Pharmaceutica Analytica Acta

Abdellah et al., Pharmaceut Anal Acta 2013, 4:9
http://dx.doi.org/10.4172/2153-2435.1000270

Research Article Open Access

Prevalence and Antibiogram Study of *Escherichia coli* and *Staphylococcus aureus* in Turkey Meat in Morocco

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Abstract

This study presents a survey of the microbiological quality of turkey meat sold in various outlets in Meknes city of Morocco and examines antimicrobial resistance of *Staphylococcus aureus* and *Escherichia coli* strains isolated to warn customers about the emergence of food poisoning. 96 samples randomly taken on different outlets, including 24 at the popular market, 24 at artisanal slaughterhouses, 24 at poulterers' shops and 24 at supermarket. According to the microbiological criteria, 83.3% of samples did not meet the standards for *E. coli*. 95.8%, 33.3%, 41.6%, 41.6% of the samples purchased from supermarket, poulterers' shops, artisanal slaughterhouses and popular market outlets, respectively, showed satisfactory quality point of view *S. aureus* among which 8.3% (8/96) of samples could be linked to a foodborne due to a concentration of *S. aureus* upper in 5 $\log_{10}$ υfc/g. The level of contamination E. coli and *S. aureus* at supermarket was recorded significantly lower ($p<0.05$) compared to other sites.

Among the 40 *E. coli* tested, the highest resistance was to amoxicillin-clavulanic acid (80%), followed by norfloxacin (67.5%), cephalothin (65%), nalidixic acid (62.5%), ampicillin (52%), trimethoprim/sulphamethoxazole (42.5%), ciprofloxacin (40%), cefoxitin (35%), ceftazidime (32.5%) and amikacin (15%). Low resistance rates were returned (between 5 and 12.5%) for ertapenem, aztreonam and gentamicin. For *S. aureus*, the highest percentage of resistance was found to the following antimicrobial agents: teicoplanin (67.5%), tetracyclin (40%) and vancomycin (30%). No resistance to the rest of antibiotics was found.

The bacterial load present on the surface of poultry carcasses reflects the general hygiene conditions in which they are prepared, stored, transported and sold. These data revealed also that the *E. coli* and *S. aureus* isolates recovered from the retail turkey meats were resistant to multiple antimicrobials, which can be transmitted to humans through food products.

Keywords: Turkey meat; Microbiological quality; Morocco; Health; Antimicrobial resistance

Introduction

Food borne diseases and poisoning are the widespread and great public health concerns of the modern world. Both developed and developing countries are largely affected by food borne infections. Food borne diseases not only affect people's health and well-being, but also have economic impacts on individuals and the countries [1], while the impact in case of developing countries is higher. It reduces markedly social and economic productivity of the countries [2]. Because of the relatively high frequency of contamination of poultry with pathogenic bacteria, raw poultry products are reported to be responsible for significant number of cases of human food poisoning [3].

Poultry meat contributes substantially to the human diet [4]. In Morocco, poultry meat is an important, low cost source of animal protein. This encourages the consumption of poultry products by a large number of consumers. Poultry meat is increasingly used by the growing rural and urban populations. This explains the high production in Morocco especially that the turkey fell from 10.5 to 50 million tonnes in recent years [5]. However, foods of animal origin, especially poultry and meat, are the major vehicles for the transmission of human salmonellosis due to cross-contamination events or inadequate cooking [6]. In Morocco, although several efforts diseases have been made to improve food safety and quality, food borne diseases still represent one of the main causes of mortality [7].

Amongst the food borne pathogens, *Salmonella* and *S. aureus* are the most common and frequent pathogens responsible for food poisoning and food related infections [8,9]. According to WHO [10], there was 25% diarrhea in foodborne illness caused by food infected with *E. coli*. In Morocco, *Salmonella*, *S. aureus* and *Clostridium perfringens* are reported to cause 42.8, 37, and 1.7% of food poisoning, respectively [11].

