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Prevalence, diversity and antimicrobial resistance of *Salmonella enterica* and *Pseudomonas aeruginosa* isolates from spring water in a rural area of northwestern Morocco

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Abstract. *Nassri I, Tahri L, Saidi A, Ameur N, Fekhaoui M. 2021. Prevalence, diversity and antimicrobial resistance of Salmonella enterica and Pseudomonas aeruginosa isolates from spring Water in a rural area of northwestern Morocco. Biodiversitas 22: 1363-1370.* The persistence and diversity of serotypes belonging to pathogens in environmental waters including surface and groundwater have been widely documented and that the aquatic environment represents a relatively stable environment for these microorganisms. Study was carried out on the prevalence, diversity, and antibiotic resistance of *Salmonella enterica* and *Pseudomonas aeruginosa* isolates taken from 102 samples of spring water in a rural region of Northwestern Morocco (Rabat-Salé-Zemour-Zaer) collected for two seasonal campaigns between March 2010 and June 2011. The search and identification of *S. enterica* and *P. aeruginosa* were carried out according to ISO 19250 and ISO 16266 methods respectively. The serotyping of *S. enterica* and *P. aeruginosa* was carried out according to the Kauffmann and White and IATS (International Antigenic Typing System) schemes respectively. Antibiotic resistance of *S. enterica* and *P. aeruginosa* were carried out by the Mueller-Hinton agar diffusion method (Biorad). *S. enterica* showed a prevalence of 10.7% with 09 different serotypes circulating including *S. paratyphi B, S. brandenburg, S. kentucky, S. group III b* (serotype 50: z52: z53,), *S. boon, S. tshiongwe, S. assinie, S. togo,* and *S. tanger*. In contrast, *P. aeruginosa* showed a prevalence of 21.6% with 07 different serotypes circulating including O6, O1, O7, O9, O4, O3, and O10. In this study, antimicrobial resistance revealed that all isolated strains of *S. enterica* and *P. aeruginosa* still exhibit a wild resistance phenotype. Contaminated water sources are reservoirs of these pathogens but do not yet present the risk factors for these bacteria to develop antibiotic resistance.

Keywords: Antimicrobial resistance, Northwestern Morocco, *Pseudomonas aeruginosa, Salmonella enteric,* spring water

INTRODUCTION

Salmonella enterica belonging to the family *Enterobacteriaceae* is considered one of the most important enteric pathogens in the world ((Xiong et al. 2020); (Mastrorilli et al. 2020)). *They* usually enter the environment via several animal feces, including mammals, reptiles, amphibians, and birds after colonizing their gastrointestinal tract (Andino and Hanning 2015). Environmental waters are contaminated by *S. enterica* via human sewage, urban and agricultural runoff, and wild and domestic animal excrement (Levantesi et al. 2012). These enteric pathogens can persist in the environment for years and can withstand periods of stress and nutrient depletion (Parker et al. 2010). Indeed, environmental factors such as rainfall, temperature, and seasonal variations are associated with the prevalence of pathogens or microbial indicators of fecal matter in water (Rodrigues et al. 2020). Moreover, there are more than 2600 *S. enterica* serotypes (Issenhuth-Jeanjean et al. 2014) but few of them have been isolated in the environment (Levantesi et al. 2012). The identification of *S. enterica* serotypes is important for understanding the environmental diversity of this kind (McEgan et al. 2014), measuring serotype trends over time in order to have information on emerging serotypes and their association with a particular host, assess the factors determining their presence and the risks associated with the incidence of water-borne diseases linked to this pathogen.

