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Antimicrobial resistance of potential pathogenic strains isolated from eggs produced by informal farms and sold in Abidjan, Ivory Coast

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The aim of this study was to investigate the microbial quality of eggs produced by informal farms and sold in most Abidjan's markets. Thus, a preliminary investigation was conducted to evaluate the frequency of eggs consumption by the population of the study area (Abidjan). After this investigation, an analysis of the eggs microbiological quality was performed both on the shell and the eatable (egg yolk) part. The eggs were categorized into two groups: i- eggs with shell covered with droppings and ii- eggs with shell not covered with droppings. Four samples of eggs were randomly taken in the same batch collected from 10 districts of Abidjan. Therefore, a total of 90 samples of each category were taken for microbial analyses. The classical method of enumeration in food bacteriology was used for the research of total aerobic mesophilic flora, *Enterococcus*, total and fecal coliforms, *Enterobacteriaceae*, the golden *Staphylococcus* and sulphite-reducing anaerobic bacteria. The present study data show that the consummation of eggs varies according to the area. None of the eatable part contained the investigated bacteria. In general, bacterial counts were higher on shells covered with droppings. Total aerobic mesophilic counts of ~5x10^7 CFU/g *vs.* 10^5 CFU/g was observed on not covered samples. Similarly, *Enterococci*, *Enterobacteriaceae* and *Staphylococcus* reached ~10^4 CFU/g on shells covered with dropping, about 1 log higher than the values observed for non-covered shells. This study suggests that the contamination of the eggs by the investigated microbial parameter is mainly observed on the shell and not on the eatable part. Also, the highest contamination levels were observed in eggs with dropping on the shells.

Key words: Bacterial load, eggs, shell, market, dropping, Abidjan.

INTRODUCTION

Eggs constitute a rich food with high quality biological protein. These egg proteins are used by Food and Agricultural Organization (FAO) of the United Nation to estimate the quality of the other protein sources

(Faverger, 2005). Besides high content in proteins, eggs also play an important part in high digestibility of denatured lipids, many vitamins and mineral salts. They also present technological, emulsifying, foaming, gelling and coagulating properties (AFSSA, 2007).

In Ivory Coast, it was reported that about 60% of consumed eggs are supplied by informal chain (FAO, 2008). In such chains, eggs are provided by traditional or family farms in which they are laid directly on the ground and most time in contact with emitted droppings. So, after their collection, these eggs are delivered on the markets for consumption without any form of hygiene such as preliminary elimination of droppings on the shells. In this case, eggs are delivered to hawkers, semi-fixed and fixed dealers in markets or roads sides. As the droppings are reported to be a source of microbial contamination (Guinebretière et al., 2009), their presence can be harmful not only to the consumer but also the eggs collector.

Thus, the presence of droppings on the egg-shell would be a vector of potentially pathogenic microorganisms to man and can represent a source of food poisoning in a population. Indeed, Since the eighties, there has been a recrudescence of food collective toxi-infections associated with egg in shell and egg product consumption (Lahellec and Salvat, 2004). The bacterium involved in those infections are *Salmonella*, *Escherichia coli* and *Staphylococcus* (De Buyser et al., 2001; Chahed et al., 2007; Kouamé-Sina et al., 2010). However, thought eggs are largely consumed in the megalopolis of Abidjan, there is a lack of data concerning the microbial risk to the populations. So, this study was conducted with the aim of evaluating the bacterial loads of eggs provided by traditional farms and sold in the various markets of Abidjan, Ivory Coast.

MATERIALS AND METHODS

Study area

All the samples were collected in ten different markets (Abobo, Adjamé, Anyama, Attécoubé, Cocody, Koumassi, Marcory, Port-Bouët, Treichville and Yopougon) of Abidjan located at the south-east of Ivory Coast (Figure 1).

Sample collection

The egg samples were collected among the dealers in various markets ("Cocovico market" of Cocody Angré, "big market" of Treichville, "big market" of Abobo, "small market" of Marcory, "Gouro market" of Adjamé, "big market" of Port-Bouët, "Banco market" of Yopougon II, "big market" of Koumassi, "local markets" of Anyama and of Attécoubé) in Abidjan. In this market, eggs supplied by the informal farmers to the dealers were randomly collected. Two categories of eggs were used as shown in Figure 2: eggs with shell covered with droppings (Figure 2a) and eggs with shell not covered with droppings (Figure 2b).

Each sample was composed of four eggs randomly taken from the same batch. So for the two kinds of collected eggs, a total of 180 (90 for each) samples were collected for microbial analyses. In terms of egg shell, 720 (180x4) were thus collected for the study. The egg collection was conducted from March 2014 to 2014 (3 months). This period represents the new-laid phase in such traditional farms. All the eggs were collected aseptically and taken to the laboratory in a clean refrigerator for proximate analysis.

Investigation

The investigation was conducted using the active participatory research methods among a specific group (young, old, men, women, etc). Semi-structured talks were used and the talks were inspired by the "snowball" method previously used by Subedi et al. (2003) and Delaunay et al. (2008). It indicates other surrounding farms apart from the first one fall back on the farm already visited (Thierry, 2009).

