Pathotyping and antibiotic resistance of porcine enterovirulent *Escherichia coli* strains from Switzerland (2014–2015)

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Abstract

A total of 131 porcine *E. coli* were isolated in 2014 and 2015 from the gut of 115 pigs raised in Switzerland and suffering from diarrhea. The isolates were tested for antibiotic resistance, serotypes, virulence factors and genetic diversity. Serotypes were assigned by agglutination tests and virulence genes were identified by polymerase chain reaction (PCR). Antibiotic resistance profile was determined by the measurement of the MIC of 14 antibiotics and by the detection of the corresponding genes using microarray and PCR approaches. Genetic diversity was determined by repetitive palindromic PCR (rep-PCR) revealing a heterogenous population. Half of the *E. coli* isolates possessing virulence factors could not be assigned to any of the 19 serotypes tested, but contained toxins and adhesins similarly to the sero-typable *E. coli* isolates. The most prevalent *E. coli* serotypes found were K88ac (18%), O139:K82 (6%), O141:K85ac (5%), O108:K`V189` (5%), O119:K`V113` (3%) and O157:K`V17` (2%). The combination of toxins EAST-1, STb and LT-I and adhesin F4 characterizing ETEC was the most frequent. The shigatoxin Stx2e (STEC) and intimin Eae (EPEC) were also detected, but less frequently. Seventy percent of the isolates were resistant to at least one antibiotic and 29% were resistant to more than 3 antibiotics. Isolates exhibited resistance to tetracycline (50%) associated to resistance genes *tet*(A), *tet*(B) and *tet*(C), sulfamethoxazole (49%) [*sul1*, *sul2* and *sul3*], trimethoprim (34%) [*dfr*], nalidixic acid (29%), ampicillin (26%) [bla_{TEM-1}], gentamicin (17%) [*aac(3)-IIc*, *aac(3)-IVa* and *aac(3)-VIa*], chloramphenicol (17%) [*catAI* and *catAIII*], and ciprofloxacin (8%) [mutations in GyrA (S83L) and ParC (S80I)]. All isolates were susceptible to 3rd generation cephalosporins, carbapenems, colistin and tigecycline. Pathogenic *E. coli* isolates from pigs in Switzerland could frequently not be assigned to a known serotype even if they contained diarrhea-causing virulence factors. They also harbor resistance mechanisms conferring resistance to antibiotics which are commonly used in pig husbandry, except for colistin. A

Pathotypiserung und Antibiotikaresistenzprofile von porcinen enterovirulenten *Escherichia coli* aus der Schweiz (2014–2015)

Im Rahmen dieser Studie wurden in den Jahren 2014 und 2015 total 131 *E. coli* Stämme aus Därmen von 115 Schweizer Schweinen isoliert, welche an Durchfall litten. Die Isolate wurden auf Antibiotikaresistenzen getestet, zudem wurden die Serotypen, die Virulenzfaktoren und die genetische Diversität bestimmt. Die Zuordnung der *E.coli*-Isolate zu den verschiedenen Serogruppen fand mit Hilfe der Serumagglutination statt und die Virulenzfaktoren wurden mit einer Polymerase-Ketten-Reaktion (PCR) bestimmt. Mit der Messung der Minimalen Hemmkonzentration von 14 Antibiotika konnten die Antibiotikaresistenzprofile bestimmt werden. Die dazugehörenden Gene wurden mittels Microarray und PCR identifiziert. Die genetische Diversität wurde mittels repetitiver palindromischer PCR (rep PCR) bestimmt. Die Hälfte der *E.coli*-Isolate, welche Virulenzfaktoren besitzen, konnten keinem der 19 getesten Serotypen zugeordnet werden, obwohl sie dieselben Toxine und Adhäsine besassen wie die serotypisierbaren Stämme. Die am häufigsten gefunden Serotypen waren K88ac (18%), O139:K82 (6%), O141:K85ac (5%), O108:K`V189` (5%), O119:K`V113` (3%) und O157:K`V17` (2%). Die Kombination der Toxine EAST-1, STb and LT-I und dem Adhäsin F4, welche charakteristisch sind für enterotoxische *E. coli* (ETEC), wurde am häufigsten gefunden. Das Shigatoxin Stx2e (STEC) und Intimin Eae (EPEC) hingegen waren eher seltener zu finden. Die Resultate der rep PCR zeigten eine heterogene Population. Siebzig Prozent der Isolate zeigten eine Resistenz gegenüber mindestens einem Antibiotikum und 29% waren resistent gegenüber mehr als 3 Antibiotika. Die Isolate wiesen Resistenzen gegenüber Tetracyclin (50%) assoziiert mit den Resistenzgenen *tet*(A), *tet*(B) und *tet*(C), Sulfamethoxazol (49%) [*sul1*, *sul2* und *sul3*], Trimethoprim (34%) [*dfr*], Nalidixinsäu-