Besides, *P. aeruginosa* is a ubiquitous opportunistic pathogen belonging to the *Pseudomonadaceae* family. The species *P. aeruginosa* has 20 different serotypes from O1 to O20; however, commercially available reagents identify only 16 of the most common serotypes from O1 to O16. *P. aeruginosa* can adapt and thrive in many ecological niches such as water, soil, air, and vegetation due to its exceptionally high metabolic versatility using many organic compounds. *P. aeruginosa* is recognized for its ability to form or join biofilms (Bédard et al. 2016) and thus contributes to bio-corrosion which is a serious problem in many contexts, such as, oil and gas industry, marine environments, water services, and medical implants (Jia et al. 2017). In intensive care units, it is the leading cause of nosocomial infections (Cabrolier et al. 2014) with 30-50% contamination associated with water (Exner, 2012). Besides, clinical isolates do not differ from environmental isolates and have no specific habitat selection. *P. aeruginosa* serotyping is the first screening for epidemiologically related strains and allows the study of the prevalence of specific serotypes and their resistance to antibiotics.

In Morocco, few studies have been conducted to investigate the prevalence, diversity, and antibiotic resistance of pathogenic strains isolated from groundwater in the absence of any sources that could introduce the character of antibiotic resistance. To our knowledge, there are no reports or publications in the literature on the diversity and resistance of pathogenic bacterial species isolated from source waters in the Rabat-Salé-Zemour-Zaer (R-S-Z-Z: region in Northwestern Morocco).

This work is the first to investigate the prevalence, diversity, and resistance of all strains isolated from *S. enterica* as a representative of fecal pathogens and from *P. aeruginosa* as an opportunistic, ubiquitous pathogenic microorganism with a high potential to acquire resistance to antimicrobial agents.

MATERIALS AND MÉTHODS

Study sites and sampling

Northwestern Morocco (Rabat-Salé-Zemour-Zaer, RSZZ) is characterized by a geographical diversity defined by a limited to a narrow coastal strip (area of Rabat Salé) oceanic domain, a semi-continental area (Zemour) and a mid-mountain area (Zaer). The water samples were collected from 51 spring water during two hydrologic campaigns March 2010 and June 2011. The coordinates of each selected source were recorded using a GPS Explorist 400.

Physical parameters

The temperature of the water sources was made using a mercury thermometer graduated to 1/10 of a degree Celsius, pH measurements were made using a pH meter ORION Research, Ionalyser model 607 ORION model 91-05 with specific electrode.

Microbiological parameters

The microbiological parameters were analyzed in Microbiology Water Laboratory, Department of Microbiology Food and Hygiene at the National Institute of Hygiene, Rabat, Morocco.

Isolation and Identification

From each water sample, *S. enterica* was isolated and characterized according to method ISO 19250 :2010. The typical colonies were biochemically identified using the Galerie API 20E system (BioMerieux)

Pseudomonas aeruginosa was isolated and characterized according to method ISO 16266: 2006 The typical colonies were confirmed using King A Agar and King B Agar (Bio-life) incubated at 42°C for 24 h. The production of specific pigments allowed the differentiation of *P. aeruginosa* and other *Pseudomonas* spp. The preliminary biochemical characterization of the strains was confirmed by using Galerie API 20NE (Bio Mérieux).

Serotyping and agglutination

Serotype *S. enterica* was performed using the slide agglutination test with specific anti-sera raised against "O" and "H" antigens of *Salmonella* according to the Kauffman-White classification scheme. This scheme is based on determining which surface antigens are produced by the *Salmonella*. The "O" antigen is the first to be determined. These are polysaccharides associated with the lipopolysaccharide of the outer membrane. Then serotyping is followed by the determination of the "H" antigen. The "H" antigens are proteins associated with the bacterial flagella.

