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Short Communication

Antimicrobial resistance profile in *Salmonella enterica* serovar Choleraesuis isolates from diseased pigs in Taiwan

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Abstract This study investigated antimicrobial resistance in *Salmonella enterica* serovar Choleraesuis (*S. Choleraesuis*) isolates from diseased pigs in Taiwan (2015–2020). Among 272 isolates, florfenicol (96.7%), enrofloxacin (96.3%), doxycycline (91.2%), gentamicin (84.6%), and tiamulin (80.5%) exhibited high resistance. 99.3% of the isolates were resistant to at least one antibiotic, and 97.8% of the isolates were multidrug resistant. This study illustrated that *S. Choleraesuis* isolates exhibited high resistance to antimicrobials currently used in the Taiwanese swine industry.

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Introduction

Salmonella enterica serovar Choleraesuis (*S. Choleraesuis*) is a highly invasive zoonotic pathogen that causes severe septicemic disease in humans and pigs.^{1,2} Human infections caused by *S. Choleraesuis* were the second most common *Salmonella* serotypes in Taiwan in 30 years ago.³ *S. Choleraesuis* is the most frequently isolated serotype from clinically ill pigs in the Animal Disease Diagnostic Center (ADDC), National Pingtung University of Science and Technology (NPUST), Taiwan. *S. Choleraesuis* infection causes septicemia, enterocolitis, pneumonia, occasionally meningitis, and encephalitis in pigs. Most *S. Choleraesuis* outbreaks occur in weaned pigs and are infrequent in adults and suckling pigs.⁴ *S. Choleraesuis* incursion usually causes unexpected economic loss in the nursery stage of pigs in Taiwan.⁵ This serovar not only causes economic losses in the pig industry but has also been known to pose a threat to human health in Taiwan for a long time.^{1,3,6}

Treatment of *S. Choleraesuis* minimizes the severity of clinical manifestations and prevents the spread of infection and pathogens in pig herds. Several studies have reported increasing antimicrobial resistance (AMR) rates in *S. Choleraesuis* isolates from both humans and pigs.^{6,7,8,9,10} In recent years, 15 *S. Choleraesuis* isolates were resistant to 8–11 antimicrobial agents.¹¹ Therefore, continuing to survey and investigate the AMR for *S. Choleraesuis* is necessary for the swine industry and human public health. The objective of the present study was to investigate the AMR of *S. Choleraesuis* isolates from diseased pigs in Taiwan by using the minimum inhibitory concentration (MIC) test. The results show AMR profiles of *S. Choleraesuis* isolates in Taiwan and provide useful information to treat salmonellosis in veterinary and human medicine.

Materials and methods

Bacterial isolates

From 2015 to 2020, a total of 272 isolates of *S. Choleraesuis* were isolated from diseased pigs. Bacterial isolation was performed at ADDC, NPUST, Taiwan. PCR assays targeting the CSR2 region were selected to conduct simplex PCR amplification of the *S. Choleraesuis* identified.¹² Before further testing, all strains were kept at -80°C in trypticase soy broth with 15% glycerol added.

Antimicrobial susceptibility testing and data analysis

The AMR of the *S. Choleraesuis* isolates was determined by MIC using the broth microdilution technique by the recommendation of the Clinical and Laboratory Standards Institute (CLSI 2022).¹³ Eight different antimicrobial agents

were tested: ceftiofur (EFT), enrofloxacin (ENR), doxycycline (DOX), florfenicol (FFC), tiamulin (TIA), gentamicin (GEN), kanamycin (KAN), and colistin (CL). Antimicrobial concentrations in twofold dilutions were evaluated, and the dilution ranges used for these antimicrobials were 0.5–256 $\mu\text{g}/\text{mL}$, except for colistin, which was 0.125–64 $\mu\text{g}/\text{mL}$. The results were interpreted using CLSI veterinary breakpoints (VET01) (CLSI, 2022), when available. *Escherichia coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 were used as the quality control strains. The isolates that were resistant to at least one agent in three or more classes of antimicrobials were defined as multidrug-resistant (MDR). The isolates were categorized as either susceptible (including isolates with moderate susceptibility that were regarded as susceptible) or resistant.

