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Assessment of human exposure to 3rd generation cephalosporin resistant *E. coli* (CREC) through consumption of broiler meat in Belgium

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ABSTRACT

Acquired resistance of *Escherichia coli* to 3rd generation cephalosporin antimicrobials is a relevant issue in intensive broiler farming. In Belgium, about 35% of the *E. coli* strains isolated from live broilers are resistant to 3rd generation cephalosporins while over 60% of the broilers are found to be carrier of these 3rd generation cephalosporin resistant *E. coli* (CREC) after selective isolation. A model aimed at estimating the exposure of the consumer to CREC by consumption of broiler meat was elaborated. This model consists of different modules that simulate the farm to fork chain starting from primary production, over slaughter, processing and distribution to storage, preparation and consumption of broiler meat. Input data were obtained from the Belgian Food Safety agencies' annual monitoring plan and results from dedicated research programs or surveys. The outcome of the model using the available baseline data estimates that the probability of exposure to 1000 colony forming units (cfu) of CREC or more during consumption of a meal containing chicken meat is ca. 1.5%, the majority of exposure being caused by cross contamination in the kitchen. The proportion of CREC (within the total number of *E. coli*) at primary production and the overall contamination of broiler carcasses or broiler parts with *E. coli* are dominant factors in the consumer exposure to CREC. The risk of this exposure for human health cannot be estimated at this stage given a lack of understanding of the factors influencing the transfer of cephalosporin antimicrobial resistance genes from these *E. coli* to the human intestinal bacteria and data on the further consequences of the presence of CREC on human health.

1. Introduction

Escherichia coli is a well studied commensal Gram negative bacterium in the gastro-intestinal tract of both humans and animals. Some specific *E. coli* strains with acquired virulence properties are considered as facultative pathogens for humans and can cause infections ranging from a self-limiting infection or bowel disturbance to a live-threatening bacteriaemia (Kaper et al., 2004; Smet et al., 2010). Also in broilers most *E. coli* strains are of commensal nature. Nonetheless some avian pathogenic strains, which are different from the human pathogenic strains, exist. Those strains can cause a variety of disease, mainly linked to the respiratory system (Persoons et al., 2011).

During the last decade *E. coli* is increasingly associated with broad spectrum β-lactam resistance mainly through the production of extended spectrum β-lactamases (ESBLs) or AmpC β-lactamases (Smet et al., 2009). This resistance has been observed in strains originating from all animal species but is significantly higher in strains isolated from intensive broiler production around the world (Bortolaia et al., 2010; Diarrassouba et al., 2007; Kojima et al., 2009; Smet et al., 2008; Verloo et al., 2003). In Belgium, although 3rd generation cephalosporins are not licensed for treatment in broilers, on average 35% of *E. coli* strains isolated from live broilers were found to be resistant to 3rd generation cephalosporins after cloacal sampling and susceptibility testing of one randomly selected colony (Persoons et al., 2010). Moreover over 60% of these cloacal swabs were positive for 3rd generation cephalosporin resistant *E. coli* (CREC) after selective isolation (Smet et al., 2008). Furthermore, CREC are increasingly isolated from humans and are causing hospital-acquired and community-acquired infections, especially urinary tract infections (Bin et al., 2011; Peirano and Pitout, 2010; Pitout, 2010; Rodriguez-Villalobos et al., 2011). Infections due to CREC are associated with a delay in initiation of

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appropriate antibacterial therapy in human medicine, which is often accompanied with increased morbidity and mortality (Pitout, 2010).

CREC can be transferred from broiler to humans, not only through direct contact but also indirectly. This indirect transfer involves mainly consumption of broiler meat or contact with surface water or vegetables contaminated with broiler excreta (Blake et al., 2003; van den Bogaard and Stobberingh, 1999). Because genes coding for ESBL's frequently are located on mobile genetic elements (Smet et al., 2009; Thomson and Moland, 2000) it is possible that there is a transfer from food-borne CREC to other commensal and pathogenic bacteria in the human intestinal tract as has recently been shown in an in vitro human gut simulation model (Smet et al., 2010). Furthermore it has been demonstrated that *E. coli* isolated form poultry, poultry meat and human clinical cases partly share the same ESBL genes, plasmids and strains. This is an additional indication of the transfer of CREC from broiler to humans (Leverstein-van Hall et al., 2011).

Given the abundant presence of CREC in broilers, the indication of common resistance genes in broilers and humans and the fact that cephalosporins are considered to be antimicrobials of critical importance for human medicine (WHO, 2007), the aim of this study was to estimate the exposure of humans to CREC through the consumption of broiler meat.

2. Materials and methods

2.1. Hazard identification

Resistance to extended-spectrum β-lactams has been associated with the production of broad spectrum β-lactamases such as extended spectrum β-lactamases (ESBL's), AmpC β-lactamases and metallo β-lactamases (Smet et al., 2009). In this study we looked at resistance via production of ESBL's and plasmid-mediated AmpC β-lactamases, described as 3rd generation cephalosporin resistance, and did not further subdivide into the different types of β-lactamases. In Belgium it has been described that 3rd generation cephalosporin resistance in *E. coli* from broilers is induced for 45% by ESBL's, 43% by AmpC β-lactamases and 12% by the combination of ESBL and AmpC β-lactamases (Smet et al., 2008).

2.2. Description of the model

The design of the farm to fork model was partially based on prior risk assessment models on *Salmonella* and *Campylobacter* (Bollaerts et al., 2009; Hartnett et al., 2001; Uyttendaele et al., 2006). The model was subdivided into three modules (Fig. 1) covering all relevant steps from the primary production up to the consumption of broiler meat and possibly contaminated vegetables. The model was mainly based on available, preferably validated data, found in literature or derived from the Belgian Food Safety agencies' annual monitoring plan or from dedicated national research programs or surveys. When no specific data was evaluable, assumptions had to be used. All assumptions were validated by an expert panel and found acceptable. The expert panel was composed by members of the Scientific Committee of the Federal Agency for the Safety of the Food Chain (FASFC) and several Belgian research institutions (Veterinary and Agrochemical Research Centre (CODA-CERVA), Ghent University, Scientific Institute of Public Health (IPH), Institute of Agricultural and Fisheries Research (ILVO)).

In the first module the production chain of broiler meat, including primary production, slaughter process and distribution was described. In the primary production the presence of CREC in healthy broilers is based on the data described by Persoons et al. (2010). These data were collected during 2007 and 2008 on 32 randomly selected broiler farms. Each farm was sampled twice in two non-consecutive production rounds (3–4 months interval) and at each sampling occasion 30 randomly selected birds were sampled by means of an individual cloacal swab. Antimicrobial susceptibility testing was performed on 1 single colony per broiler with the Kirby-Bauer disk diffusion method for 14 antimicrobial drugs amongst one was ceftiofur. In this study, over the 2 sampling rounds on average 35% of the isolated *E. coli* strains were found being resistant to 3rd generation cephalosporins. The full sampling was spread over more than 12 months and therefore covered potential seasonal effects.

