Research in Microbiology 168 (2017) 1–15

www.elsevier.com/locate/resmic

Review

Methicillin-resistant food-related *Staphylococcus aureus*: a review of current knowledge and biofilm formation for future studies and applications

Agapi I. Doulgeraki [a,*], Pierluigi Di Ciccio [b], Adriana Ianieri [b], George-John E. Nychas [a]

[a] *Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Faculty of Foods, Biotechnology and Development, Agricultural University of Athens (AUA), Iera Odos 75, Athens, 11855, Greece*

[b] *Department of Food Science, University of Parma, Via del Taglio 10, 43126, Parma, Italy*

Received 11 January 2016; accepted 5 August 2016
Available online 17 August 2016

Abstract

There is increasing concern about the public health impact of methicillin-resistant *Staphylococcus aureus*. Food and animal are vectors of transmission, but the contribution of a contaminated environment is not well characterized. With regard to this, staphylococcal biofilms serve as a virulence factor, allowing MRSA strains to adhere to surfaces and other materials used in the food industry.

Methicillin resistance and biofilm-forming capacity may contribute to the success of *S. aureus* as a human pathogen in both health care and community settings and the food production chain. This review summarizes current knowledge about the significance of food- and animal-derived MRSA strains and provides data on attachment and biofilm formation of MRSA. In addition, the impact of quorum sensing on MRSA gene expression and biofilm formation is examined.

Keywords: *Staphylococcus aureus*; MRSA; Biofilm; Quorum sensing; Virulence

1. An overview

Highly virulent strains of staphylococci are emerging which are resistant to many antimicrobic molecules, such as methicillin-resistant *Staphylococcus aureus* (MRSA). Currently, MRSA is the most commonly identified antibiotic-resistant pathogen in many parts of the world [1]. While long recognized as a nosocomial infection [2], the epidemiology of MRSA has changed in recent years with the emergence of community-acquired MRSA [3]. At present, new evidence suggests that domestic animals, including food animals, are capable of serving as reservoirs and shedders of MRSA and that transmission between host species may be possible [4]. The emergence of MRSA in food-producing animals has provoked great concern about the presence of MRSA in associated foodstuff. MRSA strains have been isolated from foods, posing a threat concerning their possible dissemination through the food production chain [5,6]. Both methicillin-sensitive *S. aureus* (MSSA) and MRSA have an inherent ability to form biofilms on various surfaces [7,8]. Staphylococcal biofilms serve as a virulence factor allowing the MRSA strains to adhere to surfaces including implanted devices and other materials used in the food sector. Scott et al. [8] reported that MRSA strains were found on various household surfaces.

2. Methicillin-resistant *S. aureus* (MRSA)

MRSA strains were first reported in humans in the 1960s and subsequently, emerged as important nosocomial pathogens with multiple healthcare-associated (HA-MRSA) clones being internationally disseminated. Until the 1980s, MRSA infections were restricted to hospitals and were primarily associated with immunocompromised individuals. In the late 1980s and early 1990s, MRSA emerged as an important agent

* Corresponding author. Fax: +30 210 5294938.
E-mail address: adoulgeraki@aua.gr (A.I. Doulgeraki).

http://dx.doi.org/10.1016/j.resmic.2016.08.001
0923-2508/

of community-acquired (CA-MRSA) infections, first in the Oceania region and later throughout the world [9,10].

CA-MRSA differ from the HA-MRSA as they show a more virulent phenotypic profile. They frequently produce the Panton-Valentine leukocidin, a toxin often associated with severe skin infections. These isolates have been reported to affect individuals without classical risk factors for MRSA infections [11,12]. In addition, nosocomial infections can now be caused by CA-MRSA lineages, making it difficult to distinguish between CA-and HA-MRSA isolates [11].

In 2010, it was estimated that MRSA caused illness in more than 150,000 persons annually in healthcare facilities in the EU [13]. Recent statistics indicate that invasive HA-MRSA infections have declined, but CA-MRSA infections are still increasing [14]. MRSA isolates display a remarkable clonal structure and pandemic clones are associated with few specific lineages [15]. Currently, MRSA is distributed worldwide and constitutes a major concern in human health because of its complex epidemiology and its ability to acquire novel antibiotic resistance mechanisms [16].

