

# Prevalence and Antimicrobial Resistance Profile of *Staphylococcus* Species in Chicken and Beef Raw Meat in Egypt

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## Abstract

Coagulase-positive (CPS) and coagulase-negative (CNS) staphylococci cause staphylococcal food poisoning. Recently, CPS and CNS have received increasing attention due to their potential role in the dissemination of antibiotic resistance markers. The present study aimed to evaluate CPS and CNS species distribution and their antibiotic resistance profile isolated from chicken and beef meat. Fifty fresh, uncooked chicken parts and 50 beef meat cuts (local  $n=27$ ; imported  $n=23$ ) were used. One hundred staphylococcal isolates belonging to 11 species were isolated and identified from chicken ( $n=50$ ) and beef ( $n=50$ ) raw meat samples. *Staphylococcus hyicus* (26/100), *lugdunensis* (18/100), *aureus* (15/100) and *epidermidis* (14/100) were dominant. *S. aureus* was 100% resistant to penicillin and sulfamethoxazole/trimethoprim. Vancomycin-resistant *S. aureus* showed intermediate resistance (51%), which might indicate the dissemination of vancomycin resistance in the community and imply food safety hazards. The percentage of resistance to  $\beta$ -lactams was variable, with the highest resistance being to penicillin (94%) and lowest to ampicillin-sulbactam (22%). Antimicrobial resistance was mainly against penicillin (94%), clindamycin (90%) and sulfamethoxazole/trimethoprim (82%). The results indicate that chicken and beef raw meat are an important source of antibiotic-resistant CPS and CNS.

## Introduction

THE POULTRY INDUSTRY in Egypt with a population of 1 billion broilers is estimated to produce 1.5 metric tons (MT) of poultry meat during the period of 2014 (Hassan, 2014). The investment in the poultry industry is around 25 billion Egyptian pounds, and ranks first in the Middle East (Hassan, 2014). The beef meat imports were around 230 MT in the year 2014 (USDA, 2014). Chicken meat contributes substantially to the diet in Egypt, and is an important, low-cost source of animal protein encouraging the consumption of chicken meat by a large number of consumers.

Today, according to the current List of Bacterial Names with Standing in Nomenclature (Euzéby, 2014), the genus *Staphylococcus* comprises 71 species, with 24 named subspecies. Staphylococcal food poisoning is believed to be greatly underreported (by about 25-fold) and underdiagnosed (by about 29-fold) (Doyle *et al.*, 2012). The consumption of chicken and beef meat was implicated in a large number of outbreaks of staphylococcal food poisoning in humans in different countries (Argudín *et al.*, 2010). Discrimination between *Staphylococcus* (*S.*) *aureus* and NSA (non-*S. aureus* staphylococci) involved in meat contamination is essential in terms of public health significance. Most staphylococci spe-

cies are coagulase-negative (CNS) and they are distinguished from coagulase-positive staphylococci (CPS) by their inability to produce the enzyme coagulase. Although CPS, and especially *S. aureus*, are regarded as important pathogens in humans and animals, CNS should not be ignored in epidemiological investigations of foodborne outbreaks based on the presence of enterotoxin genes (Rall *et al.*, 2010; Batista *et al.*, 2013). In the last 20 years, however, the interest in CNS species has significantly increased in both human and veterinary medicine (Rogers *et al.*, 2009; Braem *et al.*, 2014). Information regarding the detailed prevalence of *S. aureus* and NSA isolates was insufficient in Egypt.

The aim of this study was to evaluate the occurrence of *S. aureus* and NSA and between CPS and CNS strains isolated from retail chicken and beef marketed in Cairo, Egypt, by using cultural and biochemical tests, to determine their diversity between samples and to characterize the isolated strains based on their resistance to antibiotics.