Microbiological analysis

The plate count agar was used to count total aerobic mesophilic flora according to French standard: V08-051: 2009. Enumeration of *Enterobacteriaceae* was performed on VRBG agar media according to ISO 21528-2: 2004. For enumeration of *Salmonella*, the SS agar media was used according to the standard described in the ISO 6579: 2002 Amd 1: 2007, whereas the Baird-Parker medial was used for the identification of *Staphylococcus* (37°C for 48 h) according to the French standard V08-057-1: 2004. The TSN agar media was used for the enumeration of Clostridium and sulphite-reducing anaerobic microorganisms (ASR) following the prescriptions of the ISO 7937: 2004 standard at 45°C for 24 to 48 h. Total and fecal coliforms was enumerated (37 and 44°C for 24-48 h) with rapid *E. coli* 2 as suggested in the 2011 version of AFNOR BIO-12 / 5-01 / 99 (2) and the BEA agar was used to investigate the presence of *Enterococcus* (37°C for 24-48 h) according to the ISO 7899/1: 1998 recommendations.

For all the above cited tests, a preparation of the initial eggshells suspension was made by introducing the sample (4 eggs) in 225 ml of sterilized buffered peptone water (BPW, Merk). The four egg shells were dabbled in the diluents for about 2 min (Protais et al., 2003) to obtain the stock solution (dilution 10^{-1}). This stock solution was used to prepare work solutions (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) in aseptic conditions after decimal dilutions.

Analysis of the egg eatable part was performed after immersing the whole eggs in alcohol (70%) for 10 min to destroy the bacterial flora present all over the surface of the shells. After this operation, the content of each egg was put in a sterile glass jar and mixed with a sterile spatula. This egg solution (25 ml) was mixed with sterile distilled water (225 ml) in aseptical conditions to get a stock solution (dilution 10^{-1}).This stock solution was used to prepare work solutions (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5}) in aseptic conditions after decimal dilutions. All the different solutions were inoculated in the appropriate media for a particular test as mentioned above. This stock solution was used to prepare work solutions(10^{-2}, 10^{-3}, 10^{-4}

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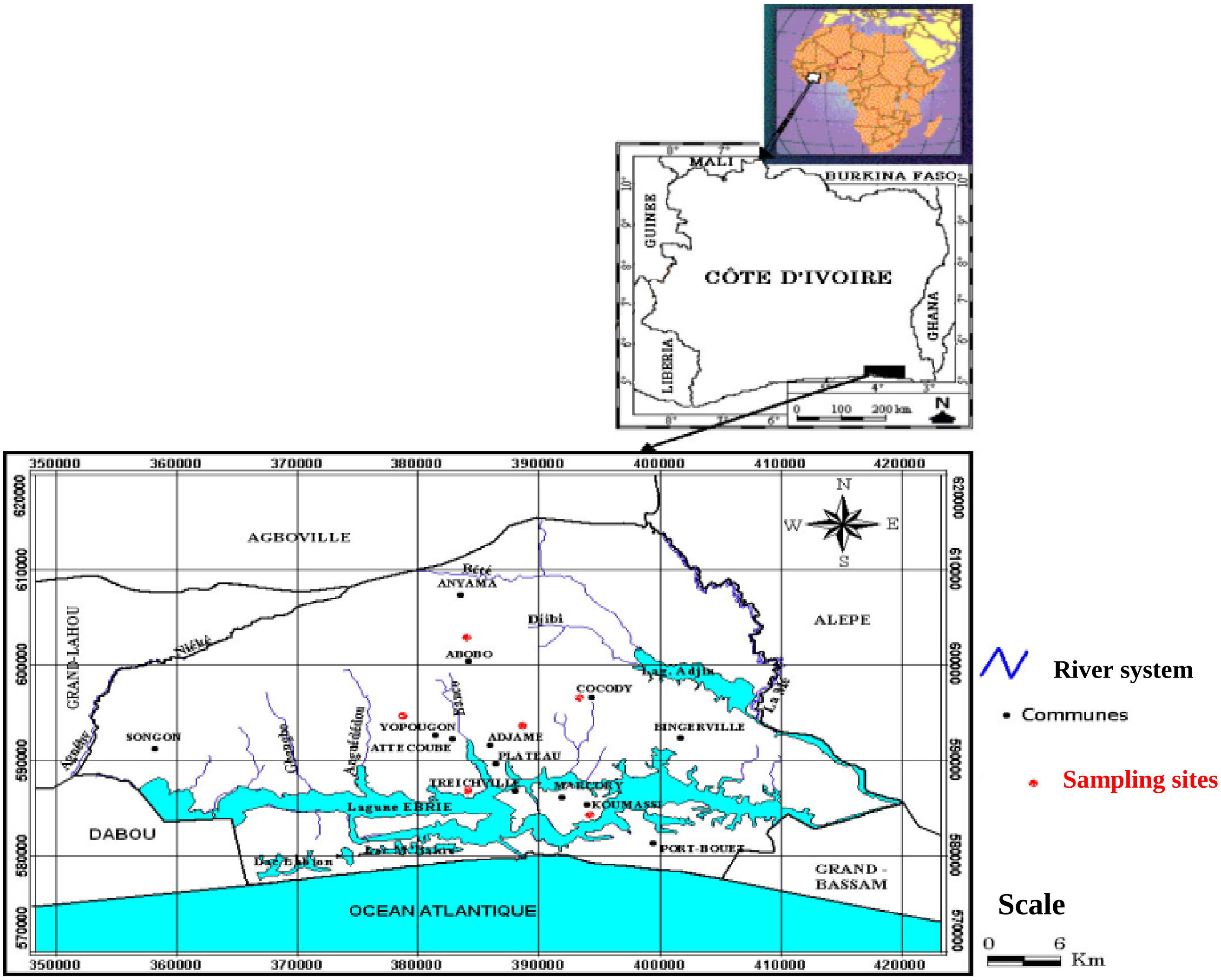
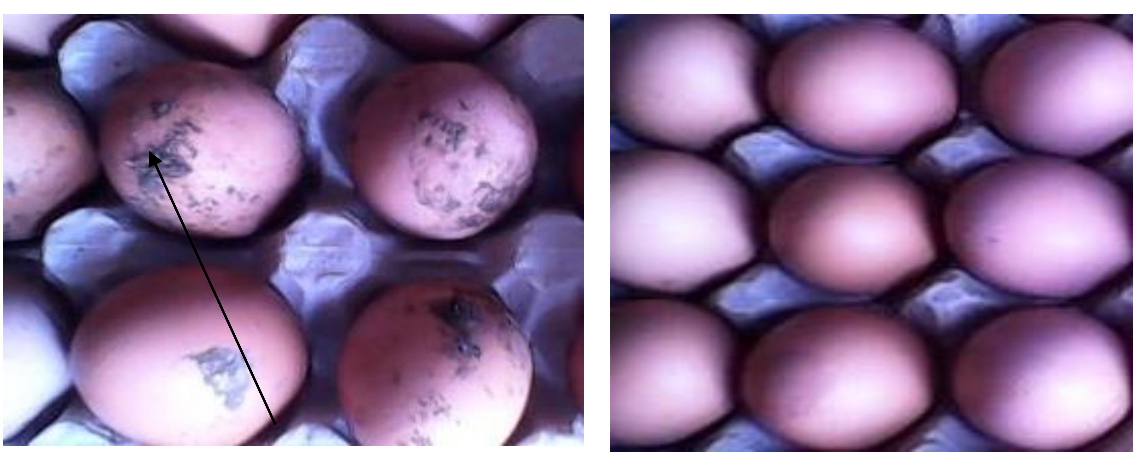


