Detection of microorganisms, endotoxins and aluminum in mobile dialysis services

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ABSTRACT. Hemodialysis water and dialysates are fundamental in the treatment of kidney disease. During just one hemodialysis session, 120 liters of dialysate are consumed. Thus, it is essential that the parameters of chemical and microbiological quality of the fluids used in dialysis systems are carefully observed. In this study, water samples were collected at 12 hospitals in the state of Rio de Janeiro. The samples were obtained at three points of fluid reservoirs: pre-, post-osmosis and dialysis solution. After collection, colony forming units (CFU), total coliforms and Escherichia coli 100 mL⁻¹ were quantified. Later, isolated colonies and endotoxin content were identified by biochemical assays. Data about total aluminum levels per sample (mg L⁻¹) were also obtained. Samples of all mobile dialysis services and points of collection were contaminated above the levels set out by national laws, in particular by Pseudomonas aeruginosa. Endotoxin levels were also above the recommended by current legislation (> 0.25 EU mL⁻¹). Only three samples contained detectable levels of aluminum, which were found to be above the recommended values for the corresponding resolution (0.01 mg L⁻¹). Finally, there were no observable amounts of total coliforms and E. coli 100 mL⁻¹ sample. The data from this study are an important step forward in the standardization and control of chemical/microbiological quality of mobile dialysis services.

Keywords: dialysis, hemodialysis units, dialysis solutions.

Detecção de microrganismos, endotoxinas e alumínio em serviços de diálise móvel

RESUMO. Águas de hemodiálise e dialisatos são peças fundamentais na terapêutica da doença renal. Durante apenas uma sessão de hemodiálise, são consumidos aproximadamente 120 litros de dialisato. Desta forma, é essencial que os parâmetros de qualidade microbiológica e química dos fluidos utilizados em sistemas de diálise sejam cuidadosamente observados. Neste trabalho, foram coletadas amostras de água em 12 hospitais do Estado do Rio de Janeiro. Amostras foram obtidas em pontos pré-osmose, pós-osmose e solução de diálise. Após coleção, quantificaram-se unidades formadoras de colônias (UFC), coliformes totais e Escherichia coli 100 mL⁻¹. Posteriormente, colônias isoladas e teor de endotoxinas foram identificados por ensaios bioquímicos. Dados acerca dos níveis totais de alumínio por amostra (mg L⁻¹) também foram obtidos. Amostras de todos os serviços de diálise móvel e pontos de coleção apresentaram contaminação acima dos níveis previstos em legislação nacional, em especial por Pseudomonas aeruginosa. Teores de endotoxinas também se mostraram acima da legislação vigente (> 0.25 EU mL⁻¹). Apenas três amostras contêm níveis detectáveis e acima dos valores preconizados por resolução correspondente (0,01 mg L⁻¹). Por fim, não foram encontradas quantidades observáveis de coliformes totais e E. coli 100 mL⁻¹ de amostra. Dados de nosso estudo são importante avanço na padronização e controle de qualidade química/microbiológica em serviços de diálise móvel.

Palavras-chave: diálise, unidades hospitalares de hemodiálise, soluções para diálise.

Introduction

Despite the presence of multiple barriers in the treatment of dialysis waters, the risk of contamination by microorganisms, pyrogens and metals should be carefully considered (Ferreira et al., 2015). Purified water predominantly contains heterotrophic bacteria, common in watery environments, especially from Pseudomonadales class, which may grow in water pipes and hemodialysis (HD) machines, therefore contaminating the dialysate (Bommer & Jaber, 2006). Analysis data indicate that Pseudomonas species are the main genus...
of bacterial contamination in dialysis waters (Favero, Alter, & Bland, 1992; Morin, 2000), and that this species should serve as microbiological quality indicators within HD centers (Vorbeck-Meister, Sommer, Vorbeck, & Hörl, 1999).

Ultrapure dialysates should contain no more than 0.1 CFU mL\(^{-1}\) (colony-forming units mL\(^{-1}\)) and 0.03 EU mL\(^{-1}\) (endotoxin units mL\(^{-1}\)). These purified dialysate solutions are efficient in preventing chronic inflammation and reducing the frequency of infection in patients under dialytic treatment (Association for the Advancement of Medical Instrumentation [AAMI], 2001; Arizono, Nomura, Motoyama, Takeshita, & Fukui, 2004; Ward, 2004; Bommer & Jaber, 2006), indicating the importance of maintaining high microbiological standards for dialysates. Hemodialysis fluids are composed of a mixture containing water and a polyelectrolyte concentrate in a 34:1 proportion, and these solutions are used for cleaning up the semipermeable membrane within dialysis equipment (Vorbeck-Meister et al., 1999).

