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Effect of Blood and Sera on Growth of *Staphylococcus aureus* in BHI media

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The aim of this study was to assess the effect of either blood or sera in BHI media on the growth rate of *S. aureus* isolates. This study was carried out on five *S. aureus* isolates including two MRSA and two MSSA isolates and COL standard isolate. Growth curve was plotted for isolates in BHI broth , BHI containing sheep blood and BHI containing calf serum then generation time of isolates in these three different growth medium was calculated. The mean of generation time of five bacteria in three media of BHI, BHIS and BHIB was 25.38, 26.2 and 26.9 min respectively which were not statistically significant (P>0.05). The generation time of standard strain was more than others. The mean generation time of MRSA was higher than MSSA isolates. This study showed that *S. aureus* which was isolated from healthy carriers had a slightly longer generation time than clinical isolates. These results suggest that addition of sheep blood and calf sera in BHI media was not able to increase the growth rate of *S. aureus*, even it slightly increased the generation time. BHI is an enriched nutritious media and is ideal for growth *S. aureus* and addition of serum and blood is not able to alter nutrition conditions in this media according to our assumption.

Key words: *Staphylococcus aureus*, Growth curve, Generation Time, Serum, Blood.

Staphylococcus aureus is a gram-positive pathogen which causes an array of diseases, ranging from minor localized skin lesions to life threatening deep tissue damage and systemic infections such as *pneumonia*, endocarditis and exotoxin syndromes[1].

Additionally, *S. aureus* is an important food-borne pathogen that causes staphylococcal gastroenteritis and food poisoning in humans[2]. *Staphylococcus aureus* is a dangerous human pathogen in both community-acquired and nosocomial infections. A fundamental biological property of this bacterium is its ability to asymptomatically colonize healthy individuals. *S. aureus* carriers are at higher risk of infection, and they are presumed to be an important source of the *S. aureus* strains that spread among individuals[3].

In many studies Brain-heart infusion broth (BHI broth) culture media is used for bacteria suspension preparation and its growth assessment rate. BHI broth is a highly nutritious general-purpose growth medium for culturing fastidious and non fastidious microorganisms. It is made by the recuperation of nutrients from boiled bovine or porcine hearts and brains. The components ofthis culture media are Brain Heart Infusion (from solids), pancreatic digest of gelatin,sodium chloride, dextrose, disodium phosphate[4,5,6]. Similar to other studies this culture media was used for growth curve plotting[7,8,9,10].

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The growth of the bacteria is affected by both physical and nutritional factors. The physical factors include the pH, temperature, osmotic pressure, hydrostatic pressure, and moisture content of the medium in which the organism is growing. The nutritional factors include the amount of Carbon, nitrogen, Sulphur, phosphorous, and other trace elements provided in the growth medium. The dynamics of the bacterial growth can be studied by plotting the cell growth (absorbance) versus the incubation time or log of cell number versus time. The microbial growth curve that represents the growth of a bacterial culture in time can be simply divided into the lag, exponential, and stationary phases[11,12]. The growth media recommended for some bacterial species may require the addition of components not already available in the base medium. These components may include, serum, blood, or chemical supplements.

Sera can serve as a source of growth factors, proteins, vitamins, hormones, Carbohydrates, lipids, amino acids, minerals, and trace elements. Additionally, serum can function as a pH buffer and can inactivate proteolytic enzymes. Sera from rabbit, horse, fetal bovine, and calf bovine sources are used to support the growth of some bacterial species in culture.

Blood is often added to growth medium to enhance the cultivation of highly fastidious microbial Species.

The aim of this study was to assess the effect of either blood or sera in BHI media on the growth rate of *S.aureus* isolated from healthy carrier and clinical sources. Comparison of growth rate of MRSA and MSSA is additional purpose of this study. The growth curves of five *S. aureus* isolates in three different growth media; including BHI, BHIB and BHIS were plotted during time of inoculation (0-time) through 24-hour incubation period determined by calculation of total number of bacteria per 0-time through to 24-hour and absorbance values. Then Generation times were calculated.

MATERIALS AND METHODS

Bacterial strains and culture media

This study was carried out on five *S.aureus* isolates including two MRSA and two MSSA isolates which comprised one isolate from healthy carrier person and one from clinical sample, in each group and COL standard isolate.

The isolates were inoculated on Blood agar, and selected single colonies were drown on BHI broth and incubated in 37°C for overnight. The concentration of each culture was standardized to 108cfu/ml equal to 0.5 McFarland. This suspension was serially diluted to 103cfuml^{-1} and this concentration was considered as 0-time, inoculation time.

