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The Microbiological Quality of Commercialized Food Products in Northwest of Morocco

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ABSTRACT: The microbiological quality analysis of 914 samples of food products, taken at restaurants and food outlets the most vulnerable to human health in the Tetouan region (Northwest of Morocco) between 2012 and 2017, were conducted at the Regional Laboratory for Epidemiological Diagnosis and Environmental Health. This analysis showed that the rate of non-compliance of different food categories reached 77.8% during 2012, and then it dropped to a percentage of 60.5% in 2014, and a rate of 62.5% during the year 2017. This non-compliance affected all the food categories studied, but it varies according to the type of food. Meat products and raw vegetables are the most contaminated, with a rate of non-compliance of 80.75% and 81.81% respectively, followed by dairy products with a rate of 63.47%, and ready meals with 60 %.

KEYWORDS: Food restaurants, food outlets, microbiological quality, Northwest of Morocco.

1 INTRODUCTION

The way of life in developed countries and developing countries such as Morocco has evolved for several decades. This evolution has significantly modified dietary habits [1]. Despite the success of our modern societies in the control of major infectious problems, they find themselves in difficulty, faced with some aspects of food hygiene [1], especially in Africa; food hygiene represents a public health problem.

In developing countries, fast food, canteens and institutional catering are becoming inevitable in the current urban development scheme [2]. In Morocco, because of rapid urbanization and the changing people's eating behavior, this type of food is becoming more and more widespread in society, so meals served in sales outlets and restaurants can be the source of collective food poisoning if hygienic precautions are not taken into consideration [3].

Foodborne illness is a public health problem for both industrialized and developing countries. In the latter case, the management of these diseases is obviously heavy, hence the need to improve surveillance programs and to set up a standard practice for this type of poisoning [4], especially the number of declared cases is below reality. This problem is common to all developing countries. The comparison of national data with data from the American Association of Poison Control Centers shows per 100 000 inhabitants in the USA there are 46 times more reports than in Morocco, with a very big difference between reporting methods [5].

The highest number of food poisoning cases in 2011 was registered in the Marrakech-Tansift-Al Hour (western region), which has more than 1400 active declarations, and more than 250 passive declarations cases, followed by the region of Sousse-Massa-Draa, the eastern region and the Doukkala-Abda (center region) [17]. The region of Tangier-Tetouan is ranked fifth in

the country, with more than 100 active cases, and the number of passive declarations does not exceed 100 cases [5]. While in 2016, the Tangier-Tetouan-Al Hoceima region, ranked first nationally in the number of reported cases (107.4 per 100 000 population), is 19.45%, followed by the Marrakech-Safi region (17.57%), Rabat-Sale-Kenitra (83.6 per 100 000 inhabitants) or (15.35%), and the Fes-Meknes region (14.39%) [15], [24]. These statements concern all types of intoxication.

Food poisoning is the third leading cause of intoxication nationwide with 17.2% among all causes. The reporting rate in the period between 2014 and 2016 is 16.63% in 2014, 19.38% during the year 2015, and 17.2% during the year 2016, with a case fatality rate of 0.3%, 0.2%, 0.1% respectively [6].

The Moroccan Poison Control Center (CAPM) recorded 2 723 cases of foodborne illness during 2016, of which 53.5% are cases of collective food poisoning. The most widely implicated foods were composite foods (12.0%), meat and meat products (7.2%), fruits and vegetables (6.2%) and dairy products (5.5%) [7]. The collective food poisoning affects all regions of Morocco, with a clear predominance in urban areas. The majority of episodes occur during the summer. The severity of the episodes is usually judged by the percentage of hospitalization cases that appear high 32% on average for the period 2008-2016. The case fatality rate remains low 0.40%. However, in 85% of cases, the confirmation could not be made [7].

In France, the mandatory declaration of collective food poisoning revealed a considerable increase in the number of outbreaks reported between 2005 and 2010. Between 2006 and 2010, the number of outbreaks of collective food poisoning in France increased significantly, reaching 116.4% increase in 2009 [8].TIACs with unknown pathogens accounted for 18.7%, and collective food poisoning for which the offending food was unknown, 26.6% of all outbreaks between 1996 and 2004. Over the same period, collective food poisoning outbreaks in commercial catering, accounted for 23.2% of households during the study period [8].

