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Salmonella in Wastewater: Identification, Antibiotic Resistance and the Impact on the Marine Environment

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Abstract

Many coastal cities around the world discharge their wastewaters into the marine environment. These wastewaters contain a high variety of pathogenic microorganisms that would have a role in the contamination of this ecosystem and may have potential risks for public health and environment. Using an environmental approach, we investigate the presence of *Salmonella* in wastewater treatment plants and its presence after the treatment in its receiving marine environment. In this environmental approach, we provide information about the inefficiency of wastewater treatment to remove *Salmonella*, especially that wastewater is considered as a good tank of high diversity of *Salmonella* serotypes. The identified *Salmonella* serotypes in the receiving marine environment almost coincide with those identified in wastewater. This characterization of *Salmonella* strains from wastewater and marine environment involves the direct impact of municipal wastewater discharges on this environment. Antibiotic susceptibility tests reveal generally the presence of multiresistant *Salmonella* strains in wastewater, which usually end up in the marine environment and may have a significant risk on the public health.

Keywords: antibiotic susceptibility, marine environment, *Salmonella*, Wastewater, wastewater treatment

1. Introduction

Many major coastal cities around the world discharge their wastewaters into the marine environment. Wastewater may consist of a combination of domestic, industrial and agricultural effluent. Generally, wastewater treatment plants (WWTPs) are mostly designed to effectively remove fecal bacteria, some chemical pollutants but are not provided to eliminate pathogenic

microorganisms [1, 2]. Furthermore, most of these treatment systems remain imperfect and continue to reject varied concentrations of enteric bacteria in the environment [1–5].

Pathogenic bacteria become normally more harmful to environment and humans when they acquire resistance to antibiotics. Moreover, many studies [5–7] confirmed the detection of antibiotic concentrations in wastewater that treatment systems generally fail to eliminate. Therefore, when there is a contact between bacteria and antibiotics traces, the wastewater could be an ideal environment to the exchange of genetic material between bacteria and consequently the acquisition of antibiotic resistance [5, 6, 8, 9].

The *Salmonella* genus is one of the pathogen bacteria that could be conveyed to the environmental waters through sewage pollution. Therefore, the determination of the presence of *Salmonella*, its levels and its antibiotic resistance at the same time in wastewaters and environmental waters are crucial and are required to assess the related health risks.

Previous works [1, 9–13] have studied the presence of *Salmonella* in wastewater but they stay insufficient to provide enough information on the diversity and the antibiotic resistance profiles of *Salmonella*. This makes transmission of *Salmonella* via wastewater, its impact on the environment and the origin of contamination poorly understood. In Morocco, *Salmonella* in wastewater is rarely discussed and its bibliography is very scarce. In this context, we are currently leading the first study about *Salmonella* in the southern region of Morocco. This work is focusing on the identification, serotyping and antibiotic susceptibility of *Salmonella* strains isolated from Agadir wastewater treatment plant.

The aim of this chapter is first to provide qualitative information on the capacity of wastewater treatment process to eliminate *Salmonella* considered among the most pathogenic bacteria easily transmissible in water. The second aim is to present the most *Salmonella* serotypes isolated from wastewater and marine environment and the possible impact of wastewater discharges on marine environment.

2. WWTPs removal efficiency of *Salmonella*

Wastewater treatment plants know a growing pressure which leads to the evacuation of untreated or insufficiently treated effluents into the environment. Thus, the efficient removal of pathogenic bacteria in wastewater is a crucial task because wastewater discharges can significantly increase the contamination of surface water and lead to water and seafood-borne infections. Generally, an optimal wastewater treatment process can attain 90–99% of reduction of microbial load [2, 14, 15]. However, in some cases, the reduction level decreases are mostly due to a nonrespective operating mode or due to bad or inadequate plant maintenance.

Several studies have shown the presence of *Salmonella* in wastewater before and after treatment even if treatment processes are different [1, 9, 10, 13]. This could be explained by the fact that WWTPs are mostly designed just to reduce microbial load but are not conceived specifically to completely eliminate the pathogenic bacteria.

An activated sludge treatment process remains unable to totally remove *Salmonella* [9, 12]. Koivunen et al. have also shown that activated sludge process coupled with phosphorus precipitation followed by a secondary settling or coupled with denitrification-nitrification fails to remove *Salmonella* from municipal wastewater [1].

Until now, no study has been conducted on the elimination of *Salmonella* by infiltration percolation treatment process through sand. However, a current study conducted by our research team (unpublished data) showed the inefficiency of this treatment system to remove *Salmonella*. Thus, treated wastewater still contains *Salmonella* which continues to be detected at the treatment plant exit.

