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Physicochemical, Bacterial and Antibioresistance in Wastewater Ibn Sina Hospital, Rabat, Morocco

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ABSTRACT

Hospital waste water contain a various elements discharged without treatment, could have an impact on the receiving environment. This study has shown that the temperature, pH and conductivity (21°C, 7 and 1000 uS/cm, respectively) are in the range of Moroccan standards. However, the COD, BOD5, SSM and VSM (750 mg/l and 352 mg / l 320 mg / l and 235 mg / l) are significantly higher than the specific limits on Moroccan urban liquid wastewater standard. The bacterial; TAMF, TC, FC and FS showed high concentrations, 10.8, 9.5, 8.6 and 7.9 ULog10, respectively compared to Moroccan standards. The ratios, especially CF/SF and COD/BOD5 respectively about 1.1 and 2.12 confirms the uncertainty of the origin of these waters and there low biodegradability. Among the eleven identified bacteria, Morganella and Providencia presented significant frequencies of antibioresistance whereas Escherichia and Staphylococcus showed low frequencies of antibioresistance in these wastewaters.

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INTRODUCTION

The Hospitals discharge a high volume of wastewater, with variable physicochemical composition, including chemicals, pharmaceutical toxic substances, radioactive elements and pathogenic microorganisms [1], L.Ferrando-Clement and [2]. Moreover, the volume of wastewater from these hospital formations varies from 400 to 1200 liters/bed/day (l/bed/day) Deloffre-Bennamour [3]; CCLIN Paris-Nord, [4]. Thus, water consumption by American hospitals is of the order of 968 l/bed/day [5] while in the French university hospitals this volume is estimated to 750 l/bed/day [6]. Mean while in developed countries the consumption seems to be arround 500 l/bed/day [7]. However the minimum domestic water consumption is about 100 l/capita/day [8].

This high volume which contains a many variable substances could generate ecological imbalances in the receiving environment [9],[1]; SIPIBEL [10]. The complexity of the effluent quality is mainly due to the use of chemicals and pharmaceuticals Tsakona [11], Huijie and [12]. L.Ferrando-Clement [2]. Several studies have shown that microorganisms may be unable to degrade these drugs (Jorgensen and Halling - Sorensen, [13]; Alexy *et al*., [14]; Carballa *et al*, [15] that can be detected in water samples, sediment and sludge in rivers and oceans [16]; Calamari *et al*., [17]; Lofter *et al*, [18], Christian *et al*, [19].

In Morocco a major wastewater volume is rejected by the rural and urban areas. Thus, in the cities the hospitals contribute to increase this volume of water discharged.

The aim of this study is the focus on the bacteriological and physicochemical characterization of liquid discharged by Ibn Sina hospital, Rabat, Morocco and, in the other hand, to collect the national reference

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elements for hospital liquid discharges. Furthermore, the screening of bacteria and their antibiotic resistance profile in these effluents during the study period proves to be of a great importance.

Methodology:

1 - Study Site:

The wastewater samples were taken from two outlets 1 and 2 which are connected to the services listed in the table below (table 1) of Ibn Sina hospital located in Rabat city in Morocco.

Table 1: Hospital Services flowing into the outlet 1 and outlet 2

Outet 1	Outlet 2
Laboratories (Anatomopathologie/transfusion/ hematology / Biochemistry/Bacteriology) A Urology / B Urology, B Medicine, Medical resuscitation / surgery resuscitation, Nephrology/Dialysis, Cardiovascular surgery, A medicine, pulmonology, Thoracic Surgery, Dermatology, Prison medicine, kitchen	Emergency medical doors / surgical emergency doors Block: Cardiac surgery, Endocrinology, Neurosurgery, Traumatology Sterilization, C Medicine, Plastic Surgery, Home nursing, Cafeteria

2 - Physicochemical sampling and analysis methods:

Composite samples taken every 2 hours were collected and analyzed once a month during year 2011-2012. Furthermore, parameters were measured on site, including temperature (T °C), conductivity (Cd), the pH. Concerning the physicochemical analyzes, especially, Suspended Matter (SSM), Suspended Volatile maters (SVM), Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD_5), Total Kjeldahl Nitrogen (TKN), Nitrate (NO_3) and total Phosphorus total (Ptot), composite samples of 24 hours, were fixed, carried and analyzed according to the AFNOR standards in the laboratory of Water Quality of the National Office of Drinking Water (NODW), Rabat, Morocco.

