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eap Gene as Novel Target for Specific Identification of *Staphylococcus aureus*▽

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The cell surface-associated extracellular adherence protein (Eap) mediates adherence of *Staphylococcus aureus* to host extracellular matrix components and inhibits inflammation, wound healing, and angiogenesis. A well-characterized collection of *S. aureus* and non-*S. aureus* staphylococcal isolates ($n = 813$) was tested for the presence of the Eap-encoding gene (*eap*) by PCR to investigate the use of the *eap* gene as a specific diagnostic tool for identification of *S. aureus*. Whereas all 597 *S. aureus* isolates were *eap* positive, this gene was not detectable in 216 non-*S. aureus* staphylococcal isolates comprising 47 different species and subspecies of coagulase-negative staphylococci and non-*S. aureus* coagulase-positive or coagulase-variable staphylococci. Furthermore, non-*S. aureus* isolates did not express Eap homologs, as verified on the transcriptional and protein levels. Based on these data, the sensitivity and specificity of the newly developed PCR targeting the *eap* gene were both 100%. Thus, the unique occurrence of Eap in *S. aureus* offers a promising tool particularly suitable for molecular diagnostics of this pathogen.

Staphylococcus aureus has been usually identified by traditional phenotypic properties based on in-house techniques or commercial assays (30). Assaying biochemical or immunologic reactions, these widely used diagnostic systems present various problems, including unsatisfying sensitivity, specificity, and assay time. The majority of the difficulties associated with these methods result from the variable expression of phenotypic features that are tested in these diagnostic procedures. Furthermore, the lack of objective criteria in the interpretation of these tests, which include slide agglutination, tube coagulation, latex agglutination, and colorimetric assays, may cause additional ambiguity. Molecular methods represent alternatives combining high sensitivity, specificity, speed, and reliability for identification. Several nucleic acid amplification-based assays have been reported for *S. aureus* that are based on specific gene targets as well as universal sequences, offering different advantages and limitations (2, 6, 12, 13, 27, 29, 31, 38, 42). Thus, additional reliable tools for detection of *S. aureus* are of major interest.

The anchorless extracellular adherence protein (Eap) of *S. aureus*, also designated major histocompatibility complex class II analogous protein (Map), selectively recognizes extracellular matrix aggregates but binds promiscuously to monomeric matrix macromolecules (16, 21, 23). This broad-spectrum adhesin shows structural homology to the C-terminal domain of bacterial superantigens but lacks superantigen activity (11, 28). Eap was recently shown to curb acute inflammatory responses, to enhance internalization of the microorganism into eukaryotic cells, to inhibit wound healing, and to function as a potent angiostatic agent (1, 15, 18, 33).

Previously, the presence of the Eap-encoding gene (*eap*) was determined to occur in only selected human clinical *S. aureus* isolates (19, 20). While sequencing results suggested that Eap sequences are highly conserved, size differences of various *eap* genes as a result of different numbers of repeats have been described (Fig. 1) (19). Due to this polymorphism of the *eap* gene, a novel PCR assay for diagnostic purposes was designed and validated in this study. Subsequently, the feasibility of an *eap*-based approach was systematically analyzed on the genomic, transcriptional, and protein levels by use of a large collection of human- and veterinary-derived *S. aureus* and non-*S. aureus* staphylococcal isolates.

MATERIALS AND METHODS

Bacterial isolates. Overall, 597 *S. aureus* isolates were tested. The collection encompassed a total of 444 German clinical *S. aureus* isolates comprising human ($n = 429$) and veterinary (bovine, $n = 10$; feline, $n = 3$; canine, $n = 1$; equine, $n = 1$) isolates as well as six reference strains of *S. aureus* (ATCC 13565, ATCC 14458, ATCC 19095, ATCC 23235, ATCC 27664, and ATCC 29213). In addition, *S. aureus* laboratory strains Newman, Wood 46, and CI7, known to be Eap-producing strains (19), were included, serving also as positive controls for the different Eap isoforms. The human isolates were collected and identified during the course of a German multicenter study comprising 219 blood and 210 nasal isolates (40). The veterinary *S. aureus* isolates were obtained from the Hannover area (Lower Saxonia, Germany). Furthermore, *S. aureus* isolates from France ($n = 12$), Hungary ($n = 21$), Italy ($n = 26$), Japan ($n = 21$), Poland ($n = 49$), and the United States ($n = 15$) were included.

