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Hazard assessment and resistance profile of *Escherichia coli* strains isolated from bovine carcasses at the main slaughterhouses of Dakar, Senegal

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Foodborne bacterial hazards and antibiotics resistance are a public health threat. This study aimed at assessing the slaughter conditions, the bacteriological quality of bovine carcasses and at determining the antimicrobial resistance of *Escherichia coli* strains isolated from bovine carcasses. A questionnaire was administered to 41 stakeholders on the slaughter line. Swabbed carcasses were analyzed according to the French standard NF ISO 17604. *E. coli* were plated on Tryptone Bile X Glucuronide and their sensitivity to 15 antibiotics was tested by the disk diffusion method. The results indicate deficiencies in slaughter practices, control of critical operations such as evisceration and also at the level of the training of stakeholders. The prevalence of contaminated carcasses was 99% with an average of 3.03 log10 CFU/cm^2. Susceptibility testing showed *E. coli* to be resistant to tetracycline (32%), colistin (26%), cefepim (12%), ceftazidime (9%) and ciprofloxacin (5%). However, all *E. coli* strains were susceptible to cefotaxime, imipenem and norfloxacin. It is concluded that bovine carcasses from Dakar slaughterhouses represent a potential risk to public health due to the occurence of *E. coli* that are possible indicators of enteropathogenic agents. It also suggests the presence of *E. coli* resistant to critical betalactamins such as third and fourth generation cephalosporins.

Key words: Antibiotic resistance, indicator bacteria, cattle, slaughter hygiene, risk factors.

INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a serious public health concern and serotype O157:H7 is one of the dangerous strains of *E. coli* that can be fatal, causing severe illness, permanent kidney and brain damage and even death in humans (Adingra et al., 2011). Cattle are known to be the main reservoir of STEC strains and the cattle bovine carrying the strain do not often show signs of clinical disease (Naylor et al., 2005).

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Epidemiological and analytical studies conducted on these pathogenic *E. coli,* in particular serotype O157:H7, have shown that human infection occurs mainly through consumption of contaminated food or water, direct contact with contaminated animal products, human carriers, or contaminated objects (Bruyand et al., 2018). Studies such as Bibbal et al. (2015) noted that the prevalence of potentially pathogenic *E. coli* varies between farms, and is estimated to be 4.5% in young dairy cattle, 2.4% in young beef cattle, 1.8% in dairy cows and 1% in beef cows.

While it is true that macroscopic inspection in slaughterhouses remains the standard means of public health protection, the fact remains that this method has its limitation in terms of detecting carcasses carrying bacterial agents responsible for foodborne diseases. In the meat value chain, previous studies have shown that carcasses from fecal contaminated cattles had a higher contamination load than those from clean cattle (Barco et al., 2015). Indeed, when slaughter hygiene is poor, dressing and evisceration operations are regarded to be stages offering multiple possibilities of contamination (Savoye, 2011). In slaughterhouses, the presence of *E. coli* is a good indicator to assess enteric contamination along the slaughter process (Ghafir et al., 2008).

Alongside the food poisoning risk posed by *E. coli* in the beef value chain, these last years have seen the emergence of extended spectrum β-lactamase (ESBLs) producing *E. coli* isolates, regarded as one of the most serious threats for animal and human health worldwide (Sihem et al., 2015). In addition, resistance to the most widely used antibacterials for the treatment of *E. coli* urinary tract infections, fluoroquinolones (FQLs) and some β lactams (3rd and 4th generation cephalosporins), have become a public health concern (Um, 2016). Given the importance of beef consumption in the Dakar region, the monitoring of the hygiene of slaughtering processes through the indicator of fecal contamination (*E. coli*) is necessary for food safety. Furthermore, monitoring of antibacterial resistance at the human-animal interface in relation with the "one health" approach provides useful knowledge at the national level to reduce the emergence and transmission of antibiotic resistant bacteria. The study aimed at assessing the slaughter conditions to evaluate the bacteriological quality of bovine carcasses and to determine the antimicrobial resistance of *E. coli* strains isolated from these carcasses in Dakar slaughterhouses.

