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Original Contribution

INVESTIGATION ON THE MICROBIAL FACTOR OF PORCINE RESPIRATORY DISEASE COMPLEX (PRDC) IN INDUSTRIAL PIG FARMING CONDITIONS

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ABSTRACT

During the last years, the incidence of respiratory pathology in pig farming has sharply increased. It is acknowledged that their etiology is frequently multifactorial. Together with viruses and mycoplasmae, a number of bacterial species were also shown to be involved. This is essential with regard to rational therapeutic control. The *in vitro* behaviour of bacterial isolates to antimicrobials is therefore particularly important.

The present study was performed in 2005-2008 on the11th pig farms. A total of 191 lung samples from different categories of pigs were investigated. The bacterial findings were positive in 139 or 72.8% of cases. In 84.2% of them, only one microbial species was involved whereas in 15.8% - more than one. In general, 16 bacterial varieties belonging to 13 different taxonomic categories were isolated and identified. The in vitro sensitivity of microbial isolates was the highest against macrolides and florphenicol.

Key words: PRDC, microbial pathogens, antimicrobial resistance

INTRODUCTION

Porcine respiratory disease complex (PRDC) affects predominantly growing and older pigs at the age of 16 to 22 weeks. It is characterized with difficult hurried breathing, fever, cough with a variable character and severity, poor feed utilization and subsequent slowed growth (1, 2, 3).

The most common etiological agents in PRDC are viruses, mycoplasmae as well as bacteria – *Pasteurella multocida*, *Actinobacillus pleuropneumoniae*, *Haemophilus* spp., *Streptococcus suis* type 2 etc. (4, 5). The appearance and the development of this disease complex is also related to the penetration of the porcine reproductive and respiratory syndrome virus (PRRSV) among pig populations, of porcine circovirus type 2 (PCV2) that causes postweaning multisystemic wasting syndrome (PMWS), and swine influenza virus (SIV). (2, 6, 7, 8). Apart the microbial agent, other risk factors such as the introduction of more intensive rearing technologies, selection for fast-growing hybrids with weaker immune system, large-scale pig farms created by introduction of animals without the obligatory quarantine, various stressors (overcrowding, excessive concentrations of harmful gases, excessive air dust, parasitoses, intestinal bacterial infections, could also be involved. (9, 10).

In Bulgaria, respiratory infections are also widely distributed among pigs and cause significant economical losses. They consist in higher mortality, poor feed conversion, mass medication costs, therapeutic treatments, etc. Growers at the end of the period and pigs in finisher groups are the most affected. Sporadic investigations carried out during epidemic outbreaks have outline a number of etiological agents, have described some epidemiologic traits and measures for prevention and control (11, 12,13).

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Similarly to what is accepted in other countries, the opinion that despite the undisputed role of viruses with respiratory tropism, the irreversible morphological changes and life-threatening functional respiratory disorders are related to the virulence of bacterial species involved in the etiology becomes more and more popular in Bulgaria as well. They are said to determine the severity of clinical manifestation of infection (1, 10, 11, 14).

The purpose of the present report was to summarize the data about the structure of the microbial etiological factor isolated throughout cases of respiratory infections in Bulgarian pig farms with different capacity, management and production technology.

MATERIAL AND METHOD

The survey included 11 different farms from Central, north, Northeast and South Bulgaria and was carried out in 2005–2008.

Epidemiological surveys were carried out by observation and epidemiologic questionnaires. In all cases, there were epidemic outbreaks of respiratory diseases in growers and/or finishers. The analysis included the parameters: morbidity, mortality, lethality rates, infection index (calculated on the basis of serological screening studies for enzootic (*Mycoplasma*) pneumonia and porcine reproductive respiratory syndrome – PRRS).

Bacteriological investigations included inoculation of altered lung tissue of dead pigs with PRDC clinical signs.

A total of 191 lung samples were investigated as followed: 77 samples from growers and 114 samples from finishers; including 33 samples from dead pigs and 81 lung samples obtained during emergency or regular slaughter.

