Supplementary Table 1. Sources of samples tested for fatty acid auxotrophy

Ascite	2
Bronchial (aspiration*, alveolar lavage* or bronchial wash)	40
Biopsy	7
Bone biopsy	14
Catheter	1
Skin (burn*, wound or infection)	16
Drain	5
Endotracheal aspiration	2
Sputum*	14
Hemoculture	2
Pleural effusion	2
Superficial pus	21
Deep pus	3
Nose	2
Feces	2
TOTAL	133

Supplementary Table 2. Triclosan resistance revealed by exogenous fatty acids in *S. aureus* clinical and veterinary isolates

81 % triclosan-sensitive	
Superficial wound	141
Biopsy	50
Carrier	38
Bronchial wash	36
Sputum	35
Hemoculture	32
Mastitis [a]	27
Urine	17
Deep wound	11
Drain	6
ORL	7
n.d	2
Catheter	1
Unknown	158
TOTAL	561

12 % triclosan-resistant	
Superficial wound	17
Drain	4
Carrier	3
Urine	3
Biopsy	2
Sputum	1
Blood culture	1
Bone biopsy	1
Mastitis [a]	29
unknown	24
TOTAL	85

7 % FA-T^R	
Superficial wound	16
Biopsy	4
Sputum	4
Deep wound	3
Blood culture	2
Carrier	2
Catheter	1
Mastitis [a]	7
unknown	10
TOTAL	49

a : Isolates from bovine mastitis cases

n.d: not determined

Supplementary Table 3. Source, Spa typing and MLST of Endo and Exo isolates

FA profile	Isolate	Source	Spa type	MLST Clonal complex
Endo	Harm 34-50	Unknown	TMDMGMK	CC5
	P^3 23	Biopsy	YHGFMBQBLO	CC8
	$P^3$138	Biopsy	YHGFMBQBLO	CC8
	$P^2$71	Carrier	YHGFMBQBLO	CC8
	Harm 47-50	Unknown	WGKAOMQ	CC8
	Harm 19-50	Unknown	WGKAOMQ	CC8
	$P^2$81	Deep	YHGFMBQBLO	CC8
	$P^2$69	Superfic	YHGFMBQBLO	CC8
	$P^2$89	Superfic	YHGFMBQBLO	CC8
	$P^3$146	Superfic	YHGFMBQBLO	CC8
	$P^3$151	Superfic	YHGFMBQBLO	CC8
	$P^3$12	Superfic	XMM	CC45
	Trsa276	Sputum	XKBQBBMM	CC45
	$P^3$1	Biopsy	XKAOAOBO	CC398
	$P^3$101	Biopsy	XKAOAOBO	CC398
	$P^3$73	Deep	XKANOAOBO	CC398
	$P^3$139	Superfic	XKAOAOBO	CC398
	$P^2$74	Superfic	UJGBBGGJ	CC15
	$P^3$49	Superfic	UJGBBGGJ	CC20
	$P^3$109	Catheter	UJGBBGJAGJ	CC20
Exo	Trsa 275	Sputum	TJMBMAMGMK	CC5
	$P^1$2	Superfic	XKAKBEBKB	CC45
	$P^1$113	Superfic	UPE	ND
	$P^1$127	Superfic	UPE	ND
	$P^1$46	Superfic	ZFGMGGM	CC25
	$P^1$76	Superfic	UJGBBGGJAGJ	CC20
	Trsa 36	Sputum	UJFMBGJAGJ	CC20
	MT8099	Mastitis	UG2MFBBLB	CC20
	MT8348	Mastitis	UG2MFBBLB	CC20
	MT8333 8333	Mastitis	UJGBBGBJAGJAGJ	CC20
	MT8343	Mastitis	UKEBPB	CC97
	MT8059	Mastitis	UJGFMBPB	CC97
	Trsa 277	Sputum	YHGFMBQBLO	CC8

Selected strains were analyzed by spa typing and Multilocus sequence typing (MLST). Repeats region of protein A gene (*spa*) is routinely used for reliable and discriminatory typing of *S. aureus*. Repeats are assigned a numerical code and spa-type is deduced from the order of specific repeats; the repeat nomenclature was that of Shopsin and colleagues (1) and spa types were retrieved from the Ridom SpaServer (http://spaserver.ridom.de). PCR primers and conditions used for the spa tandem repeat amplification were as described (2). PCR products were purified using the QIAquick PCR purification kit (Qiagen, Courtaboeuf,

France) and sequenced (Eurofins MWG Operon, Ebersberg, Germany). The primers and PCR conditions for MLST were as described at http://saureus.mlst.net/ (3). PCR products were purified as above and sequenced (Beckman-Coulter Genomics), and sequence types (STs) were identified using the MLST database (http://www.mlst.net).

