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Brief Communication

Regulation of the *erm*(C) Gene in Staphylococci from Reservoir with Different Usage of Macrolides

By *Lars B. Jensen* and *Frank. M. Aarestrup*

Danish Institute for Food and Veterinary Research, Bülowsvej 27, DK-1790 Copenhagen V, Denmark.

A high prevalence of macrolide resistant isolates has been found among staphylococci isolated from animals (4). The *erm*(C) gene is the most common gene encoding macrolide resistance in staphylococci (8). It is well known that expression of the *erm*(C) gene is normally regulated by formation of hairpin structures upstream for the *erm*(C) gene (5,9) rendering the start codon of *erm*(C) gene non-accessible. Only 14- and 15-membered macrolides like erythromycin can induce expression of the gene and induce resistance while 16-membered macrolides cannot activate expression of *erm* (C) (7). If deletions from 16 to 116 bp occur in the regulatory area, expression of the *erm*(C) gene becomes constitutive (10). Constitutive expressed *erm*(C) genes give resistance not only to 14-and 15-membered macrolides, like erythromycin, but also to 16-membered macrolides like spiramycin, tylosin and streptogramin B (6). Deletions are believed to be the result of high concentration of non-inducible macrolides like tylosin in the environment, selecting for constitutive expression of the macrolide resistance. In the presence of macrolides, like tylosin, this could give staphylococci with constitutive expressed *erm*(C) a selective advantage not only to sensitive staphylococci but also to staphylococci containing regulated *erm*(C) genes. In this article we have investigated the ratio of regulated and constitutive expressed *erm*(C) genes in human and animal reservoirs (cattle and pigs) with differences in uses of the 16-membered macrolide tylosin.

Large amounts of the macrolide tylosin have been used for pig production in Denmark for growth promotion and therapy (1). In 1996 68,350 kg of tylosin was used for growth promotion and 1,350 kg for therapy. No macrolides have been used for growth promotion for cattle but spiramycin and tylosin have been used therapeutically for treatment of mastitis (3). A total of 644 kg macrolides, primarily tylosin, was used for cattle in 1996 in Denmark. Local variations in treatment strategies exist depending on the choice of the veterinarian but due to the used strain collection this effect will be minimal. At the same time 5,934 kg of penicillin was used (Erik Jacobsen, personal communication). The usage of macrolides for treatment of infections in human in general practice constitutes approximately 20-25 percent of the total usage of antibiotics in humans. However, in human medicine 16-membered macrolides are not used. The macrolides used in human medicine in Denmark are primarily erythromycin (14-membered) and azithromycin (2).

A total of 185 macrolide resistant staphylococci were tested, twenty-nine staphylococci from cattle (8 *Staphylococcus aureus* and 21 coagulase negative staphylococci (CNS)), 111 *Staphylococcus hyicus* isolates of porcine origin and 45 *S. aureus* from non-hospitalized humans (4). All animal isolates were obtained from the DANMAP surveillance program with one isolate per herd hereby representing a broad spectrum of farms in Denmark. Human isolates were obtained from individuals of both sex and from different age groups. All human, bovine and 96 porcine isolates were collected from 1995 to 1998. The remaining 15 porcine isolates were collected in 2001, two years after the