Figure 1. Study area showing the sample collection sites in the map of Abidjan.



a. Eggs covered with droppings (Ya) **b.** Eggs not covered with droppings (Yb)

Figure 2. The two categories of eggs collected for this study.

Table 1. Percentage of eggs consumption in the municipalities of Abidjan.

Municipalities	Quantity regularly consumed per day					Trend of consumption these the last three months		
	1	2	3	4	≥5	Constant	Evolutionary	Regressive
Abobo	50.7[b]	28[c]	20[b]	1.3[d]	0.0	76.4[d]	2.8[c]	20.8[b]
Adjamé	52.4[b]	33.3[a]	14.3[d]	0.0[e]	0.0	80.9[c]	4.8[b]	14.3[c]
Yopougon	57.4[b]	25.5[c]	10.6[d]	6.4[c]	0.0	85.1[bc]	4.2[b]	10.6[d]
Attécoubé	37.5[c]	37.5[a]	12.5[d]	12.5[a]	0.0	100.0[a]	0.0[d]	0.0[e]
Koumassi	41.2[c]	35.3[a]	17.6[c]	5.9[c]	0.0	94.1[b]	0.0[d]	5.9[de]
Marcory	81.8[a]	27.3[c]	0.0[e]	0.0[e]	0.0	100.0[a]	0.0[d]	0.0[e]
Cocody	50.0[b]	33.3[b]	8.3[de]	8.3[b]	0.0	66.7[e]	8.3[a]	25.0[a]
Anyama	40.9[c]	31.8[b]	13.6[d]	13.6[a]	0.0	77.3[d]	4.5[b]	18.2[bc]
Treichville	50.0[b]	33.3[b]	16.7[c]	0.0[e]	0.0	100.0[a]	0.0[d]	0.0[e]
Port-Bouët	42.9[c]	28.6[c]	28.6[a]	0.0[e]	0.0	100.0[a]	0.0[d]	0.0[e]
Average	51.3[b]	30.4[b]	14.7[d]	4.4[c]	0.0	89.1[bc]	2.2[c]	8.6[dc]

Numbers in the table are in %; in a column, the values with the same letters are not statistically different ($p > 0.05$).

and 10^{-5}) in aseptic conditions after decimal dilutions. All the different solutions were inoculated in the appropriate media for a particular test as mentioned above.

Antibiotic susceptibility

Antibiotic susceptibility pattern of the *E. coli* and *Staphylococcus* isolates was determined using the disk diffusion method on Mueller-Hinton agar. Inhibition zone diameter values were interpreted as recommended by the Committee of Antibiogramme of the French Society of Microbiology (CASFM, 2015). The tested antibiotics are Amoxicillin (AMX), Amoxicillin + clavulanic Acid (AMC), Imipeneme (IPM), Cefuroxime (CXM), Cefepime (FEP), Cefalotine (CTN), Aztreonam (ATM), Nalidixic acid (NA) and Ciprofloxacin (CIP).

