



Antibiotic resistance profiles of coagulase-negative staphylococci in livestock environments

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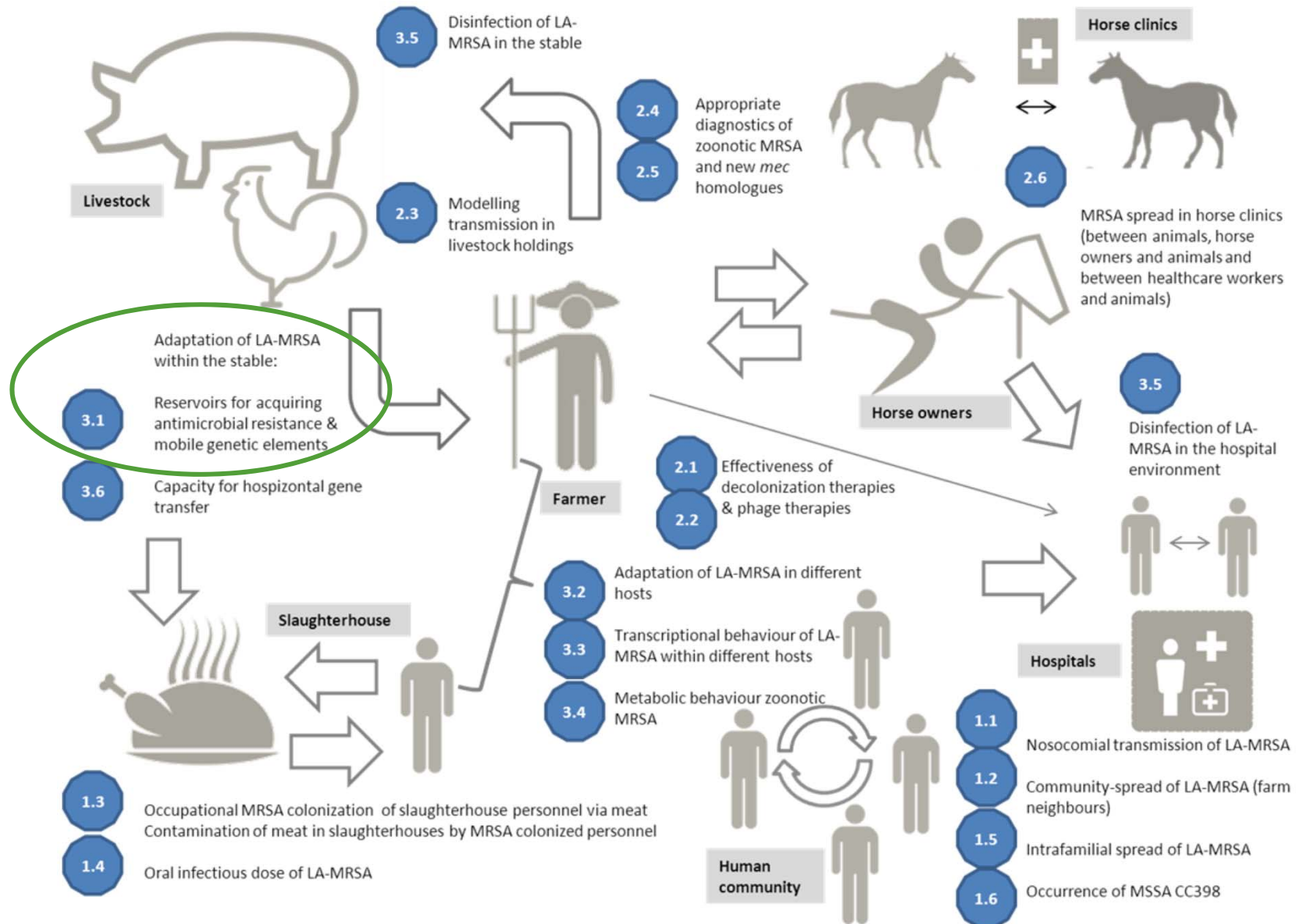
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MedVet-Staph second funding period

WP3: Influences of ecological niches & host-adaptation on LA-MRSA



The World of Staphylococci



***S. epidermidis* & other coagulase-negative staphylococci (CoNS)**

Skin & mucosa commensals

S. aureus

Broad range of infections

Methicillin resistant *S. aureus* (MRSA)

- healthcare-associated
- community-associated
- livestock-associated

Pathogens of major concern
(Antibiotic resistance, virulence)

The World of Staphylococci

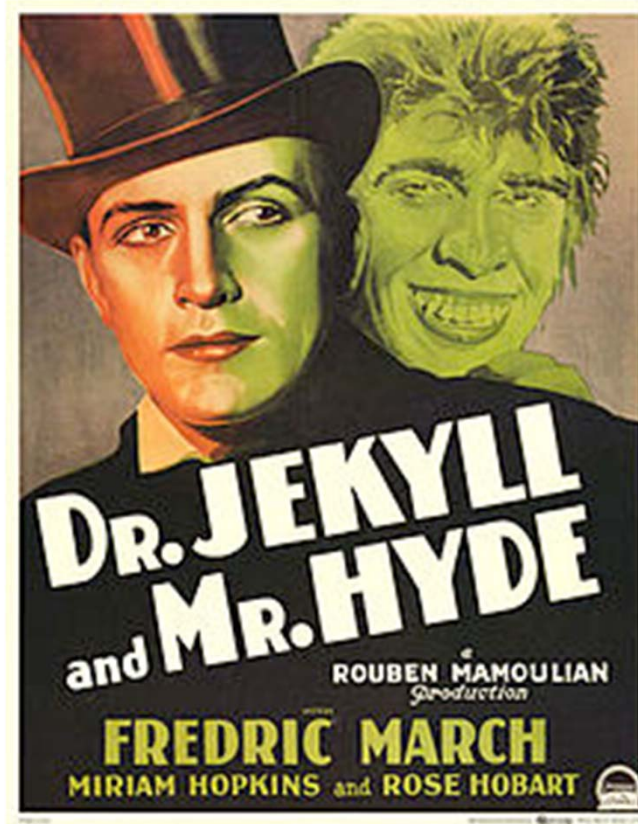


Colonisation

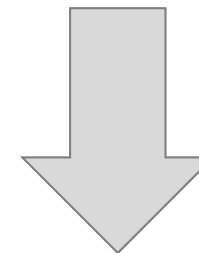


Infection

- *S. epidermidis*
- CoNS
- *S. aureus*
- MRSA



- *S. aureus*
- MRSA
- *S. epidermidis*
- CoNS



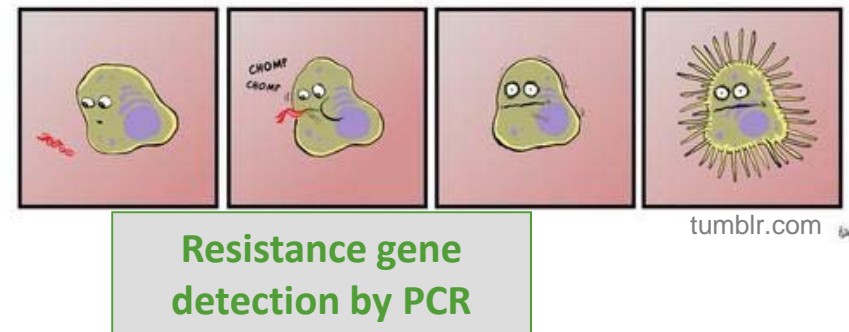
Common causes of health care-associated infections
Antibiotic multiresistance!

Setup of study

In collaboration with PD Dr. Robin Köck
and Institute for Hygiene and Microbiology, Würzburg



