Antibiotic sensitivity of common respiratory bacteria of pig from Hubei province, China

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ABSTRACT

The use of antimicrobials for feeding and treatment is crucial to animal health. However, continuous use of antibiotics is contributing to emergence and widespread of antibiotic resistance. This study aimed to investigate the antimicrobial resistance of five major respiratory pathogens in pigs of Hubei province, China, from October to December, 2019. Antibiotic susceptibility testing for *Streptococcus suis*, *Haemophilus parasuis*, *Pasteurella multocida*, *Bordetella bronchiseptica* and *Actinobacillus pleuropneumoniae* was determined to representatives of relevant antibiotic classes.

Streptococcus suis isolates were mostly sensitive to beta-lactams, whereas high levels of resistance were observed to quinolones, gentamycin, doxycycline, trimethoprime and lincomycin. For *H. parasuis*, *P. multocida* and *A. pleuropneumoniae* of *Pasteurellaceae* family, the susceptibility to beta-lactams and quinolones was displayed. Most *B. bronchiseptica* isolates were sensitive to doxycycline, azithomycin, polymycin whereas high resistance levels to beta-lactams, aminoglycosides and quinolones were recorded.

This study obtained practical data for later studies and usage to combat infections due to respiratory bacteria.

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1. Introduction

Porcine respiratory diseases complex is caused by multifactorial aetiologies, including the viral and bacterial pathogens, the environment, management and genetic factors. Within this complex, *Streptococcus suis*, *Haemophillus parasuis*, *Pasteurella multocida*, *Actinobacillus pleuropneumoniae* and *Bordetella bronchiseptica* have been known to be ubiquitous in almost all pig farms. *S. suis* is as a major respiratory commensal and pathogen of pigs and an emerging zoonotic agent of meningitis in human (Goyette-Desjardins et al., 2014). *Haemophillus parasuis* produces Glässer's disease as well as pneumonia (Nedbalcova et al., 2006). *Pasteurella multocida* causes atrophic rhinitis, particularly when combined with *B. bronchiseptica* (Jeffrey et al., 2013). *Actinobacillus pleuropneumoniae* generates contagious hemorrhagic pleuropneumonia in pigs (Brownfield, 2013). Due to their complexity and indeterminacy, bacterial diseases are very challenging to control.

Antimicrobial agents are important for effective production of food animals as growth promoter or/and disease prevention. As the world's largest pork producer and consumer, China has been reported for the massive use of antibiotic in food animal production. Zhao et al. (2011) showed antimicrobial susceptibility tests on *B. bronchiseptica* isolates from Chinese farms that were highly resistant to ampicillin, cefazolin,

streptomycin, amoxicillin and tetracycline. Zhang et al. (2015) found the most antibiotics consumed in China's swine farming were fluoroquinolones and β-lactams. Therefore, antimicrobial surveillance is necessary to provide a better understanding of antibiotic resistance in the animal population.

This study aimed to contribute the comprehension of the antibiotic susceptibility pattern of *S. suis*, *H. parasuis*, *P. multocida*, *A. pleuroplneumoniae* and *B. bronchiseptica*, the five important pathogens found in the respiratory tract of pigs in Hubei province, China, using disk diffusion test.

2. Materials and Methods

2.1. Sample collection

From October to December 2019, a total of 155 samples from 14 different pig farms in Hubei province were sent to the Animal Diagnostic Center of Huazhong University. The collected samples included lungs, spleen, synovial fluid, brain, tracheal effusion etc. Lived pigs were observed for evaluating clinical signs and endured necropsy to collect samples. For every individual pig, lung and spleen samples were sealed in a clean zipper bag; brain and synovial fluid were kept in an eppendorf tubes (EP tube). Nasal samples were collected by using sterile cotton swabs and placed in sterilized EP tubes. The samples were clearly marked.

After the period of three months, 133 strains of the five concerned bacteria species from 155 samples were isolated and identified by using multiplex PCR assays. For the identification of the five bacteria, the primers of following target genes were used: 16S rRNA to detect *S. suis* (Cheung, 2008), 16S rRNA for *H. parasuis*, apxIV for *A. pleuropneumoniae*, fla for *B. bronchiseptica* (Xue, 2009) and ktm1 for *P. multocida* (Nagai et al., 1994). The greatest number of isolated strains were obtained from *S. suis* (40%, 62/155), followed by H. parasuis (18.71%, 29/155), *P. multocida* (14.83%, 23/155), *B. bronchiseptica* (8.39%, 13/155), and *A. pleuropneumoniae* (3.87%, 6/155).

