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**Incidence and Distribution of *Salmonella* Serogroups in Some Local Food in Sana'a - Yemen**

Rihab R Taha<sup>1</sup>, Saeed M Algalibi, Yasmin N AL-Ammari.

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## Abstract

Incidence and distribution of *Salmonella* in some local food were determined in Sana'a city from April 2009 to April 2010. Three hundred and sixty two (362) different food samples were collected from local markets. The results showed that positive food samples with *Salmonella* were 26 (7.2%). The highest percent of prevalence of *Salmonella* in food were in red meat 14.7% then chicken 12.1%, eggs 11.8%, cooked foods 9.5%, raw milk and milk products 5 %, juices 4.8%, vegetables 4.4%, sandwiches 3% while the pastries recorded the lowest percent 2.6%. Thirty two (32) isolates gave positive agglutination with omnivalent antisera only 10 isolates which were serogrouped (B, C1, C2-C3, D1, E1, and E4). Some food contained more than one serogroup, red meat contained 4 serogroups (B, C2-C3, E1, and E4), eggs contained 2(B and C2-C3), chicken contained 2(C1 and C2-C3) serogroups. The percent of distribution of serogroups among the ten identified isolates were C2-C3 40%, B 20%, and 10% C1, D1, E1, E4 for each serogroups.

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Key- word: Salmonella, Food poisoning, Enteric fever.

<sup>1</sup> corresponding author Email: [rihab\\_azawii@yahoo.com](mailto:rihab_azawii@yahoo.com). Mobail: 00967-734279312. Mailing address: Biology Department, Faculty of Science, Sana'a University, republic of Yemen.

## Introduction

*Salmonella* are responsible for a variety of illnesses, including typhoid fever, food poisoning, gastroenteritis, bacteraemia and septicaemia [1]. *Salmonella* infections of humans and animals continue to be major public health problem worldwide and also have a large negative economic impact on food production [2]. Human illness due to non-typhoidal *Salmonella* is mainly caused by consumption of contaminated food, which is important pathogen in infants and children. While gastrointestinal infections caused by non-typhoidal *Salmonellas* continue to be a problem of great magnitude in different parts of the world [3]. The CDC has estimated 1.4 million non-typhoidal cases occur annually in the United States causing an estimation of 80,000-160,000 persons to seek medical attention, resulting in 16,000 hospitalizations, and nearly 600 deaths because of non-typhoidal infection and it has been also estimated that 95% of these infections are food borne were due to consumption of contaminated food [4]. The main sources of Salmonellosis in humans are food animals and their products such as raw eggs, poultry meat and pork [5]. The contamination of foods comes through several factors which include contamination water, soil, and food processing equipments, food contact surface, and the most important factor that is responsible for most cases of food borne disease is food handling also the condition of food preparation which have been effected on food through storage temperatures and poor personal hygiene [6].

At Meeting on Food Safety for the Near East, whereas, they put the Republic of Yemen with countries that are the least developed in terms of food borne disease surveillance and food safety infrastructure, while severe gastroenteritis is a major public health problem worldwide and a major contributor to mortality and morbidity in developing countries [7]. In Yemen a few studies were carried out concerning *Salmonella*, mainly on human infection [8 , 9] and from

poultry slaughter houses [10]. Unfortunately, only one (MSc) thesis was done on *Salmonella* in food but with negative results [11]. Since, the source of infection with *Salmonella* are food and water, it is important to search for presence and distribution of *Salmonella* in food . Also diagnosis of *Salmonella* down to the serogroups or serotypes level, on different types of food, may help to understand the epidemiology of this bacteria.

### Materials and methods

From April 2009 to April 2010, three hundred and sixty two (362) different local food samples including red meat, chickens, cooked food, pastries, raw milk, dairy products, eggs, vegetables and juices were collected from local supermarkets, retail markets and vendors in streets at Sana'a city. The preenrichment media used were Mannitol broth and Neutral Red Lysine Iron Cysteine broth(NRLIC) were inoculated Tetrathionate broth and Selenite Cystine broth as selective enrichment media. Then they were streak on differential media, Brilliant Green (BG) Agar and Xylose Lysine Desoxycholate (XLD) agar. All samples were incubated at 37°C for 24h . After incubation period three presumptive *Salmonella* colonies from each differential media were submitted for identification by biochemical tests. *Salmonella* isolates that gave typical biochemical reactions were submitted to serogrouping tests [12]. Serogrouping was done in Yemen using antisera from Denka Seiken CO., LTD. Tokyo, Japan.

