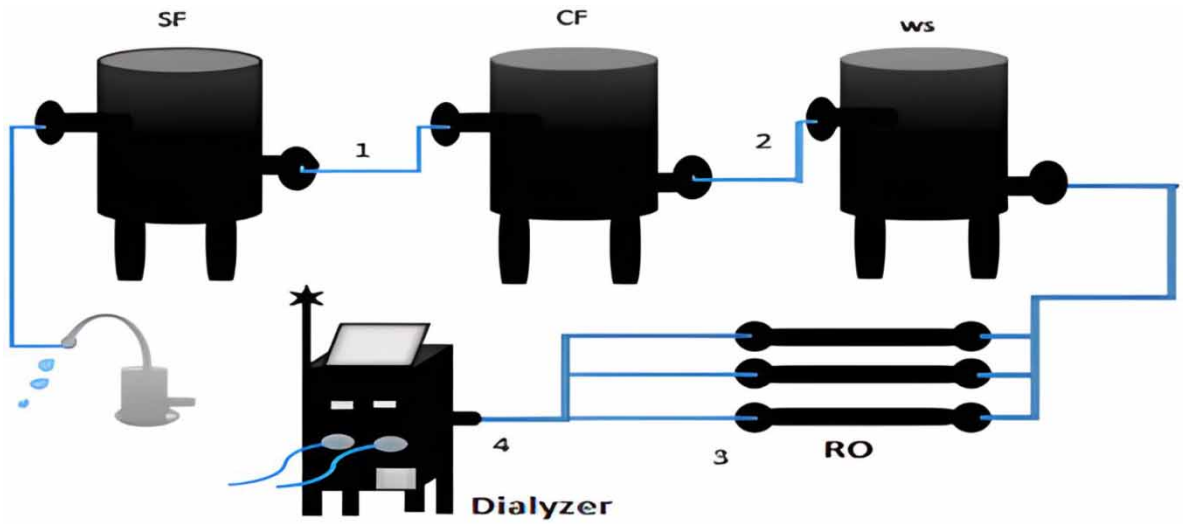

Figure 1 | Diagram of the water treatment process: 1. after sand filter (SF, *n*=14); 2. after carbon filter (CF, *n*=13); 3. after reverse osmosis (RO, *n*=14); and 4. dialysis generator input (DG, *n*=13). WS, water softener.

Distinct colonies were isolated from each positive sample and replicated on mannitol salt agar for the genus *Staphylococcus*, cetrimide agar for *Pseudomonas*, and Mac Conkey for other non-fermenting Gram-negative bacilli (NFGNB) and were incubated at 37 °C for 24 h. In total, 47 colonies were selected and checked for purity by streaking on agar plates and were identified using commercially available biochemical galleries: an API 20NE gallery (Bio Mérieux) for identifying bacteria classified as no fermentative and coagulase for staphylococcal species were used.

Molecular characterization

A molecular approach from BHI cultures performed isolate identifications. Genomic DNA was extracted using a MAGPurix extractor by following a modified protocol. The quality and quantity of the extracted materials were assessed by a Nanodrop 8000 system (Thermo Fisher Scientific, Waltham, MA, USA). The 16S rRNA gene was amplified using a Bioline HS PCR kit and universal primers, which resulted in ~1,420 bp fragments. Finally, sequencing was performed using an ABI-3130 XL Analyzer (Thermo Fisher Scientific). DNA extraction, 16S rRNA gene amplification, and sequencing were subcontracted to the Platform of Functional Genomics (Center National pour la Recherche Scientifique et Technique (CNRST) of Rabat city, Morocco). The raw data were processed, and the closest strains were identified using the BLASTN program (Altschul *et al.* 1990).

