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ORIGINAL ARTICLE

Clinical characteristics in adult patients with *Salmonella* bacteremia and analysis of ciprofloxacin-nonsusceptible isolates

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Abstract *Background/purpose:* The purpose of this study is to describe clinical characteristics of *Salmonella* bacteremia in adult patients and analyze ciprofloxacin-nonsusceptible isolates.
Methods: A total of 101 *Salmonella* blood isolates from adult patients were collected from January 2011 to December 2013 in MacKay Memorial Hospital. Eight ciprofloxacin-nonsusceptible *Salmonella* blood isolates were screened for carbapenemase and other β lactamase genes. Isolates were examined by PCR for the quinolone resistance-determining region (QRDR) of all subunits for DNA gyrase (*gyrA* and *gyrB*) genes and topoisomerase IV (*parC* and *parE*) genes.
Results: There were 22 (21.78%) *S. enterica* serovar B, 5 (4.95%) *S. enterica* serovar C_1, 7 (6.93%) *S. enterica* serovar C_2, 65 (64.36%) *S. enterica* serovar D, and 2 (1.98%) *S. enterica* serovar Typhi (*S. typhi*) isolates. β-lactamase gene screening and sequencing yielded only one bla_{CMY-2}-positive isolate. In multivariate risk factor analysis, renal insufficiency [odds ratio

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(OR) 3.774; $p = 0.020$] and heart disease (OR 2.922; $p = 0.027$) were more common among elderly patients ($\geq$65 years). Independent risk factors for ciprofloxacin-nonsusceptible strains included *S. enterica* serovar C_2 (OR 28.430; $p = 0.032$), renal insufficiency (OR 13.927; $p = 0.032$), and immunosuppression agent usage (OR 60.082; $p = 0.006$). 87.50% (7/8) of isolates had *gyrA* mutation, 62.50% (5/8) had *parC* mutation, and none had *gyrB* and *parE* mutations. Isolates with both Ser83Phe/Asp87Asn *gyrA* and Thr57Ser/Ser80Ile *parC* mutation genes were highly ciprofloxacin-resistant (minimum inhibitory concentration $\geq$4 mg/L).

Conclusions: Elderly patients with renal insufficiency and heart disease were at risk for *Salmonella* bacteremia. Those for ciprofloxacin-nonsusceptible strains included *S. enterica* serovar C_2, renal insufficiency, and immunosuppression agent usage. The 8 ciprofloxacin-nonsusceptible isolates carried *gyrA* and *parC* mutations, which cause resistance that poses a major concern.

Introduction

Salmonella infection is an important public health problem worldwide. The burden is significant, with an estimated 93.8 million cases globally and 155,000 deaths each year.[1,2] The average number of annual incidences of bacteremia due to *Salmonella* species is 103 cases per million inhabitants in southern Taiwan.[3] *Salmonella* bacteremia is usually identified by the following serogroups in microbiological laboratories: *S. enterica* serovar B, *S. enterica* serovar C_1, *S. enterica* serovar C_2, *S. enterica* serovar D, and *S. enterica* serovar Typhi.[4]

Lee et al[5] revealed that bacteremia due to nontyphoidal *Salmonella* presented with different clinical features in adults than it did in children. Nontyphoidal *Salmonella* bacteremia in children rarely caused fatalities, whereas it should be considered life-threatening in adult patients, regardless of primary or secondary bacteremia.[6] *Salmonella* bacteremia has important clinical significance because it affects patients who usually have underlying diseases, such as an immunocompromised status, connective tissue diseases, use of therapeutic immunosuppressants, systemic lupus erythematosus, malignancies, diabetes mellitus, and human immunodeficiency virus infection.[5–9]

When an invasive or severe *Salmonella* infection is encountered, ceftriaxone is recommended.[10] Much evidence indicates that the resistance to third-generation cephalosporins poses a major concern for use as an empiric antimicrobial therapy for *Salmonella* infection.[10–12]

