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Characterization of *Salmonella enterica* and Detection of the Virulence Genes Specific to Diarrheagenic *Escherichia coli* from Poultry Carcasses in Ouagadougou, Burkina Faso

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Abstract

One hundred chicken carcasses purchased from three markets selling poultry in Ouagadougou, Burkina Faso, between June 2010 and October 2010 were examined for their microbiological quality. The presence of *Salmonella* was investigated using standard bacteriological procedures, and the isolates obtained were serotyped and tested for antimicrobial susceptibility. The presence of virulence-associated genes of the five main pathogroups of diarrheagenic *Escherichia coli*—Shiga toxin–producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), enterotoxigenic *E. coli*, and enteroinvasive *E. coli*—was investigated using 16-plex polymerase chain reaction (PCR) on the mixed bacterial cultures from the poultry samples. Of the 100 chicken carcasses studied, 57 were contaminated by *Salmonella*; 16 different serotypes were identified, the most frequent being *Salmonella* Derby, found in 28 samples. Four *Salmonella* strains were resistant to tetracycline, and two were resistant to streptomycin. Based on the PCR detection of the virulence genes, in total, 45 carcasses were contaminated by three pathogroups of *E. coli*: STEC, EPEC, or EAEC. The STEC and EPEC virulence genes were detected on six and 39 carcasses, respectively. EAEC virulence genes were only detected in combination with those of EPEC (on 11 carcasses) or STEC (on two carcasses). The STEC-positive carcasses contained the genes stx_1, stx_2, *eaeA*, *esc*V, and *ent* in different combinations. None of the EPEC-positive carcasses contained the *bfp* gene, indicating that only atypical EPEC was present. EAEC virulence genes detected were *agg*R and/or *pic*. The high proportion of chicken carcasses contaminated by *Salmonella* and diarrheagenic *E. coli* indicates a potential food safety risk for consumers and highlights the necessity of public awareness of these pathogens.

Introduction

Chickens are widely consumed in Burkina Faso as well as exported to the neighboring countries. Contaminated raw or undercooked poultry products, especially chicken meat, have been shown to be an important source of foodborne pathogens for humans, resulting in numerous cases of enteric infections (Wilson, 2002). Most of the foodborne illnesses are caused by three major bacteria: *Campylobacter* spp., *Salmonella enterica*, and pathogenic *Escherichia coli* (Todd, 1997). The antimicrobial resistance of the enteric pathogens is also an important public health problem, as it is steadily increasing and can be associated with extended hospitalizations (Sackey *et al*., 2000; Varma *et al*., 2005). Multiresistant bacteria such as *Salmonella* found in humans may be of animal origin, from which they are transmitted to humans through food (White *et al*., 2001).

Diarrheagenic *E. coli* strains are commonly classified into five main heterogeneous groups based on their virulence traits (Nataro and Kaper, 1998): enterohemorrhagic *E. coli* (EHEC), also called Shiga toxin–producing *E. coli* (STEC); enteropathogenic *E. coli* (EPEC); enteroaggregative *E. coli* (EAEC); enterotoxigenic *E. coli* (ETEC); and enteroinvasive *E. coli* (EIEC). All the pathogroups can cause diarrhea and other symptoms, but especially EHEC can also cause serious complications, such as bloody diarrhea and hemolytic uremic syndrome (Nataro and Kaper, 1998).

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Traditionally, in Burkina Faso chickens roam free, scattering their feces anywhere on the house yards. More recently, also a new business activity of small-scale industrial broiler poultry production has expanded to supply the growing urban population with its demand of animal proteins. However, the hygienic conditions at the poultry markets, where slaughtering is also done, are inadequate and can lead to bacterial contamination of meat (Kagambega *et al*., 2011). The objective of this study was to determine the prevalence of *Salmonella* and diarrheagenic *E. coli* in chicken carcasses purchased from retail markets in Ouagadougou during the rainy season as well as to serotype and characterize the antimicrobial resistance of the *Salmonella* isolates.

Materials and Methods

Sampling plan

Chicken carcasses were purchased from three retail markets in Ouagadougou. Ten sellers from three markets were each visited 10 times from June to October 2010, the time period that covers the rainy season. The entire carcasses slaughtered and plucked during the visit were placed in sterile plastic bags and transported in a cool box to the laboratory, where samples were processed within an hour. There were no records available concerning the origin of the chickens. But, according to the sellers, chickens were of local breeds obtained from different areas across the country.

