Providencia in retail meats from Guangzhou, China and Osaka, Japan: prevalence, antimicrobial resistance and characterization of classes 1, 2 and 3 integrons

Huiling DI[1,2,4)#], Sisi LIANG[1)#], Qingyang LI[1)], Lei SHI[2,3)], Ayaka SHIMA[4)],
Hecheng MENG[1)], He YAN[1)] and Shinji YAMASAKI[4)*]

[1)]School of Food Science and Engineering, South China University of Technology, Guangzhou 510640, China
[2)]Research Institute of Food Safety and Nutrition, Jinan University, Guangzhou 510632, China
[3)]State Key Laboratory of Food Safety Technology for Meat Products, Xiamen Yinxiang Group Co., Ltd.,
   Xiamen 361100, China
[4)]Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Osaka 598-8531, Japan

ABSTRACT. Bacteria of the genus *Providencia* are opportunistic pathogens of clinical significance due to their association with diarrhea and urinary tract infections. The present study was conducted to examine the prevalence and antimicrobial resistance of *Providencia* spp. in retail meats sold in Guangzhou, China and Osaka, Japan. Out of 158 meat samples including beef, pork and chicken, 67 *Providencia* (42%) belonging to four species viz., *P. alcalifaciens*, *P. rustigianii*, *P. stuartii* and *P. rettgeri* were isolated, and most of them were resistant to tetracycline (91%) followed by ampicillin (69%) and streptomycin (49%). Of 67 isolates, 29 (43%) were MDR, which is defined to be resistant to more than three classes of antimicrobials. No statistically significant differences were observed between Chinese and Japanese retail meat samples regarding contamination rate of *Providencia* spp. as well as frequency of the antimicrobial resistance of the isolates including MDR. Class 1 and/or class 2 integrons were detected in six of the eight isolates that were resistant to more than 4 antimicrobials, however none of the isolates harbored class 3 integron. A *P. rustigianii* harboring the bla_{OXA-10} gene was isolated, which is the first report of *Providencia* with bla_{OXA-10} gene of food origin. These data suggest that retail meats in China and Japan are substantially contaminated with *Providencia* spp., which displayed a high frequency of antimicrobial resistance, and establishing the surveillance of *Providencia* spp., especially antimicrobial resistant one, in retail meats is imperative.

KEY WORDS: antimicrobial resistance, integron, *Providencia*, retail meat

The genus Providencia, which is a member of Enterobacteriaceae, presently includes nine species i.e. *P. alcalifaciens*, *P. stuartii*, *P. rettgeri*, *P. rustigianii*, *P. heimbachae*, *P. vermicola*, *P. sneebia*, *P. burhodogranariea* and *P. thailandensis* [24, 25, 39]. Among these, *P. stuartii* is the species most frequently attributed to urinary tract infections (UTI) in patients of advanced age [9]. *P. alcalifaciens* has been associated with diarrhea and gastroenteritis in children and travelers from developing countries [1, 2, 19, 20, 47]. Furthermore, three large outbreaks of food poisoning attributed to *P. alcalifaciens* were reported in Japan, the Czech Republic and Kenya [6, 29, 35]. In addition to *P. alcalifaciens*, *P. rettgeri* and *P. heimbachae* are also suggested to be etiological agents of diarrhea [28, 47]. The Chinese Centers for Disease Control also reported two outbreaks of food poisoning attributed to *P. rettgeri* [10, 48]. Although the increasing number of *Providencia* infections has attracted public attention, the contamination routes and prevalence of *Providencia* spp. in foods have not yet been thoroughly investigated.

Multiple drug-resistant (MDR) *Providencia* spp., especially integron-mediated MDR, are frequently detected [16, 27]. The emergence of antimicrobial resistant pathogenic bacteria, caused by antimicrobial overuse as a result of inappropriate disease treatment and growth promotion in domestic livestock, has become a significant public health threat [18, 41, 45]. It is well known that integrons, located on plasmids, transposons, or other mobile genetic elements, with the help of integrase contribute to the dissemination of antimicrobial resistance in the environment and in humans [21, 32]. Three classes of integrons, including

Table 1. Sequences and properties of the PCR primers used in this study

Name of primer	Sequence (5′-3′)	Target gene	Amplicon size (bp)	Annealing temp (°C)	Reference
INT-1U	GTTCGGTCAAGGTTCTG	*intI1*	923	50	[36]
INT-1D	GCCAACTTTCAGCACATG				
INT-2U	ATGTCTAACAGTCCATTTT	*intI2*	450	50	[13]
INT-2D	AAATCTTTAACCCGCAAAC				
INT-3U	ATCTGCCAAACCTGACTG	*intI3*	921	55	[15]
INT-3D	CGAATGCCCCAACAACTC				
qacEΔ1-F	ATCGCAATAGTTGGCGAAGT	*qacEΔ1*+*sul1* (3′-CS of class 1 integron)	800	56	[12]
sul1-B	GCAAGGCGGAAACCCGCGCC				
in-F	GGCATCCAAGCAGCAAGC	Class 1 integron variable region	Variable	52	[12]
in-B	AAGCAGACTTGACCTGAT				
Hep 74	CGGGATCCCGGACGGCATGCACGATTTGTA	Class 2 integron variable region	Variable	58	[44]
Hep 51	GATGCCATCGCAAGTACGAG				

classes 1, 2 and 3 integrons, have been reported to be associated with MDR and the dissemination of resistance genes [5, 17, 30, 32]. Some studies have shown that many of the pathogens isolated from meats or other foods are resistant to clinically important antimicrobials [43]. Thus, consumption of meats contaminated with antimicrobial resistant *Providencia* strains may not only cause infection but also serve a source for the dissemination of antimicrobial resistant genes in humans, which could have a serious impact on consequent antimicrobial therapy.