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careful identification of the causative agent and antibiotic susceptibility testing is highly recommended for targeted therapy and prudent use of antibiotics.

Keywords: *E.coli*, pigs, antibiotic resistance, virulence factors, genotyping

re (29%), Ampicillin (26%) [$bla_{\text{TEM-1}}$], Gentamicin (17%) [*aac(3)-IIc*, *aac(3)-IVa* and *aac(3)-VIa*], chloramphenicol (17%) [*catAI* and *catAIII*] und Ciprofloxacin (8%) [Mutationen in GyrA (S83L) und ParC (S80I)] auf. Alle Isolate waren empfindlich gegenüber 3. Generation Cephalosporinen, Carbapenemen, Colistin und Tigecyclin. Pathogene *E.coli* von Schweinen aus der Schweiz konnten den bekannten Serogruppen oftmals nicht zugeordnet werden, obwohl sie Durchfall verursachende Virulenzfaktoren besassen. Zudem wurden verschiedene Resistenzmechanismen gegen Antibiotika, welche routinemässig in der Schweinehaltung eingesetzt werden, entdeckt. Folglich ist eine konsequente und sorgfältige Identifikation der krankheitsverursachenen Erreger und die Antibiotikaempfindlichkeitstestung vor einer Therapie von grosser Bedeutung.

Schlüsselwörter: *E.coli*, Schweine, Antibiotikaresistenz, Virulenzfaktoren, Genotypisierung

Introduction

Pathogenic *E. coli* are among the most common causative agents of diarrhea in pigs. They are characterized by the presence of specific virulence factors including toxins and adhesins. Enterotoxigenic *E. coli* (ETEC) produce plasmid-regulated enterotoxins which are secreted proteins or peptides, classified as heat-labile (LT) and heat-stabile (STa, STb) toxins. Both toxins lead to hypersecretion of electrolytes into the intestine, resulting in fluid secretion and decreased absorption, thus causing diarrhea. Most of the ETEC strains also possess fimbrial adhesins, which are proteinaceous appendages of the outer membrane of the bacterial cell and are specific to host receptors (Nagy and Fekete, 2005). The typical adhesins found in ETEC causing acute watery diarrhea in newborn and weaned pigs are F4 (syn. K88), F5 (syn. K99), F6, F17a, F18ac and F41 (DebRoy and Maddox, 2001). Enteropathogenic *E. coli* (EPEC) which are also associated with diarrhea in pigs possess intimin (Eae) as a major adhesin. EPEC and ETEC may also produce an enteroaggregative heat-stable enterotoxin 1 (EAST-1), which consists of a small 38 amino acid protein encoded by the *astA* gene and which has been suggested to play a role in colibacillosis in pigs (Choi et al., 2001; Paiva de Sousa and Dubreuil, 2001; Osek, 2003; Veilleux and Dubreuil, 2006). Shigatoxin-producing *E. coli* (STEC) in pigs are characterized by the presence of shigatoxin Stx2e, and F18ab as major adhesin. STEC can cause both diarrhea and edema disease by damaging enterocytes followed by edema, haemorrhage and thrombosis (Quinn, 2011).

Pathogenic *E. coli* of pigs can also be grouped by serotyping based on somatic (O) and capsular (K) antigens with K88ac being the most predominant serotype (Quinn, 2011). Certain serotypes are often associated with specific virulence factors and serotyping has therefore been widely used for the identification of pathogenic *E. coli* in veterinary diagnostic. However, serotyping methods may overlook a possible absence of toxins and are frequently limited to a low number of commercially available antibodies only recognizing specific serotypes, emphasizing the need of the additional detection of the virulence factors for more accurate diagnostic.

