ANTIMICROBIAL RESISTANCE OF SHIGA TOXIN–PRODUCING *ESCHERICHIA COLI* O157:H7, O26:H11, O103:H2, O111:H8 AND O145:H28 ISOLATED FROM CATTLE SLAUGHTERED IN FRANCE

M.M. Um[1], D. Bibbal[1], M. Kérourédan[1], E. Loukiadis[2], F. Auvray[3], E. Oswald[4], H. Brugère[1]

[1] Université de Toulouse, INPT, ENVT, INSERM UMR1043, INRA USC1360, Toulouse, France

[2] Université de Lyon, VetAgro Sup, LNR STEC, CNRS UMR 5557 Microbial Ecology, Marcy l'Etoile, France

[3] Université Paris-Est, Anses, Laboratory for Food Safety, Maisons-Alfort, France

[4] INSERM UMR1043, INRA USC1360, CHU de Purpan, Toulouse, France

Abstract – The goal of this study was to investigate the antimicrobial resistance of 39 top five Shiga toxin-producing *E. coli* (STEC) isolated from slaughtered adult cattle in France. In addition, 80 atypical enteropathogenic *E. coli* (aEPEC), with same genetic characteristics as top five STEC strains except that they lacked the *stx* gene, were also tested. Seven of the 39 STEC strains (2 O157:H7, 2 O26:H11, 1 O103:H2 and 2 O111:H8) were resistant from 1 to 5 antibiotics, among ampicillin, amoxicillin+ clavulanic acid, streptomycin, tetracycline, sulfonamides and/or nalidixic acid. With regard to aEPEC strains, eight aEPEC O26:H11 were resistant. All resistant STEC and aEPEC strains were susceptible to azithromycin. This study shows that top five STEC strains isolated from French adult cattle were mainly susceptible.

Key Words – antibiotic resistance, cattle, STEC.

I. INTRODUCTION

Enterohemorrhagic *E. coli* (EHEC) strains are subset of shiga toxin-producing *E. coli* (STEC). They are responsible for hemorrhagic colitis and hemolytic uremic syndrome in humans [1]. Antimicrobial therapy for EHEC infections remains controversial [2]. Nevertheless, antibiotics are often used in clinical practice to treat patients with diarrhea. Recently, the use of azithromycin during the outbreak due to the enteroaggregative STEC *E. coli* O104:H4 resulted in decrease of STEC O104:H4 carriage [3]. The French Agency for Food Safety defined five major EHEC as STEC belonging to serotypes O157:H7, O26:H11, O145:H28, O103:H2 [4]. Cattle are known to be the reservoir of EHEC strains; and human infections mainly occur through consumption of contaminated food and water [5]. We previously reported that the prevalence of bovine carriers of top five STEC, evaluated through a weighted arithmetic mean of the prevalence by categories, was estimated to 1.8% in French slaughtered adult cattle [6]. This study also led to the isolation of atypical enteropathogenic *E. coli* (aEPEC) belonging to the top five serogroups. These strains had the same genetic characteristics as top five STEC strains except that they lacked the *stx* gene. The objective of the present study was to investigate the antimicrobial resistance of this collection of top five STEC and aEPEC isolated from adult cattle slaughtered in France.

II. MATERIALS AND METHODS

A collection of 39 top five STEC and 80 aEPEC strains [6, 7] was screened for the resistance to 16 antibiotics by the disk diffusion method (Fig. 1). Antibiotics disks used in this study were ampicillin, amoxicillin plus clavulanic acid, cefalexin, cefuroxime, cefotaxime, ceftazidime, cefepime, ertapenem, gentamicin, streptomycin, tetracycline, chloramphenicol, trimethoprim, sulfonamides, nalidixic acid, and ciprofloxacin. In a second stage, azithromycin MICs were determined for resistant strains using E-test. Antimicrobial susceptibility tests were performed and interpreted according to the recommendations and the guidelines of the French Society for Microbiology (SFM) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) [8]. Strains were considered as multidrug resistant (MDR) when they exhibited resistance to at least 3 antibiotic classes [9].