Furthermore, a total of 216 non-*S. aureus* staphylococcal isolates comprising 164 clinical isolates and 52 type and reference strains of different coagulase-negative as well as coagulase-positive and -variable species and subspecies were studied (Table 1). Except for the clinically derived *S. intermedius* (3, 5) and *S. pettenkoferi* (35) isolates, non-*S. aureus* isolates were (i) recovered from a wide range of clinical specimens derived from, e.g., human skin, respiratory tracts, urogenital tracts, and venous catheters at the University of Münster, Münster, Germany, or were (ii) collected from blood during the course of a German multicenter study and considered etiologically relevant (41). Only one isolate per patient was included in this study.

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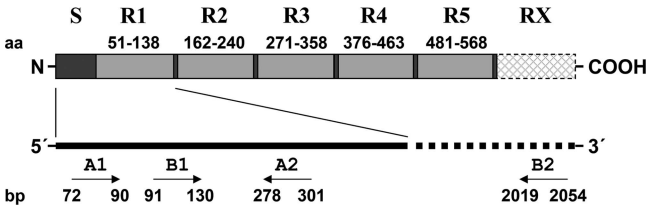

FIG. 1. Schematic representation (not to scale) of Eap and the encoding gene, showing the amino acid (aa) composition consisting of a 30-aa signal peptide (S) and 110-aa residue domains (R1, R2, . . . RX) found in multiple copies. The oligonucleotide primer binding sites of EAP-CON1 (A1) and EAP-CON2 (A2) as well as of EAP-P3wt (B1) and EAP-P2wt (B2) are indicated in base pairs (bp); sizes correspond to EAP of the Newman D2C strain.

Clinical isolates were identified on the species level by use of an ATB32 Staph biochemical test kit (BioMerieux, Marcy l'Etoile, France). When the identification of coagulase-negative staphylococci (CoNS) was ambiguous or categorized as unacceptable, universal target gene sequencing was performed as previously described (2, 7, 29). For differentiation of coagulase-positive species, in-house coagulase tube tests using rabbit plasma and rapid slide agglutination tests (Pastorex Staph-Plus, Sanofi Diagnostics Pasteur, Paris, France) were additionally performed. PCR assays for detection of *S. aureus*- and *S. intermedius*-specific *nuc* genes were applied for all included strains to confirm the differentiation of both species from other coagulase-positive or -variable and clumping factor-positive or -variable staphylococcal species (5, 6).

In addition, 30 type and reference strains of nonstaphylococcal gram-positive species mainly occurring in clusters were tested, comprising members of the genera *Dermacoccus* (*D. abyssi* DSM 17573^T; *D. barathri* DSM 17574^T; *D. nishinomiyaensis* DSM 20448^T; and *D. profundi* DSM 17575^T), *Kocuria* (*K. aegyptia* DSM 17006^T; *K. carniphila* DSM 16004^T; *K. himachalensis* DSM 44905^T; *K. kristinae* DSM 20032^T; *K. marina* JCM 13363^T; *K. palustris* DSM 11925^T; *K. polaris* DSM 14382^T; *K. rosea* DSM 20447^T; and *K. varians* DSM 20033^T), *Kytococcus* (*K. schroeteri* DSM 13884^T and *K. sedentarius* DSM 20547^T), *Macrococcus* (*M. bovicus* DSM 15607^T; *M. brunensis* CCM 4811^T; *M. carouselicus* DSM 15608^T; *M. caseolyticus* DSM 20597^T; *M. equipercicus* DSM 15609^T; *M. hajekii* CCM 4809^T; and *M. lamae* CCM 4815^T), and *Micrococcus* (*M. antarcticus* JCM 11467^T; *M. flavus* JCM 14000^T; *M. luteus* DSM 14234 and DSM 14235; and *M. lylae* DSM 20315^T).