### Results

The results showed that positive food samples with *Salmonella* were 26 (7.2%) samples out of (362) different food samples. Whereas the highest percent of prevalence of *Salmonella* in food were in red meat 14.7% then chicken 12.1%, eggs 11.8%, cooked food 9.5%, raw milk and

milk products 5 %, juices 4.8%, vegetables 4.4%, sandwiches 3% while the pastries recorded the lowest percent 2.6%, Table (1).

Table 1: Percent of *Salmonella* contamination in local food from Sana'a city.

Food kinds	No. of samples examined	No. of positive samples with <i>Salmonella</i>	(%) of total samples (362)	(%) of contamination of each food kind
Red meat	34	5	1.3	14.7
Chicken	33	4	1.1	12.1
Eggs	34	4	1.1	11.8
Cooked food	42	4	1.1	9.5
Raw milk and milk products	60	3	0.8	5
Juices	42	2	0.6	4.8
Vegetables	45	2	0.6	4.4
Sandwiches	33	1	0.3	3
Pastries	39	1	0.3	2.6
Total	362	26	7.2	-

The results of preenrichment media, selective enrichment and differential media on the isolation of *Salmonella* from food showed that Tetrathionate selective enrichment medium gave 19 positive isolates of *Salmonella* picked up from 378 presumptive isolates of *Salmonella* which represent (5%), compared to Selenite Cystine broth that gave 9 isolates of *Salmonella* out of 192 isolates that recovered by this kind of selective medium with the percent of (4.7%) see Table (2). Then total of 441 colonies including 282 colonies were collected from XLD medium which were inoculated previously from Tetrathionate broth and 159 colonies collected from XLD medium which were inoculated previously from Selenite Cystine broth, while on BG medium total of 129 colonies including 96 presumptive *Salmonella* colonies were collected from BG which were

inoculated previously from Tetrathionate broth and 33 presumptive *Salmonella* colonies inoculated from Selenite Cystine broth. The results appeared that 25 (5.7%) isolates included 16 (5.7%) isolates were positive *Salmonella* from Tetrathionate broth on XLD medium and 9 (5.7%) isolates from XLD medium which was inoculated previously from Selenite Cystine broth. Results of BG medium showed that 3 (3.1%) isolates were positive *Salmonella* from BG medium which were inoculated previously from Tetrathionate broth, while BG medium which was inoculated previously from Selenite Cystine broth failed to isolate *Salmonella*, so the total percentage of isolation *Salmonella* from BG medium was (2.3%), see Table (2).

Neutral Red Lysine Iron Cystine(NRLIC) broth works as preenrichment and selective media so samples transferred directly to differential media, one loopful from (NRLIC) broth were inoculated on XLD agar and BG agar separately. From each plate of XLD and BG a number of three colonies which were corresponded with typical *Salmonella* colonies characteristic on those media were selected for further identification. Total of 6 colonies were picked up from XLD and 3 colonies were picked up from BG medium Table (2), which indicated that 2 (33.3%) isolates were positive to *Salmonella* from XLD medium, while on BG medium no isolates were recovered Table (2). Regarding samples were inoculated to Mannitol broth, one loopful from each selective media Tetrathionate broth and Selenite Cystine broth was inoculated on XLD and BG agar plates separately then 3 colonies which were corresponded with typical *Salmonella* colonies feature were picked up for further identification. A total of 9 colonies were collected from XLD medium which were inoculated previously from Tetrathionate broth and 3 colonies collected from XLD medium which were inoculated previously from Selenite Cystine broth Table (2), while on BG medium results showed a total of 6 presumptive *Salmonella* colonies were collected from BG which were inoculated previously from Tetrathionate broth and 6 colonies of *Salmonella* inoculated from

Selenite Cystine broth. The results showed that 2 (33.3%) isolates from BG medium were *Salmonella* positive while on XLD medium no *Salmonella* isolates were recovered Table (2).