2.2. Kirby-Bauer antibiotic testing

Twenty antibiotic agents (Hangzhou Binhe Microorganism Reagent Co., Ltd) were used, including cefotazime (30 µg), cephradine (30 µg), ceftriaxone (30 µg), ceftazidime (30 µg), amoxicillin (20 µg) and ampicillin (10 µg), ofloxacin (5 µg), ciprofloxacin (5 µg), enrofloxacin (10 µg), norfloxacin (10 µg), spectinomycin (100 µg), gentamicin (10 µg), streptomycin (10 µg), amikacin (30 µg), kanamycin (30 µg), doxycycline (30 µg), lincomycin (30 µg), azithromycin (15 µg), polymyxin B (300 µg) and trimethoprim (23.75/1.25 µg).

Each purified isolates of tested bacteria were evenly spread onto a tryptic soy agar plate (TSA, BD^{TM}, USA) that had been coated with nicotinamide adenine dinucleotide liquid (NAD, Guangzhou Saiguo Biotech, China) and bovine serum (Zhejiang Tianhang Biotechnology, China). The antimicrobial discs were placed onto the surface of the agar. The plates were then incubated at 37°C for about 24 h. The inhibition zone diameter was measured and compared with standardized CLSI interpretive criteria to designate the isolate as sensitive, intermediate or resistant to the drug (CLSI, 2018). In this study, the isolates that showed intermediate were classified as resistant.

2.3. Results and Discussion

The resistant and sensitive rates of the five bacteria species to 20 antibiotic agents are presented in Table 1. Results showed the resistance rates of *S. suis* strains to quinolones, aminoglycosides, macrolides, lincomycins, tetracyclines, polymyxins and sulfonamides were all over 60%. *H. pasasuis* strains were sensitive to majority of the drugs but highly resistant to amoxicillin, streptomycin, amikacin, kanamycin and lincomycin. The resistance of *P. multocida* strains to aminoglycosides and lincosamides were apparently high compared to other antibiotic groups (Table 1).

With the small number of isolates being tested, the two purified isolates of *A. pleuropneumoniae* were sensitive to beta-lactams, quinolones and aminoglycosides. In contrast, all of the three isolates of *B. bronchiseptica* resisted to those drugs and only sensed to doxycycline, gentamicin, azithromycin and polymyxin B.