Different genetic techniques are currently used for classification of *S. aureus* strains, including pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST) and DNA sequencing of the X region of the protein A gene (spa typing). Consequently, a single *S. aureus* isolate can have more than one valid name, depending on the test used for typing. Examples of strain names are USA100, CMRSA1 or EMRSA1, based on PFGE typing, ST followed by a number (ST398) based on MLST typing, or "t" followed by a number (t011) in spa typing. MRSA are also grouped, by MLST, into clonal complexes (CC398), which contain genetically related ST types. Isolates may be identified with a combination of tests for a more complete description. The most common MRSA multilocus sequence typing (MLST) clonal complexes (CC) worldwide are CC1, CC5, CC8, CC22, CC30 and CC45 [15]. More recently, distinct lineages of MRSA have been identified from livestock, known as livestock-associated MRSA (LA-MRSA), highlighting the adaptation of the species to diverse ecological niches. Worldwide, in fact, various clones of MRSA have been reported in domestic pets, livestock, wild birds and other animals [17]. These findings revealed that companion, livestock and wildlife animals can play a major role as MRSA reservoirs. These strains (LA-MRSA) show a different profile compared to HA- and CA-MRSA. LA-MRSA has been identified as being less aggressive, not encoding many of the toxins often associated with *S. aureus* [18]. Many of the LA-MRSA strains belong to lineage multilocus sequence type 398 (ST398) and they show a broader host range compared to most other *S. aureus* lineages [10]. It has been postulated, that LA-MRSA originated as methicillin-sensitive *S. aureus* in humans and were transferred to pigs, where they acquired methicillin and tetracycline resistance via the uptake of mobile genetic elements and then transferred back to humans [19].

Methicillin resistance is, in staphylococci, conferred by the carriage of staphylococcal cassette chromosome *mec* (SCC*mec*) [20]. SCC*mec* is a mobile genetic element that includes the *mec* gene complex, composed of the *mecA* gene encoding penicillin-binding protein 2a (PBP2a), which shows low affinity for β-lactam antibiotics such as penicillin and methicillin, the two regulation genes *mecR1* (encoding the signal transducer protein MecR1) and *mecI* (encoding the repressor protein MecI) and the associated insertion sequences [21,22]. SCC*mec* also carries unique site-specific recombinases designated as cassette chromosome recombinases (*ccr*), responsible for mobility of the elements [22,23]. In addition to the *ccr* and *mec* genes, three other regions (J regions) are included in SCC*mec* typing [24]. J regions typically contain pseudogenes and truncated copies of transposons and insertion sequences and were originally designated junkyard regions. Currently, they are commonly referred to as joining regions, since they encode important functions such as resistance to additional antibiotics and to heavy metals [25].

Structural organization and the genetic content of SCC*mec* are highly diverse and the chromosomal cassettes are classified by a hierarchical system into: (i) types based on a combination of the type of *ccr* gene complex and the class of the *mec* gene complex and (ii) subtypes defined by structural differences in J-1, -2 and -3 regions [22,23]. Despite differences in size (from ~21 kb to 67 kb) and gene content, all SCC*mec* elements share common features. First of all, all SCC*mec* carry the *mecA* gene in a *mec* gene complex and the *ccr* gene(s) (*ccrAB* and/or *ccrC*) in a *ccr* gene complex. Integration of SCC*mec* at a specific site, the attB integration site sequence (ISS) present at the 3′ end of the *orfX* gene in the staphylococcal chromosome, which serves as a target for *ccr*-mediated recombination, is another shared characteristic, as is the presence of flanking direct repeat (DR) sequences containing the ISS [25].

The *ccr* gene(s) and surrounding open reading frames (ORFs) compose the *ccr* complex. To date, three phylogenetically different *ccr* genes (*ccrA*, *ccrB* and *ccrC*, with nucleotide identity of <50%) have been found in *S. aureus* strains. The *ccrA* and *ccrB* genes were classified into four allotypes, based on sequence variations between them (DNA sequence identities are between 50% and 84%) [26]. The *ccr* gene complexes reported thus far can be divided into two groups, one carrying two adjacent *ccr* genes (*ccrA* and *ccrB*) and the other carrying the *ccrC*, and five types were identified: type 1 (carrying *ccr*A1B1), type 2 (carrying *ccr*A2B2), type 3 (carrying *ccr*A3B3), type 4 (carrying *ccr*A4B4) and type 5 (carrying *ccr*C) [27].

The *mec* gene complex is classified into five classes (A to E). The prototype of the *mec* gene complex is class A *mec*, which contains *mecA*, the complete *mecR1* and *mecI* regulatory genes upstream of *mecA*, and the hypervariable region (HVR) and insertion sequence IS*431* downstream of *mecA*. The other four classes contain truncated *mecR1* genes resulting from insertion of IS1272 or IS431 and/or differences in spatial arrangement of the elements composing the complex. Several variants of the major classes have also been described [25].

Subtypes of SCC*mec* within the same *mec-ccr* complex are defined by structural differences in J regions. J1 is the region between the right chromosome junction and the *ccr* complex,