Four kinds of turkey meat outlets are used in Morocco: popular market, artisanal slaughterhouses, poulterers' shops and supermarket. They differ from each other by the level of hygiene, diet cold which is subject carcasses (ambient temperatures, refrigeration, freezing). At popular market and artisanal slaughterhouses the conditions of slaughter and sale of the product are faulty [11], indeed turkey is slaughtered and scalded in hot water. After that, the carcasses are plucked and eviscerated mostly by hand. Before and after evisceration, broil carcasses are subject to washing and other operations which my disseminate bacteria from localized sites to the rest of the carcass as

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Received August 30, 2013; **Accepted** September 29, 2013; **Published** October 03, 2013

Citation: Abdellah EA, Fouzia RF, Bouchra O (2013) Prevalence and Antibiogram Study of Escherichia coli and Staphylococcus aureus in Turkey Meat in Morocco. Pharmaceut Anal Acta 4: 270. doi: 10.4172/2153-2435.1000270

well as among carcasses. This kind of poultry is often sold in parts and the selling can take time, during which the carcasses are displayed at ambient temperatures during the day and put in the refrigerator for the night [12,13]. In these shops, the conditions are favorable to potential contamination by pathogens which may originate from the animal itself and environment factors (water, litter, air). On the contrary, poulterers' shops and supermarket are an automated poultry slaughtering process established recently, whereby automated systems are used for scalding, plucking, eviscerating and packaging carcasses. Carcasses are then stored at 4°C before sale to supermarkets and Poulterers' shops. These shops ensure the storage and sale of poultry meat under good hygienic conditions [14].

Besides, Morocco is a developing country with abuse of antibiotics in animal husbandry and it may cause antimicrobial resistance of bacteria animals. Schroeder et al. [15] proved that antibiotic resistance of bacteria isolated from humans was transferred from antibiotic resistant bacteria in animal.

The purpose of this study is to detect the contamination bacteria of retail turkey meat in retail markets in Meknes (Morocco) and examined antimicrobial resistance of *S. aureus* and *E. coli* strains isolated to warn customers about the emergence of food poisoning.

Material and Methods

Samples

Between October 2011 and October 2012, a total of Ninety-six samples of turkey breasts with skin were collected from retailers, of which 24 samples were from popular market, 24 from artisanal slaughterhouses, 24 from poulterers' shops and 24 from a supermarket in Meknes (centre-south Morocco). Each sample was placed in a separate sterile plastic bag. Samples were transported to the laboratory immediately after collection in an ice chest and tested upon arrival or stored at 2°C for no longer than 4h.

Statistical analysis

All bacterial counts were expressed as Log_{10} colony forming unit per g (Log_{10} CFU/g). To compare the log_{10} values of microbial counts, the data were analyzed using Student's t test for each type of micro-organism. Significance was determined at the 5% level.

Microbiological analysis

A 25 g sample of skin was taken aseptically by scalpel excision and stomached in a sterile stomacher bag containing 225 ml of peptone water (Biokar Diagnostics, France) for 2 min. Decimal dilutions were carried out using the same diluents.

Mesophiles were determined using plate count agar (Oxoid, England) spread plates incubated at 30°C for 72 h. *S. aureus* on Baird-Parker agar with egg yolk-potassium tellurite emulsion plates (Bio-Rad), incubated at 35 ± 1°C for 24 to 48 h and typical colonies (black surrounded by clear zones) were tested for coagulase activity using rabbit plasma (Biokar Diagnostics, France) after activation by overnight incubation in Brain Heart broth (Biokar Diagnostics, France) at 35°C. *E. coli* counts, on rapid' *E. coli* Agar (Bio-Rad, France) incubated at 37°C for 18 to 24 h, typical *E. coli* were considered as violet-to-pink.

Susceptibility to antimicrobials

Antibiotic susceptibility testing was performed by a disc diffusion method on Mueller-Hinton agar. The categories susceptible or resistant were assigned on the basis of the critical points recommended by the French committee on guidelines for susceptibility testing [16].The strains were screened for their resistance to the following antibiotics (Marnes-La-Coquette, France): nalidixic acid Na 30 µg; ciprofloxacin CIP 5 µg; ceftazidime CAZ 30 µg; amoxicillin-clavulanic acid AMC 20+10 µg; cefoxitin FOX 30 µg; cefotaxime CTX 30 µg; lincomycin, MY 15 µg; fusidic acid FD 10 µg; tetracycline TE 30 UI; teicoplanin TEC 30 µg; gentamycin CN 15 µg; vancomycin VA 30 µg; Rifampycin RD 30 µg; amikacin AK 30 µg; ertapenem ETP 10 µg; cephalothin KF 30 µg; aztreonam ATM 30 µg; ampicillin AM 10 µg; trimethoprim/sulphamethoxazole SXT 1.25/23.75 µg and norfloxacin NOR 5 µg. We used the Automated System (OSIRIS) for reading and interpreting results (Bio-Rad).