Statistical analysis

The data analysis was done according to the objectives of the study. So, for the quantitative information, the descriptive statistical analysis (average, percentage, etc) were done using the Graph pad 6.0 (Prism) software. With regards to the qualitative data, the method of analysis of contents was used. This method allows a systematic and rigorous analysis empirical results resulting from the semi-structured talks. For comparison, $p < 0.05$ was considered statistically significant.

RESULTS

The quantity of eggs regularly consumed per day and per capita was one (51.3%) followed by two (30.4%), three (14.7%) and four (4.4%). Among the interviewed people, none declared consuming more than 4 eggs daily. The trend of consumption during the last three months is dominated by a constant consumption (89.1%) (Table 1).

This study did not detect any microorganism from the eatable part of sampled eggs, suggesting bacteria were either absent or their level was below the detection limit. Consequently, further microbiological data were gotten from the shells. Total aerobic mesophilic flora of egg shells was higher among shells with droppings with an average of 4.9×10^7 CFU/g and lower in the samples of shells without dropping (1.1×10^5 CFU/g). Considering the Ya category, the highest load in total aerobic mesophilic flora was obtained in the municipality of Port-Bouët (4.3×10^8 CFU/g) and the lowest was recorded in Cocody (2.6×10^5 CFU/g). In eggs shells without dropping (Yb), the highest load was obtained in the smallest municipality of Marcory (2.8×10^5 CFU/g) and in Port-Bouët (2×10^4 CFU/g). The lowest load in total aerobic mesophilic flora among the Ya categories (10^5 CFU/g) is equivalent to the highest load of the Yb category (Table 2). The research of *Enterococcus* displays an average charge of 5.5×10^4 and 2.3×10^4 CFU/g, respectively for Ya and Yb eggs categories. However, in the Ya category, the highest load was obtained in the smallest Marcory municipality (1.3×10^5 CFU/g)

Table 2. Load of total aerobic mesophilic flora and *Enterococcus* on the egg shells collected in the markets of Abidjan.

Municipalities	Bacterial loads (CFU/g)			
	Total aerobic mesophilic flora		***Enterococcus***	
	Ya	**Yb**	**Ya**	**Yb**
Abobo	$4.1*10^{5} \pm 2.8.10^{4d}$	$2.4.10^{4} \pm 2.6.10^{3b}$	$1.5.10^{4} \pm 9.8.10^{2b}$	$5.6.10^{3} \pm 8.1.10^{1cd}$
Adjamé	$3.6*10^{6} \pm 5.2.10^{5c}$	$2.6.10^{4} \pm 7.2.10^{3b}$	$1.1.10^{5} \pm 3.2.10^{3a}$	$7.7.10^{3} \pm 2.1.10^{2}c$
Yopougon	$2.4*10^{6} \pm 2.1.10^{5c}$	$2.2.10^{4} \pm 9.8.10^{2c}$	$1.8.10^{4} \pm 5.2.10^{2b}$	$4.5.10^{3} \pm 7.2.10^{1cd}$
Anyama	$1.8*10^{6} \pm 7.8.10^{5c}$	$3.5.10^{4} \pm 1.3.10^{3b}$	$1.2.10^{5} \pm 3.7.10^{3a}$	$5.7.10^{3} \pm 7.4.10^{1cd}$
Cocody	$2.6*10^{5} \pm 1.5.10^{4d}$	$2.5.10^{4} \pm 6.4.10^{2b}$	$5.6.10^{4} \pm 1.1.10^{3b}$	$1.5.10^{3} \pm 3.8.10^{1d}$
Attécoubé	$2.7*10^{6} \pm 1,1.10^{5c}$	$3.6.10^{5} \pm 5.10^{3a}$	$7.4.10^{4} \pm 8.10^{2b}$	$1.7.10^{5} \pm 2.4.10^{4a}$
Treichville	$2.6*10^{7} \pm 5.10^{5b}$	$3.5.10^{5} \pm 1.5.10^{4a}$	$1.3.10^{4} \pm 8.8.10^{1b}$	$1.3.10^{4} \pm 2.5.10^{2b}$
Marcory	$2.6*10^{7} \pm 2.5.10^{5b}$	$2.8.10^{5} \pm 8.6.10^{3a}$	$1.3.10^{5} \pm 1.2.10^{3a}$	$1.4.10^{4} \pm 5.3.10^{2b}$
Koumassi	$2*10^{6} \pm 2.7.10^{4c}$	$2.3.10^{4} \pm 1.8.10^{3c}$	$1.4.10^{4} \pm 2.9.10^{2b}$	$1.4.10^{3} \pm 4.9.10^{1c}$
Port-Bouët	$4.3*10^{8} \pm 6.10^{6a}$	$2.10^{4} \pm 3.4.10^{2c}$	$3.7.10^{3} \pm 3.8.10^{1c}$	$1.6.10^{3} \pm 4.5.10^{1c}$
Average	$4.9*10^{7} \pm 4.2.10^{7b}$	$1.1.10^{5} \pm 4.8.10^{4}$	$5.5.10^{4} \pm 1.6.10^{4b}$	$2.3.10^{4} \pm 1.7.10^{4b}$

CFU: Colony forming unit. Ya: eggs with droppings on the shell; Yb: eggs without droppings on the shell. In a column, the values with the same letters are not statistically different (*p*>0.05).