Failure conditions and hygiene, and lack of training of officer catering sector, are often the main causes of food poisoning, for compliance against the rules of hygiene and good practices within restaurants and point-of-sale convenience stores help prevent most of these intoxications.

Considering the significant increase of cases of collective food poisoning in the region of Tetouan, surveillance and epidemiological checks carried out by the regional laboratory of epidemiological diagnosis and environmental hygiene of Tetouan during the period of 2012-2017, on four food categories: ready meals, Dairy products, meat products, and raw products. A total of 914 food samples were analyzed, and the desired parameters are enumeration of total flora, total and faecal coliforms, staphylococci, Anaerobic Sulfite-Reducing bacteria, *Salmonella* and *Listeria monocytogenes*.

2 MATERIAL AND METHODS

2.1 SAMPLING

As part of the epidemiological surveillance conducted by the Ministry of Health, routine food checks are planned at the end of which samples are taken at restaurants and food outlets the most vulnerable.

Our study was conducted in the Tetouan region located in northwest of Morocco. It covers 914 food samples from four main categories: ready meals, dairy products, meat products and raw vegetable products. These samples are taken from restaurants of different classes, traditional and modern dairies, butchers, cafes and street vendors. Once collected, between 11: 30 am and 3: 00 pm, the samples are sent to the laboratory. The transport is carried out in insulated boxes of 2 to 8°C, with accumulators of cold and in sterile bags. Delivery times do not exceed two hours after picking.

Once in the laboratory, the samples are coded and entered on the food sample receipt register and their temperature is measured using an infrared thermometer code: IHM8810SI. Then they are kept at a temperature between 2°C and 8°C. The analysis was carried out within 22 hours after their reception at the laboratory.

2.2 MICROBIOLOGICAL ANALYSIS

The searched parameters are the total coliforms (TC), fecal coliforms (FC), total germ (TG), the anaerobic sulphite-reducing bacteria (ASR), *Staphylococcus aureus*, *Salmonella* and *Listeria*.

The preparation of stock solutions and decimal dilutions for the microbiological examination is carried out according to the Moroccan standard (NM 6887) 2006 [26].

Indeed the preparation of the stock solution is made in sterile bags, weighing 25g of sample using a calibrated balance (aeADAM), whose uncertainty is 0.001, by adding 225 ml of peptone water buffered. The homogenization is carried out using

the homogenizer (BagMixer® 400, interscience, France) for 1min. A dilution series of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} in buffered peptone water was made after 20-30 min. Total coliforms, fecal coliforms, total germs and the anaerobic sulphite-reducing bacteria (ASR) are investigated by 1 ml of the successive decimal dilutions to the two-layer incorporation method into the appropriate agar plates.

For coliforms, the incubation was at 37°C for 24 hours and for faecal or thermo-tolerant coliforms the incubation was at 44°C for 24 hours. To search the total germs, the inoculum was incorporated into the Plat Count Agar (PCA) aerobic plate count (Biokard diagnostics, France), and Incubated at 30°C for 72h ± 3h. For the anaerobic sulphite-reducing bacteria, the Tryptose-Sulphite-Cycloserine (TSC) agar (Biokard diagnostics, France) egg yolk free was used. The dishes are placed in anaerobic jars and incubated at 37°C for 20h.

For Staphylococcus aureus (coagulase-positive staphylococci), 0.1 ml of the stock solution is seeded on the surface of Baird-Parker Agar (Biokard diagnostics, France), with addition of egg yolk tellurite. The dishes are incubated at 37°C for 48 hours and followed by a confirmation of colonies. Positive coagulase colonies are *Staphylococcus aureus*.

The search for Salmonella was performed on 25 g of each sample which were weighed in sterile bags (bag code) and mixed with 225 ml of peptone water, and then incubated at 37°C for 24 h. Subsequently, 0.1 ml of this pre-enrichment solution is transferred into tubes containing 10 ml of the enrichment solution Rappaport vassiliadis (Biokard diagnostics, France) and 2 ml of the same pre-enrichment solution are transferred to tubes containing 20 ml of Selenite-Cystine broth (Biokard diagnostics, France). Tubes of both batches were incubated for 24 hours at 42°C and 37°C respectively. These enrichment solutions are seeded on Hektoen Enteric Agar (Biokard diagnostics, France), and Salmonella Shigella Agar (Biokard diagnostics, France). Suspect positive tubes undergo a series of biochemical tests: Kligler-Hajna Agar seeding (Biokard diagnostics, France), urea-indole test, ONPG (Mast Diagnostics, UK) and Api 20 gallery (BioMerieu SA, France). The gallery was read by software Api 20 which determines the bacterial genus and some species of *Salmonella*. For a more accurate identification of detected *Salmonella*, the Ag-Ag serological agglutination test was carried out.

