A tandem duplication in the *ermC* translational attenuator of the macrolide-lincosamide-streptogramin B resistance plasmid pSES6 from *Staphylococcus equorum*

Lodder, G., Schwarz, S., Gregory, P., Dyke, K.G.H.
Antimicrobial Agents and Chemotherapy 40 (1996), 215-217.

A tandem duplication of 23 bp was identified in the the *ermC* gene translational attenuator of plasmid pSES6 from *Staphylococcus equorum* which mediated constitutive resistance to macrolide-lincosamide-streptogramin B antibiotics. This duplication included the ribosome binding site for the *ermC* gene as well as the first five base pairs of the *ermC* coding sequence. It was postulated that this sequence duplication affects the possible RNA conformations so that the ribosome binding site for ErmC synthesis is readily accessible to the ribosomes and thus constitutive expression of the *erm*C gene occurs.

Macrolide-lincosamide-streptogramin B resistance in *Staphylococcus lentus* results from the integration of part of a transposon into a small plasmid

Werckenthin, C., Schwarz, S., Dyke, K.G.H.

Antimicrobial Agents and Chemotherapy 40 (1996), 2224-2225.

The 8.0 kbp macrolide-lincosamide-streptogramin B resistance plasmid pSES20 from *Staphylococcus lentus* harboured part of a Tn*917*-analogous transposon including the left terminal repeat, the *erm*B gene and its regulatory region as well as the internal direct repeat. Homology between pSES20 and Tn*917* stopped at a sequence closely related to the resolution site of Tn*917* and staphylococcal recombination sites.

Molecular analysis of naturally occurring *erm*C-encoding plasmids in staphylococci isolated from animals with and without previous contact with macrolide/lincosamide antibiotics

Lodder, G., Werckenthin, C., Schwarz, S., Dyke, K.G.H.

FEMS Immunology and Medical Microbiology 18 (1997) 7-15.

A total of sixteen epidemiologically unrelated macrolide resistant staphylococcal isolates of various animal origins were investigated for the molecular basis of macrolide resistance with respect to previous contact of their host animals with macrolides and lincosamides. All isolates carried *erm*C-encoding plasmids of 2.3 - 4.0 kbp. The eight plasmids of staphylococci from animals which had not received macrolides or lincosamides showed inducible *erm*C gene expression and did not exhibit alterations in the *erm*C regulatory region. The remaining eight plasmids expressed the *erm*C gene constitutively. Six of these plasmids were from staphylococci from animals which had received tylosin or spiramycin as feed additives or lincomycin for therapeutic purposes. All constitutively expressed *erm*C genes revealed either sequence deletions or sequence duplications in their *erm*C regulatory region, as detected by a PCR assay and by sequence analysis. These sequence deletions and duplications found in naturally occurring plasmids corresponded closely to the mutations seen in the *erm*C-encoding plasmids after growth of an inducibly resistant strain in the presence of non-inducing macrolides or lincosamides under *in-vitro* conditions.

Resistance to macrolides, lincosamides and streptogramins in staphylococci from humans and animals

Werckenthin, C., Lodder, G., Schwarz, S.

Chemotherapie Journal 6 (1997) 103-110.

Macrolides, lincosamides and streptogramins (MLS antibiotics) represent three different classes of antibiotics which are mainly used in human and veterinary medicine for the control of grampositive bacterial pathogens. In addition, certain macrolides and streptogramins are used as feed additives in animal production. Resistances of staphylococci to MLS antibiotics are based on three different resistance mechanisms: (1) enzymatic inactivation, (2) efflux, and (3) target site modification. The latter mechanism is most frequently observed and based on the dimethylation of the adenine residue at position 2058 in 23S rRNA. This chemical modification prohibits binding of the antibiotics and consequently renders the ribosomes resistant to the inhibitory effects of the respective antibiotics. Three methylases, ErmA, ErmB, and ErmC, have been identified in staphylococci. The corresponding genes are located either on transposons (*erm*A, *erm*B) or on small plasmids (*erm*C). Among these *erm* genes, *erm*C has been detected most frequently in staphylococci from humans and animals. Expression of the *erm*C gene may be either inducible by certain macrolides or constitutive. Inducible *erm*C gene expression is regulated by translational attenuation. A change from inducible to constitutive *erm*C gene expression is based on structural alterations in the *erm*C regulatory region. The change of the type of *erm*C gene expression may be due to the application of MLS antibiotics which are not capable of acting as effective inducers of *erm*C gene expression. Moreover, alteration of the type of *erm*C gene expression is of clinical relevance because it is accompanied by a widening of the spectrum of antibiotics to which resistance is expressed, e.g. limited resistance to 14- and 15-membered macrolides as mediated by inducibly expressed *erm*C genes change versus extended resistance to 14-, 15-, and 16-membered macrolides, lincosamides, and B-compounds of streptogramins as conferred by constitutively expressed *erm*C genes.