Table 3. Total coliforms load and *E. coli* on the egg shells collected in the markets of Abidjan.

Municipalities	Bacterial loads (CFU/g)			
	Total coliforms		***Escherichia coli***	
	Ya	**Yb**	**Ya**	**Yb**
Abobo	$2.6.10^{3} \pm 3.9.10^{1c}$	$1.5.10^{3} \pm 1.2.10^{1b}$	0^{d}	0
Adjamé	$1.6.10^{4} \pm 7.4.10^{2b}$	$2.7.10^{3} \pm 1.3.10^{2a}$	0^{d}	0
Yopougon	$1.5.10^{3} \pm 1.1.10^{2d}$	$1.6.10^{3} \pm 3.7.10^{1b}$	0^{d}	0
Anyama	$1.5.10^{3} \pm 5.9.10^{1d}$	$1.9.10^{3} \pm 1.10^{2b}$	0^{d}	0
Cocody	$2.4.10^{4} \pm 5.8.10^{1a}$	$2.2.10^{2} \pm 6.9^{c}$	0^{d}	0
Attécoubé	$2.2.10^{4} \pm 6.2.10^{2a}$	$2.2.10^{2} \pm 1.9.10^{1c}$	0^{d}	0
Treichville	$1.9.10^{3} \pm 3.5.10^{1c}$	$1.8.10^{3} \pm 2.7.10^{1b}$	$3.3.10^{3} \pm 3.3.10^{3a}$	0
Marcory	$2.4.10^{4} \pm 3.8.10^{2a}$	$2.1.10^{3} \pm 3.5.10^{1a}$	$1.7.10^{1} \pm 1.7.10^{1c}$	0
Koumassi	$1.6.10^{3} \pm 3.8.10^{1d}$	$2.5.10^{2} \pm 2.1^{c}$	$1.6.10^{2} \pm 1.6.10^{2b}$	0
Port-Bouët	$1.7.10^{4} \pm 3.2.10^{2b}$	$2.5.10^{2} \pm 6.5^{c}$	0^{d}	0
Average	$1.1.10^{4} \pm 3.2.10^{3b}$	$1.2.10^{3} \pm 3.10^{2b}$	$1.8.10^{1} \pm 1.6.10^{1c}$	0

CFU: colony forming unit. Ya: eggs with droppings on the shell; Yb: eggs without droppings on the shell. In column, the values carrying the same letters are not statistically different (*p*>0.05).

and the lowest at Port-Bouët (3.7×10^{3} CFU/g). In the second category, Attécoubé displays the greatest average charge (1.7×10^{5} CFU/g), whereas the lowest load (1.5×10^{3} CFU/g) was observed at Cocody. The differences of *Enterococcus* counts observed between samples were not statistically significant (*p* >0.05).

The recorded load in total coliforms on the egg shells was higher in Ya samples with an average of 1.1×10^{4} CFU/g when compared with the Yb ones (average 1.2×10^{3} CFU/g). Considering the Ya category, the highest value was observed in in the municipality of Marcory (2.4×10^{4} CFU/g) and the lowest at Koumassi (1.6×10^{3} CFU/g). In the Yb category, the highest load was obtained in the municipality of Adjamé (2.7×10^{3} CFU/g). *E. coli* was detected in 3 out of the 10 collected samples and these samples were all issued from Ya category with an average value of 1.8×10^{1} CFU/g. The positive samples were collected at Treichville (3.3×10^{3} CFU/g), Koumassi (1.6×10^{2} CFU/g) and Marcory (1.7×10^{1} CFU/g) (Table 3).

Data obtained for *Enterobacteriaceae* suggest that the shells Ya categories were more contaminated (1.7×10^{4} CFU/g) than the Yb category's (1.5×10^{3} CFU/g). These germs were more detected at Marcory ($6.7x10^{4}$ CFU/g) and less in the municipality of Anyama ($1.6x10^{3}$ CFU/g). In addition, no significant difference was observed

Table 4. Load in *Enterobacteriaceae* and gilded *Staphylococcus* on the egg shells collected in the markets of Abidjan.