DNA gyrase (*gyrA* and *gyrB*) and topoisomerase IV (*parC* and *parE*) are the principal targets for antibacterial activity of quinolones. One mechanism that has been widely reported and associated with ciprofloxacin-resistance is mutations in target genes in the quinolone resistance-determining region (QRDR). Mutations at codon Ser83 or Asp87 are known to be common sites of mutation in *gyrA*, resulting in quinolone resistance. The *gyrA* mutations could be detected as Ser83Phe, Ser83Tyr, and Asp87Asn, while *parC* mutations are detected as Thr57Ser and Ser80Ile.[13–15]

The risk factors of *Salmonella* bacteremia have been reported around the world in recent years.[5,6,7,16] However, there are currently only a few articles reporting information concerning elderly patients and ciprofloxacin-nonsusceptible strains with *Salmonella* bacteremia. The purpose of this study is to describe the clinical characteristics of adult patients with *Salmonella* bacteremia and to analyze the ciprofloxacin-nonsusceptible isolates.

Methods

Bacterial isolate collection, ciprofloxacin and ceftriaxone susceptibility testing, and patient characteristics

The 101 *Salmonella* blood isolates from patients aged $\geq$18 years were collected from January 2011 to December 2013 in MacKay Memorial Hospital, a 2200-bed hospital in Taiwan. For patients with $\geq$2 positive blood cultures, only the first isolate was included. Identification was performed using the Vitek 2 system (bioMérieux Vitek Systems Inc., Hazelwood, MO, USA). O antisera was determined by the slide agglutination test by Difco antisera (Becton Dickinson, MD, USA) and used to identify the *S. enterica* isolates as serogroups A, B, C_1, C_2, D, and Typhi.

Susceptibility tests for ciprofloxacin and ceftriaxone were also performed using the Vitek 2 system. Isolates were kept frozen at -70°C in trypticase soy broth (Becton Dickinson) containing 20% glycerol (v/v) until further testing. In addition, antibiotics susceptibilities against ciprofloxacin was also determined by the agar dilution method. The minimum inhibitory concentrations (MICs) of ciprofloxacin and ceftriaxone were interpreted according to the Clinical and Laboratory Standards Institute guidelines.[17,18]

Medical records were reviewed, and the following data were collected: patient characteristics, comorbidities, invasive procedure use (Table 1), and whether or not the patient was in the intensive care unit (ICU) at the time of bacteremia onset. Central line-associated infection was defined according to the United States Centers for Disease Control and Prevention guidelines.[19] Liver cirrhosis was diagnosed by gastroenterologists based on laboratory and radiological evidence. Renal insufficiency was defined as an estimated

glomerular filtration rate less than 60 mL/min/1.73 m^2. Attributable mortality indicated that a patient died during the admission period of a *Salmonella* bacteremia episode. This study was approved by the MacKay Memorial Institutional Review Board with protocol number 14MMHIS131.

Pulse-field gel electrophoresis

The eight ciprofloxacin-nonsusceptible *Salmonella* isolates were identified by pulse-field gel electrophoresis (PFGE) following the digestion of intact genomic DNA with *Xba*I (Biolabs, Beverly, MA, USA). The DNA fragments were separated on 1% (w/v) SeaKem GTG agarose gels in 0.5% TBE [Tris-borate ethylene diamine tetra-acetic acid (EDTA)] buffer. This was done in a CHEF Mapper apparatus (Bio-Rad, Hercules, CA, USA) with a potential of 6 V/cm pulsed from 2.16 seconds to 63.8 seconds for 19 hours at 14°C.[20] The completed gels were stained with ethidium bromide and photographed with ultraviolet light.

