

# Clinical efficacy of intravaginal recombinant lysostaphin administration on endometritis in sows

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## Funding information

National Key Research and Development Program of China, Grant/Award Number: 2016YFD0501308; Key Research and Development Program of Jiangsu Province, Grant/Award Number: BE2017314

## Abstract

Recombinant lysostaphin has been used for the treatment of cow endometritis and mastitis in China. To our knowledge, no scientific effort has been made to evaluate the efficacy of lysostaphin in sows with clinical endometritis. Lysostaphin, loaded in effervescent tablets that were completely foamed and dissolved within 30 min in the presence of water or body fluids and released active lysostaphin, were administered vaginally on endometritis sows in this study. The clinical recovery, bacterial clearance and reproductive performance of sows with endometritis were investigated. We found that the 400U dosage (400U lysostaphin/pill/time, repeat once on the third day, a total of two times, with 10% oxytetracycline uterine injection as a control) is the most effective treatment. *Staphylococcus aureus* was the most prevalent finding (34%,  $n = 188$ ), followed by *Streptococcus* (32%,  $n = 181$ ), *Escherichia coli* (19%,  $n = 104$ ) and other bacilli (15%,  $n = 83$ ) before treatment by drugs. Administration of lysostaphin resulted in an extremely significant ( $p < .01$ ) reduction in *S. aureus* ( $0.18 \pm 0.25$  from  $4.57 \pm 0.33$ ) and *Streptococcus* ( $0.11 \pm 0.14$  from  $3.88 \pm 0.29$ ), as well as a significant ( $p < .05$ ) reduction in *E. coli* ( $0.55 \pm 0.42$  from  $3.11 \pm 0.14$ ). Mixed infections (83%) were predominant before treatment, in contrast to single infections (61%) after treatment. Large-scale trials were conducted to verify the clinical efficacy of lysostaphin on sow endometritis. The average cure rate of 400u lysostaphin on sow endometritis (82.5%) was higher than the antibiotic group (72.17%). In addition, our results revealed that intravaginal administration of lysostaphin had no adverse effect on the reproductive performance of sows. Thus, this lysostaphin has potential application value as a new method alternative to antibiotics to treat endometritis in sows.

## KEYWORDS

endometritis, intravaginal administration, lysostaphin, sow

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**TABLE 1** Test group

Group	Area	Quantity	Treatments
High dose group (400U)	Guandong	20	Intravaginal administration was carried out by a disposable sterile vaginal medicine applicator. The confirmed sows were promptly administered with rLysostaphin VET. One pill (100U, 200U or 400U lysostaphin/pill) each time was administered, and one pill was repeated on the third day, for a total of two times.
	Jiangsu	30	
Medium dose group (200U)	Guandong	20	
	Jiangsu	30	
Low dose group (100U)	Guandong	20	
	Jiangsu	30	
Control group (10% oxytetracycline)	Guandong	20	The confirmed sows were injected with 25 ml of 10% oxytetracycline injection once in a sterile input tube. Repeat once on the third day for a total of two administrations.
	Jiangsu	30	
The blank control group	Guandong	20	No medical treatment; observed for 14 days.

## 1 | INTRODUCTION

Endometritis is a common disease and frequently occurs in sows. It usually suffers from a lowered fertility and an aberrant vaginal discharge. The conception rate of gilts with vulval discharge drops significantly (Maclachlan & Dial, 1987). Many sows remain infertile for a longer period. Even if sows are conceived successfully, reproductive performance will be reduced, including elevated sensitivity of piglets to infection, significant depression of piglet growth, increased mortality of piglets, resulting in unfavourable economic losses (Hirsch et al., 2003). Pathogenic microorganism infected are found to be the main cause for endometritis (Dial & Maclachlan, 1988). A survey conducted in Italy during 2010–2011 showed that the incidence of bacteria-positive urine in pigs was very high—the positive rate of bacteria in the urine of sows during their first pregnancy was 9%, whereas the positive rate of bacteria in sows that have had more than one pregnancy was 21%. The bacteria-positive rate of urine in first-time lactating sows was 21%, whereas the bacteria-positive rate of urine in sows that have lactated multiple times was 40% (Bellino et al., 2013). The main pathogens of swine endometritis are *Staphylococcus*, *Streptococcus*, *Escherichia coli*, *Corynebacterium*, mould and parasites (Winter et al., 1992; Wang et al., 2020; De Winter et al., 1995). Prevention and treatment of postpartum endometritis in sows is critical to the cost savings of large-scale pig farms.