Data on the number of *E. coli* (in cfu/g) present on broiler carcasses (neck skin) and broiler parts (homogenized skin and/or meat) are based on samples taken by the Belgian Federal Agency for the Safety of the Food Chain (FASFC) collected during their annual monitoring program. In analogy with the data from primary production data for the years 2007 and 2008 were used.

Persoons et al. (2010) described a higher proportion of CREC isolates on neck skins in the slaughterhouse compared with samples from the living birds on farm. The average proportion of CREC increased from 35.13% in the live birds on farm to 43.61% in the slaughterhouse (raw data were not published). The data of the study of Persoons et al. (2010) together with the *E. coli* count on carcasses (FASFC monitoring) were used to estimate the number of CREC present on a chilled broiler carcass after slaughter, or on broiler parts.

When considering processing and distribution of the chicken meat, different pathways were possible: the carcasses can be marketed either as a complete carcass or as broiler parts (including minced meat) and the broiler meat can be sold either deeply frozen or freshly chilled. According to Halet et al. (2006) on average 68.1% of broiler meat in Belgium is purchased as broiler parts and 31.9% as a complete carcass. On average 20% of broiler parts are purchased frozen, whereas for complete carcasses this is 5% (Halet et al., 2006).

The effect of freezing on CREC is considered in module 2, since it is essentially the same process whether it is done during production or at home. Therefore the freezing effect was only taken into account at one stage to avoid double counting the effect (Fig. 1). Furthermore it was assumed that during processing and distribution in the retail the cold chain is largely respected and temperature abuse is limited to <10 °C. It was also assumed that no thawing occurred for frozen broiler meat. It is generally accepted that the minimum temperature for growth of *E. coli* is 8 °C and at 8–10 °C, growth only occurs after a long lag phase (>10 days) which was confirmed with the Combase® predictor software. Therefore it was also assumed that there is no growth of (3rd generation cephalosporin resistant) *E. coli* during processing and distribution. Moreover it was assumed that the growth characteristics of CREC are equal to cephalosporin susceptible *E. coli*. How these different steps were implemented into the model can be seen in Table 1.

In the second module transport of the meat from the retail to the consumer and storage and preparation by the consumer were modeled. During transport of the broiler meat from retail to the kitchen or refrigerator/freezer of the consumer, cooling may be insufficient and temperature may increase above 10 °C allowing growth of CREC on the broiler meat. The potential growth of CREC on broiler meat was simulated based on the model described by Bollaerts et al. (2009) taking into account the temperature of the meat in the retail, the outside temperature, the duration of the transport and the salt (NaCl) concentration of the meat. For the same reasons as described higher it was assumed that growth of CREC only occurred if the meat temperature rises above 10 °C. Again, it was assumed that the growth characteristics of CREC are equal to 3rd generation cephalosporin susceptible *E. coli*. For meat bought frozen it was assumed that no thawing occurred during transport and by consequence no growth is possible.

At the consumers' home, broiler meat can be stored either refrigerated or frozen. Based on the results of the Belgian food consumption survey (De Vriese et al., 2005) and the study of Halet et al. (2006), it is assumed that on average 39% of broiler meat is kept frozen. The latter includes fresh broiler meat frozen at home, as well as broiler meat bought already frozen. It was also assumed here that the storage of fresh broiler meat always occurs below 10 °C (with a shelf life of up

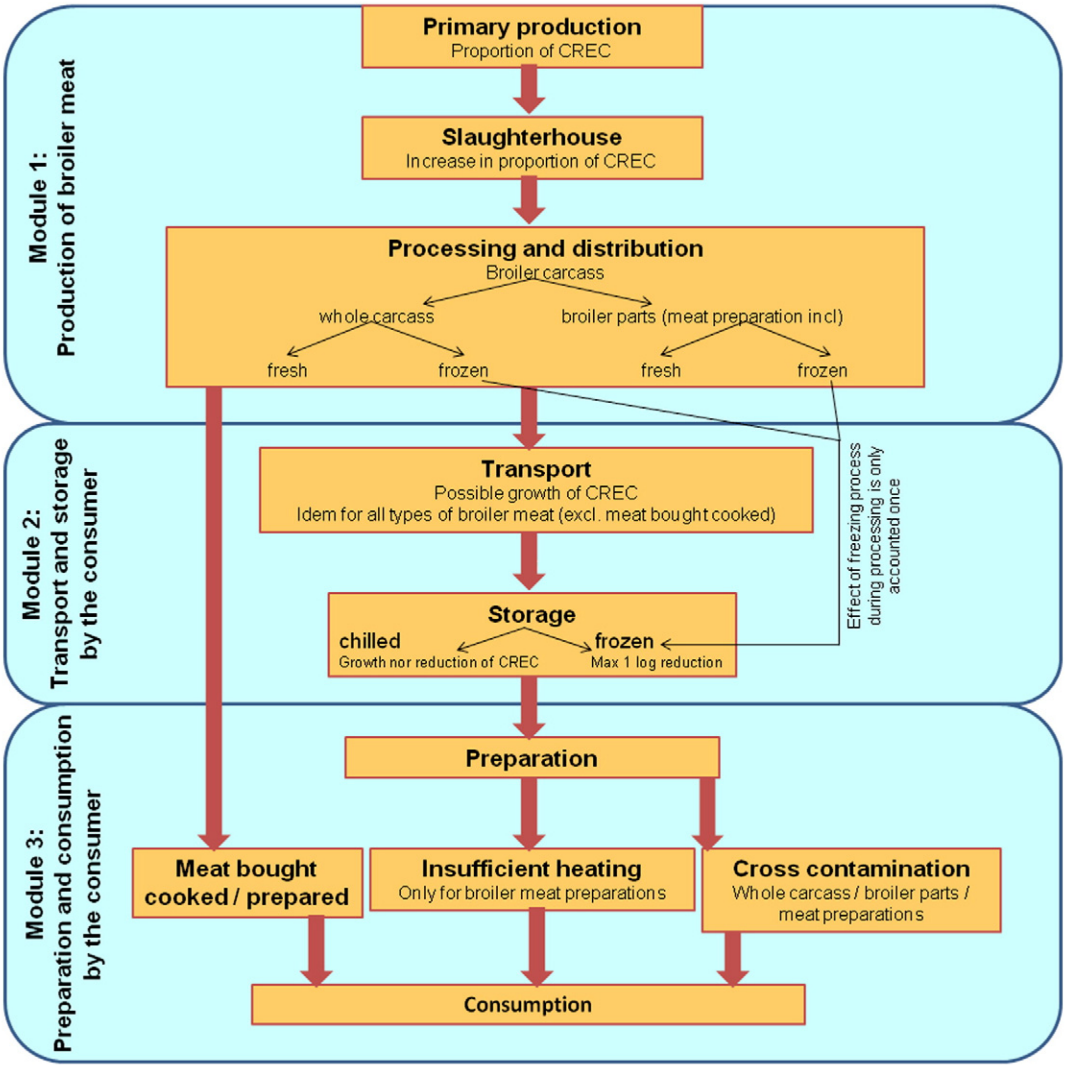

Fig. 1. Flowchart of the model.

to 5 days) so that no growth would occur. For frozen broiler meat, it was simulated that the reduction in the number of CREC was maximum 1 log after a storage of 30 days (Black et al., 2010). The duration of frozen storage of broiler meat is simulated based on the model described by Halet et al. (2006). How these different steps were implemented into the model can be seen in Table 2.