## Materials and Methods

A total of 100 samples each of chicken ( $n=50$ ) and beef (local,  $n=27$  and imported,  $n=23$ ) were randomly collected from various local supermarkets and butcher shops. All

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samples collected were unprocessed raw and fresh meat. The samples were aseptically collected, and each sample was placed in a separate, sterile plastic bag. The samples were brought under refrigeration to the laboratory and analyzed within the following 2 h. The samples (25 g) were weighed into sterile stomacher bags diluted with 225-mL sterile buffered peptone water and homogenized in a stomacher for about 5 min; 0.1 mL was streaked on Baird-Parker (BP) agar supplemented with egg yolk–tellurite emulsion and incubated at 37°C for 24 h. Strains cultured on BP agar medium were identified as *S. aureus* if growth was observed and the colonies and their surrounding showed the typical morphologic characteristics after aerobic incubation at 37°C (black colonies with an opaque precipitation halo); growth of colonies without the media-typical characteristics was considered as NSA. The tube coagulate test was determined and evaluated for coagulation after 3 and 24 h of incubation. All isolates identified as NSA and CNS based on colony morphology, Gram staining (positive), catalase reaction (positive), and coagulase test (positive) were subjected to species identification by using all the conventional biochemical methods described by Thorberg and Brandstrom (2000). For novobiocin-sensitive CNS isolates identified in samples, a simplified identification system, novobiocin-sensitive CNS built on biochemical tests was used (Thorberg and Brandstrom, 2000). To confirm the isolates as *Staphylococcus*, definitive identification of all strains included in the study was performed targeting the *Staphylococcus* genus-specific 16S rRNA gene polymerase chain reaction (fragment of 756 bp); F: AAC TCT GTT ATT AGG GAA GAA CA and R: CCA CCT TCC TCC GGT TTG TCA CC as previously described (Zhang *et al.*, 2004). For the positive control, *S. aureus* (ATCC 43300) reference strain was used as control for the test results.

The resistance pattern of *Staphylococcus* spp. isolates was determined using the disk-diffusion test (CLSI, 2012). Eleven antibiotics were chosen for the study based on the most used active principles in human medicine, national veterinary therapy, and according to their common use in research. The use of oxacillin/methicillin is not usually used in veterinary practice, and it was included in this study only for epidemiological purposes; the drugs tested are indicated in Table 1. *S. aureus* American Type Culture Collection (ATCC) 29213 and *S. aureus* ATCC 43300 were used as methicillin-susceptible and -resistant controls, respectively.

## Results and Discussion

Cairo is the largest city in the Middle East with a population of more than 16 million. Due to overcrowding and in some areas a state of poverty, inadequate sanitary conditions and poor general hygiene are seen. Raw meat is sold and available in open-air local retail shops without appropriate temperature control and is purchased by approximately 75% of households (ElNaggar *et al.*, 2006; Kader and Yahia, 2012).

Contaminated raw meat is one of the main sources of foodborne illnesses and a risk of the transmission of zoonotic infections. CPS and CNS are well established as clinical and epidemiological pathogens, and their potentially pathogenic role as foodborne pathogens should not be neglected. From the 100 samples inspected, 100 isolates (100/100; 100% isolation rate) belonging to 11 species were obtained (Table

2), from which the most dominant were *S. hyicus* (26/100), *lugdunensis* (18/100), *aureus* (15/100), and *epidermidis* (14/100). Studies have reported rates of 6% to 100% from Spain (Alvarez-Astorga *et al.*, 2002), Japan (Kitai *et al.*, 2005), Italy (Normanno *et al.*, 2007), The Netherlands (van Loo *et al.*, 2007), Nigeria (Achi and Madubuike, 2007), Jordan (Al-Tarazi *et al.*, 2009), Korea (Lim *et al.*, 2010), United States (Waters *et al.*, 2011), Turkey (Citak and Duman, 2011), India (Arul and Saravanan, 2011), China (Wang *et al.*, 2013), EFSA (2013), Egypt (EI-Jakee *et al.*, 2013), and Thailand (Akbar and Anil, 2013). The isolation rate of the *Staphylococcus* species recorded in our investigation on local beef meat (3.7%) was much lower than that recorded from the imported beef meat (47.8%), while the *S. aureus* contamination constituted a significantly higher rate in the local beef meat (33.3%) than in the imported beef meat (13.0%). The isolation rates recorded in beef were higher than those previously reported by Heo *et al.* (2008), who reported there was no significance difference in the frequency of *S. aureus* isolation between imported (10.3%) and domestic (8%) raw beef samples.

