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Mini Review

Is the Relationship between Antiviral Activity of Substances and their Properties to Restores the Antibiotic Susceptibility of Porcine "Superbug"?

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Abstract

Ukrainian veterinary drugs Amixin® (AMX, Interchem Ltd., Odesa), Izatizon® (IZT, Institute of Molecular Biology and Genetics, Kyiv) and "Zooextract"® (ZEX, DanikaFarm Ltd., Kharkiv) were tested for their antiviral activity against of the 1000 infectious units (TCID, ELD or $PFU_{50/ml}$, respectively) of Ukrainian isolates of the Pseudorabies virus (PRV), 2nd type of Porcine circovirus (PCV-2), Swine influenza virus (SIV) and associated agents of the Porcine Pasteurellosis (PAST). Their actions were tested by expositions with these agents for 14 hours at the 18 - 24°C in the active substances concentrations of 5 mg/ml for AMX and IZT, and in final dilution "1:10" for ZEX. Under these conditions, the inactivation of from 0 to100% of the agents above were resulted. The 1000 $ELD_{50/ml}$ of the SIV was inactivated with AMX and ZEX practically on 100% ($n = 12$, $P \leq 0.01$ for each), but practically not inactivated with IZT (10%, $n = 12$, $P \leq 0.01$). The 1000 $TCID_{50/ml}$ of the PRV and 1000 $PFU_{50/ml}$ of the PCV-2 were inactivated in 14 hours with IZT and ZEX on 100% ($n = 12$, $P \leq 0.01$ for each), but with AMX - on 75% only ($n = 12$, $P \leq 0.01$). At the same time the antibiotic resistant consortia PAST from the piglet's blood and nasal swabs originated from infected herds ($n = 3$), restore the sensitivity to commercial feed antibiotic Flovet (active substance Florfenicol) after *in vitro* 14hr developments by AMX, IZT and ZEX. Moreover, clinical trials in observed pigpens clear demonstrated the restore PAST sensitivity to Flovet during 1.5 - 2 weeks of application with drinking water of antiviral substances AMX ($n = 287$, $P \leq 0.000001$) and ZEX ($n = 406$, $P \leq 0.0002$). Obtained results empirically to base a new approach to overcome of Antibiotical resistance of porcine bacteria through using of antiviral substances. Theoretical base for this approach we can to see in modern proceedings of Israel and American scientists.

Keywords: *Amixin®; Izatizon®; Zooextract®; Antiviral Activity; Porcine Viruses; Porcine "Superbug" Bacteria; Restore Sensitivity to Antibiotics*

Abbreviations

AMX: Amixin®; IZT: Izatizon®; ZEX: Zooextract®; PR: Pseudorabies; PCV-2: 2nd Type of Porcine Circovirus; SIV: Swine Influenza Virus; Past: Porcine Pasteurellosis Agents; mg: Milligrams; ml: Milliliters; $TCID_{50}$: Tissue Culture Infective Dose; ELD_{50}: Chicken Embryos Lethal Dose; PFU_{50}: Plaque Forming Unites; DDT: Disk Diffusional Test; CE: Chicken Embryo; RTD: Routine Test Bacteriophages Dilution; BB/ml: Bacterial Bodies in 1 ml; RT: Room Temperature (18 - 24°C).

Results of Lab NSC IECVM for Porcine Diseases Research (Kharkiv, UA)

As it is in details described in two articles of Journal for Veterinary Medicine, Biotechnology and Biosafety (NSC IECVM eds., 2015 and in preparation-2019), our team *in vitro* tested of three Ukrainian veterinary drug AMX, IZT) and ZEX, for their antiviral activity against regional isolates of etiological agents of Pseudorabies (PR), 2nd type of Porcine circovirus (PCV-2), Swine influenza virus (SIV) and associated agents of the Avian Pasteurellosis (Past). At addition the AMX, IZT and ZEX were tested by clinical trials in three pigsties ($n = 890$ piglets in total).