The results will stand as value to the staff of the Dakar slaughterhouse, local research structures (EISMV, ISRA) Ministry of Livestock and the whole scientific community throughout the world.

MATERIALS AND METHODS

Survey and data collection

A survey on slaughter hygiene was carried out at the main modern slaughterhouse of Dakar, Senegal, which is supplied with a preparation line consisting of suspension rails, fixed or movable plateforms and elevated or lowered plateforms. It has a slaughter capacity of about 300 cattle heads per day. For this survey, observations were made on the conditions and method of slaughter, in particular the execution of the various technological operations from the holding pens of the live animals until to the obtaining of the carcasse. Then, a questionnaire was administered in the form of individual interviews. The questionnaire collected information on slaughtering practices such as the cleaning hands during the slaughter process and the knowledge and perception towards contamination factors. The target group for this survey was the staff working in the slaughter line which has an impact on the safety of prepared animals. Thus, 41 workers were selected by a simple random method and with their prior consent.

Escherichia coli sample collection

To ensure the consistency of the observed parameters over time, *E. coli* sampling was taken over a 3-month period (July to September, 2020). Samples were taken once a week, changing the collection day to be able to cover all days of the week. On each collection day, ten (10) bovine half carcasses were randomly sampled prior to chilling. The carcasses numbered 26th, 51st, 76th, 101st, 126th, 151st and so on were selected. As a sampling method, the study resorted to the non-destructive method using swabs in accordance with the provisions of French Standard NF ISO 17604: "Microbiology of foodstuffs: taking samples from carcasses for microbiological analysis". Four samples were collected per carcass, constituting a single sample as indicated by Regulation (EC) No 2073/2005. The sampling sites on the carcasses are those indicated by French Standard NF ISO 17604. Thus, four (4) anatomical sites (shoulder, flank, thigh and rump) were swabbed according to the locations indicated in Figure 1. After collecting, the samples were transported in a cooler boxes (between 0 and 5°C) and immediately analysed upon arrival at the food microbiology laboratory of the EISMV (Ecole Inter-Etats des Sciences et Médecine Vétérinaires) of Dakar/Senegal.

Analysis

The enumeration of beta-glucuronidase-positive *E. coli* was done according to ISO 16649-2: "Horizontal method for the enumeration of beta-glucuronidase-positive *E. coli*". Successive decimal dilutions (10^{-1}, 10^{-2}, and 10^{-3}) were plated on TBX agar. After 24 hours of incubation, all characteristic colonies were counted and the results were expressed in colony forming units per cm^2 (cfu/cm²) and then in $\log_{10}$ cfu/cm². Two to three characteristic *E. coli* colonies were collected per Petri dish and placed in an Eppendhorf tube containing 500µL of Luria Bertani (LB) broth (Invitrogen, Paisley, Scotland). The tubes were incubated to allow the *E. coli* to grow overnight at 37°C and transported in a cooler (between 0 and 5°C) to LNERV (Laboratoire National d'Elevage et Recherches Vétérinaires) for storage at -80°C in 20% glycerol until the time of antibiotic susceptibility testing.

Antibiogram

After thawing the strains stored at -80°C, they were incubated overnight at 37°C on regular agar to obtain fresh colonies. The Mueller-Hinton agar medium (MH) diffusion method was used according to the standards and recommendations of the antibiogram committee of the French Society of Veterinary Specialty Microbiology (CA-SFM-vet, 2019). Fifteen antibiotics (Bio-Rad) from six different families were individually added to the MH agar plates and selected on the basis of a survey of antimicrobials, of which