For the search for *Listeria monocytogenes*, a stock solution was prepared from 25g of the sample added to 225g of the Fraser II broth (Biokard diagnostics, France) and 2.25ml of the Fraser II additive were added to make a primary enrichment. The preparation was incubated at 30°C for 24 h ± 2h. For the secondary enrichment, 0.1 ml of the primary enrichment solution is taken, 10 ml of Fraser I broth (Biokard diagnostics, France) and 0.1 ml of Fraser I additive were added. The incubation is carried out at 37°C for 48h ± 2h. For the isolation and identification of *Listeria*, the primary and secondary enrichment were removed with the sterile loop and three Petri dishes containing Palcam Agar and Oxford Agar (Biokard diagnostics, France) were sequentially inoculated and incubated at 37 °C for 48h ± 2h, and Compas Listeria Agarbroth (Biokard diagnostics, France) incubated at 37°C for 24h ± 2h. For the confirmation of the genus and the species of *Listeria*, 5 colonies obtained from the three agar plates mentioned above are inoculated on Tryptone Soy Extract Yeast Agar (TSYEA) broth (Biokard diagnostics, France). After incubation at 37°C for 24h ± 2h, these colonies are then subjected to oxidase, catalase, Gram stain and mobility tests if necessary. Once the genus *Listeria* has been confirmed, the presence or absence of *Listeria monocytogenes* is checked by the hemolysis test on fresh Blood Agar. The appearance of a small β-haemolytic band informs about the suspicious presence of the *Listeria monocytogenes*, confirmed by the biochemical gallery (Api Listeria) (BioMerieu, SA, France). In this study, the food quality indicator adopted was noncompliance. A food that did not meet the recommended standards (Moroccan standards) was considered non-compliant and therefore of poor hygienic quality.

2.3 STATISTICAL ANALYSIS

The results were analyzed by SPSS software SPSS 20.IBM, version 22.

3 RESULTS AND DISCUSSION

The statistical analyses of the food samples were carried out by SPSS software.

Table 1. Percentage of non-compliance of food samples analyzed in Regional Laboratory for Epidemiological Diagnosis and Environmental Health (LRDEHM) by years (2012-2017)

		Compliance		Total
		non-compliant	Compliant	
Year	2012	77,8%	22,2%	100,0%
	2013	72,7%	27,3%	100,0%
	2014	60,3%	39,7%	100,0%
	2015	61,5%	38,5%	100,0%
	2016	64,6%	35,4%	100,0%
	2017	62,5%	37,5%	100,0%
Total		67,8%	32,2%	100,0%

Table 2. Pearson chi-square tests

	Value	ddl	Asymptotic significance (bilateral)
Pearson's chi-square	18,844[a]	5	,002
Likelihood ratio	19,258	5	,002
Linear association	12,353	1	,000
N of valid observations	914		

0 cells (0.0%) have a theoretical size less than 5. The minimum theoretical size is 36.35.

The average number of samples received at the laboratory exceeds 250 samples per year, these samples are from different food categories, but our study spread between 2012 and 2017 was devoted to the four main categories, and the total number of samples studied in each year is presented in Table 1.

Food non-conformity during the study period reached 77.8% during the year 2012. The year 2013 recorded such a high rate of non-conformity equal to 72.7%. From the year 2014, we notice a decrease in non-conformity up to 60.3% in 2014, 64.6% in 2016 and 62, 5% in 2017.

These percentages of non-conformity are comparable to those found in the Fes region, according to a study carried out during 2012, on different types of food by the regional laboratory for epidemiological diagnosis and environmental hygiene of Fes. The nonconformity rate was 75.10% [1].