In the third module the preparation and consumption of broiler meat was modeled taking into account several possible sources of cross-contamination. The routes of transfer of bacteria from broiler meat to vegetables during preparation in the kitchen by either manipulation by hands or contact with cutting boards was simulated based on the model described by Bollaerts et al. (2009). The inputs for this module were (1) food-handling behavior and (2) transfers probabilities of (3rd generation cephalosporin resistant) *E. coli* on the chicken meat to the cook's hands and cutting boards. Regarding food-handling behavior data related to the hygienic work practices in the kitchen of the Belgian consumer was used (Viaene et al., 2007; De Vriese et al., 2005). For describing the transfer probabilities of CREC, the model described by Bollaerts et al. (2009) for *Salmonella*, assuming that *Salmonella* has similar attaching characteristics as *E. coli*, was applied (Table 3).

In the chicken to hand to vegetable route the following aspects were taken into account: the proportion of CREC being transferred from broiler meat to hands when handling broiler or broiler parts (this includes the probability to touch broiler by hand); the probability that hands are washed after manipulating raw broiler meat and its effect on the number of CREC which are present on the hands; the probability and proportion of CREC being transferred from hands to raw vegetables and finally the number of CREC that are present in a certain portion of raw vegetables. The number of CREC ending up on the raw vegetables is a function of the amount of meat manipulated (portion size) and the concentration of CREC on the chicken meat at the beginning of the preparation.

The chicken to board to vegetables route of transfer takes into account the following factors: probability that both raw broiler and raw vegetables are cut on the same cutting board; proportion of CREC transferred from raw broiler meat to the cutting board; probability to wash or turn the cutting board after cutting broiler meat; proportion of CREC transferred from the cutting board to the raw vegetables; hence the number of CREC transferred onto the cutting board and, ultimately the number ending up on the raw vegetables. Also here, the number of CREC on the vegetables depends on the amount of meat manipulated (portion size) and the concentration of CREC on the chicken meat at the beginning of the preparation.

Since no data were found on the frequency of joint consumption of broiler meat and raw vegetables, it was assumed in the model as a worst case scenario that every meal with broiler meat is consumed with raw vegetables, and that these vegetables are fully consumed. On the other hand we assumed that cross contamination only occurred in 50% of the cases.

Table 1
Detailed summary of Module 1 (see Fig. 1) of the exposure model: variables, distribution of the input parameters, equations and main sources.

Process	Variable	Description	Distribution	Reference
Primary production	$P_{CREC\ prim}$	Proportion of CREC[a] isolates within the total number of *E. coli* isolates	~bpert (0.015,0.288,0.915)	Persoons et al. (2010)
Slaughterhouse	$INC_{P\ CREC\ slaught}$	Change in proportion of CREC isolates in the slaughterhouse	~bpert (−0.033,0.032,0.36)	Persoons et al. (2010)
	$N_{E.\ coli\ whole}$	Total number of *E. coli* on chilled carcasses (log cfu[b]/g meat)	~bpert (0.823,2.386,4.149)	FASFC[c] monitoring
	$N_{E.\ coli\ part}$	Total number of *E. coli* on chilled broiler parts[d] (log cfu/g meat)	~bpert (0.986,2.321,3.410)	FASFC monitoring
	$N_{CREC\ whole}$	Calculation of the total number of CREC on chilled carcasses (cfu/g meat)	$= POWER(10,N_{E.\ coli\ whole}) x (P_{CREC\ prim} x (1+INC_{P\ CREC\ slaught}))$	
	$N_{CREC\ part}$	Calculation of the total number of CREC on chilled broiler parts[d] (cfu/g meat)	$= POWER(10,N_{E.\ coli\ port}) x (P_{CREC\ prim} x (1+INC_{P\ CREC\ slaught}))$	
Processing and distribution	P_{whole}	Proportion of chicken meat bought as broiler carcass	~binomial (1,0.319)	Halet et al. (2006)
	P_{part}	Proportion of chicken meat bought as broiler parts[d]	$= 1 - P_{whole}$	Halet et al. (2006)
	$P_{fresh\ whole}$	Proportion of carcasses bought chilled	~binomial(1,0.95)	Halet et al. (2006)
	$P_{frozen\ whole}$	Proportion of carcasses bought frozen	$= 1 - P_{fresh\ whole}$	Halet et al. (2006)
	$P_{fresh\ part}$	Proportion of broiler parts bought chilled[d]	~binomial(1,0.8)	Halet et al. (2006)
	$P_{frozen\ part}$	Proportion of broiler parts frozen[d]	$= 1 - P_{fresh\ part}$	Halet et al. (2006)

[a] 3rd generation cephalosporin resistant *E. coli*.
[b] Colony forming unit.
[c] Federal Agency for the Safety of the Food Chain (Belgium).
[d] Including minced meat and meat preparations.

Consumption of raw broiler meat was not considered in the model. Since it can be assumed that for broiler carcasses and parts the contamination with CREC is exclusively located onto the surface of the meat, it was also assumed that all CREC on this meat are inactivated during cooking. Therefore, the model assumed all cooked carcasses or parts being free of viable CREC. In case of minced broiler meat preparations (e.g. minced meat, burger or sausage, etc.), the CREC can also be found in the center of the meat (protected area). In this case, the core temperature and the time during which the protected area is exposed to that temperature are critical for the survival of any bacteria. According to the Belgian food consumption survey (De Vriese et al., 2005) 9% of the broiler meat is consumed as minced broiler meat preparations. The number of CREC that are located in the protected area was simulated using a uniform proportional distribution of the bacteria in the meat. Both for the time during which the protected area is heated at a certain temperature as for the temperature reached in the protected area, two arbitrarily chosen distributions were assumed based on expert opinion and the ACMSF report on the safe cooking of burgers. The inactivation of the CREC in the protected area was calculated using the formula described by Hill et al. (2003), where heating at 65 °C during 1 min corresponds to a reduction of 3.31 log. Although this formula described by Hill et al. (2003) was developed for *Salmonella* it closely followed the data on thermal inactivation of *E. coli* O157:H7 in burgers as described in the ACMSF report. Therefore this formula was maintained in our model. The probability of broiler meat being undercooked was based on data from a study by Worsfold and Griffith (1997), where the probability of undercooking of meat was described. It has to be noted that this study considered also other meat than chicken meat.