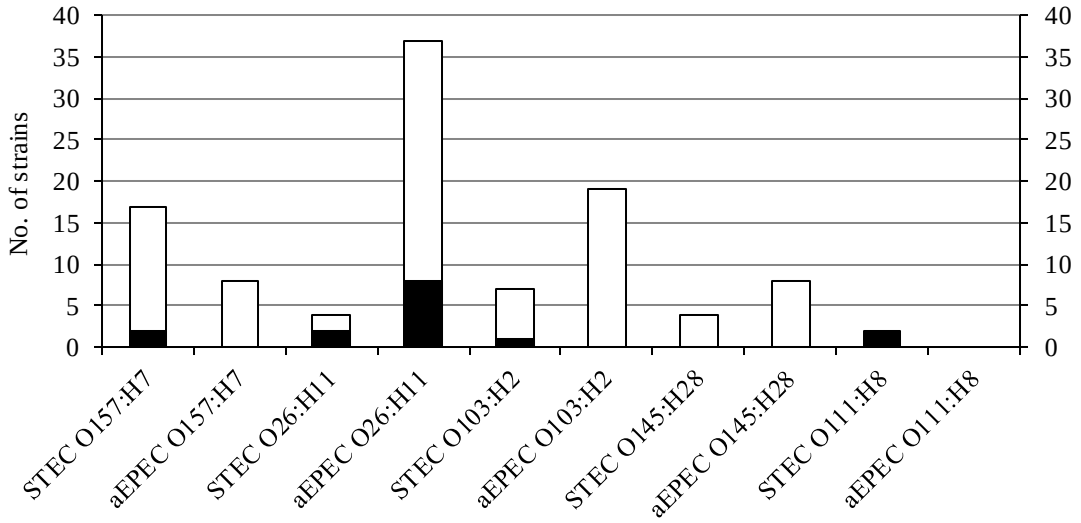

Figure 1. Number of top five STEC and aEPEC strains tested for antibiotic resistance. Filled bars indicate the number of antibiotic-resistant strains.

III. RESULTS AND DISCUSSION

Among the 39 top five STEC tested, 7 strains showed resistance to at least one of the tested antibiotics (17.9%) (Fig. 1). They belonged to serotypes O157:H7, O26:H11, O103:H2 and O111:H2 (Table 1). Six were MDR (15.4%). Resistance was mainly observed for ampicillin, streptomycin, tetracycline and sulfonamides. One strain was resistant to nalidixic acid. All the resistant strains were susceptible to azithromycin. As we observed, few studies recently reported a low prevalence of resistance in top five STEC. In Belgium, van Meervenne *et al.* [10] showed that 28.8% of STEC isolated from food, animal sources and infected humans were resistant [10]. For the top five serogroups, only 29.6% of the strains were resistant. In Japan, resistance was detected in 13.3% of the STEC O157 isolated from beef cattle [11]. In Canada, Vidovic *et al.* [12] observed that the percentage of resistant STEC O157 was significantly different between human and bovine strains: 38% *versus* 60% [12]. They also showed that resistance to tetracycline and to sulfisoxazole were the most strongly associated segregative characteristics of bovine STEC O157 strains. They suggested that these statistically differences can be explained by the mass application of antimicrobial growth promoters. In Spain, Mora *et al.* [13] also showed a higher level of resistance in STEC O157:H7 isolated from cattle (53%) compared to humans (23%) [13]. Indeed, the majority of the studies investigating the resistance of top five STEC isolated from cattle tended to show high level of resistance [14-16].

With regard to the resistance of aEPEC strains, we showed that only 10% of the strains were resistant (Fig. 1). The 8 resistant strains belonged to the serogroup O26:H11 (Table 1). They were all resistant to tetracycline, and 4 were MDR. Moreover, they were all susceptible to azithromycin. On the contrary, Medina *et al.* [17] found that at least 65% of the 206 aEPEC isolated from healthy and sick ruminants were resistant to tetracycline, streptomycin and sulfamethoxazole [17].