Results

The results of the susceptibility of 272 *S. Choleraesuis* isolates to 8 antimicrobials (7 classes) are shown in Table 1. Based on the MIC breakpoint for each antimicrobial, the isolates displayed high ratios of antimicrobial resistance to all antimicrobials examined. In all the isolates tested, florfenicol (96.7%) showed the highest resistance frequency, followed by enrofloxacin (96.3%), doxycycline (90.8%), gentamicin (84.6%), and tiamulin (80.5%). In contrast, a low resistance rate was observed for kanamycin (25.4%), ceftiofur (26.8%), and colistin (31.3%). The present results showed that most (97.8%) of the isolates were multidrug resistant.

Fig. 1 shows the AMR of *S. Choleraesuis* isolates from 2015 to 2020. More than 65% of the *S. Choleraesuis* isolates were resistant to florfenicol, doxycycline, gentamicin, enrofloxacin, and tiamulin during the study period. In the initial year of the study, colistin had the highest susceptibility (94.4%) among the tested antimicrobials, followed by ceftiofur (80.0%) and kanamycin (71.5%). However, in 2017, 68.9%, 31.0%, and 44.8% of the *S. Choleraesuis* isolates showed resistance to colistin, ceftiofur, and kanamycin, respectively (Fig. 1). In addition, the highest rates (100%) of the isolates in 2017 and 2019 were resistant to florfenicol, while 100% of isolates in 2017 and 2020 were resistant to enrofloxacin (Fig. 1).

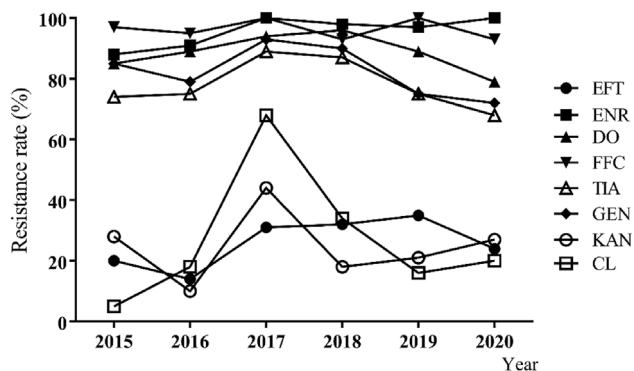
Discussion

S. enterica serovar Choleraesuis is a particularly troublesome disease that induces diarrhea, enteritis, pneumonia, and acute septicemia with high mortality in pigs; it is also an important zoonotic pathogen. A previous study suggested that *S. Choleraesuis* evolves independently in humans and pigs,¹⁴ which implies that continuing AMR investigation of *S. Choleraesuis* in pigs is necessary. We found multidrug-resistant *S. Choleraesuis* isolated from sick pigs in the

Table 1 Susceptibility and minimum inhibitory concentration (MIC) of 272 *S. Choleraesuis* isolates to eight antimicrobial agents.

AMs	No. of isolates with MIC test of ($\mu\text{g}/\text{mL}$)														MIC ₅₀	MIC ₉₀	Resistance (%)
	>256	256	128	64	32	16	8	4	2	1	0.5	0.25	0.125	0.0625			
Ceftiofur	0	1	4	16	17	26	9	4	62	130	0	3			2	32	26.8
Enrofloxacin	0	4	49	175	23	1	0	0	10	8	0	2			64	128	96.3
Doxycycline	0	0	11	37	149	50	2	3	17	2	0	1			32	64	90.8
Florfenicol	199	28	0	0	5	16	15	7	2	0	0	0			>256	>256	96.7
Tiamulin	2	0	34	107	76	15	5	0	0	8	0	25			64	128	80.5
Gentamicin	2	2	30	101	65	30	6	0	3	5	0	28			32	128	84.6
Kanamycin	62	3	3	1	1	16	93	58	22	13	0	0			8	>256	25.4
Colistin			1	0	0	15	14	55	71	94	18	0	0	4	2	8	31.3

* For the breakpoints, CLSI resistance breakpoints (M100; CLSI 2015) were basically employed (EFT, a1dd 8 a6ccg/mL; ENR, a1dd 2 a6ccg/mL; DO X, a1dd16 a6ccg/mL; FFC, a1dd 8 a6ccg/mL; GEN, a1dd16 a6ccg/mL; KAN, a1dd64 a6ccg/mL; CL, a1dd 4 a6ccg/mL; TIA, a1dd 32 a6ccg/mL). MIC₅₀ and MIC₉₀ a843 the lowest drug concentration capable of inhibiting the growth of 50% and 90% of isolates tested, respectively. In the white area are the dilution ranges tested for each antimicrobial agent. Values outside this white area indicate MIC values higher/lower than the test range concentration.