Molecular analysis of the macrolide-lincosamide resistance gene region of a novel plasmid from *Staphylococcus hyicus*

Schwarz, S., Lange, C., Werckenthin, C.

Journal of Medical Microbiology 47 (1998), 63-70.

A 4.0 kbp plasmid from *Staphylococcus hyicus*, designated pSES21, mediated resistance to macrolides and lincosamides as shown by protoplast transformation. It differed distinctly in its restriction map from all other so far reported staphylococcal macrolide resistance plasmids. Southern blot hybridization with gene probes specific for staphylococcal *erm* genes identified the resistance gene to belong to the hybridization class C. Analysis of the *erm*C gene revealed that the deduced amino acid sequence of the pSES21-encoded ErmC methylase exhibited about 93% identity to the ErmC methylases encoded by plasmid pE194. The *erm*C gene of pSES21 was expressed constitutively. Sequence analysis of the *erm*C regulatory region showed multiple base pair insertions and substitutions in the translational attenuator. As a consequence of these mutations, the reading frame of the small regulatory peptide was destroyed and a novel pair of inverted repeated sequences was generated. Previous studies identified sequence deletions and sequence duplications in the *erm*C regulatory region as the basis for constitutive *erm*C gene expression. The multiple point mutations shown in the pSES21-encoded *erm*C translational attenuator represent a novel kind of structural alterations in this regulatory region and may explain constitutive *erm*C gene expression by pairing of the newly generated inverted repeated segments in the presence of a functionally deleted reading frame for the small regulatory peptide.

Host range of the *ermF* rRNA methylase gene in human and animal bacteria

Chung, W.O., Werckenthin, C., Schwarz, S., Roberts, M.C.

Journal of Antimicrobial Chemotherapy 43 (1999), 5-14.

We screened 183 different clinical anaerobic and aerobic bacteria isolated from man and animals for the presence of the *ermF* gene using a polymerase chain reaction (PCR) assay. The *ermF* gene was detected in 107 (58%) clinical isolates, including 42 (61%) of 69 Gram-positive bacteria and 65 (57%) of 114 Gram-negative bacteria. Twenty-five ATCC isolates were also tested, and twenty (80%) carried the *ermF* gene. The gene products from the *ermF* polymerase chain reaction from 4 isolates were sequenced and showed 95 - 99 % homology at the DNA level and 98 - 99 % amino acid homology with the *ermF* gene. Eleven (58%) of the 19 Gram-negative donors tested were able to transfer the *ermF* gene, whereas all nine (100%) of the Gram-positive donors tested transferred the *ermF* gene, using either *Enterococcus faecalis* or *Haemophilus influenzae* as the recipients.

Structural alterations in the translational attenuator of constitutively expressed *ermC* genes

Werckenthin, C., Schwarz, S., Westh, H.
Antimicrobial Agents and Chemotherapy 43 (1999), 1681-1685.

Sequence deletions of 16, 59, and 111 bp as well as a tandem duplication of 272 bp with respect to the corresponding sequence of pT48 were identified in the regulatory regions of constitutively expressed *ermC* genes. Constitutive *ermC* gene expression as a consequence of these structural alterations is based on either the prevention of the formation of mRNA secondary structures in the translational attenuator or the preferential formation of those mRNA secondary structures which do not interfere with the translation of the *ermC* transcripts. A model for the development of sequence deletions in the *ermC* translational attenuator by homologous recombination is presented and experimentally tested by *in-vitro* selection of constitutively expressed mutants in staphylococcal strains deficient and proficient in homologous recombination.

Integration of pT181-like tetracycline resistance plasmids into large staphylococcal plasmids Involves IS*257*

Werckenthin, C., Schwarz, S., Roberts, M.C.

Antimicrobial Agents and Chemotherapy 40 (1996), 2542-2544.