Antimicrobial susceptibility test

The antibacterial susceptibility testing was performed using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar. Thirteen antibiotic discs (Oxoid Ltd, Basingstoke, England) were selected, which represented six classes: the penicillin consisted of ticarcillin (T/75 μg), ticarcillin–clavulanic acid (TCA/75/10 μg), piperacillin (P/100 μg), piperacillin + tazobactam (TZP, 100/10 μg), cephalosporins like ceftazidime (CAZ/30 μg), and cefepime (FEP/30 μg). The carbapenems included imipenem (IMI/10 μg), the antibiotics amikacin (AKN/30 μg), gentamycin (GEN/10 μg) among the aminoglycosides, ciprofloxacin (CIP/5 μg), and belong to the fluoroquinolone class and chloramphenicol (C/30 μg) to phenols. Trimethoprim–sulphamethoxazole (TSU, 75/25 μg) is an antagonist to folic acid biosynthesis. The results were interpreted according to the Clinical and Laboratory Standards Institute guidelines (CLSI 2014). *Pseudomonas aeruginosa* ATCC 27853 was employed as a control strain. The isolates were classified as extensively drug-resistant (XDR), multidrug-resistant (MDR), and non-MDR according to the method of Magiorakos *et al.* (2012). According to the definitions, XDR is defined as non-sensitive to at least one agent for all but two or fewer antibiotics; MDR is defined as non-sensitive to at least one of three or more resistant agents; and non-MDR isolates show no sensitivity to one or more agents in one or two antibacterial classes.

RESULTS

Counts and bacterial isolates

None of the HD patients experienced febrile/pyrogenic reactions during sample collection. According to the European standards for HD fluids and those of the Moroccan Health Ministry (ANSI/AAMI/ISO13959 2014; Ministère de santé 2018), the treated final water and the final dialysate were unacceptable if one or more of the following criteria were met: HPC of >100 CFU/ml, presence of coliforms in 100 mL, endotoxin level of >0.25 EU/mL.

Among the 54 samples, 27 (50%) presented positive cultures, and 5.5% (3/54) of the water samples were above standard, with 122, 165, and 178 CFU/mL; these three samples are from point 2 after CF. The culturable bacteria levels averaged only 10 CFU/mL in the sand filter effluents. After carbon filtration, this number increased considerably, with an average of 87 CFU/mL and a peak of 178 CFU/mL. The cultured bacteria decreased after RO treatment but were still close to 60 CFU/mL. After the entire treatment process, the treated water arrived at the generator with approximately 43 CFU/mL on average. Gram staining results showed that 79% of the isolated strains were Gram-negative bacteria, while 21% were Gram-positive bacteria isolated from mixed cultures. During the study period, monitoring the contamination level showed about the same bacterial count level before and after disinfection of the dialysate system.

Out of 54 samples from all locations at the HD center, 47 isolates were studied. According to the phenotypic characteristics and by sequencing the 16S rRNA gene, eight species of NFGNB were identified, and the frequencies of detection are listed in Table 1 according to the type of sample analyzed. The most frequently isolated species was *Stenotrophomonas maltophilia* (34%), followed by *Pseudomonas* spp. (25.5%) and 8.5% of *Burkholderia cepacia*. The identified bacterial species and their accession numbers for some sequenced 16S rRNA genes are shown in Table 2.

Table 1 | Distributions of strains according to the sampling sites

Microorganisms	No. Isolates				
	SF	CF	RO	DG	Total
Stenotrophomonas maltophilia	0	4	8	4	16
Pseudomonas aeruginosa	0	1	3	3	7
Pseudomonas sp.	0	1	1	0	2
Pseudomonas chengduensis	0	1	0	0	1
Pseudomonas fluorescens	0	1	0	1	2
Burkholderia cepacia	0	2	1	1	4
Bacillus sp.	1	0	0	1	2
Chryseobacterium meningosepticum	0	1	0	0	1
Carnobacterium inhibens	0	0	1	0	1
Other NFGNB	0	3	0	0	3
Staphylococcus warneri	1	1	2	0	4
Staphylococcus pasteuri	0	3	0	0	3
Staphylococcus capitis	0	1	0	0	1
Total	02	19	16	10	47

SD, sand filter; CF, carbon filter; RO, reverse osmosis; DG, dialysis generator input.