**Figure 1.** The antimicrobial resistance of *S. Choleraesuis* isolates obtained each year from 2015 to 2020 in Taiwan.

present study. Among 8 commonly used antimicrobial agents for Taiwanese pig herds, 5 were less susceptible: florfenicol, doxycycline, gentamicin, enrofloxacin, and tiamulin; however, some, such as colistin, ceftiofur and kanamycin, show better susceptibility than others.

Ceftiofur and enrofloxacin exhibited the broadest spectra of activity against porcine enterobacterial isolates, including *S. Choleraesuis*.¹⁵ Ceftiofur is a third-generation cephalosporin that is approved for veterinary employment in the treatment of a wide range of gram-negative bacterial infections.⁴ Ceftiofur is only administered intramuscularly because of its low oral bioavailability, and it is often used for the treatment of septicemia caused by *S. Choleraesuis* in Taiwanese pig herds. However, the results obtained in this study indicated that the resistance rate of ceftiofur against *S. Choleraesuis* was 26.8% (MIC₅₀ and MIC₉₀ values of 2 $\mu\text{g}/\text{mL}$ and 64 $\mu\text{g}/\text{mL}$, respectively.); this may influence the therapeutic effect of septicemia caused by *S. Choleraesuis* in the future. Enrofloxacin is a fluoroquinolone antimicrobial agent that is solely intended for veterinary use. The present study noted the high AMR of enrofloxacin against *S. Choleraesuis* (96.3%), with MIC₅₀ and MIC₉₀ values of 64 and 128 $\mu\text{g}/\text{mL}$, respectively. Comparison with the MIC data collected from 2002 to 2004 in Taiwan, the MIC₉₀

values of enrofloxacin of *S. Choleraesuis* isolates increased from 2^{4,16} to 128 $\mu\text{g}/\text{mL}$ (present study). Several point mutations of the quinolone resistance-determining region of the *gyrA* gene of quinolone-resistant *S. Choleraesuis* isolates have been reported.¹⁷ Therefore, a surveillance system of fluoroquinolone resistance genes for *S. Choleraesuis* isolates in Taiwanese pig herds requires further investigation.

Since 2002, chloramphenicol usage in food-producing animals has been prohibited in Taiwan. However, a new drug from the same family, florfenicol, was approved to replace chloramphenicol for therapeutic use in farm animals. Our results demonstrated alarmingly high resistance rates (96.7%) of florfenicol, rendering it ineffective against *S. Choleraesuis* over the last two decades in Taiwan.^{16,18} Gentamicin and kanamycin are aminoglycosides, and both drugs are widely used in gram-negative bacterial infections, especially enterobacterial infections. Compared with the antimicrobial susceptibility testing results of kanamycin, the sensitivity of *S. Choleraesuis* isolates ranged from 23.08%¹⁸ in the previous study to 74.6% in the present study. The sensitivity of kanamycin against *S. Choleraesuis* is increased in this study, which may imply that certain traditional antimicrobial agents are still active against *S. Choleraesuis* currently, although it was reported as having low sensitivity to *S. Choleraesuis* twenty years ago. Tiamulin and colistin are two antimicrobial agents commonly used in pig herds to control enterobacterial infection. Tiamulin is a therapeutic agent used to control respiratory and gastrointestinal pathogens. However, tiamulin had an 80.5% of resistance rate against local *S. Choleraesuis* isolates (Table 1). Comparing the MIC data, the MIC₅₀ value (64 $\mu\text{g}/\text{mL}$) of tiamulin was higher than that in a previous study (MIC₅₀ value of 8 $\mu\text{g}/\text{mL}$), whereas colistin (2 $\mu\text{g}/\text{mL}$) was much lower than that in a previous study (MIC₅₀ value of 8 $\mu\text{g}/\text{mL}$).¹⁹ The extensive use of tiamulin in veterinary medicine may exert selective pressure on *S. Choleraesuis*, favoring the survival and propagation of resistant variants over susceptible ones. Furthermore, variations in bacterial genetics, including mutations and horizontal gene transfer

that confer resistance, could also contribute to the observed high resistance rate. The combined use of tiamulin and colistin has been reported to enhance the effectiveness of each antibiotic alone¹⁹; therefore, this may be an alternative strategy for the control and/or treatment of *S. Choleraesuis* in the future.