The isolation and prevalence data are variable and differ between various countries, and comparison between studies is inadvisable. Nevertheless, comparisons could still be made, noting that there are limiting factors due to inherent differences in methodology (sampling techniques, sample size, type of samples, sampling seasons, and sites [geographical locations, supermarkets, groceries, butcher shops, vendors/hawkers], identification methods), packaging, display at the butcher shop and handling practices, food settings, slaughterhouse and butcher appliances (knives, slicing machines, wiping cloths, towels, and nail brushes), and in grocery store meatburger grinders (EC, 2011). These factors differ greatly between countries and even within the same country, especially in the underdeveloped world. As meat may originally become contaminated during or after processing, the great significance of slaughter hygiene should be emphasized due to the problem of the increased speed of current production lines (Jacob, 1989). The three slaughterhouse tasks most likely to contaminate meat are the removal of an animal's hide, the removal of its digestive system, and the spread of feedlots at slaughterhouses and slaughterhouse workers who are often illiterate and always overworked (EC, 2011). Other contamination sources of *S. aureus* are soil, water, dust and air, changes in eating habits, and mass catering; also, complex and lengthy food-supply procedures with increased international movement are additional contributing factors (Jacob, 1989).

In addition to these factors, the most important underestimated source is the asymptomatic food handlers, who may harbor *S. aureus* and can contaminate food during preparation (Todd *et al.*, 2008). *Staphylococcus* spp. are widely found as normal flora in the nose and throat (and thus on the hands and fingertips) and on the hair and skin of more than 50% of healthy individuals (FDA, 2009). Food handlers who have skin lesions, or by sneezing or coughing, are a major source of contamination of table foods (FDA, 2009). However, testing of humans (food handlers) who came into contact with the study was not an objective of this study and therefore was never attempted. Any food that requires handling in preparation may therefore easily become contaminated. Infected wounds, lesions, and boils of food handlers

TABLE 1. ANTIBIOTIC RESISTANCE PROFILE OF STAPHYLOCOCCI ISOLATES IN CHICKEN AND BEEF RAW MEAT SAMPLES

Beef isolates											
Chicken isolates						Local beef					
						Imported beef					
S. aureus			Non-S. aureus staphylococci			S. aureus			Non-S. aureus staphylococci		
No. of isolates			No. of antibiotics			No. of isolates			No. of antibiotics		
Antibiotic resistance profile											
VA	0	0	0	0	0	0	0	0	0	0	1
P, SXT	0	0	0	0	0	0	0	0	2	2	6
E, DA, TE	0	0	1	3	0	0	0	0	0	0	1
CN, E, DA, TE	0	0	1	4	0	0	0	0	0	0	0
P, E, DA, TE	0	0	2	4	0	0	0	0	0	0	0
P, E, CN, SXT	0	0	1	4	0	0	0	0	0	0	6
P, VA, SXT, CIP	0	0	0	0	0	0	0	0	0	0	4
P, TE, VA, SXT	0	0	0	0	0	0	0	0	0	0	4
P, OX, MET, SAM, SXT	0	0	1	5	0	0	0	0	0	0	0
P, CN, E, DA, TE	0	0	2	5	0	0	0	0	0	0	0
E, DA, TE, VA, SXT	0	0	1	5	0	0	0	0	0	0	6
P, CN, E, DA, SXT	0	0	0	0	0	0	0	0	0	0	5
P, OX, CN, DA, SXT	0	0	0	0	0	0	0	0	0	0	5
P, OX, CN, DA, TE, CIP	0	0	3	6	0	0	0	0	0	0	0
P, E, DA, TE, C, SXT	0	0	1	6	0	0	0	0	0	0	0
P, CN, E, DA, TE, C	0	0	1	6	0	0	0	0	0	0	0
P, OX, MET, CN, DA, SXT	0	0	1	6	0	0	2	6	0	2	6
P, CN, E, DA, VA, SXT	0	0	0	0	1	6	0	0	0	0	15
CN, E, DA, TE, C, SXT	0	0	0	0	0	0	1	6	0	0	0
CN, DA, TE, VA, SXT, CIP	0	0	0	0	0	0	1	6	0	0	0
P, OX, CN, DA, VA, SXT	0	0	0	0	0	0	1	6	0	0	0
P, OX, SAM, CN, DA, SXT	0	0	0	0	0	0	0	0	1	6	0
P, OX, CN, E, DA, TE, C	0	0	1	7	0	0	0	0	0	0	0
P, OX, MET, CN, E, DA, TE	0	0	2	7	0	0	0	0	0	0	16
P, OX, CN, E, DA, TE, CIP	0	0	1	7	0	0	0	0	0	0	0
P, CN, E, DA, TE, VA, SXT	0	0	1	7	0	0	0	0	0	0	0