Oligonucleotide primer design. Use of the published *eap*-derived primer pair P2 and P3 was hampered by the fact that amplicons of different sizes were yielded due to various repeat numbers of the *eap* gene, resulting in size variability of the gene product (19). Therefore, new primers, referred as EAP-CON1 and EAP-CON2, were designed (Fig. 1), aligning the sequences of *eap-N*, *eap-7*, and *eap-W* (EMBL accession numbers AJ290973, AJ243790, and AJ245439) originating from the strain Newman D2C (ATCC 25904), strain 7 (a clinical isolate), and strain Wood 46, respectively. These primers were compared with the primers EAP-P2wt and EAP-P3wt, which are characterized by the same target nucleotide sequences as described for the published primer pair P2 and P3 but without the added enzyme restriction site sequences (Table 2).

For verification purposes, the five clinical *S. aureus* isolates tested previously and shown to be *eap* negative by using the primer pair P2 and P3 (19) were reanalyzed by applying the EAP-CON1 and EAP-CON2 primer pair.

PCR procedures. DNA was isolated as previously described (4). The diagnostic PCR assays were performed for both target genes in a thermal cycler with a hot bonnet (iCycler; Bio-Rad, Munich, Germany). The PCR mixture consisted of 1 μg DNA, 10 mM Tris-HCl [pH 8.3], 10 mM KCl, 3 mM $MgCl_2$, 1 μM primer, 200 μM (each) dATP, dCTP, dGTP, and dTTP, 5 U of *Taq* DNA polymerase (Qbiogene, Heidelberg, Germany), and double-distilled water added to achieve a final volume of 50 μl. A total of 30 PCR cycles were run under the following conditions: DNA denaturation at 95°C for 1 min (5 min for the first cycle), primer annealing at 50°C for 1 min, and DNA extension at 72°C for 2 min. After the final cycle, the reaction was terminated by holding at 72°C for 10 min. A 10-μl volume of the PCR product was analyzed using a 1% ethidium bromide-stained agarose gel.

Southern blot analysis. DNA was restricted with EcoRI, separated on 1% agarose gel, blotted onto a nylon membrane, and probed with digoxigenin (DIG)-labeled PCR products of *eap*.

RNA isolation and Northern blot analysis. For total RNA isolation, bacteria were grown in 5 ml brain heart infusion broth for 18 h at 37°C with shaking at 160 rpm. Bacteria were pelleted by centrifugation at 4,000 rpm for 10 min at 4°C followed by RNA isolation using an RNeasy kit (Qiagen) according to the manufacturer's recommendations. Isolated total RNA was separated on a 1% agarose gel containing 15% formaldehyde, blotted onto a polyvinylidene difluoride membrane in 20× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), and fixed with UV light. The membrane was probed with an *eap*-PCR amplicon generated by primers EAP-P2wt and EAP-P3wt and prepared using DIG-labeled deoxynucleoside triphosphates. Subsequently, blots were exposed to anti-DIG antibodies (Roche) and developed with a color reaction according to the manufacturer's recommendations.

SDS-PAGE and Western blot analysis. Eap protein was detected as described earlier (19, 20). Briefly, to prepare cell surface proteins, staphylococci were grown in 5 ml brain heart infusion broth at 37°C for 18 h and then centrifuged at 10,000 × *g* for 2 min. The pellet was resuspended in extraction buffer (125 mM Tris-HCl [pH 7.0]–2% sodium dodecyl sulfate [SDS; Sigma-Aldrich]), heated at 95°C for 3 min, and then centrifuged at 10,000 × *g* for 3 min. The supernatant was passed through a Nap-10 column (Amersham Pharmacia Biotech Europe, Freiburg, Germany) containing Sephadex G-25 to remove SDS, and the eluate was stored at −20°C. A 50-μl volume of 5× sample buffer (60 mM Tris-HCl [pH 6.8], 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, and 0.1% bromophenol blue [Merck]) was added to 20 μl of cell surface extract, and the mixture was heated at 95°C for 3 min and then separated in an SDS-polyacrylamide gel electrophoresis (SDS-PAGE) minigel. For Western blot analysis, proteins separated by SDS-PAGE were electrophoretically transferred (Trans-blot SD; Bio-Rad, Munich, Germany) onto a nitrocellulose membrane (Schleicher & Schüll, Dassel, Germany), and then the membrane was blocked with 3% bovine serum albumin fraction V powder (Sigma, Taufkirchen, Germany). For probing blocked blots, anti-Eap was used, and detection was subsequently performed using alkaline-phosphatase-conjugated anti-rabbit antibody (Dako, Hamburg, Germany) developed in sheep and an alkaline phosphatase color reaction kit (Bio-Rad, Munich, Germany).