To get an efficient reduction or a total removal of *Salmonella*, a tertiary wastewater treatment is needed. Indeed, Koivunen et al. show that tertiary treatment by rapid sand contact filter allows a very good efficiency to remove *Salmonella*. Consequently, *Salmonella* was not found in wastewaters after tertiary filtration [1]. Furthermore, a current study conducted by our team showed that tertiary treatment by UV irradiation applied just on a portion of the secondary treated effluents (which are intended for the irrigation of golf courses) allows total elimination of *Salmonella* (unpublished data).

3. Microbiological risks associated with *Salmonella* in wastewater

Wastewater is known to be a common vehicle for the transport and transmission of *Salmonella* serovars and is able to pollute environment and infect humans. Indeed, several outbreaks and contaminations have been related to *Salmonella* in wastewater through irrigation of crops [16–18], infiltration and transport in soil to groundwater [19–21] or to its discharge into the marine environment [22, 23]. In this last case, although some pathogens are naturally present in the aquatic environment, *Salmonella* may be introduced through animal or human fecal and sewage pollution [24].

The risk of contamination linked to *Salmonella* is enhanced by its ability to survive longer in the environment. Indeed, contamination of groundwater, environmental water and soil by *Salmonella* is due to its ability to survive very long time in these environments [25]. Furthermore, contaminations related to *Salmonella* in seawater and seafood are increased by the capacity of *Salmonella* to survive in relatively high salt conditions [26]. Thus, the persistence of wastewater discharges in coastal waters may increase the load of *Salmonella* in marine environment, and therefore increase the higher incidence of seawater and seafood-borne infections.

4. Identification methods of *Salmonella*

Various methods have been developed for the subtyping of *Salmonella*. Each of them has its advantages and drawbacks in terms of cost, speed, robustness, and sensitivity [27]. The choice of identification techniques of bacteria is generally done according to the objectives of identifying

and the available means. Biochemical techniques are usually used to isolate and identify only species. For further characterization, determination of the origins and the relationships between different isolates, many other accurate methods are needed.

Conventional serotyping using somatic and flagellar *Salmonella* antisera is the most frequently used reference method for serotyping *Salmonella* isolated from wastewater [1, 9, 28, 29]. This method is based on the direct agglutination technique blade, involving *Salmonella* strains with different antisera to identify variants of the somatic O and flagellar H antigens. Serotyping is generally performed according to the antigenic formulae of Kauffmann-White-Le Minor scheme [30]. The main limitation of this technique is that not all O serotypes are included in Polyvalent O antisera which *Salmonella* species should agglutinate with [31]. A combination between conventional serotyping and the ribosomal spacer-heteroduplex polymorphism (RS-HP) methods was also used to characterize *Salmonella* strains from wastewater [13]. This technique based on the PCR amplification of the intergenic spacer region between the 16S and 23S rRNA genes can produce amplicon profiles allowing the discrimination of species at both serotype and intraserotype levels [13].

PFGE allows a high discrimination and it is usually used for outbreak investigations and it is also widely used for characterizing epidemic *Salmonella* strains. The capacity of PFGE to differentiate strains of bacterial pathogens makes it a standard method used to assess the epidemic spread of infectious diseases and to trace *Salmonella* outbreaks. However, even if it is reproducible and discriminatory, some strains of *Salmonella* cannot be typed by PFGE [27]. Recently, matrix-assisted laser desorption/ionization time of light mass spectrometry (MALDI-TOF MS) has been used for the identification of *Salmonella*. It is also utilized to discriminate *Salmonella Typhi* from other *Salmonella* serovars [32]. Despite its rapidity and simplicity, the preparation of MALDI-TOF requires more modifications and improvements to available protocols before being adopted as an autonomous method [32]. Other molecular methods developed as alternatives to conventional serotyping (MLST, MLVA, SNP, and molecular typing with composite microarrays) seem successful. However, these methods do not provide exactly similar results like those obtained by the current reference method which is agglutination serotyping [27].

5. *Salmonella* serotypes isolated from wastewater

As reported, wastewater is an ideal tank of *Salmonella* strains. Furthermore, identified *Salmonella* showed generally high variability of serotypes. The major serotypes isolated in wastewater from different countries are presented in **Table 1**.

