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# Antibiotic Resistance and Occurrence of Class 1 Integrons in Clinical Isolates of *Salmonella enterica*

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**Abstract:** Screening of non-typhoidal *Salmonella enterica* clinical isolates (n=20) by PCR revealed the high prevalence of class 1 integrons (45%). Multidrug-resistant (MDR) phenotypes were observed in all integron-positive isolates, regardless of the serotype. A significant correlation was found between the MDR phenotype and the presence of class 1 integrons in the isolates, especially in *S. Typhimurium*.

**Keywords:** *Salmonella enterica*, class 1 integrons, multidrug resistance.

## 1. INTRODUCTION

Multidrug-resistant (MDR) *Salmonella enterica* strains are considered as a significant threat to human health in both developed and developing countries. Association between class 1 integrons and MDR has been established for a number of *S. enterica* serotypes [1-5]. Surveillance and epidemiological studies demonstrated a considerable geographic variation in the prevalence and types of integrons among clinical isolates [1, 2, 4, 5]. However, there is a lack of information on the occurrence of class 1 integrons in *Salmonella* isolates in Armenia. Thus the aim of the present work was to investigate the prevalence of class 1 integrons and their relationship with the susceptibility to antibiotics in non-typhoidal *Salmonella* clinical isolates at this location.

## 2. MATERIALS AND METHODS

### 2.1. Isolates

The study included a collection of 20 non-typhoidal *Salmonella enterica* subsp. *enterica* strains isolated in 2011 from the patients at the “Nork” Clinical Hospital of Infectious Diseases (Yerevan, Armenia). Diagnosis was based on clinical presentations and laboratory analyses. Clinical presentations consistent with gastroenteritis were diarrhea, fever, nausea, vomiting, and abdominal cramps. Anamnesis of food consumed,

water sources, social gatherings, anybody else with a similar illness, and any recent history of travel was also recorded. For this investigation, only the patients that were not taking any type of medication including antibiotics before the hospital admission were chosen. Fecal samples were collected on the first or the second day of hospital admission. All the strains isolated from fecal samples confirmed to be *Salmonella enterica* by standard biochemical and serological tests. Biochemical tests for the identification of *Salmonella* were fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H<sub>2</sub>S production, and fermentation of galactitol (dulcitol). Serotypes of *Salmonella* were determined using the standard Kauffman-White scheme with the use of commercially available polyvalent antisera for flagellar (H) and for lipopolysaccharide (O) antigens.

For detoxification and rehydration, all patients were subjected to the standard infusion therapy. Based on the severity of the disease, 86% were placed on antibiotic therapy: (i) 38% received intravenous or intramuscular ceftriaxone (50mg/kg/day); (ii) 42% received peroral ciprofloxacin (0.5–1.0 g/day); and (iii) the rest were on the combination of one of the two drugs and trimethoprim/sulfamethoxazole. All study subjects (or parent/guardian if a child), gave their written consent to give fecal samples for the study. The study protocol was approved by the Ethics Committee of the Institute of Molecular Biology NAS RA (IORG number 0003427, Assurance number FWA00015042, and IRB number 00004079).

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## 2.2. Antibiotic (AB) Susceptibility Tests

The isolates were tested for the susceptibility to eight classes of antibiotics by disc diffusion. Standard disk diffusion method was used according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) [6]. The disks used (Micromaster Laboratories Pvt. Ltd., INDIA) contained ten antibiotics: streptomycin (S), gentamicin (G), amoxicillin/clavulanic acid (Au), ceftriaxone (Cx), trimethoprim/sulfamethoxazole (Tm/S), sulfisoxazole (Su), ampicillin (A), chloramphenicol (C), ciprofloxacin (Cp), and tetracycline (T). The isolates were classified as susceptible, intermediately resistant or fully resistant according to the CLSI guidelines [7].

## 2.3. Detection of Class 1 Integrons

Total DNA of isolates for PCR-amplification was prepared with the use of the FastDNA® Kit (Qbiogene,

Inc.), according to the manufacturer's protocols. All strains were tested for the presence of class 1 integrase-specific *int11* gene, variable segment of integrons, and 3'- conserved segment. Primers, PCR mix composition and PCR conditions used were as described previously [5]. PCR products were visualized by ethidium bromide staining after gel electrophoresis using 1.2% agarose.

