

Network Water Quality at a Hospital Center in Morocco: Bacteriological Survey and Relationship with Human Health

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ABSTRACT

Water is mandatory for the functioning of hospitals. Its consumption varies from one service to another, and its use can reduce the service quality on one hand and presents a major risk of nosocomial infection on the other hand. The health risks related to the use of water in hospitals are mostly microbiological, but sometimes they can be chemical. For this reason, the conducted work aimed to evaluate, for the first time, the quality and bacteriological efficiency of the network water of the provincial hospital center IBN BAJA in Taza, Northeast Morocco. During one year, 72 samples were analyzed in six surgery departments, one sample per month for each ward at a rate of six samples per month and per department. The results obtained showed that the water quality of the hospital network was in conformity with the requirements of the Moroccan standards. Nonetheless, some bacterial strains such as *Aeromonas salmonicida* spp *salmonicida*, *Enterococcus* spp., *Pseudomonas luteola*, *Sphingomonas paucimobilis*, *Pseudomonas stutzeri*, *Stenotrophomonas maltophilia*, *Burkholderia cepacia* and *Micrococcus luteus*, which constitute a major risk to human health, were found. Moreover, after sensitivity evaluation to the twenty-seven antibiotics, some strains have been shown to be multi-resistant, which can present a major risk of nosocomial infections in the studied hospital for human beings.

Keywords: bacteriology, water, hospital, bacteria, resistance, contamination.

INTRODUCTION

Water plays a very important role in the functioning of health care institutions; its consumption varies between 200 to 1 200 liters per day and per bed, depending on the hospital activity [D'Alessandro et al., 2016]. The water use varies depending on different utilizations, and according to the health care units for which it is intended. The health risk of water, at the hospital level, is essentially linked to the microbiological risk due to multi-resistant bacteria;

hence, hospital water must meet very precise bacteriological standards [Barbut et al., 2006].

Contaminated water leads to the transmission of waterborne diseases and hospital infections by waterborne microorganisms such as mycobacteria, Legionella, Pseudomonas..., etc causing mortality and morbidity, especially in the immuno-compromised hospitalized patients [Decker et al., 2014]. The exposure of patients to waterborne microorganisms in hospital occurs during showering, preoperative showering, skin cleansing, and water drinking. It can also occur

through the contact with contaminated medical equipment such as feeding bags, medical surgical instruments, and health care equipment that have been rinsed with tap water, or by contaminated hands of health care workers, washed with tap water, which can lead to the patient exposure to pathogens [Bhattacharjee, 2015]. The presence of microorganisms isolated from different hospital water sources indicates a potential risk to human health, especially for the immunocompromised patients suffering from serious diseases [Arroyo et al., 2020].

To this end, the *Center for Disease Control and Prevention* (CDCP) guidelines have highlighted the practices for the control of hospital waterborne infections [Chinn et al., 2003]. Hence, hospital water microbiological monitoring and surveillance is mandatory, since it ensures its good quality and compliance with standards.

For this purpose, this study aimed to monitor and analyze the microbiological quality in the different surgical departments of the provincial hospital IBN BAJA of Taza in Morocco during one year. To the best of the authors' knowledge, no previous investigation has been conducted in the same context, in this hospital center.

MATERIALS AND METHODS

A prospective study was carried out over a period of 12 months, from October 2018 to September 2019, in a provincial hospital: Ibn Baja in Taza (Northeast Morocco) which has a capacity of 317 beds. The samples were taken from six hospital departments specialized in surgery: male surgery, women surgery, central operating room, sterilization, gynecology, and child surgery. The sampling frequency adopted during the study period (October 2018-September 2019) was one sample per month for each ward.

Sampling was carried out aseptically under hygienic conditions; hence, the water samples were collected in sterile coded 500-ml vials containing sodium thiosulfate pentahydrate (0.1 %), to allow neutralization of at least 2 mg/l and up to 5 mg/l of free chlorine, depending on the neutralization dynamics [NM ISO 19458, 2009].