Table 1 Resistance phenotype of top five STEC and aEPEC strains isolated from bovine feces

Strain	Cattle category[a]	Serotype	Presence of[b]:		Resistance phenotype[c]
			stx (subtype)	*eae* (subtype)	
STEC					
C61	DC	O157:H7	+ (*stx*$_{1a}$, *stx*$_{2c}$)	+ (γ1)	AMC AMP STR TET SSS
H13	YBB	O157:H7	+ (*stx*$_{2c}$)	+ (γ1)	TET
I92	YDB	O26:H11	+ (*stx*$_{1a}$)	+ (β1)	STR TET SSS
K106	YDB	O26:H11	+ (*stx*$_{1a}$)	+ (β1)	AMP STR SSS
L24	YDB	O103:H2	+ (*stx*$_{1a}$)	+ (ε)	AMP STR TET SSS
J43	DC	O111:H8	+ (*stx*$_{1a}$)	+ (θ)	AMP STR TET SSS NAL
K50	YDB	O111:H8	+ (*stx*$_{1a}$)	+ (θ)	AMP STR TET SSS
aEPEC					
A81	YDB	O26:H11	-	+ (β1)	AMC AMP STR TET SSS
A85	YDB	O26:H11	-	+ (β1)	STR TET CHL SSS
A86	YDB	O26:H11	-	+ (β1)	AMC TET CHL SSS
A138	YBB	O26:H11	-	+ (β1)	TET
A140	YBB	O26:H11	-	+ (β1)	TET
H14	YDB	O26:H11	-	+ (β1)	TET
H52	YBB	O26:H11	-	+ (β1)	STR TET CHL SSS
I79	YDB	O26:H11	-	+ (β1)	TET

a DC, Dairy cow; YBB, Young beef bull; YDB, Young dairy bull
b +, present; -, absent
c AMC, amoxicillin plus clavulanic acid; AMP, ampicillin; CHL, chloramphenicol; NAL, nalidixic acid; SSS, sulfonamides; STR, streptomycin; TET, tetracycline

IV. CONCLUSION

In this study, we showed that the level of resistance of top five STEC and aEPEC isolated from slaughtered adult cattle in France was low. Various studies tended to show higher level of resistance. The reasons for these differences are not clear and remained to be elucidated. Moreover, it should be interesting to investigate if the low level resistance that we observed was specific to STEC and aEPEC strains among *E. coli* populations of the bovine digestive tract.

ACKNOWLEDGEMENTS

Authors pay tribute to the memory of Philippe Cartier (Livestock Institute, France), who was responsible for slaughterhouse samplings.

REFERENCES

1. Kaper, J. B., Nataro, J. P. & Mobley, H. L. (2004). Pathogenic *Escherichia coli*. Nature reviews Microbiology 2: 123-140.
2. Wong, C. S., Jelacic, S., Habeeb, R. L., Watkins, S. L. & Tarr, P. I. (2000). The risk of the hemolytic-uremic syndrome after antibiotic treatment of *Escherichia coli* O157:H7 infections. N Engl J Med 342: 1930-1936.
3. Nitschke, M., Sayk, F., Hartel, C., Roseland, R. T., Hauswaldt, S., Steinhoff, J., Fellermann, K., Derad, I., Wellhoner, P., Buning, J., Tiemer, B., Katalinic, A., Rupp, J., Lehnert, H., Solbach, W. & Knobloch, J. K.- M. (2012). Association between azithromycin therapy and duration of bacterial shedding among patients with Shiga toxin-producing enteroaggregative *Escherichia coli* O104:H4. Jama 307: 1046-1052.
4. French Agency for Food, Environmental and Occupational Health and Safety. Opinion of

the French Food Safety Agency on the advisability of revising the definition of pathogenic STEC, specified in AFSSA's Opinion of 15 July 2008. Anses. Maisons-Alfort. France. 2010.