To treat affected pig herds, antibiotics are administered via feed with tetracycline, sulphonamides, trimethoprim and colistin being the most frequently used antibiotics in pig husbandry in Switzerland (Anresis-Archvet, 2016). As a consequence, resistance to these drugs may develop among the porcine pathogens leading to therapeutic failures. A study conducted in Switzerland in 2003 by Lanz et al. already showed that a large majority of pathogenic *E. coli* isolated from pigs were resistant to those antibiotics which are commonly used for therapy except colistin. Since then, no more studies analyzing the antibiotic resistance situation in enterovirulent *E. coli* from pigs in Switzerland have been conducted. The emergence of 3rd generation cephalosporin resistance (Seiffert et al., 2013) as well as the worldwide dissemination of the recently discovered plasmid-mediated colistin resistance in *E. coli* from animals including pigs (Liu et al., 2016) prompted us to determine the actual resistance situation among pathogenic *E. coli* isolated in 2014 and 2015 from pigs with diarrhea within the PathoPig project. The PathoPig project was launched in 2014 by the Federal Food Safety and Veterinary Office (FSVO) to enable a selective clarification of diseases in piggeries

by subsidized diagnostic investigations (Hadorn, 2016). Within this project, we have been assigned the task to determine antibiotic resistance of pathogenic *E. coli* isolated from pigs suffering from diarrhea.

Animals, Material and Methods

Sample collection, species identification and growth conditions

Samples from 115 pigs suffering from diarrhea were tested for the presence of enterovirulent *E. coli* (85 samples from large intestine, 36 samples from small intestine, 10 rectal swabs) at the ZOBA, Institute of Veterinary Bacteriology, University of Bern, Switzerland. Specimens were plated onto both trypticase soy agar plates containing 5% sheep blood (TSA-SB; Becton, Dickinson and Company, New Jersey, USA) and BROLAC agar plates (Thermo Fisher Scientific, Waltham, USA) and incubated aerobically for 18 to 24 hours at 37 °C. Suspicious *E. coli* were tested for the ability to split tryptophan into indol and for the absence of oxidase. Identification was confirmed using Matrix Assisted Laser Desorption/Ionization Time of Flight (MALDI-TOF) (Microflex LT, Bruker Daltonics GmbH, Bremen, Germany).

Serotyping, genetic diversity and detection of virulence factors

Serotypes were determined by slide agglutination tests using a set of O and K rabbit antisera representing the most common porcine *E. coli* serotypes (K88ac, O149:K91, O147:K89, O8:K87, O141:K85, O138:K81, O45:K'E65', O139:K82, O157, O9:K103,987P, O64:K'V189', O10:K'V50', O35:K'V79', O115:K'V165', O119:K'V113', K91, K89, K103, K87) (APHA, Weybridge, Surrey, UK).

The virulence factors were identified by PCR targeting fimbrial (F5, F6, F18ab, F18ac, F41), enterotoxin (LT-Ia, LT-Ib, STa, STb) and shigatoxin (Stx2e) genes according to the protocol "Bosworth- PCR" (Casey and Bosworth, 2009) modified according to (Barth et al., 2007). The presence of the EAST-1 toxin was determined by the detection of the *astA* gene by PCR (Yamamoto and Na-

Table 1: Distribution of the virulence factors among the different serotypes identified in enterovirulent *E. coli* from pigs in Switzerland.