In this study, therefore, we conducted a survey regarding the prevalence of *Providencia* spp. in retail meats in a developing country, China, and a developed country, Japan, and examined the antimicrobial susceptibility and prevalence of classes 1, 2 and 3 integrons in MDR isolates.

MATERIALS AND METHODS

Sample collection

Raw meat samples (85 from Guangzhou, China, including 25 beef, 29 pork and 31 chicken and 73 from Osaka, Japan, including 23 beef, 25 pork and 25 chicken) were purchased from retail markets during 2012–2013. The meat samples were aseptically collected and transported to the laboratory in an icebox, and then used immediately for bacterial isolation.

Bacterial isolation and determination

Each sample (25 g) was placed into a sterile lateral bag containing 25 m*l* of sterilized PBS and homogenized for 1 min using a stomacher. Then, 1 m*l* of the homogenate was mixed with 4 m*l* of 1.25 × trypticase soy broth (TSB; Huankai Ltd., Guangzhou, China) and incubated in a shaker incubator overnight at 37°C. One loopful of the overnight culture was streaked onto selective polymyxin-mannitol-xylitol medium (PMXMP; containing Lab Lemco powder, sodium deoxycholate, anhydrous disodium hydrogen phosphate, phenol red dye, maltose, and xylose with polymyxin B sulfate) [47]. Presumptive *Providencia* colonies (red to pink on PMXMP medium) were streaked onto Luria-Bertani [2] agar and incubated overnight at 37°C. To identify the *Providencia*-positive isolates at the species level, the API 20E (a biochemical strip for Enterobacteriaceae; SYSMEX bioMérieux Co., Ltd., Tokyo, Japan) as well as the oxidase, adonitol and galactose utility tests were performed as previously described [34, 37, 47].

Antimicrobial susceptibility test

Antimicrobial susceptibility was determined by the disk diffusion method using 13 antimicrobial discs according to the 2012 Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antimicrobial discs (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan), corresponding to the drugs most commonly used for the treatment of infectious diseases caused by Gram-negative bacteria, were included in the experiment: tetracycline (TET) 30 *μ*g, ampicillin (AMP) 10 *μ*g, ceftazidime (CAZ) 30 *μ*g, cefoxitin (CFX) 30 *μ*g, imipenem (IPM) 10 *μ*g, streptomycin (STR) 10 *μ*g, gentamicin (GEN) 30 *μ*g, kanamycin (KAN) 30 *μ*g, nalidixic acid (NA) 30 *μ*g, norfloxacin (NFLX) 10 *μ*g, sulfamethoxazole-trimethoprim (SXT) 25 *μ*g, chloramphenicol (CM) 30 *μ*g, and fosfomycin (FOM) 50 *μ*g. *Escherichia coli* ATCC 25922 was used as a quality control. All experiments were conducted and results were interpreted according to the CLSI guidelines.

Detection and characterization of classes 1, 2 and 3 integrons in MDR Providencia spp.

Based on the antimicrobial resistance profile of the isolates, eight MDR *Providencia* spp. isolates that were resistant to at least four antimicrobials were selected for further analysis of the presence of classes 1, 2 and 3 integrons by PCR using previously described primers and protocols (Table 1). Genomic DNA was extracted from the isolates with the TIANamp Genomic DNA Kit (Tiangen, Beijing, China). *E. coli* K-12 DH1 strains containing pR388 [7], pVC2554 [8], or pSMB731 [3], which respectively carrying class 1, 2 or 3 integron, were used as positive controls. Plasmid DNA was extracted from the control strains using the

Table 2. Prevalence of *Providencia* spp. in retail meats in Guangzhou, China and Osaka, Japan

Species [a]	Percentage of contamination (n[b])								Total (158)
	China				Japan				
	Beef (25)	Pork (29)	Chicken (31)	Subtotal (85)	Beef (23)	Pork (25)	Chicken (25)	Subtotal (73)	
P. alca	44.0 (11[d])	55.0 (16)	16.0 (5)	38.0 (32[d])	35.0 (8)	0.0	48.0 (12)	27.0 (20)	33.0 (52)
P. ret	0.0	0.0	0.0	0.0	4.3 (1)	4.0 (1)	0.0	2.7 (2)	1.3 (2)
P. stu	4.0 (1[d])	3.4 (1)	3.2 (1)	3.5 (3[d])	0.0	4.0 (1)	8.0 (2)	4.1 (3)	3.8 (6)
P. rus	4.0 (1)	6.9 (2)	3.2 (1)	4.7 (4)	0.0	4.0 (1)	4.0 (1)	2.7 (2)	3.8 (6)
P. spp.	0.0	0.0	0.0	0.0	0.0	0.0	4.0 (1[c])	1.4 (1[c])	0.6 (1[c])
Total	48.0 (13[d])	66.0 (19)	23.0 (7)	45.0 (39[d])	39.0 (9)	12.0 (3)	64.0 (16)	38.0 (28)	42.0 (67[d])

a) *P. alca*, *P. alcalifaciens*; *P. ret*, *P. rettgeri*; *P. stu*, *P. stuartii*; *P. rus*, *P. rustigianii*. b) No. of positive isolates. c) API 20E indicated that this strain was *P. alcalifaciesns* or *P. rustiganii* but was positive for both adonitol and galactose utility test. d) Two species were isolated from one beef sample.

