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Characterization of Antimicrobial Resistance in *Escherichia coli* Isolates from Retail Meats and Eggs in China

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Abstract: The aim of this study was to characterize the antimicrobial resistance and mechanism of streptomycin-resistance in *Escherichia coli* isolated from retail meats and eggs during 2012-2013. A total of 76 *E. coli* isolates were assessed for antimicrobial susceptibility to 14 anti-microbial agents by Disk Diffusion Method. Resistant strains were screened by PCR for streptomycin resistance genes. Each strain was found resistant to at least one antimicrobial and 68 of 76 isolates (89.5%) showed multidrug resistance phenotypes to at least three classes of antimicrobials. The most prevalent resistances were to streptomycin (92.1%), followed by tetracycline (88.2%), ampicillin (81.6%), gentamicin (72.4%), nalidixic acid (69.7%) and sulfadimidine (63.2%). For each streptomycin-resistance gene, the predominant resistance genes were: *aadA1* (64.3%), followed by *strA/B* (51.4%) and *aadA2* (41.4%). However, the level of antimicrobial resistance and dissemination of resistance genes differed in *E. coli* strains with different sources.

Key words: *Escherichia coli*, retail food, antimicrobial resistance, resistance gene, humans

INTRODUCTION

Escherichia coli (*E. coli*) is a commensal bacterium, normally lives in the intestines of people and animals and has a wide range of hosts. Most *E. coli* are harmless and actually are an important part of a healthy human intestinal tract. However, some *E. coli* are pathogenic, meaning they can cause illness, either diarrhea or illness outside of the intestinal tract. Because of its ubiquities in environment, *E. coli* found on meat and meat products is the most important indicator for animal or human fecal contamination during the slaughter process or meat processing (Beerens, 1998).

The emergence of antimicrobial resistance among *E. coli* strains of animal origin has important public health implications (Copur-Cicek *et al*., 2014; Literak *et al*., 2009; Radhouani *et al*., 2012; Scott *et al*., 2005). Several studies showed that drug-resistant *E. coli* infections in humans were often caused by strains from animals (Altalhi *et al*., 2010; Lei *et al*., 2010; Li *et al*., 2014a; Rasheed *et al*., 2014). Since the famous report by Swann (1969) was published >40 years ago, the prevalence of antimicrobial resistant pathogens in humans has been linked to drug application in animal food production. It has been proven that the antimicrobial-resistant foodborne pathogens transferred from food to humans and that a large proportion of resistant *E. coli* isolates causing human infections in humans may be derived from food sources (Zhao *et al*., 2012). However, the frequency of resistance to different antimicrobials in *E. coli* differed to the source of the isolates. *E. coli* isolates from healthy human were resistant to ciprofloxacin (19.3%) and streptomycin (79.3%) (Li *et al*., 2014a) compared to 30.2% resistance to ciprofloxacin and 58.5% to streptomycin among *E. coli* isolates from retail meat.

E. coli is also the most common organism in studying the transfer of certain genetic elements to other species. It was found that the same resistance genes they harbored were found in diverse bacterial species from a variety of animal sources (Hannah *et al*., 2009; Islam *et al*., 2010). Karczmarczyk *et al*. (2011) investigated the underlying aminoglycoside-resistance mechanisms and revealed that the *strA/B* gene pair was the most prevalent (81%) among the determinants identified followed by aadA (77%) which was consistent with earlier reports showing that these genes are commonin isolates resistant to streptomycin and/or other aminoglycoside drugs (Sunde and Norstrom, 2006).

The objectives of this study were to determine the prevalence, antimicrobial susceptibility and mechanisms

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of streptomycin-resistance of *E. coli* isolates from retail meats and eggs collected in Jiangsu province, China during 2012-2013.

MATERIALS AND METHODS

Sample collection and *E.coli* isolation: From October 2012 to October 2013, retail meat (pork, chicken, beef and mutton) and egg were collected from retail market and supermarket of Jiangsu Province. No more than three samples were collected from one kind of product in one market for one visit in this study. All the samples were handled aseptically. The fresh products were transported in sterile container at 4°C. The bacteria strains were isolated according to methods as previously described (Karczmarczyk *et al*., 2011; Zhao *et al*., 2012). Each sample was thoroughly rinsed with 500 mL buffered peptone water. The 50 mL of the reinse was mixed with 50 mL of double-strength MacConkey broth and the mixture was incubated at 35°C for 24 h. One loopful of culture was streaked onto an eosin methylene blue agar plate and incubated at 35°C for 24 h. One presumptive *E. coli* colony per sample was transferred to a triptic soy agar plated for further purification. The colony with typical *E. coli* morphology and size was selected from each sample and then identified by classical biochemical methods and confirmed with 16S rDNA Bacterial Identification PCR Kit (TaKaRa, Dalian, China). The isolates were stored at 4°C for subsequent studies.