Serotype (n)	Virulence factors and combinations of virulence factors (n)																												
	ETEC (n=66)																						STEC (n=4)		EPEC (n=3)		other/mix (n=46)			
	LT-I	F5	F6	STa, EAST-1	STb, EAST-1	F4, EAST-1	F5, EAST-1	F6, EAST-1	STb, LT-I, F4	STb, LT-I, F4, EAST-1	STb, LT-I, F4, F5, EAST-1	STa, STb, LT-I, F4	STa, STb, F18	STa, STb, F18, EAST-1	STa, F4, EAST-1	STa, F6, EAST-1	STa, STb, F5, EAST-1	STa, F18, EAST-1	STb, LT-I, EAST-1	STa, STb, EAST-1	STa, F5, EAST-1	STx2e, F18	STx2e, F18, EAST-1	Intimin	Intimin, EAST-1	F18	EAST-1	F18, EAST-1	STa, STx2e
K88ac (23)		1							1	15	2	1	2		1												1		
O8:K87 (1)																											1		
O9:K103.987P (1)							1																						
O10:K`V50` (2)			1																								1		
O35:K`V79` (2)							2																						
O64:K`V142` (2)																											2		
O108:K`V189` (6)				1			1	2																		2			
O119:K`V113` (4)								1																			1		
O139:K82 (8)																1						2	2				1	2	
O141:K85ac (7)														2			1	1									1	1	1	
O147:K89 (1)																											1		
O157:K`V17` (3)					2													1											
Non-serotypable with virulence genes (59)	1			2	3	1	6	1	1					1		2		1	5	1	1			1			31	1	1
Non-serotypable without virulence genes (12)																													
Total	1	1	1	3	5	1	10	4	2	15	2	1	2	3	1	3	1	3	5	1	1	2	2	1	2	1	40	4	1

kazawa, 1997). Genetic diversity between the strains was determined by repetitive palindromic PCR (rep-PCR) (Vila et al., 1996).

Antimicrobial susceptibility testing and antibiotic resistance gene detection

Minimal inhibitory concentrations (MIC) were obtained for 14 antibiotics by broth microdilution in cation-adjusted Mueller-Hinton broth using EUVSEC Sensititre® plates (Thermo Fisher Scientific, Waltham, USA) following the guidelines and interpretation criteria of the European Committee of Antimicrobial Susceptibility Testing (EUCAST, online www.eucast.org) and the Clinical and Laboratory Standards Institute (CLSI, 2016). Antibiotic resistance genes were detected using AMR08 ArrayStripTM microarrays (an upgrade of (Card et al., 2013)) and the HybridisationPlus (+) Kit (Alere Technologies GmbH, Jena, Germany). A signal intensity of 0.1 or higher was considered positive. Resistance genes were confirmed by PCR as previously described (Matter et al., 2007; Endimiani et al., 2008), and using primers aac(3)-VIa-F (5'-ATTCTCGCCTTCGTCTCGTG-3') and aac(3)-VIa-R (5'-ATGCCGTTCGAATCCCAGTC-3') for the detection of the *aac(3)-VIa* gene.

Mutations in the fluoroquinolone resistance determining region of GyrA and ParC were detected from the amino acid sequence obtained from translated PCR products amplified using taq polymerase and primer pairs Ecoli_gyrA_F2 (5'-ACGTACTAGGCAATGACTGG-3') and Ecoli_gyrA_R2 (5'-ATATACGCCAGACAACCGTT-3') and Ecoli_parC_F2 (5'-CGTTGCCGTTTATTGGTGAT-3') and Ecoli_parC_R2 (5'-TATGCGGTGGAATATCGGTC-3') with an annealing temperature of 55 °C and an extension time of 30s.

Results

Serotyping and virulence factors

A total of 131 *E. coli* were isolated from 115 pigs suffering from diarrhea. Twelve *E. coli* did not contain any known virulence factors and were not further analyzed since they were likely to belong to the normal apathogenic *E. coli* flora. The remaining 119 isolates showed 85 different rep-PCR profiles. They were all found to contain virulence factors characteristic for pathogenic *E. coli* from pigs. The combination of toxins EAST-1, STb and LT-I and adhesin F4 characterizing ETEC was the most frequent (Tab. 1). The shigatoxin Stx2e

Table 2: Minimal inhibitory concentrations (MIC) of 14 antibiotics and corresponding antibiotic resistance genes for 119 *E. coli* isolated from pigs.