3 - Microbiological sampling and analysis methods:

Microbiological parameters were collected at noon in sterile bottles and immediately transported in a cooler a temperature less than 4 °C to the laboratory of the Faculty of Medicine and Pharmacy of Rabat within Mohammed V University - Souissi. Moreover, the counting of Total Aerobic Mesophilic Flora (TAMF), Total Coliforms (TC), Fecal Coliforms (FC) and Fecal Streptococci (FS) were performed according to the French standard (NF, ISO 7218, [20]) by the method of multiple tubes (MPN : Most Probable Number) using three tubes per dilution. The results expression of bacterial parameter was given by the Mac Grady table MPN/100 ml (in the text the results are expressed as $Ulog_{10}$).

The identification and research of pathogens were performed according to the conventional method by isolation on selective medium and biochemical environments identifications (Orndorf and Colwell, [21], LeMinor and Richard, [22]). This identification was confirmed by the API tests and BD-phoenix. The Antibiograms tests were performed according to the method of flooding on Müller Hinton medium.

4 – Statistical Analysis:

The statistical treatment of analytical data was performed according to the Student test using SPSS software. This test was applied to the annual averages of the parameters measured every month in two outlets 1 and 2 liquid discharges from the Ibn Sina hospital.

RESULTS AND DISCUSSIONS

1. Physicochemical characterization:

The results obtained in this study show a very fluctuating average values compared to other hospital units (Table2). Thus, the average grades in these two releases are around 21° C, 7 and 1000µs/cm, respectively, for the temperature (T°C), pH and electrical conductivity. The average concentrations of COD, BOD_5, SSM and SVM are respectively around of 750 mg / l and 352 mg / l 320 mg / l and 235 mg / l, values much higher than the Specific Limits Moroccan Values for urban liquid wastewater. The analysis of this table shows that even if the values found are low compared to other international hospital training, the COD/BOD_5 calculated ratio is 2.13 in Morocco is highly superior to reports found internationally which is about 1.6 in France and 1.4 in India and 1.9 in Brazil (Emmanuel *et al*, [23]; Gautam *et al*, [1]; J.Berto *et al*, [24]). This can only be explained by the

particular specificity of the typology of these effluents which are typical urban household. This result shows that these effluents could be biodegradable (NODW-RECMH, [25]; NODW-GTZ, [26]; NDSCLS, [27]).

Table 2: Comparison of the annual average of the physicochemical quality of the effluent from Ibn Sina Hospital in Rabat and the hospitals in some countries in the world.

Parameters	HUI, Rabat, Maroc (2013)	France (2005)	Inde (2007)	Brazil (2009)
T°	21	----	----	----
pH	7	8.8	----	7.2±0.5
Cd.....(µs/cm)	1000.8	----	----	----
BOD_5.....(mg/l)	352	1559	603	1268±155
COD.....(mg/l)	750	2516	855	2480±413
SSM.....(mg/l)	320	----	225	546.7 ±64.8
SVM.....(mg/l)	235	----	----	----
TKN.....(mg/l)	61.5	----	----	85.5 ±14.8
NO3.....(mg/l)	1	----	----	----
Tot P.....(mg/l)	7.6	----	8.8	28.6 ±7.2

Compared with Moroccan standards including Specific Limits of Domestic Wastewater Values (LSVDD, [28]) stipulated by the Joint Order No. 1607-1606 of 25 July 2006, the average annual values of global pollution parameters expressed in terms of BOD5, COD and SSM are significantly higher (table 3). These average values for the parameters mentioned above are about 2.9, 2.5 and 2.13 higher than the values of LSVDD for urbain wastewater discharge and 1.17, 1.25 and 1.28 higher than LSVDD for urbain existant wastewater discharge.

Table 3: Comparison of BOD_5, COD, SSM effluent from the Ibn Sina hospital in Rabat and Moroccan standards (LSVDD, 2006).

