AHEAD IN
ANIMAL HEALTH

Report of the 2022 International Proficiency Testing Scheme (PTS) for Antibiotic susceptibility of bacterial strains

Poultry

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Introduction

To promote a standardized performance of susceptibility tests and to improve the quality, Royal GD organizes a PTS for antibiotic susceptibility determination of bacterial strains.

This report contains the results of the 2022 International Proficiency Testing Scheme (PTS) for Antibiotic susceptibility of bacterial strains **Poultry**, that was organized by the PTS team of Royal GD, Deventer, the Netherlands.

The aim of this PTS is to enable veterinary diagnostic laboratories to assess and improve their performance of bacteriological examinations.

Samples

The sample set comprised of four freeze-dried bacterial strains. Samples were identified by sample numbers (#1 - #4) and a Bacterial code. The sample description is shown in Table 1. Participants were asked to test them under normal routine test conditions applied in their laboratory.

Table 1 – Description of samples

Sample	Code	Bacteria
Sample 1	1 ECO	*Escherichia coli*
Sample 2	2 EFU	*Enterococcus faecium*
Sample 3	3 PMU	*Pasteurella multocida*
Sample 4	4 SAU	*Staphylococcus aureus*

The national reference laboratory (NRL) for antibiotic resistance in animals in the Netherlands, WBVR (Lelystad, the Netherlands), has established reference values for these bacteria by determining the Minimal Inhibitory Concentration (MIC) for a panel of antibiotics using the broth microdilution method.
Subsequently, results (MIC values) were interpreted using clinical breakpoints depicted in the table on the WBVR website (https://www.wur.nl/nl/Onderzoek-Resultaten/Onderzoeksinstituten/Bioveterinary-Research/Diergezondheid/Antibioticaresistentie-1/Gevoeligheidstest-antibiotica.htm). This table contains internationally accepted criteria that have been taken over from EUCAST, supplemented by criteria from CLSI documents.
Results submitted by participants were compared with results obtained by WBVR.

Participants

In total, 26 different laboratories from fifteen countries participated in this PTS. One laboratory reported for all four strains both agar diffusion and broth microdilution results. Each laboratory was given a unique ID-code which was only disclosed to the laboratory itself. Laboratories are identified in this report by their ID-code. The data of the PTS and its participants are archived in a locked filing cabinet in a secured part of the GD building and also in a secured part of the network of GD that is only available for employees that are directly involved in organizing the PTS. The participating laboratories are listed in Table 2.

Table 2 – List of Participants

Participant	Country
University for Veterinary Medicine	Austria
DGZ-Vlaanderen	Belgium
Poulpharm BVBA	Belgium
Avihol Cia Ltda	Ecuador
AniCon Labor GmbH	Germany
BWE-Brüterei-Weser-Ems GmbH & Co .KG	Germany
CVUA Freiburg bacteriology	Germany
LDC Labor Diagnostik Cloppenburg GmbH	Germany
Veterinärlabor Ankum	Germany
Állat-egészségügyi Labor Kft	Hungary
Eurofins Vetcontrol Kft	Hungary
Department of Agriculture, Food and the Marine	Ireland
Israel Poultry Health Laboratory	Israel
GESCO SCA	Italy
Laboratoire De Médecine Vétérinaire de l'Etat	Luxembourg
AdVee dierenartsen	The Netherlands
Royal GD	The Netherlands
Veterinair Centrum Someren BV	The Netherlands
LAB VET Sp. Z.o.o.	Poland
SLW Biolab S.C.	Poland
Vetdiagnostica Sp. z o.o.	Poland
SMT Vet lab	Republic of South Africa
Ministry of Agriculture, Central Veterinary Laboratory	Swaziland
Ministry of Agriculture, Food Hygiene Laboratory	Swaziland
Aviagen Anadolu As	Turkey
Sci-Tech Laboratories Ltd.	United Kingdom

Results and analyses

Enclosed with the samples, each lab received a work instruction.
Results were submitted to Royal GD by entering an online form. The following results could be submitted:

- Date of receiving the samples, date of testing
- Method used for susceptibility testing (for example, tablet, disk, MIC etc.)
- Antibiotics tested
- Antibiotic load (µg) / Inhibition zone (mm) or MIC value (mg/L)
- Final results (resistant, intermediate, susceptible)

The results, as reported by the participants, are shown in Tables 3-6.

