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Screening virulence properties of staphylococci isolated from meat and meat products

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Summary

Virulence properties (biofilm formation, antibiotic susceptibility, production of extracellular enzymes and the presence of toxin genes) of staphylococci isolated from various meat and meat products were investigated. 22 *Staphylococcus* spp. (*S. aureus* n=9, *S. haemolyticus* n=4, *S. cohnii* n=3, *S. saprophyticus* n=3, *S. hominis* n=1, *S. simulans* n=1 and *S. warneri* n=1) were isolated from 120 meat and meat product samples. 10 strains were biofilm-formers. Although none of the strains was resistant to vancomycin, oxacillin, teicoplanin, ofloxacin and gentamicin, 8 strains were found to be resistant to penicillin and one strain was found to be resistant to erythromycin. In addition, all strains were negative for the *mecA* PCR. 8 strains showed lipolytic activity against Tween 80, 10 strains against Tween 20, and 18 strains against tributyrin. Moreover, 9 strains showed proteolytic activity against casein, 11 strains against milk and 17 strains against skim milk containing media. Mostly *S. aureus* strains showed positive results for *icaA*-SA, *nuc*, *geh*, *sspA*, *sspB*, *aur*, serine protease gene, *hla*, *hlb*, *set*1, and *etb*. However, 7 of coagulase-negative staphylococci strains were found to carry *see* gene. As both prevalence and concentration of this bacterium were low, and no isolate contained all virulence factors, it is concluded that common hygiene and process control measures should be sufficient to control meatborne staphylococcal intoxication.

Abbreviations: Aur = aureolysin; $CaCl_2$ = calcium chloride; cfu = colony forming unit; CNS = coagulase-negative *Staphylococci*; CRA = Congo Red Agar; DNA = deoxyribonucleic acid; DNase = deoxyribonuclease; dNTPs = deoxyribonucleotide triphosphates; HCl = hydrochloric acid; MRSA = methicillin-resistant *Staphylococcus aureus*; NA = nutrient agar; OD = optical density; PCR = polymerase chain reaction; PIA = polysaccharide intercellular adhesin; RNA = ribonucleic acid; SEA = staphylococcal enterotoxin A; SEE = staphylococcal enterotoxin E; SEs = staphylococcal enterotoxins; *sspA* = staphylococcal serin protease; *sspB* = staphylococcal cystein protease; T20 medium = Tween 20 medium; T80 medium = Tween 80 medium; TAE = tris-acetate-EDTA; TaqDNA polymerase = *Thermus aquaticus* deoxyribonucleic acid polymerase; TCA = trichloroacetic acid; Tris = tris(hydroxymethyl)-aminomethan; TSB = tryptic soy broth; UHT = ultra high temperature

Schlüsselwörter: Staphylokokken, Fleischwaren, Biofilm, extrazelluläre Enzyme.

Zusammenfassung

Untersuchung der Virulenzeigenschaften von aus Fleisch und Fleischwaren isolierten Staphylokokken

Virulenzeigenschaften (Biofilmbildung, Empfindlichkeit gegenüber Antibiotika, Produktion extrazellulärer Enzyme und Anwesenheit von Toxingenen) von *Staphylococcus*-Isolaten aus verschiedenen Proben von Fleisch und Fleischwaren wurden untersucht. 22 *Staphylococcus* spp. (*S. aureus* n=9, *S. haemolyticus* n=4, *S. cohnii* n=3, *S. saprophyticus* n=3, *S. hominis* n=1, *S. simulans* n=1 und *S. warneri* n=1) wurden aus 120 Proben von Fleisch und Fleischwaren isoliert. 10 Isolate konnten Biofilme bilden. Zwar war keiner der Stämme gegen Vancomycin, Oxacillin, Teicoplanin, Ofloxacin und Gentamicin resistent, dafür aber 8 gegen Penicillin und einer gegen Erythromycin. Zusätzlich waren alle Isolate *mecA* PCR negativ. 8 Serovare zeigten lipolytische Aktivität in Bezug auf Tween 80, 10 auf Tween 20 und 18 auf Tributyrin Nährmedium. Außerdem zeigten 9 Isolate proteolytische Aktivität auf Kasein, 11 in Milch und 17 in Magermilch enthaltenden Nährmedien. Zumeist zeigten *S. aureus*-Isolate positive Ergebnisse für *ica*A-SA, *nuc*, *geh*, *ssp*A, *ssp*B, *aur*, Serinprotease Gen, *hla*, *hlb*, *set*1 und *etb*. Jedoch wurden 7 Serovare von koagulase-negativen Staphylokokken gefunden, die das *see* Gen tragen. Die niedrige Prävalenz und Konzentration dieses Bakteriums in Fleisch und Fleischwaren, und die Tatsache, dass kein Isolat alle Virulenzeigenschaften gemeinsam aufwies, erlauben die Schlussfolgerung, dass die üblichen Hygiene- und Prozesskontrollmaßnahmen zur Verhütung fleischübertragener Staphylokokken-Intoxikationen ausreichend sein sollten.