In this era of multidrug-resistant bacteria, it is a top priority to find a new effective and safe drug for sow endometritis (Chuang & Huang, 2015). Lysostaphin is an antimicrobial agent belonging to a major class of antimicrobial peptides and proteins known as bacteriocins. It is a 27-kDa metal endopeptidase containing  $Zn^{2+}$  that cleaves specifically the cross-linking conserved pentaglycine bridges of staphylococcal peptidoglycan (Szweda et al., 2012). Compared with traditional antibiotics, lysostaphin seldom induces resistance in bacteria. Since lysostaphin kills human and animal staphylococcal pathogens, it has potential biotechnological applications in the treatment of staphylococcal infections (Fenton et al., 2010).

In this study, recombinant lysostaphin was developed into effervescent tablets and were vaginally administered in sows with endometritis. The tablets were completely foamed and dissolved within 30 min in the presence of water or body fluids releasing active

lysostaphin. The tablets contain disintegrating agents and adhesives that allow the drug to contact the endometrium for a long time and over a large area, thus achieving excellent healing results. In conjunction with the South China Agricultural University and Yangzhou University, the clinical efficacy of recombinant lysostaphin vaginal effervescent tablets (rLysostaphin VET) was determined in sow endometritis. The results of this study, including the response to lysostaphin therapy in terms of cure, bacterial clearance and reproductive performance, may provide new insights in managing endometritis in sows.

## 2 | MATERIALS AND METHODS

### 2.1 | Animals and reagents

#### 2.1.1 | Animals

This study was conducted in accordance with the guidelines for the trial of antibiotics II and III clinical efficacy evaluation (Ministry of Agriculture No. 1,247 Announcement, China) and the Guiding opinions on the treatment of experimental animals (2006, China) for animal experiments. All the animals received humane care. The study did not involve any human experimentation.

A total of 690 sows, all of which developed endometritis within a month of delivery, were selected. They came from 11 farms in the provinces of Guangdong and Jiangsu, each farm having more than 10,000 sows. Two hundred and fifty of the 690 sows selected were treated with three different dosages (i.e. 100U, 200U and 400U lysostaphin/pill/time, repeat once on the third day, a total of two times) of rLysostaphin VET according to Table 1, and 440 sows were treated with 400U rLysostaphin VET to verify the efficacy of sow endometritis. These sows were evenly, yet randomly, divided into two groups with rLysostaphin VET being administered to one group and the other being treated with an uterine injection of 10% oxytetracycline as a control. Six people were used to administer the doses and collect the field trial data in each pig farm. Since experimental drugs and control drugs were easy to distinguish in dosage form and administration method, it was difficult to achieve a complete

double-blinding. However, we had tried to achieve double-blinding as much as possible by completely separating operator and data analyst during the experiment. The operator did not participate in the data analysis, and the data analyst/supervisor did not know the product (test drug or reference drug) until the end of the trial observation and data registration.

The selection criteria were as follows: hybrids (♀Landrace♂large Yolk), parities between ~ 5 and 8, weight between ~ 175 and 186 kg. Those over 4 years old were not included in the selection. All the animals had the same feed (corn-soybean meal-based diets). The housing type and the feeding management were the same among the farms.

### 2.1.2 | Test drugs

rLysostaphin VET (batch number: 20110401, 20140201s; specifications: 100, 200 and 400U lysostaphin/pill, weighs 3 grams/pill) was provided by Shanghai Hi-Tech United Bio-Technological Research and Development Co., Ltd. The control drug, 10% oxytetracycline uterine injection (trade name: Aofulong; specification: 10g/100ml; batch number: 20110728, 20140218), was provided by Foshan Nanhai Dongfang Aolong Pharmaceutical Co., Ltd.