Municipalities	Bacterial loads (CFU/g)			
	Enterobacteriaceae		**Gilded *Staphylococcus***	
	Ya	**Yb**	**Ya**	**Yb**
Abobo	$2.7.10^3 \pm 9.10^{1c}$	$2.1.10^3 \pm 2.10^{1cd}$	$1.8.10^4 \pm 5.6.10^{2bc}$	$2.10^4 \pm 9.10^{2a}$
Adjamé	$1.8.10^4 \pm 4.10^{2b}$	$2.7.10^3 \pm 4.7.10^{1b}$	$2.4.10^4 \pm 1.5.10^{3b}$	$1.4.10^3 \pm 1.2.10^{2d}$
Yopougon	$5.4.10^3 \pm 1.6.10^{2bc}$	$1.5.10^3 \pm 1.10^{2d}$	$2.3.10^4 \pm 1.1.10^{3b}$	$1.9.10^3 \pm 2.6.10^{1dc}$
Anyama	$1.6.10^3 \pm 4.7.10^{1c}$	$2.5.10^3 \pm 45.1^b$	$1.4.10^5 \pm 5.2.10^{3a}$	$1.3.10^3 \pm 6.7.10^{1d}$
Cocody	$2.5.10^4 \pm 9.8.10^{2b}$	$2.7.10^2 \pm 1.2^c$	$2.2.10^4 \pm 5.6.10^{2b}$	$2.2.10^3 \pm 3.4.10^{1d}$
Attécoubé	$2.4.10^4 \pm 5.2.10^{2b}$	$2,5.10^3 \pm 6.4.10^{1b}$	$2.5.10^4 \pm 1.7.10^{3b}$	$4.7.10^3 \pm 9.4.10^{1c}$
Treichville	$2.10^3 \pm 3.2.10^{1c}$	$2.1.10^3 \pm 3.3.10^{1c}$	$1.3.10^5 \pm 2.2.10^{3a}$	$1.3.10^3 \pm 4.10^{1d}$
Marcory	$6.7.10^4 \pm 6.9.10^{2a}$	$3.5.10^3 \pm 2.3.10^{1a}$	$2.2.10^5 \pm 4.7.10^{3a}$	$2.3.10^3 \pm 3.7.10^{1dc}$
Koumassi	$1.7.10^3 \pm 1.7.10^{3c}$	$2.7.10^2 \pm 1.5.10^{1c}$	$1.8.10^3 \pm 2.7.10^{1c}$	$1.3.10^3 \pm 5.5.10^{1d}$
Port-Bouët	$2.5.10^4 \pm 6.10^{2b}$	$2.6.10^2 \pm 2.3^c$	$2.6.10^4 \pm 7.6.10^{2b}$	$7.3.10^3 \pm 3.3.10^{2b}$
Average	$1.7.10^4 \pm 6.4.10^{3b}$	$1.5.10^3 \pm 4.10^{2d}$	$6.4.10^4 \pm 2.4.10^{4b}$	$4.3.10^3 \pm 1.8.10^{3b}$

CFU: Colony forming unit. Ya: eggs with droppings on the shell; Yb: eggs without droppings on the shell. In a column, the values with the same letters are not statistically different (p>0.05).

between the loads of Koumassi (1.7 × 10^3 CFU/g), Treichville (2 × 10^3 CFU/g), Abobo (2.7 × 10^3 CFU/g) and Anyama (1.6 × 10^3 CFU/g) (p>0.05). Considering the Yb category, the maximal and minimal loadings of the eggs shell were obtained at Marcory (3.5 × 10^3 CFU/g) and Yopougon (1.4 × 10^3 CFU/g), respectively (Table 4). The gilded *Staphylococcus*, were detected with average charges of 6.4x10^4 (for Ya) and of 4.3x10^3 CFU/g (Yb). Among the samples of Ya category, the municipality of Marcory recorded the highest load (2.2*10^5 CFU/g). A non-significant difference was observed between the load of Marcory and the one of Treichville (1.3x10^5 CFU/g) (p<0.05). With the Yb category, the maximum loadings and minimal were obtained respectively at Abobo (2x10^4 CFU/g) and Koumassi (1.3x10^3 CFU/g). Also, it should be noted that the average charge in gilded *Staphylococcus* is higher on the eggs shells of Ya category than on the Yb one (Table 4).

The average load of the tested shells in sulphite-reducing anaerobic microorganisms, was 10 CFU/g for Ya samples and 20 CFU/g for Yb ones. The largest load (20 CFU/g) among the Ya samples was obtained at Yopougon, Koumassi, Anyama and Adjamé, whereas the largest values (50 CFU/g) with Yb series was recorded at Anyama. The Yb egg samples collected at Port-Bouët did not reveal any presence of ASR strains. The comparison between the two categories of egg show that the Yb category has a more important (20 CFU/g) average charge in germs (Table 5). A compilation of all our results on sampled egg shells shows that various kinds of microorganisms (including aerobic mesophile bacteria, *Enterococcus* and *Staphylococcus*) were present at different proportions (Figure 3). The frequency of isolation of sulphite-reducing anaerobic microorganisms was lower by 48.4% followed by *E. coli* contamination at a frequency of 5.2%. None of the *E. coli* strains isolated from chicken egg shells presented resistance to carbapenem and cephalosporins. These strains were also sensitive to other families of antibiotics tested including monobactam and quinolones. However, the recorded data shows that all the *E. coli* strains isolated from the Ya eggs shells were resistant to amoxicillin, whereas 20% were resistant to the association amoxicillin/clavulamique acid (Table 6).

All *Staphylococcus* strains isolated from chicken eggs shells were resistant to cephalosporins. These bacteria are resistant at various proportions to the other tested antibiotics such as carbapenems and quinolones.

DISCUSSION

The analysis of the egg shells shows the presence of several germs such as total aerobic mesophilic flora, total coliform and *E. coli*, *Staphylococcus*, *Enterobacteriaceae* and the anaerobic sulphite-reducing bacteria. A previous work performed on egg shell reveals that about 40 different groups of bacteria can be isolated (Saver, 1991). But, fortunately, most of those bacteria are not pathogenic. Similarly, Protais et al. (2006) showed that the most frequently microorganisms found on the egg shells are total aerobic mesophilic flora and *Enterobacteriacea*. However, among these germs, the major agents of collective food poisoning such as *Salmonella*, etc can be noted (Messens et al., 2005). It should be noted that the presence of pathogenic materials on egg shell may be dangerous to the consumers because the egg contamination is reported to be either horizontal or vertical (Fuller, 1984; Bouedo et al., 1993; Gabriel et al., 2005). The different types of

Table 5. Sulphite-reducing anaerobic microorganisms' load on the shells of egg collected in the markets of Abidjan.