The *Xba*I restriction profiles were initially compared to each other by visual inspection, and isolates were considered to be closely related if they showed differences of less than three bands.[21] Computer-assisted analysis was also performed using BioNumerics (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis was performed by the unweighted pair group method with mathematical averaging, and DNA relatedness was calculated using the band-based Dice coefficient with 1.0% band tolerance and a 1.56% optimization setting for the whole profile. Isolates were considered to belong to the same cluster when the similarity coefficient reached 85%.

Polymerase chain reaction (PCR) and sequencing

The eight ciprofloxacin-nonsusceptible *Salmonella* blood isolates were examined by PCR for QRDR of all subunits for DNA gyrase (*gyrA* and *gyrB*) genes and topoisomerase IV (*parC* and *parE*) genes using published primers.[13,15]

The PCR preparation procedure is outlined as follows. Bacteria were boiled in sterile water for 10 minutes, and the supernatant was collected and used as DNA sources for PCR. The 25 μL reaction mixture consisted of 1X S-T Gold buffer, 1.5mM $MgCl_2$, 0.2mM deoxynucleotides (dNTP), and 20 pmol of each primer. The PCR amplicons were purified

Table 1 Demographic and clinical characteristics according to variables of age and ciprofloxacin susceptibility among 101 patients with *Salmonella enterica* bacteremia.

Demographic and clinical characteristics *n* (%)	≥65 y *n* = 49 *n* (%)	<65 y *n* = 52 *n* (%)	*P*	CIP-nonsusceptible *n* = 8 *n* (%)	CIP-susceptible *n* = 93 *n* (%)	*p*
Age, y[a] 62.81 ± 18.65	78.22 ± 7.504	48.29 ± 13.627	<0.01	64.88 ± 11.544	62.63 ± 19.171	0.746
Sex male 64 (63.37)	32 (65.31)	32 (61.54)	0.852	4 (50.00)	60 (64.52)	0.460
Mortality 18 (17.82)	8 (16.33)	10 (19.23)	0.904	1 (12.50)	17 (18.28)	>0.99
Serovar B 22 (21.78)	15 (30.61)	7 (13.46)	0.065	1 (12.50)	21 (22.58)	0.682
Serovar C_1 5 (4.95)	2 (4.08)	3 (5.77)	>0.99	2 (25.00)	3 (3.23)	0.049
Serovar C_2 7 (6.93)	6 (12.24)	1 (1.92)	0.055	3 (37.50)	4 (4.30)	0.010
Serovar D 65 (64.36)	26 (53.06)	39 (75.00)	0.036	2 (25.00)	63 (67.74)	0.023
Serovar Typhi 2 (1.98)	0 (0.00)	2 (3.85)	0.495	0 (0.00)	2 (2.15)	>0.99
CIP-ns 8 (7.92)	4 (8.16)	4 (7.69)	>0.99	—	—	—
CRO-r 1 (0.99)	1 (2.04)	0 (0.00)	0.485	1 (12.50)	0 (0.00)	0.079
ICU stay 20 (19.8)	9 (18.37)	11 (21.15)	0.919	2 (25.00)	18 (19.35)	0.656
CVA 5 (4.95)	4 (8.16)	1 (1.92)	0.148	0 (0.00)	5 (5.38)	>0.99
Heart disease 30 (29.70)	20 (40.82)	10 (19.23)	0.031	3 (37.50)	27 (29.03)	0.692
COPD 7 (6.93)	5 (10.20)	2 (3.85)	0.260	2 (25.00)	5 (5.38)	0.095
Liver cirrhosis 11 (10.89)	6 (12.24)	5 (9.62)	0.917	1 (12.50)	10 (10.75)	>0.99
Renal insufficiency 20 (19.8)	14 (28.57)	6 (11.54)	0.049	4 (50.00)	16 (17.20)	0.047
Alcoholism 6 (5.94)	1 (2.04)	5 (9.62)	0.206	0 (0.00)	6 (6.45)	>0.99
Malignancy 34 (33.66)	18 (36.73)	16 (30.77)	0.672	2 (25.00)	32 (34.41)	0.714
Shock within 3 d 5 (4.95)	2 (4.08)	3 (5.77)	>0.99	0 (0.00)	5 (5.38)	>0.99
Collagen disease 2 (1.98)	0 (0.00)	2 (3.85)	0.495	0 (0.00)	2 (2.15)	>0.99
DM 29 (28.71)	18 (36.73)	11 (21.15)	0.131	4 (50.00)	25 (26.88)	0.222
Immunosuppression agent 5 (4.95)	1 (2.04)	4 (7.69)	0.363	2 (25.00)	3 (3.23)	0.049
Steroid use 6 (5.94)	1 (2.04)	5 (9.62)	0.206	1 (12.50)	5 (5.38)	0.399
Foley 21 (20.79)	10 (20.41)	11 (21.15)	>0.99	2 (25.00)	19 (20.43)	0.670
CVC 18 (17.82)	8 (16.33)	10 (19.23)	0.904	1 (12.50)	17 (18.28)	>0.99

