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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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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 [2]. 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 [14]. 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 [7]. Studies such as Bibbal et al. [6] 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 cattle had a higher contamination load than those from clean cattle [4]. 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 [10].

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 platforms and elevated or lowered platforms. 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.

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.

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*".

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)

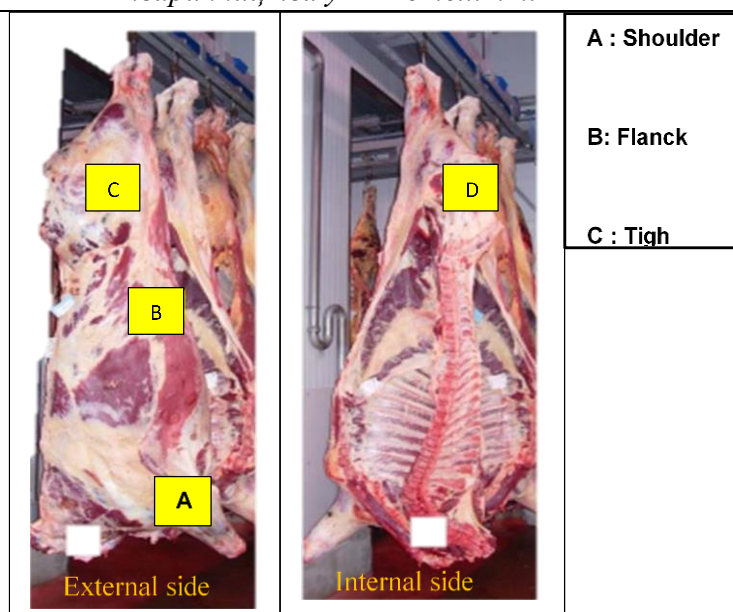


Figure 1. Carcass collection sites for *E. coli*

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.

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 $3.03 \log_{10} \text{CFU/cm}^2 \pm 0.80$. 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).

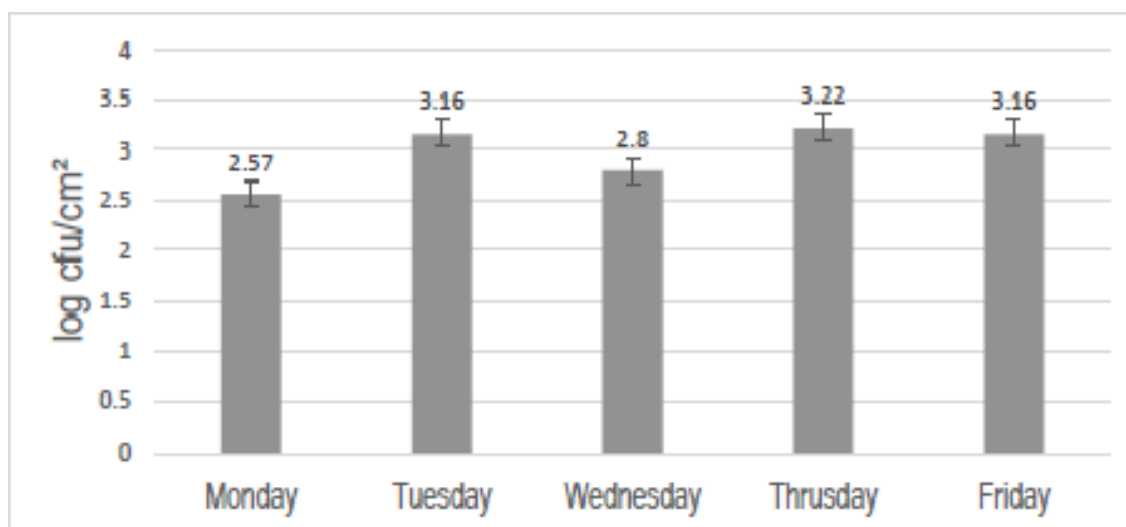


Figure 2. Average loads of bacterial flora (*E. coli*) isolated from 120 bovine carcasses according to the day of slaughter

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%), ceftazidime (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).

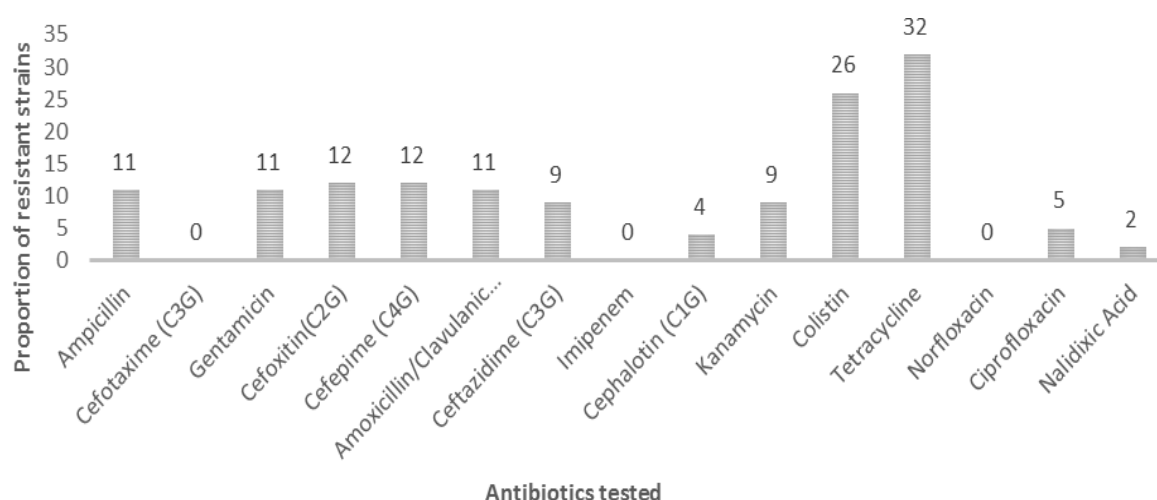


Figure 3. Variability in antibiotic resistance of *E. coli* strains isolated from bovine carcasses

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. [3] in Benin. The average contamination observed ($3.03 \log_{10}$ CFU/cm²) in this study is also much higher than the one found by Dieye [9] ($0.13 \log_{10}$ CFU/cm²). 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 ligation 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 hemolytic 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.

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 cefepime 12%, ampicillin, gentamicin and amoxicillin+clavulanic acid 11% and kanamycin 9%. These percentages are significantly lower than those reported by Kohansal and Ghanbari [11] 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 [11] because, in their study they have tested clinical strains from sick cows, which had probably 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. [3] 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. [3] 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. [12] 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. [1] 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. [5] 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. [13], 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.

Conclusion

The observed conditions during the preparation of the cattle and their consequences on the fecal contamination of the carcasses particularly *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.

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НАУКОВІ І ТЕХНОЛОГІЧНІ ВИКЛИКИ ТВАРИННИЦТВА У ХХІ СТОЛІТТІ



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