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Local *Staphylococcus aureus* Phage Groups

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Abstract

Background *Staphylococcus aureus* isolates distributed into 3 groups according to their sources, 10 isolates from each source. Each of the 30 isolates produced phage lysate. Based on our results, it has been found that these phages obtained from all isolates can be classified into 3 groups (A, B and C).

Objective To produce local phage groups from locally isolated *Staphylococcus aureus* strains to be used for epidemiological purposes.

Methods A total of 60 specimens were obtained from three different source locations, surgical theaters (instrument, walls, floor and masks), nurses and inpatients, were enrolled in a study based at Al-Sulimanyiah Teaching Hospital from December 2008 to November 2009. Each specimen was subjected to well known established microbiological. Methods for isolation and identification of *Staphylcoccus aureus.* All isolates were tested for the presence of phage employing heat method and detected by spotting method, also based on resistance or sensitivity of each isolates to give phage lysates by application of the cross-lysis technique.

Result *Staphylococcus aureus* isolates distributed into 3 groups according to their sources, 10 isolates from each source. Phages were induced from thirty Staphylococcus *aureus* isolate. Based on results obtained of the isolates, it has been found that these phages obtained from all isolates can be classified into 3 groups. Group (A) revealed that 1 and 6 phage lysates originally from isolates 1 and 6 were able to lyse all isolates in group 1 except 1 and 6 isolates and those in other groups which were unlysed. A strain was phage typeable (at least one phage produced 20 or more plaques of lysis). Isolates 15 and 16 produced phage lysates 15 and 16 in group (B) which were able to lyse all isolates in group 2 except 15 and 16 isolates and the remaining isolates in other groups which were unlysed. phage lysates 23 and 26 in group (C) which were induced from isolates 23 and 26 were able to lyse all isolates in group 3 except isolates 23 and 26 and the remaining isolates in other groups which were unlysed also.

Conclusions It is detected that 3 local phage groups from *Staphylococcus aureus* are presented to be used for epidemiological purposes in case of *Stapylococcus aureus* epidemic.

Key words *Staphylococcus aureus*, phages, epidemiology.

Introduction

Staphylococcus aureus is one of the most common agents causing infections of a wide spectrum of clinical conditions ranging from simple to life-threatening causes [1,2,3].

Staphylococcus aureus can survive on dry surfaces, increasing the chance of transmission and it is implicated in toxic shock syndrome in which some transpose allowed the rapid growth of this bacteria to release toxin that were absorbed into the blood stream [4].

Nosocomial infection is a significant epidemiological problem resulting in prolongation morbidity. *Staphylococcus aureus* species is the leading cause of nosocomial infection a methicillin resistant *Staphylococcus aureus* (MRSA). MRSA strains in the hospital are diresistant [4,5,6].

Problematically, Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Staphylococcus aureus* (VRSA) has become a major cause of hospital-

acquired infections and being recognized with increasing frequency in community acquired infections [1,5,7]. This necessitates the need for programs to prevent the spread of anti-microbial resistant microorganisms and control the use of anti-microbial drugs in health-care settings [7].

Phage typing is currently used for typing of Staphylococcus *aureus* strains in epidemiological studies. Since 1952 this technique has found widespread use, and an international system has been established for typing human strains [8, 9].

It also provides a useful information about other genera which has been particularly significant for *Staphylococci* which are difficult to distinguish on any other basis [10].

Methods

Collection of specimens:

The study was carried out between December 2008 to November 2009 at Al-Sulaimanyiah Teaching Hospital. A total of 60 specimens were collected from three different source locations; surgical theaters (instrument, walls floor and masks), nurses and inpatients. Swabs were processed for isolation of *Staphylococcus aureus*.

Specimens were screened by preliminary Gram stain and were inoculated on 10% blood agar and MocConkey's agar. *Staphylococcus aureus* was identified by conventional techniques [11].

Phage induction:

Phages were induced from each isolates using the heat method in which 10 ml of the isolate which were incubated for 16-18 h. at 37 °C, held in water bath for 2 h. at 56 °C, centrifuged or 15 minutes at 3000 RPM.