Paramètres	HUI, Rabat, Maroc (2013)	LSVDD (urbain wastewater discharge)	LSVDD (existant urbain wastewater discharge)
BOD_5...(mg/l)	352	120	300
COD.....(mg/l)	750	300	600
SSM.....(mg/l)	320	150	250

2. Microbiological characterization:

Figure 1, illustrates the evolution of the monthly concentrations of TAMF, TC, FC and FS, very large fluctuations were recorded for all microbiological parameters, and, depending on the month of the year. Thus, low values of the order of 7.02 $Ulog_{10}$, 4.11 $Ulog_{10}$ and 3 $Ulog_{10}$, respectively, for the FMAT, CF and SF, were recorded during the months of January and February when the temperature of the water is 16 ° C. Meanwhile, very high values were recorded during the months of July and August and can reach 13.54 $Ulog_{10}$, 12.62$Ulog_{10}$ 11.78 $Ulog_{10}$ and during this period where the temperature can reach 24 °C. Indeed, the comparison of averages between the summer season and winter season means of the Student t-test showed that the difference is significant for all microbiological parameters to a threshold of 95%.

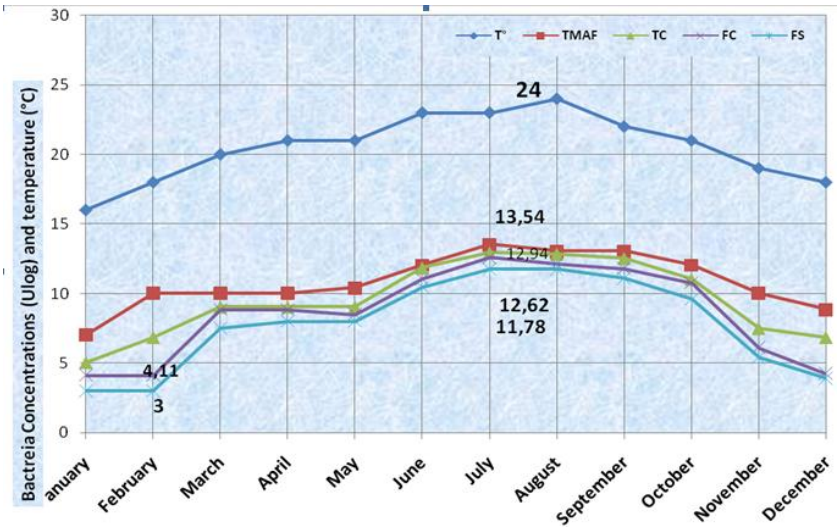

Fig. 1: Evolution of the water temperature and monthly concentrations MTAF, CT, CF and SF in the effluent from, Ibn Sina hospital, Rabat.

The microbiological quality of the annual average through the enumeration of Total Aerobic Mesophilic Flora (TAMF) showed very high values of the order of 10.8 $Ulog_{10}$. In addition, Total Coliforms (TC) , Fecal

Coliforms (FC) and Fecal Streptococci (FS) have shown the values of the order of 9.5, 8.6 and 7.9 $Ulog_{10}$ respectively (Figure 2).

Moreover, the comparison of the concentration of fecal coliforms in these waters which is around 8.6 Ulog10 is slightly higher than those found by Berto *et al*. [24], which is 8.3 $Ulog_{10}$. Also this the concentration of the bacterial flora expressed in terms of FC (8.6 $Ulog_{10}$) is significantly higher compared to that found by (Emmanuel *et al*. [23] with a value of 3.38 $Ulog_{10}$ found by Leaprat [6]) and 5.48 $Ulog_{10}$ announced by Bernet and Fine [29]). Moreover, these results are comparable similar to the value that has been found at the municipal rejection which is 8 $Ulog_{10}$ stipulated by Metcaf and Eddy [30].

The quantitative bacteriological analysis expressed by the yearly ratio of the average CF / SF which is about 1.1 value included in the range of Borrego and Romero [31], which allows to conclude that these waters are of uncertain origin. But, the anticipated results of this hospital should be higher than 2 because these care formations are typically liquid discharges of human origin. Then, these fluctuations could be attributed to higher values of FS maintained that could be explained both by the resistance of these pathogens in the water environment and by the proximity of the sampling point.