Table 2 | Predominant bacteria that were identified by 16S rDNA sequence analysis

Identified species	Sequence similarity (%)	Accession no (closest match)
Pseudomonas chengduensis	97.95	KC871534
Staphylococcus warneri	99.70	CP033098
Staphylococcus pasteuri	98.03	CP031280
Pseudomonas aeruginosa	95	LC536054
Bacillus cereus	96.5	GQ199591
Bacillus toyonensis	100	NR_121761
Carnobacterium inhibens	97.46%	CP006812
Chryseobacterium meningosepticum	97.3	MG694506
Stenotrophomonas maltophilia	99.92 97.08 99.70 100	MF354012 MN733006 MN709090 MT790731

Antibiotic resistance patterns

As shown in Table 3, *Pseudomonas* spp. was resistant to most of the tested antibiotics: piperacillin (83.33% resistance), piperacillin–tazobactam (58.33% resistance), ticarcillin (50% resistance), and ticarcillin–clavulanic acid (16.67% resistance) were the most active compounds tested and were followed by chloramphenicol (16.67% resistance), imipenem (8.33% resistance), and levofloxacin (100% susceptible). Interestingly, the *S. maltophilia* isolate exhibited high resistance to most antibiotics tested; levofloxacin was the active compound (100% susceptible). *B. cepacia* showed increased sensitivity to nearly all tested antibiotics. Almost half (43.75%) of the isolated strains tested exhibited antibiotic resistance, 31.25% (10/32) of them were classified as MDR, and 12.5% (4/32) remained susceptible to only one or two categories of antibiotic (XDR). Interestingly, *S. maltophilia* showed a very high degree of resistance to most of the evaluated antibiotics; among 16 isolated strains, 6 were non-MDR, 7 were MDR, and 3 were XDR. Among the 12 strains of *Pseudomonas* spp., 8 were non-MDR, 3 were MDR, and 1 was XDR (Figure 2).

Table 3 | Antibiotic susceptibility patterns of the predominant NFGNBs (% of resistance)

Isolated Bacterial strains	PRL	TZP	T	TCC	FEP	CAZ	AKN	GEN	CIP	LEV	IMI	C	TSU
Stenotrophomonas maltophilia (*N*: 16)	–	–	–	6	6	5	4	2	4	0	–	–	2
	–	–	–	50%	46.15%	31.25%	25%	12.5%	25%	00%	–	–	12.50%
Pseudomonas spp. (*N*: 12)	10	7	6	2	3	2	5	2	2	0	1	2	–
	83.33%	58.33%	50%	16.67%	25%	16.67%	41.6%	16.67%	16.67%	00%	8.33%	16.67%	–
Burkholderia cepacia (*N*: 4)	–	–	1	0	0	1	–	0	2	0	–	0	0
	–	–	25%	00%	00%	25%	–	00%	50%	00%	–	00%	00%

PRL, piperacillin; TZP, piperacillin+tazobactam; T, ticarcillin; TCC, ticarcillin–clavulanic acid; FEP, cefepime; CAZ, ceftazidime; AKN, amikacin; GEN, gentamicin; CIP, ciprofloxacin; Lev, levofloxacin; IMI, imipenem; C, chloramphenicol; TSU, trimethoprim–sulphamethoxazole; –, not available.

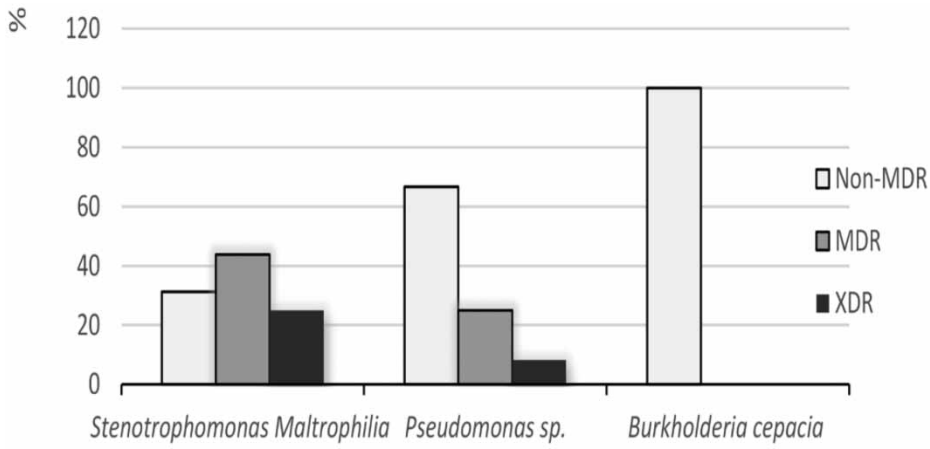

Figure 2 | Non-MDR, MDR, and XDR classification of the major isolated bacteria.