Table 2: Effect of selective enrichment media and differential media used for isolation *Salmonella* from food and milk and dairy products

	Selective enrichment and Differential media	No. of food samples		No. of presumptive <i>Salmonella</i> colonies examined				No. of confirmed <i>Salmonella</i> isolates		
		No	%	No	%	No	%	No	%	
Food	Tetrathionate broth	302		378				19	5	
	Selenite Cystine broth	302		192				9	4.7	
	Type of media	No. of colonies examined		No. of <i>Salmonella</i> isolates				Total No. of <i>Salmonella</i> isolates		
	Selective	Differential	T.T	S.C	T.T		S.C		No	%
			No	%	No	%				
	XLD	282	159	16	5.7	9	5.7	25	5.7	
	BG	96	33	3	3.1	0	0	3	2.3	
	Total	378	192	19		9		28		
Milk and milk produces	preenrichment media	Neutral Red Lysine Iron Cystine broth				Mannitol broth				
	Selective	Differential	No. of presumptive <i>Salmonella</i> colonies examined	No. of confirmed <i>Salmonella</i> isolates	No. of presumptive <i>Salmonella</i> colonies examined		No. of confirmed <i>Salmonella</i> isolates		% positive	
					No	%	T.T	S.C	T.T	S.C
	XLD	6	2	33.3	9	3	0	0	0	0
	BG	3	0	0	6	6	2	0	33.3	0
Total	9				24					

Before the biochemical tests reactions Gram stain was done to six hundred and three (603) colonies as a total colonies collected from all samples examined comprised five hundred and seventy 570 colonies including 441 colonies from XLD medium and 129 colonies from BG medium from foods samples and thirty three 33 colonies from milk and dairy products. Five hundred and ninety one 591 colonies that gave Gram stain negative and have a rod shape, were submitted for biochemical tests. Total of 591 isolates submitted first for urease test, showed that five hundred and two 502 isolates gave positive reaction for urease test, so they were excluded, while the isolates that gave negative reaction were eighty nine 89 (15.1%) isolates which were more likely to be presumptive *Salmonella*. However, 89 isolates submitted for the following biochemical tests including: Triple Sugar Iron (TSI) test, Citrate test, Mannitol fermentation test, Methyl Red test, Voges-proskauer (V-p) test, Sulphate Indole Motility (SIM) and Motility Indole Lysine (MIL) for Lysine decarboxylase test used to identify presumptive colonies of *Salmonella*.

*Salmonella* serogrouping: Identification of *Salmonella* serogroups was done by using slide agglutination procedure. Sixty three (63) isolates that supposed to be *Salmonella* according to biochemical test were submitted to serogrouping tests. The numbers of isolates, that gave positive agglutination with ominovalent antisera which contain all O somatic groups (from 1-67) were thirty two (32) isolates Table (3).

Then these 32 isolates were submitted to the reaction with polyvalent A-S, the results showed that only 15 isolates were positives with A-S antisera and 17 isolates were negative. The fifteen (15) isolates were distributed in five kinds of food as follows: red Meat 5 (33.3%) isolates, eggs 4 (26.7%), chicken 3 (20%), milk and milk products 2 (13.3%) and sandwiches 1(6.7%). From these fifteen isolates only ten (66.6%) isolates showed positive reaction with polyvalent O antisera, which were distributed in four kinds of food, red meat 5 (33.3%) isolates, eggs 2 (13.3%),



chicken 2 (13.3%), and sandwiches 1(6.7%), while the other five isolates reacted negatively with polyvalent O antisera. Then 22 (17 + 5) isolates were submitted to polyvalent O1 antisera, for another check, and the results showed all the 22 isolates were negative with polyvalent O1 antisera, Table (3). All 32 isolates were submitted to react with Vi antigen and the results showed that only one isolate was positive with Vi antisera, Table (3).The ten positive isolates with O antisera were distributed in six serogroups including B20%, C<sub>2</sub>-C<sub>3</sub>40%, C<sub>1</sub>,E<sub>1</sub>,E<sub>4</sub> and D<sub>1</sub>10% each.

