DISTRIBUTION AND PHENOTYPIC CHARACTERISTICS OF ANTIMICROBIAL RESISTANT *ESCHERICHIA COLI* ACROSS ANIMAL SPECIES FROM A SINGLE PRODUCTION UNIT

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I. INTRODUCTION

Emergence and dissemination of antimicrobial resistant bacteria is a global crisis that threatens the remarkable health benefits associated with antibiotics [1]. Antimicrobial resistance can be disseminated among animal species, and between animals and humans through direct or indirect mechanisms. For example, antimicrobial resistant *Escherichia coli* (*E. coli*) in farm animals has shown to spread through animal production units via fecal cross-contamination among groups of animals [2]. This study was conducted to determine the presence, concentration and phenotypic characteristics of antimicrobial resistant *E. coli* in different animal species housed in a single production unit.

II. MATERIALS AND METHODS

Fecal samples from cattle, dogs, pigs and sheep were collected from the Texas Tech University research farm. From each fecal sample, the total *E. coli* was enumerated and isolated on non-selective MacConkey (MAC) agar. Cephalosporin resistant *E. coli* was screened and enumerated on MAC supplemented with 8mg/L of ceftiofur (MAC+ceft) and MAC supplemented with 4mg/L of cefepime (MAC+cefp). Finally, carbapenem resistant *E. coli* was screened and enumerated on MAC supplemented with 2mg/L of ertapenem. After 24 hours of incubation at 37^{o}C, only typical lactose fermenting colonies were counted and isolated. A well isolated colony with typical morphology, was selected from both enumeration and isolation plates and confirmed through selective agar, Gram staining and indole production test as non-type specific *E. coli.* All positive *E. coli* isolates were tested for antimicrobial resistance to amoxicillin/clavulanic acid (AMC, 20µg/10µg), cefotaxime (CTX, 30µg), chloramphenicol (CHL, 30µg), ciprofloxacin (CIP, 5µg), ertapenem (ETP, 10µg), gentamycin (GEN, 10µg) and tigecycline (TGC, 15 µg) using disk diffusion assays according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) protocol [3]. Isolates that were resistant to CTX were confirmed as third generation cephalosporin resistant (3GC^{r}) *E. coli.* The latter were also tested for production of extended spectrum beta lactamases (ESBL) using the combination disk test [4]. The Fischer's exact test of independence was performed to assess the relation between the presence of cephalosporin resistant *E. coli* and the animal species using R software with a *p*-value threshold of 0.05.

III. RESULTS AND DISCUSSION

In general, cephalosporin resistant *E. coli* was present in the production unit but at a very low concentration, less than 1 log CFU/g compared to the total *E. coli* (Table 1). It was observed that, to determine the prevalence of cephalosporin resistant *E. coli* at sample level, a screening step significantly increases the detection level of resistant bacteria. In this study, no cephalosporin resistant *E. coli* was detected from isolates generated by MAC. But, using MAC+ceft and MAC+cefp plates as a screening step, 40 *E. coli* isolates were screened as cephalosporin resistant. In total, 95% of screened cephalosporin resistant *E. coli* were confirmed as 3GC^{r} *E. coli* (38 isolates). The latter isolates were from 31 fecal samples; therefore, the sample level prevalence was 32%. While all *E. coli* isolated on MAC were found susceptible to the antibiotics tested, among confirmed 3GC^{r} *E. coli* isolates, 26.3% were resistant to AMC, 21.1% were resistant to CHL and 2.6% were resistant to CIP and GEN.

The distribution of 3GC^{r} *E. coli isolates* was statistically different across the animal species (*P*= 0.0006). Furthermore, 24 out of 38 confirmed 3GC^{r} *E. coli isolates* were from cattle and only 2 were from sheep, this

suggests that in a production unit with different animal species, cattle may be expected to carry more cephalosporin resistant *E. coli,* but further research is needed to confirm this hypothesis.

Table 1. Prevalence and concentration of non-type specific *E. coli* from different animal species on distinctive MacConkey agar plates

[1]Non-type specific *E. coli* on non-selective MAC
[2]Screened cephalosporin resistant *E. coli* on MAC with ceftiofur
[3]Screened cephalosporin resistant *E. coli* on MAC with cefepime
[4]Log CFU/g
[ab] Within MAC+ceft group, proportions with uncommon letters differ ($P < 0.05$)

	Cattle (n = 30)			Dog (n = 7)			Pig (n = 30)			Sheep (n = 30)			Total (N = 97)		
	MAC[1]	MAC+ ceft[2]	MAC+ cefp[3]	MAC[1]	MAC+ ceft[2]	MAC+ cefp[3]	MAC[1]	MAC+ ceft[2]	MAC+ cefp[3]	MAC[1]	MAC+ ceft[2]	MAC+ cefp[3]	MAC[1]	MAC+ ceft[2]	MAC+ cefp[3]
Prevalence															
Percentage	100.0	46.7[a]	33.3	100.0	28.6[ab]	0.0	100.0	40.0[a]	0.0	100.0	6.7[b]	0.0	100.0	30.9	10.3
Concentration															
Range[4] Minimum	3.68	0	0	3.72	0	0	3.91	0	0	4.41	0	0	3.68	0	0
-				-			-			-			-		
Maximum	7.61	< 1	0	7.56	< 1	0	7.52	< 1	0	7.8	< 1	0	7.8	< 1	0
Mean[4]	5.6	-	-	5.4	-	-	5.6	-	-	5.8	-	-	5.7	-	-

In agreement with Winokur et al. (2001) [5], detection of 3GC^r bacteria may indicate a decrease of cephalosporin efficacy in food animals. Considering the significant threat caused by ESBL producing enterobacteriaceae, 3GC^r *E. coli* isolates were characterized for possible ESBL production. It was found that 71.1% of 3GC^r *E. coli* were, phenotypically, ESBL producers suggesting that the remaining proportion (28.9%) could be AmpC producers. The danger related to ESBL and AmpC producing bacteria is that genes related to these enzymes are mostly plasmid-associated, and therefore can easily spread among bacteria and between different animal species [2,5]. No carbapenem resistant *E. coli* was detected.

IV. CONCLUSION

The distribution of resistant bacteria among animal species housed in a single production unit may be different. Some animals may be more prone to carry resistant bacteria than others. Further studies with molecular characterization and targeting more farms are recommended to confirm the insight provided by this study.

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