The bacteriological analysis and interpretation of the sampled water was carried out in the public health laboratory at the delegation of Health Ministry of Taza according to the Moroccan standards NM 03.7.001 [Ministry of

Health, 2006] reported in Table 1. Moreover, among the exclusion criteria of this study, the toxic risk is underlined. It is certain that the water used in a health care institution is contaminated. The risks for the exposed population are of two types: the infection risk, which is the subject of this study, and the toxic risk, which is excluded from the study; because the presence of toxic substances appears more rarely in the case of dissolution of the pipe materials.

The reading and interpretation of the results were carried out following incubation. After carrying out the enumeration, the aspect and the size of the colonies found were determined; then, their purification was carried out on nutritive agar medium. The identification of the isolated germs was carried out by biochemical tests and their confirmation was performed by the API gallery. For the evaluation of the isolated germs resistance to antibiotics, these germs were tested on 27 antibiotics according to the agar diffusion method. The analysis and interpretation of strains susceptibility to antibiotics is based on a document of the French Society of Microbiology 2019 [Jehl et al., 2019].

RESULTS AND DISCUSSION

Water can carry many germs, sometimes causing serious infections in patients and health professionals, and drinking water should normally be free of bacteria. Therefore, it is essential to be familiar with the internal distribution of water within the institution in order to limit the risk of water-related infections.

Distribution and enumeration of germs isolated from network water according to the Moroccan standards

In the conducted study, the 72 water samples analyzed were of good bacteriological quality. The results obtained in Table 2 showed the absence of *Pseudomonas aeruginosa*, spores of sulfite-reducing anaerobic microorganisms, *Escherichia coli*, and coliforms in all the water points of the studied services on one hand. On the other hand, intestinal enterococci were observed in the woman's surgery service and absent in the other services. Likewise, microorganisms revivable at 37 °C were present in the sterilization service water point and absent in the other services. Moreover, the presence of microorganisms revivable at 22 °C

Table 1. Methods of bacteriological analysis of water according to the Moroccan standards NM 03.7.001

Micro-organisms researched	Bacteriological method	Incubation	Interpretation criteria
<i>Escherichia coli</i>	ISO 9308-1 - Membrane filtration		0/100 ml
	- Lactose TTC and Sodium Heptadecyl Sulfate Agar	(36 ± 2) °C during (21±3) h	
	«Indole production» test: incubation of typical colonies in tryptophan broth	(44 ± 0.5) °C during (21± 3) h	
	«Oxidase» test: incubation of typical colonies on tryptone soy agar - TSA	(36 ± 2) °C during (21 ± 3) h	
Colony Count: Oxidase - and indole +			
Intestinal enterococci	ISO 7899-2 Membrane filtration		0/100 ml
	Slanetz and Bartley Medium	(36± 2) °C during (44 ± 4) h	
	Aesculin Azide Bile Medium	(44 ± 0.5) °C during (2) h	
	Enumeration of Typical Colonies		
Coliforms	ISO 9308-1 Membrane filtration		0/100 ml
	Lactose TTC and Sodium Heptadecyl Sulfate Agar	(36 ± 2) °C during (21 ± 3) h	
	«Oxidase» test: incubation of typical colonies on tryptone soy agar - TSA	(36 ± 2) °C during (21 ± 2) h	
	Enumeration of Typical Colonies		
Spores of sulfite-reducing anaerobic microorganisms	ISO 6661- 2 Detection and enumeration of the spores of sulfite-reducing anaerobic microorganisms (Clostridia) Membrane filtration		0/100 ml
	Sulfite-Iron-Agar Medium	(37 ± 1) °C during (20 ± 4) h and (44 ± 4) h	
	Enumeration of Spores		
Revivable microorganisms at 22 °C and 37 °C	ISO 6222		20/1 mL à 37 °C 100/ 1 mL à 22 °C
	Colony Counts by Inoculation in Agar Culture Medium	Aerobic incubation at (36 ± 2) °C during (44 ± 4) h and (22 ± 2) °C during (68 ± 4) h	
	Colonial Enumerations		

Table 2. The bacterial load average in the different sites analyzed during the year 2018-2019

	Revivable microorganisms at 22 °C CFU/1 ml	Revivable microorganisms at 37 °C CFU/1 ml	Coliforms CFU/100 ml	Escherichia Coli CFU/100 ml	Intestinal enterococci CFU/100 ml	Spores of sulfite-reducing anaerobic microorganisms (clostridia) CFU/100 ml	Pseudomons aerogenosa UFC/100 ml
P1	6	0	0	0	1	0	0
P2	4	0	0	0	0	0	0
P3	3	2	0	0	0	0	0
P4	0.5	0	0	0	0	0	0
P5	3	0	0	0	0	0	0
P6	2	0	0	0	0	0	0