Results were illustrated in Table (3) indicated that some kind of food contained with more than one serogroup of *Salmonella*, for example red meat contained 4 groups (B, C<sub>2</sub>-C<sub>3</sub>, E<sub>1</sub>, and E<sub>2</sub>), eggs contained 2 groups (B, C<sub>2</sub>-C<sub>3</sub>) and two isolates were not identified, chicken contained 2 groups (C<sub>1</sub>, C<sub>2</sub>-C<sub>3</sub>) and one isolate was not identified, and sandwiches contained one isolate was belong to D<sub>1</sub> group which was the only positive isolate with Vi antigen . After somatic antigen and Vi antigen were detected, all isolates that were positive to ominovalent, polyvalent O, polyvalent A-S and Vi antisera submitted for the detection of H antigen by the available H antisera ( H-d, H-e,h, H-i, H-G, H-z29), results showed that only two isolates were positive with H-d and H-i antigens, those belonged to groups C<sub>2</sub>-C<sub>3</sub> in chicken and eggs, Table (3).

Table 3: presence and distribution of *Salmonella* serogroups in foods of Sana'a city

Monovalent ( O somatic antigens serogroups)	ominovalent (1-67)	Poly A-S	Poly O	Antigenic formula				Poly O1		
				O	H		Vi			
No. of isolates examined (63)	32V+	15V+	10 V+							
Sources of isolates	No. of isolates /		serogroups		I	II				
Red meat	7	5		0	B	4	U	U	—	2
			C2-C3		8	U	U	—		
			C2-C3		8	U	U	—		
			E1		3,10	U	U	—		
			E4		1,3,19	U	U	—		
Eggs	4	4	2	C2-C3	8	d	U	—	2	
				B	U	U	U	—		
				—	U	U	U	—		
				—	U	U	U	—		
Chicken	5	3	1	C1	7	U	U	—	3	
				—	U	U	U	—		
				C2-C3	8	i	U	—		
Raw milk	2	2	0	—	U	U	U	—	2	
				—	U	U	U	—		
Sandwich	3	1	0	1	D1	9	U	U	+	2
Cheese	2				Unidentified		U	U	—	2
Cooked food	4						U	U	—	4
Vegetables	2						U	U	—	2
Juices	2						U	U	—	2
Pastries	1						U	U	—	1
Total	31	32	15	5		10	10			

U : Unidentified because of unavailability of antisera. Ominovalent antisera: All O somatic groups (1-67). Polyvalent A-S antisera: All polyvalent O and O1 groups in addition to groups: O28, O30, O38, O 39, O40, and O41. Polyvalent O (O2, O4, O7, O8, O9. O9, 46, O3, 10, O1, 3, 19). Polyvalent O 1(O11, O13.O6.14. O16, O18, O21.O32). Available H antisera (H-d, H-e,h, H-i, H-G, H-z29).  
 +V: Positive reaction. —V: Negative reaction.

## Discussion

Isolation of *Salmonella* from food with the percent of 7.2%, are consistently with many similar reports of other countries such as; in Saudi Arabia [13] were *Salmonella* isolated from 219 (8.9%) samples out of 2474 different type of food and feed samples. In Khartoum state of Sudan it was determined the prevalence of *Salmonella* in food collected from raw and cooked food, domestic meat and fecal samples, chicken samples and water were 92 (9.2%) positives out of 996 samples( [14] . In similar study, in Yemen(11) found that out of 700 different types of foods including fresh and frozen meat, fresh and frozen chicken, milk, Taizi cheese and cheese, no *Salmonella* spp. was isolated from all these foods samples, which is inconsistently with our finding. Other study in Egypt [15] found that incidence of *Salmonella* in 200 samples of foods included; milk, cheese, sausage, and meat were 2 (1%) samples from cheese and sausage positives of *Salmonella*. In Morocco[16]found cooked meat, sausages, chicken, meat, pastry, seafood, spices, water and slaughter house collected from 2002 to 2005, overall percentage of *Salmonella* prevalence were 105 (0.91%).However the differences between *Salmonella* incidence from one country to another may be due to different risk factors of transmission of *Salmonella* to food that can be occurred, including contaminated of raw food by animal faeces, contact with animals or their environment, contaminated water and personal hygiene[17].

Most outbreaks of Salmonellosis have resulted from the consumption of contaminated meat, eggs, or dairy products [18].