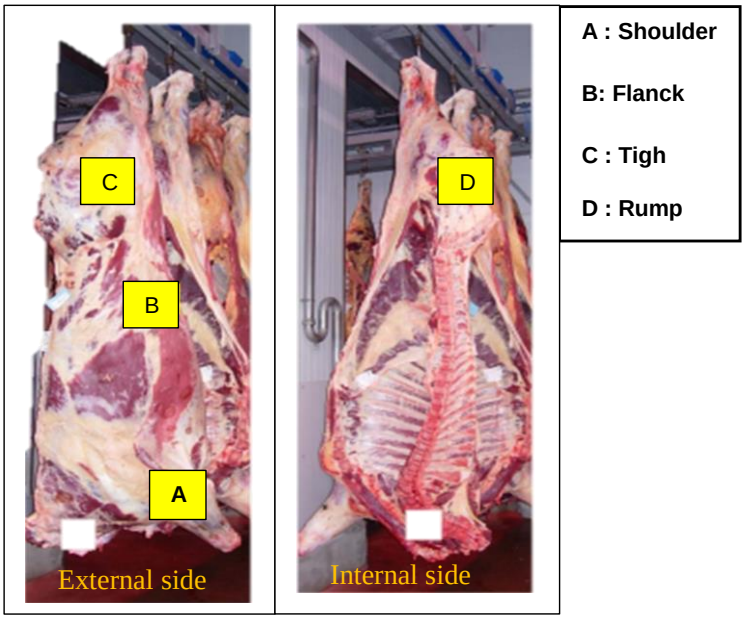

Figure 1. Carcass collection sites for *E. coli*.

some are most widely used in veterinary and human medicine in Senegal. These include: (1) Betalactamins: Ampicillin (AMP, 10 µg), Cefotaxime (CTX, 30 µg), Cefoxitin (FOX, 30 µg), Cefepime (CEF, 30 µg), Amoxicillin+clavulanic acid (AMC, 20/10 µg), Ceftazidime (CAZ, 30 µg), Cephalotin (CEF, 30 µg); (2) Carbapenems: Imipenem (IPM, 10 µg); (3) Aminosides: Gentamicin (GEN, 10 µg), Kanamycin (KAN, 30 µg); (4) Polypeptides: Colistin (COL, 50 µg); (5) Tetrayclines: Tetracycline (TET, 30 µg); (6) Quinolones and fluoroquinolones: Nalidixic acid (NAL, 30 µg) and Norfloxacin (NOR, 10 µg), Ciprofloxacin (CIP, 5 µg). The reading and measurement of the zone of inhibition diameters were done after 24 h of incubation at 37°C. Interpretation of the results was done according to the recommendations of CA-SFM-vet (2019) which classified the isolates as susceptible, intermediate and resistant. All intermediate isolates were regarded as resistant.

In addition, the study used the double disc synergy technique to detect the strains producing Extended Spectrum β-lactamases (ESBL). Agar diffusion is not the preferred method for colistin, but was used for the evaluation of colistin resistance in strains (Belloc et al., 2008).

Data analysis

The data were entered into Microsoft office Excel (2010 for windows) spreadsheet software for graphing and transforming the data into percentages. The data was then transferred to SPSS® version 24 for statistical analysis. In order to compare the rates of contaminated carcasses according to collection days, student (*t*) test and ANOVA were used. *P* value <0.05 was considered statistically significant.

Ethical consideration

For this study, authorization was obtained from slaughterhouses managers, while consent was sought before interviewing staff on the slaughter line.

RESULTS

Observations on slaughter conditions

Observations made on the cattle slaughter line revealed hygiene shortcomings in the holding pens, in particular due to the lack of water for cleaning of the floor after the passage of a herd. Cattle with diarrhea and hindquarter soiling were not sorted and separated. Animal mistreatment was observed, causing stress which eventually caused defecation. After bleeding by halal method, the evicted animals from the killing box were stacked on top of each other. The animals were also in contact during and after dressing of carcasses.

There are no functional hygiene stations to sterilize the unsuitable equipment, such as the sharp-tipped knives used at the evisceration station, which cause perforations of the intestines, pre-stomach and stomach. Ligation of the oesophagus and rectal colon is not systematic during evisceration. Partial automation of slaughter line leads to manual handling of carcasses to the refrigerating rooms. The difficulties in obtaining a supply of quality water at almost all workstations and in the sanitary facilities add to the negative conditions observed. Front of these shortcomings, nothing is done to apply preventive measures or adopt an attitude that would prevent a risk from occurring in the food chain.