P1: Woman surgery department; P2: Man surgery department; P3: Sterilization room; P4: Central operating room, P5: Gynecology department; P6: Child surgery department

was noticed. Even though these values remain well below the limits of acceptability, except for intestinal enterococci which were slightly higher than the norms in the women surgery service (1UFC/100ml), this means that 98% of the water points analyzed remain within the acceptability standards according to the Moroccan Standard 03.7.001, and to WHO [Lenntech, 2020].

These results are in contradiction with those obtained in other studies carried out in the Ibn Sina University Hospital in Rabat, Morocco [Chiguer, 2013] and in a provincial hospital in Fez (Morocco) [Bekkari et al., 2016] which found a minimum value of Total Germs of 0 CFU/100 ml and a maximum value higher than 10^6 CFU/100 ml. Furthermore, the obtained results are similar to those found in a hospital in Algeria [Amine et al., 2019] and another one in Mali [Coulinaly, 2019], which reported that none of the analyzed water samples were contaminated and that they met the drinking water standards.

Distribution of germs isolated from mains water

Concerning the biochemical identification of microorganisms isolated in the studied water points, the presence of *Aeromonas salmonicida* spp *salmonicida*, *Enterococcus* spp, *Pseudomonas luteola*, *Sphingomonas paucimobilis*, *Pseudomonas stutzeri*, and *Pseudomonas luteus* and *Stenotrophomonas maltophilia*, and *Burkholdria cepacia* was highlighted. Some of these bacteria are also found and isolated from the hospital environment that could be the source of serious hospital-acquired infections [Lalami et al., 2016].

The frequency of isolated bacterial strains varies from one health care unit to another (Table 3), due to the presence of biofilms and the adaptation of the strains to various conditions. In order to better understand the risk of these strains on humans, a bibliographical research was carried out on the different germs isolated.

Aeromonas salmonicida spp *salmonicida*, is a species of Gram-negative bacteria of the *Aeromonadaceae* family, which have the capacity to cause skin infections resembling a necrotizing fasciitis [Vincent et al., 2019]. *Enterococcus* spp. are commensal Gram-positive bacteria, and are important opportunistic pathogens that not only form biofilms on implanted catheters, and medical devices, but also cause urinary tract infections, bacteremia, endocarditis, burns and wounds at the operating site, abdominal and biliary tract infections [Garcia-Dolache et al., 2019].

Pseudomonas luteola is a Gram-negative bacterium of the genus *Pseudomonas*, which has been implicated in a variety of life-threatening infections such as: endocarditis, peritonitis, meningitis, septicaemia and cerebral abscess [Gaschet et al., 2009]. Similarly, *Pseudomonas stutzeri* is a Gram-negative bacterium of the *Pseudomonas* genus, which is also mobile. This bacterium, ubiquitous in the environment, is involved in human diseases; hence, the patients infected with *Pseudomonas stutzeri* usually present predisposing risk factors such as immune-suppression or recent surgery [Halabi et al., 2019].

In addition, *Sphingomonas paucimobilis*, of the family *Sphingomonadaceae*, is a strict aerobic Gram-negative bacterium, not very mobile with a single polar flagellum and causes bacteraemia

Table 3. Spatio-temporal distribution of isolated bacterial species

Bacterial species	Surgery department						Month
	P1	P2	P3	P4	P5	P6	
<i>Aeromonas salmonicida</i> spp <i>salmonicida</i>	+	-	-	-	-	-	November
<i>Enterococcus</i> spp	+	-	-	-	-	-	November
<i>Pseudomonas luteola</i>	+	-	-	-	-	-	December
<i>Sphingomonas paucimobilis</i>	-	-	+	-	-	-	December
<i>Pseudomonas stutzeri</i>	-	+	-	-	-	-	January
<i>Stenotrophomonas maltophilia</i>	-	+	-	-	+	-	January April
<i>Burkholdria cepacia</i>	-	+	-	-	+	-	March
<i>Micrococcus luteus</i>	-	-	-	-	-	+	March

P1: Woman surgery department; P2: Man surgery department; P3: Sterilization room; P4: Central operating room, P5: Gynecology department; P6: Child surgery department

and septicaemia; therefore it is responsible for nosocomial pneumonia with potential mortality [Tai MI and al., 2014].