Multidrug resistance patterns in *S. Choleraesuis* isolates have been observed in previous studies in Taiwan,^{1,6,20} Japan,²¹ and Spain.²² In the present study, over 90% of the *S. Choleraesuis* isolates were multidrug-resistant, and the most prevalent were enrofloxacin, doxycycline, florfenicol, tiamulin and gentamycin. Therefore, the problem of AMR of *S. Choleraesuis* is a serious issue in the swine industry in Taiwan. Since AMR *S. Choleraesuis* is a global issue, antimicrobial agent treatment alone will not be sufficient. The fluctuation of resistance rates may be influenced by factors such as shifts in antimicrobial usage, the emergence of new resistance mechanisms, and environmental conditions.^{20,22} However, the current study focused on describing the antimicrobial patterns in *S. Choleraesuis*, which might restrict further exploration. Therefore, monitoring and clarifying these issues are highly recommended in the future for effective antimicrobial stewardship and combating antimicrobial resistance. Besides, supplemental approaches such as probiotics could offer promise. For example, *bacillus*-based direct-fed microbes can modulate cells of the innate immune system and improve goblet cell function in the porcine gut mucosa.²³ The notion of tonic *bacillus*-based direct-fed microbes stimulated by gut-derived microbial products can poise innate immunity to fight infections in the respiratory tract.²³ Therefore, the usage of probiotics may be an alternative strategy for the control and/or treatment of *S. Choleraesuis*.

In conclusion, the current study updated the status of antimicrobial resistance of *S. Choleraesuis* isolates from pig farms in Taiwan. Local *S. Choleraesuis* isolates exhibited high resistance to antimicrobials currently used in the Taiwanese swine industry. These results are essential for guiding the prudent use of antibiotics in veterinary medicine; in addition, they will assist in designing and measuring effective control strategies.

Declaration of competing interest

None declared.