QIAGEN Plasmid Mini Kit (Qiagen) according to the manufacturer's instructions, and quantified by 1% agarose gel electrophoresis in 0.5 × TAE buffer (45 min, 100 V).

All PCR products were purified and sequenced by Invitrogen Biotechnology Co., Ltd. (Shanghai, China). The initial sequenced primers was the same to which were used for PCR listed in Table 1. In case the sequence was not completed, particularly variable regions of class 1/2 integron, a primer walking method was employed until sequencing was completed. The obtained sequences were analyzed by a BLAST search of the GenBank database at the National Center for Biotechnology Information via the BLAST network service (http://www.ncbi.nlm.nih.gov/).

RESULTS

Prevalence of Providencia in retail meats from China and Japan

Of the 73 retail meats purchased in Japan, 28 *Providencia* strains, belonging to four species, *P. alcalifaciens*, *P. rustigianii*, *P. stuartii*, and *P. rettgeri*, were isolated with an overall prevalence rate of 38% (Table 2). *P. alcalifaciens* was the most dominant species in beef and chicken samples. The other three species, *P. rettgeri* (2.7%), *P. stuartii* (4.1%) and *P. rustigianii* (2.7%) were detected in only two meats such as pork and beef or chicken at much lower rates. However, one strain isolated from chicken was not able to be identified species but the result of API 20E showed *P. alcalifaciens* or *P. rustigianii* because it was positive for both adonitol and galactose utility test.

On the other hand, of the 85 retail meats purchased in China, 39 *Providencia* strains belonging to three species, *P. alcalifaciens*, *P. rustigianii*, and *P. stuartii*, were isolated from 38 retail meats with slightly higher prevalence (45%) compared to that in Japanese meats. *P. alcalifaciens* was also the most prevalent species in all three tested meats, followed by *P. rustigianii* (4.7%) and *P. stuartii* (3.5%), which were also detected in all three meats. However, *P. rettgeri* was not detected in any meat samples in China. One Chinese beef sample was contaminated with both *P. alcalifaciens* and *P. stuartii*.

P. alcalifaciens, *P. rustigianii* and *P. stuartii* were detected in all three tested meats, and *P. rettgeri* was the least prevalent species which was isolated from only one beef and one pork sample purchased in Japan. *Providencia*-positive rate for pork samples in China was significantly higher than that in Japan ($P<0.01$, χ^2-test), whereas the positive rate for chicken samples in Japan was higher than that in China ($P<0.01$, χ^2-test). In the beef, the *Providencia* contamination rate was slightly higher in Chinese samples but there was no significant difference ($P>0.05$, χ^2-test).

Antimicrobial susceptibility

The 67 *Providencia* strains were characterized by antimicrobial susceptibility to 13 antimicrobials belonging to seven families (Table 3). Of 28 *Providencia* strains isolated from retail meats purchased in Osaka, Japan, 26 (93%) exhibited resistance to at least one antimicrobial (Table 4A). Antimicrobial with the highest resistant frequencies was tetracycline (82%), followed by streptomycin (61%) and ampicillin (54%). Resistance was also observed, albeit to a much lesser extent, to fosfomycin (14%), chloramphenicol (7.1%), kanamycin (3.6%) and sulfamethoxazole-trimethoprim (3.6%) as shown in Table 3.

On the other hand, of 39 *Providencia* strains isolated from retail meats purchased in Guangzhou, China, 38 (97%) were resistant to at least one tested antimicrobial (Table 4A). Of these resistant isolates, 97% were resistant to tetracycline, followed by ampicillin (80%) and streptomycin (41%). The resistant rates to other antimicrobials were much lower e.g. chloramphenicol (7.7%), sulfamethoxazole-trimethoprim (7.7%), fosfomycin (5.1%), nalidixic acid (5.1%) and kanamycin (2.6%) as shown in Table 3. The resistant rates to both tetracycline ($P<0.05$, χ^2-test) and ampicillin ($P<0.01$, χ^2-test) among isolates recovered from China were significantly higher than those of Japan. However, any *Providencia* isolated in this study did not show resistance to ceftazidime, cefoxitin, imipenem, gentamicin and norfloxacin (Table 3).

Twenty-nine MDR strains (29/67, 43%), which exhibited resistance to more than three classes of antimicrobials, were obtained (Table 4A). There was no significant difference between Japanese and Chinese strains in terms of the frequency of MDR ($P>0.05$, χ^2-test). From the viewpoint of meat source, the highest frequency of MDR was observed in isolates from chicken (52%), followed by beef (41%) and pork (36%); however, this difference was not statistically significant ($P>0.05$, χ^2-test; Table 4B).