Stenotrophomonas maltophilia is a species of aerobic Gram-negative bacteria, belonging to the *Stenotrophomonas* genus, which can cause a broad spectrum of potentially fatal infections and which is found in the environment as a commensal and in hospital settings as an opportunistic pathogen in immuno-compromised patients or a true pathogen in immune-competent patients. It is consistently implicated in bacteraemia skin infections, endocarditis, acute respiratory tract infection and meningitides [Adegoke, 2017].

In turn, *Burkholderia cepacia*, which belongs to Gram-negative bacteria, are important opportunistic pathogens that can cause variable lung infections in the patients with cystic fibrosis, resulting in asymptomatic carriage, chronic infection or ‘cepacia syndrome’, which is characterized by a rapid decline in lung function that may include invasive disease [Mahenthalingam et al., 2005],

Finally, *Micrococcus luteus* is a Gram-positive bacterium, of the family Micrococcaceae, which is an opportunistic pathogen for nosocomial infections in immunocompromised patients [Lee et al. 2020]. This species can occur in a wide variety of infections in any area of the human body, such as bacteraemia, endocarditis, ventriculitis, peritonitis, pneumonia, endophthalmos, keratolysis and septic arthritis [Han et al., 2016].

Assessment of antibiotic resistance

Evaluation of the susceptibility profile of the isolated germs, to 27 antibiotics tested (Table 4) showed that all strains have been resistant to Ticarcilin (TIC) from β -lactam. Moreover, *Aeromonas salmonicida* spp *salmonicida* is resistant to some β -lactam antibiotics such as Cefixime (CFM), Ceftriaxone (CRO), Cefoxitin (FOX), Piperacillin (PRL), and the cephalosporin family (Table 4). This resistance is similar with the results of a study conducted by Valdes et al. [2015] and Yang et al. [2019]. However, it is sensitive to Aminoglycoside, Penicillin, Phenicol, Fluoroquinolone, Macrolide, Fusidamide, Lincosamide, Quinolone, Rifamycin, Sulfamide, Cycline, Glycopeptide and other antibiotics from β - lactamine family as Cefalexin (CN), Cefotetan (CT), Cefotaxim (CTX), Imipenem (IPM). Similar results were reported by various authors [Didugu, 2016].

Regarding *Enterococcus spp.*, it was found resistant to Cephalosporin such as Ceftazidime (CAZ), β - lactamine (Cefixime), and glycopeptide (Teicoplanine) and sensitive to other antibiotics. *Pseudomonas luteola* is resistant to penicillin (Amoxicillin and amoxicillin with clavulanic acid, piperacillin), cephalosporine (Ceftazidime, Cefalotin), Lincosamide (lincomycin) and glycopeptide and sensitive for other families such as β - lactamine, macrolides, etc. Those results are contradictory with a study carried out in the