When participants used other antibiotics than tested by WBVR (see tables), this is indicated by a superscript.
Annex 1 shows the list of antibiotics the superscripts refer to.

Deviating results, from the antibiotic shown in the table, are marked in grey.
When a lab used a different antibiotic, then the deviating results are marked in orange.

Abbreviations used in the tables are:
DC = Disk content, IR = Interpreted result, IZ = Inhibition zone, MV = MIC value
S = Susceptible, I = Intermediate, R = Resistant, N = Not tested, NI = Not interpreted.

Discussion and conclusion

Eighteen participants used disk diffusion for antibiotic susceptibility testing, of which one participant additionally used broth microdilution for all four strains. Eight participants used only a MIC-method for testing the strains; seven used broth microdilution and two the VITEK system.

According to the EUCAST disk diffusion method, for *Escherichia coli*, *Enterococcus faecium*, and *Staphylococcus aureus* paper disks are used on Mueller Hinton (MH) agar (air, 35±1°C, 18±2h) and for *Pasteurella multocida* on MH agar supplemented with 5% defibrinated horse blood and 20 mg/L β-NAD (MH-F agar) (5% CO_2, 35±1°C, 18±2h), with confluent growth. For broth microdilution EUCAST refers to ISO standard 20776-1 and prescribes for *Escherichia coli*, *Enterococcus faecium*, and *Staphylococcus aureus* MH broth (sealed panels, air, 35±1°C, 18±2h) and for *Pasteurella multocida* MH-F broth (sealed panels, air, 35±1°C, 18±2h). For the quality of the antimicrobial susceptibility test, it is essential to use the correct agar/broth and incubation conditions for each bacterial species. The cut-off values used in the antibiotic susceptibility test are based on the agar and incubation condition specified for the respective bacterial species.
One participant reported to have used for all four strains sheep blood agar and another participant reported to have used also agar (not specified) with blood for all strains. One other participant did not report the agars having used. All remaining 15 participants which performed disk diffusion used the prescribed MH agar for *Escherichia coli*. Fourteen of them also used MH agar for *Staphylococcus aureus*, as prescribed; one of them used MH-F agar. Nine of these 15 participants used MH agar for the *Enterococcus faecium* strain, the prescribed agar for this bacterial species. Five used MH-F agar and one did not test this strain. Five of these 15 participants used MH-F agar for the *Pasteurella multocida*, as prescribed. Seven reported to have used MH agar, one Bovicor agar, and two reported no growth of the *Pasteurella multocida* and therefore no evaluation of the antibiotic susceptibility of this strain was possible.
All seven participants which used broth microdilution used MH broth for the *Escherichia coli* strain, and all except one used ambient incubation (one applied a CO_2-enriched atmosphere for all four strains). For the *Enterococcus faecium* strain, five used MH broth and ambient incubation and two H broth of which one used ambient incubation and one applied a CO_2-enriched atmosphere. For the *Pasteurella multocida* strain, five used MH broth, one H broth, and only one the prescribed MH-F broth. All used ambient incubation, except the one participant that used H broth applying a CO_2-enriched atmosphere for all four strains. For the *Staphylococcus aureus* strain, six used MH broth and ambient incubation, as prescribed, and one used H broth and a CO_2-enriched atmosphere.

Also the choice of antibiotics and disk contents are important for standardisation of antibiotic susceptibility tests. With respect to the beta-lactam antibiotics, the majority of the participants used the recommended antibiotics to screen *Escherichia coli* for ESBL-production and *Staphylococcus aureus* for methicillin resistance: ampicillin in combination with cefotaxime or ceftiofur and penicillin in combination with cefoxitin, respectively.

Not all individual deviations will be discussed in this report, but only findings most worth mentioning.