## 2.2 | Methods

### 2.2.1 | The inclusion criteria of suspected and diagnosed endometritis

Suspected sows with endometritis were selected by the presence of purulent or mucopurulent uterine exudate in the vagina. Arched back, lifted up tail, depressed spirit and frequent urination with low volume were the symptoms. Endometritis was diagnosed by clinical signs, such as fever and loss of appetite, and the pathogens involved in endometritis were identified from suspected sows (Jeffrey et al., 2012).

### 2.2.2 | Detection of pathogenic bacteria

Pathogens were identified from vaginal secretions to confirm clinical endometritis. Vaginal secretion samples were collected from 15% of sows with suspected endometritis.

Secretion collection process was explained as follows: (1) First the sow's vulva was cleaned with warm water, sterilized locally with 75% alcohol cotton ball. (2) Second, about 20 ml of autoclaved physiological saline was injected into the vagina by sterile vas deferens, according to artificial insemination aseptic operation. The vaginal secretions were collected and sealed in the sterilized vial, stored in a refrigerator at 4°C and detected within 2 days.

The bacterial pathogens in the collected vaginal secretion samples were identified after aerobic and anaerobic plate culture with a

variety of selective medias: enterobacteria (eosin blue agar), *staphylococci* (high-salt mannitol agar), *streptococci* (KF *streptococcus* Agar) and coryneform bacteria (blood agar base + polymyxin). Anaerobic bacteria were cultured with CDC anaerobic blood agar + sheep blood (in an anaerobic chamber). Bacteria identification was followed by API gold standards, including API20E for *Enterobacter*, API Staph for *Staphylococcus*, API Strep for *Streptococcus*, API Coryne for *Corynebacterium* and API20A for anaerobic bacteria. After 18 ~ 24 hr incubation at 37°C, the cultured colony cells were Gram stained and the cell morphologies were observed under a microscope. To perform biochemical assays, the bacterial cells from cultures were inoculated into biochemical identification microtubes, respectively, and cultured for 24 hr at 37°C according to the manufacturer's instructions.

### 2.2.3 | Methods and criteria for determining efficacy

Cure (complete recovery) refers to the sows that were recovered completely from endometritis after treatment. In order for a sow with endometritis to have achieved a complete recovery, 1) the mental state, body temperature and appetite of the sow after treatment should have returned to normal and 2) no inflammatory secretions and pus discharge in the vagina—with clear secretions and 4) the vagina returned to normal. After 7 days of weaning, estrus was normal and reproduction was normal.

Effective (sum of healing and marked improvement) refers to the sows that were fully recovered and significantly improved after treatment. Significant improvements indicates that after treatment the sow's mental state, body temperature and appetite returned to normal. They still had a vaginal secretion and discharge, but it was more transparent and clearer than that before treatment and did not contain pus or inflammatory substances.

Ineffective (no obvious improvement) refers to that the mental state, body temperature and appetite of the sows that did not return to normal after treatment, and there are still inflammatory pus discharges in the vagina.

The subsequent conception rate refers to the successful conception rate after one breeding in sows that recovered after treatment.

Re-service rate means that estrus became normal after treatment, but the sow did not successfully conceive after one breeding.

### 2.2.4 | Method of intravaginal lysostaphin administration

Three specifications (100, 200 and 400 U lysostaphin /pill) of rLysostaphin VET were used in our study. In addition to lysostaphin, each tablet contains matrix, disintegrating agent, adhesive and other auxiliary materials. The weight of one tablet was 3 grams. In the presence of water or body fluids, the tablets were completely foamed and dissolved within 30 min and to release active lysostaphin. Intravaginal administration was carried out by

a disposable sterile vaginal medicine applicator (Figure 1, total length: 65cm ± 5mm). A disposable sterile applicator was used for one tablet, and the applicator cannot be reused to prevent cross infection. The confirmed sows were promptly administered with rLysostaphin VET. Repeat administration once on the third day twice.