Introduction

Staphylococcus aureus and *S. epidermidis* are major members of the genus staphylococci and they are virulent and infectious for humans and animals. Individuals carrying colonized staphylococci in their anterior nares of nasopharynx are the main source for dissemination of staphylococci to other people and to contaminate food. Food poisoning due to contaminated red meat, poultry and their products by staphylococcal enterotoxins (SEs) is the most common type of food poisoning. SEs, mainly SEA to SEE, are heat-stable exoproteins causing gastroenteric syndromes in humans through ingestion. In fact, 53 % of the staphylococcal food poisoning cases reported in the United Kingdom between 1969 and 1990 were due to meat and meat products, and 22 % of the cases were due to poultry and poultry-based meals (WIENEKE et al., 1993; LE LOIR et al., 2003).

Biofilm formation on food-contact surfaces is a considerable interest to food hygiene (GANESH KUMAR and ANAND, 1998). Biofilm formation of staphylococci on material surfaces occurs in 2 steps. First, bacteria attach via physicochemical interactions to material surfaces (AN and FRIEDMAN, 1998). A cell surface protein (autolysin), which is encoded by *atlE* gene, plays a role in the attachment process, along with unspecific interactions (HEILMANN et al., 1997). Second is aggregation and accumulation of cells mediated by polysaccharide intercellular adhesin (PIA or slime) that is a product of the *icaADBC* locus (GÖTZ, 2002).

Staphylococci, especially *S. aureus* can produce extracellular proteases, including serine-, cysteine- and metalloproteases. Major extracellular proteases in *S. aureus* are staphylococcal serine protease (V8 protease) (*sspA*), a metalloprotease called aureolysin (Aur) and cysteine protease (*sspB*) (DUBIN, 2002). Staphylococcal proteases are able to interact with the host immune system and contribute to the infection process. Staphylococcal lipases are also virulence factors and responsible for helping colonization and invasion of bacteria especially in skin infections. Lipase also helps nutrition and dissemination of staphylococci during the infection progress (STEHR et al., 2003).

The aim of this work was to determine certain virulence properties of staphylococci isolated from various meat and meat products. For this purpose, biofilm forming abilities, antibiotic susceptibilities, production of extracellular enzymes and the presence of genes responsible for the production of virulence properties were examined.

Material and methods

Isolation and identification of staphylococci

Totally, 120 meat samples (ground beef n=20, boneless beef n=20, chicken meat n=20, turkey meat n=20) and meat products (raw hamburger meatball n=20, Turkish fermented sausage n=20) were collected from 2 cities (Edirne and Istanbul) in Turkey from July 2004 to May 2005. Staphylococci were isolated on Baird-Parker Agar (Oxoid CM275; Oxoid, Basingstoke, UK) with Egg Yolk Tellurite Emulsion (Oxoid SR 0054) and typical colonies were subcultured. Pure cultures were stored in 20 % (v/v) glycerol stocks at -80 °C. Staphylococci were identified by Gram staining, catalase, coagulase (Oxoid DrySpot Staphytect Plus Test) and deoxyribonuclease (DNase) activities. In addition, staphylococci were identified at species level by Vitek (bioMerieux S.A., Marcy l'Etoile, France) and 16S-ITS rRNA PCR-RFLP with *Taq*I restriction enzyme (SUDAGIDAN et al., 2005).