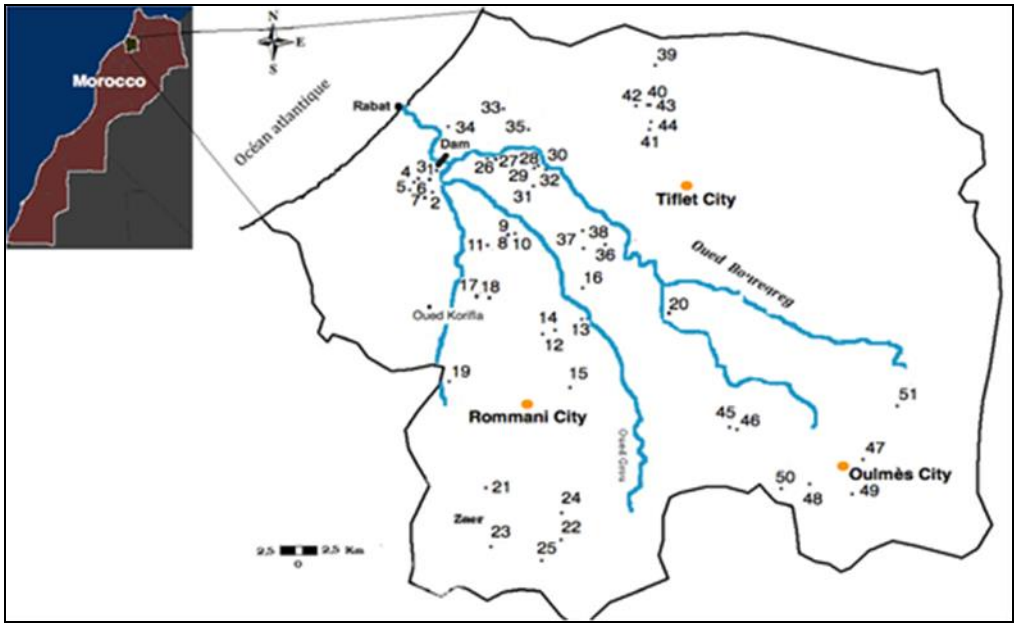

Figure1. Geographic location of survey points in Northwestern Morocco

The *P. aeruginosa* serotype was determined by slide agglutination test using polyvalent and monovalent antisera (Bio-Rad) according to the International Antigenic Typing Scheme (IATS). We first use four antisera polyvalent, then the monovalent antisera entering the composition of the polyvalent which gave agglutination; the 16 most frequent serotypes can be determined in this way (Bio-Rad).

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the disc diffusion method on Mueller-Hinton agar (MHA) and results were interpreted according to the EUCAST breakpoints (Committee of the French Society for Microbiology 2008 (CA-SFM)).

The *S. enterica* strains was screened to the fifteen antibiotics (Bio-Rad): Penicillins (Ampicillin: AMP 10 µg; Amoxicillin + clavulanate: AMC 30 µg); Phenicols (Chloramphenicol: C 30 µg); Sulfonamides-Trimethoprim (Trimethoprim/Sulfamethoxazole: SXT 25 µg; Trimethoprim: TMP 5 µg; Sulfonamides: SSS 200 µg); Quinolones (Nalidixic acid: NAL 30 µg); Tetracyclines: TE 30 µg; Aminosides (Amikacin: AN 30 µg; Gentamicin: GM 15 µg; Streptomycin: S 10 µg); 3rd generation Cephalosporins (Cefotaxime: CTX 30 µg, Ceftazidime: CAZ 30 µg, Ceftriaxone: CRO 30 µg); Fluoroquinolones (Ciprofloxacin: CIP 5 µg). *E. coli* ATCC 25922 was used as a quality control strain.

The isolated *P. aeruginosa* was screened for their resistance to the sixteen following antibiotics (Bio-Rad): Penicillins (Ticarcillin: TIC 75 µg, Ticarcillin / Clavulanic Acid: TCC 85 µg), Piperacillin: (Piperacillin: PIP 100 µg, and Piperacillin/Tazobactam: PTZ 10 µg), Cephalosporins (Cefotaxime: CTX 30 µg, Ceftazidime: CAZ 30 µg, Cefpirom: CPO 30 µg and Cefepim: FEP 30 µg); Aminoglycosides (Netilmicin: NET 10 µg, Amikacin: AN 30 µg, Tobramycin: TM 10 µg, and Gentamicin: GM 10 µg); a Monobactam (Aztreonam: ATM 30 µg), a Carbapenem (Imipenem: IPM 10 µg), a Polypeptide (Colistin: CS 10 µg) and a Fluoroquinolone (Ciprofloxacin: CIP 5 µg). The *P. aeruginosa* ATCC 27853 was used as a control reference strain.