Polyclonal antibodies. Recombinant Eap (rEap) was expressed and purified as described earlier (19, 20). Polyclonal antibodies against the recombinant protein were raised separately in two rabbits by standard procedures (17). After preimmune serum samples were collected, each rabbit was immunized subcutaneously with 50 μg of rEap derived from strain Newman in complete Freund's adjuvant (Sigma). Second and third injections of antigen in incomplete adjuvant were given subcutaneously 2 and 4 weeks later, respectively. Blood was obtained 2 weeks after the last antigen injection. Naturally occurring antistaphylococcal antibodies were complexed by mixing serum with 100 volumes of cell surface protein extract (prepared by heating whole cells in 2% SDS at 95°C for 3 min and by pelleting bacteria by centrifugation) from *S. aureus* strain AH 12 (isogenic *eap* disruption mutant of strain Newman), which does not express Eap.

RESULTS

Prevalence of *eap* in *S. aureus*. By applying the newly designed primers EAP-CON1 and EAP-CON2 in the PCR assay, a single amplification product of the *eap* gene showing the expected size of 230 bp was detected in all 588 clinical isolates and nine reference and laboratory strains of *S. aureus* tested (Fig. 2). Thus, the sensitivity of the newly developed PCR approach was 100%. Due to the primer selection targeting the first repeat domain only, different sizes of the PCR products known to be targeted by primer pairs P2 and P3 and primer pairs EAP-P2wt and EAP-P3wt, respectively, were not observed.

Moreover, the five clinical *S. aureus* isolates tested to be *eap* negative in previous study by using the primer pair P2 and P3 (19) were now found to be *eap* positive by applying the newly designed primer pair (data not shown).

Prevalence of *eap* in non-*S. aureus* bacteria. To analyze the specificity of the newly designed *eap*-specific primers, the prevalence of the *eap* gene among 216 non-*S. aureus* staphylococcal isolates comprising 47 different CoNS and coagulase-positive or -variable staphylococcal (sub-)species was investigated at

TABLE 1. Non-*S. aureus* staphylococcal (sub-)species studied

Species and subspecies	No. of clinical isolates[a]		Type and/or reference strain(s)[b]	Total no. of isolates[c]
	Blood	Other		
S. arlettae	0	0	DSM 20672^T	1
S. auricularis	0	0	DSM 20609^T	1
S. capitis subsp. *capitis*	4	1	DSM 20326^T	6
S. capitis subsp. *urealyticus*	0	0	DSM 6717^T	1
S. caprae	0	5	DSM 20608^T	6
S. carnosus subsp. *carnosus*	0	0	DSM 20501^T	1
S. carnosus subsp. *utilis*	0	0	DSM 11676^T	1
S. chromogenes	0	0	DSM 20454^T	1
S. cohnii subsp. *cohnii*	4	2	DSM 20260^T	7
S. cohnii subsp. *urealyticus*	0	0	DSM 6718^T	1
S. condimenti	0	0	DSM 11674^T	1
S. delphini	0	0	DSM 20771^T	1
S. epidermidis	45	5	DSM 20044^T	51
S. equorum subsp. *equorum*	0	0	DSM 20674^T, DSM 20675	2
S. equorum subsp. *linens*	0	0	DSM 15097^T	1
S. felis	0	0	DSM 7377^T	1
S. fleurettii	0	0	DSM 13212^T	1
S. gallinarum	0	0	DSM 20610^T	1
S. haemolyticus	14	1	DSM 20263^T	16
S. hominis subsp. *hominis*	9	0	DSM 20328^T	10
S. hominis subsp. *novobiosepticus*	0	0	ATCC 700236^T	1
S. hyicus	0	1	DSM 20459^T	2
S. intermedius	1	10	DSM 20373^T	12
S. kloosii	0	0	DSM 20676^T	1
S. lentus	0	0	DSM 20352^T	1
S. lugdunensis	1	19	DSM 4804^T	21
S. lutrae	0	0	DSM 10244^T	1
S. muscae	0	0	DSM 7068^T	1
S. nepalensis	0	0	DSM 15150^T, DSM 15151	2
S. pasteuri	0	0	DSM 10656^T	1
S. pettenkoferi	4	1	CCUG 51270^T	6
S. piscifermentans	0	0	DSM 7373^T	1
S. pseudintermedius	0	0	LMG 22219^T, LMG 22220, LMG 22221, LMG 22222	4
S. saccharolyticus	0	0	DSM 20359^T	1
S. saprophyticus subsp. *bovis*	0	0	CCM 4410^T	1
S. saprophyticus subsp. *saprophyticus*	2	12	DSM 20229^T	15
S. schleiferi subsp. *coagulans*	0	0	DSM 6628^T	1
S. schleiferi subsp. *schleiferi*	0	5	DSM 4807^T	6
S. sciuri subsp. *carnaticus*	0	0	ATCC 700058^T	1
S. sciuri subsp. *rodentium*	0	0	ATCC 700061^T	1
S. sciuri subsp. *sciuri*	2	1	DSM 20345^T	4
S. simulans	1	4	DSM 20322^T	6
S. succinus subsp. *casei*	0	0	DSM 15096^T	1
S. succinus subsp. *succinus*	0	0	DSM 14617^T	1
S. vitulinus	0	0	ATCC 51145^T	1
S. warneri	5	0	DSM 20316^T	6
S. xylosus	1	4	DSM 20266^T	6
Total	93	71	52	216