		Number of strains with MIC (µg/ml) of																	
Antimicrobial	Resistance breakpoint	<0.015	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Ampicillin	>8 (EUCAST)							5		48	35		1		2	28			
Azithromycin	NA								9		47	60	2	1					
Cefotaxime	>2 (EUCAST)					119													
Ceftazidime	>1 (EUCAST)						119												
Chloramphenicol	>8 (EUCAST)										99		6	5		4	5		
Ciprofloxacin	>1 (EUCAST)	75		8	3	4	16	2	1			6	4						
Colistin	>2 (EUCAST)							119											
Gentamicin	>4 (EUCAST)						51		42	6		1	6	2	11				
Meropenem	>8 (EUCAST)		119																
Nalidixic acid	>16 (CLSI)									81		3		1	8	8	18		
Sulfamethoxazole	>512 (CLSI)										5		14	16	17	9			2
Tetracycline	>4 (CLSI)								43		16	2	5	1	15	37			
Tigecycline	>2 (EUCAST)					104		15											
Trimethoprim	>4 (EUCAST)					39		21	15	2	1				41				

The dilution ranges tested for each antibiotic are those contained within the white area. Values situated above or below this range indicate MIC values higher than the highest concentration tested and values smaller than or equal to the lowest concentration tested respectively. Resistance breakpoints for Enterobacteriaceae (vertical lines) were obtained from the European Committee of Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI). ND, no gene detected. NA, not available. NI, not investigated.
dfrA, dihydrofolate reductase for trimethoprim resistance; *sul*, dihydropteroate synthetase gene for sulphonamide resistance; *tet*, tetracycline efflux gene; *cat*, chloramphenicol acetytransferase gene; bla_{TEM-1}, beta-lactamase gene; *aac(3)*, aminoglycoside acetyltransferase gene; GyrA (S83L), Gyrase A with a serine to leucine substitution in the fluoroquinolone-determining region at position 83; ParC (S80I), topoisomerase IV with a serine to isoleucine substitution at position 80.

(STEC), as well as adhesins F5, F6 and F18 and intimin Eae (EPEC) were also detected, but less frequently (Tab. 1). Half of the *E. coli* isolated possessing virulence factors (59 of 119 isolates) could not be assigned to any of the 19 serotypes tested. Nevertheless, they contained toxins and adhesins similarly to the *E. coli* isolates which could be assigned to a certain serotype (Tab. 1), with a majority containing EAST-1 either as a single toxin or in combination with other virulence factors. The most frequent pathotype pattern STb, LT-I, EAST-1 and F4 was associated with the ETEC serotype K88ac, whereas pathotype pattern Stx2e, EAST-1 and F18 was associated with the STEC serotype O139:K82. No specifically conserved toxin-adhesin combination could be found among the other serotypes. The most frequent *E. coli* serotypes found in this study were K88ac (18%), O139:K82 (6%), O141:K85ac (5%), O108: K`V189` (5%), O119:K`V113` (3%) and O157:K`V17` (2%). The remaining six other serotypes were only found once or twice (Tab. 1). Based on the combination of toxins and adhesins, 66 of the *E. coli* belonged to ETEC, 3 to EPEC, 4 to STEC and 46 could not be assigned to either of these groups.

>1024	% of resistant strains	% of resistance genes in resistant strains
	26	bla_{TEM-1} (87)
		NI
	0	ND
	0	ND
	17	*catAI* (67), *catAIII* (50)
	8	GyrA (S83L) (100), ParC (S80I) (100)
	0	ND
	17	*aac(3)-IIc* (15), *aac(3)-IVa* (15), *aac(3)-VIa* (10)
	0	ND
	29	NI
56	49	*sul1* (57), *sul2* (64), *sul3* (31)
	50	*tet*(A) (65), *tet*(B) (23), *tet*(C) (35), *tet*(D) (3), *tet*(E) (2)
	0	ND
	41	*dfrA1* (59), *dfrA5* (7), *dfrA7* (7), *dfrA12* (10), *dfrA13* (7), *dfrA14* (5), *dfrA17* (5), *dfrA19* (7)