[a] Data are presented as mean ± standard deviation for age. CIP-ns = ciprofloxacin-nonsusceptible; COPD = chronic obstructive pulmonary disease; CRO-r = ceftriaxone-resistant; CVA = cerebrovascular accident; CVC = central venous catheter; DM = diabetes mellitus; ICU = intensive care unit; Serovar B = *Salmonella enterica* serovar B; Serovar C_1 = *Salmonella enterica* serovar C_1; Serovar C_2 = *Salmonella enterica* serovar C_2; Serovar D = *Salmonella enterica* serovar D; Serovar Typhi = *Salmonella enterica* serovar Typhi; — = not available.

using ExoSAP-IT reagent (USB Corporation, Cleveland, OH, USA), and both strands were sequenced using the standard dideoxynucleotide method in an ABI Prism 377 DNA sequencer (Applied Biosystems, Foster City, CA, USA). Sequence similarity searches were performed with the basic local alignment search tool (BLAST; http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Scoring algorithm for predicting vascular infections

Chen et al[22] designed a simple scoring algorithm for predicting vascular infections with nontyphoidal *Salmonella* bacteremia. In this scoring system, male sex, hypertension, coronary arterial disease, and *S. enterica* serovar C_1 infections are each assigned +1 point to form the nontyphoidal *Salmonella* vascular infection score. In contrast, malignancy and immunosuppressive therapy are each assigned −1 point owing to their negative associations with vascular infections. A cutoff value of +1 represents a high sensitivity (95.0%) and an acceptable specificity (45.3%).[22]

We reviewed the medical record and imaging report such as computer tomography to find out the patient gets vascular infection or not.

Statistical analysis

Categorical variables were described as numbers and percentages. The age variable is presented as a mean value with standard deviation (SD). Categorical variables were analyzed using the Chi-square test with Yates' continuity correction or Fisher's exact test as appropriate. Continuous variables were analyzed by the Student two-sample *t* test for parametric methods. Logistic regression models were used to identify independent risk factors. Odds ratios (OR) and 95% confidence intervals (CI) were analyzed separately for each of the risk factor variables by the univariate analysis. Subsequently, all significant variables with $p \leq 0.05$ in the univariate analysis were used in the multivariate analysis of the logistic regression model. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 21.0 (SPSS Inc., Chicago, IL, USA).

Results

To describe patient characteristics, comorbidities, invasive procedure use, and serogroups of isolates, a total of 101 *Salmonella* blood isolates were collected during the study period. There were 22 (21.78%) *S. enterica* serovar B, 5 (4.95%) *S. enterica* serovar C_1, 7 (6.93%) *S. enterica* serovar C_2, 65 (64.36%) *S. enterica* serovar D, and 2 (1.98%) *S. enterica* serovar Typhi (*S. typhi*) isolates.