Table 3. Percentage and Frequency of non-compliance of different food categories analyzed during the study period

Category	Subcategory			Frequency	Percentage
	vegetables	Valid	non-compliant	103	78,0 %
			comply	29	22,0 %
			Total	132	100,0
Raw vegetables	Salad	Valid	non-compliant	68	88,3 %
			comply	19	11,7 %
			Total	87	100,0
			average		81,81%
		Valid	non-compliance	218	60,0%
A cooked dish			comply	148	40,0%
			Total	366	100,0%
	Icecream	Valid	non-compliance	36	61,0%
			comply	23	39,0%
			Total	59	100,0%
	cheese	Valid	non-compliance	11	33,3%
			comply	22	77,7%
			Total	33	100,0%
	Milk juice	Valid	non-compliance	46	76,7%
Dairy product			comply	14	23,3%
			Total	60	100,0%
	Milk	Valid	non-compliance	13	86,7%
			comply	2	13,3%
			Total	15	100,0%
			average		63,47%
	Sausage	Valid	non-compliance	41	63,0%
			comply	24	37,0%
			Total	65	100,0%
Meat	Groundmeat	Valid	non-compliance	100	90,9%
			comply	10	9,1%
			Total	110	100,0%
			average		80,57 %
Total			non compliance		70,84 %

Table 3 shows the frequency and percentage of contaminations of each food category, and the subcategory. It contains; vegetables without sauce and vegetables with sauce (salad), the percentage of non-compliance of cut vegetables was 78.0%. Vegetables with added sauces (dressing sauce) recorded a percentage of noncompliance equal to 88.3%. This rise in nonconformity in salads may be due to the acidity of acetic acid which promotes the growth of bacteria besides the desired bacteria, such as lactobacilli and clostredia bacteria that multiply in acid and whose pH is less than or equal to 4 [9]. It is also due to the multiplication of yeasts and molds that can develop at acidic pH ranging from 1.5 to 6. The values of the nonconformity of raw vegetables in general was due to the sensitivity of the hygiene technicians who target during the sampling the points of sale and the most vulnerable sites of restoration, especially with the tourist activity increases in the region of more and more

The values of non-conformity in our region represent more than double those of Fes region in a study spread between 2003 and 2006 [3].This is a percentage comparable to what was found at the level of a public hospital in the same region (Fes) during a study carried out in 2011 [10], whose rate of the non-conformity of raw plants was 32%. These percentages are still high, when compared with a study carried out during 2007 in Geneva, Switzerland, whose percentage of contamination of raw plants was only 8% [11]. This reflects the level of knowledge of the staff of the catering sector in this country, and their hygiene, food safety and the application of standards for cleaning, storage and preparation of raw plants consumed.

The category of cooked dishes studied includes all dishes of animal or vegetable origin that have been heat-treated and cooked to be ready for consumption. The percentage of non-conformity cooked dishes detected in this study is 60%, while a study in the Fes region carried out between 2003 and 2006 showed that the rate of non-conformity of this class of foods does not exceed 13.7% [3].

If we compare this percentage with that found in Tunisia during a research done on cooked dishes in the Farhat-Hached University Hospital Center of Sousse during the period spread between 2005 and 2010, whose study concerned the various stages of handling and processing of cooked dishes from receipt of the raw material, storage, cooking dishes, loading dishes to distribution and consumption. The rate of contamination ranged from 36% (storage of raw material) to 25%, which represents the percentage of non-conformity during distribution and consumption [12]. Indeed, it is a low percentage in comparison with our result, which expresses the contamination of cooked dishes in the distribution and consumption phase.

Another study carried out during 2013, concerning the microbiological quality of cooked dishes served in the university restaurants of Oran in Algeria, the results of which indicate that the non-conformity of these dishes does not exceed 19.23%. This is still a low percentage compared to our results, and knowing that cooked dishes undergo a heat treatment that destroys the majority of microorganisms. Therefore, this high level of non-conformity may be due to breakage of the cold or hot chain, inadequate or insufficient cooking method and non-compliance with food hygiene rules [13] or even the bad storage of dishes [14].

Dairy products are divided into four sub-categories, consisting of ice cream, traditional cheese, milk, and milk-based juices. All these foods are prepared from untreated raw milk. The percentage of non-compliance in this category is 63.47%, and this is a high percentage compared to that found in a study conducted in Rabat-Salé-Zemmour-Zair region during the period 2004-2009, whose percentage of nonconformity was 48.4%. This difference may be due to the fact that in the flap region the samples included both traditional and industrial dairy products (60.4% traditional and 39.6% industrial) [15], unlike the samples received in our laboratory, they are almost all traditional, and the industrial samples received are already handled and modified or transformed.

Similar study conducted in the Fez region between 2003 and 2006 showed that the rate of non-conformity of dairy products, which are all taken from traditional dairies, is 68.5% [3].