Antibiotic resistance profile

The resistance profile was further determined for those 119 isolates which contained virulence factors (Tab. 2). Seventy percent of the isolates were resistant to at least one antibiotic with 18% of the isolates being resistant to one, 13% to two, 10% to three, 7% to four, 13% to five, 5% to six, 3% to seven and 2% to eight of the antibiotics tested (Tab. 3). The most frequent combinations of resistance were those including sulfamethoxazole, trimethoprim and tetracycline. All 119 isolates were susceptible to human clinically important antibiotics such as cefotaxime, ceftazidime, colistin, meropenem and tigecycline (Tab. 2). Predominant resistances were those to tetracycline found in 50% of the isolates, sulfamethoxazole (49%), and trimethoprim (34%), followed by resistance to nalidixic acid (29%), ampicillin (26%), gentamicin (17%), chloramphenicol (17%) and ciprofloxacin (8%). Antibiotic resistance could be attributed to the presence of acquired genes of the tet (tetracyclines), bla_{TEM-1} (penicillins), *sul* (sulphonamides), *aac* (aminoglycosides) and *dfr* (trimethoprim) families, as well as to mutations in the fluoroquinolone resistance-determining region of the chromosomal topoisomerases GyrA and ParC (Tab. 2). The streptomycin resistance genes *strA* and *strB* were also detected in 8% and 16% of the isolates respectively.

Discussion

Serotyping combined with detection of virulence factors in *E. coli* isolates from pigs suffering from diarrhea showed that half of them were not serotypable using commercially available sera but contained virulence factors. The majority of the non-serotypable isolates contained the *astA* gene responsible for the production of the EAST-1 toxin, which has been suggested to play a role in the pathogenicity of *E. coli* in pigs (Choi et al., 2001; Frohlicher et al., 2008). Only a few strains did not harbor virulence factors suggesting that they were not likely to be associated with the disease, even we cannot exclude that they carry yet undescribed toxins. Detection of toxins in *E. coli* which could not be assigned to a certain serotype by agglutination indicates that serotyping is not reliable enough for the diagnostic of pathogenic *E. coli*. For an accurate identification of pathogenic *E. coli* especially in regards of prudent use of antibiotics, it is rather necessary to search for virulence factors. In this study, already more than 25% of the isolates showed resistance to tetracycline, sulfamethoxazole and trimethoprim which belong to the first choice antibiotics for the treatment of *E. coli* diarrhea in pigs in Switzerland (X. Sidler, personal communication). The high percentage of isolates resistant to antibiotics used for the treatment of diarrhea in pigs emphasizes the importance of avoiding unnecessary use of antibi-

Table 3: Distribution of multidrug resistance profile (≥ 3 antibiotics) among pathogenic *E. coli* from diarrhea in Switzerland.

Number of resistance	Antibiotic resistance profile (n)	Number of strains (%)
3	SMX, TET, GEN (2) SMX, TMP, TET (4) SMX, TET, CHL (2) SMX, TET, AMP (1) TET, GEN, AMP (1) TMP, CIP, NAL (1) TMP, NAL, CHL (1)	12 (10%)
4	SMX, TMP, TET, GEN (1) SMX, TMP, TET, CHL (3) SMX, TMP, NAL, AMP (1) SMX, TMP, TET, AMP (2) SMX, TMP, AMP, GEN (1)	8 (7%)
5	SMX, TMP, TET, AMP, GEN (2) SMX, TMP, TET, NAL, GEN (5) SMX, TMP, TET, NAL, AMP (3) SMX, TMP, NAL, CHL, AMP (2) SMX, TMP, CIP, NAL, CHL (1) SMX, CIP, TET, NAL, AMP (1) SMX, TET, NAL, AMP, GEN (1)	15 (13%)
6	SMX, TMP, CIP, TET, NAL, AMP (3) SMX, TMP, TET, NAL, AMP, GEN (1) SMX, TET, NAL, CHL, AMP, GEN (1) SMX, TMP, TET, NAL, CHL, AMP (1)	6 (5%)
7	SMX, TMP, CIP, TET, NAL, CHL, AMP (2) SMX, TMP, TET, NAL, CHL, AMP, GEN (2)	4 (3%)
8	SMX, TMP, CIP, TET, NAL, CHL, AMP, GEN (2)	2 (2%)