Table 3. Antimicrobial resistance of *Providencia* isolates obtained from retail meats in Guangzhou, China and Osaka, Japan

Antimicrobials tested [a]	Percentage of resistant (No. of resistant isolates)								Total (67)
	Chinese isolates				Japanese isolates				
	Beef (13)	Chicken (7)	Pork (19)	Subtotal (39)	Beef (9)	Chicken (16)	Pork (3)	Subtotal (28)	
Tetracycline (TET)	92.0 (12)	100.0 (7)	100.0 (9)	97.0 (8)	89.0 (8)	81.0 (1)	67.0 (2)	82.0 (23)	91.0 (61)
β-Lactams									
AMP	62.0 (8)	71.0 (5)	95.0 (18)	80.0 (31)	44.0 (4)	63.0 (10)	33.0 (1)	54.0 (15)	69.0 (46)
CAZ	0	0	0	0	0	0	0	0	0
CFX	0	0	0	0	0	0	0	0	0
IPM	0	0	0	0	0	0	0	0	0
Aminoglycosides									
STR	46.0 (6)	57.0 (4)	32.0 (6)	41.0 (16)	56.0 (5)	69.0 (11)	33.0 (1)	61.0 (17)	49.0 (33)
KAN	0.8 (1)	0	0	2.6 (1)	11.0 (1)	0	0	3.6 (1)	3.0 (2)
GEN	0	0	0	0	0	0	0	0	0
Quinolones									
NA	0.8 (1)	14.0 (1)	0	5.1 (2)	0	0	0	0	3.0 (2)
NFLX	0	0	0	0	0	0	0	0	0
Sulfamide (SXT)	0.8 (1)	14.0 (1)	5.3 (1)	7.7 (3)	11.0 (1)	0	0	3.6 (1)	6.0 (4)
Phenicol (CM)	0.8 (1)	14.0 (1)	5.3 (1)	7.7 (3)		13.0 (2)	0	7.1 (2)	7.5 (5
Other (FOM)	0	29.0 (2)	0	5.1 (2)	11.0 (1)	13.0 (2)	33.0 (1)	14.0 (4)	9.0 (6)

a) TET, tetracycline; AMP, ampicillin; CAZ, ceftazidime; CFX, cefoxitin; IPM, imipenem; STR, streptomycin; KAN, kanamycin; GEN, gentamicin; NA, nalidixic acid; NFLX, norfloxacin; SXT, sulfamethoxazole-trimethoprim; CM, chloramphenicol; FOM, fosfomycin.

Table 4. Percentage of *Providencia* isolates exhibiting antimicrobial and multi-drug resistances: (A) in country-based; (B) in meat-based

Category		(A) Country			(B) Meat			
		China (39)	Japan (28)	Total (67)	Beef (22)	Pork (22)	Chicken (23)	Total (67)
Percentage of antimicrobial resistant isolates (n[a])		97.0 (38)	93.0 (26)	96.0 (64)	91.0 (20)	96.0 (21)	96.0 (22)	96.0 (64)
Percentage of MDR isolates (n[a])	R3[b]	33.0 (13)	32.0 (9)	33.0 (22)	32.0 (7)	32.0 (7)	35.0 (8)	33.0 (22)
	R4[b]	2.6 (1)	3.6 (1)	3.0 (2)	4.5 (1)	4.5 (1)	0.0	3.0 (2)
	R5[b]	7.7 (3)	7.1 (2)	7.5 (5)	4.5 (1)	0.0	17.0 (4)	7.5 (5)
	Total	44.0 (17)	43.0 (12)	43.0 (29)	41.0 (9)	36.0 (8)	52.0 (12)	43.0 (29)

a) Number of samples analyzed. b) R3 to R5 resist to 3 to 5 different classes of antimicrobials, respectively.

Table 5. Characterization of integrons identified in MDR *Providencia* species (resistant to ≥4 kinds of antimicrobials) isolated from retail meats obtained in Guangzhou, China and Osaka, Japan

MDR isolates		Resistant phenotype[b]	Integron regions		
Species[a]	Strains		*intI* gene	*qacEΔ1-sul1*[c]	Gene cassette array[d]
P. rus	CH-b-11	TET-STR-KAN-NA-SXT-CM	1,2	+	*aadB-cat-bla*$_{OXA}$10-*aadA1*, <u>*dfrA1-sat1-aad1*</u>
P. alca	CH-c-71	TET-AMP-STR-NA-SXT-FOM	1,2	-	<u>*dfr1-sat2-aadA1*</u>
	CH-c-09	TET-AMP-STR-CM-FOM	-	-	ND
	CH-p-45	TET-AMP-STR-SXT	1,2	-	ND
	JA-b-5	TET-AMP-STR-FOM	-	-	ND
P. ret	JA-b-9	TET-STR-KAN-SXT	1	+	*dfrA12- aadA2*
P. stu	JA-c-39	TET-AMP-STR-CM-FOM	1	+	ND
	JA-c-49	TET-AMP-STR-CM-FOM	1	-	ND

a) *P. rus*, *P. rustigianii*; *P. alca*, *P. alcalifaciens*; *P. ret*, *P. rettgeri*; *P. stu*, *P. stuartii*. b) TET, tetracycline; STR, streptomycin; KAN, kanamicin; NA, nalidixic acid; SXT, sulfamethoxazole-trimethoprim; CM, chloramphenicol; AMP, ampicillin; FOM, fosfomycin. c) +, *qacEΔ1-sul1* positive; -, *qacEΔ1*-sul1 negative. d) Gene cassettes harbored by class 2 integron are underlined. ND: not detected.

Prevalence and characterization of classes 1, 2 and 3 integrons in MDR Providencia spp.

Eight MDR strains that showed resistance to at least four antimicrobials were screened for the presence of *intI1*, *intI2* and *intI3* genes, and examined antimicrobial resistant gene cassettes. The PCR results revealed that six of the eight tested strains were *intI1* gene-positive, and of these, only three harbored the classic *qacEΔ-sul1* region as shown in Table 5. Class 1 integron gene cassette was amplified from only strains JA-b-9 (*P. rettgeri*) and CH-b-11 (*P. rustigianii*), which were subsequently confirmed to contain *dfrA12-aadA2* (trimethoprim and streptomycin resistances) and *aadB-cat-bla*$_{OXA-10}$-*aadA* genes (kanamycin, chloramphenicol,

β-lactams and streptomycin resistances), respectively.

