Institut Pasteur
Microbes and Infection 14 (2012) 1352–1362
www.elsevier.com/locate/micinf

Original article

The virulence of *Staphylococcus aureus* correlates with strain genotype in a chicken embryo model but not a nematode model

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Received 27 July 2012; accepted 25 September 2012
Available online 4 October 2012

Abstract

Staphylococcus aureus infections are of major importance in human and veterinary medicine. Studies of the virulence of this bacterium are complicated by inconsistent results obtained in different animal models. We searched for an uncomplicated and inexpensive model suitable to study virulence of poultry strains of *S. aureus* using a genome-wide approach. We determined that a useful model would clearly differentiate strains of high and low virulence, and that this would generally correlate with the genetic relatedness among strains. To this end *Gallus gallus* (chicken) embryo and *Caenorhabditis elegans* (nematode) models were selected, and their response to challenge by a set of well-characterized *Staphylococcus* strains was evaluated. The chicken embryo model allowed to determine variation in virulence among strains of poultry and human origin. The survival of embryos ranged from 0% to almost 100% for the various strains. In contrast, variation in virulence of most strains in the nematode model was comparable, regardless of their origin or genotype, demonstrating limited usefulness of this model. Most importantly, a clear correlation was found between the virulence level in the embryo model and the genotype of the tested poultry strains. Our findings indicate the potential usefulness of embryo model for future identification of host-specific adaptations and virulence factors in *S. aureus*.

Keywords: *Staphylococcus aureus*; Infection model; Host–pathogen interaction; Chicken embryo; Nematode; *Caenorhabditis elegans*

1. Introduction

Staphylococcal infections are of major importance in both human and veterinary medicine. In particular, *Staphylococcus aureus* is responsible for a variety of serious diseases in a range of hosts including humans. As a consequence of the rising costs of treatment and prevention, interest in the pathobiology of this bacterium is continuing to increase. Human and zoonotic strains of *S. aureus* have been convincingly demonstrated to be genetically distinct [1–3], but it has also been established that *S. aureus* can be transmitted among different species. As the bacterium has a highly conserved core genome, host-specific adaptations are probably determined by a small number of genes carried on mobile genetic elements (MGEs) [3,4]. This implies that the bacterium may switch host specificity by acquisition or loss of MGEs, although whether these elements contribute to the ability to colonize a particular host and/or directly cause disease is unclear [5,6].

Detailed studies of host adaptation and virulence require models reflecting the complexity of host organisms. Numerous animal models for staphylococcal infection have been proposed, but those most commonly used involve laboratory rodents, especially mice, which are considered the standard for studies of host–pathogen interactions and in the development of novel therapeutic approaches [7–9]. However, this choice has largely been one of convenience, as mice are relatively

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http://dx.doi.org/10.1016/j.micinf.2012.09.006

resistant to staphylococcal infection. In most cases the amount of bacteria and its route of administration differ markedly from that which occurs naturally, raising questions about the relevance of results. Mammalian models that better reflect human infection have been proposed, including rabbit, pig and guinea pig skin infection models [10–12]. However, results obtained in different models are often inconsistent and appropriate models for multiple aspects of staphylococcal pathogenesis are lacking. In addition, relatively high costs, complexity and legal restrictions limit the use of mammals in infection studies. Alternative models for the study of staphylococcal infections have been introduced in recent years, including cell lines, tissue cultures and invertebrate hosts. Amongst insects, the fruit fly (*Drosophila melanogaster*) [13], and larvae of the greater wax moth (*Galleria mellonella*) [14] and the silkworm (*Bombyx mori*) [15] have been used as model organisms, and the nematode *Caenorhabditis elegans* has been used to investigate virulence traits of *S. aureus* and the efficacy of antibacterial treatment [16–18]. Recently a zebrafish embryo model has been proposed as an alternative for mammalian hosts being a tractable model in which the molecular and cellular mechanisms of *S. aureus* infection and non-specific host–pathogen interactions can be dissected [19]. Despite many advantages, one must be cognizant that temperature of host cultivation is not optimal for growth of *S. aureus* and other human pathogens and some virulence genes have been shown to regulate by the temperature [20]. Overall, the alternative models are even more limited than those involving mammalian hosts for drawing conclusions for human infections because of the vast differences in the physiology of the model organisms relative to higher animals.