During a hemodialysis session, approximately 120 liters of dialysate are used for blood depuration, thus, water purity is essential to avoid additional risks to the patient’s health (Bommer & Jaber, 2006). The European Pharmacopoeia recommends an upper limit of 100 CFU mL\(^{-1}\) for purified water used in dialysates (Council of Europe, 2013), once several studies have demonstrated the importance of maintaining low bacterial load within the dialysate, thus preventing clinical complications due to bacterial contaminants (Brunet & Berland, 2000; Lonnemann, 2000). Also, data indicate that endotoxins derived from Gram negative bacteria cross the semipermeable membrane during HD, inducing pyrogenic reactions in dialysis patients (Lonnemann, 1993).

In the present study, it is reported the presence of microorganisms, endotoxins and aluminum (Al) in mobile dialysis services from 12 hospitals within Rio de Janeiro State. Our data are an important advance in the standardization of chemical/microbiological quality control in mobile dialysis services.

**Material and methods**

**Sample acquisition**

Water and dialysate samples were obtained through a collaboration program between National Institute for Quality Control in Health (INQS/FIOCRUZ), Sanitary Surveillance of the state of Rio de Janeiro, Superintendence of Zoonosis Control, and Sanitary Surveillance of the City of Rio de Janeiro. Data collection in mobile dialysis stations for research and monitoring purposes has started in July 2014.

**Analysis procedures**

Sample analyses were carried out according to standardized operational procedures (POPs) from INQS: POP INQS nº 65.3210.030 – (Contagem de viáveis totais e bactérias bile tolerantes em produtos farmacêuticos, matérias primas e água para diálise); POP INQS nº 65.3210.010 – (Contagem de viáveis totais e bactérias bile tolerantes em produtos farmacêuticos, matérias primas e água para diálise); POP INQS nº 65.3210.008 – (Pesquisa de patógenos em produtos não estéreis e matérias primas de uso em sua fabricação e água para diálise); POP INQS nº 65.3330.006 – (Ensaios para endotoxinas bacterianas and POP INQS nº 65.3120.030 - Determinação de alumínio em vacinas por espectrometria de absorção atômica por chama), procedures accredited by the Institute of Metrology and Technological Quality (INMETRO) and qualified by the World Health Organization (WHO).

**Counting of colony forming units**

Water (pre- and post-osmosis) and dialysate samples of approximately 200 mL were collected using sterile flasks. After collection, 1.8% of sodium thiosulfate were added to the solution for chloride removal. Samples were stored at temperatures lower than 10°C and processed at the day of collection. Total or viable bacterial counting was accomplished using plate count agar (PCA). Samples were diluted in phosphate buffer saline (PBS) at pH 7.4, inoculating 1 mL of sample in 9 mL PBS (1:9 dilution). A volume of 1 mL was then added to 20 mL of PCA medium, melted and cooled to 45-50°C. After solidification, the medium was incubated at 30-35°C for 48 ± 3 hours. Counting of colony forming units (CFU) was carried out in plates containing up to 300 CFU.

**Presence of total coliforms and Escherichia coli**

After sample homogenization, a 100 mL volume were inoculated in 50 mL 3x-concentrated presence-absence (PA) broth and incubated at 30-35°C for up to 48 hours. Confirmatory analysis was observed in the presence of positive PA indication of bacterial growth (i.e. yellow broth). Cultured cells were then inoculated in 10 mL brilliant green bile broth (BGBB) with Durham tube and incubated for 48 ± 3 hours at 30-35°C. After incubation period, cultured cells were seeded in a plate containing eosin methylene blue agar (EMBA), MacConkey agar and tryptic soy agar (TSA) for posterior completion of biochemical proofs.
Biochemical identification of isolated bacteria

Biochemical identification was carried out according to POP INCQS/FIOCRUZ nº 65.3210.008. Using cells grown in soy casein broth, the samples were seeded in plates containing cetrimide agar, soy casein broth, mannitol salt agar, MacConkey agar and EMBA. After incubation period, the isolated colonies were submitted to biochemical identification tests.