DISCUSSION

The microbiological control of HD fluid plays a crucial role in preventing HD-associated illness. However, several studies have suggested that the potential presence of bacteria, even in small amounts, may reduce dialysate quality (Gomila *et al.* 2005). Thus, this study investigated for the first time the quality of treated water that was used for dilution of HD concentrates at a public HD unit in Mohammedia.

The results of the bacteriological analysis showed that the contamination rate was 5.5% (94.5% compliance). Oumokhtare *et al.* found a high level of compliance (100% in an HD unit in Fez, Morocco) (Oumokhtar *et al.* 2013). In Japan, HD centers in nine hospitals reported high contamination levels of dialysates, and 42.5% were above the AAMI standard (Oie *et al.* 2003). In addition, in Tuscan hospitals in Italy, seven of the nine water treatment plants for dialysis (78%) recorded negative results for all the studied parameters (Totaro *et al.* 2017).

In general, most cultivable bacterial counts were consistent with the standards. The worrying result in this study was the increased number of bacterial cultures after carbon filtration, which is mainly attributed to chlorine residue removal by carbon filtration. Microorganisms are capable of reproducing in the absence of such growth inhibitors as well as on the rough surfaces of carbon particles, which are the leading causes of biofilm formation (Mezule & Juhna 2016). In addition, the RO effluent exhibited high bacteria count levels (i.e., a peak of 178 CFU/mL), which was incompatible with its role and its capacity to retain particles; the input dialysis generator, as the last point in the loop, also recorded positive cultures of the different strains found at previous points. During this procedure, suspended bacterial reproduction and biofilm formation on tube walls may occur. The cell densities could even reach 106 CFU/cm^2 in the biofilm attached to the tubes of the HD machine (Man *et al.* 1998; Smeets *et al.* 2003). However, the presence of low flow rates and stagnation points causes the distribution network and water treatment plants located downstream of the filters to be vulnerable to bacterial growth and biofilm formation.

The identification of the isolated strains during our study revealed the predominance of *S. maltophilia* with a frequency of 34%. Our results agree with those of Rocha *et al.* (2020). The isolates of this species in this study exhibited high resistance to the antibiotics tested: 43.75% (7/16) of strains were MDR, and 18.75% (3/16) were XDR, while 12.5% were resistant to TSU, which is the antibiotic of choice for treating *S. maltophilia* infections (Anđelković *et al.* 2019). Recently, several cases of bacterial infections due to *S. maltophilia* have been reported (Thet *et al.* 2019; Rocha *et al.* 2020). This bacterium is related to a wide range of infections, with bloodstream infections and pneumonia being the two most common manifestations (Looney *et al.* 2009). In addition, infections associated with *S. maltophilia* pose serious treatment challenges; they are inherently resistant to many antibiotics and can acquire antibacterial resistance by multiple mechanisms (Chang *et al.* 2015). Therefore, the World Health Organization (WHO) has classified this bacterium as one of the MDR bacteria in hospital settings (Brooke 2014).

Pseudomonas was also isolated with a frequency of 25.53%. These results are similar to those reported by Oumokhtar *et al.* (2013). Among our 12 strains of *Pseudomonas* spp., 50% showed high resistance, mainly to piperacillin, piperacillin–

tazobactam, ceftazidime, and amikacin, while they remained sensitive at a 91.6% level to imipenem. These results present a cause for concern, given the known resistance of the bacteria of this genus to antibiotics, its virulence factors, its easy acquisition of new resistance factors, and its ability to survive in water for long periods, even without nutrients. Further measures must be implemented to remove it from dialysis water for these reasons. Regarding *B. cepacia*, the four strains identified were sensitive to most antibiotics; however, this result should not minimize its pathogenicity; it can cause bloodstream infections in HP (Rocha *et al.* 2020).