Antibiotic susceptibility testing: The antibiotic susceptibility of the *E. coli* isolates was tested against a set of antibiotic agents by antibiotic disk diffusion on Mueller-Hinton agar in accordance with the Clinical and Laboratory Standards Institute guideline. The following antimicrobials were used: ampicillin (10 μg), amoxicillin (20 μg), amoxicillin/clavulanic acid (20/10 μg), ceftriaxone (30 μg), amikacin (30 μg), streptomycin (10 μg), gentamicin (30 μg), tetracycline (10 μg), sulfamethoxazole (10 μg), sulfamethoxazole/trimethoprim (23.75/1.25 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), ofloxacin (5 μg) and chloramphenicol (30 μg). These antibiotics were selected to represent commonly used agents against *E. coli* infections in veterinary medicine. Control strains were *E. coli* ATCC25922, *Staphylococcus aureus* ATCC 25923 and *Pseudomonase aeruginosa* ATCC 27853.

Detection of streptomycin resistance genes: DNA templates of streptomycin-resistant isolate were prepared by boiling the bacterial cultures. Briefly, colonies of bacteria were suspended in 500 μL of distilled water. The mixture was then boiled at 100°C for 15 min. After centrifugation at 13,500 rpm for 1 min, supernatants were transferred to a new tube and stored at -20°C until use with PCR amplification.

Table 1: Primers used in PCR assays for streptomycin-resistance genes in Escherichia coli isolates

Primers	Target gene	Oligonucleotide primer sequences Forward/reverse (5'-3')	Size (bp)	References
StrA/B-F	*StrA/B*	AACGCCTTGCCTTCTATCTGC	645	This study
StrA/B-R		CCAAAGCCCACTTCACCGAC		
aadA1-F	*aadA1*	TTGATTTGCTGGTTACGGT	537	This study
aadA1-R		CTTCAAGTATGACGGGCTG		
aadA2-F	*aadA2*	TGTTGGTTACTGTGGCCGTA	713	Chen *et al.* (2012)
aadA2-R		GATCTCGCCTTTCACAAAGC		

To determine the presence of streptomycin resistance genes of *E. coli* strains from retail food, the following resistance genes were detected: *strA/B*, *aadA1*, *aadA2*. PCR was performed as described by Chen *et al.* (2012). The primer sequences used in the PCR reactions are listed in Table 1.

RESULTS AND DISCUSSION

***E.coli* isolation:** A total of 76 *E. coli* isolates were recovered from 373 samples, representing 20.4% (76/373) of samples tested (Table 2). The prevalence of *E. coli* in chicken products in this study is lower than that reported by Wu *et al.* (2014) (69.1% of average, range from 45.8-86.8%) and that by Xu *et al.* (2014) (≥37.4%) in China. However, the isolation rate is much higher than that reported by Lee *et al.* (2009) (9.1%) during 2004-2006 in Korea while lower than that in canada (96%) (Sheikh *et al.*, 2012). The relatively higher isolation rate (27.2%) from pork observed in the present study is consistent with previous reports from China as well as other parts of the world (Harakeh *et al.*, 2005; Jin *et al.*, 2014; Xia *et al.*, 2011). The levels of *E. coli* contamination in mutton samples were much lower (11.3%, 9/80). These data can also support the previous reports indicating that *E. coli* contamination in retail meat accounted for some of food poisoning events. Previous reports have also suggested that *E. coli* followed along with *Vibrio parahaemolyticus*, *Bacillus cereus*, *Bacillusproteus* and Salmonella, *Staphylococcus aureus* contaminated in meat-producing animal and its products mainly contributed to food poisoning in China (Li *et al.*, 2014b; Yan *et al.*, 2010; Yang *et al.*, 2010; Zhang *et al.*, 2010).

Antimicrobial susceptibility analysis: All strains were resistant to at least one of the antimicrobial agents tested, 89.5% (68/76) multi-resistant (to at least three classes of antimicrobials). The most prevalent resistances were to