Sample 1:
The *Escherichia coli* strain is resistant to enrofloxacin and tetracycline, and susceptible for ampicillin cefotaxime, and TmpS.
Most deviations obtained for this strain are related to other antibiotics being used than those recommended, other disk contents being used and not having (correctly) used the criteria in the table on the WBVR website. For example, the use of penicillin instead of ampicillin, while *Escherichia coli* is intrinsically resistant to penicillin. Or the use of cefoxitin instead of the recommended cefotaxime or ceftiofur as indicators of ESBL-production. An example of another disk content is the use of a disk with a content of 10 µg of cefotaxime instead of 5 µg. An example of interpretation discrepancy is an inhibition zone diameter of 20 mm for TmpS (disk content of 1.25-23.75 µg) being classified as intermediate-susceptible, while according to the WBVR table based on this testing result *Escherichia coli* would have been regarded as susceptible. Another example is an inhibition zone diameter of 10 mm for enrofloxacin (disk content of 5 µg) being classified as susceptible, which according to the WBVR table would have been interpreted as resistant. And inhibition zone diameters of 8 and 0 mm for tetracycline (disk content of 30 µg) were interpreted as intermediate-susceptible while according to the table it is interpreted as resistant. Another example of interpretation discrepancy is a MIC value of 2 µg/ml for ampicillin being classified as intermediate-susceptible, while according to the WBVR table based on this testing result *Escherichia coli* would have been regarded as

susceptible. And a MIC value of 4 µg/ml for enrofloxacin is interpreted according to the WBVR table as resistant and not as intermediate-susceptible.

Sample 2:

The *Enterococcus faecium* strain is resistant to TmpS, enrofloxacin and tetracycline, and susceptible for ampicillin.

The majority of the participants which performed disk diffusion tested ampicillin as representative of the group of penicillins. Nine reported a disk content of 10 µg of ampicillin, four of 2 µg, and one of 25 µg of ampicillin. One participant tested amoxicillin (disk content of 10 µg) and one tested penicillin (disk content of 10 Units). In the table on the WBVR website, disk diffusion criteria are available for enterococci for both ampicillin and penicillin, for disks with a content of 2 µg and 10 Units, respectively. Two participants classified the susceptible strain as intermediate-susceptible and resistant, respectively, based on inhibition zones obtained for ampicillin using disks with a content of 10 and 25 µg of ampicillin, respectively.

Most deviations were obtained for TmpS. Of the 17 participants that tested this strain for TmpS-susceptibility by disk diffusion, four classified the strain as susceptible and one as intermediate-susceptible. Of the eight participants that performed a MIC method, four classified the strain as resistant, of which one reported a MIC value of ≤0.5/9.5 µg/ml which according to the WBVR table is interpreted as intermediate-susceptible instead of resistant. One participant did not interpret the high MIC value found (>8 µg/ml). One classified the strain as intermediate-susceptible without reported a MIC value, and two classified the strain as susceptible of which one participant reported a MIC value of 0.25/4.75 µg/ml. Resistance to TmpS has been established based on high MIC values for both sulfamethoxazole (MIC: >1024 mg/L), trimethoprim (MIC: >32 mg/L) and the combination of these two (MIC: >8/152 mg/L). There is no explanation for the (intermediate-) susceptible result obtained for the isolate confirmed as TmpS-resistant. However, when the recommended disk diffusion criteria in the WBVR table were applied, the inhibition zone diameters reported by all four participants which classified the strain as susceptible for TmpS would have been interpreted as intermediate-susceptible. Likewise the MIC value of TmpS of 0.25/4.75 µg/ml reported by one of the participants would have been interpreted as intermediate-susceptible instead of susceptible.

One of the participants which tested the susceptibility for ciprofloxacin found the resistant strain intermediate-susceptible based on the inhibition zone diameter obtained and one other participant found the resistant strain intermediate-susceptible based on the MIC value of enrofloxacin.

No deviating results were obtained for tetracycline; all participants classified the strain as resistant to tetracycline.

Sample 3:

The *Pasteurella multocida* strain is susceptible for ampicillin, TmpS, tilmicosin, enrofloxacin and tetracycline.

The majority of the participants which performed disk diffusion tested ampicillin as representative of the group of penicillins. Nine reported a disk content of 10 µg of ampicillin, one of 2 µg, and one of 25 µg of ampicillin. One participant tested amoxicillin (disk content of 25 µg) and three tested penicillin (two using disks with a content of 10 Units and one using a disk with 1 Unit of penicillin). In the table on the WBVR website, disk diffusion criteria are available for *Pasteurella multocida* for penicillin only, for disks with a content of 1 Unit. Two participants classified the susceptible strain as resistant and one as intermediate-susceptible, respectively, based on inhibition zones obtained for ampicillin (disk content of 10 and 25 µg, respectively) and for amoxicillin (disk content of 25 µg). No deviating results for the group of penicillins were reported by participants using broth microdilution or the VITEK system.