Biofilm and slime tests

Biofilm formation was quantitatively analyzed using the microtiter plate test (STEPANOVIC et al., 2003) with Tryptic Soy Broth (TSB; Merck 1.05459; Darmstadt, Germany) containing 1% (w/v) sucrose. The adherence of staphylococci to glass tube surfaces was investigated by the tube adherence test (CHRISTENSEN et al., 1982). The Congo Red Agar (CRA) method was used to determine slime (FREEMAN et al., 1989). According to this method, black colonies were determined as slime producers, whereas red and pink colonies on CRA were evaluated as slime negative strains (ARCIOLA et al., 2002).

Antibiotic susceptibility test

Antibiotic susceptibilities against oxacillin, penicillin, vancomycin, teicoplanin, ofloxacin, gentamicin, and erythromycin were investigated by agar disc diffusion method according to Clinical and Laboratory Standards Institute (ANONYMOUS, 2006).

Extracellular enzyme production

Extracellular lipase and protease production were determined on media containing substrates specific for the enzymes. For the detection of lipase activity, Tween 20 (T20) medium (Nutrient Agar (NA; Oxoid CM 003), 0.1 g/l calcium chloride ($CaCl_2$) (Applichem A 3652; Darmstadt, Germany) and 1% (v/v) Tween 20 (Merck 817072), Tween 80 (T80) medium (NA, 0.1 g/l $CaCl_2$ and 1% [v/v] Tween 80 [Merck 822187]) (HABA et al., 2000) and tributyrin agar (Merck 1.01957) were used. During the preparation of media, T20 and T80 were autoclaved separately at 120 °C for 20 min. Skim milk agar, milk agar and casein agar media were used for the determination of extracellular protease production. Skim milk agar (NA and 1% [w/v] skim milk, Oxoid LP 031), milk agar (NA containing 10% [v/v] Ultra High Temperature [UHT] milk) and casein agar (10 g/l casein, Merck 102241), 3 g/l yeast extract (Oxoid LP 021), 5 g/l NaCl (Applichem A2942) and 20 g/l agar (Oxoid LP 011, pH 7.4) were prepared and sterilized at 121 °C for 15 min except for skim milk autoclaved separately for 5 min. After spotting the strains, the media were incubated at 37 °C for at least 3 days. The growth of bacteria from the inoculation site or halo formation was accepted as extracellular enzyme production. Proteolytic activity as halo formation in casein plates was determined after treating plates with 1% (v/v) hydrochloric acid (HCl, Merck 100317) or 5% (v/v) trichloroacetic acid (TCA, Merck 100807). HCl and TCA solutions were used to precipitate casein in the plates.

Deoxyribonucleic acid (DNA) isolation

DNA isolation procedure was adapted from ARCIOLA et al. (2001). 200 µl of culture grown overnight in 5 ml TSB were centrifuged and resuspended in 45 µl of deionized water and 15 µl of lysostaphin (100 µg/ml, Sigma L-7386, Saint Louis, Missouri, USA). After 1 hour incubation at 37 °C,