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References

- Chang CC, Lin YH, Chang CF, Yeh KS, Chiu CH, Chu C, et al. Epidemiologic relationship between fluoroquinolone-resistant *Salmonella enterica* serovar choleraesuis strains isolated from humans and pigs in Taiwan (1997 to 2002). *J Clin Microbiol* 2005;43:2798–804.
- Jean SS, Wang JY, Hsueh PR. Bacteremia caused by *Salmonella enterica* serotype choleraesuis in Taiwan. *J Microbiol Immunol Infect* 2006;39:358–65.
- Chiu CH, Su LH, Chu C. *Salmonella enterica* serotype choleraesuis: epidemiology, pathogenesis, clinical disease, and treatment. *Clin Microbiol Rev* 2004;17:311–22.
- Chang CF, Chang LC, Chang YF, Chen M, Chiang TS. Antimicrobial susceptibility of *Actinobacillus pleuropneumoniae*, *Escherichia coli*, and *Salmonella choleraesuis* recovered from Taiwanese swine. *J Vet Diagn Invest* 2002;14:153–7.
- Hsueh FC, Wang SY, Lin WH, Lin CF, Tsai CY, Huang CW, et al. Correlation of neutralizing antibodies (Nabs) between sows and piglets and evaluation of protectability associated with maternally derived nabs in pigs against circulating porcine reproductive and respiratory syndrome virus (prsv) under field conditions. *Vaccines* 2021;9:414.
- Hsu SC, Chiu TH, Pang JC, Hsuan-Yuan CH, Chang GN, Tsen HY. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. *Int J Antimicrob Agents* 2006;27:383–91.
- Chiu CH, Wu TL, Su LH, Chu C, Chia JH, Kuo AJ, et al. The emergence in Taiwan of fluoroquinolone resistance in salmonella enteria serotype choleraesuis. *N Engl J Med* 2002;346:413–9.
- Chiu CH, Su LH, Chu C, Chia JH, Wu TL, Lin TY, et al. Isolation of *Salmonella enterica* serotype Choleraesuis resistant to ceftriaxone and ciprofloxacin. *Lancet* 2004;363:1285–6.
- Hsueh PR, Teng LJ, Tseng SP, Chang CF, Wan JH, Yan JJ, et al. Ciprofloxacin-resistant *Salmonella enterica* Typhimurium and choleraesuis from pigs to humans, Taiwan. *Emerg Infect Dis* 2004;10:60–8.
- Wang JY, Hwang JJ, Hsu CN, Lin LC, Hsueh PR. Bacteraemia due to ciprofloxacin-resistant *Salmonella enterica* serotype Choleraesuis in adult patients at a university hospital in Taiwan, 1996–2004. *Epidemiol Infect* 2006;134:977–84.
- Kuo HC, Lauderdale TL, Lo DY, Chen CL, Chen PC, Liang SY, et al. An association of genotypes and antimicrobial resistance patterns among *Salmonella* isolates from pigs and humans in Taiwan. *PLoS One* 2014;9:e95772.
- Akiba M, Kusumoto M, Iwata T. Rapid identification of *Salmonella enterica* serovars, Typhimurium, choleraesuis, Infantis, hadar, Enteritidis, Dublin and Gallinarum, by multiplex PCR. *J Microbiol Methods* 2011;85:9–15.
- Clinical and Laboratory Standards Institute. Performance Standards for antimicrobial susceptibility testing. In: *Twenty-Fifth Informational Supplement*, 35; 2015.
- Tzeng JI, Chu CH, Chen SW, Yeh CM, Chiu CH, Chiou CS, et al. Reduction of *Salmonella enterica* serovar Choleraesuis carrying large virulence plasmids after the foot and mouth disease outbreak in swine in southern Taiwan, and their independent evolution in human and pig. *J Microbiol Immunol Infect* 2012;45:418–25.
- Salmon SA, Watts JL, Case CA, Hoffman LJ, Wegener HC, Yancey RJ. Comparison of MICs of ceftiofur and other antimicrobial agents against bacterial pathogens of swine from the United States, Canada, and Denmark. *J Clin Microbiol* 1995;33:2435–44.
- Huang TM, Chang YF, Chang CF. Antimicrobial susceptibility and resistance gene determinants in clinical *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis swine isolates. *Taiwan Vet J* 2004;30:116–24.
- Huang TM, Chang YF, Chang CF. Detection of mutations in the *gyrA* gene and class I integron from quinolone-resistant *Salmonella enterica* serovar Choleraesuis isolates in Taiwan. *Vet Microbiol* 2004;100:247–54.

18. Chiu YT, Lu YP, Chang IC, Chen TY. Pathologic studies and antimicrobial susceptibility of *Salmonella choleraesuis* pneumonia in pigs. *J Chin Soc Vet Sci* 1998;24:99–108.
19. Mills J, Apley M, Dau D, Bustamante A. In-vitro antimicrobial activity of tiamulin and chlortetracycline against field swine pathogens. In: *IPVS - biennial international congress - South Africa*. International Pig Veterinary Society; 2008.
20. Lee MF, Chen YH, Peng CF. Molecular characterisation of class 1 integrons in *Salmonella enterica* serovar Choleraesuis isolates from southern Taiwan. *Int J Antimicrob Agents* 2009;33:216–22.
21. Matayoshi M, Kitano T, Sasaki T, Nakamura M. Resistance phenotypes and genotypes among multiple-antimicrobial-resistant *Salmonella enterica* subspecies enterica serovar choleraesuis strains isolated between 2008 and 2012 from slaughter pigs in Okinawa Prefecture, Japan. *J Vet Med Sci* 2015;77:705–10.
22. Gil Molino M, Risco Pérez D, Gonçalves Blanco P, Fernandez Llario P, Quesada Molina A, Garcia Sanchez A, et al. Outbreaks of antimicrobial resistant *Salmonella Choleraesuis* in wild boars piglets from central-western Spain. *Transbound Emerg Dis* 2019;66:225–33.
23. Zuckermann FA, Husmann R, Chen WY, Roody R, Pfeiff J, Leistikow KR, et al. *Bacillus*-based direct-fed microbial reduces the pathogenic synergy of a coinfection with *Salmonella enterica* serovar choleraesuis and porcine reproductive and respiratory syndrome virus. *Infect Immun* 2022;90:1–16.