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Occurrence of *cfr*-encoded linezolid resistance in coagulase-negative staphylococci from livestock and exposed humans

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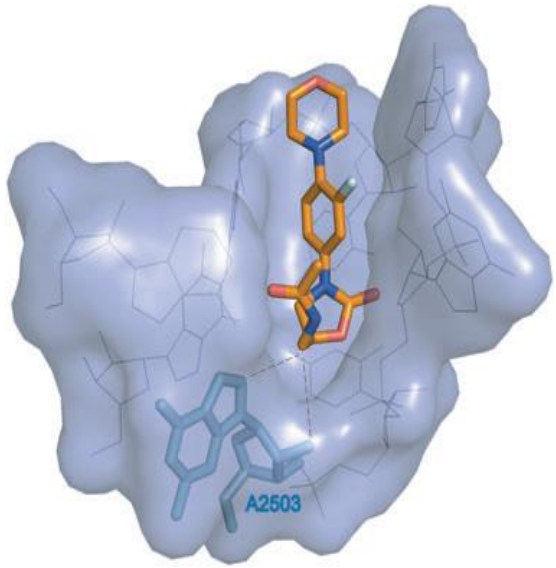
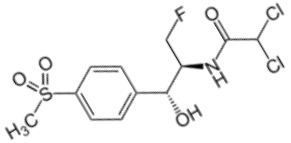
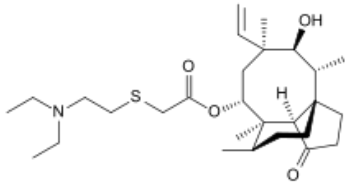
Resistance against fenicols, Lincosamids, Oxazolidinons, Pleuromutilins, Streptogramin A , „PhLOPSa“

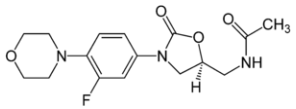




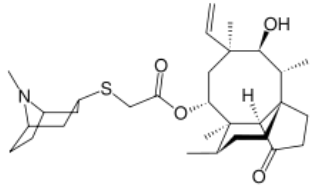
Florfenicol



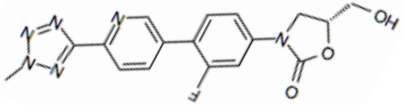
Tiamulin



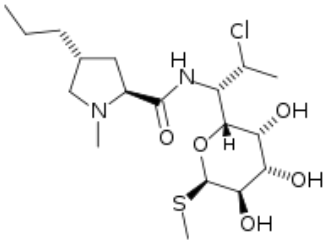
Linezolid



Retapamulin



Tedizolid



Clindamycin

Resistance Mechanisms (PhLOPSa)

- Point Mutation(s) in 23S r DNA
- Point Mutation(s), in ribosomal proteins L3, L4

Resistance against Forfenicol, Lincosamids,

- Methylation of A2503 of the23S rRNA mediated by
- *cfr*, plasmid located, transferable