Tab.1: Primers used for detection of staphylococcal genes by PCR

Gene	Primers 5′→3′	Reference	Gene	Primers 5′→3′	Reference
icaA	F-gacctcgaagtcaatagaggt R-cccagtataacgttggatacc	ZIEHBUR et al. (1999)	*sea*	F-ttggaaacggttaaaacgaa R-gaaccttcccatcaaaaaca	JOHNSON et al. (1991)
icaA-SA	F-tggctgtattaagcgaagtc R-cctctgtctgggcttgacc	KNOBLOCH et al. (2002)	*seb*	F-tcgcatcaaactgacaaacg R-gcaggtactctataagtgcc	JOHNSON et al. (1991)
icaC	F-ataaacttgaattagtgtatt R-atatataaaactctcttaaca	ZIEHBUR et al. (1999)	*sec*	F-gacataaaagctaggaattt R-aaatcggattaacattatcc	JOHNSON et al. (1991)
atlE	F-caactgctcaaccgagaaca R-tttgtagatgttgtgcccca	FREBOURG et al. (2000)	*sed*	F-ctagtttggtaatatctcct R-taatgctatatcttataggg	JOHNSON et al. (1991)
bap	F-ccctatatcgaaggtgtagaattgcac R-gctgttgaagttaatactgtacctgc	CUCARELLA et al. (2001)	*see*	F-tagataaagttaaaacaagc R-taacttaccgtggacccttc	JOHNSON et al. (1991)
mecA	F-tggtatgtggaagttagattgg R-ggatctgtactgggttaatcag	LEM et al. (2001)	*tst*	F-aagccctttgttgcttgcg R-atcgaactttggcccatacttt	BOOTH et al. (1991)
nuc	F-ggcaattgtttcaatattac R-ttttatttgcattttctacc	this study	*eta*	F-ctagtgcatttgttattcaa R-tgcattgacaccatagtact	JOHNSON et al.(1991)
geh	F-gcacaagcctcgg R-gacgggggtgtag	SAID-SALIM et al. (2003)	*etb*	F-acggctatatacattcaatt R-tccatcgataatatacctaa	JOHNSON et al. (1991)
sspA	F-gacaacagcgacacttgtga R-agtatctttacctacaactaca	KARLSSON and ARVIDSON (2002)	*hla*	F-ggtttagcctggccttc R-catcacgaactcgttcg	SALASIA et al. (2004)
sspB	F-tgaagaagatggcaaagttag R-ttgagatacactttgtgcaag	KARLSSON and ARVIDSON (2002)	*hlb*	F-gccaaagccgaatctaag R-gcgatatacatcccatggc	SALASIA et al. (2004)
aur	F-tagtagcacacgaattaacacacg R-ttccctattgcttgaatcacg	KARLSSON and ARVIDSON (2002)	*set*1	F-ggttaattcatagcgcagtatc R-caacgtttcatcgttaagctgc	SALASIA et al. (2004)
ser-prt	F-caagttgaagcacctactgg R-tagagtgtgaatcggctttgg	SMELTZER et al. (1993)			

F = forward primer; R = reverse primer

15 µl of proteinase K (100 µg/ml, Merck 124568) and 150 µl of 0.1 M tris(hydroxymethyl)-aminomethan (Tris)/HCl (pH 7.5) were added to samples and they were incubated further 1 hour at 37 °C. Finally, the bacterial lysates were boiled for 5 min and stored at -20 °C.

Polymerase chain reaction (PCR)

PCR was used to search for the presence of genes responsible for the production of methicillin resistance (*mecA*), thermonuclease (*nuc*), lipase (*geh*), protease (*sspA*, *sspB*, *aur*, serine protease gene), exfoliative toxins (*eta* and *etb*), toxic shock syndrome toxin (*tst*), α- and β-haemolysin (*hla* and *hlb*), staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed* and *see*), and staphylococcal exotoxin like protein-1 (*set*1). In addition, the presence of genes coding for biofilm formation and adhesion (*icaA*, *icaA*-SA, *icaC*, *atlE* and *bap*) was also investigated. The sequences of primers are listed in Tab. 1. PCR reactions were performed in 50 µl reaction volume containing 1.5 U *Thermus aquaticus* deoxyribonucleic acid (*Taq*DNA) polymerase (Fermentas, Vilnius, Lithuania), 5 µl of 10x reaction buffer (0.1 M Tris/HCl [pH 8.8], 0.5 M KCl, 0.8% [v/v] NP-40, and 1.5 mM $MgCl_2$), 10 µM of each of the primers (Metabion International AG, Martinsried, Germany), 0.2 mM each of the 4 deoxyribonucleotide triphosphates (dNTPs) (Fermentas), and 5 µl of the bacterial lysate as the DNA template. Amplification products were analyzed in 1.5 % (w/v) agarose gel electrophoresis in 1x tris-acetate-EDTA (TAE) buffer. PCR experiments were done twice for each strain.

