

Original Research Article

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Investigation of Bacterial Infections in Pig Farms affected by Respiratory Disease Complex in Bulgaria

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ABSTRACT

Keywords

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This study attempted to identify bacterial pathogens and determine their importance in porcine respiratory disease complex in order to identify effective and adequate measures for their control. The study was conducted in three industrial intensive pig farms in different regions of Bulgaria. Nasal swab samples from weaners and fattening pigs affected by respiratory infection were examined, as well as from the lungs of animals that had died or had been emergency slaughtered, with changes characteristic of pneumonia. The results showed that mono-infection was prevalent in the analyzed nasal samples in weaners - 54.2%, and in 70.8% *alpha hemolytic Streptococcus* was detected. In fattening pigs, associated infections dominated with 54.2%. In a significant part of the samples (62.5%) there were observed *alpha hemolytic Streptococcus* and *S. epidermidis*. The results from the microbiological examinations of the lungs showed that the prevailing cases were mono-infections in weaners pigs (70.8%), as well as in fattening pigs (75.0%). A significant proportion of lung specimens were shown to contain *E. coli*, probably due to coli septicemia potentiated by *M. hyopneumoniae*.

Introduction

Respiratory diseases remain a current problem in industrial pig farming, regardless of the herd size and the rearing technologies. These diseases very often occur with

multiple etiology, which has historically led to the coining of the term Porcine respiratory disease complex (PRDC) (Motovski, 2003; Bochev, 2007).

The PRDC etiology varies considerably in different production systems (Halbur, 1999; Motovski, 2003). It commonly involves one or two viruses, *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*, *M. hyo*) and other bacterial agents acting together to cause severe respiratory diseases and, consequently, major economic losses (Motovski, 2003).

One of the major etiological agents of PRDC is *M. hyopneumoniae*, the causative agent of enzootic pneumonia in pigs (Stipkovits *et al.*, 2001; Opriessnig *et al.*, 2004; Thacker, 2006). *M. hyopneumoniae* infection in pigs is a predisposing factor for the development of secondary bacterial infections of the respiratory tract (Bosch, 2004). According to Tielen (1995), some of the most common secondary causative agents include *Pasteurella multocida* (*P. m.*), *Streptococcus suis* (*S. suis*), *Staphylococcus aureus* (*S. aureus*) etc.

The aim of this study was to identify the bacterial causative agents and determine their role in PRDC in three different pig farms in Bulgaria in order to define effective and adequate control measures.

Materials and Methods

Samples

Samples were collected in 2014 from three pig farms located in different parts of Bulgaria with incidence of respiratory infections. The studied clinical samples included 48 nasal swabs and 48 lung samples from growing and fattening pigs.

Nasal swabs were taken by deep rubbing of dry sterile swabs against the nasal mucosa on one side. Each swab was then inserted into a container with transport medium (COPAN[®]). Lung samples were taken from

dead growing and fattening pigs and from emergency slaughtered pigs with pneumotic changes. Each collected sample was inserted into a sterile container.

Microbiological analysis

The nasal swabs and lung samples were enriched in standard broth containing 1% dextrose (BulBio-NCIPD). Then plating was done on conventional nutrient media for primary isolation of bacterial respiratory pathogens: blood agar (BioMerieux[®]); MacConkey agar (BioMerieux[®]); Chocolate agar with IsoVitalex and Bacitracin (Becton, Dickinson and Company[®]); deoxycholate citrate lactose agar (BulBio[®]- NCIPD); Kligler agar (BulBio[®]- NCIPD). Identification of the obtained isolates was done according to the classical procedures for routine microbial diagnostics and by using automated methods for identification of bacteria (Gram-staining; colony morphology; catalase test with 3% H₂O₂; H₂S production; latex slide agglutination test for *Staphylococcus aureus* and *Coagulase-negative staphylococci* (STAPHYTECT PLUS, Oxoid[®]); latex slide agglutination test for *beta-hemolytic Streptococcus* (SLIDEX Strepto Plus, BioMérieux[®]) and a VITEK 2 compact (BioMerieux[®]) automated instrument.

To determine the antibiotic sensitivity of the isolates, antibiograms were obtained by the disc diffusion assay, using the following nutrient media: Mueller Hinton 2 agar and Mueller Hinton 2 agar + 5% sheep blood (BioMerieux[®]), and BD BB Sensi-Disc Antimicrobial Susceptibility Test Discs (Becton Dickinson and Company[®]) (*Table 1*).