[a] Isolates from blood and from other specimens (skin, wound, respiratory tract, urogenital tract, and venous catheters).
[b] Type or reference strains of the respective (sub-)species as deposited in the American Type Culture Collection (ATCC); Czech Collection of Microorganisms (CCM); Culture Collection, University of Göteborg, Sweden (CCUG); German Collection of Microorganisms and Cell Cultures (DSM); or Belgian Coordinated Collections of Microorganisms (LMG).
[c] Values represent total numbers of clinical isolates plus type and/or reference strain isolates.

the DNA level. The PCR presented here showed 100% specificity; i.e., the *eap* gene was not detected in 216 clinical isolates, including a large number of CoNS isolated from blood and considered etiologically relevant, or in 52 staphylococcal type and reference strains obtained from various strain collections (Fig. 2).

In addition, all other gram-positive cocci included in this study (micrococcal and macrococcal species) were tested to be *eap* negative.

Southern blot analysis. To exclude the possibility that DNA sequence heterogeneities of the primer binding sites might cause false-negative results with non-*S. aureus* isolates, Southern blot analyses were performed. Genomic DNAs of 20 representative non-*S. aureus* staphylococcal strains, including *S. epidermidis* ($n = 10$), *S. saprophyticus* subsp. *saprophyticus* ($n = 3$), *S. haemolyticus* ($n = 3$), *S. carnosus* subsp. *carnosus* ($n = 1$), *S. hominis* subsp. *hominis* ($n = 1$), *S. capitis* ($n = 1$), and *S. xylosus* ($n = 1$) were restricted with EcoRI, separated on 1%

TABLE 2. *eap*-targeting oligonucleotide primers used in this study

Name	Oligonucleotide sequence (5′→3′)	Amplicon size (bp)	Source or reference
EAP-CON1	TAC TAA CGA AGC ATC TGC C	230	This study
EAP-CON2	TTA AAT CGA TAT CAC TAA TAC CTC		
EAP-P3wt	GCA GCT AAG CCA TTA GAT AAA TCA TCA AGT TGC TTA CAC C	Variable[a]	Derived from primer P3 (19)[b]
EAP-P2wt	TTA AAA TTT AAT TTC AAT GTC TAC TTT TTT AAT GTC		

[a] Strain-specific amplicon size depending on the number of tandem repeats in the *eap* gene.
[b] The primer sequences were modified by omitting the originally introduced restriction sites.

agarose gel, blotted onto a nylon membrane, and probed with a DIG-labeled PCR product of *eap* from strain Newman by use of primers EAP-P3wt and EAP-P2wt. All non-*S. aureus* isolates tested showed negative results with DIG-labeled *eap* PCR products, but positive results were seen with genomic DNA of *S. aureus* control strains Newman, Mu-7, and Wood 46 (Fig. 3A).