Virocidal action of drugs were tested at the at the 18 - 24°C in the active substances concentrations of 5 mg/ml for AMX and IZT, and in final dilution "1:10" for ZEX and expositions for 14 hours. Under these conditions, the inactivation of 10 - 100% of the 1000 infectious units (TCID, ELD or $PFU_{50/ml}$, respectively) of some of these viruses were resulted. Data of table 1 demonstrate that the 1000 $ELD_{50/ml}$ of the SIV was inactivated in 14 hours with AMX (5 mg/ml, n = 12, P ≤ 0.01) and ZEX (1:10, n = 12, P ≤ 0.01) practically in total. Also was estimated that IZT inactivated the SIV only at 10% in exposition under RT 14 hr (n = 12, P ≤ 0.01). The 1000 $TCID_{50/ml}$ of the PRV and 1000 $PFU_{50/ml}$ of the PCV-2 were inactivated in 14 hours with IZT and ZEX practically in total but by AMX at 70% only (See table 1).

Substances		**Inactivation level of the agents[1)], %**				
Type	**Concentration, mg/ml**	**PRV**	**PCV2**	**SIV**	**PAST**	
					Inactivation level	**Restore the PAST sensitivity to Flovet**
AMX	0.0	0^{14}	0^{14}	0^{14}	0^{14}	(-)
	5.0	100^{14}	75^{14}	100^{14}	0^{14}	(+)
IZT	0.0	0^{14}	0^{14}	0^{14}	0^{14}	(-)
	5.0	100^{14}	100^{14}	10^{14}	0^{14}	(+)
ZEX	0.0	0^{14}	0^{14}	0^{14}	0^{14}	(-)
	1:10	100^{14}	100^{14}	100^{14}	75^{14}	(+)
[1)]1000 infectious units of each viruses and 10^6 BC of PAST; high point demonstrate the hour rate; studies conducted in 3 repasts - the 4 identical samples in each.						

Table 1: *In vitro studies of goal substances' virocidal (to three porcine viruses) and "antibiotic-restore" activities (on sensitivities of Past-"superbug" to feed antibiotic Flovet, Vetsyntez company, Kharkiv).*

Because we had strong data that the AMX is effective in many cases of bacterial infections in piggery and poultry, the learning of nature of these events with goal substances were launched. For it in appropriate box 10 chickens of 10-days old were infected in 2015 by field isolate of bacterial consortia 'Con A', which by data of preview analysis contain different microorganisms including the *Pasteurella* bacteriophage and had not sensitivity to mix of gentamicin-tylosin antibiotics.

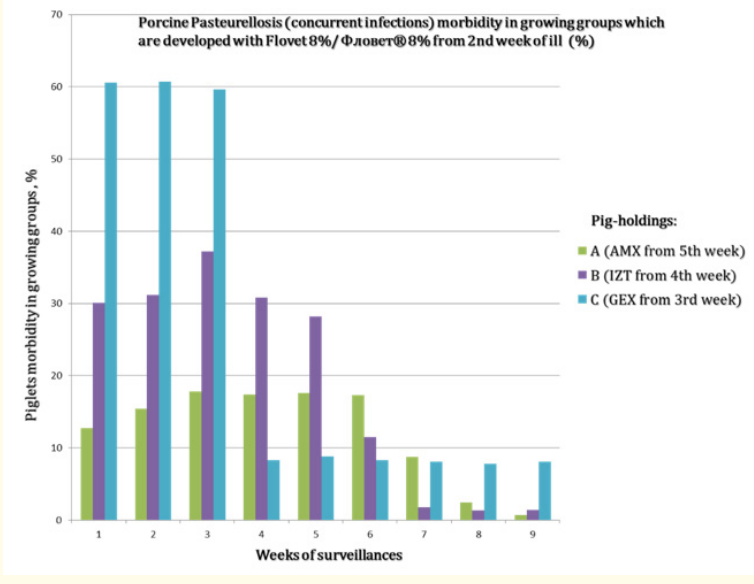
Five from those chicken were treatment with AMX by triple watering, once per-day every day, dose 15 mg/ml. 15 day apart all chickens was search on presence of antibiotic-resistant bacteria and *Pasteurella* bacteriophage in the blood samples. From chickens, which were not developed with AMX, was isolated the mixture of microorganisms (consortia) which contained *Pasteurella multocida* A type, *Mycoplasma gallisepticum* and more than 2 species of unidentified bacteria.

