# Isolates of Salmonella in Liguria from 2008 to 2009

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# **Abstract**

The ENTER-NET project is coordinated by the Istituto Superiore di Sanità (ISS) and includes Hospital and University laboratories from different areas of the country. This project has the aim to study the emergence and the spread of Salmonella spp. isolates at a national level and to evaluate their antibiotic resistance. In the period 2008-2009 the Bacteriological Laboratory of Genoa **University Department of Health Sciences** (DISSAL) collected and confirmed every year about 100 Salmonella specimens isolated by ARPAL (Azienda Regionale Protezione Ambiente Ligure) laboratories and coming from peripheral ligurian hospital laboratories. Over 204 cases of 2008-2009 period, the 50.98% had not a human origin, the 20.19% of which came from food and the 79.81% from surface streams. The remaining 49.02% were of human origin and the 65% was represented by the serotype S. typhimurium (mostly from hospitalized under 14 years old children) while S.enteritidis (12%) was mostly collected from adult hospitalized subjects and did not present pluri-resistance.

Between non-human serotypes a strain of S. bredeney, isolated from a sample of raw lamb meat in 2009, was resistant to 8 antibiotics including Chloramphenicol (CAF), Tetracycline, Ampicillin, Trimethoprim and Sulfometoxazol while a strain of S. typhymurium (from a sample of sea water) was resistant to the same last antibiotics and to the Clavulanic Acid-Amoxicillin association.

## Introduction

Still many are the diseases transmitted by contaminated 168 food, many are their different symptoms and many the

different pathogens, mostly bacteria, viruses and parasites which cause them [1, 2].

The food borne illness may result from infection with pathogenic microorganisms that colonize the intestinal mucosa or by ingestion of contaminated food or by ingestion of toxins causing disease even when the producer organism is dead [3, 4].

Salmonellosis is worldwide a common disease and is responsible for the most part of food poisoning developed in the form of outbreaks from overcrowding in closed communities (schools, nursing homes), from outbreaks in hospitals, institutions for children and from sporadic cases in public canteens and restaurants [5]. In Italy, Salmonellosis must be notified as Type II infectious disease following the DM 12. 15. 1990 and G.U 01.08.1991

The Italian Superior Institute of Health coordinates the system ENTER - NET for Italy that involves the participation of 29 diagnostic laboratories involved in clinical microbiology.

The Laboratory of Bacteriology of the Genoa University Health Sciences Department, collects data about Salmonella isolates from Liguria and carries out checks on typing and verifying antibiotic susceptibility of strains received by the network peripheral ENTER-NET Liguria laboratories [6]. The phenomenon of resistance to ampicilline, cothrimoxazole and fluoroquinolones is increasingly reported [7].

In addition, increasingly frequent cases of multiple resistance (S.typhimurium resistant to 5 antibiotics) make clear the importance and seriousness of this problem. By this way resistant bacteria can be transmitted to humans through foods, particularly those of animal origin

# **Materials and Methods**

Following the ISO6579 method, each strain was revitalized on RVB (Rappaport Vassiliadis Broth) and XLDA (Xylose, Lactose and Dextrose Agar), then was submitted to the typical Biochemical and Serological tests (BioMerieux Italia S.p.A and Biogenetics S.r.I).

The antibiotic susceptibility (Kirby - Bauer) was then evaluated submitting every strain to the action of antibiotics following the panel of the Italian Health Ministry Laboratory (ISS) including: Ampicillin, Cefotaxime.

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Chloramphenicol (CAF), Ciprofloxacyne, Gentamicin, Kanamycin, Nalidixic Acid, Streptomycin, Sulfometoxazole, Tetracycline, Cotrimoxazole, Trimethoprim, Ceftazidime, Clavulanic Acid-Amoxicyllin association.

#### Results

The strains received from 2008 through 2009 were 204 and they were all biochemically and serologically confirmed. Over 204 cases, 104 were isolated from environment (50.98%), 21 of which (20.19%) came from food and 83 (79.81%) from surface streams. The remaining 100 cases (49.02%) were of human origin divided in two classes, the one made of <14 years old patients (72%) and the other made of >30 years old age patients (28%). No strain came from the age class between 14 and 30 years old patients (Tab.1).

The most frequently all together isolated serotypes were: S. typhimurium (39.21%), S. agona (9.31%), S. enteritidis (8.33%) and S. montevideo (3.92%) (Tab. 2).

Over 21 food isolates, 5 of them came from meat food products, 4 came from herbs category, 4 from fruits and vegetables category, 3 from dairy products, 3 from raw meat samples and 2 samples from eggs (S.kottbus C2 Groups).