Municipalities	ASR Bacteria loads (CFU)	
	Ya	Yb
Abobo	4 ± 4[d]	7 ± 7[c]
Adjamé	2.10[1] ± 8[a]	2.10[1] ± 2.10[1b]
Yopougon	2.10[1] ± 2.10[1a]	2.10[1] ± 9[b]
Anyama	2.10[1] ± 8[a]	5.10[1] ± 2.10[1a]
Cocody	4 ± 4[d]	6 ± 4[c]
Attécoubé	10 ± 6[b]	2.10[1] ± 2[b]
Treichville	4 ± 4[d]	2.10.[1] ± 2.10[1b]
Marcory	8 ± 7[c]	5 ± 3[c]
Koumassi	2.10[1] ± 10[a]	4 ± 4[c]
Port-Bouët	4 ± 4[d]	0 ± 0[d]
Average values	10 ± 2	2.10[1] ± 4

ASR: Sulphite-reducing anaerobic microorganisms; CFU: colony forming unit. Ya: eggs with droppings on the shell; Yb: eggs without droppings on the shell. In column, the values with the same letters are not statistically different (*p*>0.05).

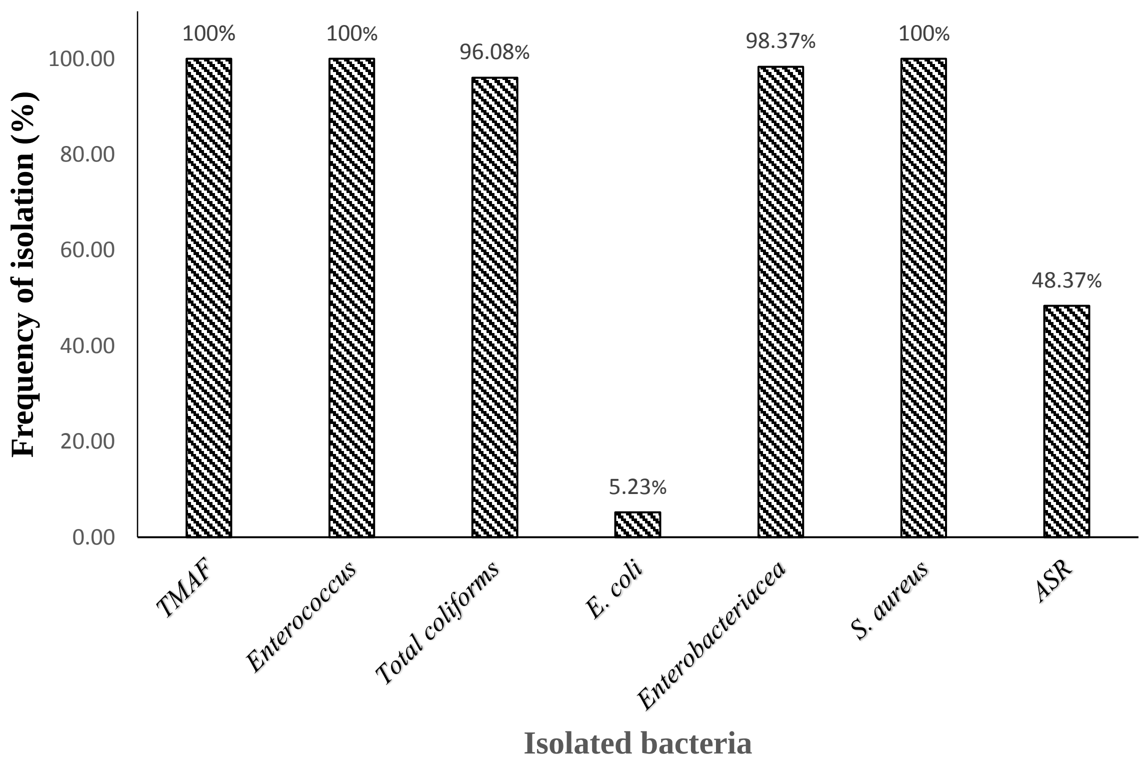


Figure 3. Frequency of some bacteria isolates on eggs shell collected in ten market of Abidjan. TMAF: total aerobic mesophilic flora, ASR: sulphite-reducing anaerobic microorganisms.

contamination are determined by the egg production environment (hen house, eggs storage room and others) and various handling (Florian and Trussel, 1958; Mayes and Takeballi, 1983; Stadelman and Cotterill, 1986; De Reu et al., 2005). Indeed, the presence of bacterial on the egg shells may be due to the intestinal flora of the good layers (Gabriel et al., 2005) because their digestive flora are a veritable nest of bacteria and fungi (Gabriel et

Table 6. Antibiotic resistance profile of *E. coli* and *Staphylococcus* isolates from egg shells collected in some markets of Abidjan.