According to the Belgian food consumption survey (De Vriese et al., 2005) 44.4% of the complete broiler carcasses purchased, is bought readily cooked. For these carcasses it was also assumed that all CREC are already inactivated at the moment of purchase and no further cross contamination will occur.

Finally the total exposure of a consumer to CREC present in one meal containing broiler meat was estimated. Therefore a distribution of the portion sizes of consumed broiler meat was elaborated. Based on the data from the Belgian food consumption survey (De Vriese et al., 2005) these portion sizes differ between 20 g and 550 g.

The model was built using @Risk® 4.5 software (Palisade Corporation®). To assess the consumer exposure to CREC through consumption of broiler meat the model was run at 10,000 iterations per simulation and for 4 arbitrarily chosen exposure doses (10, 100, 1000 and 10,000 cfu/meal) since there are no indications on the number of CREC that should be ingested to realize colonization of the human intestine and/or transfer of resistance genes to other bacteria.

After the general exposure assessment different what-if scenarios with regard to the type of chicken meat, type of storage and exposure route were elaborated to identify the important contamination routes and the main influencing factors on the exposure rate. Furthermore the role of primary production and slaughterhouse hygiene on the consumer exposure to CREC was assessed using these what-if scenarios.

3. Results

3.1. Exposure assessment to CREC via consumption of broiler meat in Belgium

Table 4 gives an overview of the average concentration (cfu/g) of CREC on/in the chicken meat during the different stages of the model. It clearly shows that the concentration of CREC after slaughter and retail is relatively high and that whole carcasses are more contaminated than broiler parts. Temperature abuse during transport by the consumer gives a slight increase in the average concentration of CREC, whereas frozen storage results in a strong decrease of the average CREC concentration. Finally, after cooking of meat preparations the average concentration of CREC is near to 0. Due to assumptions made in the design of the model all CREC are inactivated after cooking of whole carcasses and chicken parts other then meat preparations.

Table 5 gives an overview of the exposure to CREC through consumption of broiler meat for the 4 arbitrarily chosen exposure doses of CREC. The results show that the probability to be exposed to more than 10, 100, 1000 or 10,000 cfu of CREC by a serving with broiler meat is 7.0%, 3.3%, 1.5% and 0.39% respectively. Furthermore it is demonstrated that the majority of exposure is caused by cross contamination and that insufficient heating, only considered relevant for broiler meat preparations (minced meat), plays a minor role.

3.2. Impact of variables in the farm to fork chain on exposure to CREC via consumption of broiler meat (what-if scenarios)

Via what-if scenario's the effect and relative contribution of the type of broiler meat, type of storage and the exposure route were investigated (Table 6). These scenarios show that consumption of frozen broiler meat

Table 2
Detailed summary of Module 2 (see Fig. 1) of the exposure model: variables, distribution of the input parameters, equations and main sources.

Process	Variable	Description	Distribution/equation	Reference
Transport by consumer	T_{retail}	Temperature of the meat in retail (°C)	~N(3.14,7.78) truncated between −2 °C and 15 °C	Bollaerts et al. (2009)
	T_{ext}	Outdoor temperature (°C)	$\sim f=\pi\, f_1+(1-\pi)\, f_2$ with $\pi=0.64/f_1\sim N(6.7,17.9)/f_2\sim N(20.1,33.0)$ truncated between −15 °C and 35 °C	RMI[a]
	$\Delta_{max}T$	Maximal possible change in temperature (°C)	$= T_{ext}-T_{retail}$	Assumption Hill et al. (2003)
	S_T	Correction: maximal change larger than 0 (no = 0, yes = 1)	If $\Delta_{max}T>0$ than $S_T=1$ If $\Delta_{max}T\leq 0$ than $S_T=0$	
	ΔT	Change in temperature of meat during transport (°C)	~N(3.72,2.82)* x S_T *truncated between 0 °C and $\Delta_{max}T$	Hill et al. (2003)
	T_{end}	Temperature of meat at the end of transport (°C)	$= T_{retail}+\Delta T$	
	$Time_{trans}$	Duration of transport (in 15 min)	~discrete(v,w) with v = [1,2,3,4,5,6,7,8,16] and w = [0.005,0.05,0.18,0.25,…, 0.22,0.16,0.07, 0.03,0.035]	Hill et al. (2003)
	NaCl	Salt concentration of broiler meat (%)	~N(0.978,0.158)	VLAV[b]
	ΔTr_{temp}	Growth of *E. coli* in function of temperature and salt concentration (log cfu/h)	$= EXP(-6.26-(0.011\ x NaCl)+(0.32\ x T_{end})+(0.002\ x NaCl\ x\ T_{end})-(0.0085\ x POWER(T_{end},2))-(0.0045\ x POWER\ (NaCl,2)))$	Bollaerts et al. (2009)
	ΔTr	Total log growth of *E. coli* during transport time (log cfu/transport)	$= (\Delta Tr_{temp}/4)-Time_{trans}$	Bollaerts et al. (2009)
	$N_{CREC\ trans\ whole}$	Number of CREC[c] on broiler carcasses after transport[d] (log cfu/g meat)	$= (LOG10(N_{CREC\ whole}))+\Delta Tr$	
	$N_{CREC\ trans\ part}$	Number of CREC on broiler parts[e] after transport[d] (log cfu/g meat)	$= (LOG10(N_{CREC\ part}))+\Delta Tr$	
Storage by consumer	$N_{CREC\ stor\ whole}$	Growth nor reduction of CREC on broiler carcasses during refrigerated storage (log cfu/g meat)	$= N_{CREC\ trans\ whole}$	Assumption/Combase® predictor software
	$N_{CREC\ stor\ part}$	Growth nor reduction of CREC on broiler parts[e] during refrigerated storage (log cfu/g meat)	$= N_{CREC\ trans\ part}$	Assumption/Combase® predictor software
	π_{frozen}	Probability that meat is frozen[f]	~binomial (1,0.39)	Belgian food consumption survey (De Vriese et al., 2005)/ Halet et al. (2006)
	$N_{\ days\ frozen}$	Duration of frozen storage of broiler meat by the consumer (days)	~general(0,43,{0.04,0.5,1,2,7,8,42}, {0,1,2,8,80,127.5,127.5}) truncated on 30 days (Black et al., 2010)	Halet et al. (2006)
	$\Delta_{CREC\ frozen}$	Reduction of CREC during frozen storage (log cfu/g meat)	~bpert(0.5,0.75,1)	Black et al. (2010)
	$N_{CREC\ frozen\ stor\ whole}$	Number of remaining CREC of frozen storage of broiler carcass (log cfu/g meat)	$= LOG\ N_{CREC\ trans\ whole}-(\Delta_{CREC\ frozen}\, x\ N_{\ days\ frozen}/30)$	
	$N_{CREC\ frozen\ stor\ part}$	Number of remaining CREC of frozen storage of broiler parts[e] (log cfu/g meat)	$= LOG\ N_{CREC\ trans\ part}-(\Delta_{CREC\ frozen}\, x\ N_{\ days\ frozen}/30)$	

[a] Royal Belgian Meteorological Institute.
[b] Vlaams adviescentrum voor de vleesindustrie.
[c] 3rd generation cephalosporin resistant *E. coli*.
[d] Including frozen meat.
[e] Including minced meat and meat preparations.
[f] Both meat bought frozen as well as meat frozen by the consumer.

reduces the risk of exposure to CREC. Broiler meat preparations (minced meat) are the type of broiler meat with the highest contribution to exposure to CREC upon consumption which is due to the assumption that insufficient heating can play a role because the CREC may also be located in the center of the meat. Nevertheless it is demonstrated that also for broiler meat preparations cross contamination is the dominant route contributing to exposure.