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Strain      Origin                      SD-1       MetGlyIlePheSerIlePheVal
                                10        20        30        40      49
46823       human      1 ACTAATTTTATAAGGAGGAAAAAATATGGGCATTTTTAGTATTTTTGTA
9731065-8   cattle     1 ACTAATTTTATAAGGAGGAAAAAATATGGGCATTTTTAGTATTTTTGTA
9731065-7   cattle     1 ACTAATTTTATAAGGAGGAAAAAATATGGGCATTTTTAGTATTTTTGTA
9730363-2   porcine    1 ACTAATTTTATAAGGAGGAAAAAATATGGGCATTTTTAGTATTTTTGTA
39961       human      1 ACTAATTTTATAAGGAGGAAAAAATA-----------------------
39996       human      1 ACTAATTTTATAAGGAGGAAAAAATA-----------------------
9730363-6   porcine    1 ACTAATTTTATAAGGAGGAAAAAATA-----------------------
43288       human      1 ACTAATTTTATAAGGAGGAAAAAATA-----------------------
9730249-1   cattle     1 ACTAATTTTATAAGGAGGAAAAAA-------------------------
9730363-4   porcine    1 ACTAATTTTATAAGGAGGAAAAAA-------------------------
9730363-5   porcine    1 ACTAATTTTATAAGGAGGAAAAAA-------------------------
9730363-7   porcine    1 ACTAATTTTATAAGGAGGAAAAAA-------------------------
9730517-1   cattle     1 ACTAATTTTATAAGGAGGAAAAAA-------------------------
9731066-2   cattle     1 ACTAATTTTATAAGGAGGAAAAAA-------------------------

                         IleSerThrValHisTyrGlnProAsnLysLysEND Hair pin II
                                 60        70        80        90       100
46823       human     50 ATCAGCACAGTTCATTATCAACCAAACAAAAAAATAAGTGGTTATAATGAAT
9731065-8   cattle    50 ATCAGCACAGTTCATTATCAACCAAACAAAAAAATAAGTGGTTATAATGAAT
9731065-7   cattle    50 ATCAGCACAGTTCATTATCAACCAAACAAAAAAATAAGTGGTTATAATGAAT
9730363-2   porcine   50 ATCAGCACAGTTCATTATCAACCAAACAAAAAAATAAGTGGTTATAATGAAT
39961       human     50 ---------------------------------------------------
39996       human     50 ---------------------------------------------------
9730363-6   poricne   50 ---------------------------------------------------
43288       human     50 ---------------------------------------------------
9730249-1   cattle    50 ---------------------------------------------------
9730363-4   porcine   50 ---------------------------------------------------
9730363-5   porcine   50 ---------------------------------------------------
9730363-7   porcine   50 ---------------------------------------------------
9730517-1   cattle    50 ---------------------------------------------------
9731066-2   cattle    50 ---------------------------------------------------
                                     Hair pin III         SD-2      Met..
                                110       120       130       140       150
46823       human    101 CGTTAATAAGCAAAATTCATTATAACCAAATTAAAGAGGGTTATAATGAA
9731065-8   cattle   101 CGTTAATAAGCAAAATTCATTATAACCAAATTAAAGAGGGTTATAATGAA
9731065-7   cattle   101 CGTTAATAAGCAAAATTCATTATAACCAAATTAAAGAGGGTTATAATGAA
9730363-2   porcine  101 CGTTAATAAGCAAA----------------TTAAAGAGGGTTATAATGAA
39961       human    101 ---------------------------------AAGAGGGTTATAATGAA
39996       human    101 ---------------------------------AAGAGGGTTATAATGAA
9730363-6   porcine  101 ---------------------------------AAGAGGGTTATAATGAA
43288       human    101 -----------------------------------GAGGGTTATAATGAA
9730249-1   cattle   101 -----------------------------------GAGGGTTATAATGAA
9730363-4   porcine  101 -----------------------------------GAGGGTTATAATGAA
9730363-5   porcine  101 -----------------------------------GAGGGTTATAATGAA
9730363-7   porcine  101 -----------------------------------GAGGGTTATAATGAA
9730517-1   porcine  101 -----------------------------------GAGGGTTATAATGAA
9731066-2   porcine  101 -----------------------------------GAGGGTTATAATGAA
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Figure 1. Regulation of expression of the *erm*(C) gene. Deletions in the regulatory region of *erm*(C) in staphylococci from animal and human origin were identified by sequencing PCR amplicons obtained using primers RegermC-1 (5'-TAAACCGTGTGCTCTACGA C-3') and RegermC-2 (5'-CCTTTTCCTGAGCCGATTTC-3'). Origins of strains are indicated as well as Shine-Delgano (SD-1 and SD-2) sequences, sequence of the leader peptide (by amino acid translation) and start of *erm*(C) (Met…). Underlined bases indicate position of hairpin II and III.