SMX, sulfamethoxazole; TMP, trimethoprim; CIP, ciprofloxacin; TET, tetracycline; NAL, nalidixic acid; CHL, chloramphenicol, AMP, ampicillin; GEN, gentamicin

otics including colistin. Even if we did not detect any resistance to colistin in enterovirulent *E. coli* yet, the worldwide dissemination of the transferable colistin resistance genes *mcr-1/2* in *Enterobacteriaceae* in different environments including pigs underlines the need for prudent and restricted use of colistin (European Medicine Agency, 2016; Liu et al., 2016). Emergence and spread of colistin resistance in enterovirulent *E. coli* would dramatically jeopardize therapeutic options in pigs. Of note, the colistin resistance gene *mcr*-1 was recently detected in feces from healthy pigs at slaughterhouse, indicating that it is already present in the Swiss pig population and its selection should be avoided by the unnecessary use of colistin (Anresis-Archvet, 2016). Also, resistance to critically important fluoroquinolones already reached 8% and its increasing use as an alternative to colistin would also contribute to an increase of the fluoroquinolone-resistant *E. coli* population. These critically important antibiotics should only be administered after correct bacterial diagnostic and antibiotic resistance testing and only under control of a veterinarian in order to avoid random use of these antibiotics. Of note, no resistance to fluoroquinolones was detected in 2003 by Lanz et al. among pathogenic *E. coli* from pigs indicating that resistance to this class of antibiotics emerged during the past 15 years. However at that time, resistances to sulfonamides, tetracyclines and trimethoprim were already observed in a similar proportion with 81% of the *E. coli* strains being resistant to sulfonamides, 20% to sulfonamide-trimethoprim, 54% to tetracycline, 16% to ampicillin and 10% to gentamicin. Resistances were also associated with genes known to be present on transferable genetic elements (Lanz et al., 2003).

Conclusion

A correct identification of the causative agent and accurate antibiotic susceptibility testing following guidelines for prudent use of antibiotic are recommended for an adequate therapy of diseases caused by *E. coli* (European Commission, 2015; European Medicine Agency, 2016). These diagnostic measures are essential for suppressing dissemination of existing antibiotic resistance and for avoiding emergence of resistance to important antibiotics in animal husbandry such as colistin and the fluoroquinolones.

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Pathotypisation et profils de résistance aux antibiotiques des *Escherichia coli* porcins entéro-virulents en Suisse (2014–2015)

Dans la cadre de cette étude, on a isolé, durant les années 2014 et 2015, 131 souches d'*E.coli* provenant des intestins de porcs suisses souffrant de diarrhée. Ces souches ont été testées quant à leurs résistances aux antibiotiques; en outre on a déterminé leurs sérotypes, leurs facteurs de virulence et leur diversité génétique. L'attribution des isolats d'*E.coli* aux divers sérogroupes a été réalisée au moyen d'une séro-agglutination et les facteurs de virulence ont été déterminés par PCR. Les profils de résistance aux antibiotiques ont été déterminés par la mesure des concentrations inhibitrices minimales de 14 antibiotiques. Les gènes qui y étaient associés ont été identifiés par puces à ADN et PCR. La diversité génétique a été déterminée au moyen de PCR répétitives palindromiques (rep PCR). La moitié des isolats d'*E.coli* qui possédaient des facteurs de virulence n'ont pas pu être classés dans un des 19 sérotypes testés, bien qu'ils aient possédé les mêmes toxines et adhésines que les isolats qu'il a été possible de typiser. Les sérotypes les plus fréquemment trouvés étaient (18%), O139:K82 (6%), O141:K85ac (5%), O108:K`V189` (5%), O119:K`V113` (3%) et O157:K`V17` (2%). La combinaison des toxines EAST-1, STb et LT-I et de l'adhésine F4, qui est caractéristique pour les E.coli entérotoxiques (ETEC), a été le plus fréquemment trouvée. La shigatoxine Stx2e (STEC) et l'Intimin Eae (EPEC) étaient par contre plutôt rares. Les résultats des rep PCR montraient une population hétérogène. 70% des isolats présentaient une résistance face à au moins un antibiotique et 29% étaient résistants à plus de 3 antibiotiques. Les isolats montraient des résistances vis-à-vis de la tétracycline (50%) associée avec les gènes de résistance *tet*(A), *tet*(B) et *tet*(C), du sulfaméthoxazole (49%) [*sul1*, *sul*2 et *sul*3], du trimethoprime (34%) [*dfr*], de l'acide nalidixique (29%), de l'ampicilline (26%) [$bla_{\text{TEM-1}}$], de la gentamicine (17%) [*aac(3)-IIc*, *aac(3)-IVa* et *aac(3)-VIa*], du chloramphenicol (17%) [*catAI* and *catAIII*] et de la ciprofloxacine (8%) [mutations dans GyrA (S83L) et ParC (S80I)]. Tous les isolats étaient sensibles aux céphalosporines de troisième génération, au carbapénèmes, à la colistine et à la tigecycline. Les *E.coli* pathogènes des porcs en Suisse n'ont souvent pas pu être attribués aux sérogroupes connus, bien qu'ils possèdent les facteurs de virulence causant la diarrhée. En outre on a découvert divers mécanismes de résistance contre des antibiotiques qui sont régulièrement utilisés dans la production porcine. En conséquence, une identification rigoureuse et soignée des germes responsables de maladie et un testage de la sensibilité avant traitement est de première importance.