Results and Discussion

Mesophiles

It is important to determine the aerobic total criterion which is used as hygienic indicator in the slaughter- process. In total of 96 samples, the number of bacteria was 4.15 Log_{10} CFU/g in minimum registered in samples from the supermarket and in maximum 8.66 Log_{10} CFU/g registered in samples from slaughterhouses (Table 1). From this point of view parameter (mesophiles), the percentage of unacceptable samples was 48.95% (Table 1). This result was higher than the result obtained by Cohen et al. [17] in Morocco with 29.2% unacceptable samples of poultry meat poultry. In Hanoi, Nguyen Van Ton [18] found 54.65% meat poultry samples over national standards (Figure 1a).

	Mesophiles		***E. coli***		***S. aureus***	
	Min-Max	A	Min-Max	A	Min-Max	A
Samples from	Log_{10} ufc/g	N%	Log_{10} ufc/g	N%	Log_{10} ufc/g	N%
slaughterhouses	6.05-8.66	10/24	1.8- 4.88	5/24	2.62-5.65	10/24
Poulterers' shops	5.69-7.81	9/24	2.02-5.49	2/24	2.30-5.70	8/24
Super market	4.15-7.60	19/24	2.65- 4.78	5/24	0- 4.48	23/24
Popular market	4.88-8.26	15/24	1.67- 4.96	4/24	2.3- 5	10/24
Range*	<6.7 log_{10} ufc/g		<2.2 log_{10} ufc/g		<3.7 log_{10} ufc/g	

A: Acceptable, N%: Rate of compliance, Min: Minimum, Max: Maximum, Range*: According to the microbiological criteria for raw meat poultry [37].

Table 1: Bacterial counts (Log_{10} cfu/g) found in retail turkey outlets (n=96).

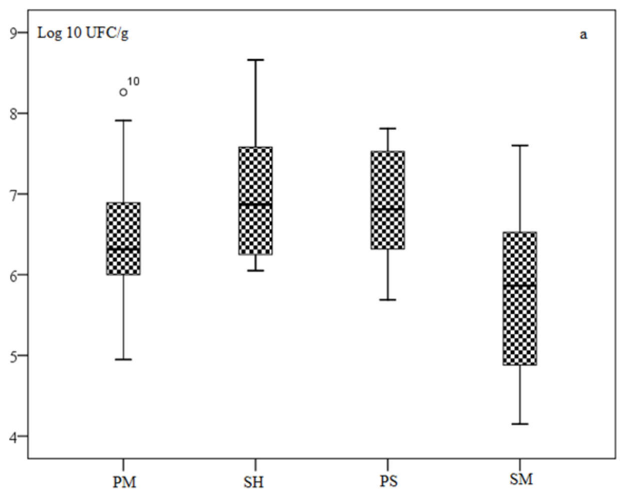


Figure 1a: Mesophiles.

Escherichia coli

Coli form bacteria are indicator organisms as enterobacteriaceae are of intestinal origin. Indicator organisms may be employed to reflect the microbiological quality of foods relative to product shelf life or their safety from food borne pathogens. Microbial indicators are more often employed to assess food safety and sanitation than quality [19,20]. According to WHO [10], there was 25% diarrhea in food borne illness caused by food infected with *E. coli*. In this study, for these bacteria, average counts were 3.43 Log_{10} CFU/g in samples purchased from super market and 3.85 log_{10} CFU/g in those purchased from artisanal slaughterhouses (Figure 1c). In this study 100% of food samples were infected with *E. coli*. There was 5.49 Log_{10} CFU/g in maximum and 1.67 Log_{10} CFU/g in minimum (Table 1). Proportion of unacceptable sample was 83.3% (Table 1), higher than some results announced. Tran Thi Hanh et al [21] found 68.75% samples of poultry meat sold in Hanoi's market. In America, 38.7% meat poultry samples in Washington infected with *E. coli* (Cuiwei Zhao et al.) [22]. Cohen N et al. [17] indicated 48.4% samples infected with E.coli in Morocco, 22.4% among these was unacceptable. Levels of contamination of samples were significantly (P<0.05) higher in poulterers' shops, slaughterhouses and in popular market than in supermarkets, possibly due to the good hygienic conditions in the supermarkets at the time of the previous stages (Figure 1c).