The use of 16S rDNA sequence analysis (for the first time) in the dialysis water in Morocco not only allows us to confirm the strains that we identified but also other strains (not identified by conventional tests), such as *Bacillus cereus*, *Staphylococcus warneri*, *S. pasteuri*, and *Carnobacterium inhibens*. Therefore, the presence of all these bacterial varieties with resistant strains in treated water is a matter of concern in HD centers and should be taken seriously when assessing HD water quality by performing routine monthly disinfections with high-level disinfectants. Other solutions to improve the quality of HD water were recommended: disinfecting machines frequently by updating the disinfection protocol (Asserraji *et al.* 2014; Verma 2015), biofilm analysis on tubing dialysis machines (Marion-Ferey *et al.* 2003), improving nursing education on dialysis water (Chamney & James 2008), and conducting monthly quality assurance for risk analysis to the progress of water quality. The choice of piping materials is also essential. International standards (BS EN 12201-3 2003; BS EN 806-5 2012) suggest the use of AISI Type 304/316 stainless steel, copper, polyvinyl chloride, or polypropylene (used in the water treatment system in the present study) in water distribution systems, all of which can withstand physical and chemical shock disinfection treatments.

Limitations

Our study was set in one dialysis center and there may not be enough data points to generalize our findings to other centers. The other limitation was the shortage of some antibiotics which prevented us from doing the antibiotic test for all the strains in a homogeneous way.

CONCLUSIONS

In this study, the hemodialysis water treatment system has been colonized by MDR Gram-negative bacteria. The CFU values for dialysis water exceeded the limit of 100 CFU/mL in three samples after CF. Therefore, strict monitoring of the water delivery system eliminates all bacteria, even those protected in biofilm. To elucidate the bacterial community in dialysis water with precision, we recommend that future studies apply culture-independent protocols to detect viable but non-culturable bacteria (VBNC).

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AUTHOR CONTRIBUTIONS

L.C., T.C., I.Z., R.A.M., and F.M. conceived and designed the study. N.R., L.C., and R.A.M. undertook experimental procedures. L.C., T.C., and N.R. undertook the analysis. L.C. and R.A.M. drafted the manuscript. All authors approved the final manuscript.

ETHICS APPROVAL AND CONSENT

Ethical permission was not required and was not necessary for this study. However, written approval was granted by the Provincial Hospital Center (PHC) Mohammedia administration and by the head of the laboratory where this work was done.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W. & Lipman, D. J. 1990 Basic local alignment search tool. *Journal of Molecular Biology* **215** (3), 403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

Anđelković, M. V., Janković, S. M., Kostić, M. J., Živković Zarić, R. S., Opančina, V. D., Živić, M. Ž., Milosavljević, M. J. & Pejčić, A. V. 2019 Antimicrobial treatment of *Stenotrophomonas maltophilia* invasive infections: systematic review. *Journal of Chemotherapy (Florence, Italy)* **31** (6), 297–306. https://doi.org/10.1080/1120009X.2019.1620405.

ANSI/AAMI/ISO13959 2014 *American National Standard Institute: New AAMI Water Quality Standard for Hemodialysis*. Association for the Advancement of Medical Instrumentation, Arlington, VA, p. 4.

Asserraji, M., Maoujoud, A., Belarbi, M. & Elfarouki, R. 2014 Monitoring the microbiological quality of dialysate and treated water. *Saudi Journal of Kidney Diseases and Transplantation* **25** (1), 91. https://doi.org/10.4103/1319-2442.124499.

Brooke, J. S. 2014 New strategies against *Stenotrophomonas maltophilia*: a serious worldwide intrinsically drug-resistant opportunistic pathogen. *Expert Review of Anti-Infective Therapy* **12** (1), 1–4. https://doi.org/10.1586/14787210.2014.864553.

BS EN 806-5 2012 *Specifications for Installations Inside Buildings Conveying Water for Human Consumption*. Operation and Maintenance, British Standards Institution – Publication Index | NBS. Available from: https://www.thenbs.com/PublicationIndex/documents/details?Pub=BSI&DocId=300211.

BS EN 12201-3 2003 *Plastic Piping Systems for Water Supply – Polyethylene (PE)*. Fittings, British Standards Institution – Publication Index | NBS. Available from: https://www.thenbs.com/PublicationIndex/documents/details?Pub=BSI&DocID=267005.