## 3. RESULTS

Among 20 *Salmonella* clinical isolates tested nine isolates demonstrated the presence of *int11* (Table 1). PCR-amplification with the set of primers for 3'- conserved segment indicated that all *int11*<sup>+</sup> strains produce the amplicons of the expected size (800bp) and thus were positive for the 3'-conserved segment too. The remaining *Salmonella* isolates did not generate PCR amplicons with any of the primer sets

**Table 1: Presence and Type of Class 1 Integrons, and Resistance Profile of *Salmonella* Clinical Isolates**

Isolate number	<i>Salmonella</i> Serotype	PCR-amplification target (primers set)			Resistance profile	Number of AM classes <sup>c</sup>
		<i>int11</i> (1U-1D)	VS <sup>a</sup> (inF-inB) b.p.	3'-CS <sup>b</sup> ( <i>qacEΔ1-F sul1-B</i> )		
1	S. Enteritidis	-	-	-	-	0
2	S. Enteritidis	-	-	-	-	0
3	S. Enteritidis	-	-	-	-	0
4	S. Enteritidis	-	-	-	-	0
5	S. Typhimurium	-	-	-	-	0
6	S. Enteritidis	-	-	-	A	1
7	S. Enteritidis	-	-	-	T*	1
8	S. Typhimurium	-	-	-	AAu	2
9	S. Typhimurium	-	-	-	AAu	2
10	S. ser. I 4,[5],12:i-	-	-	-	ASuTm/ST*Cx	4
11	S. Enteritidis	+	1000	+	ASSuTm/SAu*	4
12	S. Enteritidis	-	-	-	CpCSGTSuCx	6
13	S. Enteritidis	+	1000	+	ACSSuTm/SAuCx*	6
14	S. Enteritidis	+	1000	+	ACSSuTm/SAuCx	6
15	other <i>Salmonella</i>	+	1000	+	ACSSuTm/SAuCx	6
16	S. Typhimurium	+	1000	+	ACSSuTm/STAu*	6
17	S. Typhimurium	+	1500	+	ACSSuTm/STAuCx	7
18	S. Typhimurium	+	1500	+	ACSSuTm/STAu*Cx	7
19	S. Typhimurium	+	1500	+	ACSSuTm/STAu*Cx	7
20	S. Typhimurium	+	1500	+	ACSSuTm/STAuCx	7

<sup>a</sup>variable segment of integron; <sup>b</sup>3'-conserved segment of integron; <sup>c</sup>number of antimicrobial classes to which the isolate is resistant; \*intermediate resistance to the drug.

used (Table 1) and, therefore, they were considered as integron-negative (*intI1*<sup>-</sup>).

Thus, the results showed the presence of class 1 integrons in 45% of the isolates (9 out of 20), five of which were identified as *S. Typhimurium* (55.6%), three isolates were identified as *S. Enteritidis* (33.3%), and one strain belonged to another *Salmonella* serotype (Table 1).

PCR-amplification with the set of primers targeting the variable segment of integrons indicated the putative gene cassettes in all *intI1*<sup>+</sup> strains (Table 1, Figure 1) since there were no “empty” integrons among them. All variable segments were represented by a single amplicon, and these amplicons were represented by two types: (i) those producing a 1000 bp amplicon (5 isolates: 3 – *S. Enteritidis*, 1 – *S. Typhimurium*, and 1 – other *Salmonella*), and (ii) those producing an amplicon of about 1500 bp (4 isolates, all *S. Typhimurium*).