(continued)

TABLE 1. (CONTINUED)

Chicken isolates										Beef isolates					
										Local beef			Imported beef		
S. aureus		Non-S. aureus staphylococci		S. aureus		Non-S. aureus staphylococci		S. aureus		Non-S. aureus staphylococci					
No. of isolates	No. of antibiotics	No. of isolates	No. of antibiotics	No. of isolates	No. of antibiotics	No. of isolates	No. of antibiotics	No. of isolates	No. of antibiotics	No. of isolates	No. of antibiotics				
Antibiotic resistance profile															
P, OX, CN, E, DA ,TE, SXT	0	0	1	7	0	0	0	0	0	0	0				
P, E, DA, TE, VA, C, SXT	0	0	1	7	0	0	0	0	0	0	0				
P,CN,DA,TE,VA,SXT,CIP	0	0	0	0	0	0	1	7	0	1	7				
P, OX, CN, DA,VA, SXT, CIP	0	0	0	0	0	0	1	7	0	0	0				
P, OX, MET, SAM, CN, DA, SXT	0	0	0	0	0	0	1	7	0	0	0				
P, OX, MET, CN, DA, SXT, CIP	0	0	0	0	0	0	1	7	0	1	7				
P, OX, MET, CN, DA,VA, SXT	0	0	0	0	0	0	0	0	0	1	7				
P, OX, E, DA, C, SXT, CIP	0	0	0	0	0	0	0	0	0	1	7				
P, OX, CN, DA, TE, SXT, CIP	0	0	0	0	0	0	0	0	0	1	7				
P, OX, CN, E, DA, TET , SXT, CIP	0	0	1	8	0	0	0	0	0	0	0				
P, OX, MET, SAM, CN, E, DA, TE	0	0	1	8	0	0	0	0	0	0	0				
P, OX, MET, CN, E, DA, TE, SXT	0	0	2	8	0	0	0	0	0	0	0				
P, MET, CN, E, DA, TE, SXT, CIP	0	0	0	0	1	8	0	0	0	0	0				
P, OX ,CN, DA, TE, VA, SXT, CIP	0	0	0	0	2	8	0	0	0	0	0				
P, OX, MET, CN, DA, TE, VA, SXT	0	0	0	0	0	0	1	8	0	0	0				
P, OX, MET, CN, DA, VA, SXT, CIP	0	0	0	0	0	0	0	0	0	2	8				
P, OX, MET, CN, E, DA, TE, VA, SXT	1	9	2	9	0	0	0	0	0	0	0				
P, Ox, MET, SAM, CN, E, DA, TE, SXT	1	9	1	9	0	0	0	0	0	0	0				
P, Ox, MET, SAM, CN, E, DA, TE, VA	0	0	1	9	0	0	0	0	0	0	0				
P, Ox, MET, SAM, CN, E, DA, TE, CIP	0	0	1	9	0	0	0	0	0	0	0				
P, OX, MET, CN, E, DA, TE , SXT, CIP	0	0	2	9	0	0	1	9	0	0	0				
P, OX, MET, CN, E, DA, TE, VA, SXT	0	0	3	9	0	0	0	0	0	1	9				

(continued)

[illegible]

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TABLE 2. PREVALENCE OF COAGULASE-POSITIVE AND COAGULASE-NEGATIVE STAPHYLOCOCCI IN CHICKEN AND BEEF RAW-MEAT SAMPLES