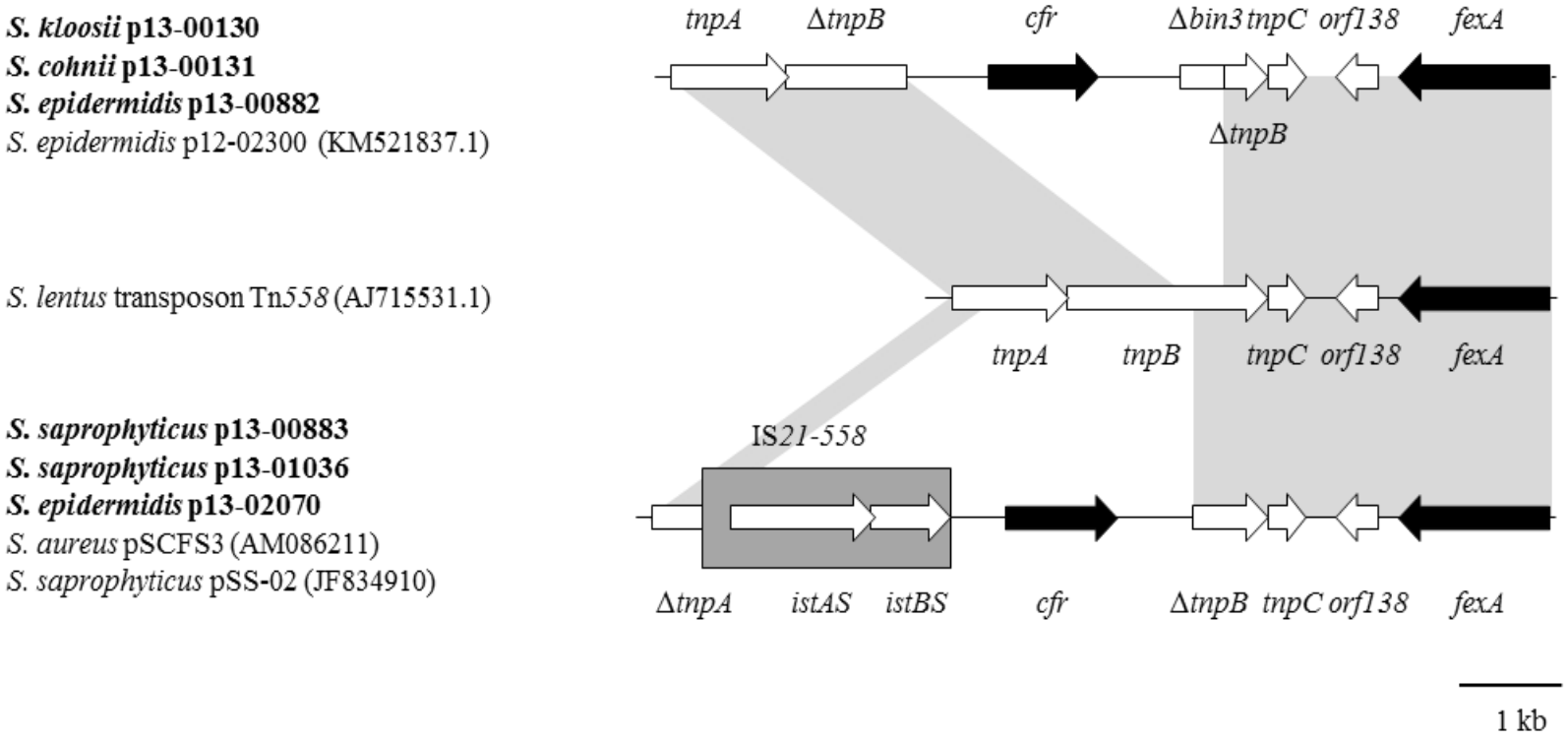
} Florfenikol, Clindamycin, Linezolid,Tiamulin, Retapamulin StreptograminA

- Efflux, ABC-porter,
  optrA, plasmid located , transferable

Resistance against Forfenicol, Clindamycin, Linezolid, Tedizolid, Tiamulin, Retapamulin, StreptograminA

