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Letter to the Editor

Characterization and clonal grouping of pathogenic *Escherichia coli* isolated from intestinal contents of diarrheic piglets in Villa Clara province, Cuba, according to their antibiotic resistance and ERIC-PCR profiles

Dear Editor,

Enteric infections with pathogenic *Escherichia coli* are important causes of diarrhoea or oedematous disease in young pigs, which are responsible for tremendous economic losses to the swine industry due to mortality, costs of medication, and growth retardation (Francis, 1999). Studies aimed on the differential identification of enteropathogens have reported enterotoxigenic *E. coli* as a commonly associated pathogen with piglet's diarrhoea worldwide (Katsuda et al., 2006; Hong et al., 2006). However, in Cuba, where diarrheic diseases are responsible for 31% and 37% of pig's mortality during the pre-weaning and post-weaning periods, respectively (Cabrera and García, 2009), the identification and characterization of enteropathogens by the Veterinary Diagnostic Laboratories are very limited. Recently, a survey conducted in the frame of an Interuniversity Cooperation Program among the Flemish Interuniversity Council of Belgium and the Central University of Cuba determined a high seroprevalence of specific antibodies against the F4 and the F18 fimbriae in the Cuban swine herd, suggesting that $F4^+$ and $F18^+$ pathogenic *E. coli* are widespread as potential etiologic agents of colibacillosis (de la Fé Rodríguez et al., 2011); also, enterotoxigenic *E. coli* was the most frequently detected enteropathogen (25.6%) in diarrheic pigs from Villa Clara province, Cuba, during another survey aimed on the differential identification of *E. coli*, *Salmonella*, toxigenic *Clostridium perfringens*, Rotavirus A, TGEV, PEDV, *Cryptosporidium*, *Eimeriidae*, and helminths (de la Fé Rodríguez et al., in preparation).

As important contribution to the epidemiological characterization of swine colibacillosis in Cuba, 26 pathogenic *E. coli* previously isolated from intestinal contents of pigs suffering pre-weaning or post-weaning diarrhoea in six large indoor piggeries located in Villa Clara province, were assayed for their antibiotic resistance by the disc diffusion method according to the NCCLS (M2-A7, M100-S10) and for their genetic relatedness by the ERIC-PCR fingerprinting analysis using primers ERIC1R and ERIC2 (Versalovic et al., 1991; Osek, 2000).

Different from reports showing high resistance of *Enterobacteriaceae* to a wide range of antibiotics (Hendriksen et al., 2008; Smet et al., 2009), herein were exclusively displayed high resistance rates (number of resistant and intermediate-resistant isolates * 100/26) to antibiotics routinely used for treating diarrhoea and other diseases in Cuban piggeries: tetracycline-30 μg (69%), ampicillin-10 μg (54%), sulphonamide compounds-300 μg (50%), and kanamycin-30 μg (50%). Vieira et al. (2009) reported a positive association between tetracycline treatment incidence rate and the probability of isolating a tetracycline resistant *E. coli* from pigs. Gentamicin-10 μg resistance rate was low (4%) despite being frequently administered in Cuba. Except for chloramphenicol-30 μg and nalidixic acid-30 μg, for which 12% resistance rates were displayed, all isolates were susceptible to antibiotics less or practically never administered in Cuban piggeries (i.e. trimethoprim-5 μg, trimethoprim:sulphamethoxazole/1:19–25 μg, amoxicilline-clavulanic acid-30 μg, ciprofloxacin-5 μg, cefotaxime-30 μg, cefazolin-30 μg, and amikacin-30 μg). Maynard et al. (2003) concluded that the genes behind phenotypic resistance are not static but are rather in a state of flux driven by various selection forces such as the use of antimicrobials.

Multi-drug resistance was present in 65% of isolates: 4 (15%) were resistant to two antibiotics, 3 (12%) to three, 8 (31%) to four, and 1 (4%) to five and six. $F18^+/LT^+/STb^+$ and $F4^+/STa^+/STb^+$ virotypes were the most multi-resistant among fimbriated *E. coli*. Chloramphenicol resistance was mostly found in $F18^+/LT^+/STb^+$ *E. coli*, and verotoxigenic $F18^+$ isolates were susceptible to all antibiotics, except to sulphonamides (Fig. 1).