Microbial analyses

Salmonella. The whole carcass was placed in a large plastic bag containing 225 mL of buffered peptone water, and the bag was vigorously massaged and shaken for 1 min at room temperature. Fifty milliliters of the rinse fluid from the bag was incubated at 37°C for 18–20 h, and then 0.1 mL was added to 10 mL of Rappaport-Vassiliadis broth (Oxoid, Basingstoke, UK) and incubated for an additional 24 h at 42°C before a loopful (10 μL) was plated on xylose-lysine-deoxycholate agar (Oxoid). Colonies exhibiting typical *Salmonella* morphology were preliminarily confirmed biochemically using lysine and triple sugar iron agars. Final verification was done at the National Institute for Health and Welfare (Helsinki, Finland) with API 20E (Biomerieux, Marcy l'Etoile, France), and the strains were serotyped according to the Kauffman-White scheme (Kauffmann, 1971).

All *Salmonella* strains were tested for susceptibility to 12 different antimicrobial agents using the disk diffusion method on Mueller-Hinton agar (Oxoid) at 37°C for 24 h. The antibiotic disks (Oxoid) used were ampicillin (10 μg), chloramphenicol (30 μg), streptomycin (10 μg), sulfonamide (300 μg), trimethoprim (5 μg), ciprofloxacin (5 μg), tetracycline (30 μg), gentamicin (10 μg), nalidixic acid (30 μg), cefotaxime (5 μg), mecillinam (10 μg), and imipenem (10 μg).

Diarrheagenic *E. coli*. Fifty milliliters of the buffered peptone water rinse fluid was incubated at 37°C for 18–20 h. A loopful (10 μL) of the enriched sample was streaked onto sorbitol MacConkey's agar (Oxoid) and incubated at 37°C overnight. All the bacterial mass and colonies growing on a plate were collected, conserved in 1.8-mL tubes containing trypticase soy agar at 4°C, and subsequently sent to the National Institute for Health and Welfare. There, a plastic stick was used to retrieve some bacterial mass from the tubes, and bacteria were recultivated on cystine–lactose electrolyte-deficient agar (Difco, Sparks, MD) at 36°C for 18 h. A loopful (10 μL) of the bacterial mass from the cysteine-lactose electrolyte-deficient plate was boiled in 300 μL of sterile water for 10 min, and the supernatant containing DNA was used for polymerase chain reaction (PCR) detection of the virulence genes of diarrheagenic *E. coli*.

The presence of the specific virulence genes for STEC, EPEC, EAEC, ETEC, and EIEC in the chicken carcasses was studied using 16-plex PCR targeting the genes *uid*A, *stx*$_1$, *stx*$_2$, *hly*A, *eae*A, *esc*V, *ent*, *bfp*, *agg*R, *pic*, *elt*, *est*Ia, *est*Ib, *inv*E, *ipa*H, and *ast*A. The primers, PCR conditions, and control strains used were previously described by Kagambega *et al*. (2012). The following criteria were used for identification of *E. coli* pathogroups: for STEC, the presence of *stx*$_1$ and/or *stx*$_2$ and possibly *eae*A, *esc*V, *ent*, and EHEC-*hly*; for EPEC, the presence of *eae*A and possibly *esc*V, *ent*, and *bfp* (the absence of *bfp* indicated atypical EPEC); for EAEC, the presence of *pic* and/or *agg*R; for ETEC, the presence of *elt* and/or *est*Ia or *est*Ib; and for EIEC, the presence of *inv*E and *ipa*H. Because *ast*A was not specific for a certain pathogroup, it was not used in the final analysis.

Results

Of the 100 chicken carcasses examined, 57 were contaminated by *S. enterica* and, based on the presence of the virulence genes, 45 by diarrheagenic *E. coli*. Sixteen different serotypes of *Salmonella* were identified—Derby (28 isolates), Chester (5), Hato (4), Banana, Monschui, Senftenberg (3 of each), and Adelaide, Agona, Anatum, Brancaster, Eastbourne, Galiema, Nima, Nottingham, Saarbruecken, and Typhi (1 of each)—along with one strain of Group B (4,12:e,h:–) and one of Group C (6,7,14:d:–). Most of the isolates were sensitive to the tested antimicrobials; only four Derby isolates were resistant to tetracycline, and one Derby isolate and one Anatum isolate were resistant to streptomycin. Among the 45 samples containing the virulence genes of diarrheagenic *E. coli*, EPEC genes were detected in 28 samples, STEC genes were detected in 4 samples, EPEC genes together with EAEC genes were detected in 11 samples, and STEC genes together with EAEC genes were detected in 2 samples (Table 1). No ETEC or EIEC genes were detected. The STEC-positive carcasses contained the genes *stx*$_1$, *stx*$_2$, *eaeA*, *esc*V, and *ent* in different combinations. None of the EPEC-positive carcasses harbored the *bfp* gene, indicating that only atypical EPEC was present. In addition, they carried *escV* and/or *eae* and/or *ent* as virulence markers. The detected EAEC virulence genes were *aggR* and/or *pic*.