Because of the high prevalence of CREC amongst Belgian broilers we investigated the impact of this prevalence on the consumer exposure to CREC. From this what-if scenario it could be clearly derived

Table 3
Detailed summary of Module 3 (see Fig. 1) of the exposure model: variables, distribution of the input parameters, equations and main sources.

Process	Variable	Description	Distribution/equation	Reference
General	Serving Size	Serving size when consumption of broiler meat (g)	~cumul(20,550,{20,…,500}, {0.028,…,0.999})	Belgian food consumption survey (De Vriese et al., 2005)
	$\pi_{\text{cross cont}}$	Probability of cross contamination	~binomial(1,f) With f~uniform(0.4,0.6)	Assumption
Cross contamination through manipulation	(tC,H)	Proportion of CREC[a] transferred from broiler meat to hands	~beta(1.78,41.1)	Bollaerts et al. (2009)
	π_{WH}	Probability to wash or rinse hands after touching raw broiler meat and before touching raw vegetables	~binomial(1,f) with f~discrete({0.862,0.8174}, {0.8649,0.1351})	Belgian food consumption survey (De Vriese et al., 2005)/ Viaene et al. (2007)
	(tH,H)	Proportion of CREC removed by rinsing and washing of hands	If $\pi_{\text{WH}}=0$ than (tH,H) = 1 If $\pi_{\text{WH}}=1$ than (tH,H)~ beta(0.24,6.67)	Bollaerts et al. (2009)
	(tH,S)	Proportion of CREC transferred from hands to raw vegetables	~beta(0.6,2.3)	Bollaerts et al. (2009)
	$N_{\text{CREC veg hands}}$	Number of CREC transferred to raw vegetables through manipulation for the different types of broiler meat[b] (cfu/portion)	= (POWER(10,$N_{\text{CREC stor whole}}$) x (tC,H) x (tH,H) x (tH,S) x Serving Size x $\pi_{\text{cross cont}}$ = (POWER(10,$N_{\text{CREC stor part}}$) x (tC,H) x (tH,H) x (tH,S) x Serving Size x $\pi_{\text{cross cont}}$ = (POWER(10,$N_{\text{CREC frozen stor whole}}$) x (tC,H) x (tH,H) x (tH,S) x Serving Size x $\pi_{\text{cross cont}}$ = (POWER(10,$N_{\text{CREC frozen stor part}}$) x (tC,H) x (tH,H) x (tH,S) x Serving Size x $\pi_{\text{cross cont}}$	
Cross contamination through cutting board	(tC,B)	Proportion of CREC transferred from raw broiler meat to cutting board	= POWER(10,f)/100 With f~N(0.171,0.16)	Bollaerts et al. (2009)
	$N_{\text{CREC board}}$	Number of CREC transferred to the cutting board for the different types of broiler meat[b] (cfu/cutting board)	= ((1 − (tC,H)) x POWER (10,$N_{\text{CREC stor whole}}$) x (tC,B) x Serving Size = ((1 − (tC,H)) x POWER (10,$N_{\text{CREC stor part}}$) x (tC,B) x Serving Size = ((1 − (tC,H)) x POWER(10,$N_{\text{CREC frozen stor whole}}$) x (tC,B) x Serving Size = ((1 − (tC,H)) x POWER (10,$N_{\text{CREC frozen stor part}}$) x (tC,B) x Serving Size	
	$\pi_{\text{board handling}}$	After cutting the raw broiler meat the cutting board can have 3 different treatments for which the probability is given	Same cutting board, not washed~ discrete({0.031,0.2429}, {0.8748,0.1252}) Same cutting board, washed or other side~discrete({0.022,0.1937}, {0.8748,0.1252}) Different cutting board~discrete ({0.947,0.5634},{0.8748,0.1252})	Belgian food consumption survey (De Vriese et al., 2005)/ Viaene et al. (2007)
	$N_{\text{CREC board wash}}$	Number of CREC on cutting board for the 3 different treatments (cfu/cutting board)	Same cutting board, not washed: = $N_{\text{CREC board}}$ x 1 Same cutting board, washed or other side: = $N_{\text{CREC board}}$ − f With f~bpert(1,4.5,7)) Different cutting board: = $N_{\text{CREC board}}$ x 0	
	(tB,S)	Proportion of CREC transferred from cutting board to raw vegetables	POWER(10,f)/100 With f~N(1.535,0.32)	Bollaerts et al. (2009)
	$N_{\text{CREC veg board}}$	Number of CREC on raw vegetables through contact with cutting board (cfu/portion)	= $N_{\text{CREC board wash}}$ x (tB,S) x $\pi_{\text{cross cont}}$	
Cross contamination (total)	$N_{\text{CREC veg}}$	Total number of CREC on raw vegetables through cross contamination (cfu/portion)	= $N_{\text{CREC veg hands}}$ + $N_{\text{CREC veg board}}$	

(continued on next page)

Table 3 (*continued*)

Process	Variable	Description	Distribution/equation	Reference
Heating of minced broiler meat and meat preparations	$\pi_{\text{minced meat}}$	Probability of consuming minced meat and meat preparations	~binomial(1,0.09)	Belgian food consumption survey (De Vriese et al., 2005)
	$\pi_{\text{undercooking}}$	Probability of unsufficient heating	~bpert(0.05,0.1,0.2)	Worsfold and Griffith (1997)
	$\text{Temp}_{\text{cooking}}$	Temperature of protected zone (°C)	~bpert(60,65,70)	Assumption/expert opinion/ ACMSF report
	T_{cooking}	Time of exposure of protected zone to heating (min)	~bpert(0.5,1,1.5)	Assumption/expert opinion/ACMSF report
	$P_{\text{CREC protected}}$	Proportion of CREC in protected zone	~uniform(0,0.1)	Assumption
	$N_{\text{CREC protected}}$	Number of CREC in protected zone for fresh and frozen meat (log cfu/g meat)	$= N_{\text{CREC stor part}} \text{ x } P_{\text{CREC protected}}$ $= N_{\text{CREC frozen stor part}} \text{ x } P_{\text{CREC protected}}$	
	$N_{\text{CREC cooking}}$	Number of CREC remaining after heating (log cfu/g meat)	$= \text{POWER}(10,(N_{\text{CREC protected}} - (T_{\text{cooking}}/\text{POWER}(10,(-0.14 \text{ x} \text{Temp}_{\text{cooking}}) + 8.58)))) \text{ x } \pi_{\text{undercooking}}$	Hill et al. (2003)
	$N_{\text{CREC minced}}$	Number of CREC remaining after heating (log cfu/portion)	$N_{\text{CREC cooking}} \text{ x }$ Serving size	
Exposure during consumption	$S_{\text{broiler meat}}$	Proportion of type of broiler meat that is bought	Meat preparations = 0.09 Broiler meat bought cooked = 0.14 Broiler meat bought raw = 0.77	Belgian food consumption survey (De Vriese et al., 2005)
	$N_{\text{ CREC meat}}$	Exposure to CREC through consumption of broiler meat (log cfu/portion)	- for meat preparations = $N_{\text{CREC minced}}$ - for broiler meat bought cooked = 0 - for broiler meat other than meat preparations = 0	/
	$N_{\text{CREC cross cont}}$	Exposure to CREC through cross contamination[c] (log cfu/portion)	= log $N_{\text{CREC veg}}$	
	$N_{\text{CREC meal}}$	Total exposure to CREC during consumption of a meal containing broiler meat (log cfu/meal)	$= N_{\text{CREC meat}} + N_{\text{CREC cross cont}}$	