Results

Staphylococcal strains

22 *Staphylococcus* sp. from 120 meat and meat product samples were identified as *S. aureus* (n=9), *S. haemolyticus* (n=4), *S. cohnii* (n=3), *S. saprophyticus* (n=3), *S. hominis* (n=1), *S. simulans* (n=1) and *S. warneri* (n=1). Isolates originated from ground beef (n=5), chicken meat (n=5), boneless beef (n=4), raw hamburger meatball (*n*=3), turkey meat (n=3), and Turkish fermented sausage (n=2). *S. aureus* counts were below the maximum acceptable limits (5.0x 10^3 cfu/g for minced meat and meat products) in all minced meat (TURKISH FOOD CODEX, 2006) and meat product samples (TURKISH FOOD CODEX, 2000).

Biofilm formation

Optical density (OD) measurements at 570 nm were used to determine biofilm formation in the microtiter plate test. 10 staphylococcal strains showed strong adherence ($OD_{570} \geq 0.5$). With the tube adherence test, only 1 strain (*S.*

Tab.2: Biofilm formation, antibiotic resistance, the presence of the genes and extracellular enzyme production by isolated *Staphylococcus* species

Strain code	Species	Biofilm	Antibiotic resistance	*icaA* -SA	*nuc*	*geh*	*sspA*	*sspB*	*aur*	Ser Prt	*hla*	*hlb*	*set*1	*etb*	SEs	Lipase positives	Protease positives
AAG-29B	*S. aureus*	-		+	+	+	+	+	+	+	+	+	+	-	-	T20, T80, Tribut.	SM, milk, casein
AAG-34A	*S. aureus*	-		+	+	+	+	+	+	+	+	+	+	+	-	T20, T80, Tribut.	SM, milk
AAG-36A	*S. aureus*	-		+	+	+	+	+	+	+	+	-	+	+	-	T20, T80, Tribut.	SM, milk
AAG-36C	*S. aureus*	+	Pen.	+	+	+	+	+	+	+	+	-	+	-	-	T20, T80, Tribut.	SM, milk, casein
AAG-63	*S. aureus*	-	Pen.	+	+	+	+	+	+	+	+	+	+	-	-	T20, Tribut.	SM
AAG-67	*S. aureus*	-	Pen.	+	+	+	+	+	+	+	+	+	+	-	-	T20, T80, Tribut.	SM, casein
AAG-68	*S. aureus*	-	Pen.	+	+	-	+	+	+	+	+	-	+	-	-	T20, T80, Tribut.	SM, milk
AAG-74	*S. aureus*	-	Pen., Eryt.	+	+	-	+	+	+	+	+	+	+	-	-	T20, Tribut.	SM, milk, casein
AAG-76	*S. aureus*	-		+	+	+	+	+	+	+	+	+	+	-	-	T20, T80, Tribut.	SM, milk, casein
AAG-3B	*S. haemolyticus*	+		-	-	-	-	-	-	-	-	-	-	-	see	Tribut.	
AAG-5A	*S. haemolyticus*	+		-	-	-	-	-	-	-	-	-	-	-	-	Tribut.	SM, casein
AAG-56A	*S. haemolyticus*	+		-	-	-	-	-	-	-	-	-	-	-	-		
AAG-78A	*S. haemolyticus*	-		-	-	-	-	-	-	-	-	-	-	-	-		SM, milk, casein
AAG-7	*S. cohnii*	+		-	-	-	-	-	-	-	-	-	-	-	-	Tribut.	
AAG-8B	*S. cohnii*	+		-	-	-	-	-	-	-	-	-	-	-	-	Tribut.	
AAG-34B	*S. cohnii*	+		-	-	-	-	-	-	-	-	-	-	-	see		SM, casein
AAG-3A	*S. saprophyticus*	-	Pen.	-	-	-	-	-	-	-	-	-	-	-	see	Tribut.	SM, milk
AAG-14	*S. saprophyticus*	-	Pen.	+	-	-	-	-	-	-	-	-	-	-	see	Tribut.	SM
AAG-24B	*S. saprophyticus*	+		-	-	-	-	-	-	-	-	-	-	-	see	Tribut.	SM
AAG-83	*S. hominis*	-		-	-	-	-	-	-	-	-	-	-	-	see		SM, milk, casein
AAG-16A	*S. simulans*	+	Pen.	-	-	-	-	-	-	-	-	-	-	-	see	Tribut.	SM
AAG-4A	*S. warnerii*	+		-	-	-	-	-	-	-	-	-	-	-	-	T20, T80, Tribut.	milk