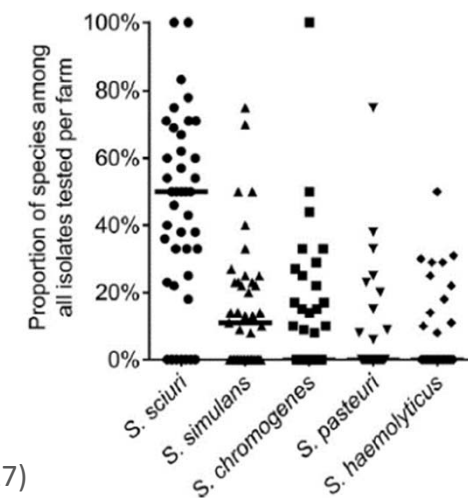
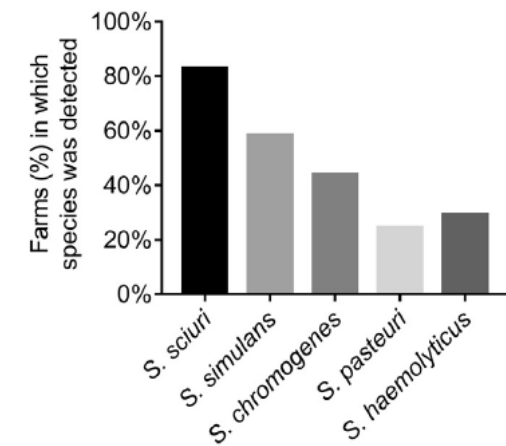
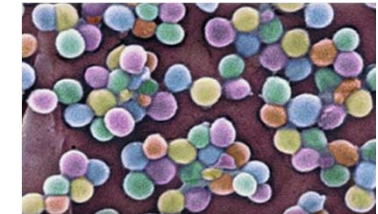
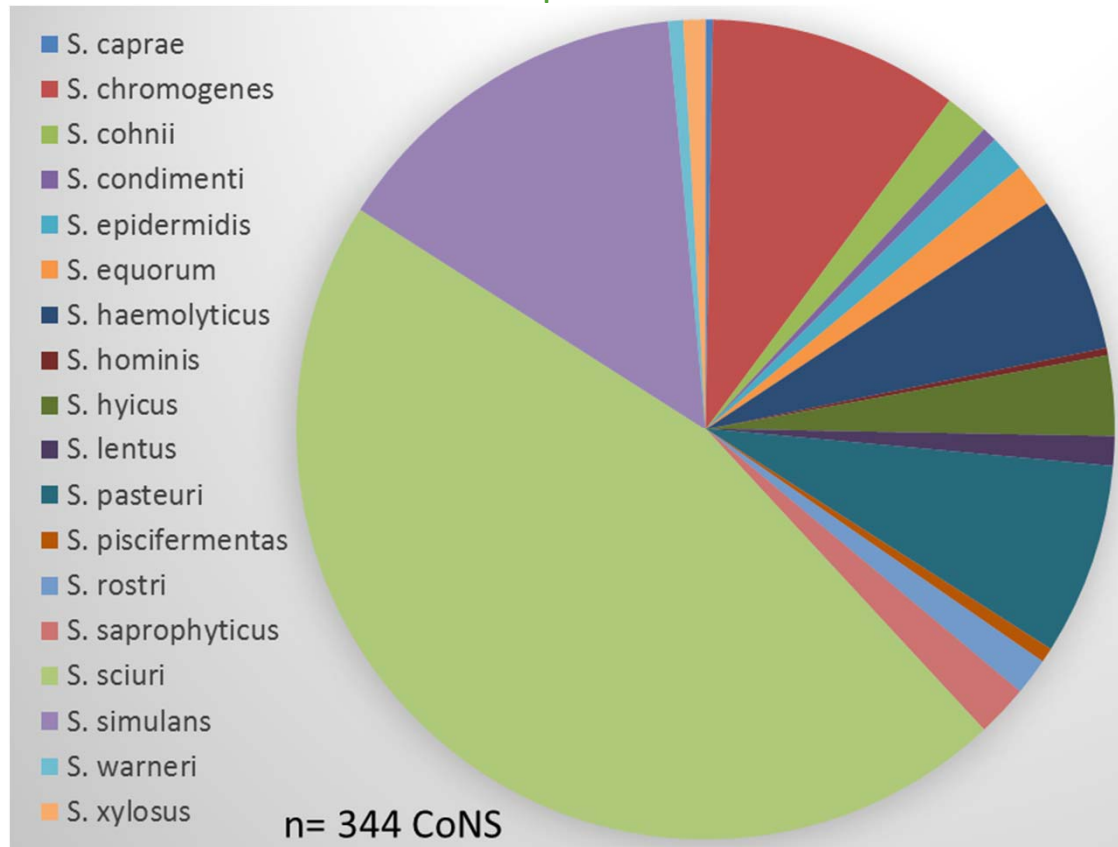
- Feb to Sep 2013: Testing of pig holdings in North Rhine-Westphalia and Lower Saxony for presence of ESBL-producing enterobacteria
Garcia-Cobos *et al.*, 2015
- 6 dust and 6 manure samples collected (41 farms)
- Samples tested for MRSA/MSSA and CoNS
- CoNS further analysed by us:





Species composition by 16S rRNA sequencing

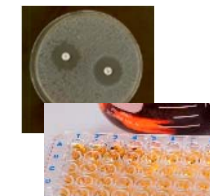
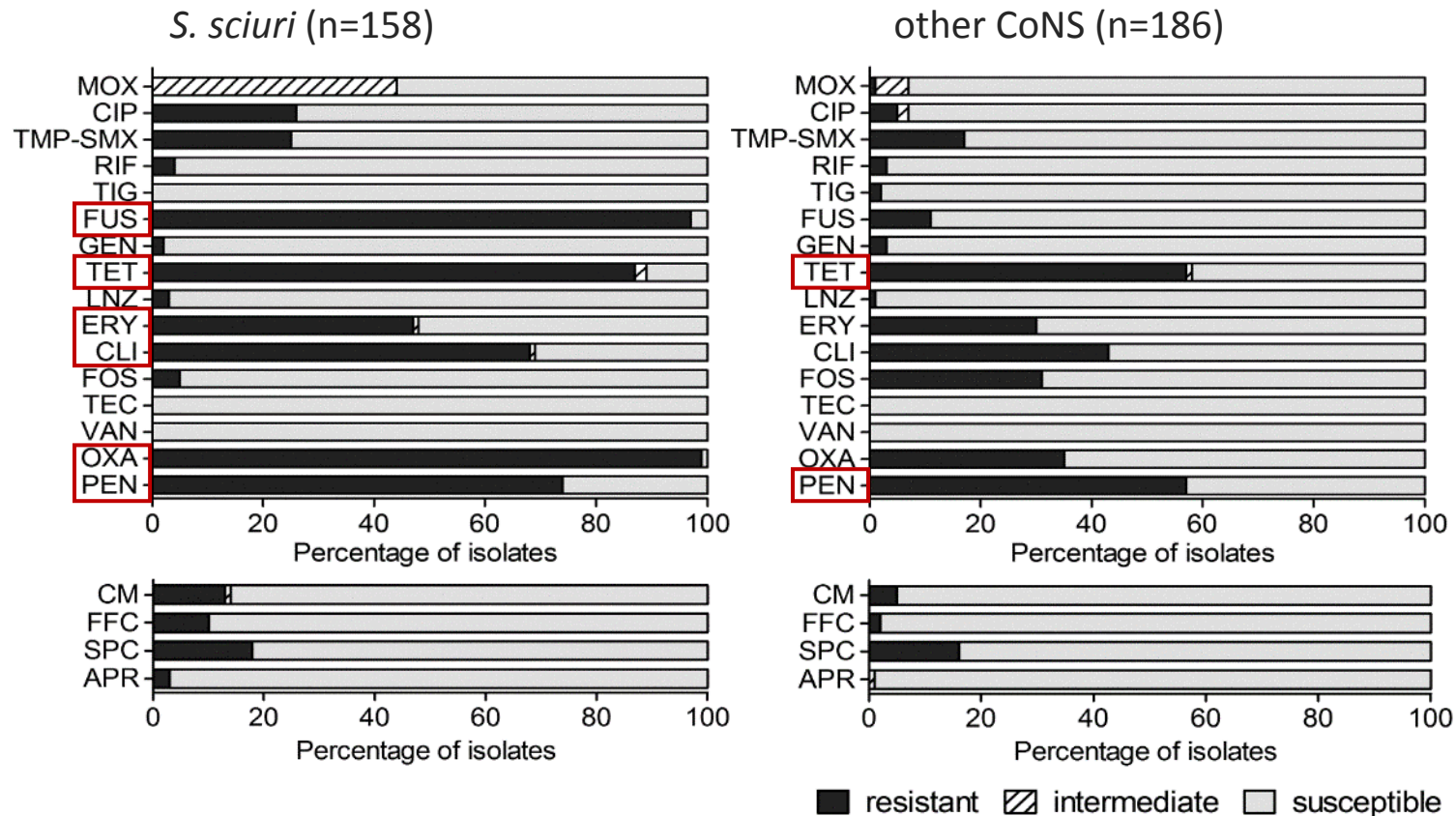
- 18 different staphylococcal species could be identified
- *Staphylococcus sciuri* was detected in almost half of all isolates, in over 80% of farms
- *S. sciuri* is the most common species in livestock environment



(Schoenfelder *et al.*, 2017)

Antimicrobial Susceptibility Tests

- *S. sciuri* shows highest resistance rates (especially for FUS, TET, ERY, CLI, OXA, PEN)
- Resistance to TET and PEN highest among other CoNS



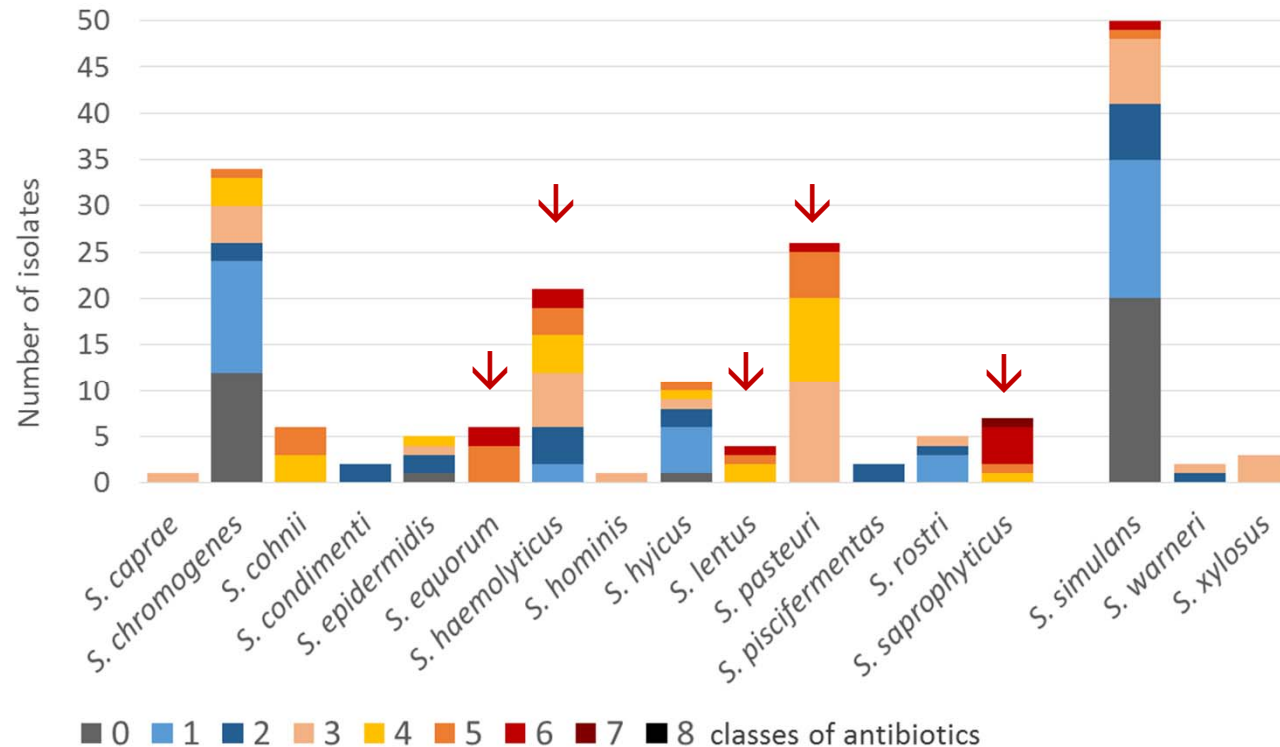
(Schoenfelder *et al.*, 2017)

