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Prevalence, molecular and antimicrobial resistance of *Salmonella* isolated from sausages in Meknes, Morocco

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ABSTRACT

Salmonella is among the most important food borne pathogens worldwide contaminating a wide range of animal products including meat products. The aims of this study go through two steps: The first step is to estimate the proportion of sausages products contaminated with *Salmonella* in Meknes city (Morocco), which were collected from various shopping sites: butchery, street vendors, supermarket and souk (Weekly market combines the population of the small villages around Meknes city). The second one is to identify serovars, to determine the antimicrobials resistance patterns of isolates and to detect the *invA* and *spvC* genes. 34 (21.79%) *Salmonella* were isolated, recovered 4 serogroups and 12 serotypes. The most prevalent serotypes were *Salmonella* Corvallis (23.53%) and *Salmonella* Kentucky (17.65%). All *Salmonella* isolates were tested for their susceptibility to 18 selected antimicrobials agents, of which 100% were resistant to at least one antimicrobial, 85.30% (29/34) were resistant to two or more antimicrobials and 44.12% (15/34) were resistant to at least three antimicrobials. All *Salmonella* are resistant to ampicillin, 76.47% to streptomycin, 20.59% to sulfonamides, 17.65% to Tetracycline and 11.77% to Ofloxacin. The "ACSSuT" penta-resistance pattern was observed in tow of the *Salmonella* Typhimurium strains. In addition, this study showed that all *Salmonella* strains (34) were positive for invasion gene *invA* and negative for the virulence gene *spvC*.

1. Introduction

Food-borne diseases imposes a substantial economic burden and threatens the public health on society causing an acute morbidity and chronic sequelae [1,2]. Worldwide, the consumption of contaminated food is responsible for two million deaths per year [3]. Furthermore, the non-typhoidal *Salmonella* is the main cause of food-borne diseases, which pose a big problem for medicine and agribusiness; it's associated in most cases with the consumption of animal products [4,5]. However, some studies show that even of plant origin may be a vector for transmission of *Salmonella* to humans [6,7].

Worldwide, *Salmonella* is responsible for the millions cases of foodborne diseases each year [8]. The incidence of salmonellosis ranged between 14.53 and 17.55 cases per 100,000 people in the United States [9]. Moreover, *Salmonella* was the second most commonly reported zoonoses in the European Union with a total of 91,034 confirmed cases of human salmonellosis in 2012 [10]. The true incidence of salmonellosis in both humans and animals is difficult to evaluate in developing countries because of the lack of epidemiological surveillance systems [11]. The bacterial resistance to antimicrobial agents, including *Salmonella* is a major problem of food safety; it affects both the veterinary and public health by transfer of resistant strains to humans through the food chain [12,13]. The multi-resistant strains can sometimes be the cause of international outbreaks raising fears therapeutic impasses and increased morbidity and mortality for simple food poisoning [4].

In Morocco, the consumption of meat products has experienced a sharp increase in recent years and the presence of *Salmonella* in these products is usual [14–16], which increases the population exposure to the pathogenic agent. Also, the manufacture of sausages is done under conditions that do not comply with good

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hygiene practices. In Meknes city, sausages are sold in supermarkets, butchery, street vendors and souk. The street vendors are located mainly in neighborhoods, they have generally a very low cultural level and are unconscious of good hygiene practices and work in illegal conditions without control and without regulatory legislation. As regards the souk, sausages are presented on parts in contact with air and dust at ambient temperatures, without any respect for good hygiene practices. Those who make a deadly food and a potential transmission vector of pathogenic bacteria to humans.

The aim of this study is to determine the prevalence of *Salmonella* in sausages made from beef meat, turkey meat and artisanal sausages "Merguez" trades in Meknes city (Morocco), using the conventional method, by biochemical and serological means. The molecular identification by PCR was performed to detect the *invA* and *spvC* genes. The sensitivity and resistance of *Salmonella* to antimicrobials agents were studied. It should be mentioned that "Merguez" is a small raw sausage generally made of lean and fat beef mixed with condiments and stuffed into natural casings of sheep and beef; it is generally sold in street vendors.

2. Material and methods

2.1. Samples collections

From March 2014 until February 2015, 156 samples of sausages (60 of Turkey sausages, 60 of Beef sausages, 36 of Artisanal sausages "Merguez") were collected from various sites: 72 from butchery, 36 from street vendors, 24 from Supermarket and 24 from Souk (Weekly market) in Meknes city in Morocco, with a frequency of 13 samples per month (5 of Turkey sausages, 5 of Beef sausages, 3 of Artisanal sausages "Merguez"). The collected amount of each sample of sausage is about 40 g, which they were transferred immediately in a cooler to microbiology laboratory at the Faculty of Science Meknes.