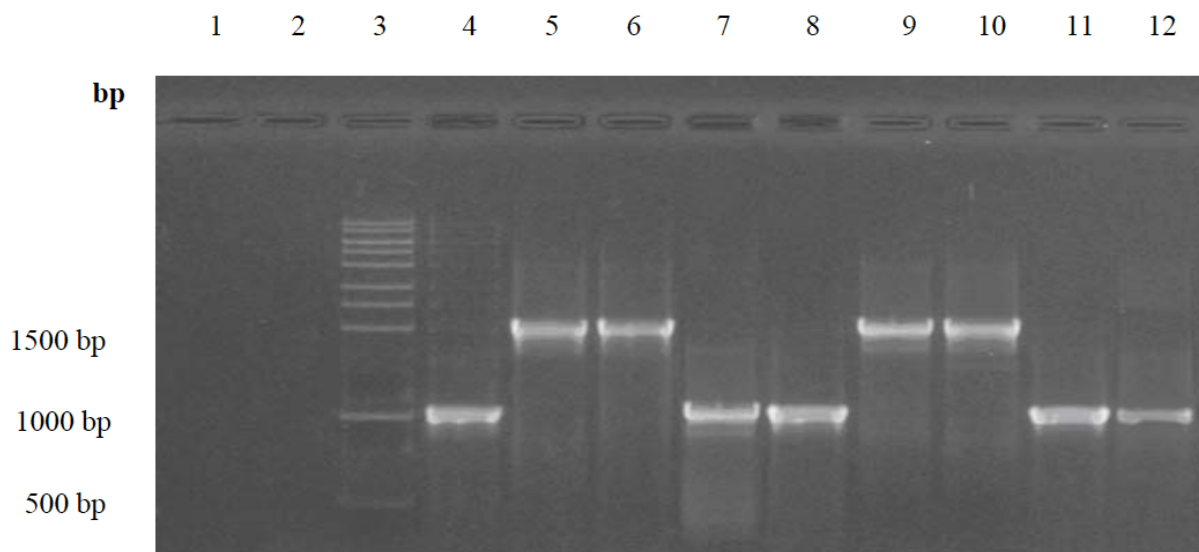
All *Salmonella* clinical isolates were tested for the susceptibility to ten antibiotics. The highest level of susceptibility among the isolates tested was registered towards ciprofloxacin and gentamicin (95%). Resistance profiles and the number of antibiotic classes to which bacterial isolates were simultaneously resistant are summarized in Table 1. Based on these results, a comparative analysis of *intI1*<sup>+</sup> and *intI1*<sup>-</sup> isolates was performed in order to reveal the association of antibiotic resistance phenotypes with integrons.

Five isolates (25%) were sensitive to all antibiotics tested (Table 1, isolates 1-5). It is noteworthy that all these sensitive isolates were *intI1*<sup>-</sup>, with the majority belonging to the *S. Enteritidis* serotype (isolates 1-4). Resistance to one or two classes of antibiotics was observed in four isolates (20%), and they were also *intI1*<sup>-</sup> (isolates 6-9).

The results revealed that 55% of the isolates tested (11 of 20) were resistant to four or more antibiotics (isolates 10-20) thus displaying an MDR phenotype. Notably, about 82% of MDR isolates were *intI1*<sup>+</sup> (9 out of 11) and the MDR phenotypes were observed in all integron-positive isolates, regardless of the serotype. Our study revealed only two *intI1*<sup>-</sup> strains with MDR phenotypes (isolates 10 and 12), and their resistance profiles were markedly different from the profiles of other MDR strains.

Currently, *S. Enteritidis* and *S. Typhimurium* are the predominant *Salmonella* serotypes encountered in clinical isolates in the region. Therefore, it was interesting to consider the MDR phenotype in these serotypes separately.

Five out of eight *S. Typhimurium* isolates tested (62.5%) were resistant to at least six classes of antibiotics thus displaying an MDR phenotype (isolates 16-20). Moreover, the isolate 16 exhibited ACSSuT resistance profile, typical of *S. Typhimurium* phage type DT104 [1, 2, 4]. It is known that among the *S. Typhimurium* DT104 strains the genes encoding for the



**Figure 1:** Electrophoretic separation of PCR amplicons generated with the inF-inB primer set (specific for variable segment of class 1 integrons): 1 – H<sub>2</sub>O control; 2 – *E. coli* K12 (negative control), 3 – DNA mol. weight markers (DNA-Leiter kombi, CL 22.1); 4-12 – *Salmonella* clinical isolates (4, 7, 8 – *S. Enteritidis*; 5, 6, 9, 10, 11 – *S. Typhimurium*; 12 – another *S. enterica* serotype).

mentioned penta-resistance profile are located in complex class 1 integrons, named In104, within 3' end of the chromosomal genomic island SG11 [1, 8]. SG11 is an integrative mobilizable element (IME), which can be transferred horizontally to other serotypes [9, 10]. The different types of SG11 MDR complex integrons have been described in a wide variety of *S. enterica* serotypes [11-13, 18].