MOX (moxifloxacin), **CIP** (ciprofloxacin), **TMP-SMX** (trimethoprim/sulfamethoxazole), **RIF** (rifampicin), **TIG** (tigecycline), **FUS** (fusidic acid), **GEN** (gentamicin), **TET** (tetracycline), **LNZ** (linezolid), **ERM** (erythromycin), **CLI** (clindamycin), **FOS** (fosfomycin), **TEC** (teicoplanin), **VAN** (vancomycin), **OXA** (oxacillin), **PEN** (benzylpenicillin), **SPC** (spectinomycin), **FFC** (florfenicol), **CM** (chloramphenicol), **APR** (apramycin)



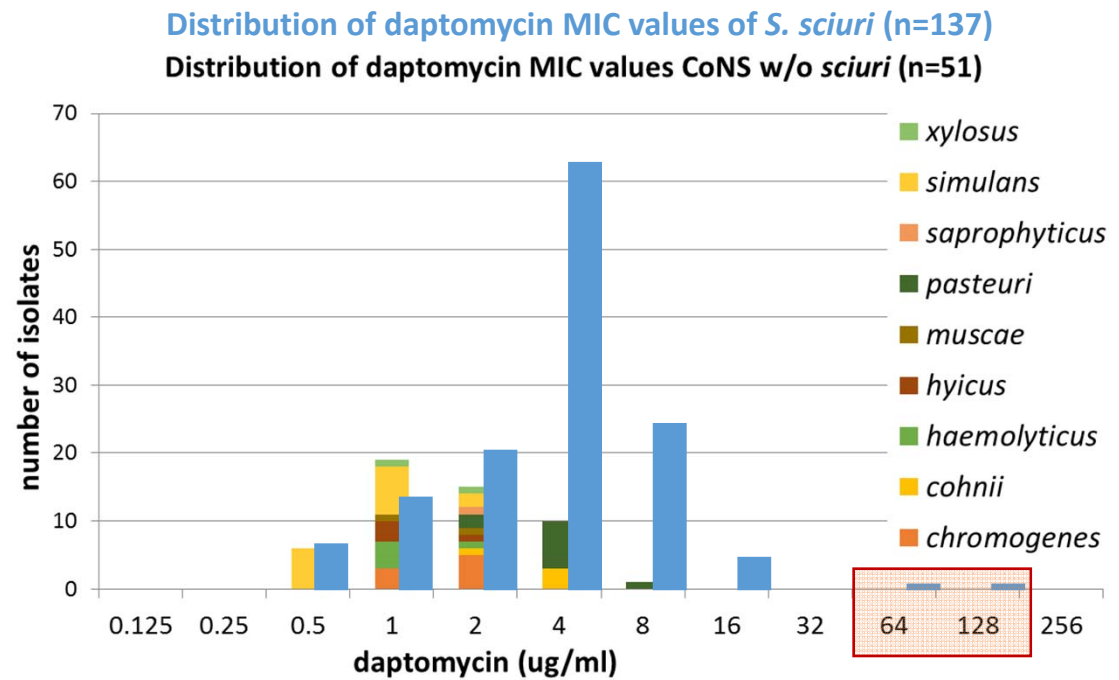
Antimicrobial Susceptibility Tests

- All 158 *S. sciuri* isolates were resistant to ≥ 2 antibiotic classes
- Next to *S. sciuri*, also *S. saprophyticus*, *S. pasteurii*, *S. haemolyticus*, *S. equorum* and *S. lentus* showed high level of multiresistance



Antimicrobial Susceptibility Tests

- MIC of last resort antibiotic daptomycin range from 1 to 16 mg/L for *S. sciuri*
- Two *S. sciuri* isolates show high level resistance against daptomycin (MIC ≥ 64)



(Schoenfelder *et al.*, 2017)

- Experimental indication of daptomycin inactivation (collaboration with Prof. Dr. Holzgrabe, Uni Würzburg)
- Preliminary data suggesting proteolytic cleavage of daptomycin



Inactivation of the Lipopeptide Antibiotic Daptomycin by Hydrolytic Mechanisms

Vanessa M. D'Costa,^a Tariq A. Mukhtar,^a Tejal Patel,^a Kalinka Koteva,^a Nicholas Waglechner,^a Donald W. Hughes,^{b†} Gerard D. Wright,^a and Gianfranco De Pascale^a

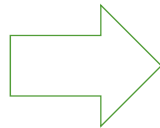
M. G. DeGrootte Institute for Infectious Disease Research, Department of Biochemistry, and Biomedical Sciences, McMaster University, Hamilton, ON, Canada,^a and Department of Chemistry, McMaster University, Hamilton, ON, Canada^b



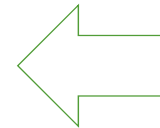
Antimicrob Agents Chemother 2012, 56: 757–764

- Detection of daptomycin hydrolytic activity in a **soil actinomyces** library
- Proof of enzymatic function
- No gene or protein assigned so far

Streptomyces roseosporus
(soil-dwelling bacterium)



Daptomycin



Resistance genes in soil
microorganisms?



Detection of AB resistance genes by PCR

Antibiotic tested	Number of non-susceptible isolates tested	Resistance genes tested for	Number of isolates positive in PCR
Oxacillin	156/ 158 (<i>S. sciuri</i>)	<i>mecA_{SCC}</i>	105
Spectinomycin	59/ 344	<i>spc</i>	15
		<i>spw</i>	13
		<i>spd</i>	34
Apramycin	4/ 344	<i>apmA</i>	2 → MIC 64 (<i>sciuri</i>)
Linezolid	8/ 344	<i>cfr</i>	2 → simultaneous resistance to
Florfenicol	19/ 344	<i>fexA</i>	18 Linezolid
Chloramphenicol	30/ 344	<i>cat</i> (pC194)	1 Phenicols
		<i>cat</i> (pC221)	6 Lincosamides
		<i>cat</i> (pC223)	4 Pleuromutilins Streptogramin A

(Schoenfelder *et al.*, 2017)



Genetic localisation and cross-species occurrence of resistance genes

	Gene	Genetic localisation/ association with MGEs	Cross-species detection	Species
Aminoglycosides	<i>spc</i>	Transposons (Tn554) Plasmids	yes	<i>S. aureus</i> <i>S. sciuri</i> other CoNS
	<i>spw</i>			
	<i>spd</i>			
	<i>apmA</i>	Plasmids	yes (this study)	<i>S. aureus</i> <i>S. sciuri</i>
Phenicol	<i>cat</i> genes	Plasmids	yes	Broad host range
	<i>fexA</i>	Tn558 (plasmids, chromosome)	yes	<i>S. aureus</i> CoNS <i>Streptococcus suis</i> <i>Bacillus</i> sp.
Oxazolidinones	<i>cfr</i>	Plasmids	yes	<i>S. aureus</i> <i>S. epidermidis</i> <i>S. sciuri</i> & other CoNS



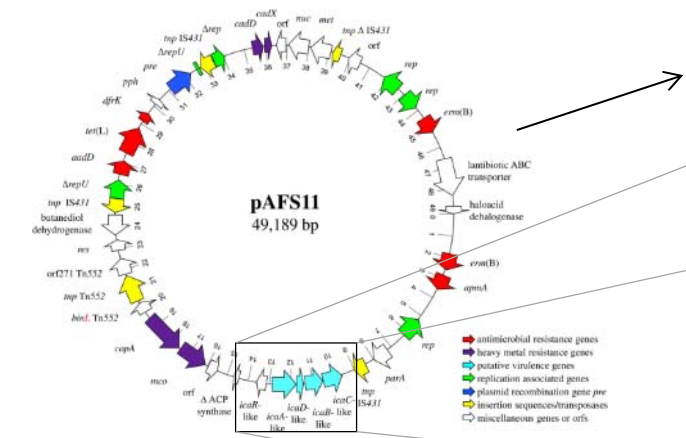
Summary

- High prevalence of *S. sciuri* in environmental samples of livestock
- High levels of antibiotic resistance in *S. sciuri* and other CoNS (multiresistance)
- Detection of (novel) resistance genes *cfr*, *apmA*, *fexA* with possibility for horizontal gene transfer (HGT)
- Alarming low susceptibility levels against daptomycin

- Detection of *ica*-like gene cluster on *S. aureus* plasmid (pAFS11)
 - *influence of horizontally acquired genes on virulence (biofilm) and regulatory crosstalk*
(collaboration with Prof. Dr. Stefan Schwarz)

