

Prevalence and patterns of antimicrobial resistant of fecal *Escherichia coli* isolates among pigs on eight farrow-to-finish farms and their production environments in Shandong Province, China

Hong-Chao ZHOU¹, Lin LIU², Zhao-Fang TIAN², Wen-Juan ZHANG^{2,*}

¹College of Veterinary Medicine, Northwest A&F University, Yangling, 712100 Shanxi – CHINA

²Central Laboratory of Animal Disease Diagnosis and Surveillance, Yantai, 26400 Shandong – CHINA

Received: 28.08.2010 • Accepted: 28.07.2011

Abstract: The main objectives of this study were to determine the prevalence and antimicrobial resistance patterns of *Escherichia coli* isolates in feces of pigs and their production environments on farms. A total of 560 isolates of *Escherichia coli*, from 112 samples obtained from weaner and finisher pigs, feed, water, and house sparrows on 8 farrow-to-finish farms, were tested for susceptibility to 16 antimicrobials. The prevalence of resistance varied widely (0.0% to 69.5%) among the antimicrobials tested. From the tested swines, 75.75% of the isolates were resistant to one or more antimicrobials. Resistance was significantly more frequent ($P = 0.005$, $P < 0.01$) in swine isolates compared to those from feed, water, and sparrows and significantly more frequent ($P = 0.007$, $P < 0.01$) in weaner pigs compared to finisher pigs. The findings indicate that resistance to a broad range of antimicrobials was prevalent among fecal *E. coli* isolates of swine on the study farms. Fortunately, *E. coli* was not isolated from the feed, water, and house sparrows.

Key words: Swine, commensal *Escherichia coli*, antimicrobial resistance, production environments

Antimicrobials are valuable tools to treat clinical diseases and to maintain healthy and productive animals (1), but their use can lead to selection for resistant bacteria (2–4). Antimicrobial resistance among commensal *E. coli* of swine is important because it reflects the selective pressure exerted on *E. coli* to develop resistance and is one of the potential reservoirs of resistance genes available for dissemination to pathogens (5,6). Therefore, the World Health Organization (WHO) and World Organization for Animal Health (OIE) recommend monitoring antimicrobial resistance in commensal *E.*

coli (7,8). Unfortunately, data on the antimicrobial-resistant commensal *E. coli* are sparse in developing countries, particularly in China where antimicrobials are overused in veterinary medicine and food animals.

The purpose of this study was to determine antimicrobial resistance profiles of *E. coli* isolates in healthy swine herds, weaner and finisher pigs, and their production environments on 8 purposefully selected farrow-to-finish farms in the Shandong Province of China.

* E-mail: wenjuanzhangzx@163.com

All of the samples were collected randomly from each of the 8 farms, for culture and isolation of *E. coli*, during 2009 and 2010. On each farm, 10 fecal samples were collected from each of the weaner and finisher pig groups and pooled, respectively, 2 samples were collected from 2 healthy house sparrows, and 2 samples were collected from feed and water, giving a total of 112 samples. Within 24 h of collection, the samples were streaked for isolation on MacConkey agar plates. The plates were incubated at 37 °C for 18–24 h. From each sample, 5 colonies with typical *E. coli* morphology were picked and streaked again on MacConkey agar plates and then restreaked on Miller agar plates (Difco, Becton, Dickinson). The isolates were tested for indole production and use of citrate as the sole carbon source. Lactose positive, indole positive, and citrate negative isolates were considered to be *E. coli*.

Antimicrobial susceptibility testing was performed according to the National Committee for Clinical Laboratory Standards methods (9). Antimicrobial minimum inhibitory concentrations (MICs) for the *E. coli* isolates were determined via broth microdilution using the PASCO MIC/ID system. Antimicrobials included in the panels are listed in Table 1. *E. coli* ATCC 25922 was used as a quality control strain.

Overall, 112 samples were obtained for culture. From each sample, 5 isolates (independent colonies) were obtained, giving a total of 560 isolates that were tested for susceptibility to 16 antimicrobials. Out of 560 isolates, 200 were from weaner pigs, 200 were from finisher pigs, 80 were from feed and water, and 80 were from sparrows.

The results of antimicrobial susceptibility in *E. coli* isolates are summarized in Table 2. The overall prevalence of resistance was as follows: 75.75% (303/400) of the isolates from the weaner and finisher pigs were resistant to at least 1 antimicrobial. Of the 80 isolates from feed and water, 12 (15%) were resistant, each of them to only 1 antimicrobial agent tested. Meanwhile, 8 of the 80 (10 %) *E. coli* isolates from the house sparrows were also resistant. In the *E. coli* isolates from swine, the point prevalence was higher in isolates from weaners than from finishers, and the highest prevalence of resistance was to tetracycline (69.5%) and sulfamethoxazole (68%) in weaners, but the isolates were sensitive to neomycin, ceftriaxone, and ceftazidime. The result was less serious than the study by Yang (1).