This non-conformity of dairy products is related to the contamination of each subcategory that constitutes this group. According to Table 3, the raw milk analyses show a nonconformity rate of this subcategory equal to 86.7%, which is a high percentage compared to the results of a research carried out in the Fez region between the 2003-2006, concerning the microbiological quality of raw milk, and the non-conformity rate of raw milk was 65.3% [3]. Another study in the same region of Fez during 2013 showed that the contamination rate of raw milk was 69% [16]. These results are comparable to the previous study in the region of Fez. But another study conducted in the eastern region of Morocco spread over 3 years has shown that the rate of non-conformity of raw milk was 75% [17], and this result is similar to ours.

This difference of almost percentage (20%) between the results obtained in our laboratory and that of Fez, is probably due to non-compliance with hygiene conditions by professionals in the field in traditional dairies, since milking, and collection until transport and storage, but also to the type of milk analyzed which was mainly the traditional raw milk of the cow, unlike the one studied in Fes, it mainly concerned industrialized raw milk [3, 16]. A study conducted in Fes during 2013, showed that the contamination rate of artisanal cheese is 50% [16].

The percentage of the non-conformity of the traditional cheese analyzed during the study period in our laboratory was 33.3% and it is a percentage lower than that found during a study carried out in Fes between 2009 and 2010, with a percentage of non-conformity of 50% [18], and also lower than the result obtained during a study carried out by a network of 6 laboratories of the REQUASUD network on artisanal cheeses prepared from different types of raw milk (cow, goat and sheep), in the Wallonie region of Belgium. This study has shown that the percentage of non-conformity was around 40% [19].The production of artisanal cheese of poor hygienic quality is probably the result of the use of contaminated raw milk and/or non-compliance with the rules and hygiene measures of premises, equipment and handlers [18].

Ice and ice cream have become a consumable throughout the year and in every country in the world. Samples and analyzes of this class of food concerned traditional products. All the ice creams analyzed are milk-based and not industrialized. In fact, the rate of non-conformity was 61%. Other studies carried out on industrialized ice and ice cream in the region of Fez (Morocco) and in Bejaia (Algeria) have shown that the quality of these frozen products comply with Moroccan and Algerian standards, respectively [20-21]. On the other hand, the same study carried out in Fes on traditionally produced frozen products has shown that 83% of these products are unfit for consumption [20]. These percentages prove that the production of this type of food is done in poor hygienic conditions.

Microbiological analyses were also carried out on the category of meat products, consisting mainly of raw ground meat and sausages. These analyses showed that this type of food is nonconforming as a percentage of 80.57%. In another study carried out in the region of Fez during the period between 2003 and 2006 showed that the average of non-conformity of this food category was 59.5% [3].

The ground meat defines a considerable non-conformity represented by a percentage of 90.9%, and it is a very high percentage compared to a study carried out at the University of Liege in Belgium, which revealed that the percentage of products meat incriminated in food poisoning was 27% [22].

Our percentage remains high compared to another study carried out in the laboratory of epidemiological diagnosis and hygiene of the environment of Fez in 2016 which revealed that 73.38% of the samples of the minced meat marketed in this region is unfit for consumption [23].

Sausages in the Tetouan region are also non-compliant by a percentage of 63, 0%, this percentage of nonconformity remains lower than that found during a study carried out at various points of sale in Meknes city (Morocco) during the period between 2014 and 2015, which showed that 80.77% of sausages sold do not meet microbiological standards [24]. Another study carried out in Dakkar (Senegal) in 1992 showed that the rate of non-conformity of sausages sold on supermarkets and at points of sale was 31% [25].

4 CONCLUSION

The total non-conformity of all the samples of different food categories analyzed is 70.84% which reflects a very bad handling of food at the level of the different classes of restaurants, and the different outlets for ready-to-eat products, that it is at the level of production, storage, handling and distribution. The lack of respect for the hygiene conditions of the catering staff is due to their ignorance and lack of training and awareness programs by the sectors responsible for food hygiene and the health of the population, because this percentage of non-conformity (70.84%) can be considered a threat to public health at the regional level. Moreover, some foods received in the laboratory were not considered in this study because of the lack of a standard for interpreting the results obtained. So it is important to give classifications and acceptability standards for some foods that are not included in the 2004 ministerial order. Other studies assessing the microbiological quality of these foods according to the desired parameters and the influence of spatiotemporal changes are being developed.

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