Different integration sites of the *cfr* gene in *fexA*-carrying transposons

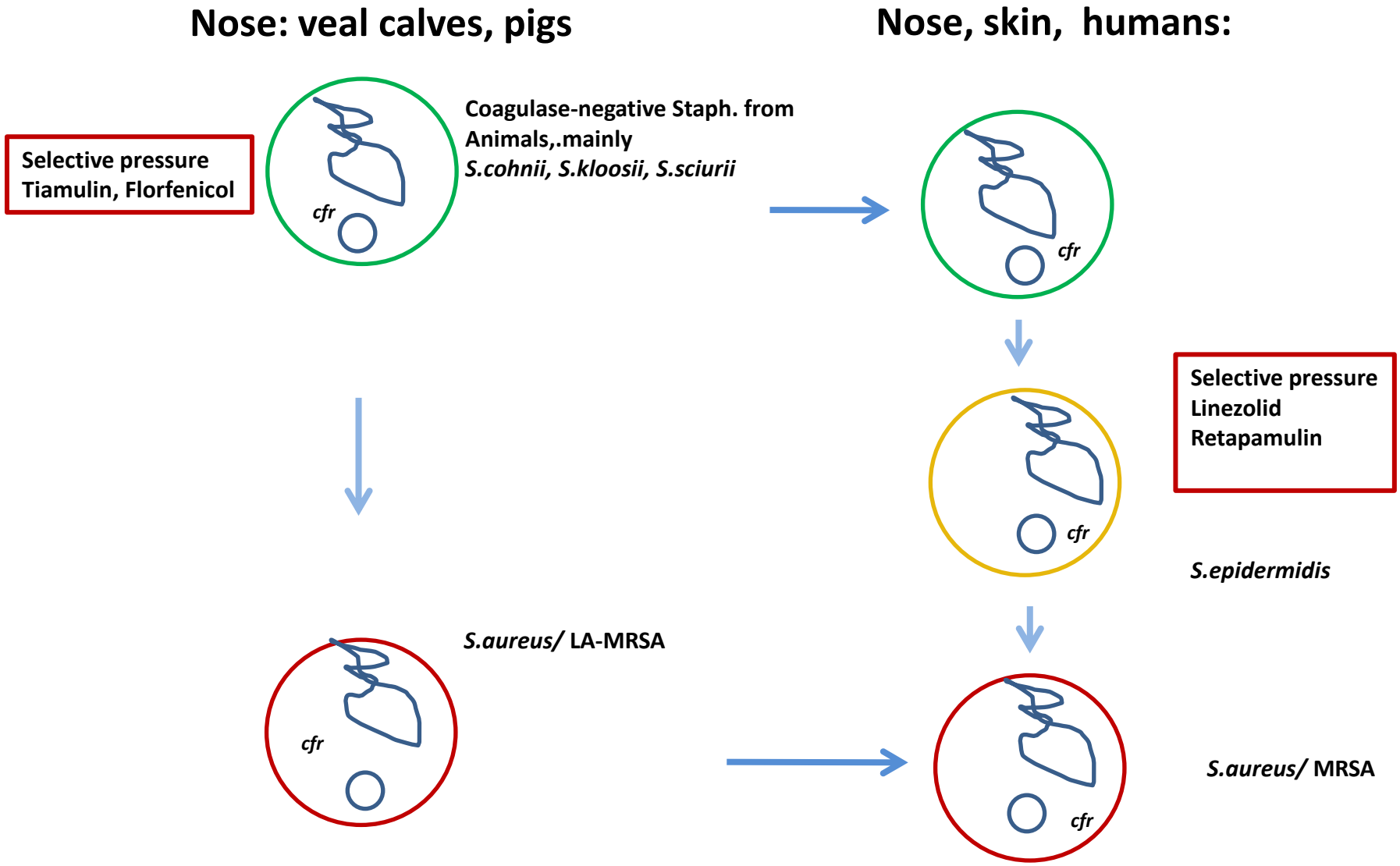
S.Schwarz and coworkers



cfr mediated resistance in staphylococci

- First description of plamids containing *cfr* in coagulase-negative staphylococci (CoNS) of livestock origin (Kehrenberg and Schwarz, 2006)
- Obviously frequent in livestock in China (Wang et al.,2012)
- Singular observations in LA-MRSA in Europe (Kehrenbereg et al., 2009)
- Emergence in nosocomial *S.epidermidis* (ST2) from infections in humans in many countries; intrahospital and interhospital spread
- Clusters of nosocomial infections with HA-MRSA ST125 containing *cfr* in Spain.
- Role of an animal reservoir of *cfr* for ist emergence in *Staphylococcus spp.* from humans ?