Staphylococcus species	Chicken meat (n=50)	Beef meat (n=50)			Total (n=100)
		Local (n=27)	Imported (n=23)	Total (n=50)	
Coagulase-positive <i>aureus</i>	3/50 (6%)	9/27 (33.3%)	3/23 (13.0%)	12/50 (23.1%)	15/100 (15%)
Coagulase-positive non- <i>S. aureus</i> staphylococci (NSA)					
<i>hyicus</i>	10/50 (20%)	10/27 (37%)	6/23 (26.1%)	16/50 (30.8%)	26/100 (26%)
<i>intermedius</i>	1/50 (2%)	3/27 (11.1%)	3/23 (13.0%)	6/50 (11.5%)	7/100 (7%)
<i>schleiferi</i> subsp. <i>coagulans</i>	—	4/27 (14.8%)	—	4/50 (7.7%)	4/100 (4%)
Total	14/50 (28%)	26/27 (96.3%)	12/23 (52.2%)	38/50 (76%)	52/100 (52%)
Coagulase negative non- <i>S. aureus</i> staphylococci (NSA)					
<i>epidermidis</i>	13/50 (26%)	—	1/23 (4.3%)	1/50 (2.1%)	14/100 (14%)
<i>lugdunensis</i>	15/50 (30%)	—	3/23 (13.0%)	3/50 (6.3%)	18/100 (18%)
<i>haemolyticus</i>	1/50 (2%)	—	1/23 (4.3%)	1/50 (2.1%)	2/100 (2%)
<i>hominus</i>	3/50 (6%)	—	1/23 (4.3%)	1/50 (2.1%)	4/100 (4%)
<i>Schleiferi</i> subsp. <i>coagulans</i>	2/50 (4%)	—	—	—	2/100 (2%)
<i>simulans</i>	—	—	1/23 (4.3%)	1/50 (2.1%)	1/100 (1%)
<i>cohnii</i>	1/50 (2%)	—	—	—	1/100 (1%)
<i>lentus</i>	1/50 (2%)	1/27 (3.7%)	—	1/50 (2%)	2/100 (2%)
<i>scuri</i>	—	—	4/23 (17.4%)	4/50 (8.3%)	4/100 (4%)
Total	36/50 (75%)	1/27 (3.7%)	11/23 (47.8%)	12/50 (25%)	48/100 (48%)

may also be sources of contamination, as well as coughing and sneezing by individuals with respiratory infections (McEntire, 2004). *S. aureus* contaminations are often associated with institutions such as hospitals, schools, and prisons, where food is often prepared in mass quantities and held until consumption (McEntire, 2004). Lack of sanitation by workers and improper time-temperature combinations during storage can lead to contamination of the product and growth of the microorganism to levels at which toxin is produced (McEntire, 2004).

Podkowik *et al.* (2012) showed that raw chicken and beef meat are an important source of CNS and that they received increasing attention due to their potential role in the dissemination of antibiotic-resistance markers in the community and imply food-safety hazards. The antimicrobial resistance profile of the tested CPS and CNS isolates to different antibiotics is recorded in Table 1. All investigated CPS and CNS isolates were resistant to at least one antibiotic. A high overall resistance to antibiotics was observed, again possibly from using antibiotics as growth promoters in animal feed (Wise, 2007), especially those commonly used for both human and animal care, which should consequently be avoided. Antimicrobial resistance was mainly against penicillin (94%), clindamycin (90%), and sulfamethoxazole/trimethoprim (82%). Vancomycin-resistant *S. aureus* showed intermediate resistance (51%), which might indicate the dissemination of vancomycin resistance in the community and imply food-safety hazards (Martins *et al.*, 2013). The percentage of resistance to  $\beta$ -lactams was variable, with the highest resistance being to penicillin (94%) and lowest to ampicillin-sulbactam (22%). CNS are more resistant than *S. aureus* and may be a source of  $\beta$ -lactam resistance genes (de Medeiros *et al.*, 2011).

These findings highlight the high potential risk for consumers in the absence of strict hygienic and preventative measures to avoid the presence of CPS and CNS isolates in foods. Emphasis should be placed on implementing The

Codex Alimentarius Code of Hygienic Practice for Meat (CHPM), which includes the primary international standard for meat hygiene and incorporates a risk-based approach to application of sanitary measures throughout the meat production chain (OIE, 2014). Also, contamination during the distribution and consumption of the final food products must be taken into account. In addition, the potential trade impacts of beef meat garners attention to the multidrug-resistant characteristics of the isolated CPS and CNS and their possible international spread, which poses a threat to public health through their ability to be transferred to humans. Our results show the need for and importance of improving monitoring data and global cooperation in controlling food contamination, raising the sense of urgency to prevent a more threatening drug-resistant bacteria from emerging and unleashing a pandemic. Our findings also provide useful information for public health projects in Egypt, and indicate that the implementation of the Codex Committee on Food Import and Export Inspection and Certification Systems (CCFICS) and the OIE Handbook on Import Risk Analysis for Animals and Animal Products publication, which provide guidelines useful to bilateral parties to develop principles and guidelines in this area, has become a must and that food control should cover both export and import.

#### Disclosure Statement

No competing financial interests exist.

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