Table 4. Results of sensitivity and resistance to the applied antibiotic discs

Gensu	Antibiotic resistance type	Antibiotic sensible type
<i>Aeromonas salmonicida</i> spp <i>salmonicida</i>	CAZ ;CFM; CRO; FOX; KF; OX; TIC	AK; AMC; AX; C; CIP; CN; CT; CTX; E; F; IPM; L; NA; OFX; PRL; RA; SXT; TE; TOB; TEC
<i>Enterococcus</i> spp	CAZ; CFM; TIC; TEC	AK; AMC; AX; C; CIP; CN; CRO; CT; CTX; E; F; FOX; IPM; KF; L; NA; OFX; OX; PRL; RA; SXT; TE; TOB ;
<i>Pseudomonas luteola</i>	AMC; AX; CAZ; KF; L; PRL; TEC; TIC	AK; C; CFM; CIP; CN; CRO; CT; CTX; E; F; FOX; IPM; NA; OFX; OX; RA; SXT; TE; TOB;
<i>Sphingomonas paucimobilis</i>	E; KF; TEC; TIC	AK; AMC; AX; C; CIP; CN; CAZ; CFM; CRO; CT; CTX; F; FOX; IPM; L; NA; OFX; OX; PRL; RA; SXT; TE ; TOB;
<i>Pseudomonas stutzeri</i>	AMC; AX; E; TIC	AK; C; CAZ; CFM; CIP; CN; CRO; CT; CTX; F; FOX; IPM; K; L; NA; OFX; OX; PRL; RA; SXT; TE; TOB; TEC
<i>Stenotrophomonas maltophilia</i>	AMC; AX; CAZ; CFM; CRO; CTX; E; FOX; IPM; KF; OX; PRL; TIC; TEC;	AK; C; CIP; CN; CT; F; L; NA; OFX; RA; SXT; TE; TOB;
<i>Burkholderia cepacia</i>	AMC; AX;CAZ; CRO; CTX; E; F; FOX; KF; L; OX; TIC; TEC	AK; C; CFM; CIP; N; CT; IPM; NA; OFX; PRL; RA; SXT; TE; TOB
<i>Micrococcus luteus</i>	AK, E, SXT, TOB, TIC	AMC; AX; C; CIP; CN; CAZ; CFM; CRO; CT; CTX; F; FOX; IPM; KF; L; NA; OFX; OX; PRL; RA; TE; TEC

same context [Odjadjare, 2012]. *Sphingomonas paucimobilis* is resistant to Macrolide (Erythromycine), cephalosoprine (Cefalotine), and glycopeptide and sensitive for other antibiotics. *Pseudomonas stutzeri* is penicillin-resistant (Amoxicillne + Clavulanic Acid, amoxicilline), resistant to Macrolide (E), and cephalosporin (TIC), but sensitive to other antibiotics.

In addition, *Stenotrophomonas maltophilia* is resistant to Penicillin (Amoxicillne + Clavulanic Acid, Amoxicillin, Piperacillin), Cephalosporin (Ceftazidime, Cefalotine), and β -lactams (Cefixime, Ceftriaxoplon, Ceforyefillory).

Regarding *Burkholdria cepacia*, it is resistant to penicillin (Amoxicillne + Clavulanic Acid, amoxicilline), cephalosporin (Ceftazidime, Cefalotine), β -lactamine (Ceftriaxone, Cefotaxime, Cefoxitine, Oxacilline), Fusidamine, Macrolide, lincosamide TEC. These results are similar with another study concerning resistance to Ceftriaxone but contradictory for sensitivity to Ciprofloxacin [Rastogi et al., 2019].

Finally, *Micrococcus luteus* is resistant to Amikacine, Erythromycine, Triméthoprime-Sulfametoxazole, Tobramycine and sensitive to the other antibiotics.

CONCLUSIONS

This study aimed to determine the water bacteriological quality from the network of the provincial hospital of Ibn Bajja Taza (Morocco) in various surgery departments. For this purpose, 72 samples were taken according to the Moroccan standards NM 3.07.001. The samples showed compliance with regard to enterococci, intestinal coliforms, and revivable germs in addition to the absence of *Escherichia coli* and *Pseudomonas aeruginosa*. Therefore, the results of the bacteriological analysis show that this hospital's network waters are of good quality according to Moroccan standards NM 3.07.001 and those of the WHO. However, the identification of isolated germs and the evaluation of their sensitivity to antibiotics revealed that they present a major risk for hospitalized patients and healthcare professionals, given their multi-resistance to several antibiotics on the one hand, and clinically dangerous effects for humans on the other hand. Identification of the isolated germs revealed the presence of bacteria of environmental and human origins, such as *Aeromonas salmonicida* spp. salmonicida,

Enterococcus spp., *Pseudomonas luteola*, *Sphingomonas paucimobilis*, *Pseudomonas stutzeri*, *Pseudomonas luteus*, *Stenotrophomonas maltophilia*, and *Burkholdria cepacia*, according to the different studied departments.

Therefore, it is necessary to establish a regular supervision system to control the critical water points, then to carry out periodic microbiological analyses for the identification and isolation of opportunistic bacterial strains in order to avoid exceeding an alert threshold by implementing the actions to eliminate and reduce the severity of water contamination. This will allow total or partial control of the contamination risks and dangers.

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