RESULTATAND DISCUSSIONS

Physical parameters

The analysis of physical parameters shows water temperatures ranging from 19 °C to 25°C except for the source S50 which recorded a temperature of 42°C. The measured pH is generally neutrality with values varying between 6.4 (S24) and 7.79 (S18) with the exception of sources (S46, S22, S50, S23), which respectively show pH of 5.38; 5.5; 5.87 and 6.06.

Microbiological parameters

Salmonella enterica. was isolated in 12 samples (1x S8, 2 x S10, 2 x S18, 1x S19, 1x S21, 1x S28, 2 x S36, 1x S46, 1x S47) out of 102 samples of water from sources analyzed thus showing a prevalence of 10.7%. All strains are serotypable. 09 different circulating serotypes were identified from which virulent strains of *Salmonella* were isolated (*S. paratyphi B, S. kentucky, S. brandenburg*). The distribution of serotypes did not show a predominance of one serotype over another. Indeed, during the two companions *S. boon, S. tshiongwe* and *S. assinie* were isolated twice while *Salmonella brandenburg, S. togo, S. kentucky, S. group III b* (serotype 50: z52: z53), *S. paratyphi B* and *S. tanger* each have a single isolate. The generated results are shown in (Table 1).

The antimicrobial activity of *S. enterica* strains revealed a low level of resistance among all anti *Salmonella* drugs tested.

Pseudomonas aeruginosa was isolated in 22 samples out of 102 source water samples analyzed, for a percentage of 21.6% (Table. 2). 100% of the strains are serotypable and belong to seven serotypes, the most dominant of which is serotype O6. We identified 07 different circulating serotypes among which O6 (31.8%), O1 (27%), O7 (13.6%), O9 (9%) while serotypes O4 and O10 are isolated only one time during this study period. The distribution of serotypes showed a predominance of serotypes O6 and O1 over other serotypes. The generated results are shown in (Table 2). The antimicrobial activity of *P. aeruginosa* strains revealed a low level of resistance among all anti-*Pseudomonas* drugs tested.

Table 1. Distribution of serotypes of *Salmonella enterica* in Northwestern Morocco area during the two campaigns

	Survey points	2010			2011		
		pH	**T°**	**Serotypes (***S. enterica***)**	**pH**	**T°**	**Serotypes** (*S. enterica*)
Zaer	S8	6.63	22	*S. brandenburg*	6.76	25	-
	S10	7	23.1	*S. boon*	7.29	25	*S. tshiongwe*
	S18	7.79	21	*S. togo*	7.79	19.7	*S. boon*
	S19	7.5	20	-	7.93	21	*S. kentucky*
	S21	7.49	19.9	-	6.88	21	*S. groupe III b*
Sehoul-Tiflet	S28	7.26	19.9	-	7.34	23	*S. tshiongwe*
	S36	7.25	23	*S. assinie*	7.32	23.3	*S. assinie*
Oulmes	S46	5.86	20.8	-	5.38	22.4	*S. paratyphi B*
	S47	7	21.4	*S. tanger*	6.89	22.4	-

Table 2. Distribution of serotypes of *Pseudomonas aeruginosa* in Northwestern Morocco area during the two campaigns