Data concerning *Salmonella* serotyping (**Table 1**) show high heterogeneity of serotypes isolated from wastewater. This high diversity can be explained by the variety of the origin of effluents carrying these *Salmonella* strains. The most frequent serotypes identified in France wastewater were Newport, Saintpaul, and Brandenburg [13]. Indeed, Serotypes Mbandaka, Virchow, Hadar, Indiana, Infantis, Saintpaul, and Senftenberg are commonly isolated from poultry farms. Also, Typhimurium and Indiana serotypes are generally isolated in human pathology and can derive from healthy carriers [13]. Espigares et al. have also explained this

diversity by the variety of the origin of *Salmonella* which may be of human or animal origin [9]. *Salmonella* Agona, Saintpaul, Virchow, and Corvallis are the most frequent serotypes identified in Finnish wastewater [1]. According to Koivunen et al., this diversity may be reported to the size of the population suggesting, therefore, that larger populations produce larger spectra of serovars [1]. However, despite these explanations, the origin of this diversity remains unknown. The large variability in serovars identified in USA wastewater indicates multiple sources of the isolates. This variability may be due to fecal shedding in clinical salmonellosis or to animal agriculture [33]. Our current study (not shown data) concerning isolated *Salmonella* from wastewater in Agadir, a coastal city in Morocco, show high variability of serotypes. Thus, among 52 *Salmonella* strains we identified 18 different serotypes. *Salmonella* Muenster was the major serotype which showed a high incidence with 14 isolates followed by *Salmonella* Infantis, *Salmonella* Senftenberg, *Salmonella* Montevideo, and *Salmonella* Kentucky as the most frequent serotypes identified. This high diversity of serotypes in Agadir wastewater can be explained by its various origins including domestic and industrial effluents especially those coming from poultry slaughterhouse and fish processing plants very widespread in the city.

Country	France	Spain	Finland	Morocco	Mexico	USA
Serotypes	Agona	Anatum	Agona	Anatum	Typhimurium	Agona
	Bardo	Enteritidis	Corvallis	Give	Vejle	Banana
	Brandenburg	Hadar	Blockley	Newport	Corvallis	Montevideo
	Derby	London	Enteritidis	Senftenberg	Nchanga	Derby
	Hadar	Newport	Hadar	Typhimurium	Nitra	Edinburg
	Indiana	Typhimurium	Infantis		Stanleyville	Hadar
	Infantis		Lexington			Havana
	Manhattan		Mbandaka			Infantis
	Newport		Muenster			Saintpaul
	Saintpaul		Panama			Senftenberg
	Senftenberg		Saintpaul			Typhimurium
	Typhimurium		Typhimurium			Newport
	Virchow		Virchow			Thompson
References	[13]	[9]	[1]	[28]	[29]	[33]

Table 1. *Salmonella* serotypes isolated from wastewater.

6. *Salmonella* serotypes isolated from a wastewater receiving marine environment

In coastal cities, the marine environment is usually the final end of treated and untreated sewage. Table 2 shows some studies throughout the world carried in this regard.

Country	France	Spain	Mexico	Morocco	USA
Serotypes	Cerro	Anatum	Stanley	Altona	Adelaide
	Newport	Enteritidis	Galiema	Anatum	Agona
	Typhimurium	Goldcoast	Bulovka	Corvallis	Arizona
	Virchow	Hadar	Othmarschen	Hadar	Bardo
		London	Tonev	Senftenberg	Hartford
		Newport	Subaru	Typhimurium	Newport
		Typhimurium	Typhimurium	Kentucky	Poona
			Vejle	London	Reading
			Winnipeg	Mbandaka	Typhimurium
				Muenster	
References	[13]	[34]	[29]	[35, 36, 38]	[47]

Table 2. *Salmonella* serotypes isolated from marine environment.

As reported in wastewater, data from marine environment also reveal a large diversity of *Salmonella* serotypes. However, the origin of these serovars remains little known. Following to data in **Tables 1** and **2**, it appears that the most serotypes found in wastewater are also isolated in the surrounding marine environment. Furthermore, studies from France [13] and Mexico [29] showed that Newport and Typhimurium were respectively the major *Salmonella* serotypes isolated in wastewater. These serotypes were also the same major serotypes isolated from marine environment [13, 29]. Serovar Typhimurium has been shown to be the most common serovar isolated from marine environment in different parts of the world [13, 29, 34–36]. It was suggested previously that this serotype shows an excellent adaptation to the marine water stress after passing through wastewater [37]. In Agadir, study conducted by our team [38] in marine environment has identified *S. Muenster* as the major serotype (13 strains among 46 isolates). This high occurrence of serotype Muenster coincides with its high incidence in Agadir wastewater. Therefore, these findings reveal that wastewater discharges into the marine environment could be the main source of contamination and the principal origin of *Salmonella* strains found in this environment.

7. Antimicrobial resistance of *Salmonella* isolated from wastewater

Urban wastewater treatment plants (UWTPs) are suspected to be among the main anthropogenic sources for antibiotics, antibiotic resistant genes (ARGs) and antibiotic resistant bacteria (ARB) spread into the environment [14, 39, 40]. The presence of antibiotics in effluents

is generally due to incomplete metabolism in humans or because of the mismanagement of unused antibiotics [4, 41].

