Microbial Pathogenesis xxx (2016) 1–7

Contents lists available at ScienceDirect

Microbial Pathogenesis

journal homepage: www.elsevier.com/locate/micpath

# Fitness and competitive growth comparison of methicillin resistant and methicillin susceptible *Staphylococcus aureus* colonies

Emine Durhan [a], Safiye Elif Korcan [b], Mustafa Altindis [c, *], Muhsin Konuk [d]

[a] Department of Biology, Afyon Kocatepe University, 03200, Afyonkarahisar, Turkey
[b] Health Care Vocational College, Uşak University, Uşak, Turkey
[c] Department of Medical Microbiology, Sakarya University, School of Medicine, Turkey
[d] Department of Molecular Biology and Genetics, Üsküdar University, 34662, Istanbul, Turkey

ARTICLE INFO

*Article history:*
Received 17 May 2016
Accepted 6 June 2016
Available online xxx

*Keywords:*
MRSA
mecA
Fitness cost
Antibiotic resistance
Growth rate

ABSTRACT

Exponential developments of both Methicillin resistant *Staphylococcus aureus* (MRSA) 3R ve 36R and methicillin susceptible *Staphylococcus aureus* (MSSA) 27S were evaluated in the presence and absence of oxacillin. The strains were isolated from the specimens collected in microbiology department. It was also determined the transfer of mecA gene from 3R to 27S strain by using the replica plate technique. It was observed that the presence of antibiotics in the preliminary culture had a positive impact on the growth of the secondary culture of MRSA isolates. Comparison results of Rt bacteria in three different mixed cultures, assessed with Tukey's HSD test, showed a significant statistical difference among the groups. The values were as following; on the first day; Df: 2, F: 60.90, P: 0.0001, second day; Df:2, F:90.56, P: 0.0000, and third day; Df:2, F:4.86, P:0.0557. As a result of the study, we can suggest that the gene expression levels of the transferred antibiotic resistance genes could help us in both controlling hospital originated sickness and developing new strategies to prevent the spread of resistant bacteria.



## 1. Introduction

Methicillin (oxacillin)-resistant *Staphylococcus aureus* (MRSA) is known to be one of the most prevalent nosocomial pathogens throughout the world and capable of causing a wide range of hospital-linked infections [1]. Such organisms are also frequently resistant to most of the commonly used antimicrobial agents, including the aminoglycosides, macrolides, chloramphenicol, tetracycline, and fluoroquinolones [2]. In addition, MRSA strains should be considered as resistant to all cephalosporins, cephems, and other β-lactams. *In vitro* test results obtained with those agents contain a staphylococcal cassette chromosome mec (SCCmec) element harbouring the *mecA* gene that codes for an alternative penicillin binding protein (PBP), PBP2a, which confers resistance to beta-lactams, the antibiotic class of first choice for treating *S. aureus* infections [3].

MRSA have been implicated increasingly in complicated infections with higher mortality related to difficulties or delay in initiating effective antibiotic therapy [4]. Therefore the increasing rates of recovery of antimicrobial-resistant microorganisms in hospital and community settings are of growing concern [5]. Resistance may emerge from a mutation in an intrinsic chromosomal gene or by acquisition of exogenous genetic material bearing resistance determinants. Resistance to antibiotics frequently reduces the fitness of bacteria in the absence of antibiotics; this is referred to as the "cost" of resistance [6].

There are several known factors to affect the fitness of circulating strains: drug pressures, environmental changes, genotype of the strain and the stress induced by the competing strains. All these factors either independently or together put the bacterial population under fluctuating selection pressure. The fitness of a pathogen is composed of a number of interrelated parameters. The most important of these is the relative rates at which antibiotic-susceptible and resistant bacteria (i) grow in infected hosts and the environment, (ii) are transmitted between hosts, and (iii) are cleared from the infected hosts [7]. Because of these reasons pathogen work had to be done.

Gene transferring studies has been carried out between mutant-resistant strain and sensitive strains up to now. However, antibiotic sensitive or public and hospital originated isolates can carry various mutations, and this state could affect their resistance development

* Corresponding author.
*E-mail address:* maltindis@sakarya.edu.tr (M. Altindis).

http://dx.doi.org/10.1016/j.micpath.2016.06.009
0882-4010/

even if they perform gene transfer among them. Therefore, both the fitness and gene transfer ratios of the resistant and sensitive strains from hospital specimens should be evaluated separately. This evaluation is thought to help us in predicting the resistance development of the strains in different media or environment.

Considering the major public health problem that would result from MRSA dissemination, our goals were to determine the fitness of clinical isolates 3R and 36R, and to investigate the genetic alterations occurred in 27S MSSA strain.