Solid agar and liquid nutrient media were employed – blood agar (base Bulbio - National Centre for Infections and Parasitic Diseases; NCIPD) – for coryneform bacteria, streptococci and *Arcanobacterium pyogenes*, McConkey's agar (Bulbio - NCIPD) – for enterobacteria; Bordet-Gengou (Becton Dickinson) agar – for Bordetella and blood agar supplemented with Vitox (Oxoid) – for isolation of *Actinobacilus pleuropneumoniae*.

For microbiological diagnostics of *Mycoplasma* organisms (*M. hyopneumoniae* and *M. hyothinis*), liquid and solid Hy-Labs media (Hy Laboratories) – Israel were used.

The identification of isolates was done by means of the semi-automated system CRYSTAL (Becton Dickinson).

Serological investigations included immunoenzymatic test (blocking ELISA) for detection of antibodies against PRRSV as well as against *M. hyopneumoniae*. Commercial kits K 0043 (Dako Cytomation) and Bomeli Diagnostic were respectively used.

The sensitivity of isolated and identified strains to antimicrobials was tested by the disc diffusion method and interpreted in the three score Bauer-Kirby system, as per the requirements of CLSI - Performance Standards for Antimicrobial Disk and Dilution Susceptibility Tests for Bacteria isolated from Animals - Approved Standard – Third Edition, Document M31-A3,vol.8 replaced №31-A2, vol. 22, №6 (15).

Antibiogrammes were performed on Mueller-Hinton agar (NCIPD, Sofia). Filter paper discs and tablets of 10 antimicrobial drugs with the following concentrations **(Table 1)** were used:

Table 1. *Antibacterial discs used*

	Antimicrobial disc	Code	μg/disc	Manufacturer
1.	Amoxicillin	Amx	25	Pfizer
2	Cefuroxime	Cxm	30	NCIPD
3	Gentamicin	G	10	NCIPD
4	Spectinomycin	Spt	30	CEVA
5	Doxycycline	D	30	CEVA
6	Chloramphenicol	C	30	NCIPD
7	Colistin	Col	50	CEVA
8	Tulathromycin	Tul	30	Pfizer
9	Enrofloxacin	Enr	5	Bayer
10	Trimethroprime/sulfamet oxazole	SXT	1.25-23.75	CEVA

* NCIPD=National Centre for Infections and Parasitic Diseases

RESULTS

The bacteriological investigations of 191 samples detected bacterial findings in 139 (72.8%). Out of them, a single microbial species was found out in 117 (84.2%) cases and in 22 samples (15.8%) the finding was polymicrobial – 17 samples with two species and 4 with three different species. As a whole, 162 bacterial strains belonging to 13 different taxonomic categories were isolated and identified.

Table 2 presents the data about discovered microbial species for the entire period of the survey and their relative proportion.

It is evident that the most frequently involved bacterial species was *P. multocida*, with a share of 22% from all isolates, detected in 7 of surveyed farms. A relatively high was the percentage of isolated alpha-haemolytic streptococci (16.7%), and that of enterobacteriae, especially *E. coli* (13,6%). In Bulgaria, the species *A. pyogenes,* has known mainly as an opportunistic microbial species, causing secondary purulent infections, was also relatively frequently isolated from lungs of pigs (15.6%).

Table 2. *Microbial species isolated from the lungs of swine with respiratory signs in 11 pig farms*

	Microbial species	Number of isolates	%	Farm №№
1	*Pasteurella multocida*	34	22,0	1,2,3,6,9,10,11
2	*α-haemolytic streptococci*	27	16,7	3,6,9,10,11
3	*β-haemolytic streptococci*	13	8,0	1,2,4,3,6,10,
4	*Staphylococcus aureus*	8	4,9	3,,9
5	*Coagulase-negative staphylococci (CNS)*	3	1,9	2,3,9,10
6	*Klebsiella* spp.	5	3,1	3,5
7	*Bordetella bronchiseptica*	9	5,6	8,9,12
8	*Actinobacilus pleuropneumoniae*	23	14,2	1,3,6,8
9	*Arcanobacterium pyogenes*	9	15,6	4,5,9,11
10	*Esherichia coli*	22	13,6	1,4,8,10
11	*S. Choleraesuis*	3	1,9	1
12	*Haemophilus parasuis*	4	2,5	9,11
13	Gram-negative nonfermentative spp.	2	1,2	7

The causative agent of *Actinobacillus* pleuropneumonia (APP) was evidenced in 14.2% of samples originating from 4 infected farms whereas *Haemophilus parasuis* – causing the so-called Glesser's disease was present in 4 samples (2.5%), obtained from two farms with respiratory pathology.