The age distribution of the study population was 62.81 ± 18.65 years. The male-to-female ratio was 64:37 (males, 63.37%, 64/101). There were 49 patients aged ≥65 years. Table 1 shows the demographic and clinical characteristics according to variables of age and ciprofloxacin susceptibility among 101 adult patients with *Salmonella enterica* bacteremia. The mean (± SD) age of adult patients <65 years old was 48.29 ± 13.627 years. The mean (± SD) age of elderly patients ≥65 years old was 78.22 ± 7.504 years. Compared with the <65-year-old adult patients, more patients older than 65 had heart disease (40.82% vs. 19.23%; $p = 0.031$) and renal insufficiency (28.57% vs. 11.54%; $p = 0.049$). In contrast, fewer people ≥65 years old had *S. enterica* serovar D bacteremia (53.06% vs. 75.00%; $p = 0.036$). In patients with ciprofloxacin-nonsusceptible bacteremia, there were more *S. enterica* serovar C_1 and *S. enterica* serovar C_2 isolates than in the ciprofloxacin-susceptible groups (C_1, 25.00% vs. 3.23%, $p = 0.049$; C_2, 37.50% vs. 4.30%, $p = 0.010$). Comorbidities such as renal insufficiency (50.00% vs. 17.20%, $p = 0.047$) and immunosuppressive agent usage (25.00% vs. 3.23%; $p = 0.049$) were more common in the ciprofloxacin-resistant bacteremia group.

All variables with a *p* value of ≤0.05 in the univariate analysis were considered for inclusion in the logistic regression model for the multivariate analysis. Table 2 shows the multivariate analysis of risk factors in patients ≥65 years old and ciprofloxacin-nonsusceptible strains among adult patients with *S. enterica* bacteremia determined through the logistic regression analysis. Multivariate analysis revealed that the independent factor associated with ≥65-year-old elderly patients included renal insufficiency (OR 3.774; $p = 0.020$) and heart disease (OR 2.922; $p = 0.027$). The independent risk factors for ciprofloxacin-nonsusceptible strains included *S. enterica* serovar C_2 (OR 28.430; $p = 0.032$), renal insufficiency (OR 13.927; $p = 0.032$), and immunosuppression agent usage (OR 60.082; $p = 0.006$).

Table 3 shows the clinical characteristics of patients, serogroups, QRDR of all subunits for the DNA gyrase (*gyrA*) gene and topoisomerase IV (p*arC*) gene, and MICs among the ciprofloxacin-nonsusceptible *Salmonella* bacteremia. The most common serogroup in ciprofloxacin-nonsusceptible *Salmonella* isolates was *S. enterica* serovar C (62.50%, 5/8), including two *S. enterica* serovar C_1 and three *S. enterica* serovar C_2 isolates.

Isolates with the *gyrA* mutation comprised 87.50% (7/8), while 62.50% (5/8) of the isolates had the *parC* mutation, but none had *gyrB* or *parE* mutations. The *gyrA* mutations were manifested as Ser83Phe, Asp87Asn, and Ser83Tyr. The *parC* mutations were manifested as Thr57Ser and Ser80Ile. The two isolates of *S. enterica* serovar C_1 with Ser83Phe/Asp87Asn *gyrA* mutation and Thr57Ser/Ser80Ile *parC* mutations exhibited high-level resistance to ciprofloxacin (MIC ≥ 4 mg/L). The other isolates harboring either the *gyrA* mutation or *parC* mutation gene presented low-level resistance to ciprofloxacin (MIC = 2 mg/L).

The similarity of PFGE patterns in eight ciprofloxacin-nonsusceptible *Salmonella* blood isolates is presented in Figure 1. Isolates No.5 and No.6 of *S. enterica* serovar C_1 were considered to belong to the same cluster. Isolates No.7 and No.8 of *S. enterica* serovar B were considered to belong to the same cluster when their similarity coefficient reached 85%.