Class 2 integron was also detected in three of the six *intI1* gene-positive *Providencia* strains, which were all isolated from Chinese samples. Variable regions, containing a similar set of antimicrobial resistant genes, i.e., *dfrA1-sat1-aad1* and *dfrI-sat2-aadA1*, were detected in *P. rustigianii* and *P. alcalifaciens* strains (CH-b-11 and CH-c-71, respectively). However, no class 3 integron was detected in any *Providencia* strains analyzed in this study (Table 5).

DISCUSSION

Since isolation of *Providencia* and its association with diarrhea and UTI have been increasingly reported in developing and developed countries [2, 6, 7, 9–11, 19, 20, 23, 27, 29, 35, 37, 38, 42, 47, 48], in this study we therefore examined the prevalence of *Providencia* in retail meats purchased from markets in Guangzhou, China and Osaka, Japan. Our results indicate that retail meats in both China and Japan were substantially contaminated with *Providencia* spp., especially *P. alcalifaciens*, and *Providencia* contamination rate of retail meats purchased in China (45%) was higher than that in Japan (38%). On the other hand, the *Providencia* contamination rate in same kind of meats sold in Thailand was much higher (68%) [39] than that in China and Japan. These findings may indicate why diarrhea attributed to *Providencia* is more common in developing countries and travelers from developing countries [20, 33, 38, 47]. However, another study showed a significantly lower *Providencia* contamination rate in retail foods purchased in Guangzhou, China (2.9%) [46]. One possible reason for this difference is the selective media used for each study. For example, our study used selective medium for *Providencia* while their study used selective medium for Enterobacteriaceae. Alternatively, the different rates could be due to samples analyzed. Our study analyzed retail meats only but their study analyzed not only retail meats but also other food samples such as vegetables, aquatic products, frozen foods and so on.

One more thing, which we have to discuss the result of this study, is the high prevalence rate of *Providencia* in Japanese beef. In slaughterhouse in Japan the strict regulation such as HACCP has been introduced. Thus, the contamination of the fecal bacteria must be very less. At this moment, however, we have no idea why the contamination rate of *Providencia* was so high in beef in Japan. One possible explanation would be cross contamination of *Providencia* to beef from other souses such as pork and chicken in the backyard of the supermarket. Further studies are required to understand the real situation of contamination rate of beef in Japanese.

P. alcalifaciens, *P. rettgeri*, *P. rustigianii*, and *P. stuartii* were isolated from meat samples in both this study and study in Thailand [38]. The fact that *P. alcalifaciens*, *P. rettgeri*, and *P. stuartii* were isolated from both patients with diarrhea/urinary tract infections and meat samples suggests that food animals, and in particular their meat, might be an important source of *Providencia* transmission to humans. Further studies are required regarding prevalence of *Providencia* in the environment, food animals, pet animals and so on.

In this study, we also examined antimicrobial susceptibility of the *Providencia* strains isolated from retail meats purchased in China and Japan. Our findings are similar to a previous report from Nigeria [1], showing that *Providencia* isolates in farm animals are commonly resistant to multiple antimicrobials including tetracycline, ampicillin, and streptomycin. On the other hand, the frequency of MDR was not statistically significant among *Providencia* strains isolated between China and Japan. Since usage of antimicrobials in veterinary settings must be different between China and Japan, there is no explanation why there was no significant different between these two countries. Although 43% (29/67) of the *Providencia* strains were MDR, it is reassuring that all the *Providencia* strains were susceptible to clinically important antimicrobials such as norfloxacin, imipenem, cefoxitin and ceftazidime. However, detection of resistance to sulfamethoxazole-trimethoprim, fosfomycin, and nalidixic acid is alarming because these antimicrobials are also important for the treatment of urinary tract infections and diarrhea. Therefore, rational use of antimicrobial agents should be emphasized in both veterinary and medical settings. Although our results cannot be used to ascribe the presence of resistance phenotypes to antimicrobials used in livestock, it does provide support for the idea that foods of animal origin are a potential source of antimicrobial resistant human pathogens [4, 14, 44].

Since integrons are often associated with MDR, we also examined the presence of integrons in MDR *Providencia* isolated in this study. Class 1 and/or class 2 integrons were detected in six of eight MDR *Providencia* strains with resistance to ≥4 antimicrobial agents. This finding supports the idea that class 1 and/or class 2 integrons present in MDR bacteria from meat samples most probably contaminated from food-producing livestock are a possible reservoir for the dissemination of resistance genes in the environment or in humans [5, 17, 40]. In particular, it should be noted that classes 1 and 2 integrons were detected in one MDR *P. rustigianii* isolated from a Chinese meat sample, which was also reported in another study that examined prevalence of *Providencia* spp. in animal feces [1, 26]. Machado *et al.* [26] suggested that various recombinational events may occur in these genetic platforms. However, additional detail investigations are necessary to support this hypothesis.