Discussion

Our study revealed a common occurrence of *Salmonella* (57%) and the virulence genes of diarrheagenic *E. coli* (45%) in chicken carcasses sold at the retail markets in Ouagadougou. The proportion of *Salmonella* in this study was comparable to that observed in chickens in Cameroon (60%) (Nzouankeu *et al*., 2010) and in Ethiopia (68%) (Tibaijuka *et al*., 2003). The high prevalence of *Salmonella* in chicken may be due to asymptomatic carriage of *Salmonella* in avian caeca, which can lead into cross-contamination of the carcass during or after slaughter (Tibaijuka *et al*., 2003; Dione *et al*., 2011). This is especially true when considering the poor hygienic conditions

Table 1. Virulence Genes Associated with the Pathogroups of Diarrheagenic *Escherichia coli* Detected by 16-Plex Polymerase Chain Reaction in Chicken Carcasses

	Virulence genes														
Control strains	stx_1	stx_2	*EHEC*-hly	eae*A*	esc*V*	ent	bfp	elt	est*Ia*	est*Ib*	agg*R*	pic	ipa*H*	inv*E*	uid*A*
RH4270 (STEC)	+	+	+	+	+	+	−	−	−	−	−	−	−	−	+
RH4283 (EPEC)	−	−	−	+	+	+	−	−	−	−	−	−	−	−	+
IH56822 (EAEC)	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+
RH3533 (ETEC)	−	−	−	−	−	−	−	+	+	+	−	−	−	−	+
RH6647 (EIEC)	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+
Identified pathogroups (n[a])															
STEC (2)	+	−	−	−	+	−	−	−	−	−	−	−	−	−	+
STEC (1)	-	+	−	−	+	−	−	−	−	−	−	−	−	−	+
STEC (1)	+	−	−	+	+	−	−	−	−	−	−	−	−	−	+
STEC + EAEC (1)	+	−	−	−	+	−	−	−	−	−	−	+	−	−	+
STEC + EAEC (1)	−	+	−	+	+	+	−	−	−	−	+	−	−	−	+
aEPEC (16)	−	−	−	−	+	−	−	−	−	−	−	−	−	−	+
aEPEC (1)	−	−	+	−	+	−	−	−	−	−	−	−	−	−	+
aEPEC (7)	−	−	−	−	+	+	−	−	−	−	−	−	−	−	+
aEPEC (4)	−	−	−	+	+	−	−	−	−	−	−	−	−	−	+
aEPEC + EAEC (4)	−	−	−	−	+	−	−	−	−	−	−	+	−	−	+
aEPEC + EAEC (1)	−	−	−	+	+	−	−	−	−	−	−	+	−	−	+
aEPEC + EAEC (1)	−	−	−	+	+	−	−	−	−	−	+	−	−	−	+
aEPEC + EAEC (3)	−	−	−	−	+	−	−	−	−	−	+	−	−	−	+
aEPEC + EAEC (1)	−	−	−	−	+	+	−	−	−	−	+	−	−	−	−

Positive (+) and negative (−) polymerase chain reaction findings are indicated.
[a]Number of the samples with the indicated virulence gene profile.
aEPEC = atypical enteropathogenic *E. coli*; EAEC, enteroaggregative *E. coli*; EIEC, enteroinvasive *E. coli*; EPEC, enteropathogenic *E. coli*; ETEC, enterotoxigenic *E. coli*; STEC, Shiga toxin–producing *E. coli* (also called enterohemorrhagic *E. coli*).

at the popular open markets where the slaughter of chickens often takes place (Kagambega *et al*., 2011). The study conducted in Accra, Ghana, on chicken carcasses purchased from supermarkets detected a lower proportion (7%) of *Salmonella* (Sackey *et al*., 2000).