We also attempted to match our study participants with the simple scoring algorithm designed by Chen et al[22] to predict vascular infections with nontyphoidal *Salmonella* bacteremia in our study. We found a high sensitivity [100%, 5/(5+0)] and an acceptable specificity [47.44%, 37/(41+37)] of vascular infections in patients.

Table 2 Multivariate analysis of risk factors for ≥65-year-old elderly patients and ciprofloxacin-nonsusceptible strains among patients with *Salmonella enterica* bacteremia.

Demographic and clinical characteristics	≥65 y elderly patients (n = 49)			Ciprofloxacin-nonsusceptible (n = 8)		
	Odds ratio	(95% CI)	p	Odds ratio	(95% CI)	p
S. enterica serovar C_1	—	—	—	6.036	0.243–150.202	0.273
S. enterica serovar C_2	—	—	—	28.430	1.328–608.699	0.032
S. enterica serovar D	0.557	0.161–1.923	0.354	0.430	0.028–6.600	0.545
Renal insufficiency	3.774	1.230–11.576	0.020	13.927	1.254–154.653	0.032
Heart disease	2.922	1.132–7.543	0.027	—	—	—
Immunosuppression agent	—	—	—	60.082	3.146–1147.441	0.006

CI = confidence interval; — = not available.

Discussion

This study had two main objectives. The first objective was to elucidate the prevalence of *S. enteric*a serogroups. The second objective was to investigate the difference between patients with *Salmonella* bacteremia in those <65 and ≥65 years old and in those afflicted with ciprofloxacin-susceptible strains versus those with ciprofloxacin-nonsusceptible strains.

The serogroup distribution of *Salmonella* bacteremia may vary in different geographical localities. In our study, the most common serogroup of *Salmonella* bacteremia was *S. enterica* serovar D (64.36%, 65/101). The second most common serogroup was *S. enterica* serovar B (21.78%, 22/101), followed by *S. enterica* serovar C_2 (6.93%, 7/101), *S. enterica* serovar C_1 (4.95%, 5/101), and *S. enterica* serovar Typhi (1.98%, 2/101). These results corroborate those of Lin et al,[4] who analyzed patients from northern Taiwan in 2010–2012 and found that the most common *Salmonella* serogroup was *S. enterica* serovar D (68.8%), followed by *S. enterica* serovar B (23.4%), *S. enterica* serovar C_2 (6.3%), and *S. enterica* serovar C_1 (1.6%).

Our findings had some differences from previous reports. Parry et al[2] revealed that serogroups causing bacteremia included *S.* Enteritidis (47.6%) and *S.* Typhimurium (14.3%) for patients from Liverpool, UK. Li et al[6] demonstrated that the serogroups of nontyphoidal *Salmonella* isolates included *S. enterica* serovar B (40.2%), *S. enterica* serovar D (30.9%), *S. enterica* serovar C (26.5%), and *S. enterica* serovar E (1.5%) in patients with malignancy from southern Taiwan. Yen et al[8] showed that infections in 31 patients (39.24%) were due to *S. enterica* serovar D. Infections were due to *S. enterica* serovar B in 24 patients (30.38%) and *S. enterica* serovar C in 22 patients (27.85%) from northern Taiwan in 2001–2003.

Interestingly, Zaidenstein et al[23] found that males ≥60 years of age were more likely to have bacteremia than females of the same age. They also showed that *S. enterica* serovar Enteritidis had the highest incidence in patients' ≥60 years old. Some studies suggested that the risk factors were associated with underlying diseases, such as diabetes mellitus, human immunodeficiency virus infection, and solid tumor.[8,24] However, we found that heart disease and renal failure were more common in those with *Salmonella* bacteremia who were ≥65 years old. The discrepancies may vary between different geographical localities or different study periods. We suggest that separate outbreak investigations should be performed in each hospital setting,

Table 3 The clinical characteristics of patients, serogroups, quinolone resistance determining region of all subunits for DNA gyrase (*gyrA)* gene, topoisomerase IV (*parC*) gene and MICs among the ciprofloxacin-nonsusceptible *Salmonella enterica* bacteremia.