## 2. Materials and methods

### 2.1. Selection of bacterial isolates

Two MRSA (3R-36R) isolates and a MSSA (27S) isolate were obtained over a period of one year from 2012 to 2013 in the Department of Medical Microbiology of Afyon Kocatepe University, TURKEY. Identification of *S. aureus* was confirmed by standard laboratory methods based on colony morphology in sheep blood agar catalase, and tube coagulase tests. CHROMagar MRSA is a new chromogenic medium for the identification of MRSA. 3R MRSA, 36R MRSA and 27S MSSA isolates were inoculated on CHROMagar MRSA. The growth of colonies showing any pink or mauve coloration was considered to be positive. The procedure was performed as recommended by the manufacturer. Methicillin resistance was assessed in accordance to Clinical and Laboratory Standards Institute guidelines (CLSI) [8]. Oxacillin (Sigma-Aldrich Company, USA) susceptibility test of isolates by using broth dilution method was performed in accordance to National Committee for Clinical Laboratory Standards (NCCLS) guidelines. For molecular confirmation of both MRSA and MSSA were performed by real time (rt)-PCR. The presence of the *mec*A and SCCmec genes were verified by Xpert MRSA/SA PCR. Modification of this method was developed for clinical specimens were also performed. Isolates (0.5 McFarland) suspended in sterile distilled water and were diluted with the ratio of 1:1000. The following procedure was performed as recommended by the manufacturer.

### 2.2. Determination of growth rates

After MICs determination by diluting in Mueller-Hinton broth (MHB), growth rates were measured at 37 °C in MHB, with or without oxacillin presence. 3R, 36R and 27S strains were grown for 24 h at 37 °C with 1/50 the MIC with or without oxacillin (Sigma). The cultures were then diluted 1:20 into Brain Hearth Infusion broth (BHI) with or without oxacillin and grown at 37 °C with shaking. After incubation, $10^5$ CFU bacteria containing inoculate were transferred onto 200 μl of BHI micro plates at the beginning of the stationary phase. These were incubated at 37 °C with regular shaking (125 rpm). Bacterial growth was measured every 20 min by recording the absorbance at 600 nm using a micro plate reader [9].Growth rates were then calculated by using the equation given below.

$\mu = [\ln(N_t) - \ln(N_0)]/(t - t_0)$, where

N: natural logarithm of population density.t: against time.

Relative growth rates were also calculated as the ratio of the growth rate of resistant transconjugant strain 3R and 36R versus recipient strain 27S.

### 2.3. Mixed growth experiments

Growth competition experiments were performed according to Foucault et al. [9]. In this assay, a comparison between strain 3R (methicillin and ciprofloxacin-resistant) and susceptible strain 27S (methicillin and ciprofloxacin-susceptible) was carried out by inoculating $10^5$ CFU of each strain into BHI broth at ratios of 1:1, 1:100 and 100:1 in the absence of oxacillin [10,11].

Susceptible, resistant and transconjugant strains (Rt) number of bacteria were determined by using the replica plate technique in mixed cultures at ratios of 1:1, 1:100, and 100:1. We used filter-papers to make replica inoculations from the initial plate on to a series of plates with different media. Three different media were used for determination of the antibiotic susceptibility of isolates. The first medium was antibiotic-free Blood agar. In this medium, both antibiotic-sensitive and −resistant isolates could grow. Their numbers were determined at nonselective antibiotic-free Blood plates. The second medium was containing only oxacillin and in this medium only antibiotic resistant Rt and 3R isolates could grow. The third medium was Blood agar containing 4 μg/ml oxacillin and 0.25 μg/ml ciprofloxacin, and 3R.strain might grow in it. After determining the numbers of both antibiotic-resistant and sensitive ones, Relative fitness was expressed as the competition index (CI). CI values were calculated mathematically by the following formula:

CI = (antibiotic resistant bacteria/antibiotic-sensitive bacteria ($t_1$)/(antibiotic resistant bacteria/antibiotic-sensitive bacteria) $t_0$.

Possible or Rt(s) determined colonies were chosen randomly to examine both their antibiotic, oxacillin, and ciprofloxacin, susceptibility and if they have the mecA gene. While their antibiotic susceptibility was determined by disc diffusion method, presence of *mecA* was confirmed by Gene*Xpert MRSA/SA PCR* method TM.

### 2.4. Statistical analysis

Mean values and standard deviations were calculated using Excel version 11.3.7 software. Student's *t*-test and TukeyHSD analysis were used to evaluate differences between means, with a significant probability at a P value of $\leq$0.05.

### 2.5. Ethical approvement

Ethical endorsement was ratified by the Medical Faculty Ethical Committee of Afyon Kocatepe University, TURKEY.

## 3. Results

### 3.1. Definition of the isolates

The catalase and coagulase tests of the 3R and 36R isolates were determined as positive. It was determined that these isolates could also reproduce in CHROMagar and their resistance to oxacillin was at 64 μg/ml concentration. The presence of Staphylococcal protein A (*spa*), staphylococcal cassette chromosome (SCC) and the gene for methicillin resistance (*mecA*) were verified with rt-PCR (Fig. 1).

Although we established *spa* genes by qualitative rt-PCR in MSSA 27S isolates, it was observed that these genes in SCC were not *mecA*. Ciprofloxacin and oxacillin resistant 3R isolate, oxacillin-sensitive and ciprofloxacin-resistant 36R isolate, and both ciprofloxacin and oxacillin sensitive 27S MSSA isolates has been used in further research.

### 3.2. Determination of growth rate

Two different pre-culture conditions have been applied in the evaluation of growth kinetics. The first one was BHI medium without oxacillin and the second one was a BHI medium with oxacillin. They were then transferred on to second antibiotic

Please cite this article in press as: E. Durhan, et al., Fitness and competitive growth comparison of methicillin resistant and methicillin susceptible *Staphylococcus aureus* colonies, Microbial Pathogenesis (2016), http://dx.doi.org/10.1016/j.micpath.2016.06.009