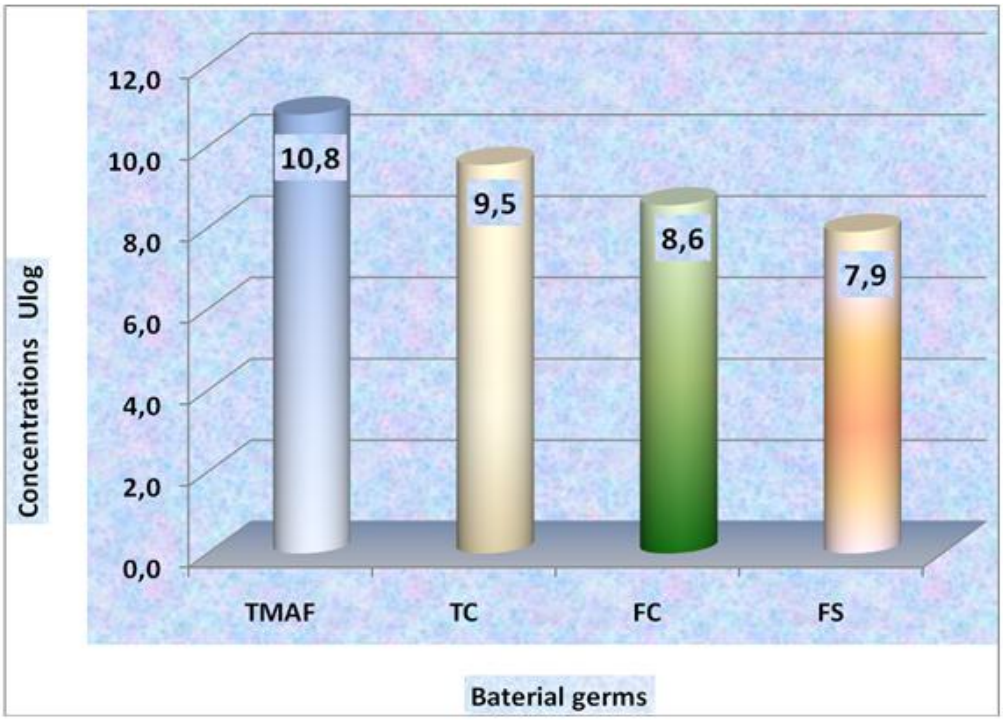

Fig. 2: Average annual concentrations of TAMF, TC, FC and FS in the effluent of the Ibn Sina Hospital, Rabat

Table 4: Bacterial species found in the effluent from the Ibn Sina Hospital in Rabat.

Espèce	Famille	Caractéristique de l'espèce
Morganella moganii	Enterobacteriaceae	Bacille, Glucose+, gram-, non fermentant
Providencia rettgeri	Enterobacteriaceae	Bacille, Glucose+, gram-, facultatif aerobie
Enterococcus faecalis	Enterococcaceae	Cocci, gram+
Acinetobacter baumannii	Moraxellaceae	Coccoides, gram-, aerobie, microaerophile
Pseudomonas aeruginosa	Pseudomonadaceae	Bacille gram-, oxydase +, aerobie,
Proteus mirabilis	Enterobacteriaceae	Bacille, Glucose+, gram-, facultatif aerobie
Klebsiella pneumonia	Enterobacteriaceae	Bacille, Glucose+, gram-, facultatif aerobie
Enterobacter colacae	Enterobacteriaceae	Bacille, Glucose+, gram- , facultatif aerobie
Proteus vulgaris	Enterobacteriaceae	Bacille, Glucose+, gram-, facultatif aerobie
Echerichia coli	Enterobacteriaceae	Bacille, Glucose+, lac+, gram- , facultatif aerobie
Staphylococcus aureus	Staphylococcaceae	Cocci, gram+, catalase +

3. Diversity of the bacterial population and antibiotic resistance profile:

Bacterial species found in the effluent from the Ibn Sina hospital are about 11 species, most of them are the bacilli belonging to the Enterobacteriaceae family which are gram negative, oxidase negative except *pseudomonas* which is oxidase positive (Table 4). The Gram positive bacteria were also encountered including *Enterococcus faecalis* and *Staphylococcus aureus* belonging to the family of the Enterococcaceae and Staphylococcaceae respectively (Carlos N. [32]).

The similar results were found in Brazil with about 20 species among which7 are highly pathogenic (J.Berto [24]).