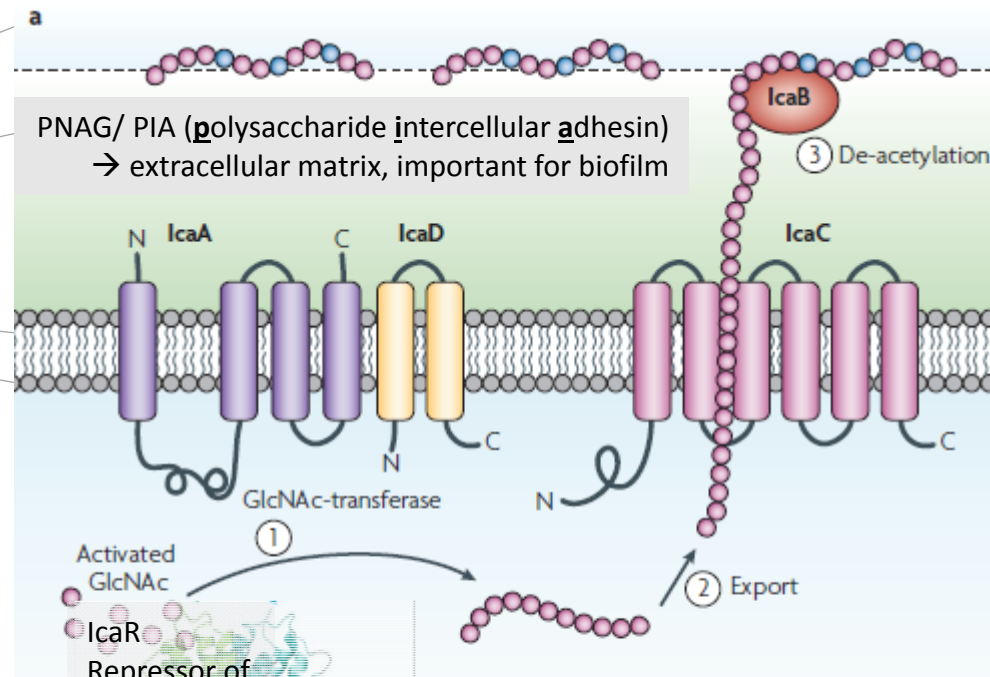


S. aureus Rd11 with *ica*-like genes on a plasmid (pAFS11)



5 antibiotic resistance genes
2 heavy metal resistance gene operons

(Feßler et al., 2017)

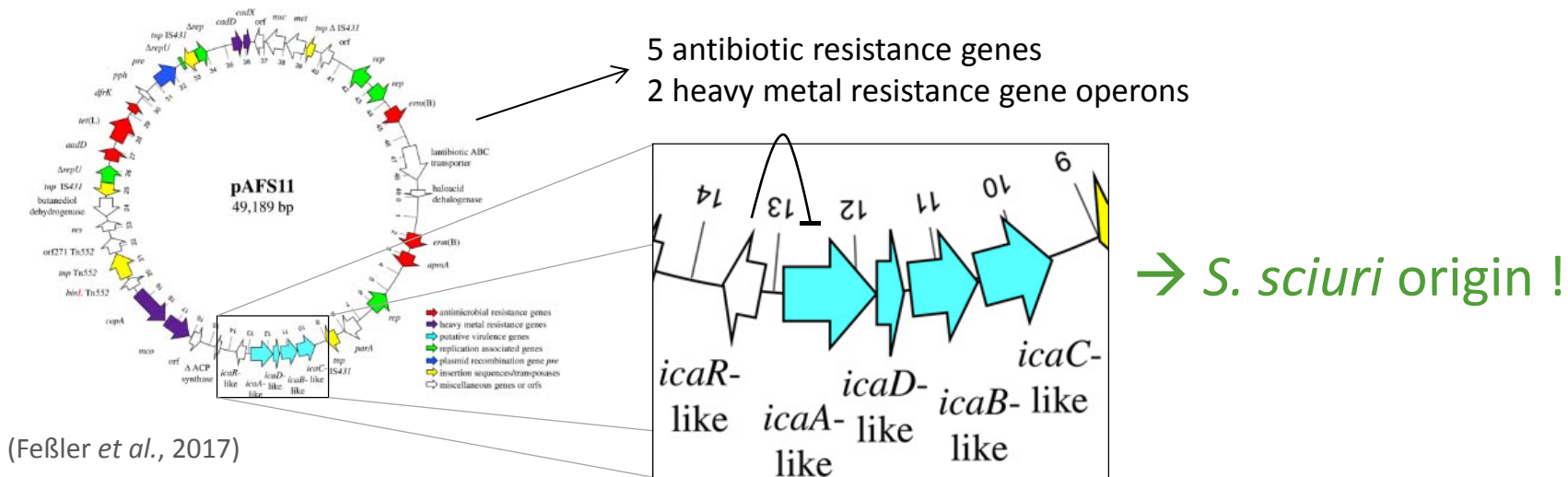


(Otto M., 2009)

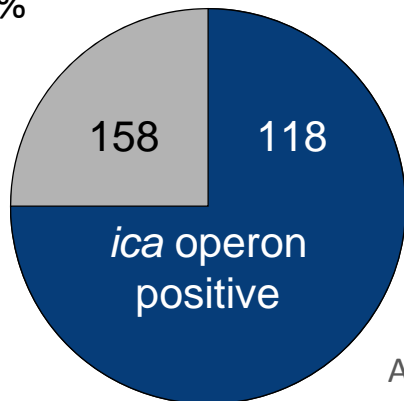
(Jeng et al., 2008)



S. aureus Rd11 with *ica*-like genes on a plasmid (pAFS11)



homology of pAFS11 proteins to *S. sciuri*:
IcaADBC: 96-99 %
IcaR: 76 %

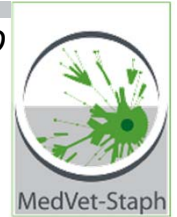


Ann-Katrin Kieninger, MSc

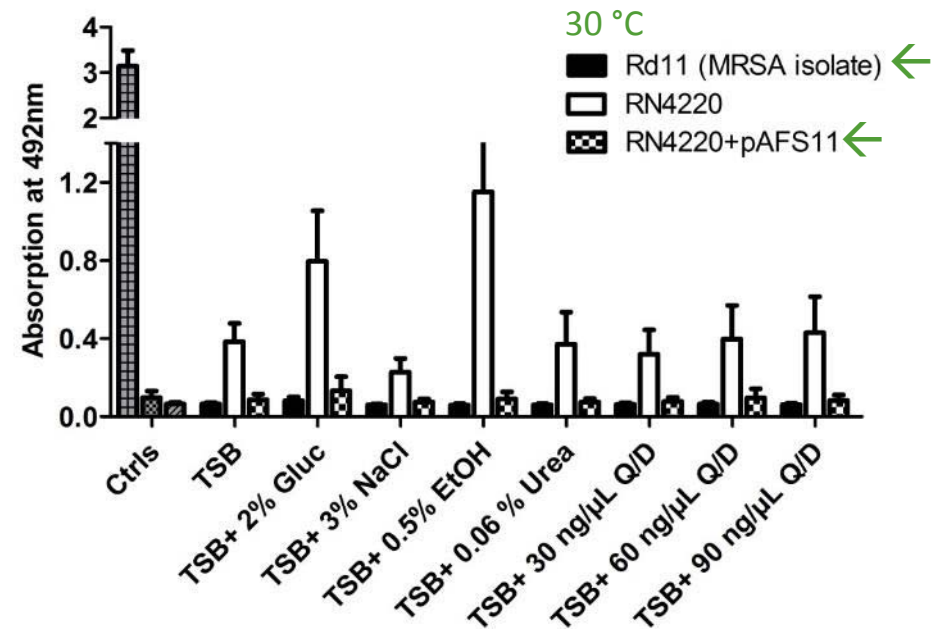
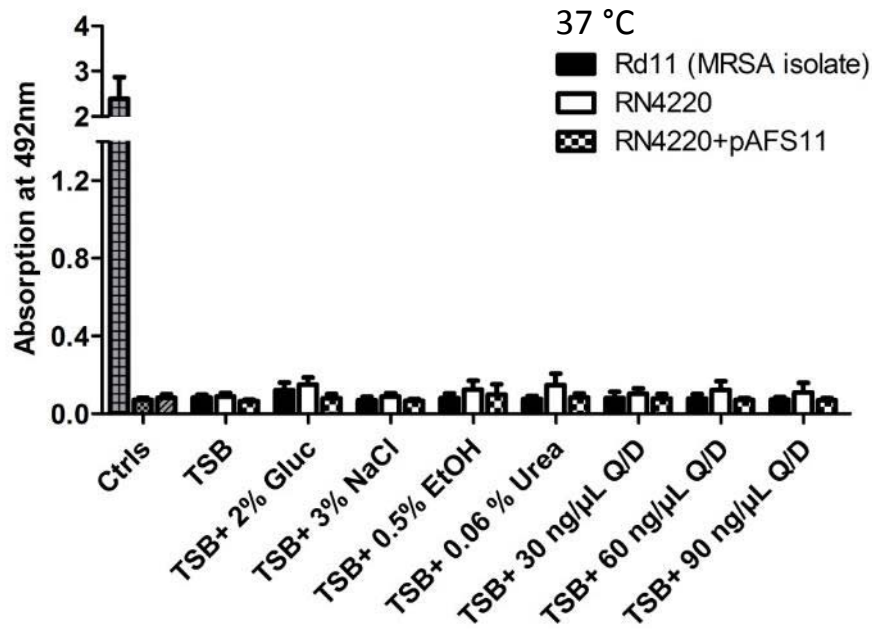
	<i>S. sciuri</i> isolates						epi	aur
<i>icaADBC</i>	TS78	TS82b	TS129a	TS147	TS180a	pAFS11	RP62A	COL
TS78	-	99.0	96.1	100.0	97.0	96.5	60.2	60.2
TS82b	35.0	-	95.7	99.0	97.8	96.1	60.2	60.3
TS129a	133.0	146.0	-	96.0	97.8	98.6	60.2	60.3
TS147	1.0	36.0	134.0	-	97.0	96.5	60.2	60.2
TS180a	100.0	73.0	75.0	101.0	-	98.0	60.4	60.4
pAFS11	118.0	132.0	49.0	119.0	64.0	-	60.1	60.4
RP62A	1368.0	1367.0	1368.0	1368.0	1361.0	1370.0	-	71.5
COL	1366.0	1365.0	1367.0	1367.0	1361.0	1361.0	973.0	-