Transcription analysis of *eap* gene. The same 20 non-*S. aureus* strains used for Southern blot analysis were selected for Northern blot analysis of *eap* gene transcripts. Whereas *eap* mRNA signals for strains Newman, CI7, and wood 46 were positive, mRNA signals of all non-*S. aureus* strains were absent (Fig. 3B).

SDS-PAGE and Western blot analysis. A set of 20 representative strains was analyzed for the expression of Eap protein. In Coomassie blue-stained SDS-PAGE gels, non-*S. aureus* strains tested produced a range of proteins in quantities similar to those seen with *S. aureus* strains Newman, CI7, and Wood 46. However, the band corresponding to Eap was not detected in any of the 20 tested non-*S. aureus* strains (Fig. 4A).

In Western blot analyses, anti-Eap antiserum used at a dilution of 1:20,000 recognized a 60-kDa Eap protein in cell surface extracts from strains Newman and CI7 and a 70-kDa Eap protein in cell surface extracts of strain Wood 46. In contrast, non-*S. aureus* staphylococcal isolates did not produce any proteins detectable by anti-Eap antiserum (Fig. 4B).

DISCUSSION

For *S. aureus*, secreted proteins such as Eap have pleiotropic effects, mediating adherence to host extracellular matrix components (e.g., fibronectin, fibrinogen, vitronectin, collagens, and elastin) and thereby potentially contributing to bacterial colonization of host tissues (10) and, in part, to invasion of nonprofessional phagocytes (19–21). In addition, Eap has anti-inflammatory and immunomodulatory functions in the host, indicating that Eap constitutes a potent virulence factor in the course of staphylococcal infections (1, 8, 15, 18, 25). Thus, Eap acts as a secretable expanded repertoire adhesive molecule (9).

In this study, the Eap-encoding gene (*eap*) was studied as a diagnostic target for specific identification of *S. aureus* by PCR amplification. Whereas sequencing results suggest that the *eap* sequences are in general highly conserved, size differences are known for the encoding gene, as a result of different numbers of repeats, as well as for the translated product, as additionally determined by assays using an insertional point mutation or ribosomal slippage at the site of a poly(A) region located at the 3′ end of the respective repeat region (19). Thus, the described PCR primer pairs generated to study the size variants (19) do not fulfill requirements (i) to detect *eap* homologs in other staphylococcal species and (ii) to use this gene as a target for specific and reliable identification of *S. aureus*. Consequently, a novel primer pair more suitable for diagnostic purposes was generated based on all known *eap* nucleotide sequences.

Based on previous observations that this gene is highly conserved among *S. aureus* isolates and that it was shown to be absent in a small collection of *S. epidermidis* isolates (19, 20), large collections of staphylococcal species and subspecies comprising different CoNS as well as coagulase-positive or -variable and/or clumping factor-positive or -variable staphylococci from various clinical and geographical sources were analyzed

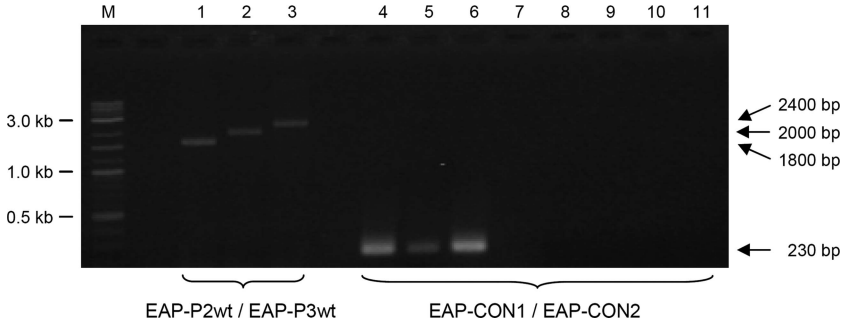

FIG. 2. Agarose gel (1%) electrophoresis patterns showing PCR products amplified with *eap* primers by use of genomic DNA of *S. aureus* ATCC 29213 (lanes 1 and 4), *S. aureus* Newman (lanes 2 and 5), and *S. aureus* Wood 46 (lanes 3 and 6) as well as genomic DNA of *S. epidermidis* DSM 20044 (lane 7), *S. haemolyticus* DSM 20263 (lane 8), *S. hominis* subsp. *hominis* DSM 20238 (lane 9), *S. intermedius* DSM 20373 (lane 10), and *S. lugdunensis* DSM 4804 (lane 11). Lane M, DNA molecular mass marker (1-kb/100-bp DNA ladder); lanes 1 to 3, *eap* gene amplified with primers EAP-P2wt and EAP-P3wt; lanes 4 to 11, *eap* gene amplified with newly designed primers EAP-CON1 and EAP-CON2 (see Table 2).