5. Karmali, M. A., Gannon, V. & Sargeant, J. M. (2010). Verocytotoxin-producing *Escherichia coli* (VTEC). Vet Microbiol 140: 360-370.
6. Bibbal, D., Loukiadis, E., Kérourédan, M., Ferre, F., Dilasser, F., Peytavin de Garam, C., Cartier, P., Oswald, E., Gay, E., Auvray, F & Brugère, H. (2015). Prevalence of carriers of Shiga toxin-producing *Escherichia coli* serotypes O157:H7, O26:H11, O103:H2, O111:H8 and O145:H28 in French slaughtered adult cattle. Appl Environ Microbiol 81: 1397-1405.
7. Bibbal, D., Loukiadis, E., Kérourédan, M., Peytavin de Garam, C., Ferre, F., Cartier, P., Gay, E., Oswald, E., Auvray, F. & Brugère, H. (2014). Intimin gene (*eae*) subtype-based real-time PCR strategy for specific detection of Shiga toxin-producing *Escherichia coli* serotypes O157:H7, O26:H11, O103:H2, O111:H8, and O145:H28 in cattle feces. Appl Environ Microbiol 80: 1177-1184.
8. CA-SFM/EUCAST (2014). Comité de l'antibiogramme de la Société Française de Microbiologie. Recommandations 2014. V.2.0 Décembre 2014, Paris, France.
9. Schwarz, S., Silley, P., Simjee, S., Woodford, N., van Duijkeren, E., Johnson, A. P. & Gaastra, W (2010). Editorial: assessing the antimicrobial susceptibility of bacteria obtained from animals. J Antimicrob Chemother 65: 601-604.
10. Van Meervenne, E., Boon, N., Verstraete, K., Devlieghere, F., De Reu, K., Herman, L., Buvens, G., Pierard, D. & Van Coillie, E. (2013). Integron characterization and typing of Shiga toxin-producing *Escherichia coli* isolates in Belgium. Journal of medical microbiology 62: 712-719.
11. Sasaki, Y., Usui, M., Murakami, M., Haruna, M., Kojima, A., Asai, T. & Yamada, Y. (2012). Antimicrobial resistance in Shiga toxin-producing *Escherichia coli* O157 and O26 isolates from beef cattle. Japanese journal of infectious diseases 65: 117-121.
12. Vidovic, S., Tsoi, S., Medihala, P., Liu, J., Wylie, J. L., Levett, P. N. & Korber, D. R. (2013). Molecular and antimicrobial susceptibility analyses distinguish clinical from bovine *Escherichia coli* O157 strains. J Clin Microbiol 51: 2082-2088.
13. Mora, A., Blanco, J. E., Blanco, M., Alonso, M. P., Dhabi, G., Echeita, A., Gonzalez, E. A., Bernardez, M. I. & Blanco, J. (2005). Antimicrobial resistance of Shiga toxin (verotoxin)-producing *Escherichia coli* O157:H7 and non-O157 strains isolated from humans, cattle, sheep and food in Spain. Research in microbiology 156: 793-806.
14. Karama, M., Johnson, R. P., Holtslander, R., Gyles, C. L. (2008). Phenotypic and genotypic characterization of verotoxin-producing *Escherichia coli* O103:H2 isolates from cattle and humans. J Clin Microbiol 46: 3569-3575.
15. Srinivasan, V., Nguyen, L. T., Headrick, S. I., Murinda, S. E. & Oliver, S. P. (2007). Antimicrobial resistance patterns of Shiga toxin-producing *Escherichia coli* O157:H7 and O157:H7- from different origins. Microb Drug Resist 13: 44-51.
16. Cergole-Novella, M. C., Pignatari, A. C., Castanheira, M. & Guth, B. E. (2011). Molecular typing of antimicrobial-resistant Shiga-toxin-producing *Escherichia coli* strains (STEC) in Brazil. Research in microbiology 162: 117-123.
17. Medina, A., Horcajo, P., Jurado, S., De la Fuente, R., Ruiz-Santa-Quiteria, J. A., Dominguez-Bernal, G. & Orden, J. A. (2011). Phenotypic and genotypic characterization of antimicrobial resistance in enterohemorrhagic *Escherichia coli* and atypical enteropathogenic *E. coli* strains from ruminants. Journal of veterinary diagnostic investigation : official publication of the American Association of Veterinary Laboratory Diagnosticians, Inc 2011 23: 91-95.