Noticeably, four of the remaining *S. Typhimurium* isolates with the MDR phenotype exhibited the same resistance profile to seven classes of antibiotics, ACSSuTAuCx (isolates 17-20). It is known as another important MDR phenotype of non-typhoidal *Salmonella* [14]. This profile includes resistance to amoxicillin/clavulanic acid (Au) and ceftriaxone (Cx) in addition to the penta-resistance profile of *S. Typhimurium* phage type DT104.

Conspicuously, all MDR *S. Typhimurium* isolates were *intl1*<sup>+</sup> while the remaining *S. Typhimurium* isolates were resistant to no more than two classes of antibiotics and all of them were *intl1*<sup>-</sup>. Thus, the strong association between MDR phenotype and the presence of class 1 integrons in the *S. Typhimurium* isolates was observed.

Among the ten *S. Enteritidis* isolates tested, four (40%) were resistant to at least to four antibiotic classes thus exhibiting an MDR phenotype. Moreover, three of them (isolates 12-14) exhibited the profiles of resistance to six antibiotic classes, closely resembling the aforementioned penta-resistance profile, typical of *S. Typhimurium* DT104. The differences were observed in resistance to one antibiotic (Table 1). It is notable that all 3 *intl1*<sup>+</sup> *S. Enteritidis* isolates were MDR, similarly to *intl1*<sup>+</sup> *S. Typhimurium*. However, we revealed one *S. Enteritidis* isolate with the MDR phenotype, which was *intl1*<sup>-</sup> (isolate 12).

#### 4. DISCUSSION

In this work we focused on the prevalence of class 1 integrons and comparative analysis of resistance profiles between integron-positive and integron-negative isolates. Screening of *Salmonella* clinical isolates (n=20) detected the high prevalence of class 1 integrons (45%) with the two types of embedded gene cassettes of about 1000 bp (predominantly in *S. Enteritidis*) and 1500 bp (*S. Typhimurium* only), occurring separately.

Susceptibility testing indicated the high prevalence of MDR phenotypes in *Salmonella* clinical isolates

(55%), with the higher percentage of *S. Typhimurium* (62.5%) compared to *S. Enteritidis* (40%). Notably, the MDR phenotypes were observed in all integron-positive isolates, regardless of the serotype. Our results revealed the more frequent occurrence of the MDR phenotype in integron-positive isolates compared to integron-negative, especially for *S. Typhimurium* isolates. The results indicated a strong association between the MDR phenotype and the presence of class 1 integrons in the *S. Typhimurium* isolates. Still, not all MDR *Salmonella* isolates were *intl1*<sup>+</sup>, two of them were found to be *intl1*<sup>-</sup>. The latter suggests that the repertoire of mobile genetic elements (MGEs) carrying the battery of antibiotic resistance genes in our *Salmonella* isolates is not limited to integrons but may be represented by other MGEs as well.

In general antibiotic resistance profiles displayed certain serotype-specific features. Our results revealed that 25% of the isolates tested exhibited MDR phenotypes typical of non-typhoidal *Salmonella*, either with penta-resistance, ACSSuT, as in *S. Typhimurium* DT104, or resistance to 7 (ACSSuTAuCx) antibiotics [14]. It is of note that the latter isolates were *S. Typhimurium*, which is of great concern. Notably, among the *S. Enteritidis* isolates tested this MDR phenotype was not detected, although the closely related resistance patterns were observed. Although the resistance level in *S. Enteritidis* isolates is not as high as in *S. Typhimurium*, the MDR phenotype has become increasingly common in *S. Enteritidis*, which may compromise the therapy.

#### 5. CONCLUSIONS

Thus, the results indicated the high prevalence of class 1 integrons and associated MDR among the clinical *Salmonella enterica* isolates in Armenia, which may complicate the choice of antibiotics for empirical therapy. Further studies are necessary to reveal the genetic background of MDR phenotypes and to estimate the genetic kinship among the isolates. More rigorous regulations should be imposed on antibiotic use, together with a vigilant epidemiological surveillance, to prevent the emergence and spread of MDR non-typhoidal *Salmonella* isolates.

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