[a] 3rd generation cephalosporin resistant *E. coli*.
[b] Respectively chilled broiler carcasses, chilled broiler parts, frozen broiler carcasses and frozen broiler parts.
[c] Only for meat preparations and broiler meat bought raw.

that the proportion of CREC within the total number of *E. coli* present in live broilers at primary production, plays a significant role in the exposure rate (Table 7). Fixation of this proportion at a high level (0.75) clearly results in an increase of the probability of consumer exposure for the 4 arbitrarily chosen exposure doses whereas fixation of this proportion at a low level (0.1) results in a decrease.

Finally, the impact of hygiene practices during slaughter and processing of broiler meat is presented (Table 8). It is demonstrated that the overall contamination with *E. coli* of broiler meat after slaughter and processing is of major influence on the influx of CREC into the production and consumption chain and therefore also on the consumer exposure to CREC.

Table 4
Evolution of the average CREC concentration on/in the chicken meat (cfu/g) during the different stages of the model.

Process	Whole carcass	Broiler parts	Broiler meat preparations
Chicken meat in retail prior to transport by the consumer	256.1	117.4	117.4
Chicken meat after transport by the consumer	294.5	137.8	137.8
Chicken meat after chilled storage by the consumer	294.5	137.8	137.8
Chicken meat after frozen storage by the consumer	126.5	58.1	58.1
Chicken meat after cooking	0	0	0.7

4. Discussion

The model used in this study provides a quantitative probabilistic estimation of exposure describing the different steps in the production, processing and consumption of broiler meat and the possible influence of the different aspects on the consumer exposure to CREC.

The risk of exposure to CREC through the consumption of broiler meat for humans particularly lies in the transfer of resistance genes of *E. coli* to other, potentially pathogenic, bacteria during passage or colonization of the human intestinal tract (Leverstein-van Hall et al., 2011; Smet et al., 2010). If these resistance genes end up in pathogenic bacteria that might cause serious illness for which antimicrobial treatments are needed, the presence of cephalosporin resistance

Table 5
Origin of the exposure to cephalosporin resistant *E. coli* during the consumption of a meal containing broiler meat for 4 arbitrarily chosen exposure doses.

Exposure dose (cfu/meal)	10,000 cfu	1000 cfu	100 cfu	10 cfu
Exposure via insufficient heating [a]	0%	0%	0%	0.03%
Exposure via cross contamination [b]	0.39%	1.5%	3.3%	6.97%
Total exposure	0.39%	1.5%	3.3%	7.0%

[a] Only for broiler meat preparations (minced meat).
[b] For chilled or frozen broiler carcasses and parts (broiler meat preparations included).

Table 6
Elaboration of scenario-analysis for the probability of exposure to cephalosporin resistant *E. coli* in one meal containing broiler meat with regard to the type of meat, type of storage and exposure route for 4 arbitrarily chosen exposure doses.

Exposure dose (cfu/meal)		10,000 cfu	1000 cfu	100 cfu	10 cfu
Whole carcass	Only consumption of whole carcasses (all types)	0.63%	2.1%	4.3%	9.3%
	Only consumption of whole chilled carcasses	0.81%	2.1%	4.5%	9.8%
	Only consumption of whole frozen carcasses	0.32%	1.3%	3.4%	7.6%
Broiler parts	Only consumption of broiler parts (all types)	0.56%	1.8%	3.9%	8.0%
	Only consumption of chilled broiler parts	0.62%	1.8%	4.1%	8.3%
	Only consumption of frozen broiler parts	0.22%	1.1%	3.0%	6.3%
Broiler meat preparations	Only consumption of broiler meat preparations	1.6%	3.2%	5.7%	11.2%
	Exclusion of cross contamination	0.96%	1.3%	1.6%	2.5%
Total exposure for the entire model (Table 5)		0.39%	1.5%	3.3%	7.0%

will likely hamper the treatments with the conventionally used antimicrobials (Smet et al., 2010).

In modeling the exposure to CREC the farm to fork continuum has been modeled, partially making use of information and modules elaborated in prior risk assessment on *Salmonella* and *Campylobacter* (Bollaerts et al., 2009; Hartnett et al., 2001; Uyttendaele et al., 2006). We used specific input data on the prevalence of CREC (and generic *E. coli*) in broilers and broiler meat which were obtained from the Belgian Food Safety agencies' annual monitoring plan or available as results from dedicated national research programs or surveys. For some aspects for which no specific knowledge was present some assumptions were made i.e. identical growth and survival characteristics of cephalosporin resistant and generic *E. coli*; the absence of temperature abuse (>10 °C for several hours) during production, processing, distribution and display in the retail; the consistent consumption of vegetables with every serving of broiler meat. Some of these assumptions may have resulted in an overestimation of consumer exposure to CREC (e.g. the co-consumption of vegetables with broiler meat and thus ability for cross-contamination) whereas other might result in an underestimation (e.g. no temperature abuse). Further assembling data on these knowledge gaps may contribute to more accurate risk estimates.

The model shows that the majority of exposure is caused by cross contamination in the kitchen. This is partly the consequence of the structure of the model, because it is assumed that for broiler meat other than minced meat preparations the only exposure route is cross contamination. However, cross contamination is also the main route of exposure for minced broiler meat preparations and is more important than insufficient heating. This is in agreement with risk assessment for other food borne pathogens such as *Campylobacter* (Uyttendaele et al., 2006) and *Salmonella* (Uyttendaele et al., 2009). Thus, in accordance to the prevention of food born infections by zoonotic pathogens such as *Salmonella* and *Campylobacter*, also this exposure assessment of CREC via broiler meat consumption points out the importance to comply with good hygiene practices during preparation of broiler meat, as well by consumers as in professional catering or food service operations.