Intravaginal lysostaphin administration process was explained as follows: The vulva was cleaned along with the surrounding area with warm water and sterilized locally with 75% alcohol cotton ball. The piston of the disposable sterile applicator was pulled out, put the tablet into the horn end of the applicator, and lifted slowly the applicator with a 30–45° angle upward into the vagina. After insertion to about 10–15 cm, turned to level. When you felt a little resistance in the front part of the applicator, stopped moving forward (approximately inserted 25–30 cm), pushed the piston gently to push the tablet into the vagina.

### 2.2.5 | The concentration of lysostaphin in rLysostaphin VET

Each batch of tablets had passed strict product quality inspection. The following were the detection steps and methods of lysostaphin concentration. One rLysostaphin VET was dissolved with 0.05mol/L Tris-HCl buffer (pH 7.5) to make a solution containing about 10 units of lysostaphin per ml. Solution was filtered by 0.45µm filter. Supernatant was saved as a lysostaphin sample.

The concentration of lysostaphin was analysed by a modified dye release assay as described previously (Zhou et al., 1988). Dye group was linked to *Staphylococcus aureus* dead cells to form dye-pentaglycine (KNR-PG) compound as substrate. When lysostaphin contacted the substrate, the enzyme cut off the link and released the dye group which can be detected at 595 nm (Liu et al., 2014). Briefly, 50 µl lysostaphin sample, 130 µl substrate and 720 µl Gly-NaOH buffer (0.2mol/L, pH10.0) were added into Eppendorf centrifuge tube and incubated at 37°C for 20 min. Then 300 µl 95% ethanol was added to stop the reaction. The tubes were centrifuged at 10,000 r/min for 10 min. Supernatant was measured at 595nm. Different volumes of standard lysostaphin solution (9U/mL) were measured following the same procedure mentioned above. A standard curve was calculated. The lysostaphin concentration of the samples was determined based on the standard curve. Data were calculated as the average ± standard deviation of three samples ( $n=3$ ).

The standard lysostaphin enzyme comes from the SIGMA company, and a unit of lysostaphin activity is defined as follows In

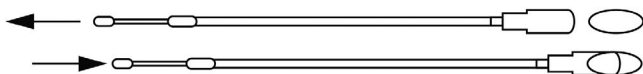


FIGURE 1 The disposable sterile vaginal medicine applicator

a 6.0ml reaction system with pH 7.5, at 37°C, the amount of enzyme required for reducing the turbidity of *Staphylococcus aureus* (A620nm) from 0.250 to 0.125 in 10 min.

### 2.2.6 | Detection of lysostaphin in vaginal secretions after administration

After lysostaphin (400U) administration at 12h, 24h, 36h, 48h and 72h, vaginal secretions of sick sows and healthy sows were collected aseptically using 10 ml disposable syringes with needles removed, and kept in sterilized vials, placed in a refrigerator at -18°C for laboratory test. Vaginal secretions before lysostaphin administration were tested as the control. The minimal limits of detection ever attained by the modified dye release assay were only at the microgram range and of low specificity. These are far from lysostaphin pharmacokinetics demands in its clinical applications for detection of lysostaphin in vaginal secretions and serum. So lysostaphin in vaginal secretions were detected by the double antibody sandwich enzyme-linked immunoadsorbent assay (ELISA, the limit of detection is 0.98 ng of rLysostaphin/mL), which was described in the published work of Huang et al., 2007.