Patotipizzazione e profili di resistenza agli antibiotici dell'*Escherichia coli* enterovirulenta nei suini in Svizzera (2014-2015)

In questo studio tra gli anni 2014 e 2015, sono stati isolati un totale di 131 ceppi di *E. coli* dal budello di 115 suini svizzeri che soffrivano di diarrea. Gli isolati sono stati testati alla resistenza antibiotica e quindi si è determinato il sierotipo, i fattori di virulenza e la diversità genetica. L'assegnazione degli isolati di *E. coli* tra i diversi gruppi sierologici ha avuto luogo con l'aiuto di agglutinazione sierica e i fattori di virulenza sono stati determinati utilizzando una reazione a catena della polimerasi (PCR). Misurando la minima concentrazione inibente di 14 antibiotici, è stato possibile determinare i profili di resistenza agli stessi. I geni corrispondenti sono stati identificati mediante microarray e PCR. La diversità genetica è stata determinata da ripetitivo palindromico PCR (rep PCR). Per la metà degli isolati di *E. coli* che possedevano dei fattori di virulenza, non si è potuto assegnare uno qualsiasi dei 19 sierotipi testati, anche se le stesse tossine e adesine possedevano gli stessi ceppi sierotipizzati. I sierotipi più comunemente rilevati erano: K88ac (18%), O139: K82 (6%), O141: K85ac (5%), O108: K`V189` (5%), O119: K`V113` (3%) e O157: K`V17` (2%). La combinazione di tossine EST-1, B ST e LT-I e l'adesina F4, caratteristiche dell'enterotossigena *E. coli* (ETEC), si sono rilevate più di frequente. La tossina Shiga Stx2e (STEC) e l'intimina Eae (EPEC), tuttavia, erano più rare. I risultati della rep PCR hanno mostrato una popolazione eterogenea. Settanta per cento degli isolati erano resistenti ad almeno un antibiotico, e il 29% erano resistenti a più di tre antibiotici. Gli isolati erano resistenti alla tetraciclina (50%) associata con i geni di resistenza *tet*(A), *tet*(B) e *tet*(C), sulfametossazolo (49%) [*sul1*, *sul2* e *sul3*], trimetoprim (34%) [*dfr*], acido nalidixico (29%), ampicillina (26%) [$bla_{\text{TEM-1}}$], gentamicina (17%) [*aac(3)-IIc*, *aac(3)-IVa* e *aac(3)-IVa*] cloramfenicolo (17%) [*catAI* e *catAIII*] ciprofloxacina (8%) [mutazioni in GyrA (S83L) e ParC (S80I)]. Tutti gli isolati erano sensibili alla 3a generazione di cefalosporine, carbapenemici, colistina e tigeciclina. I patogeni *E. coli* dei suini provenienti dalla Svizzera, spesso non potevano essere suddivisi nei sierogruppi conosciuti anche se possedevano i fattori di virulenza che causavano la diarrea. Inoltre, sono stati identificati vari meccanismi di resistenza agli antibiotici, che vengono abitualmente amministrati ai suini. Concludendo possiamo affermare che un'identificazione coerente e accurata del patogeno che provoca la malattia e i test di sensibilità agli antibiotici prima del trattamento sono di grande importanza.

Pathotyping and antibiotic resistance of porcine enterovirulent *Escherichia coli* strains from Switzerland (2014–2015)

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