Interpretation of the Antibiogramme with Neo-Sensitabs
MIC breakpoints according to CLSI (VET01S 2024)
Inoculum according to Kirby-Bauer / confluent colonies.

Mueller-Hinton agar and McFarland 0.5 inoculum.

ONLY VETERINARY SPECIFIC BREAKPOINTS ARE SHOWN

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Breakpoints MIC (µg/ml) S	R
	Amoxicillin+Clav.	20+10 µg	AMC30					
	Dogs, cats			-	-	-	≤ 0.25	≥ 1
	Staphylococcus spp.				Use cefoxitin		≤ 4/2	≥ 8/4
	Other organisms	(UTI only)		≥ 18	-	-	≤ 8/4	≥ 32/16
	Amikacin	30 µg	AMI30					
	Enterobacteriales			≥ 20	17-19	≤ 16	≤ 4	≥ 16
	Pseudomonas			-	-	-	≤ 4	≥ 16
	Ampicillin	10 µg	AMP10					
	Enterobacteriaceae			-	-	-	≤ 0.25	≥ 1
	Staphylococcus spp.				Use penicillin			
	Enterococcus spp.			≥ 17	-	≤ 16	≤ 8	≥ 16
	Streptococcus spp. (not *S. pneumoniae*)			-	-	-	≤ 0.25	
	Mannheimia haemolytica			-	-	-	≤ 0.03	≥ 0.25
c)	Apramycin	40 µg	APRAM	≥ 23	22-20	≤ 19	≤ 4	≥ 16
c)e) h)	Cefadroxil	30 µg	CDX30	≥ 23	22-20	≤ 19	≤ 8	≥ 32
e)h)	Cefazolin	30 µg	CFZ30	≥ 23	22-20	≤ 19	≤ 2	≥ 8
	E. coli, K. pneumoniae, P. mirabilis			≥ 15	.	≤ 14		
	Streptococcus spp.			-	-	-	≤ 2	≥ 8
f)	Cefoxitin	30 µg	CFO30					
	S. aureus			≥ 22	-	≤ 21	OxaS	MecA pos
	Coag. neg. staph.			≥ 25	-	≤ 24	OxaS	MecA pos
a)c) h)	Ceftriaxone	30 µg	CTR30					
	(Cefovecin, *Streptococcus*)			≥ 24	23-21	≤ 20	≤ 0.12	≥ 0.5
h)	Cefpodoxime (dogs)	10 µg	CPD10	≥ 21	20-18	≤ 17	≤ 2	≥ 8
c)h)	Cefquinome	30 µg	CFQUI	≥ 23	22-20	≤ 19	≤ 2	≥ 8
h)	Ceftiofur	30 µg	CFTIO	≥ 21	20-18	≤ 17	≤ 2	≥ 8
	S. equi subsp. *zooepidemicus* (horses)			≥ 22	-	-	≤ 0.25	
c)h)	Cefuroxime	30 µg	CXM30	≥ 18	17-15	≤ 14	≤ 8	≥ 32
e) h)	Cephalexin	30 µg	CFLEX	-	-	-	≤ 16	≥ 32
c)e) h)	Cephalothin	30 µg	CEP30	≥ 23	22-20	≤ 19	≤ 2	≥ 8
	Cephapirin	30 µg	CFP30	≥ 18	17-15	≤ 14	≤ 8	≥ 32
	Chloramphenicol	30 µg	CLR30					
	Staphylococcus			-	-	-	≤ 2	≥ 8
i)	Clindamycin	2 µg	CLIN2					
	Staphylococcus spp.			≥ 21	20-15	≤ 14	≤ 0.5	≥ 4
c)	Doxycycline	30 µg	DOX30					
	Enterobacteriales (dogs)			-	-	-	≤ 0.12	≥ 0.5
	Enterobacteriales (humans)			≥ 14	13-11	≤ 10	≤ 4	≥ 16
	Staphylococcus (dogs)			≥ 25	24-21	≤ 20	≤ 0.5	≥ 0.5
	Staphylococcus (horses)			-	-	-	≤ 0.12	≥ 0.5
m)	Enrofloxacin (dogs)	5 µg	ENR.5	-	-	-	≤ 0.06	≥ 0.5
	Enrofloxacin (cats)	5 µg	ENR.5	≥ 23	22-17	≤ 16	≤ 0.5	≥ 4
m)	Enrofloxacin	10 µg	ENROF	-	-	-	-	-
	Florfenicol	30 µg	FFC30					
	Cattle			≥ 19	18-15	≤ 14	≤ 2	≥ 8
	Swine			-	-	-	≤ 4	≥ 16

	NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm S	I	R	Breakpoints MIC (µg/ml) S	R
	Streptococcus suis, P. multocida.			≥ 22	21-19	≤ 18	≤ 2	≥ 8
	Actinobacillus spp.							
	Bordetella bronchoseptica			≥ 22	21-19	≤ 18	≤ 2	≥ 8
c)	Fish pathogens			≥ 28	27-24	≤ 23	≤ 2	≥ 8
c)	Flumequine	30 µg	FLUME	≥ 20	19-17	≤ 16	≤ 2	≥ 4
c)	Fosfomycin (U)	200 µg	FO200	≥ 18	-	< 18	≤ 32	> 32
c)	Fucidin	100 µg	FUCID					
	Plain agar			≥ 28	27-24	≤ 23	≤ 1	≥ 4
	Blood agar			≥ 26	25-23	≤ 22	≤ 1	≥ 4
c)	Fucidin	10 µg	FUC10	≥ 21	-	≤ 20	≤ 1	≥ 1
c)	Furazolidone	50 µg	FURAZ	≥ 23	22-20	≤ 19	≤ 4	≥ 8
	Gentamicin	10 µg	GEN10	≥ 16	15-13	≤ 12	≤ 2	≥ 8
c)	Kanamycin	30µg	KAN30	≥ 18	17-14	≤ 13	≤ 16	≥ 64
c) m)	Marbofloxacin (dogs)	5 µg	MAR.5	-	-	-	≤ 0.12	≥ 0.5
	Marbofloxacin (cats)	5 µg	MAR.5	≥ 20	19-15	≤ 14	≤ 1	≥ 4
b) c)	Metronidazole (anaerobes)	16 µg	MTR16	≥ 28	27-24	≤ 23	≤ 4	≥ 8
c)	Naf-Pen-Strep	5+2+20 µg	N+P+S	≥ 20	19-17	≤ 16	≤ 1/1/4	≥ 2/2/16
c)	Neomycin	120 µg	NEOMY	≥ 23	22-20	≤ 19	≤ 6	≥ 25
c)	Nitrofurantoin	300 µg	NI300	≥ 17	16-15	≤ 14	≤ 32	≥ 128
	Nitrofurantoin	100 µg	NI100	≥15	14-13	≤ 12	≤ 32	≥ 128
f)	Oxacillin	1 µg	OXA.1					
	S. intermedius (pseud)			-	-	-	-	-
c)	Oxolinic acid	10 µg	OXOLI	≥ 20	19-17	≤ 16	≤ 4	≥ 8
	Penicillin/Novo	10 U + 30 µg	PEN+N					
	Mastitis			≥ 18	17-15	≤ 14	≤ 1/2	≥ 4/8
	Penicillin	10 units	PEN10					
k)	*Staphylococcus* spp.			-	-	-	≤ 0.5	≥ 2
	Streptococcus			-	-	-	≤ 0.5	≥ 2
	Piperacillin/tazobactam	100/10 µg						
	Enterobacteriales			≥ 25	24-21	≤ 20	≤ 8/4	≥ 32/4
	Pseudomonas, Staphylococcus			-	-	-	≤ 8/4	≥ 32/4
	Tetracyclines	30 µg	TET30					
	Staphylococci			≥ 19	18-15	≤ 14	≤ 4	≥ 16
	Cattle (BRD)			≥ 23	22-19	≤ 18	≤ 2	≥ 8
	Swine (BRD)			≥ 27	26-24	≤ 23	≤ 0.5	≥ 2
	Dogs			≥ 23	22-18	≤ 17	≤ 0.25	≥ 1
	Streptococci (Swine), P. multocida			-	-	-	≤ 0.5	≥ 2