The analysis of the antibiotic resistance bacteria profile found in the effluent of the Ibn Sina hospital has no special resistance or sensitivity in the water environment compared to that commonly found in the literature (Table 5). This profile showed that among the 11 identified bacteria, *Morganella morganii*, *Providencia rettgeri* showed significant frequencies of resistance to antibiotics. In addition, *Escherichia coli* and *Staphylococcus aureus* showed low frequencies of resistance to commonly used antibiotics. Then, studies on the antibiotic resistance of bacteria in wastewater some of hospitals showed genetic resistance (Iversen *et al*., [33]; Fuentefria *et al*, [34], Tumeo *et al*, [35], Yang *et al*, [36]). Thus, studies of the resistance of *Pseudomonas aeruginosa* in liquid discharges from two hospitals located in Rio Do Sul, Brazil hospitals showed a high resistance to wastewater strains multidrug resistance genotype of these strains (Fuenteria *et al*., [37]).

Table 5: Antibiotic resistance of bacteria found in the effluents from the Ibn Sina Hospital, Rabat, Morocco.

		Morganella Morganii	Providencia rettgeri	Enterococcus faecalis	Acinetobacter baumannii	Pseudomonas aeruginosa	Proteus mirabilis	Klebsiella pneumonia	Enterobacter colacae	Proteus vulgaris	Echerichia coli	Staphylococcus aureus
Penicillines A	Amp	R	R	S	R	--	R	R	R	R	R	R
	Amx	R	R	S	R	--	R	R	R	R	R	R
	Amx+ ac clavu	R	R	S	R	--	R	R	R	S	R	--
	Amx + Slbactam	R	R	R	R	R	R	R	R	S	R	S
Penicillines M	Oxacillin	--	--	--	--	--	--	--	--	--	--	S
Carbapenemes	Imp	S	S	--	S	S	S	S	S	S	S	--
Cephalosporines	Ceftriaxone	S	S	--	R	R	S	S	S	S	S	S
	Ceftazidime	S	S	--	S	R	S	S	S	S	S	S
Aminosides	Ami	S	R	R	S	S	S	S	S	S	S	S
	Gen	S	R	R	S	S	S	S	S	S	S	S
Sulfamides	Sulfamide + Trimethoprime	R	--	R	R	R	S	R	--	--	S	S
Phenicoles	Chlora	S	S	S	--	R	S	--	--	--	--	S
Macrolides	Eryt	--	--	S	--	--	--	--	--	--	--	S
Quinolones	Ciproflo	R	S	R	S	S	--	S	S	S	S	S
	Ofloxacine	R	S	R	--	S	--	S	S	S	S	S
Divers	Colistine	R	R	--	S	S	R	S	S	R	S	--
	Ac fusi	--	--	--	--	--	--	--	--	--	--	S

Conclusion:

The outcome of this study shows that the average concentrations of the year at the two outlets on the parameters studied, temperature (T ° C) , pH and conductivity are respectively about 21 ° C , 7 and 1000 us / cm. The physicochemical analysis expressed in terms of COD, BOD_5, SSM and SVM respectively 750 mg / l, 352 mg / l, 320 mg / l and 235 mg / l significantly higher than Moroccan Specific Limits on Urban Liquid Waste. These physicochemical concentrations are low compared to the values found in wastewater that some international hospital units. Indeed, regarding the COD/BOD_5 ratio showed values in the range of 2.13 showing that these waters are typical urban household with the lower limit of biodegradability.

Microbiological analysis expressed in terms of TAMF, TC, FC, and FS respectively 10.8, 9.5, 8.6 and 7.9 $ULog_{10}$, very high compared to national norms values and to those found in on the international scale. Moreover, the quantitative bacteriological analysis expressed by the ratio of CF/SF which is about 1.1 stipulating an uncertain origin and those fluctuations could be attributed to high values of SF . This statement could be explained by the resistance of these bacteria group in the water environment and the proximity of the point of sampling.

The antibiotic resistance profile showed that among the 11 identified bacteria, *Morganella morganii, Providencia rettgeri* showed significant frequencies of resistance to antibiotics. However, *Escherichia coli* and *Staphylococcus aureus* showed low frequencies of resistance to commonly used antibiotics.

So finally to better understand the quantitative and qualitative fluctuations in the bacterial diversity found in this hospital, further studies by means of the molecular biology of resistance of these organisms must be deeper.

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