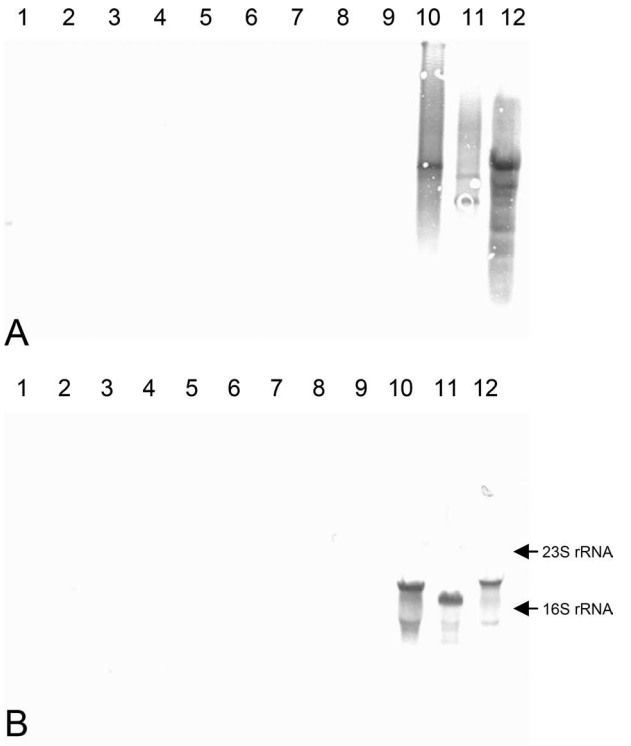

FIG. 3. Southern blot analysis of genomic DNA (A) and Northern blot of total RNA (B). Lanes 1 to 4, *S. epidermidis*; lane 5, *S. carnosus* subsp. *carnosus*; lane 6, *S. haemolyticus*; lane 7, *S. hominis* subsp. *hominis*; lanes 8 to 9, *S. saprophyticus* subsp. *saprophyticus*; lanes 10 to 12, *S. aureus* (lane 10, strain Newman; lane 11, strain CI7; lane 12, strain Wood 46). Blots were prepared as described in Materials and Methods.

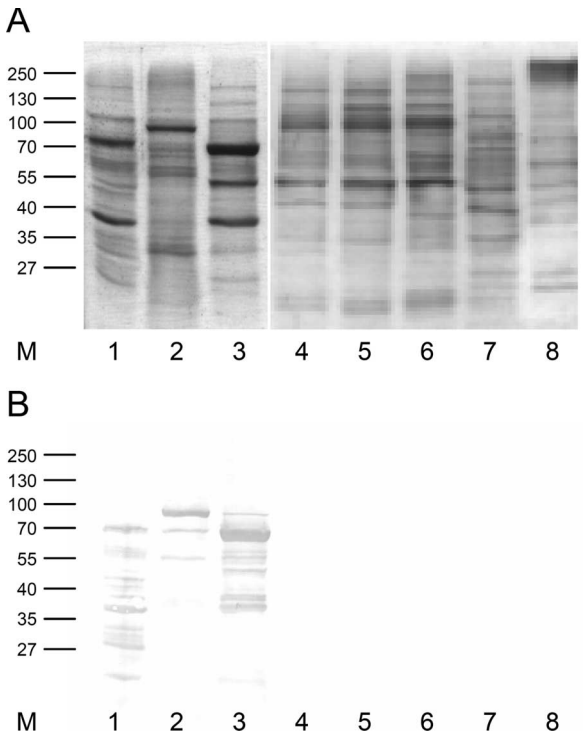

FIG. 4. SDS-PAGE (A) and Western immunoblot (B) analysis of SDS surface protein extracts. Lane M, marker; lanes 1 to 3, *S. aureus* strains CI7 (lane 1), Wood 46 (lane 2), and Newman (lane 3); lanes 4 to 8, strains of non-*S. aureus* staphylococcal species *S. epidermidis* (lanes 4 and 5), *S. carnosus* subsp. *carnosus* (lane 6), *S. haemolyticus* (lane 7), and *S. hominis* subsp. *hominis* (lane 8). Blots were prepared as described in Materials and Methods.