These cultures incubated at 37°C in 24 test tubes which were sampled at hourly intervals from the time of inoculation of the culture, 0-time, through 24-hour incubation period. Each sample were inoculated on the Muller Hinton agar medium was purchased from Merck Company and incubated at 37°C, the number of colonies were counted after 24-hour incubation. The total number of bacteria per hour was calculated by the following equation: The number counted colony ×1/dilution index and the growth curve was plotted. The generation time of each *S.aureus* isolates was determined by choosing two points on the exponential phase of the growth curve, using the following formula:

$$g = 0.301 \times t / \log Nt - \log No$$

(g; Generation time, No; the initial population number (The first point), Nt; population at second point, t; the time interval (in hours) between the 2 points)

On the other hand, growth curve and generation time were determined by absorbance values at 578 nm (OD 578 nm) and were recorded for each suspension at hourly intervals.

This process also carried out for tested bacteria on two different media; BHI broth containing 5% sheep blood (BHIB) and BHI broth containing 5% Calf serum (BHIS). Each experiment was performed in triplicate and the mean was calculated subsequently.

The comparison of generation times among different isolates and different culture media were tested by comparing means of ANOVA method and $p<0.05$ was considered as significant.

RESULTS

The growth curves of five *S. aureus* isolates in three different growth media; including

BHI , BHIB and BHIS were plotted during time of inoculation (0-time) through 24-hour incubation period. Fig 1 shows growth curve of Clinical isolate methicillin-resistant *S. aureus* (2P) in three growth media; BHI, BHIB and BHIS at 24 h (Fig. 1). The growth curves of other isolates were similar to 2P, in which the corresponding data were not shown.

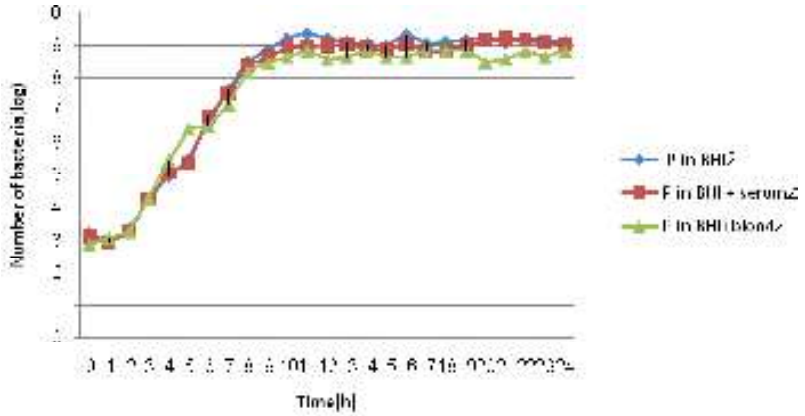

Fig. 1. Growth curve Clinical isolate MRSA (2P) in three medium, BHI broth , BHI broth containing 5% sheep blood and BHI broth containing5% Calf serum at 24 h.

The mean of generation time of five bacteria in three media of BHI broth , BHI broth containing 5% calf serum and BHI broth containing 5% sheep blood was 25.38, 26.2 and 26.9 min respectively which were not statistically significant (P>0.05). (Table 1)

In Fig. 2, growth pattern (growth curve) of five isolates have been shown in BHI broth medium which demonstrate all isolates having relatively similar patterns.

Table 1. Themean of generation time of five *S. aureus* isolates in three different growth medium

Media	N	Mean	Std. Deviation (±)
BHI broth	5	25.4	4.2
BHI broth+ calf serum	5	26.2	1.2
BHI broth +Blood	5	26.95	4.7
Total	15	26.18	3.5

P>0.05

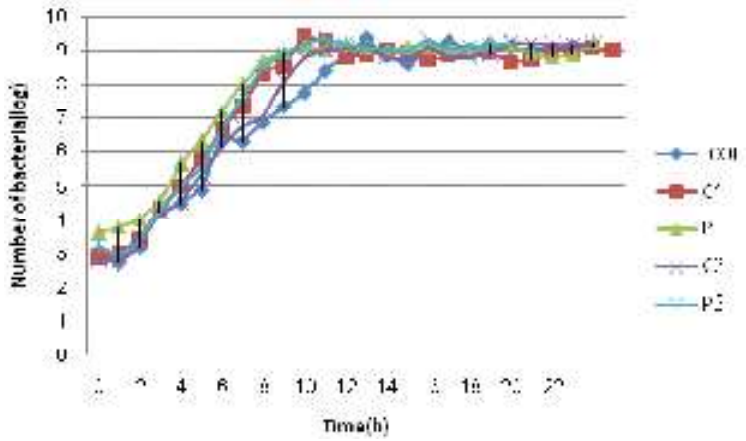

Fig. 2. Growth curves of *S. aureus* strains in BHI broth medium which demonstrate all isolates having relatively similar patterns. Standard strain(COL),Clinical isolate MRSA(2P), Clinical isolate MSSA(1P), Carrier isolate MRSA (2C),Carrier isolate MSSA (1C).