Site	Survey points	2010			2011		
		Serotypes *P. aeruginosa*	pH	T°	Serotypes *P. aeruginosa*	pH	T°
Zaer	S 2	PMA P1 (O1)	6.8	20.5	PMA P1(O1)	7.15	24
	S 3	PME P4 (O4)	6.94	21	-	7.16	23.6
	S 6	-	6.99	21	PMC P10 (O10)	7.19	25
	S 10	-	7	23.1	PMA P6 (O6)	7.29	25
	S 15	PMF P7 (O7)	7.11	25.7	-	6.82	25
	S 16	-	7.25	22	PMA P1(O1)	7.3	25
	S 17	PMA P6 (O6)	7.3	21	PMA P6 (O6)	6.94	19.1
	S 19	PMA P6 (O6)	7.5	20	PMF P7 (O7)	7.93	21
	S 21	PMA P6 (O6)	7.49	19.9	PMA P3 (O3)	6.88	21
	S 23	-	6.12	20	PMA P1(O1)	6.06	21.8
Sehoul-Tiflet	S 32	PMA P3 (O3)	7.23	23.9	PMA P1(O1)	7.23	20.7
	S 36	PMC P9 (O9)	7.25	23	PMC P9 (O9)	7.32	23.3
	S 39	PMF P7 (O7)	7.21	24	PMA P6 (O6)	6.6	22.4
Oulmes	S 46	PMA P6 (O6)	5.86	20.8	PMA P1(O1)	5.38	22.4

The incidence of antibiotic resistance in *P. aeruginosa* was predicted to be greater than the. incidence in *S. enterica* isolated from the same waters. In contrast, it was observed that the resistance to the antibiotics tested in our isolates was very low both for P.aeruginosa and for *S. enterica*, thus showing the presence of strains of wild phenotype.

Discussion

The survey points in this area (Figure 1) identified 09 different circulating *S. enterica* serotypes, including *S. paratyphi B, S. brandenburg, S. kentucky, S. group III b* (serotype 50: z52: z53,), *S. boon, S. tshiongwe, S. assinie, S. togo*, and *S. tanger* (Table 1). The use of certain spring waters as a watering house favors their contamination by the feces of livestock and poultry. The latter has a very high level of *Salmonella* contamination and are frequently among the most important reservoirs for a variety of *Salmonella* serotypes transmitted to humans (Jackson et al. 2013; Andino and Hanning 2015; Wang et al. 2020).

Salmonella enterica contamination depends largely on its resistance to environmental factors that control its survival, and its ability to be carried by water when it moves in nature. In the present study, samples were collected during the relatively dry months as the majority of precipitation in the RSZZ region occurs in winter and the highest temperatures occur in summer. These samples showed a prevalence of *Salmonella* of 10.7% which may be on the rise during periods of precipitation because its abundance in the environment is strongly influenced by seasonal precipitation and water temperature. Indeed, this survival in these aquatic environments is achieved either by its entry into the viable but nonculturable (VBNC) state and/or as being a free-living protozoan (Liu et al. 2018). The presence of clinically important *S. enterica* serotypes in natural waters has been well documented, however, the isolation of serotypes in water follows the same trends as infection in humans and wildlife that are found in the same area suggesting a common local origin.

In our work, we demonstrated the presence of *S. paratyphi B* responsible for paratyphoid fever. The latter is common in less industrialized countries, mainly due to the problem of non-potable drinking water, inadequate effluent disposal, and flooding. *S. enteritidis, S. typhimurium*, and *S. typhi* are not among the serotypes isolated during our study period, although they represent the most frequently isolated serotypes from humans and animals (Alio Sanda, A et al. 2017). This might reflect the lower survival rates of these serotypes in these water samples, even though common hosts of these serotypes were present in the RSZZ region (cattle, poultry, and others) and these low rates are due to adverse or stressful conditions. The serotypes in our study can be classified according to the target animal species. Some are exclusively adapted to humans, mainly *S. paratyphi B*, an agent of paratyphoid fevers. The others (*S. kentucky, S. brandenburg, S. boon, S. tshiongwe, S. togo, S. group III b* (serotype 50: z52: z53), *S. assinie, S. tangier*) can cross the barrier of species. They are present in many animal species, usually latent or causing subclinical disease, and they can affect humans. However, analysis of the serotypes isolated in this study shows a mixed human and animal origin, thus highlighting their role in the contamination of these water sources.