Serological tests

The etiological role of *M. hyopneumoniae* and *A. pleuropneumoniae* in respiratory

diseases in pigs was diagnosed using enzyme immunoassays for detection of specific antibodies in blood sera of growing and fattening pigs from the studied pig farms.

Blocking ELISA was used for detection of specific antibodies against *M. hyopneumoniae* (Ingezim M. hyo compact, INGENASA®) and indirect ELISA, for detection of specific antibodies against *A. pleuropneumoniae* (Ingezim APP mix, INGENASA®). All procedures were strictly based on the manufacturer's instructions.

Results and Discussion

The data from the microbiological analysis of the nasal swabs from growing and fattening pigs originating from pig farms with respiratory symptoms are presented in *Fig. 1* and *Fig. 2*. The obtained results showed that, in the studied farms, mono-infection was predominant in the growing pigs (54.2%). The majority of the isolates were identified as *alpha-hemolytic Streptococcus* (70.8%). There was also high

prevalence of infections caused by *S. epidermidis* (37.5%). In 12.5% of the cases, *E. coli* strains were identified. *Klebsiella* spp. and *S. aureus* had similar prevalence of 8.3%. In the fattening pigs, there were predominantly associated infections (54.2%). In this group of pigs, the microbiological tests did not reveal pathogenic *E. coli* and *S. aureus* in the three farms examined by us. The most prevalent pathogens in the fattening pigs were identified to be *alpha hemolytic Streptococcus* and *S. epidermidis*, both with 62.5% prevalence. In 33.3% of the cases, *Klebsiella* spp. were isolated.

The microbiological analysis of the lung samples taken from dead pigs with clinical signs of respiratory disease (*Fig. 3* and *Fig. 4*) showed that the infections that prevailed in the growing pigs were mono-infections (70.8%). Most of the isolates were identified as *E. coli*; then *Klebsiella* spp., *S. aureus* and *Coagulase-negative staphylococci* (CNS) ranked second, with 8.3% each. There was a comparatively low prevalence of *alpha hemolytic Streptococcus* (4.2%).

Table.1 Antibacterial discs used

Antibiotic group	Antimicrobial disc	Code	µg/disc
1. Aminopenicillines	Amoxicillin	AMX	25
2. Cephalosporines	Cephalexin	CN	30
3. Aminosides	Gentamicin	GM	10
	Amikacin	AN	30
4. Tetracyclines	Tetracyclin	TE	30
5. Quinolones	Ciprofloxacin	CIP	5
6. Sulphonamides	Trimethoprim/Sulfamethoxazole	SXT	1,25-23,75

Table.2 Results of serological tests for proof of specific antibodies against *M. hyopneumoniae* and *A. pleuropneumoniae*

swine farms	Antibody against		Antibody against	
	<i>M. hyopneumoniae</i>		<i>A. pleuropneumoniae</i>	
	growing pigs	fattening pigs	growing pigs	fattening pigs
1	+	+	-	+
2	+	+	-	+
3	+	+	+	+

Table.3 Sensitivity of microorganisms isolated from nasal swab samples from growing and fattening pigs to certain antibiotic agents

Antibiotic	<i>E. coli</i>			<i>Klebsiella spp.</i>			<i>S. aureus</i>			CNS			<i>α-Strept.</i>		
	(n=3)			(n=10)			(n=2)			(n=24)			(n=32)		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
AMX - 25 µg	3	-	-	7	1	2	-	1	1	2	5	17	-	8	24
CN - 30 µg	-	2	1	3	-	7	-	-	2	-	2	22	-	2	30
GM - 10 µg	-	-	3	-	-	10	-	-	2	-	1	23	-	5	27
AN - 30 µg	-	-	3	-	-	10	-	-	2	17	4	3	22	8	2
TE - 30 µg	3	-	-	4	1	5	2	-	-	21	1	2	30	2	-
CIP - 5 µg	-	-	3	-	-	10	-	-	2	7	-	17	5	4	23
SXT - 1.25-23,75 µg	3	-	-	3	3	4	-	1	1	9	3	12	15	2	15

Table.4 Sensitivity of microorganisms isolated from lungs of dead and slaughtered growing and fattening pigs to certain antibiotic agents