No	Year	Sex	Age	Outcome	ICU stay	DM	Heart disease	Renal insufficiency	Poly-microbial	Serovar	*gyrA* mutation	*parC* mutation	MICs to CIP by Agar dilution	MICs to CIP by Vitek 2
1	2012	F	77	Survived	No	Yes	No	Yes	No	C_2	NP	T57→S	2	2
2	2013	M	51	Survived	No	No	No	No	No	C_2	D87→N	T57→S	2	2
3	2012	F	72	Survived	Yes	Yes	No	Yes	*E. coli*	C_2	S83→F	T57→S	2	4
4	2013	M	80	Survived	Yes	No	No	Yes	No	D	D87→N	NP	2	2
5	2011	M	52	Survived	No	No	Yes	No	No	C_1	S83→F, D87→N	T57→S, S80→I	≥ 4	8
6	2013	M	70	Died	No	No	No	No	No	C_1	S83→F, D87→N	T57→S, S80→I	≥ 4	8
7	2012	F	63	Survived	No	Yes	Yes	Yes	No	B	D87→N	NP	2	2
8	2013	F	54	Survived	No	Yes	Yes	No	No	B	S83→Y	NP	2	2

*ICU, intensive care unit; DM, diabetes mellitus; *E. coli*, *Escherichia coli*; Serovar C_2, *Salmonella enterica* serovar C_2; Serovar D, *Salmonella enterica* serovar D; Serovar C_1, *Salmonella enterica* serovar C_1; Serovar B, *Salmonella enterica* serovar B; S, serin ; F, phenylalanine; D, aspartic acid; N, asparagine; Y, tyrosine; T, threonine; I, isoleucine; NP, not present; CIP, ciprofloxacin.
*MICs (mg/L)

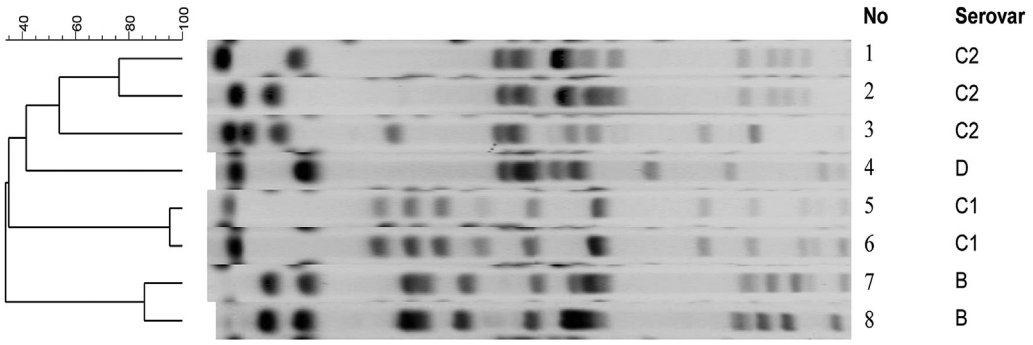

Figure 1. Pulse-field gel electrophoresis of ciprofloxacin-nonsusceptible strains of *Salmonella* blood isolates (eight isolates).

and infection control strategies need to be developed to limit the spread of *Salmonella* infection.