Chamney, M. & James, R. 2008 Should renal nurses be aware of water quality? *Journal of Renal Care* **34** (2), 68–76. https://doi.org/10.1111/j.1755-6686.2008.00015.x.

Chang, Y.-T., Lin, C.-Y., Chen, Y.-H. & Hsueh, P.-R. 2015 Update on infections caused by *Stenotrophomonas maltophilia* with particular attention to resistance mechanisms and therapeutic options. *Frontiers in Microbiology* **6** (September). https://doi.org/10.3389/fmicb.2015.00893.

CLSI 2014 Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement (M100-S24) | AACC.Org. Available from: https://www.aacc.org/store/books/9200/performance-standards-for-antimicrobial-susceptibility-testing.

Coresh, J., Selvin, E., Stevens, L. A., Manzi, J., Kusek, J. W., Eggers, P., Van Lente, F. & Levey, A. S. 2007 Prevalence of chronic kidney disease in the United States. *JAMA* **298** (17), 2038–2047. https://doi.org/10.1001/jama.298.17.2038.

Damasiewicz, M. J., Polkinghorne, K. R. & Kerr, P. G. 2012 Water quality in conventional and home haemodialysis. *Nature Reviews Nephrology* **8** (12), 725–734. https://doi.org/10.1038/nrneph.2012.241.

DES 2018 *Monitoring and Sampling Manual: Environmental Protection (Water) Policy*. Department of Environment and Science Government, Brisbane.

Gomila, M., Gascó, J., Busquets, A., Gil, J., Bernabeu, R., Buades, J. M. & Lalucat, J. 2005 Identification of culturable bacteria present in haemodialysis water and fluid. *FEMS Microbiology Ecology* **52** (1), 101–114. https://doi.org/10.1016/j.femsec.2004.10.015.

Heidarieh, P., Shahraki, A. H., Yaghoubfar, R., Hajehasani, A. & Mirsaeidi, M. 2016 Microbiological analysis of hemodialysis water in a developing country. *ASAIO Journal (American Society for Artificial Internal Organs: 1992)* **62** (3), 332–339. https://doi.org/10.1097/MAT.0000000000000353.

Junglee, N. A., Rahman, S. U., Wild, M., Wilms, A., Hirst, S., Jibani, M. & Seale, J. R. C. 2010 When pure is not so pure: chloramine-related hemolytic anemia in home hemodialysis patients. *Hemodialysis International* **14** (3), 327–332. https://doi.org/10.1111/j.1542-4758.2010.00454.x.

Looney, W. J., Narita, M. & Mühlemann, K. 2009 *Stenotrophomonas maltophilia*: an emerging opportunist human pathogen. *The Lancet Infectious Diseases* **9** (5), 312–323. https://doi.org/10.1016/S1473-3099(09)70083-0.

Magiorakos, A.-P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., Harbarth, S., Hindler, J. F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D. L., Rice, L. B., Stelling, J., Struelens, M. J., Vatopoulos, A., Weber, J. T. & Monnet, D. L. 2012 Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* **18** (3), 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.

Man, N.-K., Degremont, A., Darbord, J.-C., Collet, M. & Vaillant, P. 1998 Evidence of bacterial biofilm in tubing from hydraulic pathway of hemodialysis system. *Artificial Organs* **22** (7), 596–600. https://doi.org/10.1046/j.1525-1594.1998.06195.x.

Marion-Ferey, K., Enkiri, F., Pasmore, M., Husson, G. P. & Vilagines, R. 2003 Methods for biofilm analysis on silicone tubing of dialysis machines. *Artificial Organs* **27** (7), 658–664. https://doi.org/10.1046/j.1525-1594.2003.07148.x.

Mezule, L. & Juhna, T. 2016 Effect of labile organic carbon on growth of indigenous *Escherichia coli* in drinking water biofilm. *Chemical Engineering Transactions* **49** (April), 619–624. https://doi.org/10.3303/CET1649104.

Ministère de santé 2018 Cadre de Référence Des Centres d'hémodialyse: Générateurs – Unités de Traitement et de Distribution d'eau Dispositifs Médicaux et Médicaments d'hémodialyse.