nucleotide differences

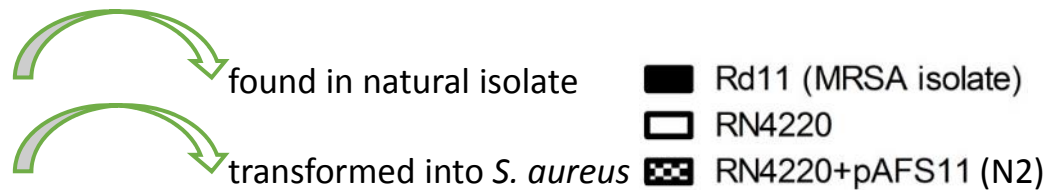
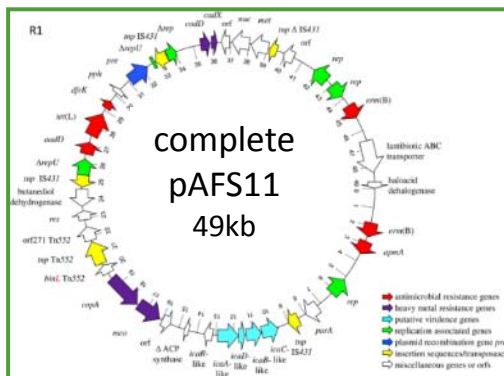




Reduction of biofilm in the presence of pAFS11

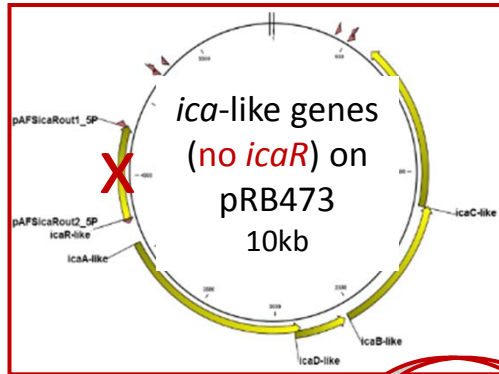
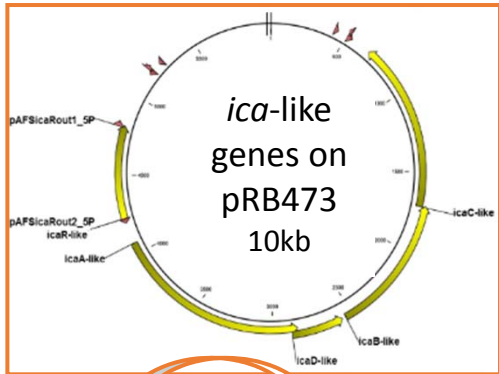
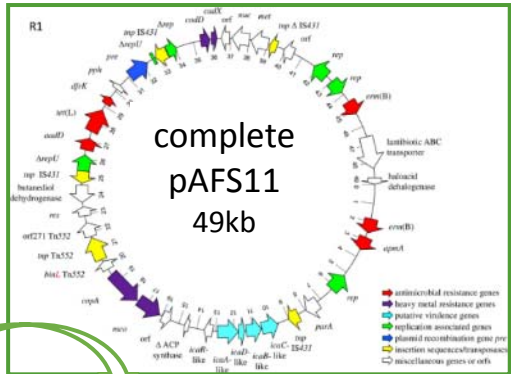
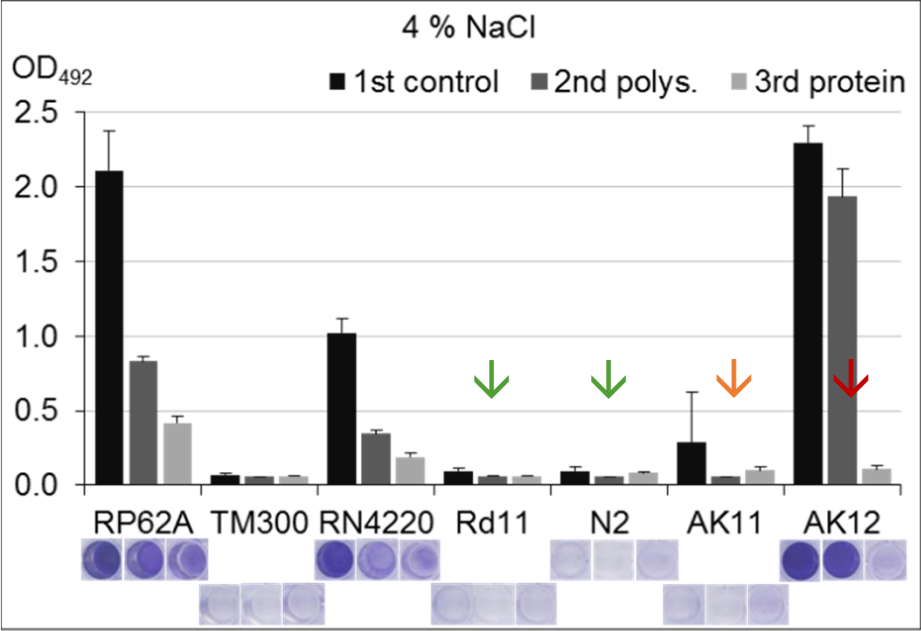
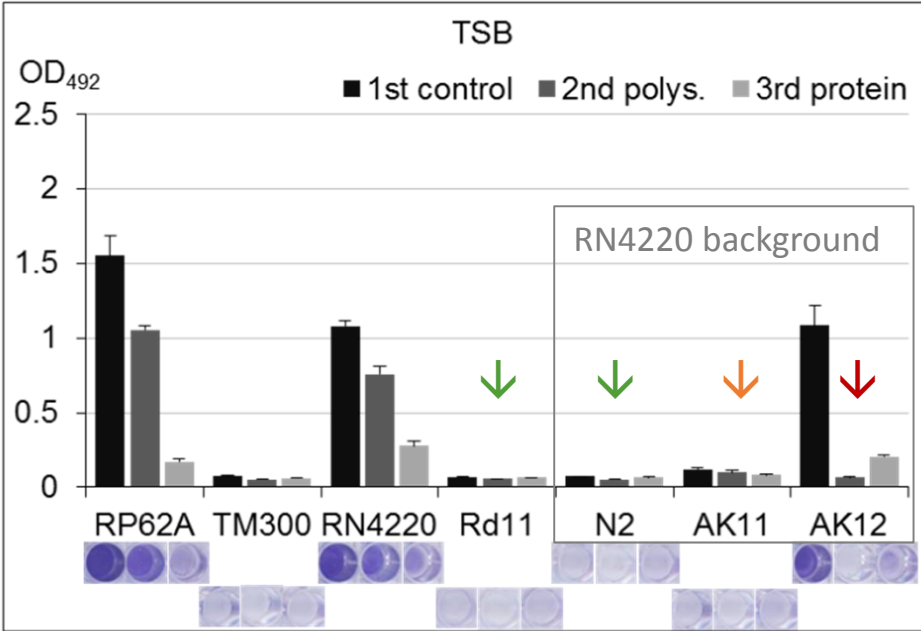


(Feßler et al., 2017)





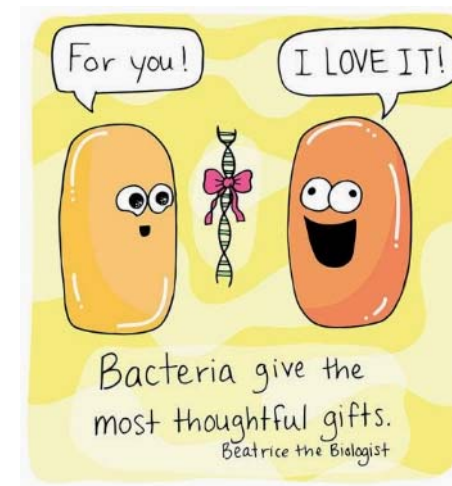
Reduction of biofilm in the presence *ica*-like genes alone