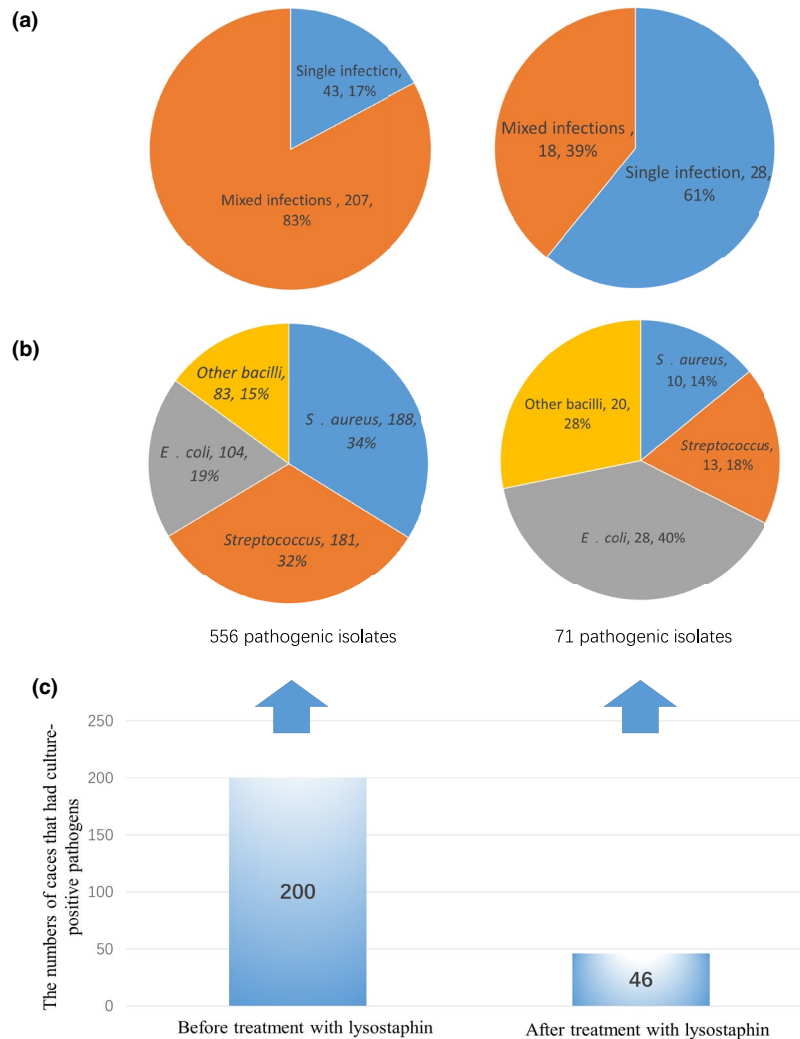
### 2.2.7 | Experimental data processing

The results of the examinations were statistically analysed by  $\chi^2$  analysis. Statistical significance was indicated at two levels: \*  $p < .05$  and \*\*  $p < .01$ .

## 3 | RESULTS

### 3.1 | Distribution of strains

A total of 556 pathogenic isolates were collected from the 200 sows included in the study before treatment. *S. aureus* was the most prevalent isolate (34%,  $n = 188$ ), followed by *Streptococcus* (32%,  $n = 181$ ), *E. coli* (19%,  $n = 104$ ) and other bacilli (15%,  $n = 83$ ). *S. aureus* and *Streptococcus* accounted for more than 60%, which was consistent with results from a previous study (Wang et al., 2017). Mixed infections (83%) were the most prevalent infection, which added to the difficulty of clinical treatment. After treatment with lysostaphin, only 71 pathogenic organisms were isolated from 46 cases. There were no pathogens detected in the other 154 cases. The types and distribution of pathogenic bacteria in the secretions from the sows changed significantly before and after treatment. *E. coli* was the most prevalent finding (40%,  $n = 28$ ), followed by other bacilli (28%,  $n = 20$ ), *Streptococcus* (18%,  $n = 13$ ) and *S. aureus* (14%,  $n = 10$ ). Furthermore, single infections (61%) were the most prevalent infection. These results are shown in Figure 2.



**FIGURE 2** The distribution of major pathogens and types of infection. (a) The types of infection. (b) The distribution of the main pathogens. (c) The numbers of culture-positive pathogens that were detected

### 3.2 | Effect of lysostaphin on bacterial clearance

Secretions from sows with endometritis were collected by Yangzhou University after one course of treatment in each group. The pathogens in the secretions were then identified. The growth of each type of bacteria was evaluated on plates before and after treatment (two repetitions). The results are presented in Table 2.