Montanari, L. B., Sartori, F. G., Cardoso, M. J. d. O., Varo, S. D., Pires, R. H., Leite, C. Q. F., Prince, K. & Martins, C. H. G. 2009 Microbiological contamination of a hemodialysis center water distribution system. *Revista Do Instituto De Medicina Tropical De Sao Paulo* **51** (1), 37–43. https://doi.org/10.1590/s0036-46652009000100007.

Oie, S., Kamiya, A., Yoneda, I., Uchiyama, K., Tsuchida, M., Takai, K. & Naito, K. 2003 Microbial contamination of dialysate and its prevention in haemodialysis units. *The Journal of Hospital Infection* **54** (2), 115–119. https://doi.org/10.1016/s0195-6701(02)00402-4.

Oumokhtar, B., Lalami, A. E. O., Mahmoud, M., Berrada, S., Arrayhani, M. & Houssaini, T. S. 2013 Prevent infection linked to the dialysis water in a hemodialysis center in Fez city (Morocco). *The Pan African Medical Journal* **16**, 122. https://doi.org/10.11604/pamj.2013.16.122.2877.

Pontoriero, G., Pozzoni, P., Andrulli, S. & Locatelli, F. 2004 [The quality of dialysis water]. *Giornale Italiano Di Nefrologia: Organo Ufficiale Della Societa Italiana Di Nefrologia* **21** (Suppl 30), S42–S45.

Rocha, V. F. D., Cavalcanti, T. P., Azevedo, J., Leal, H. F., Silva, G. E. O., Malheiros, A. R. X., Ataide, L. A., Filho, J. A. L., Almeida, A. R. P., Khouri, N. dA., Reis, M. G. & Reis, J. N. 2020 Outbreak of *Stenotrophomonas maltophilia* and *Burkholderia cepacia* bloodstream infections at a hemodialysis center. *The American Journal of Tropical Medicine and Hygiene*. https://doi.org/10.4269/ajtmh.20-1035.

Shahryari, A., Nikaeen, M., Hatamzadeh, M., Dastjerdi, M. V. & 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.

Smeets, E., Kooman, J., van der Sande, F., Stobberingh, E., Frederik, P., Claessens, P., Grave, W., Schot, A. & Leunissen, K. 2003 Prevention of biofilm formation in dialysis water treatment systems. *Kidney International* **63** (4), 1574–1576. https://doi.org/10.1046/j.1523-1755.2003.00888.x.

Thet, M. K., Pelobello, M. L. F., Das, M., Alhaji, M. M., Chong, V. H., Khalil, M. A. M., Chinniah, T. & Tan, J. 2019 Outbreak of nonfermentative gram-negative bacteria (*Ralstonia pickettii* and *Stenotrophomonas maltophilia*) in a hemodialysis center: gram-negative bacteria outbreak in hemodialysis unit. *Hemodialysis International* **23** (3), E83–E89. https://doi.org/10.1111/hdi.12722.

Tong, M. K.-H., Wang, W., Kwan, T.-H., Chan, L. & Au, T.-C. 2001 Water treatment for hemodialysis. *Hong Kong Journal of Nephrology* **3** (1), 7–14. https://doi.org/10.1016/S1561-5413(09)60050-8.

Totaro, M., Casini, B., Valentini, P., Miccoli, M., Giorgi, S., Porretta, A., Privitera, G., Lopalco, P. L. & Baggiani, A. 2017 Evaluation and control of microbial and chemical contamination in dialysis water plants of Italian nephrology wards. *The Journal of Hospital Infection* **97** (2), 169–174. https://doi.org/10.1016/j.jhin.2017.05.011.

Verma Dr., S. 2015 Bacteriological quality of treated water and dialysate in haemodialysis unit of a tertiary care hospital. *Journal of Clinical and Diagnostic Research*. https://doi.org/10.7860/JCDR/2015/14681.6692.

Ward, R. A. 2011 Avoiding toxicity from water-borne contaminants in hemodialysis: new challenges in an era of increased demand for water. *Advances in Chronic Kidney Disease* **18** (3), 207–213. https://doi.org/10.1053/j.ackd.2011.01.007.

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