Special tests

Cloxacillin		CLOXA	Detection of plasmid mediated AmpC
Phenylboronic acid		BORON	Detection of AmpC and KPC beta lactamases
Imipenem+EDTA	10+750 µg	IM10E	Detection of metallo-β-lactamases
Dipicolinic acid		D.P.A	Detection of metallo-β-lactamases

Kits

ESBL, AmpC, ESBL + AmpC Confirm kit	ESBL + AmpC
ESBL + AmpC Screen Kit	ESBL + AmpC
KPC, MBL, OXA-48 Confirm Kit	Carbapenemases

Remarks:

a) Results with Ceftriaxone are valid for Cefoperazone and Cefovecin.

b) Metronidazole 16 µg is the representative of the Nitroimidazole-group, including Ronidazole, Ornidazole, Ipronidazole, and Moxnidazole. Results obtained with Metronidazole are applicable to the others.

c) MIC breakpoints have not been given by the CLSI.

d) From August 2005, the FDA no longer allows the use of Enrofloxacin for treating infections in poultry (to avoid development of resistance in *Campylobacter* spp.).

e) Results of Cephalothin susceptibility tests are used to predict susceptibility to the first generation cephalosporins, such as Cephadroxil and Cephalexin.

f) Results of Cefoxitin and Oxacillin with staphylococci are used to predict susceptibility to Cloxacillin. Cefoxitin resistant staphylococci should be reported as resistant to all beta-lactams. In case of discordant results between Cefoxitin and Oxacillin, report the strains as resistant (R). Use Cefoxitin for testing S. aureus. For non S. aureus staphylococci use Oxacillin 1 µg: R ≤ 17 mm (MIC ≥ 0.5 µg/ml)

g) The results of Trimethoprim+Sulfa can be used to predict the susceptibility of other potentiated sulphomanides with Trimethoprim.

h) For detection of ESBL (CTX-M) and AmpC beta-lactamases (CMY) in *Salmonella* spp. see user's guide **"Detection of resistance mechanisms using Neo-Sensitabs™ and Diatabs"** (www.rosco-diagnostica.com) on "Detection of Beta-Lactamases" (7,9).

i) Routine screening of Clindamycin inducible resistance in staphyloccci/streptococci should be performed (double disk/induction test). Results are also valid for Lincomycin.

j) *Salmonella spp.* Resistant to Ciprofloxacin 1 µg should be reported as resistant to other fluoroquinolones (Enrofloxacin, Marbofloxacin etc.).

l) High level Aminoglycoside resistance if zone is < 14 mm.

m) Zone diameter breakpoints have been removed because no correlate data exists for the revised MIC breakpoints. Zone diameter breakpoints will be reassessed once data becomes available.

Staphylococci in animals

a) Screening of beta-lactamase production in S. aureus: Look at the inhibition zone around Penicillin 10 U Neo-Sensitabs. If the zone edge is sharp (cliff) the isolate produces beta lactamase. If the zone edge is fuzzy (beach) the isolate is beta-lactamase negative. This test is only valid for Staphylococcus aureus.

b) Screening for Methicillin (Oxacillin) resistance: Test S. aureus using both Cefoxitin 30 µg and Oxacillin 1 µg Neo-Sensitabs. In case of discordant results between Cefoxitin and Oxacillin, report the strain as resistant. For non- S. aureus staphylococci use Oxacillin 1 µg Neo-Sensitabs. R <= 17 mm (MIC >= 0.5 µg/ml).

c) Test for Clindamycin induction: Place Clindamycin 2 µg and Erythromycin 15 µg Neo-sensitabs 20 mm (edge to edge) apart from each other (for staphylococci). For streptococci the distance should be 12 mm. Flattening of the Clindamycin zone adjacent to Erythromycin 15 µg indicates inducible Clindamycin resistance. Result is reported as Clindamycin R.

d) MRSA and similar:

Bemis et al. (11) found that the Cefoxitin disk diffusion test had low sensitivity for detection of Oxacillin resistance in members of the S. intermedins (*S. intermedius, S. schleiferi subsp schleiferi* and *S. schleiferi subsp coagulens*). Oxacillin disk diffusion had a high sensitivity and specificity for detecting *mecA* mediated Oxacillin resistance.