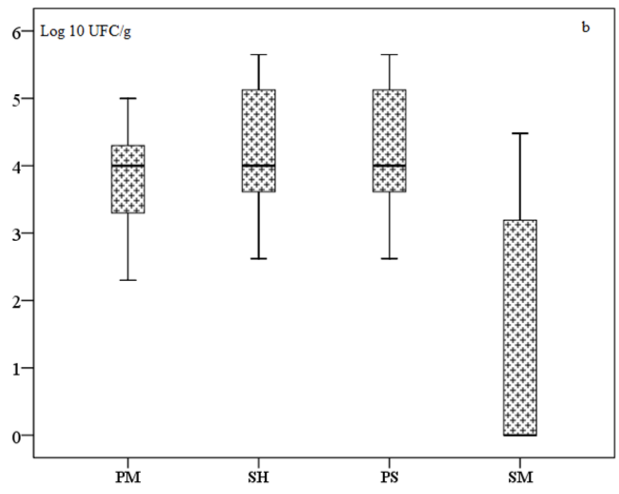
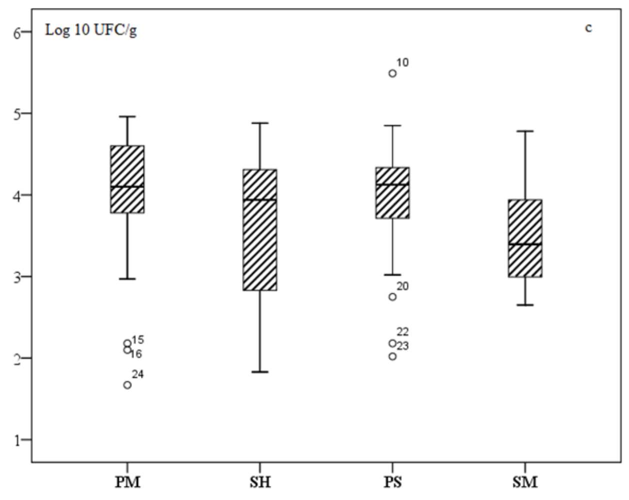


Figure 1b: *Staphylococcus aureus.*



PM: Popular market; SH: Artisanal slaughterhouses; PS: Poulterers' shops; SM: Supermarket; (On): Samples "n" to extreme values not included in the curve box mustache.

Figure 1c: *Escherichia coli.*

Staphylococcus aureus

S. aureus has long been recognized as one of the food poisoning bacteria of concern to human health worldwide [23]. Average counts of *S. aureus* in samples purchased from popular market and artisanal slaughterhouses outlets were 3.85 and 4.19 Log_{10} CFU/g respectively (Figure 1b). These levels of contamination were higher to those obtained by Álvarez-Astorga et al. [24] in chicken legs. Similarly, Waldroup AL [25] obtained values ranging from 3 to 5 Log_{10} CFU/g. Counts found by other authors are very variable. However, in samples purchased from supermarket, *S. aureus* counts were lower than those of other outlets. On the basis of the CNERNA-CNRS guidelines [26], 95.8%, 33.3%, 41.6%, 41.6% of the samples purchased from supermarket, poulterers' shops, artisanal slaughterhouses and popular market outlets, respectively, showed satisfactory quality. The rest of the samples were considered of the unacceptable quality (Table 1). Enumeration of *S. aureus* revealed that the count of pathogen exceeded 5 Log_{10} CFU/g in 8 out of the 96 analysed samples (8.3%). Such high level of contaminated with *S. aureus* has been associated with increased risk for staphylococcal food poisoning [27]. High contamination of food with *S. aureus* has been related to improper personal hygiene of employees during handling and processing [28].

This comparison should be made with caution because several factors must be taken into account when making such comparisons, including differences in country and origin, type of meat samples, sampling seasons, slaughterhouse sanitation, and isolation methods.

Antibioresistance

Food is an important factor for the transfer of antibiotic resistances. Such transfer can occur by means of antibiotic residues in food, through the transfer of resistant food-borne pathogens or through the ingestion of resistant strains of the original food microflora and resistance transfer to pathogenic microorganisms [29,30]. *S. aureus* strains are known to be frequently resistant to antibiotic therapy due to their capacity to produce an exopolysaccharide barrier and because of their location within micro abscesses, which limit the action of drugs [31].

Antibiotic resistance in *S. aureus* strains to 8 antimicrobial agents is shown in Table 2. Overall, the highest percentage of resistance was found to the following antimicrobial agents: teicoplanin (67.5%), tetracyclin (40%) and vancomycin (30%). No resistance to the rest of antibiotics was found. Otalu et al. [32] reported 100% resistance in *S. aureus* isolates from poultry meat against tetracycline and 61.5% against methicillin in Nigeria [32]. Multidrug resistant *S. aureus* have been reported several times [33]. Extensive uses of these antibiotics are thought to be the major cause of drug resistance in food borne pathogens [32].