Possibilities of dissemination of *cfr* coded PhLOPSA resistance



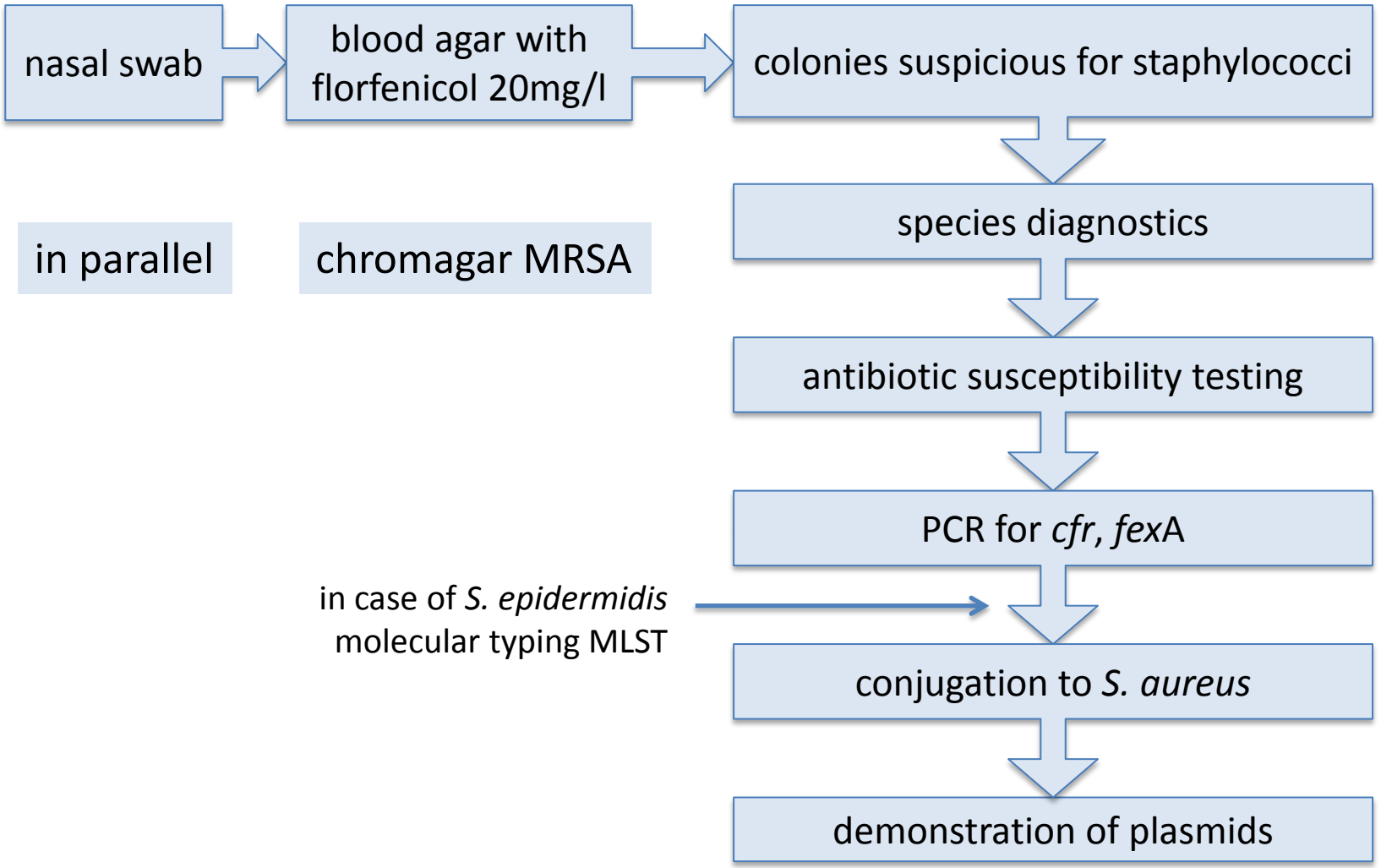
Farms enrolled into the study and demonstration of Linezolid resistant, *cfr* containing staphylococci (2014-2015)