Interview information

As for the staff, a total of 41 workers were interviewed on the knowledge, practices and perception of the cattle preparation hygiene. Nearly all of them (95.83%) noted

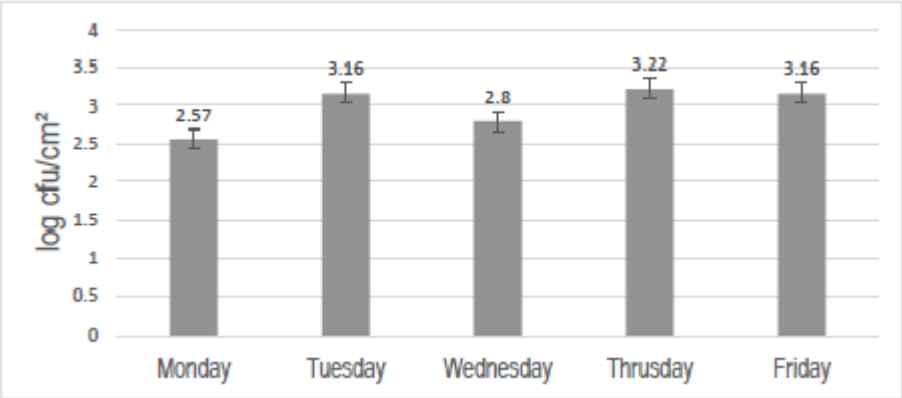

Figure 2. Average loads of bacterial flora (*E. coli*) isolated from 120 bovine carcasses according to the day of slaughter.

the importance of awareness-raising and training sessions on Good Hygiene Practices (GHP), in order to maintain the hygiene of the preparation at an acceptable level. However, most of them indicated that they did not receive any capacity building training to reinforce their knowledge, particularly regarding hygiene standards and food-related hazards. With regard to compliance with GHP, all respondents stated that the lack of hygiene in the beef meat preparation chain can be a source of contamination that can have negative consequences on the quality of the carcasses. As for the hygiene conditions of the premises, 51.2% found them acceptable. With regard to the risk of contamination, 54.2% indicated that contact with the carcasses on the slaughter line was conducive to contamination. The same is true for the absence of water at the work stations, which is considered to lead to the contamination of carcasses. The presence of water and soap in the sanitary facilities was regarded by more than 62.5% to be very important in reducing the risk of cross-contamination of carcasses. The respondents also stated that the hygiene of the premises (62.5%) and the sanitary facilities (66.7%), the cleanliness of the hands of the staff (2.5%) and of the equipment (66.7%), are factors that help to reduce the contamination of the carcasses. During the work, if the hands of the handlers were soiled, 79.2% said that they washed their hands before continuing the work. In relation to the slaughtering/dressing operations, 66.7% of the respondents indicated that the presence of leather on the carcass is a source of contamination. The same was true for 79.2% and 41.7% of the respondents who respectively confirmed that the presence of stomach contents and feces on the carcasses are sources of contamination. The automatic conveying of carcasses was considered by 20% of the respondents to be a less risky practice compared to manual conveying, that almost 40% of the staff considered this practice to have no impact on the contamination of carcasses. The others had no opinion on the matter. Animal mistreatment was considered by almost 57.14% of them having no impact on carcass quality.

Occurrence of *E. coli* in samples

Bacteriological analyses were performed on a total of 120 bovine carcasses. The prevalence of *E. coli* β-glucuronidase-positive carriage on the sampled carcasses was 99% for an overall average contamination of 3.03 log10 CFU/cm^2 +/- 0.80. The analysis of the average bacterial loads isolated per day indicated that overall carcass contamination rates were low at the beginning of the week and climbed throughout the week. (Figure 2).

The analysis revealed a statistically significant difference ($P<0.05$) between the contamination averages of Monday and the rest of the days (Tuesday, Wednesday, Thursday and Friday). However, there was no statistically significant difference ($P>0.05$) between the Thursday and Friday averages.