Pen. = penicillin; Eryt. = erythromycin; genes = *icaA*-SA = PIA, *nuc* = thermonuclease, *geh* = lipase, *sspA* = serine protease, *sspB* = cysteine protease, *aur* = metalloprotease, Ser Prt = serine protease gene, *hla* = α-haemolysin, *hlb* = β-haemolysin, *set*1 = staphylococcal exotoxin like protein-1; *etb* = exfoliative toxin B; SEs = staphylococcal enterotoxin positive genes; T20 = Tween 20; T80 = Tween 80; Tribut. = tributyrin; SM = skim milk

cohnii AAG-8B) showed strong adherence, while 13 strains were weakly adherent to glass surfaces. 1 strain (*S. haemolyticus* AAG-5A) formed dark bordeaux colonies on CRA, while 17 strains formed red colonies and 4 strains formed pink colonies.

Antibiotic susceptibility

All strains were susceptible to vancomycin, oxacillin, teicoplanin, ofloxacin and gentamicin. Only 1 *S. aureus* strain (AAG-74) was resistant to erythromycin and 8 strains (5 *S. aureus* and 3 Coagulase-Negative Staphylococci [CNS]) were resistant to penicillin.

Extracellular enzyme production

8 strains had lipolytic activity on T80 medium, while 10 strains displayed lipolysis on T20 medium. Moreover, 18 strains formed halo on tributyrin agar. Protease production on skim milk, milk agar and casein agar also differed. 11 strains formed halo on milk agar and 17 strains on skim milk agar, respectively. In addition, 9 strains displayed proteolytic activity on casein agar. The distribution of the extracellular enzyme productions is presented in Tab. 2.

Presence of the genes

None of the strains was found to carry *icaA*, *icaC*, *atlE* and *bap* by both biofilm forming and non-biofilm forming strains. However, when the specific primers were used for *icaA* sequence of *S. aureus* (*icaA*-SA), 10 strains (9 of them *S. aureus*) gave positive results by PCR. None of the strains contained *mecA*, *eta*, *tst*, *seb*, *sec* and *sed* genes. In the detection of *sea*, very weak bands were obtained in 9 strains (2 of them *S. aureus*). The prevalence of the other genes in *S. aureus* and CNS strains is shown in Tab. 2.

Discussion

Formation of biofilms

Biofilms protect bacteria from antibiotics, disinfectants and mechanical cleaning processes in respect to their free-living counterparts (LEWIS, 2001; STEWART and COSTERTON, 2001). In this study, 10 strains were identified as biofilm formers by the microtiter plate test. Among these, only 1 strain was *S. aureus* and the others were CNS. The experiments showed that the results of the microtiter plate test were not consistent with the tube adherence test. Biofilm formers in the microtiter plate showed weak or no adherence to glass tube surfaces. Except for the *S. cohnii* strain, AAG-8B adhered strongly to glass surfaces. The reason of this might be due to surface charge, hydrophobicity and other surface properties of bacteria responsible for the attachment of bacteria to surfaces (MEI et al., 1989; AN and FRIEDMAN, 1998). None of our biofilm forming staphylococcal strains formed black colonies on CRA. Similar results were also reported by other researchers. KNOBLOCH et al. (2002) found that CRA test results did not correlate with the microtiter and the tube adherence tests.