It is interesting that similar to this report, Maynard et al. (2003), Hariharan et al. (2004), Varga et al. (2008), and Akwar et al. (2008) found high resistance rates to tetracycline, ampicillin, and sulphonamide compounds, as well as high susceptibility to gentamicin and cephalosporins in porcine enteric *E. coli* from Canada. In Cuba, thousands of breeding stock pigs have been imported from Canada during last decade, and they could be the source of pathogens of non-obligatory declaration like *Enterobacteriaceae* carrying genes encoding drug resistance, but other epidemiological studies are needed to prove it. Previous information about antibiotic resistance of porcine *E. coli* from Cuba is not available (Blanco et al., 2006).

There were high degrees of polymorphism in the DNA sequences of *E. coli* tested herein, suggesting that most of them are from different clones: 17 clonal groups were

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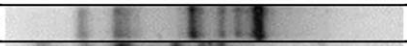
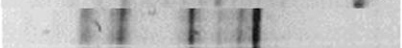
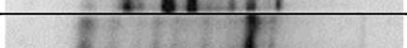
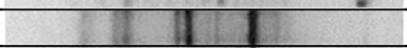
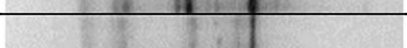
Virotype	Piggery/pig	ERIC-PCR profile	Antibiotic resistance profile	Group
STa$^+$	D/s		Amp-Sul	I
STb$^+$	F/w		Tet-Amp-Sul-Kan-Chl-Gen	II
	A/w		None	III
	A/s		None	III
STa$^+$/STb$^+$	F/s		Tet-Amp-Kan	IV
	D/w		Tet-Kan-Nal	V
	D/w		Tet-Amp-Kan-Nal	V
	D/s		Tet-Kan	VI
	D/s		Kan	VII
				
	B/s		Tet	VIII
	B/s		Tet	VIII
	B/s		Tet-Amp	IX
LT$^+$/STb$^+$	C/s		Tet-Amp-Sul-Kan	X
F18$^+$/LT$^+$/STb$^+$	E/w		Tet-Amp-Sul-Chl	XI
				
	F/w		Tet-Sul-Kan-Chl-Nal	XI
F18$^+$/STx2e$^+$	D/w		Sul	XII
	D/w		None	XII
	D/w		Sul	XII
F6$^+$/STa$^+$	D/w		None	XIII
				
F4$^+$	C/w		Tet-Amp-Sul-Kan	XIV
F4$^+$/STa$^+$/STb$^+$	E/s		Tet-Amp-Sul-Kan	XV
	E/s		Tet-Amp-Sul	XV
	C/s		Tet-Amp-Sul-Kan	XV
	C/w		Tet-Amp-Sul-Kan	XV
	C/w		Tet-Amp-Sul-Kan	XVI
F5$^+$/F41$^+$/STa$^+$	E/s		Tet-Amp	XVII
S. Newport	F/w		Amp-Sul	S-I
	A/w		Sul	S-I
				

Fig. 1. Clonal groups of pathogenic *E. coli* associated with piglet's diarrhoea in Villa Clara province, Cuba, according to their virotype, ERIC-PCR and antibiotic resistance profiles. S, suckling piglet; W, weaned pig; M, 100 bp marker run in the same gel of ERIC-PCR products visualized on its top; Amp, ampicillin; Sul, sulphonamide compounds; Tet, tetracycline; Kan, kanamycin; Chl, chloramphenicol; Gen, gentamicin; Nal, nalidixic acid; *S.* Newport, *Salmonella enterica* subspecie *enterica* serovar Newport isolated from intestinal contents of weaned pigs.

distinguished based on the virotype of the isolates as well as on the similarity of their ERIC-PCR electrophoretic banding pattern and their antibiotic resistance. Overall, the ERIC-PCR profiling correlated with the antibiotic resistance and with the virotype of isolates (Fig. 1). For instance, 3 verotoxigenic isolates from piggery D belonged to a very homogeneous group (XII) as they generated almost an identical fingerprinting and resistance profiles; other isolates from groups III, XI, and XV had the same condition. There appeared a genetic diversity among 8 STa$^+$/STb$^+$ isolates, which were categorized into 6 clonal groups. Among herds some isolates (e.g. F4$^+$ or F18$^+$) were found to be clonally related. The often trading of pigs among piggeries and other ways affecting piggery's biosafety could lead the spreading of pathogenic *E. coli* in Villa Clara. Clonal relations were found inside herds for STb$^+$, STa$^+$/STb$^+$, F18$^+$/STx2e$^+$ or F4$^+$/STa$^+$/STb$^+$ virotypes. These findings confirm that ERIC-PCR, by which pathogenic *E. coli* isolated in Cuba were tested for the first time, is an accurate and reliable technique for studying the molecular epidemiology of *E. coli* (Dalla-Costa et al., 1998; Namvar and Warriner, 2006; Yuan et al., 2010).