found in natural isolate: Rd11
transformed into *S. aureus*: N2

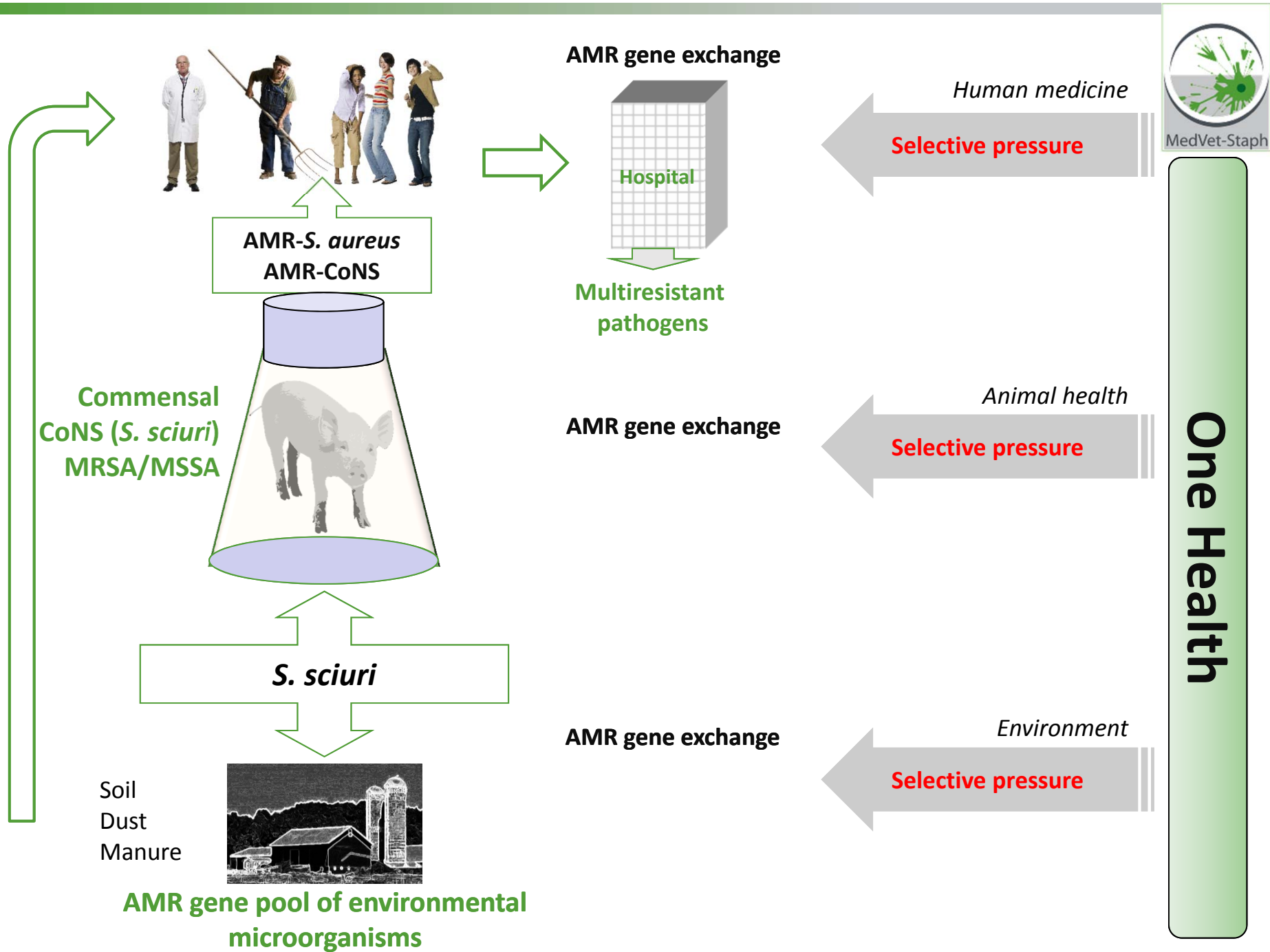
transformed into *S. aureus* RN4220:
AK11

AK12



Summary

- Detection of *ica*-genes from *S. sciuri* origin on a *S. aureus* plasmid pAFS11
→ *horizontal gene transfer event*
- Plasmid copy of *ica*-operon *represses* biofilm formation in *S. aureus*
→ *influence on virulence*
- *ica*_{pAFS}-genes are transcribed and seem functional
- Presence of additional IcaR_{pAFS} on plasmid represses transcription of chromosomal copy of *icaA*_{aur} → *crosstalk of virulence regulators*



Acknowledgements/ Collaborations



PD Dr. med. Wilma Ziebuhr
Dr. med. Dong Ying
Katharina Hufgard
Ann-Katrin Kieninger
IMIB, University of Würzburg

PD Dr. med. Robin Köck
Institute of Hygiene, University Hospital Münster (IP4)
(Schoenfelder *et al.*, Vet Microbiol. 2017 Feb;200:79-87)

PD Dr. med Christoph Schoen
Institute for Hygiene and Microbiology, University of Würzburg

Prof. Dr. med. vet. Stefan Schwarz
Friedrich-Loeffler-Institute, Neustadt-Mariensee (IP6)
(Feßler *et al.*, Vet Microbiol. 2017 Feb;200:95-100)

Dr. med. vet. Birgit Walther
Institute of Microbiology and Epizootics, Berlin (IP8)

Prof. Dr. Wolfgang Witte
Robert Koch Institute, Wernigerode (IP9)
(Cuny *et al.*, Vet Microbiol. 2017 Feb;200:88-94)

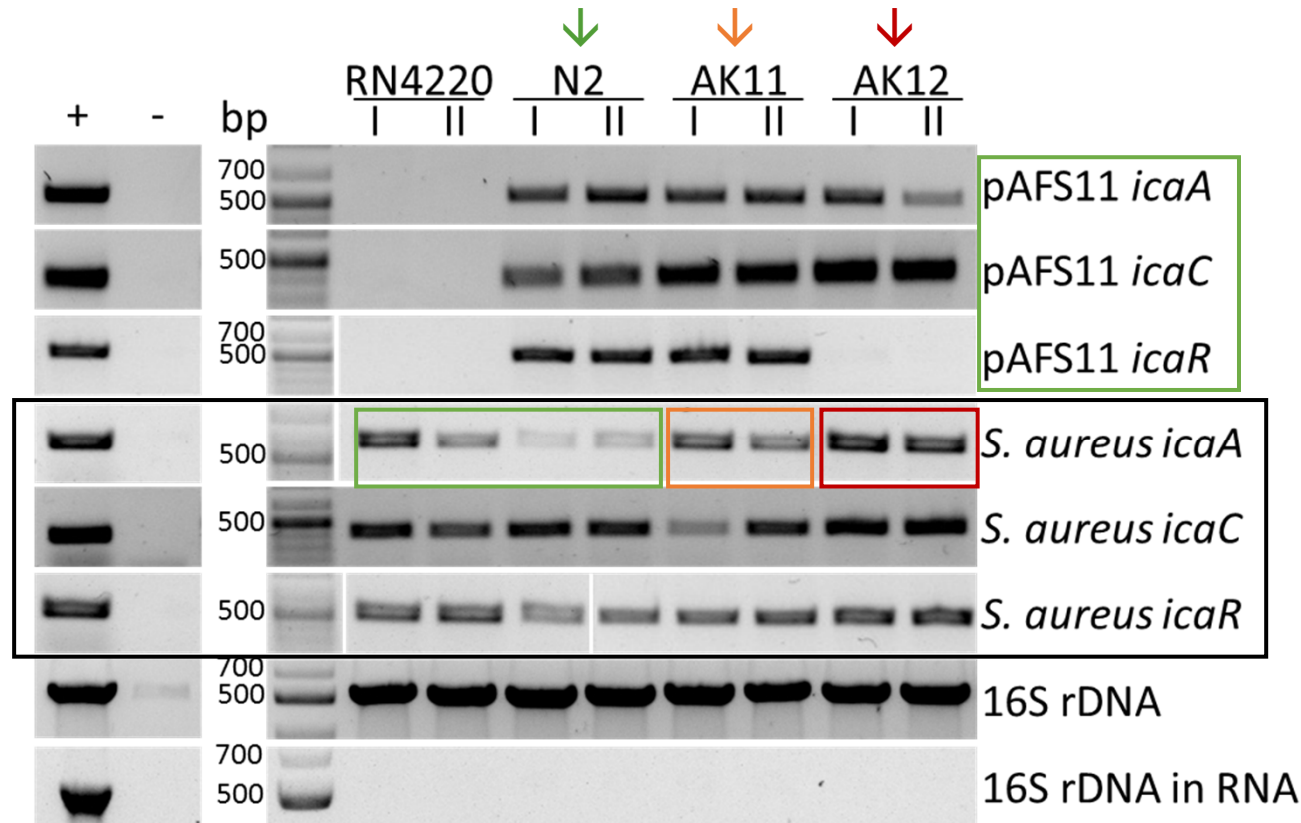


Thank you
for your attention!

Vielen Dank für Ihre
Aufmerksamkeit!