Results of this study revealed that the highest percentage of incidence and isolation of *Salmonella* were from red meat 5 (14.7%) samples, then chicken 4 (12.1%), eggs 4 (11.8%), cooking food 4 (9.5%), raw milk and milk products 3 (5%), juices 2 (4.8%), vegetables 2 (4.4%) , sandwiches 1 (3%) and pastries 1(2.6%) Table (1). It was published that more than 95% of cases of *Salmonella*

infection are foodborne [19]. The primary pathogenic for human infected with *Salmonella* are serovars that caused contamination of foods from animal sources such as meat, poultry, eggs, milk and milk products. Increasing attention has been focused on the prevention and control of *Salmonella* in animal production, which is the main source of outbreaks in humans. Other reason may be meat samples that contaminated by *Salmonella* appeared high sensitivity for cultures methods, which are able to detect at least 0.4 CFU of *Salmonella* spp. [20]. In addition cross contamination of carcasses with *Salmonella* can also occur during slaughtering operations. The important route of transmission of *Salmonella* organism from animals to man is via food products of animal origin which may be contaminated at the source or during handling and contamination of meat by *Salmonella* may occur at the abattoir from symptomless animal excretes, contaminated an abattoir equipment and floors, where *Salmonella* gains access to meat at any stage during slaughtering operations [21]. We noticed in Yemen's that the slaughter houses lacking to any substantial efforts to prevent *Salmonella* contamination of various foods from farm to consumers which play main role in meat contamination. In this study chicken showed 12.1% incidence of *Salmonella* from different parts of chicken carcasses. *Salmonella* infections in chicken continue to be a major problem worldwide, according to U.S. Department of Agriculture estimates, nearly 40 percent of the poultry supplies are contaminated with *Salmonella* [22]. Then 11.8% of eggs samples were contaminated with *Salmonella*, generally, eggs can get contaminated by *Salmonella* bacteria via transmission means that the eggs get contaminated by penetration of *Salmonella* through the egg shell during or after oviposition, with the bacteria coming from the colonized gut or from contaminated feces [23]. We observed during the collection of eggs sample, most of eggs were contaminated with chicken feces. Regarding cooking foods 9.5% of these foods carrying *Salmonella*, food is contaminated in the

kitchen after it has been cooked may be when using stuff cooking contaminated by *Salmonella* before and after cooking, cooked food that stands at room temperature for a long time, especially poultry, is at risk [22].

*Salmonella* was isolated from milk and milk products with 5% ,it is indicated that milk and other dairy products is other potential route for *Salmonella* infection of humans, *S. Enteritidis* as well as *S. Typhimurium* were occasionally isolated from milk and dairy product that contaminated from feedstuffs to dairy cow and milk [24]. Food and agricultural organization defines street foods as ready-to-eat foods including cooked food, sandwiches, pastries, salads and juices [25]. In our study ready-to-eat foods samples included cooked food, sandwiches, pastries and juices appeared different level of *Salmonella* incidence, in cooked foods (9.5%), juices (4.8%), sandwiches (3%) and pastries (2.6%).

Isolation of *Salmonella* by using two different types of selective media showed that there were no differences between Tetrathionate broth and Selenite Cystine broth for isolation of *Salmonella* from foods in this study, Table (2). *Salmonella* present in small numbers in foods and some of selective media tend to inhibit the growth of certain types of *Salmonella* [9]. Regarding differential media used in this study it was observed that XLD medium more effective than BG medium for *Salmonella* isolation, since *Salmonella* recovered by a percent of (5.7%) from XLD medium, while from BG medium the percent of recovery was (2.3%), Table (2). Another study in Yemen indicated that XLD medium was more differentiate and specific for *Salmonella* than SSA medium, the percent of *Salmonella* isolated on XLD (77.6%), while on SSA (22.9%) [9].

In milk and dairy products results of differential media showed that from Nutrient Red Lysine Iron Cystine broth on XLD were 2 (33.3%) out of 6 colonies examined, while on BG medium

failed to detect *Salmonella* from milk and dairy products from Nutrient Red Lysine Iron Cystine broth, see Table (2). Isolation of *Salmonella* from differential media with Mannitol broth show that Tetrathionate broth was successfully with BG medium to detect *Salmonella* from Mannitol broth which were 2 (33.3%) out of 6 colonies were examined, while Selenite Cystine broth and XLD medium failed to detect *Salmonella* from Mannitol broth, Table (2). Our results were in agreement with [26].