Quantification of aluminum levels

Quantification of Al in samples were accomplished through emission spectrometry (ICP-OES), adapted from Medeiros et al. (2012) and POP INCQS nº 65.3120.030 - “Determinação de alumínio em vacinas por espectrometria de absorção atômica por chama”.

Results and discussion

Counting of CFU and aluminum levels

The presence of microorganisms was detected in all hospital units (1-12) and collection points, i.e. pre-osmosis (hospital’s water supply), post-osmosis (treated water, used in dialysis) and dialysate solution (electrolyte aqueous solution, with or without glucose). In the hospitals 8 and 12 (see Table 1), however, dialysate solutions were unavailable at the time of collection. Table 1 presents the total number of aerobic bacteria from collected samples at each acquisition point. Table 1 also presents quantification of Al levels, which were detected in only three pre- and post-osmosis samples, however, all three samples contained Al values above resolution (RDC 11/2004) limits of 0.01 mg L⁻¹.

Presence of total coliforms and Escherichia coli

Detection of total coliforms and E. coli showed absence in 100 mL of samples from all hospital and collection points, thus indicating low coliform counting in waters used in these dialysis units.

Table 1. Counting of heterotrophic bacteria and quantification of aluminum levels.

| HOSPITALS | Pre-osmosis | Post-osmosis | Dialysis solution | Pre-osmosis | Aluminium levels | Post-osmosis |
|-----------|-------------|--------------|-------------------|-------------|-----------------|-------------|
| 1         | 1.2 x 10⁶ CFU mL⁻¹ | 1.5 x 10⁶ CFU mL⁻¹ | 2.0 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 2         | 1.1 x 10⁶ CFU mL⁻¹ | 1.3 x 10⁶ CFU mL⁻¹ | 2.3 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 3         | 1.4 x 10⁶ CFU mL⁻¹ | 2.5 x 10⁶ CFU mL⁻¹ | 2.0 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 4         | 1.3 x 10⁶ CFU mL⁻¹ | 1.6 x 10⁶ CFU mL⁻¹ | 2.5 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 5         | 1.5 x 10⁶ CFU mL⁻¹ | 2.1 x 10⁶ CFU mL⁻¹ | 2.1 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 6         | < 10 CFU mL⁻¹ | 6.1 x 10⁵ CFU mL⁻¹ | 1.5 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 7         | < 10 CFU mL⁻¹ | < 10 CFU mL⁻¹ | < 10 CFU mL⁻¹ | (0.156 ± 0.007) mg L⁻¹ | < 0.009 mg L⁻¹ | < 0.009 mg L⁻¹ |
| 8         | 2.2 x 10⁶ CFU mL⁻¹ | 2.8 x 10⁶ CFU mL⁻¹ | (0.22 ± 0.04) mg L⁻¹ | TR | (0.24 ± 0.04) mg L⁻¹ | TR |
| 9         | < 10 CFU mL⁻¹ | < 10 CFU mL⁻¹ | < 10 CFU mL⁻¹ | > 0.27 mg L⁻¹ | TR | > 0.01 mg L⁻¹ |
| 10        | < 10 CFU mL⁻¹ | 1.8 x 10⁶ CFU mL⁻¹ | 3.0 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 11        | 1.2 x 10⁶ CFU mL⁻¹ | 1.9 x 10⁶ CFU mL⁻¹ | 3.0 x 10⁶ CFU mL⁻¹ | TR | TR | TR |
| 12        | 2.1 x 10⁶ CFU mL⁻¹ | 1.0 x 10⁶ CFU mL⁻¹ | Al | TR | TR | TR |

*UFC = colony forming units. Al = unavailable sample. TR = trace/undetectable.

Aluminum toxicity may affect bone health and induce encephalopathic syndromes, and previous works have evaluated the effect of Al present in haemodialysis waters on patient’s health (Charhon, Pascale, Meunier, & Accominotti, 1985). From the 12 mobile dialysis services, from pre- and post-osmosis water samples, only three contained Al levels above the Brazilian limits of 0.01 mg L⁻¹ (RDC 11/2004). Aluminum concentrations from these samples, however, were found to be ten to twenty-fold (~ 0.215 mg L⁻¹) higher than legally preconized. It’s important to question whether these Al levels could affect patients if continuously present in the dialysis solutions.
Al-Naseri, Mahdi, and Hashim (2013) found that in 60% of hemodialysis centers from Baghdad (Iraq), the bacterial counting was between 50 and 100 CFU mL\(^{-1}\). Also, the presence of \textit{P. aeruginosa} was correlated with higher average endotoxin levels. Here, it was found relatively low but significant levels of heterotrophic bacteria (\(\sim 2.00\) CFU mL\(^{-1}\)). Also, these microorganisms were isolated from all mobile services (hospitals) and points of collection (dialysis solution, pre- and post-osmosis water); only samples 7, 9 and 10 showed \(<10\) CFU mL\(^{-1}\) on at least one collection point, however, samples 7 and 9 were among those that had higher Al levels.