1. **Shopsin B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE, Bost DA, Riehman M, Naidich S, Kreiswirth BN.** 1999. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. J Clin Microbiol **37:**3556-3563.
2. **Harmsen D, Claus H, Witte W, Rothganger J, Claus H, Turnwald D, Vogel U.** 2003. Typing of methicillin-resistant *Staphylococcus aureus* in a university hospital setting by using novel software for spa repeat determination and database management. J Clin Microbiol **41:**5442-5448.
3. **Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG.** 2000. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. J Clin Microbiol **38:**1008-1015.

Supplementary Table 4. FabI changes in FA-T^R isolates with a functioning FASII

Isolate	FabI	Additional FabI (sh-FabI [a][1])
$P^2$81	Phe204Cys [1-3]	+
$P^3$12	Ala198Gly [1,4]	+
Harm 3450	Ala198Gly [1,4]	ND
Trsa 277[b]	Ala198Gly [1,4]	-
NL 36[c]	Ala198Gly [1,4]	ND
MT 8333[c]	Ala95Val [1,5]	-
Harm 4750	Ala95Val [1,5]	ND
$P^1$171[c]	His61Tyr[d]	+
$P^1$179[c]	His61Tyr[d]	+
$P^1$190[c]	His61Tyr[d]	+
$P^1$130[c]	His61Tyr[d]	+
$P^3$1	His61Tyr[d]	+
$P^3$73	His61Tyr[d]	+
$P^3$101	His61Tyr[d]	+
$P^3$139[b]	His61Tyr[d]	+
$P^2$69	WT	+
$P^2$71	WT	+
$P^2$74	WT	+
$P^2$89	WT	+
$P^3$23	WT	+
$P^3$49	WT	+
$P^3$109	WT	+
$P^3$138	WT	+
$P^3$146	WT	+
$P^3$151	WT	+
Harm 1950	WT	+
MT 8059	WT	+
Trsa 276	WT	+
Harm 2950	WT	+
$P^1$19	WT	ND
$P^1$39	WT	ND

a: sh-FabI likely transferred from *Staphylococcus haemolyticus* [1]. b: profile evolved from an Exo-FA profile c: profile evolved from an Intermediate profile,. d: variant likely

unrelated to triclosan resistance as it is present in hundreds of published *S. aureus* genome sequences and the amino acid position is not structurally conserved. ND: not determined.

1. **Ciusa ML, Furi L, Knight D, Decorosi F, Fondi M, Raggi C, Coelho JR, Aragones L, Moce L, Visa P, Freitas AT, Baldassarri L, Fani R, Viti C, Orefici G, Martinez JL, Morrissey I, Oggioni MR.** 2012. A novel resistance mechanism to triclosan that suggests horizontal gene transfer and demonstrates a potential selective pressure for reduced biocide susceptibility in clinical strains of *Staphylococcus aureus*. Int J Antimicrob Agents **40:**210-220.
2. **Nielsen LN, Larsen MH, Skovgaard S, Kastbjerg V, Westh H, Gram L, Ingmer H.** 2013. *Staphylococcus aureus* but not *Listeria monocytogenes* adapt to triclosan and adaptation correlates with increased *fabI* expression and *agr* deficiency. BMC Microbiol **13:**177.
3. **Fan F, Yan K, Wallis NG, Reed S, Moore TD, Rittenhouse SF, DeWolf WE, Jr., Huang J, McDevitt D, Miller WH, Seefeld MA, Newlander KA, Jakas DR, Head MS, Payne DJ.** 2002. Defining and combating the mechanisms of triclosan resistance in clinical isolates of *Staphylococcus aureus*. Antimicrob Agents Chemother **46:**3343-3347.
4. **Brenwald NP, Fraise AP.** 2003. Triclosan resistance in methicillin-resistant *Staphylococcus aureus* (MRSA). J Hosp Infect **55:**141-144.
5. **Skovgaard S, Nielsen LN, Larsen MH, Skov RL, Ingmer H, Westh H.** 2013. *Staphylococcus epidermidis* isolated in 1965 are more susceptible to triclosan than current isolates. PLoS One **8:**e62197.