Molecular Diagnostics of Clinically Important Staphyloccoci

J. ŠTĚPÁN, R. PANTUČEK, J. DOŠKAŘ*

Department of Genetics and Molecular Biology, Faculty of Science, Masaryk University, 611 37 Brno, Czechia
fax   + 420 549 492 570  
e-mail doskar@sci.muni.cz

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ABSTRACT. Bacterial species of the genus Staphylococcus known as important human and animal pathogens are the cause of a number of severe infectious diseases. Apart from the major pathogen Staphylococcus aureus, other species until recently considered to be nonpathogenic may also be involved in serious infections. Rapid and accurate identification of the disease-causing agent is therefore prerequisite for disease control and epidemiological surveillance. Modern methods for identification and typing of bacterial species are based on genome analysis and have many advantages compared to phenotypic methods. The genotypic methods currently used in molecular diagnostics of staphylococcal species, particularly of S. aureus, are reviewed. Attention is also paid to new molecular methods with the highest discriminatory power. Efforts made to achieve interlaboratory reproducibility of diagnostic methods are presented.

Abbreviations
EI A enzyme immunoassay
HVR hypervariable region
IS insertion sequence
MIC minimum inhibitory concentration
MLEE multilocus enzyme electrophoresis
MRSA methicillin-resistant Staphylococcus aureus
MSCRAMM microbial surface components recognizing adhesive matrix molecule
MSSA methicillin-sensitive Staphylococcus aureus
PCR polymerase chain reaction
PFGE pulsed-field gel electrophoresis
RAPD randomly amplified polymorphic DNA
REA restriction endonuclease analysis
RFLP restriction fragment length polymorphism
SCCmec staphylococcal chromosome cassette methicillin-resistance island
SRFH selective restriction fragment hybridization
SSCP single-strand conformation polymorphism
VNTR variable number of tandem repeat

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*Corresponding author.
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1 INTRODUCTION

Staphylococci are among the most widespread pathogenic and opportune pathogenic bacteria (Kloos et al. 1991; Holt et al. 1994). At present, 37 staphylococcal species are known, 9 of them including 2 subspecies, and 1 of them including 3 subspecies. Some taxa of the genus Staphylococcus are relevant to human medicine, others have been found only in other animals. Based on their capacity to produce free coagulase, staphylococci are traditionally divided into two groups: coagulase-positive species, considered to be pathogenic, and less dangerous coagulase-negative species (Table I).

Staphylococcus aureus, pathogenic to both humans and other animals, is the most relevant of the coagulase-positive species. It is a major clinical pathogen responsible for many serious infections among the general population and a leading cause of nosocomial infections. Non-human animals are the hosts of the non-S. aureus coagulase-positive species (Table I).

The complex cell-wall and extracellular proteins of S. aureus are constituted by multiple antigens and biologically active substances acting as pathogenicity factors: capsule (Sau et al. 1997), peptidoglycan, teichoic acids, agglutinogens, staphylococcal protein A, clumping factor (bound coagulase) (Ni Eidhin et al. 1998), other adhesins of the MSCRAMM family (Foster and Höök 1998), staphylocoagulase, staphylokinase, hylauronidase, staphylococcal V8 proteinase (Dur et al. 1997), hemolysins, leucocidins (Gravet et al. 1998), exfoliative toxins, enterotoxins, toxic shock syndrome toxin 1 (Dinges et al. 2000), epidermal cell differentiation inhibitor (Yamaguchi et al. 2001, 2002), and staphylococcal exotoxin-like proteins (Williams et al. 2000). Strains producing more exoproteins, such as exfoliative toxins or enterotoxins, are characterized by greater pathogenicity, pose a higher risk to humans, and cause more serious diseases. A central role in the pathogenesis of staphylococci has been assigned to the staphylococcal accessory gene regulator (agr), which increases the expression of many secreted virulence factors (Yarwood and Schlievert 2003).

The most clinically important coagulase-negative staphylococci are S. epidermidis, S. haemolyticus, S. hominis, S. sciuri and S. saprophyticus. S. epidermidis is a typical opportunist pathogen known to cause a number of infections in weakened patients. Certain types of hospital-acquired infections (e.g., those associated with the use of catheters) are due to strains producing slime, i.e., an adherence factor. S. haemolyticus is the second most frequently isolated species of coagulase-negative staphylococci after S. epidermidis. It was detected in patients with infected wounds, urinary tract infections, endocarditis, and bacteremia. Interestingly, vancomycin resistance was reported in this species (Biavasco et al. 2000). S. hominis is the third most commonly isolated species whose multiresistant subspecies S. hominis subsp. novohiosepticus (Kloos et al. 1998), mostly isolated from hemocultures or catheters, causes severe infections of the bloodstream (Petráš et al. 2002). S. saprophyticus is the causative agent of urinary tract infections. The species S. sciuri may cause wound infections and peritonitis and is considered to be the natural reservoir of the mecA gene – the methicillin resistance determinant (Wu et al. 1998; Marsou et al. 1999). S. lugdunensis and S. schleiferi subsp. schleiferi are opportunist pathogens, commonly associated with endocarditis, septicemia, and prosthetic device infections. Diseases caused by other species such as S. cohnii, S. simulans, or S. warneri are less fre-