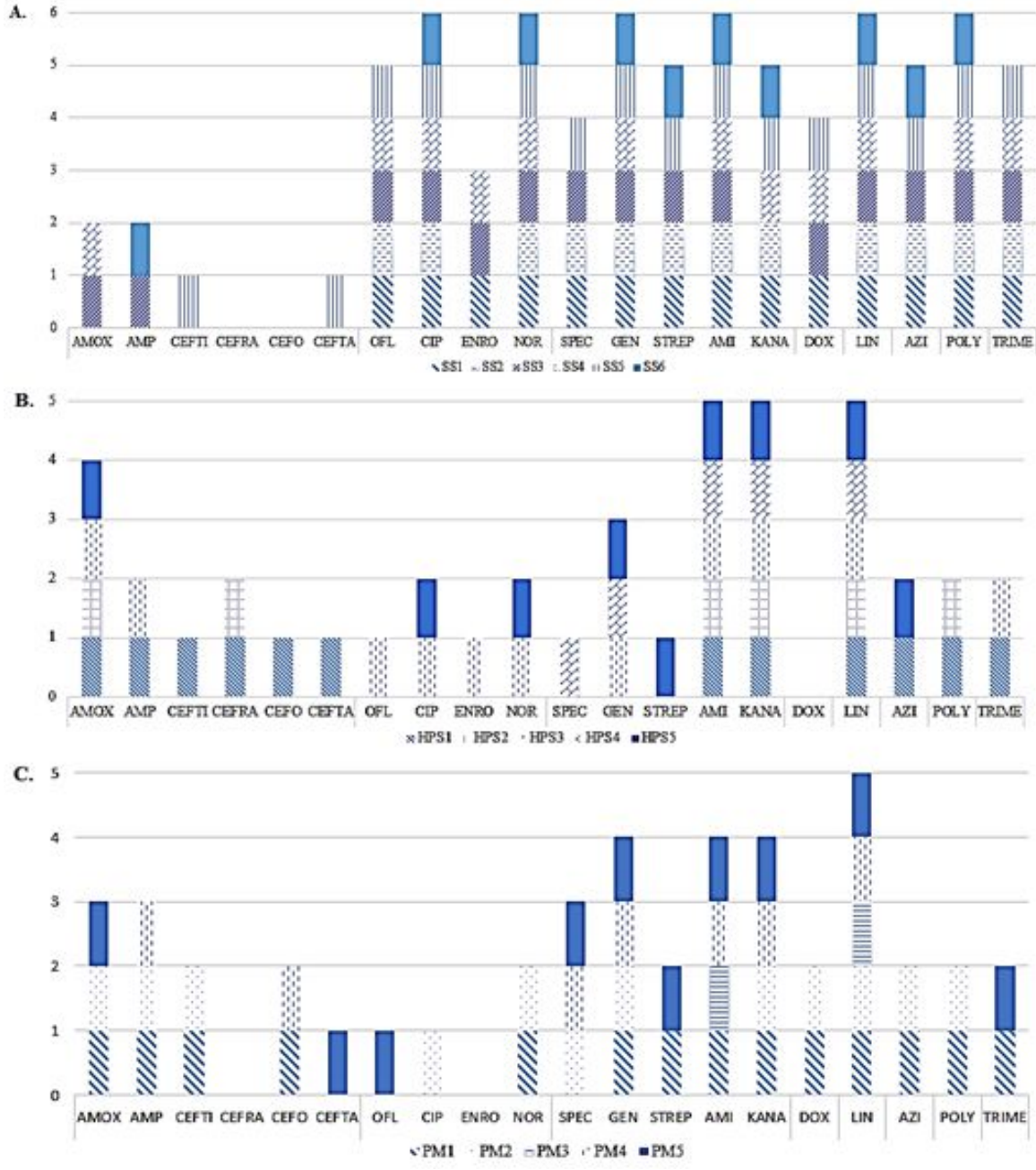
The drug-resistance pattern of bacterial isolates obtained in this study indicates that *S. suis*, *H. parasuis*, *P. multocida*, *B. bronchiseptica* and *A. pleuropneumoniae* displayed high antibiotic resistance rates to 8 tested antibiotics/antimicrobial classes. The resistance proportion of *S. suis* to these antibiotics were all

Table 1. Antibiotic susceptibility rates (%) and number of *S. suis*, *H. parasuis* and *P. multocida* isolates (in brackets) from infected pigs of Hubei province

Antibiotics	*S. suis*		*H. pasasuis*		*P. multocida*	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Amoxicillin	62.5 (5)	37.5 (3)	20.0 (1)	80.0 (4)	25.0 (1)	75.0 (3)
Ampicillin	75.0 (6)	25.0 (2)	50.0 (2)	50.0 (2)	40.0 (2)	60.0 (3)
Ceftiaxone	75.0 (6)	25.0 (2)	80.0 (4)	20.0 (1)	60.0 (3)	40.0 (2)
Cefotaxime	100.0 (6)	0.0 (0)	75.0 (3)	25.0 (1)	50.0 (2)	50.0 (2)
Ceftazidime	25.0 (1)	75.0 (3)	66.7 (2)	33.3 (1)	0.0 (0)	100.0 (1)
Cefradine	80.0 (4)	20.0 (1)	60.0 (3)	40.0 (2)	100.0 (4)	0.0 (0)
Ofloxacin	25.0 (2)	75.0 (6)	80.0 (4)	20.0 (1)	80.0 (4)	20.0 (1)
Ciprofloxacin	0.0 (0)	100.0 (8)	50.0 (2)	50.0 (2)	80.0 (4)	20.0 (1)
Enrofloxacin	50.0 (4)	50.0 (4)	75.0 (3)	25.0 (1)	100.0 (4)	0.0 (0)
Norfloxacin	0.0 (0)	100.0 (8)	60.0 (3)	40.0 (2)	60.0 (3)	40.0 (2)
Spectinomycin	37.5 (3)	62.5 (5)	80.0 (4)	20.0 (1)	40.0 (2)	60.0 (3)
Gentamicin	0.0 (0)	100.0 (8)	40.0 (2)	60.0 (3)	20.0 (1)	80.0 (4)
Streptomycin	0.0 (0)	100.0 (6)	0.0 (0)	100.0 (1)	0.0 (0)	100.0 (2)
Amikacin	0.0 (0)	100.0 (8)	0.0 (0)	100.0 (5)	20.0 (1)	80.0 (4)
Kanamycin	0.0 (0)	100.0 (7)	0.0 (0)	100.0 (5)	20.0 (1)	80.0 (4)
Doxycycline	25.0 (2)	75.0 (6)	100.0 (5)	0.0 (0)	60.0 (3)	40.0 (2)
Lincomycin	0.0 (0)	100.0 (8)	0.0 (0)	100.0 (5)	0.0 (0)	100.0 (5)
Azithromycin	12.5 (1)	87.5 (7)	60.0 (3)	40.0 (2)	60.0 (3)	40.0 (2)
Polymyxin B	0.0 (0)	100.0 (8)	60.0 (3)	40.0 (2)	60.0 (3)	40.0 (2)
Trimethoprim	12.5 (1)	87.5 (7)	60.0 (3)	40.0 (2)	60.0 (3)	40.0 (2)