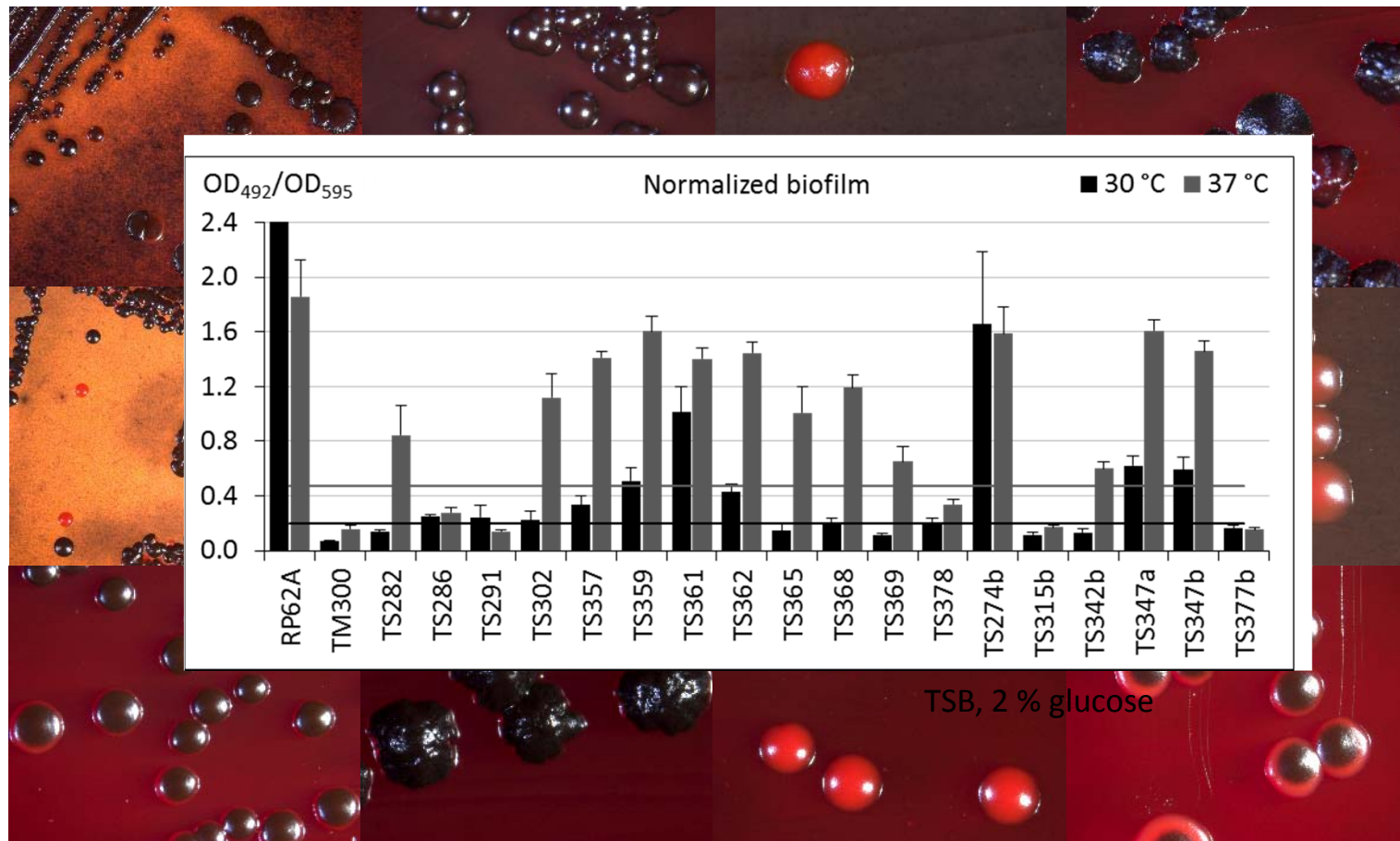
Transcription of pAFS11 *ica*-like genes and chromosomal *ica* operon



- Similar transcript levels of pAFS11 *ica* genes in AK11 and AK12, despite *icaR* deletion in AK12
- Repression of *S. aureus* *ica* genes in N2 (pAFS11 *icaR* present)
- Up-regulation of *S. aureus* *ica* genes upon lack of pAFS11 *icaR* → crosstalk?

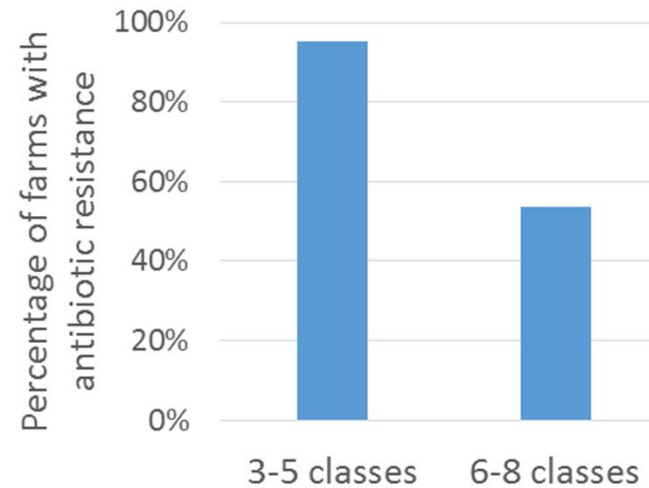
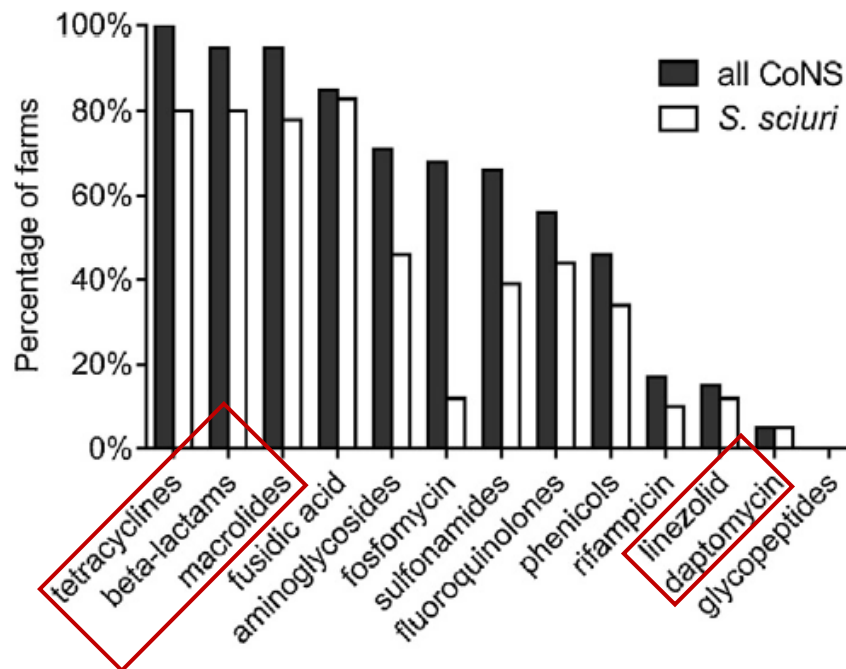
Versatile biofilm phenotypes in *S. sciuri*

- *S. sciuri* isolates tested for biofilm formation (CRA and plate assay)
- Presence of *ica*_{*sciuri*} genes alone not sufficient for biofilm formation



Antimicrobial Susceptibility Tests

- Multiresistant isolates (≥ 3 AB classes) were found in 98% of tested farms (40/41)
- Multiresistance profile is mainly driven by high occurrence of *S. sciuri*

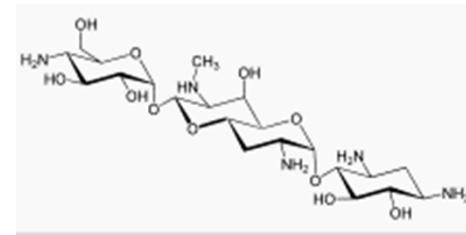
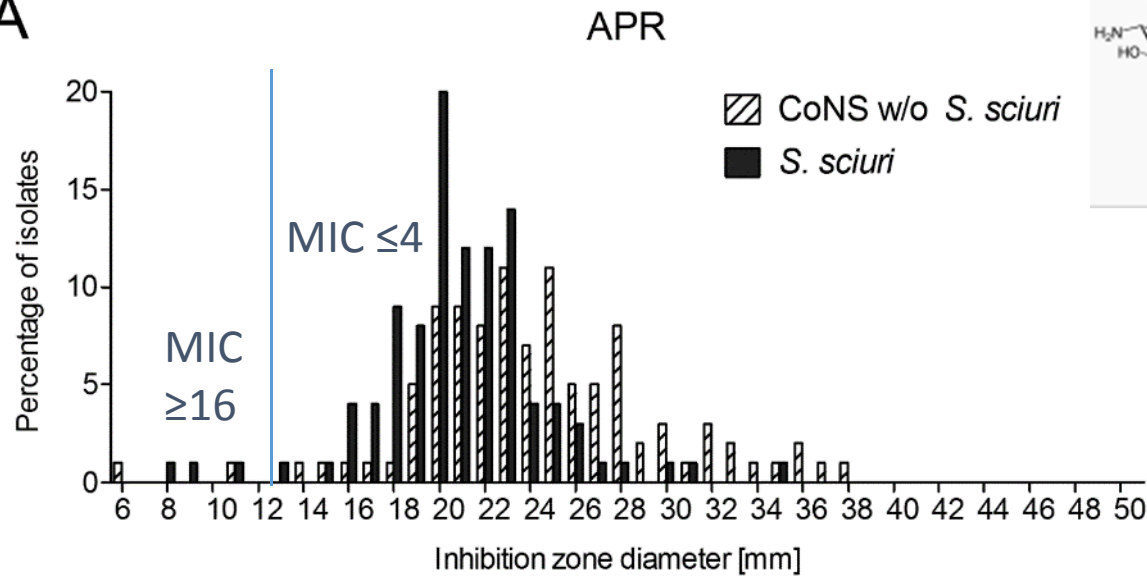


(Schoenfelder *et al.*, 2017)