- 3 veal calf farms in LUX which used florfenicol (no other antibiotics)
  52 animals, 10 humans, 1 dog

- 14 veal calf farms in LUX and 2 in DE with no use of florfenicol
  142 animals 8 pig farms in the south of Lower Saxony, DE

- 8 pig farms in the south of Lower Saxony, DE
  67 animals, 39 humans

Procedure for the investigation of nasal swabs from animals and humans on staphylococci containing *cfr*



Farms enrolled into the study and demonstration of Linezolid resistant, *cfr* containing staphylococci (2014-2015)

- 3 veal calf farms in LUX which used florfenicol (no other antibiotics)
  - 52 animals, 10 humans, 1 dog
  - 12 animals positive for *cfr* containing CoNS; no demonstration in swabs from humans and from the dog

- 14 veal calf farms in LUX and 2 in DE with no use of florfenicol

  142 animals, no demonstration of *cfr* containing staphylococci

- 8 pig farms in the south of Lower Saxony, DE
  - 67 animals, 39 humans

    3 farms, 11 animals and 1 human were positive for *cfr* containing CoNS

Florfenicol resistant CoNS,

Veal calfe farms using florfenicol in Luxembourg

Farm	Animal positive for FLO + R CoNS	Species	Resistance genes			Resistance traits of transconjugants[1]
			cfr	*fexA*	*optrA*	
1	6/17	*S. lentus* (4)	+	+	-	ClI, FLO, LNZ
		S. sciuri (2)	+	+	-	
2	3/17	*S. auricularis* (3)	+	+	-	
3	3/18	*S. simulans* (1)	+	+	-	
		S. cohnii (1)	+	+	-	
		S. sciuri (1)	+	+	-	

10 humans working on these farms were negative.

[1] Transconjugants harboured plasmids of 38 – 40 kb

[2] All isolates were also resistant to **retapamulin** (MIC > 16 mg/l).

Nasal colonization of farmers and veterinarians with linezolid resistant , *cfr* containing staphylococci, cross sectional sample from a meeting of the agricultural association, Luxembourg, November 2016

Farmers, (n= 29)

Veal calfes	19	two of them positive: *S.cohnii* (GEN, ERY, CLI, FUS ,**LNZ)** *S.haemolyticus* (PEN, OXA, TET, PHO**, LNZ**)
Veal calfes, pig poultry	6	
Pig	3	
Dairy cattle	1	

Veterinarians (n= 16)

Veal calfes, other livestock	14
Dairy cattle	1
Laboratory	1

Veal calf farms in Luxembourg (14) and in Germany (2) not using Florfenicol

Nasal swabs from calves (20 animals at each farm) and from humans (n=48) at these farms were negative for linezolid resistant staphylococci.

Florfenicol resistant CoNS

Pig farms in Germany

Farm	Individuals positive	Species	Resistance genes			Resistance traits of transconjugants
			cfr	*fexA*	*optrA*	
1, pigs	7/11	*S. cohnii* (4)	+	+	-	
		S. simulans (1)	+	+	-	
		S. kloosii (2)	+	+	-	
dust	2/7	*S. kloosii* (2)	+	+	-	CLI, FLO, LNZ
humans	2/3	*S. xylosus* (1)	+	+	-	
		S. cohnii (1)	+	+	-	
2, pigs	2/12	*S. saprophyticus* (2)	+	+	-	CLI, FLO, LNZ
humans	0/3					
3, pigs	2/6	*S. kloosii* (2)	+	+	-	CLI, FLO, LNZ
humans	0/3					

All isolates were also resistant to retapamulin (MIC > 16 mg/l).