Table 1. Identification of presence and regulation of the *erm*(C) gene was done using PCR. Classification of genes as regulated or constitutive was based on size of the obtained amplicon. Consumption of antimicrobial agents in the three reservoirs is indicated.

	Presence and regulation of *erm*(C) among staphylococci of human and animal origin			
Origin	Human *S. aureus*	Cattle staphylococci	Pigs *S. hyicus*	
Year	1995-1998	1995-1998	1995-1998	2001
Usage	low	moderate	high	low
n =	45	29	96	15
erm(C) positive*	69	100	99	47
regulated	81	31	9	43
constitutive	19	69	91	57

* All numbers are given in percentage

discontinued usage of growth promoters in Denmark.

The presence of *erm*(C) was confirmed using previous described primers (4). Among the animal isolates from 1995-98, all except one porcine isolate contained the *erm*(C) gene (Table 1). *erm*(C) was found in 23 (69%) of the human isolates and 7 (47%) of the porcine isolates from 2001. PCR for erm(A) and erm(B) was performed for porcine isolates from 2001. No positive amplicons were obtained (data not shown). A set of PCR primers (RegermC-1: 5'-TAAACCGTGTGCTCTACGAC-3' and RegermC-2: 5'-CCTTTTCCTGAGCCGATTTC-3') was constructed spanning the regulatory region upstream the *erm*(C) gene and PCR amplification was performed. Fourteen amplicons from selected strains from the three different reservoirs were sequenced. Results are presented in Figure 1.

Deletion of 16 bp, 107 bp, 109 bp and 111 bp was found in the regulatory region of *erm*(C). Based on the obtained sequences, the size of the PCR amplicons could be used to determine whether an *erm*(C) gene was expressed constitutive or regulated. Results on regulation of the *erm*(C) gene in the three reservoirs are presented in Table 1.

The differences in occurrence of regulated *erm*(C) between isolates from the different reservoirs were statistically significant (chi-square test). Significant difference could be demonstrated between *S. hyicus* from pig from 1995-98 and 2001 ($p=0.034$) and between staphylococcal isolates from pigs and cattle ($p=0.013$), isolates from cattle and humans ($p<0.001$) and isolates from humans and pigs ($p<0.001$).

In a reservoir with high usage of tylosin constitutive expressed *erm*(C) genes were dominant (91% in porcine isolates from 1995-98). In a reservoir with moderate usage of tylosin constitutive expressed genes was still most prevalent (69% in cattle and 57% in pigs from 2001) while in a reservoir with no usage of tylosin regulated *erm*(C) genes was most prevalent (81% in human isolates). When comparing porcine *erm*(C) positive S. hyicus isolates from 1995-98 with isolates from 2001 a change in the ratio could be observed between constitutive and regulated genes. This change to a higher prevalence of regulated *erm*(C) genes could reflect the changes in usage of tylosin introduced by the discontinuous usage of growth promotion in 1998 in Denmark. Results presented here indicate that the ratio of constitutive to regulated *erm*(C) genes could be related to the amount of tylosin used in the different reservoirs. Statistically significant differences in occurrence of constitutive and regulated *erm*(C) genes were demonstrated for reservoirs with different usage of tylosin. This indicates that not only have the usage of tylosin selected for macrolide resistant staphylococci (2) but regulation of expression of the *erm*(C) gene has also been changed. Since regulated *erm*(C) do not give resistance to tylosin and only very limited amount of spiramycin and tylosin has been used for human therapy, the higher prevalence of constitutive expressed resistance genes in animal isolates compared to human isolates could be associated to the usage of tylosin as growth promoter and prevalence of constitutive expressed *erm*(C) in the human reservoir could indicate an animal origin of the resistance.

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Reprints may be obtained from: Lars B. Jensen, Danish Institute for Food and Veterinary Research, Bülowsvej 27, DK-1790 Copenhagen V, Denmark. E-mail: lje@dfvf.dk, tel: (+45) 72 34 60 00, fax: (+45) 72 34 60 01.