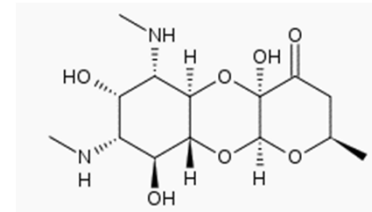
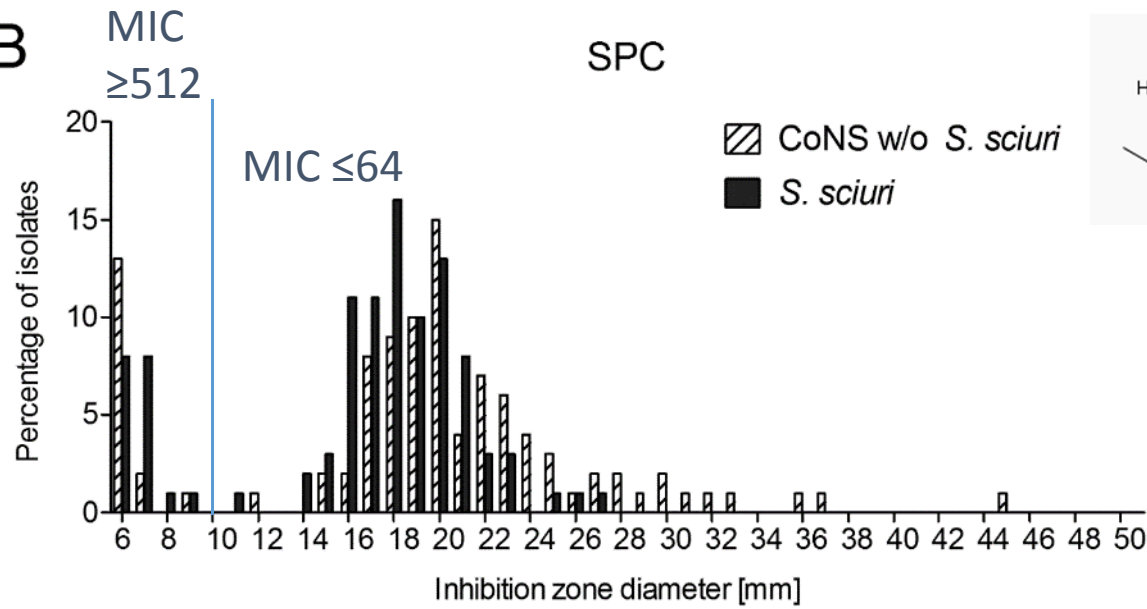
Resistance against different antibiotic classes among all tested farms

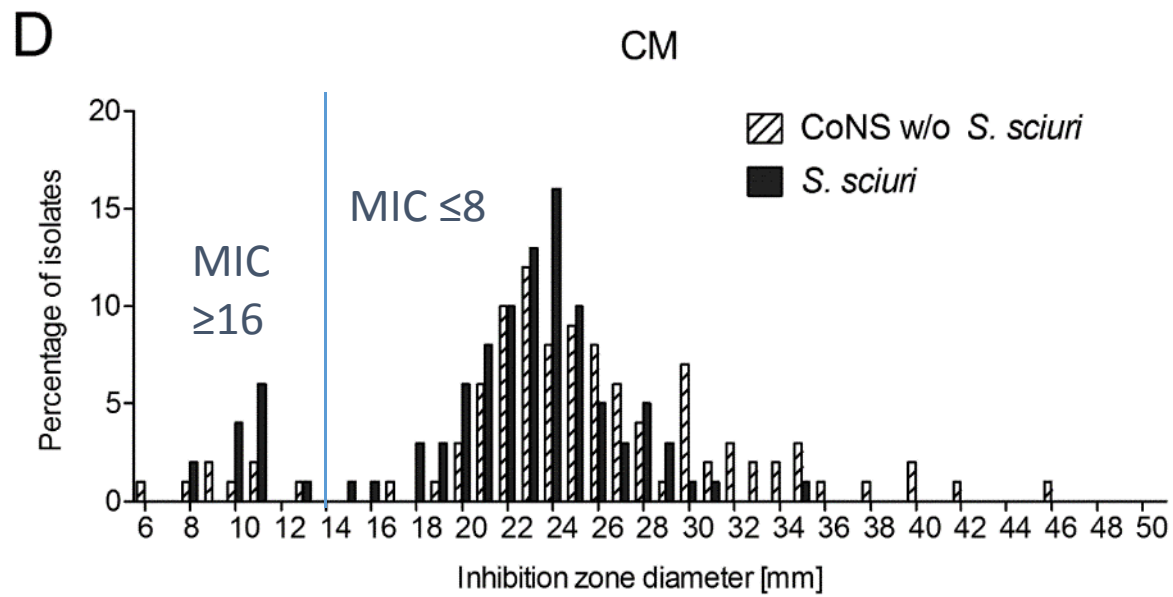
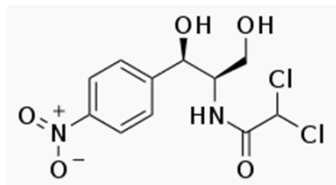
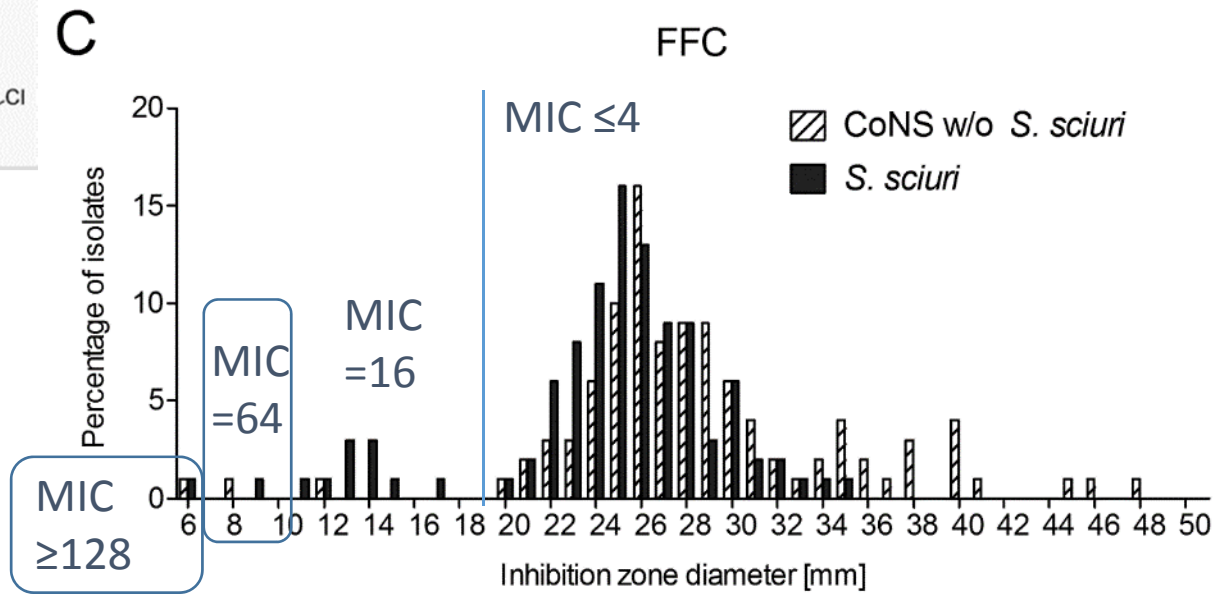
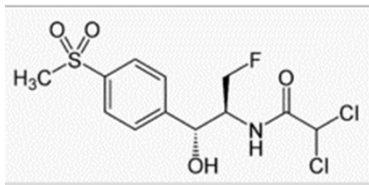
- Resistant isolates not only present in few, exceptional farms, but in all farms

A



B

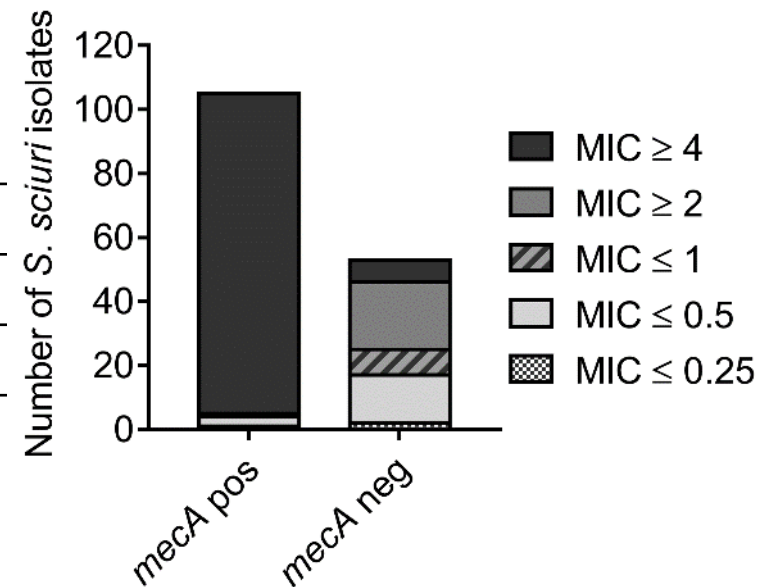






Detection of AB resistance genes by PCR

Antibiotic tested	Number of non-susceptible isolates tested	Resistance genes tested for	Number of isolates positive in PCR
Oxacillin	156/ 158 (<i>S. sciuri</i>)	<i>mecA_{SCC}</i>	105
Spectinomycin	59/ 344	<i>spc</i> <i>spw</i> <i>spd</i>	
Apramycin	4/ 344	<i>apmA</i>	
Linezolid	8/ 344	<i>cfr</i>	
Florfenicol	19/ 344	<i>fexA</i>	
Chloramphenicol	30/ 344	<i>cat</i> (pC194) <i>cat</i> (pC221) <i>cat</i> (pC223)	



(Schoenfelder *et al.*, 2017)



Presence of AB resistance genes by PCR

mecA, mecC (Oxacillin, Cefoxitin screen)

spc, spw, spd (Spectinomycin) → spectinomycin 9-O-adenyltransferase (*spc*)
novel spw likely to be of enterococcal origin

Wendlandt *et al.*, 2013
Jamrozy *et al.*, 2014

apmA (Apramycin)

Fessler *et al.*, 2011

cat (Chloramphenicol) → chloramphenicol acetyltransferase

Florfenicol is resistant to inactivation by CAT enzymes

fexA (Chloramphenicol, Florfenicol) → exporter gene

Kehrenberg and Schwarz, 2006

cfr (Chloramphenicol, Florfenicol, Clindamycin, Linezolid) → rRNA methylase



Antibiotics tested

*tested manually,
rest tested by automated system