Fig. 1 illustrates the Gram staining-based distribution of isolated bacterial microflora

Gram-negative representatives predominated: 64.1% of isolates. This fact largely predetermined the approach of empirical therapy and mass medication when selecting an antimicrobial drug. The choice should however take into consideration the data about the involvement of mycoplasmae in the etiology of PRDC in our country.

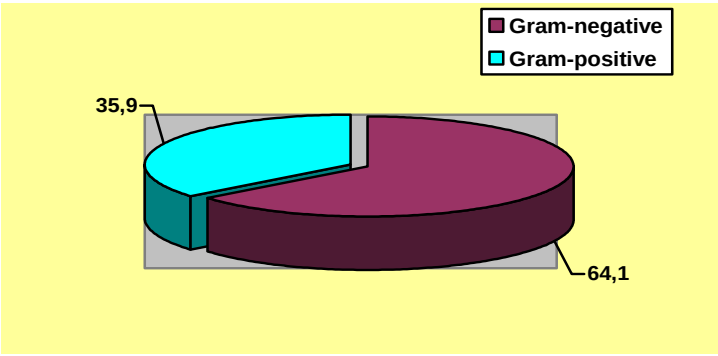

Fig. 1. Percentage distribution of Gram-positive and gram-negative microbial species which were isolated from the pigs with porcine respiratory disease complex

The data from **Table 3** showed that from the 11th surveyed farms; only 4 were infection-free, whereas the other 7 were positive for *Mycoplasma* infections; furthermore, in 4 farms, antibodies against the two commonest species - ***M. hyopneumoniae*** and *M. hyorhinis,* were simultaneously encountered.

Table 3. *Farms, serologically positive for Mycoplasma infections*

M. hyopneumoniae	***M. hyorhinis***	**Negative**
1, 3, 6, 9	1, 3, 5, 6, 8, 9,11	2, 4,7, 10

Another important factor that was serologically proved in three of surveyed farms was the reproductive respiratory syndrome (PRRS) **(Table 4)**. It should be outlined that in these 3 farms, the most numerous and most different secondary bacterial agents (pasteurellas, alpha and beta haemolytic streptococci) were detected at a time, and in two of them: No. 6 and No. 9, the APP agent as well. In these farms, morbidity and lethality rates were the highest and the most considerable economic losses were formed.

Table 4. *Farms, serologically positive for PRRSV*

positive	**negative**
2, 6, 9,	1, 3, 4, 5, 7, 8, 10, 11

Table 5. *Distribution of microbial isolates among pigs with evidence of PRDC in 11 different pig farms.*

Farm	**Microbial species**
1	*M. hyopneumoniae M. hyorhinis P. multocida; β-haemolytic streptococci; A.pleuropneumoniae; E. coli ; S. Choleraesuis*
2	*P. multocida; β-haemolytic streptococci; CNS;*
3	*M. hyopneumoniae; M. hyorhinis; P. multocida; α-haemolytic streptococci; β-haemolytic streptococci; S. aureus; Klebsiella* spp.; *A. pleuropneumoniae; H. parasuis*
4	*β-haemolytic streptococci; A. pyogenes; E. coli; H. parasuis*
5	*M. hyorhinis; A. pyogenes; Klebsiella* spp.
6	*M. hyopneumoniae; M. hyorhinis; P. multocida; α-haemolytic streptococci; β-haemolytic streptococci; A.pleuropneumoniae*
7	*Haemophilus parasuis;* Gram-negative nonfermentative spp.
8	*M. hyorhinis; A. pleuropneumoniae; E. coli ;*
9	*M. hyorhinis; P. multocida; α-haemolytic streptococci; β-haemolytic streptococci; S. aureus; CNS*; *A. pyogenes; H. parasuis*
10	*P. multocida; α-haemolytic streptococci; α -haemolytic streptococci; CNS*; *E. coli;*
11	*M. hyopneumoniae; M. hyorhinis; P. multocida; α -haemolytic streptococci; A. pyogenes; H. parasuis*

The data for microbial species isolated in the 11 pig farms are presented in **Table 5**.