Extended spectrum β-lactamases (ESBL), which can degrade a variety of β-lactamases, is also one of the most important antimicrobial resistances in clinical settings. bla_{OXA-10} is one of the genes encoding β-lactamases. bla_{OXA-10} has been so far detected in only clinical *Providencia* strains such as *P. rettgeri* (Guangzhou, China, GenBank accession no. KJ488989.1), *P. rettgeri* (Nigeria, GenBank accession no. GU056843.2) and *P. stuartii* (Tunisian Burn Unit, GenBank accession no. JN193567.1). In this study, bla_{OXA-10} gene was detected in *Providencia* spp. isolated from a retail meat sample. This is the first report, to the best of our knowledge, regarding isolation of *Providencia* spp. a carrying bla_{OXA-10} gene from meats. The bla_{OXA-10} gene encodes β-lactamases with a serine residue as an active site belonging to class D β-lactamases, which was found in a variety of Gram-negative bacteria, especially *Pseudomonas aeruginosa* [31]. The OXA-10 β-lactamase has a narrow spectrum that can hydrolyze cephalosporins, cefotaxime, ceftriaxone, and aztreonam at low levels but not ceftazidime, cephamycins, and carbapenems [22]. Likewise, in

this study, a *P. rustigianii* strain (CH-b-11) harboring bla_{OXA-10} was susceptible to all four tested β-lactam antibiotics (Table 2). However, the importance of this β-lactamase-integron-carrying organism should not be neglected, as mutant derivatives of OXA-10, such as OXA-11, OXA-13, OXA-16, OXA-28, OXA-35 and OXA-74, possess increased activities toward expanded-spectrum cephalosporins [31].

In summary, this is the first report focusing on the prevalence and antimicrobial resistance of *Providencia* spp. in retail raw meats in China and Japan. Our results showed that retail meats in both China and Japan are substantially contaminated with *Providencia* spp., especially *P. alcalifaciens*, which displayed a high frequency of antimicrobial resistance. In addition, class 1 and/or 2 integrons were also associated with MDR in *Providencia* spp. These data suggest that meats may be a potential source of *Providencia* for human infections, which highlights the importance of *Providencia* spp. in retail meats as a reservoir for antimicrobial resistance genes. Although we detected only a small number of *Providencia* isolates, our data suggested that establishing a surveillance program for resistant *Providencia* spp. in retail meat is imperative, as well as supervision for prudent use of antimicrobials in food animals to prevent the dissemination of MDR *Providencia* spp. among animals and humans.