Antibiotic resistance profile

All 57 isolates of *E. coli* were tested for their antibiotic resistance profiles against 15 different antimicrobial agents (Figure 3). All *E. coli* isolates were non-ESBL-producers. Among non-ESBLs-producing strains, 9% (5/57) were resistant to third generation cephalosporin, in particular to ceftazidime but none were resistant to cefotaxime. The data revealed also a higher prevalence of *E. coli* strains resistant to tetracycline (32%), followed by colistin (26%), cefepime (12%), cefoxitin (12%), ampicillin, gentamicin and amoxicillin + clavulanic acid with a proportion of 11%, and kanamycin (9%). Low rates of resistance were observed with nalidixic acid (2%), cephalotin (4%) and ciprofloxacin (5%). In contrast all of them were susceptible to cefotaxime, imipenem and norfloxacin (Figure 3).

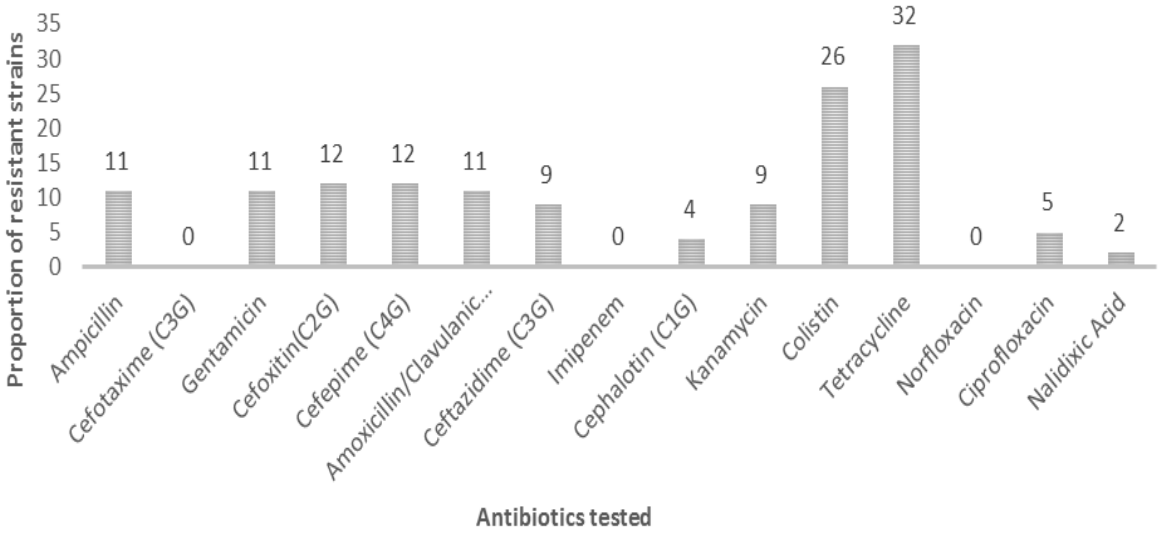

Figure 3. Variability in antibiotic resistance of *E. coli* strains isolated from bovine carcasses.

DISCUSSION

Observations on slaughter conditions

The slaughtering conditions of cattle observed at the Dakar slaughterhouses are not adequate to prevent contamination of carcasses, particularly due to poor slaughtering practices. According to the FAO/OIE (2009) guidelines on food safety, there are hygiene standards to be complied with. Indeed, these standards cover all essential aspects of production such as cleanliness and welfare of the animals, adequate facilities for hand washing, cleaning and disinfection of instruments, water supply, control of sensitive operations (dressing/ evisceration) and adequate sanitary facilities. However, in our context the conditions do not meet the pre-established standards to ensure the production of safe meat for human consumption. Similar results were also reported by Dieye (2011) and Yougbare (2014) in slaughterhouses in Dakar. Therefore, these observations suggest that the carcasses of slaughtered animals represent a risk to public health. This indicator should lead to the implementation of actions to improve the general hygiene of slaughtering conditions, in order to prevent and reduce the factors that contaminate cattle carcasses in Dakar slaughterhouses.