Embryonated egg model offers the potential to bridge the gap between invertebrate and mammalian host models for studies of systemic infection. This technically uncomplicated and low cost approach has been successfully used to investigate the virulence of *S. aureus* [21,22], other bacteria [23,24], and fungi [25,26]. Nonetheless, an important difference between mammalian and avian models is the immune status of the host. Systemic infections are predominantly studied in immunocompetent animals, whereas the adaptive immune system of chicken embryos is not functional until late embryonic development.

The aim of this study was to assess whether there is a correlation between the genotype of particular *S. aureus* strains and their virulence in chicken embryo and nematode models. We assumed that a useful model would show that closely related strains have comparable virulence, and would show variation in the level of virulence among at least some unrelated strains. The relatedness of poultry strains was determined using multiple state-of-the-art typing methods, and their virulence was evaluated in *Gallus gallus* (chicken) embryo and *C. elegans* models. The chicken embryo model enabled to pinpoint variation in the level of virulence of different strains. Moreover the virulence correlated with the genotype of tested strains. The variation in virulence of strains in the nematode model was low, highlighting the limited usefulness of this model for the study of virulence determinants among poultry and human strains of *Staphylococcus*.

2. Materials and methods

2.1. Ethical statement

All experiments were performed in compliance with the animal protection laws of Poland. Experiments utilizing chicken embryos were terminated on developmental day 17 at the latest, four days prior to hatching.

2.2. Bacterial and nematode strains and growth conditions

The characteristics of bacterial and nematode strains used in this study are summarized in Table 1. All *Staphylococcus* strains were maintained at −70 °C in tryptic soy (TS) medium containing 10% glycerol, and cultured in TS broth (TSB) at 37 °C with aeration. *C. elegans* strain N2 was maintained at room temperature (RT) on nematode growth medium plates spread with *Escherichia coli* strain OP50 as a food source. The nematodes were manipulated as described previously [17,27].

2.3. C. elegans *infection model*

The nematodes were manipulated using established techniques [17,28], with the following modifications. To synchronize the growth of worms, eggs were collected via the alkaline hypochlorite method and incubated in S buffer (129 ml 0.05 M K_2HPO_4, 871 ml 0.05 M KH_2PO_4, 5.85 g NaCl) for 16–22 h at RT. Worms arrested at L1 larval stage were plated on nematode growth medium agar spread with *E. coli* strain OP50, and incubated at 25 °C for 24 h to generate the L4 larvae stage. *Staphylococcus* assay plates were prepared as follows. The bacterial strains were grown overnight with aeration at 37 °C in TSB. A 1:9 dilution was made in TSB, and 10 µl of the diluted culture was spread on 3.5 cm diameter plates containing TS agar (TSA) supplemented with 5 µg/ml nalidixic acid. The agar plates were incubated at 37 °C for 3 h, equilibrated to room temperature for 30–60 min, and then seeded with worms. For each replicate approximately 50 L4-stage nematodes were added to each plate seeded with the *S. aureus* strain to be tested. Each experiment consisted of four replicates for each strain, and a single unseeded control. For each *Staphylococcus* strain three independent experiments were performed, in total comprising 12 seeded replicates and 3 unseeded controls. The plates were incubated at 25 °C for 7 days and examined at least every 24 h using a stereomicroscope. The number of live and dead worms was recorded. A worm was considered dead when it failed to respond to the plate being tapped or a gentle touch with a platinum wire. Worms that died as a result of becoming stuck to the wall of the plate were excluded from the analysis.

2.4. Chicken embryo infection model

Hatching eggs were obtained from a commercial hatchery, and originated from chickens bred without the use of antibiotic