Administration of lysostaphin resulted in an extremely significant ( $p < .01$ ) reduction in the *S. aureus* and *Streptococcus* count and a significant ( $p < .05$ ) reduction in the *E. coli* count—compared to the blank control. After administration of 400U rLysostaphin VET, the bacterial evaluation index of *S. aureus*, *Streptococcus* and *E. coli* dropped to  $0.18 \pm 0.25$  from  $4.57 \pm 0.33$ ,  $0.11 \pm 0.14$  from  $3.88 \pm 0.29$  and  $0.55 \pm 0.42$  from  $3.11 \pm 0.14$ , respectively. The rate of *S. aureus* and *Streptococci* clearance did not differ between the 200U lysostaphin group, 400U lysostaphin group and 10% oxytetracycline control group. These results revealed

that the primary pathogenic bacteria in the secretions of endometritis sows were effectively cleared by rLysostaphin VET.

### 3.3 | Clinical efficacy of lysostaphin on sow endometritis

After a course of treatment, the sows were observed for a clinical observation period of 14 days. The results are shown in Table 3. In Guangdong, the cure rate of high doses of rLysostaphin VET (400U) was 85.0%, which was comparable to the control group (85%), and the efficacy rate was 90.0%, which was comparable to the control group (85%). In Jiangsu, the cure rate of high doses of rLysostaphin VET (400U) was 83.33%, which was comparable to the control group (83.33%), and the efficacy rates were 86.63%, which was comparable to the control group (90.00%). There was no statistical difference

**TABLE 2** Distribution of intravaginal pathogenic bacteria before and after treatment

Number	Group	Cases	Bacteria	Bacterial evaluation index before treatment	Bacterial evaluation index after treatment
1	High dose group (400U)	30	<i>S.aureus</i>	4.57 ± 0.33	0.18 ± 0.25**
			<i>Streptococcus</i>	3.88 ± 0.29	0.11 ± 0.14**
			<i>E.coli</i>	3.11 ± 0.14	0.55 ± 0.42**
2	Medium dose group (200U)	30	<i>S.aureus</i>	4.44 ± 0.57	0.25 ± 0.15**
			<i>Streptococcus</i>	3.59 ± 0.39	0.17 ± 0.21**
			<i>E.coli</i>	3.22 ± 0.18	0.81 ± 0.56**
3	Low dose group (100U)	30	<i>S.aureus</i>	4.53 ± 0.41	0.38 ± 0.31**
			<i>Streptococcus</i>	3.74 ± 0.27	0.28 ± 0.15**
			<i>E.coli</i>	3.10 ± 0.21	1.90 ± 0.72 <sup>+</sup>
4	Control group (10% oxytetracycline)	30	<i>S.aureus</i>	4.48 ± 0.41	0.19 ± 0.36**
			<i>Streptococcus</i>	3.64 ± 0.48	0.13 ± 0.24**
			<i>E.coli</i>	3.15 ± 0.22	0.35 ± 0.18**
5	The blank control group	30	<i>S.aureus</i>	4.61 ± 0.34	4.43 ± 0.77
			<i>Streptococcus</i>	3.53 ± 0.54	3.31 ± 0.88
			<i>E.coli</i>	3.03 ± 0.15	2.88 ± 0.67

a: Bacterial evaluation index refers to the average number of logarithms of the total number of bacteria (*S. aureus*, *Streptococcus* and *E. coli*, respectively) in the vaginal secretions. The higher the number, the more bacteria that grew.

\*indicates a significant difference as compared with the blank control group ( $p < .05$ );

\*\*indicates that the difference is extremely significant as compared with the blank control group ( $p < .01$ ); unlabelled data indicate that the difference is not significant ( $p > .05$ ).