The mean of generation time of COL strain in the three different growth mediums have been 30.5 minutes and the generation time of another isolates was different from 22.55 min (22 min and 30 second) to 27.52 min (27 min and 30 second). The COL generation time was more than others.

Table 2. The mean of generation time of five *S. aureus* isolates

Isolate	N	Mean	Std. Deviation(±)
COL	3	30.49	2.4
Carrier MSSA	3	27.53	2.3
Patient MSSA	3	22.55	2.5
Carrier MRSA	3	24.16	2.8
Patient MRSA	3	26.2	2.4
Total	15	26.18	3.5

P=0.02*

The mean generation time of MRSA was higher than MSSA isolates, which was 26.9±3.55 min and 25±3.47 min for MRSA and MSSA respectively but these differences were not statistically significant (P>0.05).

The mean generation time of clinical and healthy carrier isolates were 25.8±2.9min and 24.4±2.96 min respectively, were not significant.

Fig. 3 shows generation time of *S.aureus* strains in three different growth media.

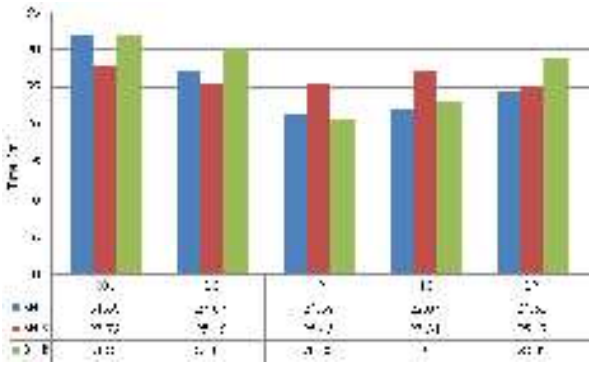

Fig. 3. Generation time of *S. aureus* strains in three different growth media.standard strain (COL), Clinical isolate MRSA(2P),Clinical isolate MSSA(1P),Carrier isolate MRSA (2C), Carrier isolate MSSA (1C)

DISCUSSION

This study showed that addition of sheep blood and calf sera in BHI media was not able to increase the growth rate of S.aureus, even it slightly increased the generation time. BHI is an enriched nutritious media and is ideal for growth S.aureus and addition of calf serum and sheep blood is not able to alter nutrition conditions in this media according to our assumption.

On the other hand , the sera inhibitory role on growth bacteria and particularly on S.aureus has been discussed in other studies[13,14]. Sera thermal treatment at 56°C reduces the sera bactericidal effect and increase the bacterial growth simultaneously[9]. Blood antibacterial effect against some bacteria previously reported[15].

Stratton *et al.,* found that human serum was less supportive than Mueller -Hinton broth for both growth and antimicrobial-induced lysis and killing of both methicillin-sensitive and methicillin -resistance *Staphylococcus aureus*[16]

Wiltshire *et al*., demonstrated that the growth rate of *S. aureus* in BHI is more than Pig sera culture medium[10], but on the other hand Oogai *et al.,* showed the growth rate of this bacteria in TSB and calf sera culture medium was similar[17].

The other aim of this study was to identify whether different isolates of *S. aureus* have similar growth pattern in particular culture media. As Fig. 2 elaborates, all the isolates of *S. aureus* in our work have relatively similar growth patterns. Although the growth rate of COL strain was lower than other isolates but all of them had the similar growth patterns ultimately.

Laurent and Rozgonyi, in two different independent studies found that MRSA isolates have longer generation time compared with MSSA

strains[8,18,19], which is similar to our findings.

MRSA isolates contain mecA gene encoding PBP2 Aprotein located on the bacterial cell surface, which might prevent the nutrient entry into *S.aureus.* If this hypothesis is correct, then it could be the reason for drugs and disinfectant agent's hard entry in MRSA isolates and therefore we expect MRSA to be more resistant than that of MSSA.

Finally , this study showed that *S.aureus*, which isolated from healthy carriers had a slightly longer generation time than clinicalisolates which is similar to Chen *et al.,* investigation showing mean doubling times for nasal carriage isolates of *S. aureus* were higher compared to infection isolates[20].

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