REFERENCES

1. Aibinu, I. E., Pfeifer, Y., Ogunsola, F., Odugbemi, T., Koenig, W. and Ghebremedhin, B. 2011. Emergence of β-lactamases OXA-10, VEB-1 and CMY in Providencia spp. from Nigeria. *J. Antimicrob. Chemother.* **66**: 1931–1932. [Medline] [CrossRef]
2. Albert, M. J., Faruque, A. S. and Mahalanabis, D. 1998. Association of *Providencia alcalifaciens* with diarrhea in children. *J. Clin. Microbiol.* **36**: 1433–1435. [Medline]
3. Arakawa, Y., Murakami, M., Suzuki, K., Ito, H., Wacharotayankun, R., Ohsuka, S., Kato, N. and Ohta, M. 1995. A novel integron-like element carrying the metallo-β-lactamase gene *bla*IMP. *Antimicrob. Agents Chemother.* **39**: 1612–1615. [Medline] [CrossRef]
4. Barlow, R. S., Fegan, N. and Gobius, K. S. 2009. Integron-containing bacteria in faeces of cattle from different production systems at slaughter. *J. Appl. Microbiol.* **107**: 540–545. [Medline] [CrossRef]
5. Byrne-Bailey, K. G., Gaze, W. H., Zhang, L., Kay, P., Boxall, A., Hawkey, P. M. and Wellington, E. M. 2011. Integron prevalence and diversity in manured soil. *Appl. Environ. Microbiol.* **77**: 684–687. [Medline] [CrossRef]
6. Chlibek, R., Jirous, J. and Beran, J. 2002. Diarrhea outbreak among Czech Army Field Hospital personnel caused by *Providencia alcalifaciens*. *J. Travel Med.* **9**: 151–152. [Medline] [CrossRef]
7. Collis, C. M., Recchia, G. D., Kim, M. J., Stokes, H. W. and Hall, R. M. 2001. Efficiency of recombination reactions catalyzed by class 1 integron integrase IntI1. *J. Bacteriol.* **183**: 2535–2542. [Medline] [CrossRef]
8. Cordano, A. M. and Virgilio, R. 1996. Evolution of drug resistance in Salmonella *panama* isolates in Chile. *Antimicrob. Agents Chemother.* **40**: 336–341. [Medline]
9. Cornaglia, G., Frugoni, S., Mazzariol, A., Piacentini, E., Berlusconi, A. and Fontana, R. 1995. Activities of oral antibiotics on *Providencia* strains isolated from institutionalized elderly patients with urinary tract infections. *Antimicrob. Agents Chemother.* **39**: 2819–2821. [Medline] [CrossRef]
10. Cui, K., Fu, X., Li, Z. and Li, C. 2000. An investigation into food poisoning caused by *Providencia rettgeri*. *Lit. Inf. Prev. Med.* **6**: 116.
11. Douka, E., Perivolioti, E., Kraniotaki, E., Fountoulis, K., Economidou, F., Tsakris, A., Skoutelis, A. and Routsi, C. 2015. Emergence of a pandrug-resistant VIM-1-producing *Providencia stuartii* clonal strain causing an outbreak in a Greek intensive care unit. *Int. J. Antimicrob. Agents* **45**: 533–536. [Medline] [CrossRef]
12. Dalsgaard, A., Forslund, A., Serichantalergs, O. and Sandvang, D. 2000. Distribution and content of class 1 integrons in different Vibrio cholerae O-serotype strains isolated in Thailand. *Antimicrob. Agents Chemother.* **44**: 1315–1321. [Medline] [CrossRef]
13. Falbo, V., Carattoli, A., Tosini, F., Pezzella, C., Dionisi, A. M. and Luzzi, I. 1999. Antibiotic resistance conferred by a conjugative plasmid and a class I integron in *Vibrio cholerae* O1 El Tor strains isolated in Albania and Italy. *Antimicrob. Agents Chemother.* **43**: 693–696. [Medline]
14. Fey, P. D., Safranek, T. J., Rupp, M. E., Dunne, E. F., Ribot, E., Iwen, P. C., Bradford, P. A., Angulo, F. J. and Hinrichs, S. H. 2000. Ceftriaxone-resistant salmonella infection acquired by a child from cattle. *N. Engl. J. Med.* **342**: 1242–1249. [Medline] [CrossRef]
15. Fonseca, E. L., Vieira, V. V., Cipriano, R. and Vicente, A. C. 2005. Class 1 integrons in *Pseudomonas aeruginosa* isolates from clinical settings in Amazon region, Brazil. *FEMS Immunol. Med. Microbiol.* **44**: 303–309. [Medline] [CrossRef]
16. Giakkoupi, P., Tryfinopoulou, K., Polemis, M., Pappa, O., Miriagou, V. and Vatopoulos, A. 2015. Circulation of a multiresistant, conjugative, IncA/C plasmid within the nosocomial *Providencia stuartii* population in the Athens area. *Diagn. Microbiol. Infect. Dis.* **82**: 62–64. [Medline] [CrossRef]
17. Gillings, M., Boucher, Y., Labbate, M., Holmes, A., Krishnan, S., Holley, M. and Stokes, H. W. 2008. The evolution of class 1 integrons and the rise of antibiotic resistance. *J. Bacteriol.* **190**: 5095–5100. [Medline] [CrossRef]
18. Gómez-Lus, R. 1998. Evolution of bacterial resistance to antibiotics during the last three decades. *Int. Microbiol.* **1**: 279–284. [Medline]
19. Guth, B. E. and Perrella, E. 1996. Prevalence of invasive ability and other virulence-associated characteristics in *Providencia alcalifaciens* strains isolated in São Paulo, Brazil. *J. Med. Microbiol.* **45**: 459–462. [Medline] [CrossRef]
20. Haynes, J. and Hawkey, P. M. 1989. *Providencia alcalifaciens* and travellers' diarrhoea. *BMJ* **299**: 94–95. [Medline] [CrossRef]
21. Heuer, H., Binh, C. T., Jechalke, S., Kopmann, C., Zimmerling, U., Krögerrecklenfort, E., Ledger, T., González, B., Top, E. and Smalla, K. 2012. IncP-1epsilon plasmids are important vectors of antibiotic resistance genes in agricultural systems: diversification driven by class 1 Integron gene cassettes. *Front. Microbiol.* **3**: 2. [Medline] [CrossRef]
22. Huovinen, P., Huovinen, S. and Jacoby, G. A. 1988. Sequence of PSE-2 beta-lactamase. *Antimicrob. Agents Chemother.* **32**: 134–136. [Medline] [CrossRef]
23. Janda, J. M., Abbott, S. L., Woodward, D. and Khashe, S. 1998. Invasion of HEp-2 and other eukaryotic cell lines by *Providenciae*: further evidence supporting the role of *Providencia alcalifaciens* in bacterial gastroenteritis. *Curr. Microbiol.* **37**: 159–165. [Medline] [CrossRef]
24. Juneja, P. and Lazzaro, B. P. 2009. *Providencia sneebia* sp. nov. and *Providencia burhodogranariea* sp. nov., isolated from wild Drosophila

melanogaster. *Int. J. Syst. Evol. Microbiol.* **59**: 1108–1111. [Medline] [CrossRef]

25. Khunthongpan, S., Sumpavapol, P., Tanasupawat, S., Benjakul, S. and H-Kittikun, A. 2013. *Providencia thailandensis* sp. nov., isolated from seafood processing wastewater. *J. Gen. Appl. Microbiol.* **59**: 185–190. [Medline] [CrossRef]

26. Machado, E., Coque, T. M., Cantón, R., Sousa, J. C. and Peixe, L. 2008. Antibiotic resistance integrons and extended-spectrum beta-lactamases among Enterobacteriaceae isolates recovered from chickens and swine in Portugal. *J. Antimicrob. Chemother.* **62**: 296–302. [Medline] [CrossRef]

27. Mahrouki, S., Chihi, H., Bourouis, A., Ben Moussa, M. and Belhadj, O. 2014. First characterization of a *Providencia stuartii* clinical isolate from a Tunisian intensive care unit coproducing VEB-1-a, OXA-2, qnrA6 and aac(6′)-Ib-cr determinants. *Braz. J. Infect. Dis.* **18**: 211–214. [Medline] [CrossRef]

28. Mohr O'Hara, C., Steigerwalt, A. G., Green, D., McDowell, M., Hill, B. C., Brenner, D. J. and Miller, J. M. 1999. Isolation of *Providencia heimbachae* from human feces. *J. Clin. Microbiol.* **37**: 3048–3050. [Medline]

29. Murata, T., Iida, T., Shiomi, Y., Tagomori, K., Akeda, Y., Yanagihara, I., Mushiake, S., Ishiguro, F. and Honda, T. 2001. A large outbreak of foodborne infection attributed to *Providencia alcalifaciens*. *J. Infect. Dis.* **184**: 1050–1055. [Medline] [CrossRef]