Perreten et al. (12) describe the spread of Methicillin resistant *S. pseudointermedius* in Europe and North America. Besides MR, the isolates showed resistance to Trimethoprim 90.3%, Gentamicin 88.3%, Streptomycin 90.3%, Macrolides 89.3%, Floroquinolones 87.4%.

High occurrence of MRSA and Methicillin resistant S pseudintermedius (15,16) was found in horses and small animals respectively.

Petersen et al (24) mention that the recent discovery of human and bovine MRSA isolates carrying a new mecA gene (now designated mecC) caused concern, because they are not detected by conventional confirmatory tests for MRSA. They conclude, that that mecC carrying MRSA can be exchanged between humans and ruminants.

Walther et al (25) found that the new MRSA variant (mec C) is not restricted to ruminants or humans, but it was also found in companion animals (dogs and cats). The isolates were resistant to 6 µg/ml Cefoxitin but were not detected by the mecA test.

Vanderhaegen et al (26) isolated MR non-aureus staphylococci from bovines. They were S. sciuri, S. lentus and S. fleuretti. Both mecA and mecC genes were detected. Cefoxitin MIC was a poor indicator for mecA mediated resistance in non-aureus staphylococci from animals. Both Oxacillin and Cefoxitin should be tested. Resistance to one or both indicates Methicillin resistance.

Enterococci

Detection of High-Level resistance:
Use Gentamicin 250 µg Gentamicin and Streptomycin 500 µg Neo-Sensitabs.

Enterococci showing zones of inhibition < 14 mm around Gentamicin 250 µg Neo-sensitabs should be reported as High Level Resistant to Gentamicin (no synergism with penicillins).

Enterococci showing zones of inhibition < 14 mm with Streptomycin 500 µg Neo-Sensitabs should be reported as High level resistant to Streptomycin (no synergism with penicillins).

ESBL, AmpC and carbapenemases

Vo et al. (13) found that 17 Ceftiofur resistant isolates from horses (4 *E. coli* and *3 K. pneumoniae)* were multidrug resistant. 5 produced ESBL and 1 produced AmpC with integrons in 6 isolates.

Hopkins et al. (14) report a marked increase of *Salmonella enterica serovar* resistant to Ampicillin, Streptomycin, Sulfonamides and Tetracycline, has been noted in food-borne infections, and in pigs, pig meat in several European countries. To prevent a global epidemic of these newly emerging clones or strains, as occurred with *S. tiphimurium* DT104, intervention strategies are needed as soon as possible.

CTX-M, ESBL enzymes have been found in egg, bovine mastitis, raw chicken, broiler chickens and turkeys in the UK and Europe (17,18,19,20).

Madec et al (21) found multidrug resistant Salmonella enterica serovar tiphimurium DT104 in cattle in France. They were Ceftiofur resistant and Cefoxitin susceptible, showing a typical Cephalosporin/Clavulanate synergy, indicating ESBL production.

Leverstein et al (22) conclude that intestinal carriage with ESBL-producing bacteria in food-producing animals and contamination of retail meat may contribute to increased incidences of infections with ESBL-producing bacteria in humans. Transmission of ESBL genes from poultry to humans takes place most likely through the food chain.

Fischer et al (23) isolated an E. coli producing VIM-1 (MBL) carbapenemase on a pig farm and conclude that the prevalence of carbapenemases in bacteria from livestock is probably underestimated.

Dierikx et al (27) found a high prevalence (6.8%) of ESBL/AmpC producing E. coli at Dutel broiler farms and a high prevalence of ESBL/AmpC in E. coli from farmers. Most common genes were CTX-M1, SHV-12 (ESBLs) and CMY-2 (AmpC).

Sunde et al (28) reports the first isolate (E. coli 1248) of animal origin (broiler) detected in Norway, with reduced susceptibility to cephalosporins. The ESBL was detected using Ceftazidime and Cefepime Neo-sensitabs with and without Clavulanate. The detection was unexpected and, probably, it has been part of the bacterial flora of animals taken to Norway for breeding purposes.