Four large staphylococcal plasmids ranging in size from 31 to 82 kbp have been shown to mediate tetracycline resistance via an integrated copy of the *tet*(K)-encoding plasmid pT181 which was flanked by copies of the insertion element IS*257.* In two cases, IS*257* elements interrupted the *repC* reading frame of pT181 and a 8 bp sequence from within the *repC* gene was duplicated at the interrupted site. In the third plasmid, the IS*257* elements interrupted the pT181 DNA immediately upstream of the *repC* coding sequence with a 8 bp duplication. In the fourth case, the IS*257* elements flanked a pT181-like plasmid with one IS*257* in the *repC* coding sequence and the other within the recombinase (*pre*) coding sequence, so that a section of the pT181 sequence was deleted. All four integration sites detected in this study differ from those previously described for the IS*257*-mediated integration of pT181 like plasmids into large plasmids or into the chromosomal DNA.

A novel plasmid from *Staphylococcus epidermidis* specifying resistance to kanamycin, neomycin and tetracycline

Schwarz, S., Gregory, P.D., Werckenthin, C., Curnock, S., Dyke, K.G.H.

Journal of Medical Microbiology 45 (1996), 57-63

The naturally occurring plasmid pSTS7 from *Staphylococcus epidermidis* mediated resistance to tetracycline via a *tet*L gene and to kanamycin and neomycin via an *aad*D gene. Plasmid pSTS7 showed partial restriction map and sequence homology to the previously described tetracycline resistance plasmid pNS1981 from *Bacillus subtilis* and to the kanamycin/neomycin/bleomycin resistance plasmid pUB110 from *Staphylococcus aureus*. Sequence analysis of the regions flanking the two resistance genes in pSTS7 led to the identification of a novel site for interplasmid recombination which could explain the derivation of pSTS7 from the incompatible pNS1981- and pUB110-like parental plasmids under tetracycline-selective pressure.

Tetracycline resistance in *Salmonella enterica* subsp. *enterica* serovar Dublin

Frech, G., Schwarz, S.

Antimicrobial Agents and Chemotherapy 42 (1998), 1288-1289

The 47 kbp plasmid pGFT1 from *Salmonella enterica* subsp. *enterica* serovar *dublin* mediated tetracycline resistance via a *tet*(A) gene located on an integrated copy of a Tn*1721*-analogous transposon. The integration site of the transposon was located within the reading frame of a *fip* gene. Plasmid pGFT1 was shown to be conjugative and to be able to replicate and express tetracycline resistance in *Escherichia coli*.

Plasmid-encoded tetracycline resistance in *Salmonella enterica* subsp. *enterica* serovars Choleraesuis and Typhimurium: identification of complete and truncated Tn*1721* elements

Frech, G., Schwarz, S.

FEMS Microbiology Letters 176 (1999), 97-103.

During routine screening of *Salmonella enterica* subsp. *enterica* (*S.*) isolates of animal origin for plasmid-encoded tetracycline resistance, two tetracycline resistance plasmids, the 50 kbp plasmid pGFT3 of *S.* Choleraesuis and the 9.5 kbp plasmid pGFT4 of *S.* Typhimurium var. Copenhagen DT002, were detected. The respective tetracycline resistance genes (*tet*) were identified by hybridization and PCR analysis to belong to hybridization class A. Conjugation experiments identified plasmid pGFT3 as a conjugative plasmid. Molecular analysis of the *tet*(A) gene area and the flanking regions identified a complete Tn*1721*-like transposon on plasmid pGFT3 and a truncated Tn*1721*-like element on plasmid pGFT4. The complete Tn*1721*-like element was integrated into a transposase reading frame of a truncated Tn*3* transposon also located on plasmid pGFT3. The truncated Tn*1721*-like element of plasmid pGFT4 lacked the entire transposase part. This Tn*1721*-relic was integrated in an unknown reading frame which on amino acid level showed homology to the Rop protein of *E. coli*. A model for the deletion of the transposase part was developed on the basis of the sequences present at the termini of the truncated Tn*1721*-like element.

Tn*5706*, a transposon-like element from *Pasteurella multocida* mediating tetracycline resistance

Kehrenberg, C., Werckenthin, C., Schwarz, S.
Antimicrobial Agents and Chemotherapy 42 (1998), 2116-2118.