Interview information

The evaluation of the knowledge, attitudes, practices and perceptions of the slaughterhouse workers revealed a significant need for capacity building on GHP. Indeed, some obvious contamination situations are considered by some workers as having no impact on the bacteriological quality of carcasses. Furthermore, nothing is done by the workers to apply preventive measures or adopt an attitude that would prevent a risk from occurring in the food chain, as indicated in the results of observations section. These findings were also noted by Cadmus et al. (2008) who indicated that slaughterhouse workers in Nigeria were engaging in unhygienic practices that put meat consumers directly at risk. The low level of hygienic training may be the cause, as well as the lack of training in the hygiene of slaughter processes for butchers. In fact, there is no training plan established and executed according to a predefined periodicity to reinforce the level of knowledge of the staff, particularly with regard to hygiene standards and food-related hazards. These results are in line with those of Okoli et al. (2006), who found that most butchers lack professional training in Nigeria. In principle, staff undertaking meat hygiene activities should be trained or instructed so that they have the required knowledge and skills (FAO, 2006). Failure to do so can lead to serious consequences for public health. Hence, there is a need to implement a training plan for staff to change their slaughtering practices.

Occurrence of *E. coli* in samples

The many shortcomings observed during cattle slaughtering operations have consequences on the contamination of cattle carcasses. The present study showed that the carriage rate of cattle carcasses with *E. coli* was of 99%. This is significantly higher than the 10% reported by Phillips et al. (2001) in Australia and the 57.92% by Ahouandjinou et al. (2016) in Benin. The average contamination observed (3.03 $\log_{10}$ CFU/cm^2) in this study is also much higher than the one found by Dieye (2011) (0.13 $\log_{10}$ CFU/cm^2). This level of

contamination reflects poor hygiene practices during slaughtering operations. According to Ray (2001) and Savoye (2011), the main source of *E. coli* contamination of meat is the intestinal tract of animals. Their presence corresponds to a defect in the slaughter technique. Consequently, the *E. coli* isolated in this study probably originate from the absence of ligature of the oesophagus and rectal colon during evisceration, which favours the soiling of carcasses by feces, or by the leakage of gastric contents after accidental perforation of the gastric sacs by the operator. But *E. coli* strains can also come from meat handlers. The variability observed in the average bacterial loads isolated according to the day of slaughter has made it possible to identify two main trends: Firstly, a level of contamination that is lower at the beginning of the week and higher at the end of the week. This can be explained by the state of fitness of the workers, who resume work after the Sunday break (slaughterhouses operate 6 days a week), as well as the low throughput slaughtering at the beginning of the week. Secondly, we can mention the purchases which are more often made on weekend for most households. Despite this development, the end of the week is marked by the fatigue among workers who will have been working since the beginning of the week, but also by the increase in slaughtering at the end of the week in order to ensure the availability of meat on the weekend. The contamination of bovine carcasses on the weekend represents a significant human health threat, especially if the meat is consumed undercooked. This could lead to foodborne outbreaks and sporadic cases of benign diarrhea, but some can progress towards severe disease such as hemolitic uremic syndrome (HUS). In order to protect public health, the consumers should sufficiently cook the meat from the slaughterhouses of Dakar, and two different teams of well-trained workers can alternate for slaughter operations to avoid having only one team to do the work the whole week. This can improve the quality of carcasses throughout the week, especially on Fridays.