2.2. Isolation of Salmonella

Twenty-five grams of sausages were pre-enriched into 225 mL of sterile buffered peptone water (Oxoid, England) and incubated at 37 °C for 18–24 h 0.1 mL of pre-enrichment culture was inoculated into 10 mL of Rappaport-Vassiliadis broth (Biokar) and 1 mL was inoculated into 10 mL of Selenite Cysteine Broth (Biokar), the cultures were incubated at 42 °C and 37 °C, respectively, for 24 h. From them, a streak culture is performed on Hektoen enteric agar (Biokar) and *Salmonella-Shigella* agar (Biokar), respectively; the inoculated plates were incubated at 37 °C for 24 h. The suspect colonies of *Salmonella* were inoculated into Hanja Kligler agar (Biokar), Simmons citrate agar (Oxoid), mannitol motility agar (Bio-Rad), lysine decarboxylase broth (Scharlab), Indole urea (bio Merieux RSA) and incubated at 37 °C for 18–24 h. The oxidase discs (in vitro diagnostics) and ONPG (Oxoid Limited) are also tested. All the isolates were confirmed by biochemical tests using an API 20E (Bio Mérieux RSA).

2.3. Molecular identification of Salmonella

All *Salmonella* strains collected in this study were tested for the invasion gene *invA* and virulence gene *spvC* (Table 1), using the conventional polymerase chain reaction (PCR) as described by Karraouan et al. [15].

2.4. Determination of Salmonella serogroup and serotype

The serogroup and serovars of the *Salmonella* isolates were determined by slide agglutination tests with O antigen and H antigen antiserums obtained from Pasteur Institute of Casablanca city in Morocco. The results were interpreted according to the Kauffmann-White scheme [17].

2.5. Antimicrobial susceptibility testing

The agar dilution method with Mueller-Hinton agar was used to test the susceptibility of *Salmonella* to 18 antimicrobials (Table 2). The results were interpreted according to the recommendation of the Clinical and Laboratory Standards Institute [18]. In this study, the isolates showing a decrease in susceptibility (intermediate) were considered as resistant and *Escherichia coli* ATCC 25922 was used as control strains.

3. Results

3.1. Prevalence of Salmonella in sausage samples

Salmonella was detected in 21.79% (34/156) of samples. Artisanal sausages "Merguez" are the most contaminated 30.55% (11/36), followed by Turkey sausages 23.33% (14/60) and Beef sausages 15% (9/60). Considering the area of marketing, sausages sold in Street Vendors are the most contaminated (30.55%), followed by souk (29.16%), butchery (18.05%) and supermarket (12.5%). Invasion gene operon *invA* was detected in all *Salmonella* isolates in this study. However, *spvC* was not detected in any strain (Fig. 1).

3.2. Determination of serogroups and serotypes of isolated Salmonella

The diversity of raw material and the conditions of manufacturing sausages influence the distribution of serogroups and serotypes. In this study, 4 serogroups and 12 serotypes were identified in 34 *Salmonella* isolates with an abundance of

Table 1
PCR primers used for amplification of virulence genes in *Salmonella* isolates.

Virulence genes	Primer sequence 5′-3′	Size (bp)	Reference
invA	F- TATCGCCACGTTCGGCAA R- TCGCACCGTCAAAGGAACC	275	[43]
spvC	F- CGGAAATACCATCAAATA R- CCCAAACCCATACTTACTCTG	669	[44]

Table 2
Antimicrobial agents and the range of concentrations tested.

Antimicrobial agents	Code	Concentration Disc
Ampicillin	AMP	10 µg
Amoxicillin	AML	25 µg
Ticarcillin-clavulanic acid	TIM	85 µg
Imipenem	IPM	10 µg
Ceftriaxone	CRO	30 µg
Cefuroxime sodium	CXM	30 µg
Ceftazidime	CAZ	30 µg
Cefamandole	MA	30 µg
Colistin sulfate	CT	50 µg
Kanamycin	K	30 µg
Ofloxacin	OFX	5 µg
Nalidixic acid	NA	30 µg
Sulfonamide	SSS	200 µg
Gentamicin	CN	30 µg
Chloramphenicol	C	30 µg
Tetracycline	TE	30 µg
Streptomycin	S	10 µg
Trimethoprim–Sulfamethoxazole	SXT	1.25 µg/23.75 µg