The nonsusceptible rate of ciprofloxacin resistance in our study was 7.92% (8/101). In contrast, Wang et al[25] showed that the overall rate of ciprofloxacin resistance among their *S.* Choleraesuis isolates was 59%, and the annual rate increased with time from 0% prior to 2000 to 80% in 1996—2004 in northern Taiwan. Liao et al[26] found that there was a significant reduction in the incidence of *S.* Choleraesuis bacteremia during 2005—2006 since its peak in 2004 and that the incidence remained low at both institutions during 2006—2011 in northern Taiwan. In contrast, the percentage of *S.* Choleraesuis isolates with ciprofloxacin resistance was approximately 80% during 2001—2004 and 100% during 2006—2011.[26]

Chiou et al. revealed that different ciprofloxacin resistance rates of Salmonella enterica serovar Typhi isolates from Bangladesh and Taiwan. Among the isolates from Bangladesh, 39.5 % (15/38) were resistant to ciprofloxacin, and from Taiwan, 0% (0/36) were resistant. We presented that the two Salmonella enterica serovar Typhi isolates were susceptible to ciprofloxacin in this study. Our results were in accord with that reported previously in Taiwan.[27] However, Lee et al. presented that there were two Salmonella enterica serovar Typhi isolates with complete resistance to ciprofloxacin (MIC >32 mg/L) from Taiwan in 2011. The two isolates were from one patient who had recently travelled to India.[28]

We also found that the clinical characteristics of patients harboring ciprofloxacin-nonsusceptible isolates were significantly different from those harboring ciprofloxacin-susceptible isolates. Patients with ciprofloxacin-nonsusceptible isolates were more likely to have renal insufficiency comorbidity and immunosuppressive agent usage. Chen et al[7] reported the highest risk in the elderly from southern Taiwan with age-related disorders and younger patients receiving immunosuppressive therapy for their underlying diseases.

In this study, the eight ciprofloxacin-nonsusceptible isolates were associated with mutations *gyrA* and *parC* to ciprofloxacin within QRDRs in *Salmonella*. 87.50% (7/8) of the isolates had *gyrA* mutation and 62.50% (5/8) of the isolates had *parC* mutation, but none had *gyrB* and *parE* mutations. In contrast, in Malaysia, Karunakaran et al[29] detected the *qnrS* gene in 17/23 (73.91%), and single *gyrA* mutations were detected in 6/23 [26.09%; Asp87Tyr ($n = 3$), Asp87Asn ($n = 2$), and Ser83Phe ($n = 1$)]. A *parC* (Thr57Ser) mutation was detected in 13/23 (56.52%) of the isolates coexisting with either a *qnrS* gene or a *gyrA* mutation. Although the detected rate of *gyrA* mutation was different in our report, the coexisting *gyrA* and *parC* mutations presenting high-level ciprofloxacin resistance had the same results. A first-step mutation reduced the susceptibility of DNA gyrase to a low level, and additional mutations in *gyrA* or mutations in *parC* could further augment resistance. The *parC* alterations played a complementary role in the development of higher-level fluoroquinolone-resistance.[14,29]

One limitation of this study is that it was conducted in a single medical center and the results may not be applicable to other hospitals. Another limitation is that the isolates were only identified as serogroups, such as crude *S. enterica* serovar B, *S. enterica* serovar C_1, *S. enterica* serovar C_2, *S. enterica* serovar D, or *S.* Typhi by the conventional biochemical tests and "O" Group typing in the microbiological laboratory.

In conclusion, this study demonstrates that among adult patients with *Salmonella* bacteremia, the risk factors of ≥65 year-old elderly patients included renal insufficiency and heart disease. The risk factors of ciprofloxacin-nonsusceptible strains included *S. enterica* serovar C_2, renal insufficiency, and immunosuppression agent usage. Furthermore, the eight ciprofloxacin-nonsusceptible isolates carried *gyrA* and *parC* mutations, whereas *gyrB* and *parE* mutations were not found. The isolates with coexisting Ser83Phe/Asp87Asn *gyrA* mutation and Thr57Ser/Ser80Ile *parC* mutation genes exhibited high-level resistance to ciprofloxacin (MIC ≥ 4 mg/L). Resistance caused by *gyrA* and *parC* mutations among ciprofloxacin-nonsusceptible isolates poses a major concern.

Conflicts of interest

The authors declare that they have no conflicting interests.

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