Cottell et al (29) detected ESBL, CTX-M32 in 29 of 88 steers over a 26-day period. Besides, CTX-M positive bacteria were found in feces in greater numbers than previously reported in the United States. The authors fear that the CTX-M genes may spread among animals.

Detection of ESBL, AmpC and carbapenemases in animals

Screening:

Enterobacteriaceae showing the following zones of inhibition:

Cefpodoxime 10 µg Neo-Sensitabs: <= 17 mm and/or

Cefotaxime 30 µg or Cefquinome 30 uµ Neo-Sensitabs: <= 27 mm and/or

Ceftazidime 30 µg and/or Ceftiofur 30 uµ Neo-Sensitabs: <= 22 mm

Should be suspected of possessing an **ESBL** and should be tested to confirm it.

Isolates producing zones of inhibition <= 16 around Cefoxitin 30 µg Neo-Sensitabs should be suspected of possessing an **AmpC** beta-lactamase and should be tested to confirm it.
Use the ESBL + AmpC Screen kit or the Total ESBL + AmpC Confirm Kit from Rosco Diagnostica.

Isolates producing zones of inhibition < 23 mm around Imipenem 10 µg Neo-Sensitabs should be suspected of possessing a **Carbapenemase** and should be tested to confirm it. Use the KPC, MBL OXA-48 Confirm Kit from Rosco Diagnostica.

Quality Control of Veterinary Antibiotics

Zone diameter in mm

NEO-SENSITABS	POTENCY	CODE	*E. coli* ATCC 25922	*S. aureus* ATCC 25923	*Ps. aeruginosa* ATCC 27853	*S. pneumoniae* ATCC 49619
Amoxicillin-clavulanate	20/10 µg	AMC30	18-24	28-36	-	-
Apramycin	40 µg	APRAM	21-28	22-30	18-24	-
Ceftiofur	30 µg	CFTIO	26-31	27-31	14-18	32-34
Enrofloxacin	5 µg	ENR.5	32-40	27-31	15-19	-
Florfenicol	30 µg	FFC30	22-28	22-29	-	24-31
Marbofloxacin	5 µg	MAR.5	29-37	24-30	20-25	-
Penicillin/Novo	10/30 µg	PEN+N	-	30-36	-	24-30
Pirlimycin	10 µg	PIRLI	-	19-25	-	-
Tiamulin	30 µg	TIAMU	-	25-32	-	-

Acceptable QC ranges for *Campylobacter jejuni* ATCC 33560.
Incubation at 36-37° C for 24 hours:

NEO-SENSITABS	POTENCY	CODE	Zone diameter in mm
Nalidixan	30 µg	NAL30	25-34
Ciprofloxacin	5 µg	CIPR5	32-45
Erythromycin	15 µg	ERY15	26-38

References:

1) CLSI VET01S. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals. 7th edition. 2024.
3) Jones R. N. et al: Tiamulin activity against fastidious and non-fastidious veterinary and human bacterial isolates: initial development of in vitro susceptibility test methods. J. Clin. Microbiol., **40**, 461-5, 2002.
4) Petersen A et al: Harmonization of antimicrobial susceptibility testing among veterinary diagnostic laboratories in the five Nordic countries. Microbial Drug Resistance, **9**, 381-388, 2003.
5) Gray J.T. et al: Antibiotic susceptibility testing of bacteria isolated from animals. Clin. Microbiol. Newsletter, **27**, 131-5, 2005.
6) Rich M. et al: Clindamycin-resistance in MRSA isolated from animals. Veterinary Microbiology, **111**, 237-40, 2005.
7) Xian-Zhi Li: Beta lactam resistance and beta-lactamases in bacteria of animal origin. Vet. Microbiol., **121**, 197-214, 2007.
8) Lüthje P. et al: Molecular basis of resistance to macrolides and lincosamines among staphylococci and streptococci from various animal sources collected in the resistance monitoring program B of T-Germ. Vet. Int. J. Antimicr. Agents, **29**, 528-535, 2007.
9) Smet A. et al: Diversity of ESBL and Class C beta-lactamases among cloacal E. coli isolates in Belgian broiler farms. Antimicr. Ag. Chemother **52**, 1238-43, 2008.
10) Hunter P.A. et al: Antimicrobial-resistant pathogens in animals and man: prescribing, practices and policies. J. Antimicr. Chemother. **65**, suppl1, 3-17, 2010.
11) Bemis et al.: Comparison of Test to Detect Oxacillin Resistance in *S. intermedius, S. schleiferi and S. aureus* isolates from canine hosts. J. Clin. Microbiol., 44, 3374-3376, 2006.
12) Perreter V. et al.: Clonal spread of MR *S. pseudointermedius* in Europe and North America: An International Multicenter Study. J. Antimicr. Chemother., 65, 1145-1154, 2010.
13) Vo ATT et al.: Characteristics of Extended-Spectrum Cephalosporin Resistant *E. coli* and *K. pneumonia* isolates from horses. Vet. Microbiol., 124, 248-255, 2007.
14) Hopkins K. L. et al.: Multiresistant *Salmonella enterica serovar 4,* in Europe: a new pandemic strain?. Eurosurveillance, 15, 3, June, 2010
15) Van den Eede A et al: High occurrence of MRSA ST398 in equine nasal samples. Accepted manuscript. Vet. Microbiology 2008.
16) Ruscher C et al: Widespread rapid emergence of distinct Methicillin and multidrug-resistant S. pseudintermedius (MRSP) genetic linkage in Europe. Vet. Microbiol. **144**, 340-346, 2010.
17) Bortolaia V et al: E. coli producing CTX-M-1,-2 and -9 group beta-lactamases in organic chicken egg production. Antimicrob. Ag. Chemother. **54**, 3527-3528, 2010.
18) Locatelli C et al: CTX-M1 ESBL-producing K. pneumonia isolated from cases of bovine mastitis. J. Clin. Microbiol. **48**, 3822-3823, 2010.
19) Dhanji H et al: Cephalosporin resistance mechanisms in E. coli isolated from raw chicken imported into the UK. J. Antimicr. Chemother. **65**, 2534-2537, 2010.
20) Randall LP et al: Prevalence of E.coli carrying ESBLs (CTX-M and TEM-52) from broiler chickens and turkeys in Great Britain between 2006 and 2009. J. Antimicr. Chemother. **66**, 86-95, 2011.

21) Madec JY et al: ESBL's CTX-M1 gene carried on an IncI1 plasmid in multidrug resistant S. enterica serovar Typhimurium DT104 in cattle in France. J. Antimicr, Chemother. **66**, 942-943, 2011.
22) Leverstein MA et al: Dutch patients, retail chicken, meat and poultry share the same ESBL genes, plasmids and strains. Clin. Microbiol, Infect. Febr. 2011 (accepted article)
23) Fischer J et al: E. coli producing VIM-1 carbapenemase isolated on a pig farm. J. Antimicr. Chemother. **67**, 1793-1795, 2012.
24) Petersen A et al: Epidemiology of MRSA carrying the novel mecC gene in Denmark, corroborates a zoonotic reservoir with transmission to humans. Clin. Microbiol. Infect. **19**, E16-E22, 2013.
25) Walther B. et al: MRSA variant in companion animals. Emerg. Infect. Dis. **18**, 2017-2019, 2012.
26) Vanderhaegen W. et al: Characterization of MR non-S. aureus staphylococci carriage isolates from different bovine populations. J. Antimicr. Chemother. **68**, 300-307, 2013.
27) Dierkx C et al: ESBL and AmpC producing E. coli in Dutch broilers and broiler farmers. J. Antimicr. Chemother. **68**, 60-67, 2013.
28) Sunde M et al: E. coli of animal origin in Norway contains a TEM-20 carrying plasmid closely related to TEM-20 and TEM-52 plasmids from other European countries. J. Antimicr. Chemother. **63**, 215-216, 2009.
29) Cottell et al: CTX-M32 an IncN plasmid in E. coli from beef cattle in the United States. Antimicr. Ag. Chemother. **57**, 1096-1097, 2013.