Antibiotic	<i>E. coli</i>			<i>Klebsiella spp.</i>			<i>S. aureus</i>			CNS			<i>α-Strept.</i>		
	(n=40)			(n=2)			(n=4)			(n=10)			(n=2)		
	R	I	S	R	I	S	R	I	S	R	I	S	R	I	S
AMX - 25 µg	26	1	13	2	-	-	-	2	2	-	1	9	-	-	2
CN - 30 µg	4	13	23	2	-	-	-	-	4	-	-	10	-	-	2
GM - 10 µg	-	4	36	2	-	-	-	-	4	-	-	10	-	-	2
AN - 30 µg	-	1	39	-	-	2	-	-	4	-	3	7	2	-	-
TE - 30 µg	39	-	1	2	-	-	4	-	-	8	2	-	2	-	-
CIP - 5 µg	6	1	33	-	-	2	1	2	1	-	-	10	-	-	2
SXT - 1.25-23,75 µg	17	-	23	2	-	-	-	2	2	-	1	9	-	-	2

Fig.1 Percent of pigs in growing and fattening affected by mono infection and associated infections (nasal samples)

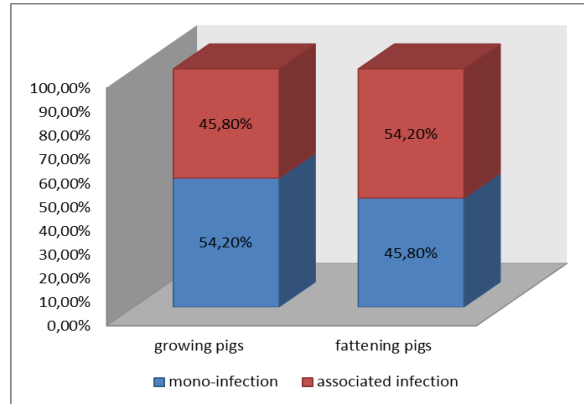


Fig.2 Results of microbiological studies on nasal swab samples from growing and fattening pigs (%)

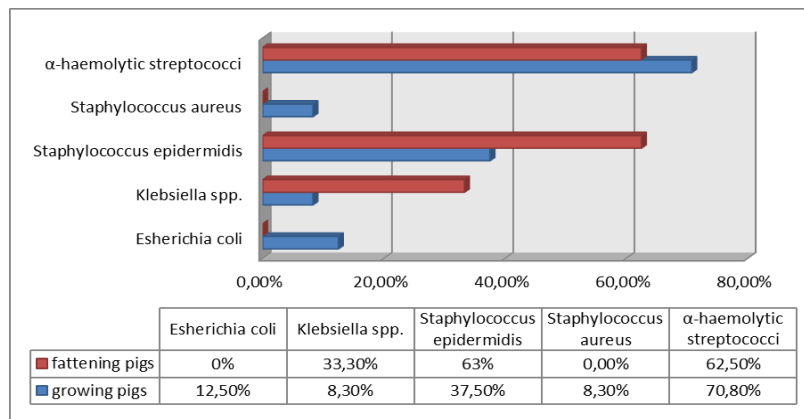


Fig.3 Percent of pigs in growing and fattening affected by mono infection and associated infections (lung samples)

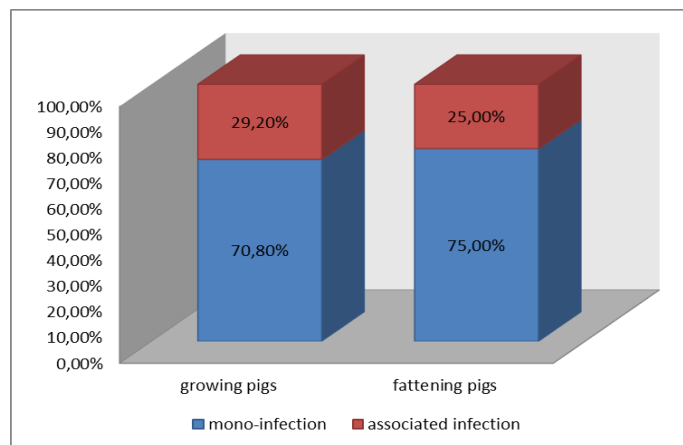
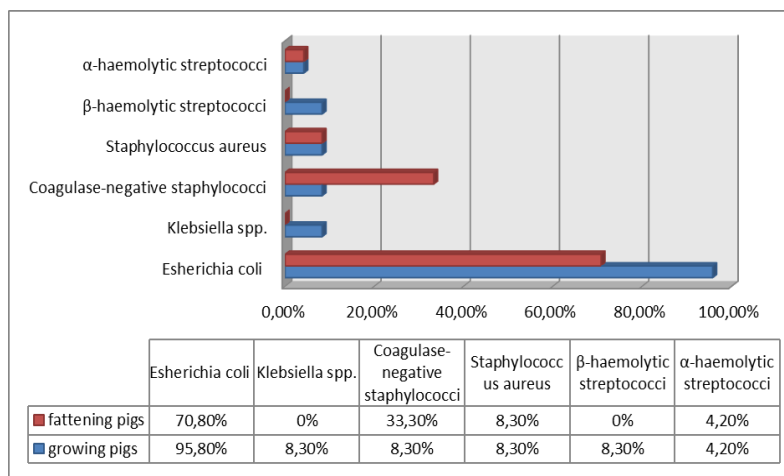