In the present study, *Salmonella* Derby was the predominant serotype, as it was also in our previous study (Kagambega *et al*., 2011). Other serotypes that we found previously during the dry season (that is, *Salmonella* Agona and *Salmonella* Tilene) were not common this time. In other Western African countries several different serotypes have been found to be most common in chicken-related samples: in Gambia, *Salmonella* Poona (Dione *et al*., 2011); in Senegal, *Salmonella* Brancaster (Dione *et al*., 2009); in Nigeria, *Salmonella* Virchow and *Salmonella* Hiduddify (Raufu *et al*., 2009; Fashae *et al*., 2010); and in Cameroon, *Salmonella* Enteritidis (Nzouankeu *et al*., 2010; Wouafo *et al*., 2010). These data indicate that there is no specific *Salmonella* serotype typical for chicken. Isolation of *Salmonella* Typhi in our study was of particular significance because this serotype is of human origin and is responsible for typhoid fever, which is still of major concern in developing countries (Kariuki, 2008). *Salmonella* Typhi can be transmitted by the fecal-oral route through contaminated food or water, but its only reservoir is humans. Thus, the detection of *Salmonella* Typhi in a chicken carcass can probably be explained by the poor hygienic practices of the seller.

Salmonella Derby strains isolated in the present study were mostly susceptible to the tested antimicrobials; only five strains were resistant—four to tetracycline and one to streptomycin. None of the strains was resistant to fluoroquinolones. The study conducted in Nigeria found fluoroquinolone-resistant *Salmonella* Derby strains and concluded that they may constitute a public concern because of the presence of the fluoroquinolone-mediating *qnr* genes (Fashae *et al*., 2010). The location of the *qnr* genes on mobile genetic elements coupled with the indiscriminate use of antimicrobials facilitates selection and the potential spread of resistance genes to other serotypes (Fashae *et al*., 2010).

The virulence genes that indicate the presence of EPEC were the most prevalent among the chicken carcasses. The EPEC pathogroup usually causes diarrhea in infants, and its prevalence was 16% among diarrheagenic children under 5 years old in Burkina Faso (Bonkoungou *et al*., 2011). The proportion of EPEC in this study (39%) and in our previous study during the dry season (29%) (Kagambega *et al*., 2012) was higher than that observed in Cameroon, where 11% of the chickens were contaminated by EPEC (Nzounkeu *et al*., 2010). In the United States, a very low proportion (1%) of atypical EPEC in chicken breasts was reported (Xia *et al*., 2010). Only virulence genes carried by atypical EPEC were detected in this study. Atypical EPEC appears to be more closely related to STEC and as such is considered as an emerging pathogen (Trabulsi *et al*., 2002).

STEC virulence genes were detected in this study in 6% of the chicken carcasses, whereas in our previous study during the dry season, we did not detect any STEC virulence genes among the 30 chicken carcasses studied (Kagambega *et al*., 2012). The Shiga toxin–encoding genes were also absent from chicken carcasses studied in the United States (Zhao *et al*., 2001), but in Korea 7% prevalence was detected (Lee *et al*., 2009). We detected the gene profiles stx_1, *eaeA*, *esc*V and stx_2, *eaeA*, *esc*V in chicken carcasses. However, the *eaeA* gene detected may belong to either STEC or EPEC or both, but this can be confirmed only if the strains are isolated.

EAEC virulence genes were detected in 13% of the chicken carcasses examined, in contrast to the studies conducted in Korea (Lee *et al*., 2009) and in Burkina Faso during the dry season (Kagambega *et al*., 2012), where no EAEC was detected in chicken carcasses. In the present study, EAEC virulence genes were detected only in combination with those of EPEC or STEC from the same samples, probably because both pathogroups were present. However, this could also indicate the presence of a so-called hybrid strain that has gained virulence genes from another diarrheagenic *E. coli*, as was the case with the strain that carried genetic determinants of both STEC and EAEC and caused the recent large outbreak in Germany (Mellmann *et al*., 2011).

The results of our present and previous (Kagambega *et al*., 2011, 2012) studies suggest that the prevalences of *Salmonella* and diarrheagenic *E. coli* as well as the number of different serotypes and pathogroups present on retail chickens might be higher during the rainy season than during the dry season. Also, the study on the etiology of childhood diarrhea in Burkina Faso suggested that the infections caused by enteropathogenic bacteria were more common during the rainy season (I.J.O. Bonkoungou, personal communication). In any case, the contamination of chickens by *Salmonella* and diarrheagenic *E. coli* was found to be common in Burkina Faso, which raises a public health concern. Therefore, efforts should be made to educate producers, retailers, and consumers on the proper handling and cooking of chicken meat. Follow-up studies should be carried out to monitor the situation in the future.

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Disclosure Statement

No competing financial interests exist.

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