Three deviations were found for TmpS: the susceptible strain was classified as resistant by two participants based on inhibition zone diameters and by one participant based on the MIC value obtained. These deviating results cannot be explained.

With respect to the macrolides it can be concluded that different macrolides were tested (erythromycin, tylosin, and tulathromycin). There are no official interpretation criteria for *Pasteurella multocida* and erythromycin and tylosin. For *Pasteurella multocida* and tilmicosin there are only criteria for pigs. Based on these criteria, the strain is classified as susceptible for tilmicosin.

No deviating results were obtained for enrofloxacin; all participants classified the strain as susceptible for fluoroquinolones (ciprofloxacin and enrofloxacin).

For tetracycline there were two deviations: two participants classified the susceptible strain as resistant based on the inhibition zone diameters found (0 mm and 19 mm, respectively).

Sample 4:

The *Staphylococcus aureus* strain is resistant to penicillin, cefoxitin, clindamycin, erythromycin, and susceptible for tetracycline.

Of the 18 participants which performed disk diffusion 11 tested ampicillin as representative of the group of penicillins. Nine reported a disk content of 10 µg of ampicillin, one of 2 µg, and one of 25 µg of ampicillin. One participant tested amoxicillin (disk content of 10 µg) and six tested penicillin (four using disks with a content of 10 Units and two using disks with 1 Unit of penicillin). In the table on the WBVR website, disk diffusion criteria are available for *Staphylococcus aureus* for penicillin only, for disks with a content of 1 Unit. One participant classified the resistant strain as susceptible based on the inhibition zone obtained for ampicillin (disk content of 10 µg). No deviating results for the group of penicillins were reported by the nine participants using broth microdilution or the VITEK system; eight of these nine participants tested penicillin and the remaining participant tested ampicillin.
Ten participants that used disk diffusion determined the susceptibility of the *Staphylococcus aureus* strain for the recommended cefoxitin. Two tested ceftiofur and another two cefotaxime. Of the eight participants that determined the susceptibility using a MIC-method, three tested cefoxitin, three ceftiofur, one cefalotin, and one cefotaxime. All eight classified the strain correctly as resistant.
All participants that tested the susceptibility of the *Staphylococcus aureus* strain to lincosamides and macrolides correctly classified the strain as resistant. Different representatives of these groups were tested. In the WBVR table staphylococci criteria are given only for clindamycin and erythromycin disks, both with a content of 15 µg of the antibiotics.
Two deviating results were reported for tetracycline. Two participants classified the strain as intermediate-susceptible instead of susceptible. One used disk diffusion and the other used broth microdilution. The latter determined a MIC value of 0.25 µg/ml which according to the WBVR table is interpreted as susceptible instead of intermediate-susceptible. On the other hand, a MIC value of 2 and ≤2 µg/ml as reported by two of the participants classifying the strain as susceptible is interpreted according to the table as intermediate-susceptible instead of susceptible.

In conclusion, generally, the quality of the susceptibility tests is rather good. Permanent attention is needed for the choice of agar, antibiotics and disk contents, and the correct interpretation of the inhibition zone diameters and MIC values.

Acknowledgements

We would like to thank all participants for their enthusiasm, their quick response and their valuable comments.

Results

Table 3a – Paperdisk/Tablet Method: Sample 1 ECO (*Escherichia coli)*

Lab code	Used method
ABSP6560	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6582	paperdisk on Columbia sheep blood agar with confluent growth
ABSP6583	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6606	Paperdisk on agar + blood
ABSP6626	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6629	Kirby-Bauer Disk diffusion
ABSP6643	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6645	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6690	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6737	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6741	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6770	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6773	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6790	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6794	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6806	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6857	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6984	Paperdisk on Mueller Hinton agar (MH) with confluent growth

Table 3b – Results Paperdisk/Tablet method, **Sample 1 ECO** (*Escherichia coli)*