**TABLE 3** Clinical efficacy of different doses of lysostaphin on sow endometritis

Number	Group	Area/Cases	Cure rate/%	Effective rates /%
1	High dose group (400U)	Guangdong /20	85.00	90.00 <sup>b</sup>
		Jiangsu /30	83.33	86.63 <sup>b-</sup>
2	Medium dose group (200U)	Guangdong /20	80.00	80.00 <sup>b-</sup>
		Jiangsu /30	76.67	83.33 <sup>b-</sup>
3	Low dose group (100U)	Guangdong /20	60.00	65.00 <sup>b-</sup>
		Jiangsu /30	63.33	70.00 <sup>b</sup>
4	Control group (10% oxytetracycline)	Guangdong /20	85.00	85.00 <sup>b</sup>
		Jiangsu /30	83.33	90.00 <sup>b</sup>
5	The blank control group	Guangdong /20	10.00	25.00 <sup>++</sup>
		Jiangsu /30	10.00	20.00 <sup>++</sup>

Cure rate: The rate of cured cases. Cases with complete recovery, where body temperature and appetite returned to normal, no evidence of inflammatory secretions and pus discharge in the vagina, and secretions were clear.

Effective rates: The rate of effective cases. Cases with improved recovery, where body temperature and appetite returned to normal. Although there was a certain secretion in the vagina, it was more transparent and clearer, without pus or inflammatory substances.

The results of the examinations were statistically analysed by  $X^2$  analysis. Compared with the blank control group (the fifth group), a indicates extremely significant difference, b indicates significant difference, c indicates no significant difference. Compared with the oxytetracycline control group (the fourth group), ++ indicates extremely significant difference, + indicates significant difference, - indicates no significant difference.

**TABLE 4** Large-scale trials of lysostaphin on sow endometritis

Area	Drug	Cases	Cure rate /%	Effective rate /%
Guangdong	rLysostaphin VET (400U)	120	85.00	91.67
	Control group (10% oxytetracycline)	120	63.33	75.00
Jiangsu	rLysostaphin VET (400U)	100	80.00	89.00
	Control group (10% oxytetracycline)	100	81.00	91.00

**TABLE 6** Detection of lysostaphin in vaginal secretions after administration

The time after administration (h)	Detection of lysostaphin in vaginal secretions after administration (ng/mL)	
	healthy sows (n = 8)	sick sows (n = 8)
0	ND	ND
12	45.56 ± 10.76	33.27 ± 8.01
24	25.74 ± 4.42	21.5 ± 5.29
36	7.34 ± 2.09	4.45 ± 1.17
48	ND	ND
72	ND	ND

"ND" means lower than the detection limit of 0.98ng/ml.

**TABLE 5** Reproductive performance of lysostaphin on sow endometritis

Area	Drug	Cases	The subsequent conception rate /%	Re-service rate%
Guangdong	rLysostaphin VET (400U)	102	100% (102/102)	0% (0/102)
	Control group (10% oxytetracycline)	76	84.21% (64/76)	15.79% (12/76))
Jiangsu	rLysostaphin VET (400U)	80	93.75 (75/80)	6.25 (5/8)
	Control group (10% oxytetracycline)	81	95.06 (77/81)	4.94 (4/81)

between the groups of 400U lysostaphin and the control group of 10% oxytetracycline. This inspired us to use lysostaphin for clinical research in sow endometritis and, more importantly, encouraged us to further conduct large-scale trials.

### 3.4 | Large-scale trials of lysostaphin on sow endometritis

Large-scale trials were conducted to verify the effect of lysostaphin on sow endometritis (Table 4). Four hundred and forty sows with clinical manifestations of endometritis were selected from 10 scale farms in two different areas. Of the 440 positive sows, 220 were administered 400U rLysostaphin VET, whereas the remaining 220 cases were treated with 10% oxytetracycline uterine injection as control.

The cure rate of lysostaphin in Guangdong and Jiangsu was 85.00% and 80.00%, respectively. Thus, the average cure rate of lysostaphin on sow endometritis (82.5%) was higher than the antibiotic group (72.17%). In addition, the average effective rate of lysostaphin on sow endometritis exceeded 90.00% (91.67% in Guangdong and 89% in Jiangsu). These indicate that the effect of rLysostaphin VET on sow endometritis was better than the control oxytetracycline injections.