Additional investigations allow estimating the presence of the lytic *Pasteurella* bacteriophage in this consortium. The same lytic *Pasteurella* bacteriophage was revealed previously in initial consortium, which used for infection of these chickens. Titer of this bacteriophage in initial and passaged consortium was the same - 10^9 by RTD: this is evidence that studied consortium is stable. The isolated *Pasteurella multocida* A type from untreated flock was insensitive to mix of gentamicin-tylosinon test-disks (1 mg/ml every) like to this bacterium in initial consortium.

At the same time from chicken, which were developed with AMX was isolated analogous consortium contained the same microorganisms with exclusion of the lytic *Pasteurella* bacteriophage. Moreover, there was revealed that isolated *Pasteurella multocida* A type from treated flock had high sensitivity to mix of gentamicin-tylosin on test-disks (1 mg/ml every) that unlike to this bacterium in initial consortium.

Based on the above results, in period 2016 - 2019 we performed the clinical trials of goal drugs in complex with commercial feed antibiotic Flovet (Vetsyntez company, Kharkiv: active substance the Florfenicol, 200 mg/g) to control of porcine emergent reproductive and neonatal mix-infections (PRNI) in three industrial pigsties where pigs were early developed with Flovet without of any effect. There was isolated the antibiotic resistant consortia PAST from the piglet's blood and nasal swabs originated from infected herds in these pigsties. These consortia restored the sensitivity to this antibiotic after *in vitro* 14 hr-developments by AMX, IZT and ZEX as show above (See table 1).

On figure 1 show the clinical results of 9-weeks application (with drinking water) of AMX, IZT and ZEX apart by 5, 4 and 3 weeks, respectively, after diagnosis estimation. As we can see, there critical melioration of porcine clinical status was lunched from 4th week of goal substances application in all cases - till to drop of piglet's morbidity to "technological level" (to 1 - 3% with AMX and IZT and to 8% with ZEX) on 7th - 9th weeks of AMX, IZT and GEX application, in correspondent pigpens.



These clinical results were analyses by retrospective "Evans' county" approach. The piglets which were diseased on 9th week of goal substances drinking were considered as exposed, E(+) and diseased, D(+) in each observed pigsty (See table 2).

Tables - 2-by-2 unstratified
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Pigsty A (at week 9th)	D+	D-	Total
E AMX+	2	259	261
E AMX-	5	21	26
Total	7	280	287

Tests of significance
Fisher exact test (one tailed) : 0,000078
Fisher exact test (two tailed) : 0,000078
Uncorrected chi-square : 33,88
p-value : 0,000000
Yates corrected Chi-square : 26,56
p-value : 0,000001
Measures of exposure effect [99% CI]
Risk ratio : 0,04 [0,00; 0,32]
Odds ratio : 0,03 [0,00; 0,30]
Risk difference : -0,18 [-0,38; 0,02]
Proportional attributable risk : -24,10 [-202,38; -2,10]
Population proportional attr. risk : -6,88 [-9,52; -1,60]
Substance efficacy [99% CI]
Substance AMX efficacy : 0,96 [0,68; 1,00]
Screening [99% CI]
Prevalence : 0,02 [0,01; 0,06]
Sensitivity : 0,29 [0,04; 0,77]
Specificity : 0,97 [0,94; 0,13]
Accuracy : 0,08 [0,05; 0,13]
Predictive value of +ve result : 0,01 [0,00; 0,04]
Predictive value of -ve result : 0,81 [0,54; 0,94]
Matched data
Z : 15,57
One-sided p-value : 0,000000
Two-sided p-value : 0,000000
McNemar Chi-square : 242,46
p-value : 0,000001
McNemar odds ratio [99% CI] : 51,80 [16,26; 184,04]
Difference in proportions [99% CI] : 0,89 [0,74; 1,03]

Tables - 2-by-2 unstratified

Pigstay C (week 9th)	D+	D-	Total
E_{ZEX}+	30	342	372
E_{ZEX}-	10	24	34
Total	40	366	406