Also, *S. kentucky* is a common inhabitant of the gastrointestinal systems of poultry, cows, and other animals, and is considered to be an occasional pathogen in humans (Haley et al. 2019). ST152 and ST198, are among the most frequently isolated and characterized *S. kentucky* sequence types (Alikhan et al. 2018). In regions of Africa, the Middle East, Europe, and South Asia, ST198 is frequently isolated from poultry and livestock, (Le Hello et al. 2011; Le Hello et al. 2013a; Le Hello et al. 2013b) and generally associated with human infections (Haley et al. 2019; Xiong et al. 2020).

Salmonella brandenburg has been a major cause of abortions in sheep (Gill et al. 2016), leading to a considerable economic impact on farmers. The potential risk of spreading the disease was sheep carrying the disease through their excreta and contaminated water sources. Indeed, when the cases of abortion on farms reach a peak, there will probably be heavy contamination of the environment and human exposure to the risk of infection.

Salmonella of group III, of which *S. group III b* (serotype 50: z52: z53,) is a part, is generally isolated from cold-blooded animals, especially reptiles and from the environment, but are only 'exceptionally the cause of pathological disorders in humans (Pui et al. 2011). Some studies show a high prevalence in the tonsils of sheep at the slaughterhouse (Bonke et al. 2012).

According to the literature, the serotypes: *S. tshionqwe, S. boon, S. togo, S. assinie*, and *S. tanger* are not among those most frequently isolated (Alio Sanda et al. 2017). To our knowledge, none of these species present any specific peculiarity and we can therefore content ourselves with reporting them without further comment.

In our study, the antimicrobial resistance of *S. enterica* showed that all the strains isolated show a wild phenotypic profile of resistance to the antibiotics tested. Our results coincide with the work of (Gorski et al. 2011) which showed the presence of a single isolate (*S. montevideo*) resistant to streptomycin and gentamicin on fifty-four strains sensitive to 12 antibiotics tested. In Morocco, our results are different from those of other studies that have presented a variable rate of antibiotic resistance of 45.2% of isolates from wastewater (Oubrim et al. 2012) against, 100% resistance in surface waters

like the Oued Khoumane (Ben Moussa et al.2014) and surface waters of the Hassan II dam (Chahboune et al. 2014). The resistance of *S. enterica* to antibiotics in these studies is due to the choice of the study area which has high contamination of *Salmonella* from the wastewater. On the other hand, there is a difference in the rate of resistance to antibiotics ranging from 45.2% to 100%. This is probably due to the methodology recommended by these studies on the choice of antibiotic used (variety of the activity spectrum) and the geographical variety of the study area. Also, the resistance of *S. enterica* to antibiotics is mainly ensured by the production of extended-spectrum beta-lactamase (ESBL) and plasmid cephalosporins which are the main cause of resistance to β-lactams in members of the Enterobacteriaceae family (Liakopoulos et al. 2016). In Morocco, this resistance has been reported in several serotypes of *S. enterica* mainly *S. typhimurium, S. kentucky, S. enteritidis, S. senftenberg*, and S. *agona*. The occurrence of *S. enterica* serotypes resistant to quinolones, fluoroquinolones, and third-generation cephalosporins has continued to increase (Andino and Hanning 2015) and has been observed in several studies in Morocco (Ohmani et al. 2010; Ziyate et al. 2016; Allaoui et al. 2014; Amajoud et al. 2017). Other studies have shown a fairly high prevalence of multidrug-resistant strains (Oubrim et al. 2012; Le Hello et al. 2013b; Chahboune et al. 2014; Ed-dra et al. 2017). It should be noted that *S. enterica* resistant to ciprofloxacin is generally resistant to several antibiotics.