The 4378 bp putative tetracycline resistance transposon Tn*5706* of *Pasteurella multocida* is composed of an internal *tet*(H)-*tetR* resistance gene region which is flanked by almost identical insertion elements IS*1596* and IS*1597*. Two reading frames for proteins of 70 and 228 amino acids were detected in each of these IS elements. The 228 aa protein revealed homology to transposase proteins of Gram-positive bacteria.

Tetracycline resistance in *Staphylococcus* spp. from domestic animals

Schwarz, S., Roberts, M.C., Werckenthin, C., Pang, Y., Lange, C.

Veterinary Microbiology 63 (1998), 217-227.

A total of 838 staphylococcal isolates representing nineteen different species were obtained from cattle, cats, dogs, ducks, guinea pigs, horses, mink, pigeons, pigs, rabbits, and turkeys. From these 228 (27.2 %) isolates were shown to be resistant to tetracycline and to carry one or two of the tetracycline resistance (*tet*) genes *tet*(K), *tet*(L), *tet*(M), or *tet*(O) with seven different distribution patterns. Additional resistances to one or more antibiotics were observed in 153 (67.1 %) of the tetracycline resistant isolates. The *tet*(M) gene was found in 94.3 % of the *S. intermedius* isolates while the *tet*(K) gene predominated in most of the other staphylococcal species independently of the host animal. The *tet*(K) and *tet*(L) genes were located on plasmids while the *tet*(M) and *tet*(O) genes appeared to be associated with the chromosome.

Resistances to tetracyclines, macrolides and chloramphenicol in staphylococci of animal origin

Schwarz, S., Werckenthin, C., Lodder, G., Lange, C.
BIO*spectrum* Suppl. 1/97 (1997), 5-8.

Genes conferring resistances to tetracyclines, macrolides and chloramphenicol are widely distributed among staphylococci from animals. So far, three tetracycline resistance genes, *tet*K, *tet*L, and *tet*M, another three macrolide resistance genes *erm*A, *erm*B, and *erm*C as well as two chloramphenicol *cat* genes have been identified. Since all these resistance genes are often located on plasmids and/or transposons, they are easily interchanged between the various staphylococcal species from humans and animals, but also between staphylococci and other Gram-positive bacteria.

Resistances to protein biosynthesis inhibitors in staphylococci: resistance genes and their spread

Werckenthin, C., Schwarz, S.

Berliner und Münchener Tierärztliche Wochenschrift 109 (1996), 348-354.

The rapid spread of antibiotic resistances in a wide variety of bacteria is mainly due to the location of antibiotic resistance genes on mobile genetic elements such as plasmids and transposons. Principal ways of transfer of plasmid- and transposon-encoded resistance genes are presented using examples of the predominant genes mediating resistances to protein biosynthesis inhibitors such as tetracyclines, aminoglycosides, macrolide-lincosamide-streptogramin B antibiotics, and chloramphenicol in staphylococci. Transfer between different staphylococcal cells is substantially based on transduction, transformation, conjugation and mobilization while transfer of resistance genes within the same bacterial cell often includes interplasmidic recombination events and chromosomal integration of resistance plasmids or transposons. The abilities of the transferred resistance plasmids or transposons to integrate or to be integrated into DNA molecules, plasmids or chromosomal DNA, of the new host cell are of major importance to circumvent strain-, species- or genus-specific barriers such as restriction/modification systems, plasmid incompatibilities or deficiencies of plasmid replication which may limit efficient resistance gene transfer.

Aspects of bacterial resistance to antimicrobials used in veterinary dermatological practice

SCHWARZ, S., NOBLE, W.C.

Veterinary Dermatology 10 (1999), 163-176.

Aspects of bacterial resistance to the major classes of antimicrobials used in veterinary dermatology are presented in this review. Resistance of Gram-positive and Gram-negative bacteria to tetracyclines, macrolide-lincosamide-streptogramin antibiotics, chloramphenicol, mupirocin, sulfonamides, trimethoprim, aminoglycosides, fluoroquinolones and ß-lactam antibiotics are depicted with respect to the different mechanisms of acquired and intrinsic resistance. Examples are given for the three major resistance mechanisms, enzymatic inactivation, decreased intracellular drug accumulation and target modification. In addition, basic informations about mobile genetic elements which carry resistance genes, such as plasmids, transposons and gene cassettes, and their modes of spreading via transduction, conjugation, mobilization and transformation are provided.