Lab code	Ampicillin (S)			Cefotaxime (S)			Trimethoprim / Sulfamethoxazole (S)			Enrofloxacin (R)			Tetracycline (R)		
	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ
ABSP6560	10	S	20	30	S[8]	30	1.25/23.7	S	25	5	R	0	30	R	10
ABSP6582	10	S	18		N		25	I	20	5	S	10	30	I	8
ABSP6583	10	S	18		N		1.25/23.75	S	22	5	R	0	30	R	0
ABSP6606	10	S	14	30	S	26	25	S	20	5	R[14]	10	30	R	0
ABSP6626	10	S	23	30	I[7]	19	1,25/23,75	S	17	5	R[14]	0	30	R	0
ABSP6629	10	S	20	30	S[7]	22	25	S	20	5	R[14]	10	30	R	8
ABSP6643	25	S[1]	21	30	S[8]	27	1.25+23.75	S	23	5	R	16	30	R	7
ABSP6645	10	R[3]	10	10	I	16	5	S	23	5	R[14]	14	30	R	11
ABSP6690	10	R	10	30	S	32	25	S	22	10	R[14]	13	30	R	0
ABSP6737	10	S	21	5	S	22	25	S	25	5	R	10	30	R	6
ABSP6741	25	R	6	30	S	36	25	S	24	30	R[15]	6	30	R	6
ABSP6770	10	S[1]	21		N		25	S	23	5	R	7	30	R	0
ABSP6773	10	S	19	30	S[7]	20	1.25/23.75	S	20	5	R[14]	8	30	R	9
ABSP6790	10	S	18	5	S	26	25	S	22	5	R	0	30	R	0
ABSP6794	10	S	17	30	S[8]	28	25	S	20	5	R	0	30	R	0
ABSP6806		N			N		1.25/23.75	S	19	5	R[14]	10	30	R	0
ABSP6857	10	S	20	5	S	30	1.25-23.75	S	22	5	R	0	30	R	0
ABSP6984	10	S	19	30	S[8]	24	1/24	S	20	5	NI	0	30	I	0

Table 3c – MIC method, **Sample 1 ECO** (*Escherichia coli)*

Lab code	Used system	Broth	Atmosphere
ABSP6568	Micro-Naut	Müller-Hinton	CO2
ABSP6606	AviPro-Plate MCN6	Mueller-Hinten	aerob
ABSP6632	Vitek 2 compact		
ABSP6752	Mikrodilutionsverfahren	MHB	aerob
ABSP6799	SENSITITRE	CAMHB	Ambient
ABSP6918	MicroNaut	CAMHB	Ambient
ABSP6945	VitekII		
ABSP6982	MicroNAUT	CAMHB	ambient
ABSP6986	Micronaut	CAMHB	Ambient

Table 3d – Results MIC method, **Sample 1 ECO** (*Escherichia coli)*

Lab code	Ampicillin (S)		Cefotaxime (S)		Trimethoprim / Sulfamethoxazole (S)		Enrofloxacin (R)		Tetracycline (R)	
	IR	MV	IR	MV	IR	MV	IR	MV	IR	MV
ABSP6568	S	=2	S	≤1	S	≤0.5/9.5	R	>2	R	>8
ABSP6606	N		S[8]	<=2, <=1	S	<=0,5/9,5	R	>2	R	>8
ABSP6632	N		R[4]		S		N		R	
ABSP6752	R[3]	>8 µg/ml	S[8]	<=0,25 µg/ml	S	<=0,5/9,5 µg/ml	R	>2 µg/ml	R	>8 µg/ml
ABSP6799	S	8	S[8]	≤1	S	≤0.5	R	>2	R	>8
ABSP6918	S	=2	S	<=0.125	S	<=0.25/4.75	R	>2	R	>16
ABSP6945	S		S[8]		S		R		R	
ABSP6982	S	=2	S[8]	≤0.25	S	≤0.5/9.5	R	>1	R	>8
ABSP6986	I	=2	S[8]	=0,25	S	≤0,25/4,75	I	=4	R	>8

Table 4a – Paperdisk/Tablet Method: Sample 2 EFU (*Enterococcus faecium)*

Lab code	Used method
ABSP6560	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6582	paperdisk on Columbia sheep blood agar with confluent growth
ABSP6583	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6606	Paperdisk on agar + blood
ABSP6626	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6629	Kirby-Bauer disk diffusion
ABSP6643	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6645	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6690	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6737	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6741	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6770	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6773	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6790	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6794	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6806	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6857	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6984	Not tested

Table 4b – Results Paperdisk/Tablet method, **Sample 2 EFU** (*Enterococcus faecium)*