Antibiotic resistance in *E. coli* strains to 14 antimicrobial agents is shown in Table 3. Overall, the highest percentage of resistance was found to the following antimicrobial agents: amoxicillin-clavulanic acid (80%), norfloxacin (67.5%), cephalothin (65%), nalidixic acid (62.5%), ampicillin (52%), trimethoprim/sulphamethoxazole (42.5%), ciprofloxacin (40%), cefoxitin (35%), ceftazidime (32.5%) and amikacin (15%). Low resistance rates were returned (between 5 and 12.5%) for ertapenem, aztreonam and gentamycin. In Morocco, Chaiba et al.

Antibiotic	Concentration Disc	S (Sensitive)			R (Resistant)		
		diameter (mm)	n	%	diameter (mm)	n	%
FOX	30 µg	≥ 27	40	100	<25	0	0
MY	15 µg	≥ 22	40	100	<17	0	0
FD	10 µg	≥ 24	40	100	<24	0	0
TE	30 UI	≥ 23	24	60	<21	16	40
TEC	30 µg	≥ 17	13	32.5	-	27	67.5
CN	15 µg	≥ 20	40	100	<13	0	0
VA	30 µg	≥ 17	28	70	-	12	30
RD	30 µg	≥ 29	40	100	<24	0	0

Total 40 *S. aureus* strains were examinated.

Table 2: Result of antibiotic resistance of *S. aureus* isolated.

Antibiotic	Concentration Disc	S (Sensitive)			R (Resistant)		
		diameter (mm)	n	%	diameter (mm)	n	%
NA	30 µg	≥ 20	15	37,5	<15	25	62,5
CN	15 µg	≥ 18	38	95	<16	2	5
CIP	5 µg	≥ 25	24	60	<22	16	40
AK	30 µg	≥ 17	34	85	<15	6	15
FOX	30 µg	≥ 22	26	65	<15	14	35
ETP	10 µg	≥ 28	35	87,5	<26	5	12,5
CTX	30 µg	≥ 26	40	100	<23	0	0
CAZ	30 µg	≥ 26	17	42,5	<19	13	32,5
KF	30 µg	≥ 18	14	35	<12	26	65
AMC	20/10 µg	≥ 21	8	20	<16	32	80
ATM	30 µg	≥ 27	32	80	<21	2	5
AM	10 µg	≥ 19	19	47,5	<16	21	52,5
SXT	1.25/23.75µg	≥ 16	23	57,5	<13	17	42,5
NOR	5 µg	≥ 25	13	32,5	<22	27	67,5

Total 40 *E. coli* strains were examinated.

Table 3: Result of antibiotic resistance of *E. coli* isolated.

[34] obtained resistance in *E. coli* strains of poultry meat to tetracyclin (80%), chloramphenicol (6.6%), amoxiline (20%), acid nalidixic (26.6%), gentamycin (0%), Neomycin (6.6%) and trimethoprim/sulphamethoxazole (33.33%). Antunes et al. [35] also reported a high antimicrobial resistance of *Salmonella* isolates recovered from poultry products including chicken and turkey to nalidixic acid, tetracycline and streptomycin (ranging from 36% to 50%) but low resistance rate to trimethoprim (3%) in Portugal. We noticed also an increase of resistance to ciprofloxacin (40%), that represent the treatment of choice of severe non typhoidal *Salmonella* infection in adults [36,37]. This could be related to the use of some fluoroquinolones form methaphylactic or therapeutic purposes in poultry feed and drinking water.

Conclusion

The results of this study indicate the lack of unsatisfactory sanitary conditions and quality control during manufacturing and/or post production handling of the turkey meats, and a possible health safety problem. Also, the results clearly indicate that attempts have to improve the sanitary conditions in traditional turkey meat production procedure and we recommend more restrictions on the irrational use of antibiotics and public awareness activities should be undertaken to alert the public to the risks of the unnecessary use of antibiotics.

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Citation: Abdellah EA, Fouzia RF, Bouchra O (2013) Prevalence and Antibiogram Study of Escherichia coli and Staphylococcus aureus in Turkey Meat in Morocco. Pharmaceut Anal Acta 4: 270. doi: 10.4172/2153-2435.1000270