30. Plante, I., Centrón, D. and Roy, P. H. 2003. An integron cassette encoding erythromycin esterase, ere(A), from *Providencia stuartii*. *J. Antimicrob. Chemother.* **51**: 787–790. [Medline] [CrossRef]

31. Poirel, L., Naas, T. and Nordmann, P. 2010. Diversity, epidemiology, and genetics of class D beta-lactamases. *Antimicrob. Agents Chemother.* **54**: 24–38. [Medline] [CrossRef]

32. Sajjad, A., Holley, M. P., Labbate, M., Stokes, H. W. and Gillings, M. R. 2011. Preclinical class 1 integron with a complete Tn402-like transposition module. *Appl. Environ. Microbiol.* **77**: 335–337. [Medline] [CrossRef]

33. Sen, R. 1962. Isolation of strains of the Providence group from cases with diarrhoea in Ibadan, Nigeria, West Africa. *Indian J. Med. Res.* **50**: 622–626. [Medline]

34. Senior, B. W. 1997. Media for the detection and recognition of the enteropathogen *Providencia alcalifaciens* in faeces. *J. Med. Microbiol.* **46**: 524–527. [Medline] [CrossRef]

35. Shah, M. M., Odoyo, E., Larson, P. S., Apondi, E., Kathiiko, C., Miringu, G., Nakashima, M. and Ichinose, Y. 2015. First report of a foodborne *Providencia alcalifaciens* outbreak in Kenya. *Am. J. Trop. Med. Hyg.* **93**: 497–500. [Medline] [CrossRef]

36. Shi, L., Fujihara, K., Sato, T., Ito, H., Garg, P., Chakrabarty, R., Ramamurthy, T., Nair, G. B., Takeda, Y. and Yamasaki, S. 2006. Distribution and characterization of integrons in various serogroups of *Vibrio cholerae* strains isolated from diarrhoeal patients between 1992 and 2000 in Kolkata, India. *J. Med. Microbiol.* **55**: 575–583. [Medline] [CrossRef]

37. Shima, A., Hinenoya, A., Asakura, M., Nagita, A. and Yamasaki, S. 2012. Prevalence of *Providencia* strains among children with diarrhea in Japan. *Jpn. J. Infect. Dis.* **65**: 545–547. [Medline] [CrossRef]

38. Shima, A., Hinenoya, A., Samosornsuk, W., Samosornsuk, S., Mungkornkaew, N. and Yamasaki, S. 2016. Prevalence of *Providencia* strains among patients with diarrhea and in retail meats in Thailand. *Jpn. J. Infect. Dis.* **69**: 323–325. [Medline] [CrossRef]

39. Somvanshi, V. S., Lang, E., Sträubler, B., Spröer, C., Schumann, P., Ganguly, S., Saxena, A. K. and Stackebrandt, E. 2006. *Providencia vermicola* sp. nov., isolated from infective juveniles of the entomopathogenic nematode Steinernema thermophilum. *Int. J. Syst. Evol. Microbiol.* **56**: 629–633. [Medline] [CrossRef]

40. Stalder, T., Barraud, O., Casellas, M., Dagot, C. and Ploy, M. C. 2012. Integron involvement in environmental spread of antibiotic resistance. *Front. Microbiol.* **3**: 119. [Medline] [CrossRef]

41. Tollefson, L., Altekruse, S. F. and Potter, M. E. 1997. Therapeutic antibiotics in animal feeds and antibiotic resistance. *Rev. - Off. Int. Epizoot.* **16**: 709–715. [Medline] [CrossRef]

42. Tshisevhe, V. S., Lekalakala, M. R., Tshuma, N., Janse van Rensburg, S. and Mbelle, N. 2016. Outbreak of carbapenem-resistant *Providencia rettgeri* in a tertiary hospital. *S. Afr. Med. J.* **107**: 31–33. [Medline] [CrossRef]

43. White, D. G., Zhao, S., Sudler, R., Ayers, S., Friedman, S., Chen, S., McDermott, P. F., McDermott, S., Wagner, D. D. and Meng, J. 2001. The isolation of antibiotic-resistant *salmonella* from retail ground meats. *N. Engl. J. Med.* **345**: 1147–1154. [Medline] [CrossRef]

44. White, P. A., McIver, C. J. and Rawlinson, W. D. 2001. Integrons and gene cassettes in the enterobacteriaceae. *Antimicrob. Agents Chemother.* **45**: 2658–2661. [Medline] [CrossRef]

45. World Health Organization. 2012. The evolving threat of antimicrobial resistance options for action. Geneva.

46. Ye, Q., Wu, Q., Zhang, S., Zhang, J., Yang, G., Wang, H., Huang, J., Chen, M., Xue, L. and Wang, J. 2017. Antibiotic-resistant extended spectrum β-lactamase- and plasmid-mediated AmpC-producing Enterobacteriaceae isolated from retail food products and the pearl river in Guangzhou, China. *Front. Microbiol.* **8**: 96. [Medline] [CrossRef]

47. Yoh, M., Matsuyama, J., Ohnishi, M., Takagi, K., Miyagi, H., Mori, K., Park, K. S., Ono, T. and Honda, T. 2005. Importance of *Providencia* species as a major cause of travellers' diarrhoea. *J. Med. Microbiol.* **54**: 1077–1082. [Medline] [CrossRef]

48. Zhang, G. 2005. An outbreak of foodborne infection attributed to *Providencia rettgeri*. *Sh. J. Prev. Med.* **17**: 128.