Lab code	Ampicillin (S)			Trimethoprim / Sulfamethoxazole (R)			Enrofloxacin (R)			Tetracycline (R)		
	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ
ABSP6560	10	S	30	1.25/23.7	R	0	5	R	12	30	R	0
ABSP6582	10	S	22	25	R	0	5	R	0	30	R	0
ABSP6583	10	S	26	1.25/23.75	R	0	5	R	13	30	R	0
ABSP6606	10	S	24	25	R	0	5	R[14]	14	30	R	0
ABSP6626	10	S	19	1,25/23,75	R	0	5	R[14]	10	30	R	0
ABSP6629	10	S	30	25	S	26	5	R[14]	14	30	R	7
ABSP6643	2	S	25	1.25+23.75	S	24	5	R	16	30	R	6
ABSP6645	10	I	14	5	R	14	5	I[14]	20	30	R	14
ABSP6690	10	S	25	25	R	0	10	R[14]	16	30	R	0
ABSP6737	2	S	22	25	R	6	5	R	11	30	R	6
ABSP6741	25	R	6	25	S	32	30	R[15]	6	30	R	6
ABSP6770	10	S[1]	28	25	R	0	5	R	12	30	R	0
ABSP6773	10	S	30	1.25/23.75	S	25	5	R[14]	13	30	R	6
ABSP6790	2	S	23	25	R	0	5	R	11	30	R	0
ABSP6794	2	S	28	25	R	0	5	R	0	30	R	0
ABSP6806		N		1.25/23.75	R	0	5	R[14]	13	30	R	0
ABSP6857	10	S[3]	25	1.25-23.75	I	23	5	R	0	30	R	0
ABSP6984		N			N			N			N	

Table 4c – MIC method, **Sample 2 EFU** (*Enterococcus faecium)*

Lab code	Used system	Broth	Atmosphere
ABSP6568	Micro Naut	H-Medium	CO2
ABSP6606	AviPro-Plate, MCN6	H-Medium	aerob
ABSP6632	Vitek 2 compact		
ABSP6752	Mikrodilutionsverfahren	MHB	aerob
ABSP6799	SENSITITRE	CAMHB	Ambient
ABSP6918	MicroNaut	CAMHB	Ambient
ABSP6945	Vitek II		
ABSP6982	MicroNAUT	CAMHB	ambient
ABSP6986	MicroNaut	LH-CAMHB	Ambient

Table 4d – Results MIC method, **Sample 2 EFU** (*Enterococcus faecium)*

Lab code	Ampicillin (S)		Trimethoprim / Sulfamethoxazole (R)		Enrofloxacin (R)		Tetracycline (R)	
	IR	MV	IR	MV	IR	MV	IR	MV
ABSP6568	S	<=0.25	R	>2/38	R	>2	R	>8
ABSP6606	N		R	>2/38	R	>2	R	>8
ABSP6632	S[3]		N		R		R	
ABSP6752	S[3]	=0,25 µg/ml	R	<=0,5/9,5 µg/ml	R	=2 µg/ml	R	>8 µg/ml
ABSP6799	S	≤0.5	NI	>8	S[14]	4	R	>8
ABSP6918	S	=0.0625	S	<=0.03125/0.59375	I	=2	R	=32
ABSP6945	S		I		R		R	
ABSP6982	S	=0.25	S	≤0.25/4.75	R	>2	R	>8
ABSP6986	S	≤0,25	R	>2/38	R[16]	>2	R	>8

Table 5a – Paperdisk/Tablet Method: Sample 3 PMU (*Pasteurella multocida)*

Lab code	Used method
ABSP6560	blood agar - no growth
ABSP6582	paperdisk on Columbia sheep blood agar with confluent growth
ABSP6583	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6606	Paperdisk on agar + blood
ABSP6626	Bovicor agar
ABSP6629	Kirby-Bauer disk diffusion
ABSP6643	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6645	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6690	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6737	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6741	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6770	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6773	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6790	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6794	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6806	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6857	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6984	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth

Table 5b – Results Paperdisk/Tablet method, **Sample 3 PMU** (*Pasteurella multocida)*