over 60% except for β-lactam group. Some antibiotics that used to effectively deal with Gram-negative bacteria (*H. parasuis*, *P. multocida*, *B. bronchiseptica* and *A. pleuropneumoniae*) such as macrolides and beta-lactams were indicated to be less sensitive, especially lincomycin could not be used for any bacterial isolates. Polymycin B, which is known to use in human treatment, presented 100% resistance by *S. suis* and *A. pleuropneumoniae*, and 40% by *H. parasuis* and *P. multocida*. As a result, only a narrow spectrum of effective antibiotic drugs can be used for the treatment of infection in Hubei pigs.

This study also revealed the number of bacte-



*Note: Beta-lactams (AMOX: amoxicillin, AMP ampicillin, CEFTI: ceftriaxone, CEFRA: cefradine, CEFO: cefotaxime, CEFTA: ceftazidime). Quinolones (OFL: ofloxacin, CIP: ciprofloxacin, ENRO: enrofloxacin, NOR: norfloxacin). Aminoglycosides (SPEC: spectinmycin, GEN: gentamycin, STREP: streptomycin, AMI: amikacin, KANA: kanamycin). Tetracyclines (DOX: doxycycline). Lincosamide (LIN lincomycin). Macrolides (AZI: azithromycin). Polymycin (POLY: polymyxin B). Sulfonamide (TRIME: trimethoprime). SS1 – SS6: *S. suis* isolates number 1 to 6. HPS1 – HPS6: *H. parasuis* isolates number 1 to 5. PM1 – PM5: *P. multocida* isolates number 1 to 5.

Figure 1. The number of bacterial isolates resistant to antimicrobial agents (A) *S. suis* isolates.

rial isolates that exhibited multi-drug resistance (MDR) (Figure 1). According to these data, each isolate of *S. suis* were resistant to at least one antimicrobial drug in more than six antimicrobial categories. Each isolate of *H. parasuis* and *P. multocida* were resistant to at least one antimicrobial drug in two or more antimicrobial categories. The three *B. bronchiseptica* isolates were

also against to at least one antimicrobial agent of beta-lactams, quinolones, aminoglycosides and lincosamides. Similarly, *A. pleuropneumoniae* isolates were resistant to at least one antimicrobial agent of seven tested drug classes, except for macrolides.

The results suggested that five species of bacteria were highly multi-resistant to the eight common drug classes. Multi-drug resistance is a problem that continues to challenge the healthcare sector. Different countries have reported the widespread of clinical resistance due to the massive of antimicrobial drugs (Jong et al. 2018). The transmission of MDR bacteria into the community is seriously associated with increased morbidity, mortality, healthcare costs and antibiotic use. Together with many European countries and the USA, China is preparing a national action to deal with antibiotic resistance. Current technology makes possible the identification of new drugs or inhibitors of resistance mechanisms to extend the life of existing antibiotics, or alternatives like plant extracts (Laxminarayan, 2013). However, these tend to take time and require further efforts. Initial steps to prevent the spreading of MDR is use antibiotics only when needed and correctly, control the usage by reducing antibiotics in livestock management.

Due to different antibiotic usage of different farms, more difficulty and complication have raised in the aspect of antibiotic control of the area. The temporary solution is giving drug regimen based on susceptibility result of individual farms. Long-term plan with a detailed guideline of antibiotic implication should be developed for the control of bacterial disease and protect public health from antimicrobial resistance.

3. Conclusions

The results demonstrated high multi-resistance among the five bacterial species to the eight tested antimicrobial classes. The results emphasize the need for continuous surveillance of resistance patterns. Antibiotic prescription guidelines and infection control through the early detection of clinical should be carried out to prevent transmission of pathogens, as well as in the possible incorporation of the prevalent serotypes in the development of new vaccines.

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