Tests of significance
Fisher exact test (one tailed) : 0,000646
Fisher exact test (two tailed) : 0,000646
Uncorrected chi-square : 15,98
p-value : 0,000064
Yates corrected Chi-square : 13,67
p-value : 0,000218
Measures of exposure effect [99% CI]
Risk ratio : 0,27 [0,12; 0,62]
Odds ratio : 0,21 [0,07; 0,63]
Risk difference : -0,21 [-0,42; -0,01]
Proportional attributable risk : -2,65 [-7,29; -0,60]
Population proportional attr. risk : -1,99 [-4,15; -0,53]
Substance ZEX efficacy [99% CI] : 0,73 [0,38; 0,88]
Screening [99% CI]
Prevalence : 0,10 [0,07; 0,14]
Sensitivity : 0,75 [0,53; 0,89]
Specificity : 0,07 [0,04; 0,11]
Accuracy : 0,13 [0,09; 0,18]
Predictive value of +ve result : 0,08 [0,05; 0,13]
Predictive value of -ve result : 0,71 [0,47; 0,87]
Matched data
Z : 17,64
One-sided p-value : 0,000000
Two-sided p-value : 0,000000
McNemar Chi-square : 311,25
p-value : 0,000002
McNemar odds ratio [99% CI] : 34,20 [14,79; 82,27]
Difference in proportions [99% CI] : 0,82 [0,70; 0,94]

Table 2: *Statistical account by "Evance' county" method (prints of EpiCalc 2000) of clinical data for AMX- and ZEX- activity in correspondly pigpens on week 9th after application with drink water in mix with feed antibiotic Flovet (E+); negative control (E-) is pig groups with Flovet but not AMX- and ZEX- application*

These data we can interpret as clinical evidences of antiviral action directed on the phages of mentioned bacteria consortia. The hypothesis of acquires the antibiotic sensitivity by pathogenic bacteria through bacteriophages inhibition is brightly explained by Tzipilevich E., *et al* [1]. AMX' antiviral activity and literature data on interferonogenic activities of it and its analogues [2,3], we can assume that the therapeutic effect of this drug can be achieved by both direct (contact) antiviral action and by mediation of interferon induction. We believe that 'antibacterial effect' of the Amixin® complex application consist in the elimination of bacteriophages that controlled the sensitivity of its bacteria-host to antibiotics. Clue significance of the substances' antiviral activity is bolded by our clinical results on Florfenicol-sensitivity' restore of the porcine "superbug" PAST-consortia through influence of absolutely different antiviral drugs - AMX, IZT and ZEX. This data can help to look the new approaches to restore self-control of the infected herds over the "superbug" circulated in Ukrainian piggery and poultry farming [4-7].

Conclusion

Obtained results empirically to base a new approach to overcome of Antibiotical resistance of porcine bacteria through using of antiviral substances. Theoretical base for this approach we can to see in modern proceedings of Israel and American scientists.

Bibliography

1. Tzipilevich E., *et al*. "Acquisition of Phage Sensitivity by Bacteria through Exchange of Phage Receptors". *Cell* 168.1-2 (2017): 186-199.
2. Lyakhov SA and Litvinova LA. "Mechanisms of the antiviral activity of amixin (review of literature). 2. Antiviral activity and influence on some biochemical processes' [Mekhanizmy protivovirusnoy aktivnosti amiksina (obzor literatury). 2. Protivovirusnaya aktivnost' i vliyanie na nekotorye biokhimicheskie protsessy]". *Modern Infection [Suchasni infektsii]* 2 (2008): 112-116.
3. Zholobak NM., *et al*. "Effect of tilorone and its analogues on the change in rat hepatocyte mitochondrial potential". *Fiziolohichnyi zhurnal* 58.2 (2012): 39-43.
4. Buzun AI., *et al*. "Guidelines for control of stable form of RNPI". Kharkiv: SOP of National Scientific Center 'Institute of Experimental and Clinical Veterinary Medicine' (2001).
5. Demchenko EV., *et al*. "Chlamydial laryngitis: clinical picture and amixine treatment". *Vestnik Otorinolaringologii* 5 (2000): 58-60.
6. Drew T. "The emergence and evolution of swine viral diseases: To what extent have husbandry systems and global trade contributed to their distribution and diversity?" Revue Scientifique et Technique *(International Office of Epizootics)* 30.1 (2011): 95-106.
7. Zhu L., *et al*. "Mechanisms of suppression of interferon production by porcine reproductive and respiratory syndrome virus". *Acta Virologica* 56.1 (2012): 3-9.

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