Antibiotic resistance profile

The results of the antibiotic susceptibility testing of *E. coli* strains showed a variability among the fifteen antibiotics tested. The *E. coli* strains were resistant to tetracyclines (32%), colistin 26%, cefoxitin and cefepime12%, ampicillin, gentamicin and amoxicillin+clavulanic acid 11% and kanamycin 9%. These percentages are significantly lower than those reported by Kohansal and Ghanbari (2018) who obtained on a total of 52 isolates of bovine origin a resistance to ampicillin of 73% and tetracycline of 65%. The present results are not similar with those of Kohansal and Ghanbari (2018) because, in their study they have tested clinical strains from sick cows, which had probabily been in contact with antibiotics. The resistance to tetracyclines can be explained by their wide range of uses in treating animal diseases due to their broad spectrum of activity. The results are also lower than those of Ahouandjinou et al. (2016) who obtained ampicillin 87.77%, cefoxitin 20.80% and amoxicillin+clavulanic acid 66.19%. This difference in the results can be explained by the fact that fewer *E. coli* strains (57) were tested than in the study by Ahouandjinou et al. (2016) who used 150 strains of *E. coli*. However, our results are higher than those of Sarr (2012) who noted very low levels of resistance to kanamycin (2.5%) and tetracycline (2.5%). This may be related to the higher number of *E. coli* strains tested compared to the study of Sarr (2012). They also do not agree with the results of Martel et al. (1983) in France who obtained very high levels of resistance exceeding 50% for all *E. coli* strains of bovine origin tested against ampicillin, kanamycin and tetracycline. In the previous study, clinical *E. coli* strains from cows and their newborn calves treated with prophylaxis were also tested. That is why, levels of resistance in this study are higher than our context. Low resistance to ceftazidime (9%) and sensitivity of all isolates to cefotaxime (0%) were noted both of which are third generation cephalosporins. Studies, such as the one by Sarr (2012), have shown that *E. coli* isolates sensitive to ceftazidime were also sensitive to cefotaxime. However, Abayneh et al. (2019) reported in their study that among the seven non-ESBL producing strains, six were resistant to ceftazidime and only one was resistant to cefotaxime. Our results seem to be closer to this second study. Therefore, the low resistance of *E. coli* strains to third generation cephalosporins could be explained by the high cost of the latter, which would limit the related prescriptions to veterinary settings or extensive farming systems. Furthermore, we did not detect ESBL producing strains in this study, which could be due to the small number of tested strains. Resistance to colistin was determined by the disk diffusion method. According to Belloc et al. (2008) this method can be used for the evaluation of colistin resistance. However, Wasyl et al. (2013) found a prevalence of 0.9% of colistin resistant strains by the reference method. This rate is much lower than the 26% observed in the present study. This may be due to the fact that we did not use the same method. All strains tested were sensitive to imipenem and norfloxacin. They were most active on *E. coli* strains. According to Mendes et al. (2009), the frequency of carbapenem resistance is low, affecting less than 2% of the strains isolated. Our results are similar to this observation. This can be explained by the fact that these are last-line molecules and therefore practically not used in human and veterinary medicine. The low rates of resistance observed with quinolones/fluoroquinolones show that despite their use in cattle farming, these molecules remain effective on *E. coli* strains. The profile of resistance to antibiotic observed in this study can help to determine the origin of the animals farming system.

Because of the low resistance rates, these animals

probabily come from extensive farming systems, where antibiotic therapy is not used as in intensive farming systems. According to Van Boeckel et al. (2015), the intensive system is marked by the widespread and unprecedented use of antimicrobials at subtherapeutic doses to stimulate growth or prevent disease. This leads to the emergence of resistant bacteria in this system. In terms of human health, data indicate on the one hand a less alarming resistance phenomenon and on the other hand some significant classes of antibiotics which have been most active, as third generation cephalosporins, carbapenem and quinolones. These latter can be used in the case of *E. coli* diseases. However, their rational use should be ensured by veterinary and human health professionnals in order to combat the spreading of antibiotic resistance.

Conclusion

The observed conditions during the preparation of the cattle and their consequences on the fecal contamination of the carcasses particulary *E. coli*, seem to be a public health concern. This study highlights the place of *E. coli* as fecal indicator in the processing of beef cattle at the main abattoir in Dakar during the warmer months. Thus, to reduce their impact on public health, slaughtering conditions should be improved by upgrading the general hygiene of cattle slaughter preparation. It is also important to identify the training needs of the staff and to define a training plan enabling each staff member to be trained in food hygiene. Furthermore, the analysis of antimicrobial profile concluded to a phenomenon of antibiotic resistance of *E. coli* isolated from beef carcasses. This finding implies that the meat prepared in Dakar slaughterhouses is likely to play the role of a vector in the dissemination of resistant bacteria to humans. It is necessary to break the chain of fecal contamination of carcasses. Then, antimicrobial surveillance plans should also be implemented at the primary production and slaughterhouse levels to better understand the risks of human exposure to resistant *E. coli* via meat of beef cattle. Finally, in research, it would be necessary to characterize the virulence genes of the isolated *E. coli* strains and to search for serogroups that are potentially dangerous for humans, such as: O103, O145, 308 O26 and O111.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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