### 3.5 | Reproductive performance of lysostaphin on sow endometritis

The experimental results in Jiangsu Province showed that the subsequent conception rate of the treated group was 93.75% and 95.06% in the control group. It was surprising that the subsequent conception rate of the treated group in Guangdong was 100%, but only 84.21% in the control group (Table 5). This indicates that lysostaphin has no adverse effect on the reproductive performance of sows.

## 4 | DISCUSSION

Both in vitro and in vivo studies performed with lysostaphin have shown that there is much potential for lysostaphin to be used solely or in combination with other antibacterial agents to prevent or treat bacterial *Staphylococcal* infections (Desbois et al., 2010; von Eiff et al., 2003; Kokai-Kun et al., 2007; Yang et al., 2007). Lysostaphin is effective against not only *Staphylococcus* but also *Streptococcus*. The in vitro activities of lysostaphin on clinical bacterial isolates from sows with endometritis were studied (data not show). The minimal inhibitory concentration (MIC) values for lysostaphin was assessed for 20 *S. aureus*, 28 *Streptococcus*, and 40 *E. coli* clinical isolates. The

MIC90 for *S. aureus*, *Streptococcus* and *E. coli* was 0.25 U/mL, 0.125 U/mL and > 128 U/mL, respectively. These results supported the in vivo results, in which the clearance rate of *S. aureus* and *Streptococci* was better than the clearance rate for *E. coli*.

The vaginal secretion samples from the healthy sows and the sick sows that have been treated with lysostaphin were collected at different time points to detect enzymatic activity by ELISA. ELISA, benefited its high sensitivity and extremely low detection limit (0.98 ng rLysostaphin/mL), was useful for both research and for the study of pharmacokinetics of lysostaphin in tissue and in blood and other body fluids. The test results are shown in Table 6. Enzyme activity was detected in the vaginal secretions of healthy sows and diseased sows 36 hr after administration (7.34 ng/ml and 4.45 ng/ml, respectively), but neither was detected at 48 hr, indicating that the drug remains active in the vagina for at least 36 hr. This is the reason for which the drug was repeated every other day.

In the field of livestock and poultry, there are also studies on transgenic technology where the breast tissue of transgenic cows express lysostaphin to help prevent mastitis (Wall et al., 2005). Lysostaphin is secreted into the milk through a breast epithelial cell-specific expression system. However, these studies are in the experimental research stage and no clinical application has been observed. Luckily, lysostaphin was licensed to treat cow endometritis and mastitis in China, which significantly promotes the application of lysostaphin in agriculture. To our knowledge, no scientific effort has been made to evaluate the efficacy of lysostaphin in the treatment of sow endometritis. Our results showed that the clinical efficacy of lysostaphin on sow endometritis was better than the antibiotic group (Table 4, in the Guangdong group). Lysostaphin showed great application value, as it did not have any adverse effects on the reproductive performance of sows, and even improved the subsequent conception rate. Lysostaphin is milder and less irritating than the antibiotic oxytetracycline. In addition, lysostaphin has the potential to accelerate wound healing rate (Cui et al., 2011; Johnson et al., 2018; Miao et al., 2011), which may be conducive to the recovery of uterus and the improvement of reproductive performance. The advantages of lysostaphin on the reproductive performance of sows still need to be future investigated with the experiments designed to collect valid data, such as farrowing rates, numbers of piglets born alive, weaning to service intervals. This is the current research direction of lysostaphin in the field of sow breeding. In conclusion, our results showed that lysostaphin has potential application value as a new method alternative to antibiotics to treat endometritis in sows.

#### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

#### FUNDING INFORMATION

This work was supported by National Key Research and Development Program of China (2016YFD0501308), Key Research and Development Program of Jiangsu Province (BE2017314).

#### PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1002/vms3.417>.

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**How to cite this article:** Ye G, Huang J, Li G, et al. Clinical efficacy of intravaginal recombinant lysostaphin administration on endometritis in sows. *Vet Med Sci*. 2021;7:746–754. <https://doi.org/10.1002/vms3.417>