streptomycin (92.1%), tetracycline (88.2%), ampicillin (81.6%), gentamicin (72.4%), nalidixic acid (69.7%), sulfadimidine (63.2%), amikacin (52.6%), sulfamethoxazole-trimethoprim (51.3%), chloramphenicol (60.5%), ofloxacin (50%) and ciprofloxacin (46.1%) (Table 3) Which is consistent with other reports in China (Jin *et al*., 2014; Lei *et al*., 2010; Xia *et al*., 2011; Xu *et al*., 2014). *E. coli* isolates commonly showed resistance to tetracycline, trimethoprim-sulphamethoxazole, nalidixic acid and ampicillin (Harakeh *et al*., 2005; Johnson *et al*., 2005; Lee *et al*., 2009; Lei *et al*., 2010; Xia *et al*., 2011; Xu *et al*., 2014). The resistances were relatively low to amoxicillin (30.3%), ceftriaxone (40.8%) and amoxicillin-clavulanate (13.2%). Most of *E. coli* strains had the higher susceptibility to amoxicillin-clavulanate (66.7-89.5%) especially in *E. coli* isolates from chicken samples (89.5%). Significant differences were found in the percentage resistance of isolates from different type of products. Strains from chicken and mutton showed no susceptibility to most antimicrobials such as streptomycin, sulfadimidine, trimethoprim-sulfamethoxazole, tetracycline, nalidixic acid, ciprofloxacin and ofloxacin in chicken strains; ampcilin, gentamicin, sulfadimidine, trimethoprim-sulfamethoxazole, tetracycline, nalidixic acid and ofloxacin in mutton strains.

Table 2: Isolation of Escherchia coli in food samples in Jiangsu Province, China, 2012-13

Foods	No. of positive samples/No. of total sample (%)
Retail meat	52/298 (17.5)
Pork	22/81 (27.2)
Chicken	19/77 (24.8)
Beef	11/60 (18.3)
Fish	9/80 (11.3)
Egg	15/75 (20.0)
Total	76/373 (20.4)

Among the strains isolated from beef, the resistance rates reached >45.5%, except for resistance to amoxicillin-clavulanate, ciprofloxacin and ofloxacin reaching frequencies 18.2%; the resistance rates were >57.9%, except for amoxicillin, ceftriaxone and amoxicillin-clavulanate; the strains isolated from egg showed resistance to all the antimicrobials (≥33.3%) except for amoxicillin-clavulanate (6.7%). Strains isolated from pork in contrast, showed resistance to amoxicillin, amoxicillin-clavulanate, trimethoprim-sulfamethoxazole, nalidixic acid, ciprofloxacin and ofloxacin (<45.3%). These differences indicated that the antimicrobial resistance was prevalent in chicken products. The results were in accordance with other studies conducted in China (Lei *et al*., 2010; Xu *et al*., 2014). The antimicrobial susceptibility results of the research showed the *E. coli* isolates from retail meat and egg in Jiangsu Province of China exhibited the high level of antibiotic resistance to antimicrobials. Among these antimicrobials, the resistance rates of the isolates to streptomycin, tetracycline, ampcillin and gentamicin which were widely used to control the bacteria infection were was very high (>50%). It is clear that the wider use of antimicrobial drugs is pivotal in the selection of bacterial resistance (Zhao *et al*., 2011).

Prevalence of streptomycin-resistance genes: The molecular investigations on the underlying streptomycin resistance mechanisms showed that identical resistance phenotypes were based on different genes (Table 4): *aadA1*, *aadA2* and *strA/B* and combination of them. Two of the genes (*aadA1* and *strA/B*) were widely spread (frequency >50%) among the resistant strains. In one