Study on nasal colonization with Linezolid-resistant, *cfr* containing staphylococci of 169 veterinarious and 263 of their family members from a cohort study performed in Germany in 2012

4/169 (2,3%) of the veterinarians positive

3/263 (1,1%) of the family members positiv

Florfenicol-resistant CoNS from nasal swabs of veterinarians (n = 171) and from their family members (n = 263) in Germany

Species		Resistance genes			MLST	Resistance traits of transconjugants
		cfr	*fexA*	*optrA*		
S. epidermidis (4)	fm	+	+	-	5	CLI, FLO, LNZ
	vt	+	+	-	10	CLI, FLO, LNZ
	vt	+	+	-	2	CLI, FLO, LNZ
	vt	+	+	-	19	CLI, FLO, LNZ
S. saprophyticus (2)	fm	+	+	-		CLI, FLO, LNZ
S. saprophyticus (1)	vt	+	+	-		

Nasal swabs from persons without occupational animal contact (2013)

Swab from 363 inhabitants of the city of Braunschweig (250.000 inhabitants) taken in 2013 (Mehraij et al., 2014) were checked for florfenicol resistant CoNS, they all were negative.

cfr in CoNS species as usual colonizers/infectious agents in humans

S.epidermidis: 4 isolates from veterinarians and their family members
MLST: ST2, ST5, ST10 are known from hospitals
(Kozitskaya et al., 2005)

S.saprophyticus: 3 isolates from humans, 2 isolates from cattle
host-specificity ?

An old study from Scaninavia reported congruent seasonality of *S.Saprophyticus* UTI in humans and demonstration in cattle, pigs, and fodder
(Hedman et al., 1993)

In vitro transferability of *cfr*-carrying plasmids to S. aureus by filter mating (conjugation)

Transfer to *S.aureus :* Strain 8325-4, rif-r, mup-r was used as recipient.

Conjugation frequencies ranged from 3×10^{-6} to 5×10^{-5} .

Transconjugants contained *cfr* and *fex*A and exhibited resistance to clindamyin, linezolid, and florfenicol.

Transconjugants contained plasmids with sizes ranging between 38 to 40 kb.

Transferable wide dissemination of *cfr*

- Transconjugants from this study harboured plasmids of 38-40 kb
- The *cfr* containing segments from *S.epidermidis* (ST10), *S.cohnii,* and
- *S.kloosii* showed 99% identity with the segment of a plasmid of *S.epidermidis from a G*erman hospital (Bender et al., 2015)
- The segment contained by plamids in *S.saprophyticus* from a veterinarian showed 100% identity with that of a plasmid contained by an *S.saprophyticus* isolate from China (Cui et al., 2013).

Cfr mediated linezolid resistance in MRSA

- **Surprisingly rare in LA-MRSA CC398**
  Singular clinical case of VAP in Germany (Cuny et al., 2017)
  Singular clinical case in Belgium (Paridens et al., 2017)

- **Well known from a cluster of nosocomial infections in Madrid hospitals**
  HA-MRSA ST125,IV; (Morales et al., 2010, Sanchez et al., 2010)
- **Recent reports from Ireland (HA-MRSA ST22,IV; Shore et al., 2016)**
  Italy (HA-MRSA ST5,II, Antonelli et al., 2016)

Conclusions

- **Linezolid resistant CoNS containing *cfr* were detected in nasal swabs from veal calfes at farms that used florfenicol in Luxembourg and from pigs at conventional farms in Germany. The isolates did`nt contain *optrA.***
- **More rarely *cfr* containing CoNS were also detected in humans with occupational exposure to livestock.**
- ***cfr* containing plasmids were transferred to *S. aureus* 8325-4 by filter mating.**
- **In a population based sample from humans in the Braunschweig area in DE florfenicol/linezolid resistant staphylococci were not isolated from nasal swabs.**
- **Further surveillance is needed for timely detection of spread to humans.**

One health approach and antibiotic consumption

- **At least in cattle the emergence of *cfr* mediated resistance seems to be associated with florfenicol usage.**
- **There is most likely also selective pressure by linezolid usage in hospitals.**
- **Is topical use of retapamulin (dermatology: acne, surgery: wound infetions) really wise ?**

It is well known that topial use of antibiotics particularly favours emergence of resistance in staphylococci (fusidic acid, mupirocin, gentamicin).