Table 7
Elaboration of scenario-analysis for the probability of exposure to 4 arbitrarily chosen doses of cephalosporin resistant *E. coli* with regard to the prevalence of cephalosporin resistant *E. coli* in primary production.

Exposure dose (cfu/meal)	10,000 cfu	1000 cfu	100 cfu	10 cfu
Proportion[a] of CREC[b] in primary production = 0.75	0.58%	1.9%	4.2%	9.3%
Proportion[a] of CREC[b] in primary production = 0.1	0.14%	0.78%	2.2%	4.8%
Total exposure for the entire model (Table 5) with average proportion of CREC in primary production = 0.36	0.39%	1.5%	3.3%	7.0%

[a] Within total number of *E. coli*.
[b] 3rd generation cephalosporin resistant *E. coli*.

Table 8
Elaboration of scenario-analysis for the probability of exposure to 4 arbitrarily chosen doses of cephalosporin resistant *E. coli* with regard to the overall contamination of broiler meat with *E. coli* after slaughter and processing for 4 arbitrarily chosen exposure doses.

Exposure dose (cfu/meal)	10,000 cfu	1000 cfu	100 cfu	10 cfu
Maximal contamination with *E. coli* of broiler carcasses and broiler parts: $N_{E.\ coli\ whole} = 4.15$ en $N_{E.\ coli\ part} = 3.41$ (Table 1)	1.9%	4.3%	8.9%	16.7%
Minimal contamination with *E. coli* of broiler carcasses and broiler parts: $N_{E.\ coli\ whole} = 0.82$ en $N_{E.\ coli\ part} = 0.99$ (Table 1)	0%	0.16%	1.1%	2.5%
Total exposure for the entire model (Table 5)	0.39%	1.5%	3.3%	7.0%

In the different what-if scenarios it is clearly shown that both the proportion of CREC (within the total number of *E. coli*) in primary production and especially the overall contamination of broiler carcasses or broiler parts with *E. coli* during the slaughtering process have a major impact on the consumer exposure to elevated numbers of CREC. This means that a sound antibiotic drug policy in primary production and respect of good hygiene practices in the slaughterhouse and cutting plant could reduce significantly the probability of exposure to CREC during consumption of broiler meat. This last statement is in accordance with a recent *Campylobacter* infection risk assessment which states that reducing the level of *Campylobacter* at carcass level is an efficient intervention method for reducing the risk of campylobacteriosis (EFSA, 2011).

As for the total exposure of humans to CREC, the model only describes one possible transmission route, namely the transfer of CREC from broilers to humans through consumption of broiler meat. It is clear that also meat from other origin (e.g. pork) might carry CREC and that direct contact between animals and humans can cause exchange of cephalosporin resistant bacteria. Moreover, animals as a reservoir of CREC can introduce the resistant bacteria in the environment (water, vegetation) and thus may be responsible for an indirect way of transfer of cephalosporin resistance to humans. Bonnet (2004) described the occurrence of ESBL resistance in *Kluyvera* spp. in the soil and Ruimy et al. (2009) found that 13% of the studied vegetables and fruits carried *Rahnella* spp. that showed ESBL resistance. Given that consumption of vegetables is not always accompanied by a preliminary heating step or other reduction step, this transmission route is also to be considered. Yet, it is currently impossible to determine the relative importance of these different transmission routes due to a lack of data on the presence of CREC in other reservoirs and food products than broiler and broiler meat.

The present model was restricted to an exposure assessment because current knowledge on risk characterization of the ingestion of CREC via food consumption and thus its consequences for human health is insufficient. The model estimates that the probability to be exposed to more than 1000 cfu of CREC by a serving with broiler meat is about 1.5%. It can be expected that especially people who are treated with cephalosporins are at risk because this treatment results in an increased population size of the cephalosporin resistant microbial population which facilitates the transfer of cephalosporin resistance genes as has been demonstrated *in vitro* by Smet et al. (2010). Although no data is available, it is likely that also other factors have an impact on the transfer of cephalosporin resistance genes in the human digestive tract.

Finally there are hardly any data on the prevalence of CREC in the intestinal flora of humans in Belgium. Moreover, the data that are available (Meex et al., 2008) only take clinical patients into account. This is again an important piece of missing data to quantify the impact of the exposure from animal origin on the presence of CREC in the human population.

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References

Advisory Committee on the Microbiological Safety of Food (ACMSF), a. Report on the safe cooking of burgers http://www.sofht.co.uk/downloads/acmsfburgers0807%5B1%5D.pdf.

Bin, L., Jing-Yong, S., Qing-Zhong, L., Li-Zhong, H., Xin-Hong, H., Yu-Xing, N., 2011. High prevalence of CTX-M β-lactamases in faecal *Escherichia coli* strains from healthy humans in Fuzhou, China. Scandinavian Journal of Infectious Diseases 43, 170–174.

Black, E.P., Hirneisen, K.A., Hoover, D.G., Kniel, K.E., 2010. Fate of *Escherichia coli* O157:H7 in ground beef following high-pressure processing and freezing. Journal of Applied Microbiology 108, 1352–1360.

Blake, D.P., Hillman, K., Fenlon, D.R., Low, J.C., 2003. Transfer of antibiotic resistance between commensal and pathogenic members of the Enterobacteriaceae under ideal conditions. Journal of Applied Microbiology 95, 428–436.

Bollaerts, K.E., Messens, W., Delhalle, L., Aerts, M., Van der Stede, Y., Dewulf, J., Quoilin, S., Maes, D., Mintiens, K., Grijspeerdt, K., 2009. Development of a quantitative microbial risk assessment for human Salmonellosis through household consumption of fresh minced pork meat in Belgium. Risk Analysis 29, 820–840.

Bonnet, R., 2004. Growing group of extended-spectrum beta-lactamases: the CTX-M enzymes. Antimicrobial Agents and Chemotherapy 48, 1–14.

Bortolaia, V., Guardabassi, L., Trevisani, M., Bisgaard, M., Venturi, L., Bojesen, A.M., 2010. High diversity of extended-spectrum beta-lactamases in *Escherichia coli* isolates from Italian broiler flocks. Antimicrobial Agents and Chemotherapy 54, 1623–1626.

Combase® predictor software, a. http://www.combase.cc.

De Vriese, S., Debacker, G., de Henauw, S., Huybrechts, I., Kornitzer, M., Leveque, A., Moreau, M., Van Oyen, H., 2005. The Belgian food consumption survey: aims, design and methods. Archives of Public Health 63, 1–16.

Diarrassouba, F., Diarra, M.S., Bach, S., Delaquis, P., Pritchard, J., Topp, E., Skura, B.J., 2007. Antibiotic resistance and virulence genes in commensal *Escherichia coli* and *Salmonella* isolates from commercial broiler farms. Journal of Food Protection 70, 1316–1327.

European Food Safety Authority (EFSA), 2011. Scientific opinion on *Campylobacter* in broiler meat production: control options and performance objectives and/or targets at different stages of the food chain. EFSA Journal 9, 2105–2246.