Table 3: Antimicrobial susceptibility of *E. coli* isolates from retail food

	No. of resistant and susceptible isolates (%)											
Antibiotics	----Pork (n = 22)----		--Chicken (n = 19)--		---Beef (n = 11)---		--Mutton (n = 9)--		---Egg (n = 15)---		----Total (n = 76)----	
β-lactams												
Ampicillin	19 (86.3)	1 (4.5)	15 (78.9)	11 (5.3)	9 (81.8)	0	7 (77.8)	0	12 (80)	2 (13.3)	62 (81.6)	6 (7.9)
Amoxicillin	6 (27.3)	16 (72.7)	3 (15.8)	12 (63.2)	6 (54.5)	3 (27.3)	2 (22.2)	5 (55.6)	6 (40)	7 (46.7)	23 (30.3)	43 (56.6)
Ceftriaxone	10 (45.3)	11 (50)	1 (5.3)	18 (94.7)	7 (63.6)	3 (27.3)	5 (55.6)	3 (33.3)	8 (53.3)	3 (20)	31 (40.8)	38 (50)
Amoxicillin-clavulanate	4 (18.2)	16 (72.7)	0	17 (89.5)	2 (18.2)	8 (72.7)	3 (33.3)	6 (66.7)	1 (6.7)	13 (86.7)	10 (13.2)	60 (78.9)
Aminoglycosides												
Gentamicin	17 (77.3)	2 (9.1)	15 (78.9)	2 (10.5)	6 (54.5)	4 (36.3)	7 (77.8)	0	10 (66.7)	2 (13.3)	55 (72.4)	10 (13.2)
Amikacin	10 (45.3)	7 (31.8)	11 (57.9)	7 (36.8)	9 (81.8)	1 (9.1)	5 (55.6)	2 (22.2)	5 (33.3)	9 (60)	40 (52.6)	26 (34.2)
Streptomycin	22 (100)	0	17 (89.5)	0	10 (90.9)	0	6 (66.7)	2 (22.2)	15 (100)	0	70 (92.1)	2 (2.6)
Sulfonamides												
Sulfadimidine	15 (68.2)	5 (22.7)	17 (89.5)	0	5 (45.5)	5 (45.5)	6 (66.7)	0	5 (33.3)	6 (40)	48 (63.2)	16 (21.1)
Trimethoprim-sulfamethoxazole	9 (40.9)	3 (13.6)	11 (57.9)	0	6 (54.5)	3 (27.3)	9 (100)	0	4 (26.7)	6 (40)	39 (51.3)	12 (15.8)
Tetracycline	20 (90.9)	0	19 (100)	0	8 (72.7)	1 (9.1)	7 (77.8)	0	13 (86.7)	0	67 (88.2)	2 (2.6)
Chloramphenicol	14 (63.6)	6 (27.3)	12 (63.2)	5 (26.3)	10 (90.9)	0	5 (55.6)	3 (33.3)	5 (33.3)	9 (60)	46 (60.5)	23 (30.3)
Quinolones and fuoroquinolone												
Nalidixic acid	11 (40.9)	6 (27.3)	19 (100)	0	5 (45.5)	0	9 (100)	0	9 (60)	4 (26.7)	53 (69.7)	10 (13.2)
Ciprofloxacin	9 (40.9)	10 (45.5)	15 (78.9)	0	2 (18.2)	7 (63.6)	2 (22.2)	7 (77.8)	7 (46.7)	5 (33.3)	35 (46.1)	29 (38.2)
Ofloxacin	6 (27.3)	7 (31.8)	18 (94.7)	0	2 (18.2)	5 (45.5)	7 (77.8)	0	5 (33.3)	7 (46.7)	38 (50)	19 (25)

Table 4: Streptomycin-resistance genes screened with PCR in *E. coli* isolates tested

	No. of resistance gene in *E. coli* (%)					
Resistance genes	Pork (n = 22)	Chicken (n = 16)	Beef (n = 10)	Mutton (n = 6)	Egg (n = 15)	Total (n = 70)
StrA/B	9 (40.9)	14 (87.5)	0	2 (33.3)	11 (73.3)	36 (51.4)
aad A1	19 (86.4)	10 (62.5)	6 (60)	4 (66.7)	6 (40)	45 (64.3)
aad A2	10 (45.5)	3 (18.8)	8 (80)	4 (66.7)	4 (26.7)	29 (41.4)
StrA/B +aad A1	7 (31.8)	9 (56.3)	0	4 (66.7)	5 (33.3)	22 (31.4)
StrA/B +aad A2	3 (9.1)	2 (12.5)	0	4 (66.7)	2 (13.3)	11 (15.7)
aad A1+ aad A2	8 (37.5)	2 (12.5)	5 (50)	3 (50)	1 (6.7)	19 (27.1)
StrA/B +aad A1+ aad A2	3 (9.1)	1 (6.3)	0	3 (50)	1 (6.7)	8 (11.4)

streptomycin-resistance isolate from beef, the genes responsible for resistance could not be identified, indicating other resistance genes or possible resistance mechanisms.

Of the 70 *E. coli* isolates tested, 69 (98.6%) carried at least one of the resistance genes examined with 36 (51.4%), 45 (64.3%) and 29 (41.4%) isolates carrying strA/B, aad A1and aad A2, respectively (Table 4). Although the *strA/B*, *aad A1* and *aad A2* genes has been reported to be predominant in *E. coli* isolates from animals in many countries (Ahmed *et al*., 2009; Zhang *et al*., 2014) it was still remarkable that in this study, 87.5% of the *E. coli* isolates from chicken, 86.4% from pork, 80% from beef carried strA/B, aad A1and aad A2, respectively. It was demonstrated that the *strA/B*, *aad A1* and *aad A2* genes were highly endemic in China. The distribution of gene combination was as follows: the combination of strA/B+aad A1 was found in 22 isolates, strA/B+aad A2 in 11 isolates, aad A1+aad A2 in 19 isolates and strA/B+aad A+aad A 2 in 8 isolates. It was reported in many studies that *E. coli* (including other bacteria, Salmonella) from clinical samples, retail meat and healthy animal carried multiple streptomycin-resistance genes (Ahmed *et al*., 2014; Tang *et al*., 2011; Xia *et al*., 2013; Yu *et al*., 2014).

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CONCLUSION

This study highlights the prevalence of resistant *E. coi* in retail meats and eggs in China with the possibility of their transfer to humans leading to therapeutic failure. Therefore, *E. coli* transmission from animal products could be responsible for human infections and retail meats and eggs most probably serve as a reservoir.

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