Antibiotic resistance profile		***Escherichia coli*(n = 5)**		***Staphylococcus*(n = 180)**	
		Resistance	**Sensibility**	**Resistance**	**Sensibility**
β-lactams	**Penicillin**				
	AMX	100	0	65	35
	AMC	20	80	55	45
	Carbapenem				
	IMP	0	100	0	100
	Cephalosporin				
	CXM	0	100	45	55
	FEP	0	100	60	40
	CTN	0	100	35	65
	Monobactam				
	ATM	0	100	30	70
Quinolone	NA	0	100	15	85
	CIP	0	100	0	100

AMX: Amoxicillin; AMC: Amoxicillin + clavulanic acid; IPM: Imipénem; CXM: Cefuroxime; FEP: Cefepime; CTN: Cefalotine; ATM: Aztreonam; NA: Nalidixic acid; CIP: Ciprofloxacin.

al., 2003). So, there is an established link between the layers breeding system and the produced eggs microbial load (Mallet et al., 2005). For example, the free-range farming are favorable to a high contamination of egg shell and its eatable part (Wall et al., 2008).

The present study results show that various kinds of bacteria are found on the egg shell. The diversity of bacterial loads on the sampled eggs could also be related to the use of old cells because the conditioned eggs bacterial loads increase when the cells are used too many times (Huneau-Salaün et al., 2009). In addition, the level of eggs-laying surfaces cleanliness could affect the bacterial load of these eggs (Mallet et al., 2005). Attention should be paid during the manipulations of eggs as it was established that the bacterial load of egg shell influences the penetration of potentially harmful pathogens in egg through the shell (Schoeni et al., 1995; Braun et al., 1999; Raghiante et al., 2010).

However, in spite of the remarkable presence of various bacteria on the analyzed shells, their eatable parts were free from microorganism. This result is contradictory to that of other authors who observed an *E. coli* prevalence between 26.29 and 90% in the eatable part of eggs (Lakehal, 2006; Protais et al., 2006). Such differences between the present study result and those reported by other authors may be due to the age of the analyzed eggs. Indeed, the inside content of old eggs is more likely to be contaminated by contaminants from the shell. One of the reasons that can explain the absence of microbial contaminants in the present study samples may be the bactericidal effect of the white eatable part of egg (Vidal et al., 2003). So, microorganisms may be destroyed by this barrier with aim to protect the content of the eggs. Considering the shells, total coliform and of *E. coli* contaminants were present on the analyzed samples. The coliforms are naturally present in the intestinal flora of chicken and thus can easily contaminate egg shell via droppings (Gabriel et al., 2005). This result corroborates that of Bouedo et al. (1993).

The presence of *Staphylococcus* and anaerobic sulfite-reducing germs in this investigation is similar to the result reported by Elasri and Afilal (2014). These authors also suggest that the droppings may be the source of egg shells contamination with such microorganisms. These results show that the presence of dropping on egg shells is correlated to a higher contamination level. So with this high contamination level among samples with dropping on shells, their use without any sanitary precautions can be dissuaded. These high loads show the extent of the fecal contamination in the cases of free-range farming (Wall et al., 2008; Bouedo et al., 1993) because droppings are very charged in microorganisms in such kind of farming (Dougnon et al., 2014). The *Staphylococcus* load is higher in this study among egg shells with droppings.

The incidence observed is lower than that reported by several authors with 45% of *Staphylococcus* reported on samples from poultry farms or chicken meat (Hassen et al., 2003; Ifesan et al., 2009).

Antimicrobial susceptibility analysis showed *Staphylococcus* strains resistant to several antibiotics. These strains of *Staphylococcus*, regardless of their origin, are mostly resistant to methicillin. Indeed, *Staphylococcus* strains isolated during this study showed a general resistance to β-lactam family and high sensitivity to carbapenems and monobactams. Thus, 65% of the *Staphylococcus* strains are resistant to oxacillin, 55% to oxacillin + clavilamique acid.

Egg shell isolates displayed a methicillin-resistant suggesting a human (clinical or environmental) origin. Indeed, the presence of methicillin-resistant strains in foods was reported in Germany by van den Broek (2003). The detection of resistant *Staphylococcus* in this study is of concern for food safety as this genus encompasses pathogenic species and in particular *Staphylococcus aureus* which can be resistant to antibiotics such as oxacillin and can produce toxin (Baba-Moussa et al., 2011, Attien et al., 2014). In 2010, an EFSA study at Abidjan reported similar results by showing that the main reservoir of methicillin-resistant *Staphylococcus* are pigs, calves and broilers (EFSA, 2010). This presence of methicillin-resistant *Staphylococcus* in food could be of human origin and linked to contamination of meat products during processing, transportation or sale.

Conclusion

The microbiological analyses of the egg shells, independently of the collection site showed the presence of many bacteria such as total aerobic mesophilic flora, *Enterococci*, total coliforms, *E. coli*, *Enterobacteriaceae*, gilded *Staphylococcus* and anaerobe sulfite-reducer (ASR) germs. Although, the eggs shells without droppings are less contaminated than those with dropping, the two groups of egg remain “not conforms” because of the presence of ASR germs that represent food poisoning risk. So, these results suggest a systematic cleaning (with adequate tools) of eggs in the traditional (informal) farms before markets supply.

Conflict of Interests

The authors have not declared any conflict of interests.

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