Biochemical identification (see Table 2) of isolated bacteria shows the presence of indicator microorganisms (\textit{Pseudomonas}, especially \textit{P. aeruginosa}), which were previously shown to negatively affect dialysis patients (Ferreira et al., 2015). Also, \textit{S. maltophilia} was shown to induce bacteremia in a dialysis patient with long-term central venous catheter (Kara, YilmazM, Şit, Kadiroğlu, & Kokoğlu, 2006) and in end-stage renal disease patients receiving maintenance hemodialysis (Gnanasekaran & Bajaj, 2009). Strateva, Kostyanev, and Setchanova (2012) reported a case of sepsis induced by \textit{R. pickettii} in a hemodialysis patient from Bulgaria. Kaitwatcharacha, Silpapojakul, Sirojitsurong, & Kanhawakul (2000) reported a subclavian catheter-related \textit{B. cepacia} outbreak in nine patients undergoing hemodialysis; authors also observed that \textit{B. cepacia} formed biofilms while growing in the catheters.

\textit{Sphingomonas. paucimobilis}, also detected in the water samples of this study, is an uncommon cause of peritonitis and other dialysis-related infections. Recent reports, however, have found this microorganism in co-infections caused by opportunistic catheter growth. One case of \textit{S. paucimobilis}-induced peritonitis was reported by Lee et al. (2013). There are little or no evidence of \textit{B. diminuta} infection in dialysis patients; other species, \textit{B. vesicularis}, was previously shown to cause peritonitis (Choi et al., 2006). Badrising, Bakker, Lobatto, and Van Es (2014) reported a peritonitis case due to a co-infection by \textit{R. radiobacter} and \textit{M. osloensis}. Other \textit{Moraxella} species have also been shown to cause dialysis-related infections.

In 2009, \textit{A. xylosoxidans} was shown, in a case report, to induce prosthetic valve endocarditis, where it was also characterized as an emerging pathogen in catheter-related infections used in dialysis (Ahmed, Nistal, Jayan, Kuduvalli, & Anijeet, 2009). Finally, \textit{Acinetobacter} species are among the main opportunistic microorganisms in nosocomial infections, and \textit{A. baumannii} strains are usually characterized as highly dangerous pathogens due to its mechanisms of antibiotic resistance, clinical impact and co-infection potential (Peleg, Seifert, & Paterson, 2008).

The monitoring of clinics and mobile dialysis units by sanitary surveillance, in collaboration to INCQS, demonstrates that lowering tolerance levels for chemical and microbiological quality of HD fluids is effective in optimizing quality standards within those dialysis services. Medical centers should, however, try to surpass such minimum standards, thus ensuring patient safety and accordance to legal determinations. Application of ultrapure dialysates, for instance, may represent a significant advance in the treatment of patients with kidney disease.

Although a great variety of microorganisms have been detected in this study, \textit{P. aeruginosa} was predominant (data not shown), corroborating with previous results (Favero et al., 1992; Morin, 2000). This species produces stable biofilms, resistant to several disinfection treatments, thus it’s important to consider determining a legal parameter (e.g. presence/absence in 10 mL) for dialysates. After exhaustive analysis (2014-2016), data strongly indicates that microbiological contamination found in samples from the hospital water supplies (i.e. pre-osmosis) are resistant to disinfection and filtration procedures, allowing the presence of pyrogens (i.e. lipopolysaccharides) and live bacteria throughout the dialytic process.

**Conclusion**

Our data indicate the need for establishing novel quality standards within national legislation, determining more restrict parameters for evaluation and analysis of the water used in mobile dialysis in the state of Rio de Janeiro. In addition, it is important to highlight, as previous reviewed in Ferreira et al. (2015), that bacterial contamination in HD centers is a worldwide reality, therefore mobile dialysis units should also be verified for such parameters in other Brazilian States and overseas.

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