Serological formula was done for each *Salmonella* isolates according to available imported O, H and Vi antisera. In this study results indicated that the presumptive sixty three (63) *Salmonella* isolates according to biochemical test were submitted to serotyping tests according to Kauffman-white scheme, these isolates were submitted to omnivalent antisera which contain all O somatic groups (from 1-67), 32 isolates gave positive agglutination with omnivalent antisera. Then these thirty two (32) isolates were submitted to polyvalent A-S antisera 15 isolates were positives with polyvalent A-S antisera and distributed in five types of foods red meat, eggs, chicken, raw milk and sandwiches, 10 isolates of them were positive with polyvalent O antisera. These isolates were serogrouped as the following: serogroup C2-C3 4( 40%) isolates, followed by serogroup B were two isolates(20 %) and then the serogroups C1, D1, E1 and E4 one isolates(10 %) for each Table (3), another study in Sanaa the serogroups isolated from Yemeni children were group C(60.5%) and group B (29%). While in Tamar city [9] *Salmonella* serogroups were isolated from patients were serogroup D1(70%), serogroup B(27.7%) and serogroup A(2.2%).

While 5 isolates were negative with polyvalent O antisera were not identified by the available antisera Table (3). On other hand 17 isolates were negative with polyvalent A-S antisera but were positive with omnivalent which mean that these isolates are *Salmonella* but polyvalent A-S antisera lacked other somatic serogroups from O42 to O67. In Saudi Arabia [27] the adjacent

country to Yemen, eleven *Salmonella* serogroups were identified, approximately 96.88% of all *Salmonella* serogroups isolated from poultry and poultry environments are members of the antigenic groups B71 (24.6%), C197 (24.7%), D186 (31.9%) and C<sub>2</sub>-C<sub>3</sub> 3 (5.2%).

The predominance and distribution percentage of *Salmonella* serotypes in food are different in different region of the world according to methods of isolation, quality of the sample and growth characteristics of the serovars, particularly those adapted to a host species [20]. It is important to mentioned that *Salmonella* continue to increase the new serotypes annually there are 2,463 serotypes (serovars) of *Salmonella* in 2000 while according to WHO collaborating center for reference and research on *Salmonella*, which mentioned the present number of total *Salmonella* serovars were 2579 serovars, which caused *Salmonella* nomenclature so complex and confusion and new serotypes are listed in annual updates of the Kauffmann-White scheme [28,29].

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## تواجد وانتشار المصليّة لجنس السالمونيلا في بعض الأغذية المحليّة في صنعاء - اليمن

رحاب رشيد طه ، سعيد منصر الغالبي ، ياسمين ناجي العماري

تم تحديد تواجد وانتشار بكتيريا السالمونيلا في بعض الاغذية المحليّة في مدينة صنعاء من أبريل 2009 إلى أبريل 2010. تم جمع ثلاثمائة واثنان وستون (362) عينة غذائية مختلفة من الأسواق المحليّة. أظهرت النتائج أن العينات الغذائية الموجبة لبكتيريا السالمونيلا 26 (7.2%). كانت أعلى نسبة لانتشار السالمونيلا في الغذاء هي في اللحوم الحمراء 14.7% ثم الدجاج 12.1% والبيض 11.8%، والأطعمة المطبوخة 9.5%، الحليب الخام ومنتجات الألبان 5% والعصائر 4.8% والخضروات 4.4%، السندويشات 3% في حين سجلت أقل نسبة منوية في المعجنات 2.6%. أعطت اثنان وثلاثين (32) عزلة تفاعل موجب مع الأمصال المضادة ominovalent . وقد تم تحديد المصليّة لعشرة (10) عزلات منها فقط وهي المجموعة المصليّة B ، C1 ، C2-C3 ، D1 ، E1 ، وE4. أحتوت بعض المواد الغذائية على أكثر من مجموعة مصليّة كما في اللحوم الحمراء 4 (B ، C2-C3 ، E1 ، وE4) والبيض 2 (B ، C2-C3) والدجاج 2 (C1 ، C2-C3) . وكانت النسبة المنوية لانتشار المصليّة بين العزلات العشرة المشخصة هي 40% C2-C3 ، 20% B ، و10% لكل من المصليّة C1 ، D1 ، E1 ، E4 .