In our study, we showed the presence of a strain of *S. kentucky* sensitive to the antibiotics tested. While today we are witnessing a global emergence of multi-resistant *S. kentucky*. In fact, from 2002 to 2008, the *S. kentucky* ST198-X1-SGI1 strain resistant to ciprofloxacin isolated from different hosts and different sources including the environment was reported in Egypt and distributed throughout Africa and the Middle -Orient (Le Hello et al. 2013a; Le Hello et al. 2013b). This resistance to ciprofloxacin is probably due to the current use of fluoroquinolones in poultry sectors in North Africa (Le Hello et al. 2011). The ST198-X1-SGI1 strain resistant to ciprofloxacin, considered as a clone of epidemic interest, has shown since 2009 the acquisition of extended-spectrum β-lactamase genes (CTX-M-1, CTX-M-15), from cephalosporins (CMY-2) or carbapenemase (OXA-48, VIM-2) genes encoded by the plasmid. (Le Hello et al. 2013b). *S. kentucky* multidrug-resistant (MDR) belonging to a single line, emerged following the acquisition of the genomic island of *Salmonella* (SGI 1) conferring besides resistance to ampicillin, streptomycin, gentamicin, sulfamethoxazole, and tetracycline (Hawkey et al. 2019). As a result, most MDRs of *S. enterica* serotype *Kentucky* circulating in the world result from the clonal expansion of a single line which acquired AMR chromosomal genes 30 years ago (Hawkey et al. 2019), and continues to diversify and accumulate additional resistance to last-line antimicrobials under the stress of antibiotics; (Haley et al. 2019; Xiong et al. 2020). Recently, *S. kentucky* ST198, multidrug-resistant (MDR) has spread widely, following the travel of infected people (Haley et al. 2019).

Also, *P. aeruginosa* presented a prevalence of 21.6% of water samples. Our results are different from those obtained from the work carried out in Morocco. Indeed, a study of Moorish bath well water showed a prevalence of 57% (El Ouardi et al. 2013), another study reported a very high prevalence of *P. aeruginosa* in 61.15% (Hafiane et al. 2019) of well water, while another hospital study showed a very low prevalence of 7.40% (Frikh and Maleb,2017). The explanation for the widely varying levels of *P. aeruginosa* in these studies reflects the difference in the conditions of their environments. A meta-analysis of samples from environments with intense human contact shows a higher prevalence of *P. aeruginosa* compared to those with less human contact (Crone et al. 2020). This explains the rather high prevalence of *P.aeruginosa* in these well waters. While in hospitals the low prevalence observed is probably due to the rigorous application of measures to prevent contamination in this environment.

In this study, all the strains isolated from *P. aeruginosa* are serotypes and belong to seven serotypes among which are O6, O1, O7, O9, O3, O4, and O10 (Table 2). The most dominant serotypes are O6 and O1, this coincides with studies that have reported the predominance of the same serotypes (Nedeljković et al. 2015). While the other serotypes (O7, O9, O3, O4, and O10) were less frequent and show variable prevalence between different works. This variability is probably due to the environmental conditions of their ecological niche thus affecting the antigenic structure of these strains of *P. aeruginosa*.

Recently, nosocomial infections and outbreaks of *P. aeruginosa* O: 12 and O: 11 are reported to be a major problem in hospitals due to the multidrug-resistant nature (Nedeljković et al. 2015). Indeed, human-induced changes in the environment, such as the introduction of antimicrobials, affect the population structure of *P.aeruginosa* and lead to rapid clonal expansion. The O12 MDR serotype considered exclusively as a clinical isolate was recently isolated from the waters of the Moorish baths (El Ouardi et al. 2013) probably contaminated by their users. Indeed, colonized or infected people are the main reservoirs of the O12 clone and are considered sources of environmental contamination.

Moreover, the resistance levels of *P. aeruginosa* in this study were relatively low compared to the antibiotics tested. However, it is difficult for us to compare our results with other studies carried out in Morocco which have shown the fairly common presence of multi-resistant species of *P. aeruginosa*. Most of these studies have been carried out using strains of clinical origin (Maroui et al. 2016; Frikh and Maleb, 2017) or strains isolated from an area of intensive agriculture (Hafiane et al. 2019) or strains isolated from well water in Turkish baths (El Ouardi et al. 2013). However, the prevalence rate of multi-resistant strains varies from study to study. In a hospital environment, Mr. Frikh has shown a prevalence of multi-resistant strains of *P. aeruginosa* of 8.4%, whereas this rate was 88.8% in a study in Cameroon. This same study estimates that this discrepancy is probably due to the bacterial ecology of each of them, and also to the fact that these are not the same antibiotics that were used (Frikh and Maleb 2017). While the groundwater study (Hafiane et al. 2019) reported a 68% prevalence of multi-resistant strains (Hafiane et al. 2019). This rather high rate is probably due to the excessive use of antibiotics in this region.