Generally, bacteria in wastewater are in permanent contact with various elements including antibiotics and other chemical products. Furthermore, antibiotic resistance was positively correlated with the occurrence of antibiotic residues. In addition, a relationship between antibiotic residues, bacterial community structure and antibiotic resistance was demonstrated [42]. Therefore, the elimination of these antibiotics from wastewater is a major concern. Sorption and hydrolysis could be a degradation pathway that may lead to the removal of antibiotics in wastewater [39]. However, in previous reports, it has been demonstrated that UV radiation is not effective to remove antibiotics [43]. This indicates that photolysis is not an important mechanism for degradation of these compounds in wastewater. A combination of physico-chemical and biological treatment and the optimization of their operating conditions might prove an effective removal increase of persistent antibiotic residues in wastewater [39].

According to previous studies [6, 44, 45], ARB have been detected widely in wastewater samples and comparatively to surface water, higher proportion of ARB was reported in raw and treated wastewater than in surface water [4, 40]. It has also been reported that *Salmonella* strains show a high proportion among other multidrug resistant bacteria [46]. According to these studies, conditions in wastewater treatment plants (WWTPs) seem to be favorable for the proliferation of ARB.

Antibiotic resistance (ABR) of *Salmonella* isolated from wastewater has been discussed in some studies. A wide variety of antibiotic resistance patterns of *Salmonella* serotypes have been found in USA wastewater [33]. Indeed, 86% of 647 *Salmonella* isolates were susceptible, 4% were monoresistant and 10% were multiresistant. *Salmonella* serovars multiresistant to at least four antibiotics were *Salmonella Braenderup*, Derby, Edinburg, Hadar, Saintpaul, Typhimurium, Uganda and Virchow. A resistance to the third generation of cephalosporins considered as one of the first drugs of choice to treat human salmonellosis has also been reported [33]. From Finnish wastewater [1], among 197 isolates, 44% of the *Salmonella* strains were resistant to antimicrobial agents, almost 20% were multiresistant. The most multiresistant serotype was *Salmonella Saintpaul* (to 6 antibiotics). Moreover, 32% of strains were resistant to nalidixic acid which can indicates the reduction of sensitivity to ciprofloxacin considered as the selected drug in severe infections [1]. The most resistant serotype isolated in wastewater from Spain was *Salmonella Hadar* with a pattern of multiresistance to six antibiotics [9]. In Morocco, among 42 strains isolated from wastewater, 19 (45.2%) were resistant at least to two antibiotics. The most multiresistant serotype was *S. Typhimurium* followed by *S. Hadar* and *S. Senftenberg*. *S. Typhimurium* was resistant to ampicillin, chloramphenicol, streptomycin, sulfamethoxazole/trimethoprim, and tetracycline. Serovars Give, Newport and Anatum were susceptible to all used antibiotics [28]. Antibiotic susceptibility test (ATS) of *Salmonella* serotypes from Agadir wastewater treatment plant carried out by our team showed that among 52 isolates 23% of strains were multiresistant. ATS was carried out by the disk diffusion method, with a panel of 32 antimicrobial drugs (Bio-Rad) and interpreted according to the EUCAST clinical guidelines (http://www.eucast.org/clinical_breakpoints/). Multiresistant serovars were Chester, Kentucky, and Typhimurium. The most multiresistant serotype identified in this study was Typhimurium with a pattern of multiresistance to 18 antibiotics.

All these data showed that wastewater is a tank of a wide variety of *Salmonella enterica* serovars and ABR patterns. Other data showed that multiresistant bacteria have been detected extensively in wastewater samples which pass through WWTPs and arrive to the receiving environment [14, 40, 44]. Consequently, this problem is of great concern for the wastewater management of coastal cities because multiresistant bacteria have become a significant public health problem. The caused infection is much more difficult to be treated because the panel of effective antibiotics will be reduced.

8. Conclusions

- Conventional wastewater treatment without efficient tertiary treatment, like filtration or disinfection by UV, is generally insufficient and consequently constitutes a risk for public health.
- Wastewater is an ideal tank of high diversity of *Salmonella* serotypes and ABR patterns which usually end up in the marine environment in coastal cities.
- The presence of the same major *Salmonella* serovars simultaneously in sewage and in the marine environment confirms that the principal source of contamination of marine environment by *Salmonella* is wastewater discharges.
- Improvement in the efficiency of treating antibiotics residues in WWTPs is the first line of defense against the potential ecological impacts of these chemicals in the environment.
- On the one hand, wastewater treatment must adopt effective methods for treatment, and on the other hand, there should be a rigorous approach for surveillance and monitoring effluents before and after treatment and discharge into the environment.

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