Table 6 shows the results from the tests on the behaviour of most commonly isolated species to antimicrobial drugs. It could be seen that tested isolated were in general most sensitive to tulathromycin (except for *E. coli*), florphenicol and the member of cephalosporin group – cefuroxime.

Table 6. *Sensitivity to antimicrobials of some microbial species, isolated from pigs with PRSC (number/%).*

Microbial species	number of strains	Amx	Cxm	G	Spt	Dox	Flo	Col	Tul	Enr	SXT
P. multocida	34	30 (88,2)	30 (88,2)	27 (79,4)	28 (82,3)	22 (64,7)	29 (85,3)	-	33 (97,1)	26 (76,3)	24 (70,6)
Actinobacillus pleuropneumoniae	23	21 (91,3)	21 (91,3)	16 (69,6)	18 (78,3)	16 (69,6)	22 (95,6)	-	23 (100)	21 (91,3)	18 (78,3)
α-haemolytic streptococci;	27	25 (92,3)	23 (85,2)	18 (66,7)	22 (81,5)	23 (85,2)	24 (88,9)	-	25 (92,3)	16 (59,3)	19 (70,4)
β-haemolytic streptococci	13	11 (84,6)	11 (84,6)	9 (69,2)	10 (76,9)	11 (84,6)	12 (92,3)	-	13 (100)	8 (61,5)	10 (76,9)
Esherichia coli	22	16 (72,7)	17 (77,3)	17 (77,3)	20 (90,9)	11 (50,0)	20 (90,9)	21 (95,5)	12 (54,5)	19 (86,4)	14 (63,6)

The relatively lowest sensitivity percentages were detected against doxycycline and potentiated sulphonamides, whereas the behaviour against amoxicillin and the aminoglycosides gentamicin and spectinomycin were in a medium position.

The relatively low percentage of streptococcal isolates, sensitive to enrofloxacin should be emphasized, as it confirmed the well known fact that fluoroquinolones were not the drugs of choice for treatment of infections caused by these microorganisms.

DISCUSSION

The data obtained in the present survey suggest that bacterial species, involved in the etiology of porcine respiratory disease complex were numerous, belonged to various taxonomic categories and varied among farms. The most commonly detected PRDC etiological agent in Bulgaria were *Mycoplasma* species *M. hyopneumoniae* and *M. hyorhinis*, that could induce outbreaks of porcine enzootic pneumonia on their own (16). This fact confirms the findings of Burch, 2004 and is essential for elaborations of prevention and restriction measures (17).

The similar incidence of *P. multocida,* often incriminated as a secondary infectious agent of *Mycoplasma* pneumonia and observed in respiratory viral infections, deserves a special attention (2; 18). That is why its emergence in the three farms where PRRSV was detected, was not unusual. It should be however mentioned that in most surveyed farms (all of them using contemporary industrial rearing technologies) no antibodies against PRRSV have been detected although the reproductive respiratory syndrome in Bulgaria is acknowledged since 2001 by Yordanov & Chenchev (19).

According to our investigations, *Pasteurella* infection was almost always present in farms, serologically positive for enzootic pneumonia, thus supporting the view of Ciprian et al., 1988, that *Mycoplasma* infection results in increased susceptibility to *Pasteurella*-induced pneumonia.

The more frequent isolation of enterobacteriae and particularly of *E. coli,* should be carefully appraised as an indication for a poor level of the hygiene in some of the farms.

The isolation of causative agents of *Actinobacillus* pleuropneumonia (APP) and Glesser's disease are important facts showing that these illnesses are a problem for some of the farms, requiring a strict control and better management practices.

The results about the behaviour of bacterial isolates to antimicrobial drugs discovered relatively high levels of resistance to tetracyclines as well as the presence of strains, resistant to fluoroquinolones. At the same time, a preserved sensitivity to tulathromycin and florphenicol – able to concentrate and persist in respiratory organs, was observed, making them very suitable for therapeutic control.

These results should be taken into consideration throughout the development of

individual programmes for monitoring and control of respiratory diseases in pig farms with regard to minimizing related economic losses.

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