Overall, the isolates from weaners were significantly ($P = 0.007$, $P < 0.01$) more likely to be

Table 1. The MIC distributions (range) and also breakpoints of antimicrobials.

Antimicrobials	Resistance breakpoint ($\mu\text{g/mL}$)	MIC50 ($\mu\text{g/mL}$)	MIC90 ($\mu\text{g/mL}$)
Ampicillin	32	>32	>32
Amoxicillin	32.16	8.4	16.8
Streptomycin	64	>256	>256
Gentamycin	16	2	>16
Kanamycin	500	>64	2048
Nalidixic acid	32	>256	>256
Neomycin	4	4	>8
Tetracycline	16	>32	>32
Chloramphenicol	32	4	>32
Florfenicol	32	16	>32
Sulfamethoxazole	512	>512	>512
Levofloxacin	8	8	>16
Ciprofloxacin	4	16	>16
Ceftriaxone	64	<0.025	<0.025
Ceftazidime	32	16	>32
Cephalothin	32	16	32

Prevalence and patterns of antimicrobial resistant of fecal *Escherichia coli* isolates among pigs on eight farrow-to-finish farms and their production environments in Shandong Province, China

Table 2. Percentage of antimicrobial-resistant *E. coli* isolates.

Antimicrobials	Percentage of resistance (%)				
	Weaners (n = 200)	Finishers (n = 200)	Combined ^a	Feed and water (n = 80)	Sparrows (n = 80)
Ampicillin	58.5	43	50.75	1.25	0
Amoxicillin	48.5	30	39.25	1.25	1.25
Streptomycin	57.5	50	53.75	0	3.75
Gentamycin	20.5	14.5	17.5	0	0
Kanamycin	26	3.5	14.75	0	0
Neomycin	3.5	3	3.25	0	0
Nalidixic acid	58	47	52.5	1.25	0
Tetracycline	69.5	52	60.75	6.25	3.75
Chloramphenicol	23.5	14	18.75	1.25	0
Florfenicol	9.5	3	6.25	0	0
Sulfamethoxazole	68	42	55	3.75	1.25
Levofloxacin	38	17.5	27.75	0	0
Ciprofloxacin	45.5	30	37.75	0	0
Ceftriaxone	0.5	0	0.25	0	0
Ceftazidime	0	0	0	0	0
Cephalothin	48.5	32	40.25	0	0

^aThe results combined weaners and finishers.

resistant to antimicrobials than the isolates from the finisher pigs. That might be due to the more extensive use of antimicrobials in weaners for growth promotion, treatment, and prophylaxis than in finisher pigs (10). Furthermore, weaners are younger and at risk of enteric infections, perhaps because of waning passive immunity, mixing with pigs from other litters or farms, and colonization by resistant organisms (11). Antimicrobials tend to be used less frequently in finishers, in part because they are close to marketing for human consumption and there is a need to avoid antimicrobial residues in pork (12).

With regards to the multidrug resistance profiles, the majority of isolates from swine were resistant to

at least 2 antimicrobials from the 16 tested. Patterns of resistance to more than 2 antimicrobials are presented in Table 3. Of the isolates, 1 was resistant to 15 antimicrobials (0.18% of the isolates; the highest in this study), and 57 (10.18% of the isolates; the most in the study) were resistant to 6 antimicrobials. The isolates were mostly resistant to between 4 and 8 antimicrobials in this study.

Resistance to the aminoglycosides and penicillins, sulfonamides, and tetracyclines were most common among the observed patterns. The most common resistance pattern was Tetracycline + Sulfonamide + Amino glycoside + Penicillin.

Table 3. Percentage of multi-antimicrobial resistant *E. coli* isolates.

Number of antimicrobials isolates resistant to	Prevalence of resistance (%) ^{a,b}			
	Weaners isolates (n = 200)	Finishers isolates (n = 200)	Feed and water isolates (n = 80)	Sparrows isolates (n = 80)
2	4	4	0	0
3	6	4.5	0	0
4	8	6	0	0
5	10	8	0	0
6	16	12.5	0	0
7	13.5	10	0	0
8	13	10	0	0
9	2.5	1	0	0
10	1.5	1.5	0	0
11	3	2	0	0
12	1.5	0.5	0	0
13	0	0	0	0
14	0	0	0	0
15	0.5	0	0	0
16	0	0	0	0

^aOverall, significantly different between weaners and finishers ($P = 0.007$, $P < 0.01$).

^bOverall, significantly different between isolates from swine and feed, water and sparrows ($P = 0.005$, $P < 0.01$).

Although the use of chloramphenicol was forbidden in China, resistance to the antimicrobial persisted in the fecal *E. coli* of swine (Table 2). The persistence of resistance to chloramphenicol might result from the process of co-selection by other antimicrobials still being used in pigs (13). This occurs when genes encoding for these withdrawn antimicrobials are linked with those encoding for other antimicrobials still being used (6). Another pathway for persistence of antimicrobial resistance is the linkage of chromosomally located antimicrobial resistance genes that are not readily lost by bacteria (14).