Fig.4 Results of microbiological studies on lungs from growing and fattening pigs (%)



Interestingly, *beta hemolytic Streptococcus* was isolated in one of the studied farms. In the fattening pigs, the dominant infections were also mono-infections (75.0%), with *E. coli* being the leading causative agent, and *CNS* ranking second. All the tested lung samples were negative for *Klebsiella* spp. and *beta hemolytic Streptococcus*. Other etiological agents that were isolated included *S. aureus* and alpha hemolytic *Streptococcus*, in 8.3% and 4.2% of the cases, respectively.

Our results concerning the detection of *M. hyopneumoniae* and *A. pleuropneumoniae* infections in the studied farms (Table 2) showed that antibodies against *M. hyopneumoniae* were found in different technological groups of pigs in all the three studied farms. In two of the farms, none of the tested samples from the growing pigs was positive for *A. pleuropneumoniae*. Conversely, among the fattening pigs, such antibodies were detected in all the three farms. In pigs, infection with *M. hyopneumoniae* and *A. pleuropneumoniae* leads to suppression of the phagocytic activity of alveolar macrophages, resulting in immunosuppression and thus predisposing the animals to secondary bacterial infections of the respiratory tract.

The patterns of *in vitro* antibiotic sensitivity of the microbial isolates from the nasal swabs of growing and fattening pigs are presented in Table 3. The obtained results showed that the *E. coli* isolates were resistant to amoxicillin, tetracycline and sulfametoxazol+trimetoprim and susceptible to gentamicin, amikacin and ciprofloxacin. Similar patterns of antibiotic sensitivity to gentamicin, amikacin and ciprofloxacin were observed in all *Klebsiella* spp. isolates. Most strains showed amoxicillin resistance. *S. aureus* demonstrated good sensitivity to cephalexin, gentamicin, amikacin and ciprofloxacin, but was absolutely resistant to tetracycline. The antimicrobial activity of amoxicillin, cephalexin, gentamicin and ciprofloxacin against most *alpha hemolytic Streptococcus* isolates was good, but that of amikacin and tetracycline was poor.

The response of the microbial isolates from the lung samples of the growing and fattening pigs to different antimicrobial agents are shown in Table 4. The majority of the *E. coli* isolates from lung samples had good sensitivity to gentamicin, amikacin and ciprofloxacin, but were resistant to amoxicillin and tetracycline. All the tested *Klebsiella* spp. isolates showed resistance to most of the antibiotic agents included in this

study, except amikacin and ciprofloxacin. In our study, *S. aureus* was observed to have good sensitivity to cephalexin, gentamicin and amikacin and resistance to tetracycline. A lot of CNS isolates exhibited sensitivity to the tested antimicrobial agents, except tetracycline. Our data indicate that the prevailing type of antibiotic resistance among all the tested *alpha hemolytic Streptococcus* strains was that to amikacin and tetracycline, whereas the sensitivity to the other antimicrobial agents included in this study was preserved.

In conclusion, the prevalent pathogen in the majority of the studied nasal swabs from growing and fattening pigs with clinical signs of respiratory infection, was *alpha hemolytic Streptococcus*. The prevalent infections in the affected lungs of weaning and fattening pigs were shown to be mono-infections, with *E. coli* as the leading cause. The majority of the Gram-positive and Gram-negative bacterial isolates showed high levels of resistance against tetracycline antibiotics. *M. hyopneumoniae* and *A. pleuropneumoniae* were demonstrated to play a role in the multiple etiology of respiratory diseases in pigs.

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