Lab code	Penicillin (S)			Trimethoprim / Sulfamethoxazole (S)			Tilmicosin (S)			Enrofloxacin (S)			Tetracycline (S)		
	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ
ABSP6560		N			N			N			N			N	
ABSP6582	10	S[2]	28	25	R	0	15	S	14	5	S	10	30	R	0
ABSP6583	10	S[2]	27	1.25/23.75	S	26	15	I[11]	16	5	S	25	30	S	26
ABSP6606	10	S	26	25	S	28	15	S	12	5	S[14]	30	30	S	28
ABSP6626	10	S[2]	25	1,25/23,75	R	16	15	R	15	5	S[14]	22	30	R	19
ABSP6629	10	S[2]	26	25	S	28	15	R[11]	18	5	S[14]	34	30	S	28
ABSP6643	25	I[1]	17	1.25+23.75	S	23	15	S	14	5	S	26	30	S	25
ABSP6645	10	S[2]	28	5	S	34	15	S[11]	21	5	S[14]	30	30	S	28
ABSP6690	10	S[2]	23	25	S	34	15	S	13	10	S[14]	37	30	S	25
ABSP6737	1	S	22	25	S	25	15	NI[11]		5	S[14]	28	30	S	28
ABSP6741	25	R[2]	6	25	S	30	15	I[11]	20	30	S[15]	32	30	S	31
ABSP6770	10	S[1]	26	25	S	31		N		5	S	31	30	S	26
ABSP6773	10	S[2]	34	1.25/23.75	S	35	15	I[11]	20	5	S[14]	40	30	S	30
ABSP6790		N			N			N			N			N	
ABSP6794	10	S[2]	21	25	S	28	15	R[11]	17	5	S	25	30	S	25
ABSP6806	10	S	26	1.25/23.75	S	26	15	R[11]	19	5	NI[14]	31	30	NI	26
ABSP6857	2	S[2]	23	1.25-23-75	S	31	15	R[11]	19	5	S	33	30	S	24
ABSP6984	10	R[2]	28	1/24	S	30	15	S	15	5	S	30	30	S	26

Table 5d – MIC method, **Sample 3 PMU** (*Pasteurella multocida)*

Lab code	Used system	Broth	Atmosphere
ABSP6568	Micro Naut	H-Medium	CO2
ABSP6606	AviPro- Plate, MCN6	Mueller-Hinton	aerob
ABSP6632	Vitek 2 compact		
ABSP6752	Mikrodilutionsverfahren	MHB	aerob
ABSP6799	SENSITITRE	CAMHB	Ambient
ABSP6918	MicroNaut	CAMHB	Ambient
ABSP6945	Vitek II		
ABSP6982	MicroNAUT	H-medium	ambient
ABSP6986	MicroNaut	LH-CAMHB	Ambient

Table 5d – Results MIC method, **Sample 3 PMU** (*Pasteurella multocida)*

Lab code	Penicillin (S)		Trimethoprim / Sulfamethoxazole (S)		Tilmicosin (S)		Enrofloxacin (S)		Tetracycline (S)	
	IR	MV	IR	MV	IR	MV	IR	MV	IR	MV
ABSP6568	S	≤0.125	S	<0.5/9.5	S	≤8	S	≤0.25	S	≤2
ABSP6606	S	<=0,125	S	<=0,5/9,5	S	<=8	S	<=0,25	S	<=2
ABSP6632	N		S		N		S		S	
ABSP6752	S	<=0,0625 µg/ml	S	<=0,5/9,5 µg/ml	S[13]	/	S	<=0,125 µg/ml	S	<=0,25 µg/ml
ABSP6799	S[2]	0.25	S	≤0.5	NI	≤8	S	≤0.12	S	≤0.5
ABSP6918	S[2]	=0.125	S	<=0.25/4.75	R	>16	S	<=0.125	S	=1
ABSP6945	S[2]		R		R[11]		S		S	
ABSP6982	S[2]	=0.125	S	≤0.5/9.5	S	≤4	S	≤0.125	S	≤0.5
ABSP6986	S	≤0,125	S	≤0,25/4,75	I[11]	=2	S	≤ 0,25	S	<1

Table 6a – Paperdisk/Tablet Method: Sample 4 SAU (*Staphylococcus aureus)*

Lab code	Used method
ABSP6560	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6582	paperdisk on Columbia sheep blood agar with confluent growth
ABSP6583	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6606	Paperdisk on agar + blood
ABSP6626	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6629	Kirby-Bauer disk diffusion
ABSP6643	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6645	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6690	Paperdisk on Mueller Hinton agar + blood (MH-F) with confluent growth
ABSP6737	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6741	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6770	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6773	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6790	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6794	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6806	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6857	Paperdisk on Mueller Hinton agar (MH) with confluent growth
ABSP6984	Paperdisk on Mueller Hinton agar (MH) with confluent growth