Halet, D., Viaene, J., Gellynck, X., 2006. Consumer behavior towards fresh poultry meat. Department Agricultural Economics Report 2006. .

Hartnett, E., Kelly, L., Newell, D., Wooldridge, M., Gettinby, G., 2001. A quantitative risk assessment for the occurrence of *Campylobacter* in broilers at the point of slaughter. Epidemiology and Infection 127, 195–206.

Hill, A.A., England, T.J., Snary, E.L., Kelly, L.A., Cook, A.J.C., Wooldridge, M., 2003. A "farm-to-consumption" risk assessment for the adverse effects to human health of *Salmonella* Typhimurium in pigs. Centre for Epidemiology and Risk Analysis, Veterinary Laboratories Agency, UK report. .

Kaper, J.B., Nataro, J.P., Mobley, H.L., 2004. Pathogenic *Escherichia coli*. Nature Reviews. Microbiology 2, 123–140.

Kojima, A., Asai, T., Ishihara, K., Morioka, A., Akimoto, K., Sugimoto, Y., Sato, T., Tamura, Y., Takahashi, T., 2009. National monitoring for antimicrobial resistance among indicator bacteria isolated from food-producing animals in Japan. Journal of Veterinary Medical Science 71, 1301–1308.

Leverstein-van Hall, M.A., Dierikx, C.M., Cohen Stuart, J., Voets, G.M., van den Munckhof, M.P., van Essen-Zandbergen, A., Platteel, T., Fluit, A.C., van de Sande-Bruinsma, N., Scharinga, J., Bonten, M.J.M., Mevius, D.J., 2011. Dutch patients, retail broiler meat and poultry share the same ESBL genes, plasmids and strains. Clinical Microbiology and Infection 17, 873–880.

Meex, C., Melin, P., Docquier, J.D., Kabasele, T., Huynen, P., Tulkens, P.M., Giet, D., De Mol, P., 2008. Presence of extended-spectrum beta-lactamase-producing Enterobacteriaceae in the fecal flora of patients from general practice. 18th ECCMID Congress Barcelona 19–22 April 2008 (Poster 631). http://www.facm.ucl.ac.be/posters/2008/ECCMID/Meex-et-al-ECCMID-2008-P631.pdf.

Peirano, G., Pitout, J.D.D., 2010. Molecular epidemiology of *Escherichia coli* producing CTX-M β-lactamases: the worldwide emergence of clone ST131 O25:H4. International Journal of Antimicrobial Agents 35, 316–321.

Persoons, D., Dewulf, J., Smet, A., Herman, L., Heyndrickx, M., Martel, A., Catry, B., Butaye, P., Haesebrouck, F., 2010. Prevalence and persistence of antimicrobial resistance in broiler indicator bacteria. Microbial Drug Resistance 16, 67–74.

Persoons, D., Callens, B., Dewulf, J., Haesebrouck, F., 2011. Een update van colibacillose bij kippen. Flemish Veterinary Journal 80, 161–166.

Pitout, J.D.D., 2010. Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae: changing epidemiology and drug treatment choices. Drugs 70, 313–333.

Rodriguez-Villalobos, H., Bogaerts, P., Berhin, C., Bauraing, C., Deplano, A., Montesinos, I., de Mendonça, R., Jans, B., Glupczynski, Y., 2011. Trends in production of extended-spectrum beta-lactamases among Enterobacteriaceae of clinical interest: results of a nationwide survey in Belgian hospitals. The Journal of Antimicrobial Chemotherapy 66, 34–47.

Ruimy, R., Brisabois, A., Bernede, C., Skurnik, D., Barnat, S., Arlet, G., Momcilovic, S., Elbaz, S., Moury, F., Vibet, M.A., Courvalin, P., Guillemot, D., Andremont, A., 2009. Organic and conventional fruits and vegetables contain counts of Gram-negative bacteria expressing resistance to antibacterial agents. Environmental Microbiology 12, 608–615.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Catry, B., Herman, L., Haesebrouck, F., Butaye, P., 2008. Diversity of extended-spectrum beta-lactamases and class C beta-lactamases among cloacal *Escherichia coli* isolates in Belgian broiler farms. Antimicrobial Agents and Chemotherapy 52, 1238–1243.

Smet, A., Martel, A., Persoons, D., Dewulf, J., Heyndrickx, M., Herman, L., Haesebrouck, F., Butaye, P., 2009. Broad-spectrum beta-lactamases among Enterobacteriaceae of animal origin: molecular aspects, mobility and impact on public health. FEMS Microbiology Reviews 34, 295–316.

Smet, A., Rasschaert, G., Martel, A., Persoons, D., Dewulf, J., Butaye, P., Catry, B., Haesebrouck, F., Herman, L., Heyndrickx, M., 2010. *In situ* ESBL conjugation from avian to human *Escherichia coli* during cefotaxime administration. Journal of Applied Microbiology 110, 541–549.

Thomson, K.S., Moland, E.S., 2000. Version 2000: the new β-lactamases of Gram-negative bacteria at the dawn of the new millennium. Microbes and Infection 2, 1225–1235.

Uyttendaele, M., Baert, K., Ghafir, Y., Daube, G., De Zutter, L., Herman, L., Dierick, K., Pierard, D., Dubois, J.J., Horion, B., Debevere, J., 2006. Quantitative risk assessment of *Campylobacter* spp. in poultry based meat preparations as one of the factors to support the development of risk-based microbiological criteria in Belgium. International Journal of Food Microbiology 111, 149–163.

Uyttendaele, M., Baert, K., Grijspeerdt, K., De Zutter, L., Horion, B., Devlieghere, F., Heyndrickx, M., Debevere, J., 2009. Comparing the effect of various contamination levels for *Salmonella* in chicken meat preparations on the probability of illness in Belgium. Journal of Food Protection 72, 2093–2105.

van den Bogaard, A.E., Stobberingh, E.E., 1999. Antibiotic usage in animals: impact on bacterial resistance and public health. Drugs 58, 589–607.

Verloo, D., Butaye, P., Dierick, K., Imberechts, H., 2003. Descriptive epidemiology of the resistance observed in *Escherichia coli* isolated from healthy cattle, pigs and broilers, their meat and meat products. Proceedings of the Flemish Society for Veterinary Epidemiology and Economics, Torhout, Belgium, p. 67 (December 11).

Viaene, J., Gellynck, X., Messens, W., 2007. The economics of reducing *Campylobacter* in the Belgian poultry meat chain. Biotechnology in Animal Husbandry 23, 155–167.

Vlaams Adviescentrum voor de Vleesindustrie (VLAV), 2011. Written answer to the question which is the average salt concentration of different types of chicken meat in Belgium.

World Health Organisation (WHO), 2007. Critically Important Antimicrobials for Human Medicine, 3rd edition. . (May).

Worsfold, D., Griffith, C.J., 1997. Assessment of the standard of consumer food safety behavior. Journal of Food Protection 60, 399–406.