The most frequently in food isolated serotypes were S. typhimurium (14.42%) and S.agona (9.62%) followed by serotypes S.arizona, enteritidis and montevideo.

The most representative non-human serotypes resisting to more than 3 antibiotics were: *S. agama, S. typhimurium* and *S. bredeney*. In particular, *S. bredeney* isolated from a sample of raw lamb meat was resistant to 8 antibiotics, including Chloramphenicol (CAF), Tetracycline, Ampicillin, Trimethoprim and Sulfometoxazol.

A strain of S. typhymurium (from a sample of sea-water) was resistant to the same last antibiotics and to the Clavulanic Acid - Amoxicillin association.

The 79% of humans isolated strains was represented mainly by serotype S.typhimurium (65 isolates) while S.enteritidis (12%) was mostly collected from adult hospitalized subjects and did not present pluri-resistance.

S.typhimurium however, was frequently isolated (72%) from children under 14 years old who were hospitalized (61%) at the Institute G. Gaslini and was resistant to more than three antibiotics.

An adult isolated strain was resistant to CAF too.

## **Discussion**

Through 2009, human isolates decreased from 56 (2008) to 44 and environmental isolates were only 32 compared to 72 of 2008. A reduction to 41.98% of the isolates notifications was observed.

Also in 2009, as in previous years, S. typhimurium was isolated mostly on humans (60.71% in 2008 and 70.45% in 2009) while in non-human cases (environment and foods)

	Patients <14aa	Patients >30aa	All patients	Surface streams	Food samples	Environment	All samples
2008	42	14	56	56	16	72	128
2009	30	14	44	28	4	32	76
2008-09	72 72.00%	28 28.00%	100 49.02%	83 79.81%	21 20.19%	104 50.98%	204

Table 1. Isolations of Salmonella genus in Liguria from 2008 to 2009.

2008-2009	Enviromental origin	%	Human origin	%	Environmental and Human Origin	%
S.typhimurium	15	14.42	65	65	80	39.21
S.agona	10	9.62	9	9	19	9.31
S.arizona	7	6.73			7	3.43
S.enteritidis	5	4.80	12	12	17	8.33
S.montevideo	4	3.85	4	4	8	3.92
S.thompson	3	2.88	1	1	4	1.96
S.S.paul	3	2.88			3	1.47
S.kottbus	3	2.88			3	1.47
S.braenderup	3	2.88			3	1.47
S.lagos	3	2.88			3	1.47
Others	48	46.15	9	9	57	27.94
Total	104	100	100	100	204	100
serotypes	58		14			

Table 2. Salmonella serotypes isolated during 2008 and 2009 in Liguria.

its presence was very low (12.5% in 2008 and 18.75% in 2009) on the benefit of many other serotypes with the prevalence of serum group C in both years.

Compared to 2008, the strains resistant to more than 3 antibiotics and regarding serotypes isolated from the environment, doubled from 4.18% (2008) to 9.375% (2009), while the serotypes of human origin decreased significantly from 14.29% to 4.5% in 2009 (8 out of 56 in 2008 and 2 out of 44 in 2009).

The presence of pluriresistant strains inside hospital environment represents one of the principal cause of nosocomial infections persistence (Italian Health Minister Doc. N.52/85).

In fact, an excessive utilization of extended-spectrum antibiotics, easily causes the emergence of mutant strains often resistant to them.

The most important and valid action to control and stop nosocomial infections spreading, is represented by a continuous surveillance on hospital wards specific infections.

With this aim, an inside hospital technical commission including ward physicians, microbiologists and pharmacologists would be very useful especially if surveillance schedules specific for every patient would be adopted to be analyzed and periodically compared between them [9].

The genus Salmonella is often isolated in hospital infections; infirmary-patient or patient-patient interhuman contacts or instruments colonization represent quite always their real reason.

Outside hospital, Salmonellas remain the leading cause of notified environmental (surface water and foods) outbreaks [10].

A special risk regards the "ready" food for consumption, having not been submitted to any healing process. New foods such as centrifuged vegetables and fruits or fruit juices have been accused to vehicle *Salmonella* [11]. In both the cases a strictly application of HACCP recommendations in controlling the various production flux diagram phases would reduce the risk basically.

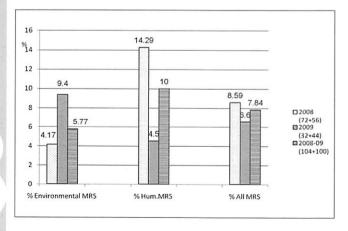


Figure 1. Ligurian Multi Resistant Salmonellae (MRS) isolates percentage values.

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