IcaADBC genes are present most often in virulent and biofilm forming strains and they are not found in saprophytic and non-biofilm forming strains (ZIEBUHR et al., 1997). However, *ica* genes have also been found in non-biofilm forming *S. epidermidis* strains (MØRETRØ et al., 2003). Particularly, *ica* genes have been reported in both *S. aureus* and other *Staphylococcus* sp. (CRAMTON et al., 1999; ALLIGNET et al., 2001). In screening of the *ica* genes, we used the primers designed for *S. epidermidis icaADBC* locus. However, we could not find any *icaA* and *icaC* in both biofilm forming and non-biofilm forming strains. In addition, none of the strains carried *atlE* and *bap* genes. When we used specific primers designed for *S. aureus icaA* gene (KNOBLOCH et al., 2002), all *S. aureus* and one non-biofilm forming *S. saprophyticus* strains displayed positive results. Hence, this might be due to sequence differences in the *icaADBC* locus among staphylococci. KNOBLOCH et al. (2002) indicated that both biofilm positive and biofilm negative *S. aureus* strains, isolated from blood cultures and nasal specimens, could carry the *icaADBC* locus. These results support our positive *icaA* results obtained in non-biofilm forming *S. aureus* strains. On the other hand, biofilm forming ability of *S. epidermidis* has been shown to be switched off by insertion sequences inactivating *icaA* or *icaC* genes (ZIEBUHR et al., 1999).

Antibiotic resistance

Methicillin-resistant *S. aureus* (MRSA) and CNS are considered as important public-health problems. Direct detection methods of *mecA* by PCR demonstrated increased sensitivity, specificity and accuracy compared to phenotypic tests (LEM et al., 2001). In this study, none of the strains was found to be resistant to oxacillin and none of them carried *mecA* gene. The prevalence of MRSA in food is low. Recently, KITAI et al. (2005) investigated the prevalence of MRSA in 444 raw chicken meat samples in Japan and they showed that only 2 *mecA*-positive MRSA strains were present in the samples.

Lipolytic and proteolytic activity

Lipolytic activity of our staphylococcal strains was analyzed on Tween 20, Tween 80 and tributyrin containing media. From these lipolytic strains, 7 *S. aureus* strains contained the *geh* gene and in 3 strains (2 of them identified as *S. aureus*) not carrying this gene also showed lipolytic activity. Although there are similarities in the structures and sequences of staphylococcal lipases, they showed significant differences in their biochemical and catalytic activities (ROSENSTEIN and GÖTZ, 2000). In this study, only *S. aureus* strains were found to carry *sspA*, *sspB*, *aur* and serine protease gene. Whereas, CNS as well as *S. aureus* strains showed proteolytic activity (Tab. 2).

Staphylococcal enterotoxins

In the examination of enterotoxin genes, only 7 CNS strains showed positive results for *see*, but *seb, sec* and *sed* genes were not detected. In the detection of *sea* gene very weak bands were obtained in 9 strains and they were accepted as negative. It has been also reported that 9 of 50 *S. aureus* strains, originating from raw minced meat and raw sausages, were found to carry SEs (SEA-SEE) genes (BANIA et al., 2006). Our PCR results showed that all *S. aureus* strains were positive for *hla* and *set*1 genes (Tab. 2) and only 6 *S. aureus* strains displayed positive results for *hlb*. Furthermore, *S. aureus* strains (AAG-34A and AAG-36A) carried *etb* gene, but the toxin production profiles and the expression of *etb* gene were not investigated in this study.

Impact on the hygiene of meat and meat products

In conclusion, screening virulence properties of staphylococci from meat and meat products purchased in 2 Turkish cities gives important results for the presence of pathogenic strains. In this study, mainly *S. aureus* isolates carried virulence properties. The results of our study suggest that staphylococci with virulence genes are present in some, but not all Turkish meat and meat products at levels of <100 cfu/g. There was no correlation between factors which would facility survival (biofilm formation, extracellular enzymes) and factors which determine pathogenicity (antibiotic resistance, staphylococcal enterotoxin genes). Thus, the main focus in prevention of foodborne staphylococcal intoxication should be in keeping staphylococcal numbers in foods as low as possible by adequate control of process factors (low storage temperature, proper heating processes) and hygiene measures (no possibilities for recontamination) (ICMSF, 1998).

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Anatomie der Vögel. Klinische Aspekte und Propädeutik Zier-, Greif-, Zoo-, Wildvögel und Wirtschaftsgeflügel. Von H.E. KÖNIG, R. KORBEL und H.-G. LIEBICH. Schattauer, Stuttgart, 2009. 2. Aufl., 384 Seiten, 663 Abbildungen, davon 592 in Farbe, 14 Tabellen, gebunden, EUR 132,70, ISBN 978-3-7945-2578-2.