Due to their high consumption, antibiotics are ubiquitous in groundwater, through anthropogenic activities such as wastewater discharges, animal husbandry, fertilization, and landfill leachate (Zhang et al. 2015, Sui et al. 2015). The concentration of antibiotics, the exposure time, the added substrates, as well as the combined effects of several antibiotics are all factors that influence the effects of antibiotics on the microbial communities of the soil and the aquatic environment (Ding and He, 2010). In addition to the misuse of antibiotics, there is the transmission of resistance from one bacteria to another. The presence of horizontal gene flow between different species and genera has been shown to help carry resistance genes (Szekeres et al. 2018). Under the effect of selective pressure, resistant strains will survive and reproduce by transferring resistance to other generations and to other pathogens and commensals that may come into contact. Nowadays, the presence of ARGs in groundwater has been well documented in different sites including groundwater near municipal solid waste landfill, groundwater under leaking sewers, groundwater underlying water treatment facilities livestock and groundwater under a highly urbanized area, and that this contamination has been affected by direct or indirect local anthropogenic activities(Wu et al. 2020)

This contamination thus induces the propagation of resistance genes in species that, previously, were not known to possess them, in particular the β-lactamase genes. The latter has seen a fairly high abundance in groundwater compared to other resistance genes (Wu et al. 2020). Their expression is spread in many families and species of *Enterobacteriaceae* and *P. aeruginosa* and worldwide in isolates from different epidemiological contexts in humans, animals, and the environment (Liakopoulos et al. 2016). Indeed, the emergence of the expression of ESBL-type beta-lactamases (extended-spectrum beta-lactamases) is considered to be an important cause of resistance worldwide (Nasreen et al. 2015) because it confers high resistance to most therapeutic beta-lactams; and their genes, especially located on plasmids, spread very easily between bacteria (En-nassiri et al. 2017). While the most important targets of fluoroquinolones for microorganisms are DNA gyrase (gyrA and gyrB) and topoisomerase IV (parC and parE); mutations in the gyrA and parC genes are generally the mechanisms of bacterial resistance to quinolone antibiotics (Xiong et al. 2020).

In this study, resistance in *S. enterica* and *P. aeruginosa* is low compared to the antibiotics tested showing that the acquisition of resistance in isolates is strictly dependent on the geographical area of isolation. Indeed, the latter is a predominantly rural area (Figure1) with a high concentration of the population in the urban environment, especially in the Rabat-Salé conurbation and a tendency to depopulate the countryside. Of a useful agricultural area, 92.10% is non-irrigated land with insufficient popularization of the use of modern techniques and means to improve production and yields. The region (R-S-Z-Z) has a potential for poorly performing livestock due to the predominance of the local breed and a forest area of 30% concentrated in the province of Khemisset. It is therefore easy to understand that the strains isolated in this study did not acquire the resistance characteristics because they were neither exposed to one or more families of antibiotics nor in direct contact with newly multi-resistant strains introduced into their environment.

In this area, contaminated water sources are the reservoirs of a diversity of *S. enterica* and *P. aeruginosa* serotypes but still exhibit a wild resistance phenotype. This shows that the geographic location of water sources, as well as land use and activities of the indigenous population, probably played an important role in the absence of antibiotic resistance. On the other hand, the emergence of multidrug-resistant strains in the environment today raises fears of development towards resistance shortly. To conclude on the diversity and antimicrobial resistance of these microorganisms, actually, a study is in progress.

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