Table 6b – Results Paperdisk/Tablet method, **Sample 4 SAU** (*Staphylococcus aureus*)

Lab code	Penicillin (R)			Cefoxitin (R)			Clindamycin (R)			Erythromycin (R)			Tetracycline (S)		
	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ	DC	IR	IZ
ABSP6560	10	R[2]	10	30	R[8]	10	15	R[9]	0	15	R[12]	0	30	S	30
ABSP6582	10	R[2]	0		N		15	R[9]	0	15	R[12]	0	30	S	26
ABSP6583	10	R[2]	10		N		109	R[9]	16	15	R	7	30	S	28
ABSP6606	10	R	0	30	R	18	2	R	0	15	R	0	30	S	22
ABSP6626	10	R[2]	11	30	R[8]	19	2	R	0	15	R[12]	0	30	S	27
ABSP6629	10	R[2]	12	30	R	14	2	R	7	15	R	7	30	S	26
ABSP6643	10	R	10	30	R	15	15	R[9]	6	15	R	6	30	S	28
ABSP6645	10	R[2]	11	10	I[6]	15	2	R	0	15	R	0	30	S	24
ABSP6690	10	R[2]	0	30	R	16	10	R	0	15	R[12]	0	30	S	27
ABSP6737	10	R	6	30	R	10	2	R	6	15	R	6	30	S	27
ABSP6741	25	R[2]	6	30	R[6]	18	109	R[9]	6	15	R	6	30	S	26
ABSP6770	10	R[1]	0		N			N			N		30	S	34
ABSP6773	10	S[2]	30	30	R	16	2	R	6	15	R	6	30	S	25
ABSP6790	1	R	0	30	R	11	2	R	0	15	R	0	30	S	25
ABSP6794	2	R[2]	0	30	R	20	2	R	0	15	R	0	30	S	25
ABSP6806	10	R	11		N		2	R	0	15	R	0	30	S	22
ABSP6857	1	R	0	30	R	17	2	R	0	15	R	0	30	I	20
ABSP6984	10	R[2]	11	30	R	17	2	R[10]	0	15	R	0	30	S	24

Table 6c – MIC method, **Sample 4 SAU** (*Staphylococcus aureus)*

Lab code	Used system	Broth	Atmosphere
ABSP6568	Micro Naut	H-Medium	CO2
ABSP6606	AviPro- Plate, MCN6	Mueller- Hinton	aerob
ABSP6632	Vitek 2 compact		
ABSP6752	Mikrodilutionsverfahren	MHB	aerob
ABSP6799	SENSITITRE	CAMHB	Ambient
ABSP6918	MicroNaut	CAMHB	Ambient
ABSP6945	Vitek II		
ABSP6982	MicroNAUT	CAMHB	ambient
ABSP6986	MicroNaut	CAMHB	Ambient

Table 6d – Results MIC method, **Sample 4 SAU** (*Staphylococcus aureus)*

Lab code	Penicillin (R)		Cefoxitin (R)		Clindamycin (R)		Erythromycin (R)		Tetracycline (S)	
	IR	MV	IR	MV	IR	MV	IR	MV	IR	MV
ABSP6568	R	>2	R[6]	>1	N		R[12]	>16	S	≤2
ABSP6606	R	>2	N		N		R	>4, >16	S	<=2
ABSP6632	R		R[5]		R		R		S	
ABSP6752	R	>8 µg/ml	R[8]	>4 µg/ml	R	>4 µg/ml	R[13]	/	S	<=0,25 µg/ml
ABSP6799	R[2]	32	R	>16	R	> 4	R	>8	S	≤0.5
ABSP6918	R	=32	R	<22 mm/>8	R	>8	R	>8	S	=0.25
ABSP6945	R		R[8]		R		R		S	
ABSP6982	R	>8	R	>4	R	>2	R	>4	S	≤0.25
ABSP6986	R	>8	R[8]	>4	R[9]	> 32	R	>4	I	=0,25

Appendix 1

List of antibiotics used

	Penicillins
1	amoxicillin
2	ampicillin
3	penicillin

	Cephalosporins
4	cefalexin
5	cefalotin
6	cefotaxime
7	cefoxitin
8	ceftiofur

	Lincosamides
9	lincomycin
10	pirlimycin

	Macrolides
11	erythromycin
12	tilmicosin
13	tylosin

	Quinolones
14	ciprofloxacin
15	flumequine
16	marbofloxacin