Supernatants were collected to represent the phage lysates. Filter sterilized through 0.45 ul filters. Each phage lysate was labeled to carry the number of the original isolates. [12]

Phage detection:

Cross lysis technique was followed in which each phage lysate was crossed against all isolates on plates seeded confluent by 0.1 ml of culture of 16-18 h. at 37 °C by spotting a drop (0.01 ml) of phage lysate on designated position on plaque plate of trypticase soy agar incubated for 6-8 h. and examined for plaque formation which demonstrate the sensitivity of certain isolate to certain phage lysate [8,12]. 3 phage typing of all strains were performed according to standard methods [8,9].

Initially, strains were typed in routine test dilution (RTD, the highest dilution producing confluent lysis). In the cases of negative reactions, 100 concentration (100 RTD) was also used. The lytic reactions ×RTD were read as follows: + + = more than 50 plaques (strong lysis); + = 20-50 plaques (moderate lysis); ± = less than 20 plaques (weak lysis); - = no plaques (no lysis). A strain was considered phage typeable when it was lysed strongly (+ +) or moderately (+) by at least one phage.

Results

In this study 60 samples were collected from three different source locations, surgical theaters (instrument, walls, floor and masks), nurses and inpatients in Al-Sulaimanyiah Teaching Hospital, yielded 30 *Staphylococcus aureus* isolate grouped into 3 groups according to their sources and distributed as followed:

Group 1: 10 isolates (1-10) from surgical theaters.

Group 2: 10 isolates (11-20) from nurses.

Group 3: 10 isolates (21-30) from inpatients.

Based on results obtained of the isolates, it has been found that these phages obtained from all isolates can be classified into 3 groups in which isolate 1 and 6 represent phage group (A) which is able to lyse all isolates in group 1 except isolate 1 and 6.

Lysis is indicated by the formation of plaques, a strain was considered phage type able when it was lysed strongly or moderately by at least one phage (at least one phage produced 20 or more plaques of lysis). It has been found also that all other isolates in the other groups were resistant to this phage group.

Phage group (B) which represents the isolates 15 and 16 has been found to lyse all isolates in

group 2 except isolates 15 and 16 which were resistant also.

In this work it has been found also that phage group (C) which is represented by isolates 23 and 26 was able to lyse all isolates in group 3 except isolates 23 and 26 which were resistant in addition to all other isolates and the remaining groups of isolates were resistant to this phage group.

Discussion

Several workers around the world agreed that phage typing for *Staphylocoocus aureus* to trace the source of an epidemic is a very important technique [13,14,15,16].

Our study seems to confirm this approach and it could be the first attempt in our country during the period from December 2008 to November 2009, to present 3 phage groups of *Staphylococcous aureus* which can be used locally for epidemiological purposes. This fact coincides with results of other authors [17,18], while our results were higher than those reported by others [13,19,20].

It is possible that some variations could be present due to changes in phage lytic patterns which can be affected by geographical distribution or also the abuse of antimicrobials which lead to drug resistance which in turn lead finally to change the behavior of both the *staphylococcus aureus* isolates and their phages [13,14].

The reason behind taking equal number of isolates from each source is to reduce any chance of variation in lytic pattern between phage lysates.

It is a fact that a bacterium is resistant to its own phages [21], this can be observed in our results in which the isolates 1, 6, 15, 16, 23 and 26 were resistant to their own phages. This could be either that the specific locus on the DNA donor bacterium is already occupied by another phage particle [22,23,24] or the receptors are missed.

Lysis as shown by the agar method of spotting supernatant materials on lawns can be caused by (i) high phage multiplicity's leading to strongly lysis, (ii) cell wall enzymes. Lysis sometimes found in phage containing crude supernatant fluid, (iii) infection by low phage multiplicities followed by lytic cycles and release of phage progeny that are mutated or defective. [25,26,27,28].

It is concluded that 3 local phage groups from *Staphylococcus aureus* are presented to be used for epidemiological purposes in case of *Stapylococcus aureus* epidemic.

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