8 Jahre seit der letzten Auflage liegt die „Anatomie und Propädeutik des Geflügels" nunmehr in der zweiten Auflage vor. Schon die Änderung des Titels, mithin die Konzentration auf die Anatomie, impliziert eine gewisse Neuausrichtung dieses Lehrbuches. Die Propädeutik hat den Stellenwert eines Untertitels erfahren zusammen mit den „klinischen Aspekten bei Zier-, Greif-, Zoo-, Wildvögel und Wirtschaftsgeflügel". Alle aufgeführten Aspekte bei den einzelnen Vogelgruppen adäquat zu bearbeiten, ist sicherlich eine besondere Schwierigkeit für jedes Werk und letztlich nicht möglich, da die einzelnen Themenkreise doch sehr heterogen sind. Mithin hätte sich das Werk wohl selbst einen Gefallen getan, auf die wenigen Stellen zu verzichten, die auf das Wirtschaftsgeflügel verweisen sollen, was sich auch im Titel niederschlagen könnte. Zu konträr sind mittlerweile die Vorgehensweisen in der klinischen Betreuung von Wirtschaftsgeflügel, im Vergleich zu Zier-, Greif-, Zoo- und Wildvögeln. Beispielhaft können hier die Möglichkeiten der Euthanasie anfgeführt werden, die beim Wirtschaftsgeflügel und den Ziervögeln erheblich variieren. Auch die beiden neu eingefügten Kapitel über die Geschichte der Vogelanatomie sowie die Falknerei und Greifvogelmedizin sind für den Kliniker, der sich mit dem Wirtschaftsgeflügel beschäftigt, von minderer Bedeutung. Eine Konsequenz könnte aber sein, dass sich der Kreis der Interessierten, die sich ausserhalb der Veterinärmedizin mit Vögeln beschäftigen, z.B. Personen mit dem Schwerpunkt Biologie, erweitert.

Das Werk ist in Organsystemen aufgeteilt, was aus didaktischer Sicht sehr zu begrüßen ist. Die Kombination von anatomischen Präparaten, schematischen Darstellungen und histologischen Aufnahme sind sehr gut geeignet, dem Leser die entsprechenden Sachverhalte nahe zu bringen, was der ausdrücklichen Erwähnung bedarf. Sie stellt die Grundlage dar, um funktionale Zusammenhänge zwischen den einzelnen Strukturen besser aufzuzeigen und für den Interessierten erfassbarer zu machen. Grundlage des Werkes sind somit die zahlreichen Abbildungen (weit über 600 werden angeführt), die ein bestechendes Charakteristikum des vorliegenden Werkes darstellen. Die Qualität der Präparate und Aufnahmen werden sicherlich jeden Betrachter faszinieren. Hier tritt ein Fundus von Wissen zu Tage, dem sich niemand, der sich mit Vögeln beschäftigt, entziehen kann. Darüber hinaus spiegelt sich in ihnen die Sorgfalt der Herausgeber wider, mit der sie sich der Aufgabe zur Verfassung des vorliegenden Werkes gestellt haben. Insbesondere bei histologischen Abbildungen, aber auch bei anderen Darstellungen, wären Angaben zu den Größenverhältnissen wünschenswert, was vielleicht eine Anregung für zukünftige Ausgaben sein kann.

Abgerundet wird das Buch durch die Auflistung anatomischer Schlüsselwörter, welche den schnellen Zugriff auf die wichtigsten Fachbegriffe ermöglicht.

Die Anatomie der Vögel ist jeder/m Tierärztin/Tierarzt und jedem, der in seinem beruflichen Umfeld mit Vögeln zu tun, hat sehr zu empfehlen. Insbesondere für den Studierenden ist es ein unverzichtbares Werk, um die grundlegenden Informationen zur Anatomie der Vögel in übersichtlicher Form, aus erster Hand und in ausgezeichneter Darstellung zu erhalten.

M. Hess