for the presence of the *eap* gene and gene product homologs on the DNA, transcriptional, and protein levels. To our knowledge, this is currently one of the most comprehensive collections of staphylococcal isolates tested for validation of a molecular diagnostic target. It is noteworthy that the clinical isolates tested previously to be *eap* negative (19) have now in fact been shown to harbor the *eap* gene, demonstrating the superiority of the newly designed primer pair to former applied primers. It also stresses the so far unexceptional possession of this gene by *S. aureus*.

For detection of putative differences between CoNS strains recovered from the physiological skin floras and those strains involved in clinical significant infections, isolates were included from a German multicenter study in which only CoNS strains considered etiologically relevant were collected. However, when the newly designed PCR primers were used, all of the non-*S. aureus* staphylococci were shown to be *eap* negative. In order to exclude negative PCR results due to potential DNA sequence heterogeneities of the primer binding sites, Southern blot analyses were performed with DIG-labeled *eap* PCR amplicons. Of particular interest, Southern blot analyses as well as Northern and Western blot analyses gave no positive results for non-*S. aureus* isolates. Hence, the existence of Eap homologs in other staphylococcal species is highly unlikely. Given the pleiotropic biologic effects of Eap and the prevalence value of 100% for the *eap* gene, one may speculate that this secretable expanded repertoire adhesive molecule may be of crucial importance to *S. aureus*. However, currently it is unclear why only 70% of the isolates tested expressed Eap under in vitro conditions (19) and whether this correlates with specific requirements in a given ecological niche.

In the light of the ongoing increase of the prevalence of methicillin-resistant *S. aureus* strains in hospitals and other health care facilities worldwide (14) and the advent, dissemination, and emergence of the highly virulent Panton-Valentine leukocidin-positive *S. aureus* clones (39), rapid and accurate identification of *S. aureus* infections and/or colonization is therefore a prerequisite for adequate and timely therapy, disease control, and effective epidemiological surveillance. Presently, phenotypic detection and identification of *S. aureus* are standard procedures in routine clinical microbiology. However, identification based on morphological characteristics, metabolic pathways, growth factor requirements, or antigenic composition depends fundamentally on expression of genetic information influenced by various factors and often involves nonobjective criteria for interpretation of results. Consequently, manifold molecular strategies have been developed to overcome the limitations of the traditional identification approaches. In addition to universal gene targets based on the ribosomal gene cluster or on the RNA polymerase B (*rpoB*) (2, 29, 31, 34), several specific targets, such as the staphylococcal coagulase gene (*coa*), a thermostable nuclease gene (*nuc*), a superoxide dismutase gene (*sodM*), the HSP60 gene, specific

genomic DNA fragments of unknown function, and the *fem* factor-encoding genes (6, 13, 24, 26, 32, 37), were used for molecular detection and identification of *S. aureus*. However, some of these studies concentrated on only a limited number of staphylococcal species. Additionally, target gene-negative *S. aureus* strains as well as target gene homologs in CoNS were observed, hampering both the sensitivity and the specificity of these molecular approaches (22, 24, 36). Here, we demonstrate that targeting the *eap* gene allows appending the diagnostic toolbox. This gene appears to be particularly suitable for molecular diagnostics of *S. aureus*. Based on these data, the sensitivity and specificity values for the *eap* gene were both remarkably high (i.e., 100%).

In conclusion, Eap was shown to be a virulence factor specifically restricted to *S. aureus* and occurring in all isolates of this species tested. In contrast, CoNS and other coagulase-positive or -variable staphylococcal species were shown to miss the encoding gene as well as homologs of this factor as shown at the DNA, transcriptional, and protein levels. Thus, the presence of Eap seems to be a core trait of *S. aureus*, offering a promising target for sensitive and specific detection of *S. aureus*.

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