As far as public health is concerned, we should note that 1 isolate from weaners was resistant to ceftriaxone, which is the third-generation cephalosporin used to treat *E. coli* and *Salmonella* infections in children (15).

Fortunately, the results showed that swine antimicrobial-resistant *E. coli* was not isolated from feed, water, and sparrow samples and their antimicrobial resistance may reflect a persistence of commensal bacterial microbiota.

In conclusion, the findings of this study reflect the on-farm selection pressure for antimicrobial resistance and the potential food-safety risk from near-market animals. Describing antimicrobial resistant *E. coli* in the 8 swine farms provides baseline information for monitoring on-farm antimicrobial resistance in *E. coli*.

Acknowledgment

This study was funded by grant no. SDGP2008-54-O of the Shandong Province of China.

Prevalence and patterns of antimicrobial resistant of fecal *Escherichia coli* isolates among pigs on eight farrow-to-finish farms and their production environments in Shandong Province, China

References

1. Yang, H.C., Cheng, S., White, D.G., Zhao, S.H., McDermott, P., Walker, R., Meng, J.H.: Characterization of multiple-antimicrobial-resistant *Escherichia coli* isolates from diseased chickens and swine in China. *J. Clin. Microbiol.*, 2004; 42: 3483–3489.
2. Akwar, T.H., Poppe, C., Wilson, J.: Risk factors for antimicrobial resistance among fecal *Escherichia coli* from residents on forty-three swine farms. *Microb. Drug. Resist.*, 2007; 13: 69–76.
3. Harada, K., Asai, T., Kojima, A., Sameshima, T., Takahashi, T.: Contribution of multi-antimicrobial resistance to the population of antimicrobial resistant *Escherichia coli* isolated from apparently healthy pigs in Japan. *Med. Microbiol. Immun.*, 2007; 51: 493–499.
4. Stine, O.C., Johnson, J.A., Keefer-Norris, A.: Widespread distribution of tetracycline resistance genes in a confined animal feeding facility. *Int. J. Antimicrob. Agents.*, 2007; 29: 348–352.
5. Van den Bogaard A.E., London N., Stobberingh E.E.: Antimicrobial resistance in pig fecal samples from the Netherlands (five abattoirs) and Sweden. *J. Antimicrob. Chemother.*, 2000; 45: 663–671.
6. Summers, A.O.: Generally overlooked fundamentals of bacterial genetics and ecology. *Clin. Infect. Dis.*, 2002; 34–92.
7. Franklin, A., Acar, J., Anthony, F et al.: Antimicrobial resistance: harmonisation of national antimicrobial resistance monitoring and surveillance programmes in animals and in animal-derived food. *Rev. Sci. Tech.*, 2001; 20: 859–870.
8. World Health Organization. WHO Global Principles for the Containment of Antimicrobial Resistance in Animals Intended for Food. Geneva Switzerland: World Health Organization, 2000. [homepage on the Internet]. Available from http://whqlibdoc.who.int/hq/2000/WHO_CDS_CSR_APH_2000.4.pdf.
9. National Committee for Clinical Laboratory Standards. 2003. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals; approved standard. NCCLS M31-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Wayne, Pa.
10. Dunlop, R.H., McEwen, S.A., Meek, A.H., Black, W.D., Friendship, R.M., Clarke, R.C.: Prevalence of resistance to seven antimicrobials among fecal *Escherichia coli* of swine on thirty-four farrow-to-finish farms in Ontario, Can. *Prev. Vet. Med.*, 1998; 34: 265–282.
11. Langlois, B.E., Dawson, K.A., Leak, I., Aaron, D.K.: Effect of age and housing location on antibiotic resistance of fecal *coli* forms from pigs in a non-antibiotic-exposed herd. *Appl. Environ. Microb.*, 1988; 54: 1341–1344.
12. Akwar, H.T., Poppe, C., Wilson, J., Reid-Smith, R.J., Dyck, M., Waddington, J., Shang, D., McEwen, S.A.: Prevalence and patterns of antimicrobial resistance of fecal *Escherichia coli* among pigs on 47 farrow-to-finish farms with different in-feed medication policies in Ontario and British Columbia. *Can. J. Vet. Res.*, 2008; 72: 195–201.
13. Harada, K., Asai, T., Kojima, A., Ishihara, K., Takahashi, T.: Role of co-resistance in the development of resistance to chloramphenicol in *Escherichia coli* isolated from sick cattle and pigs. *Am. J. Vet. Res.*, 2006; 67: 230–235.
14. Sandvang, D., Aarestrup, F.M.: Characterization of aminoglycoside resistance genes and class 1 integrons in porcine and bovine gentamicin-resistant *Escherichia coli*. *Microb. Drug. Resist.*, 2000; 6: 19–27.
15. Fey, P.D., Safranek, T.J., Rupp, M.E., Dunne, E.F., Ribot, E., Iwen, P.C.: Ceftriaxone-resistant *Salmonella* infection acquired by a child from cattle. *New Engl. J. Med.*, 2003; 342: 1242–9.