Phage .typing of staphylococcus aureus using locally produced phages

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ABSTRACT

Out of 60 specimens, 30 *staph aureus* 1solates were obtained from different source location, surgical theater (instrument, walls floor and masks), nurses and in patients in S.T.H

Ten Isolates from each source of the total specimens were able to produce phage lysates after induction by heat method and detected by spotting method

By application of the spotting method it has been found that these phage lysates were able to be classified into 3 groups according to their sources:

- Phage lysates from isolates 1 and 6 represent phage group A which were originally from isolates from surgical theater.
- Phage lysates from isolates 15 and 16 represent phage group B which were originally from isolates from Nurses.
- Phage lysates from isolates 23 and 26 represent phage group C which were originally from isolates from Inpatients.

Objective and background: The main objective of this study was to produce local phage groups from local isolates of staph aureus to be used for epidemiological purposes .staphylococcus aureus is widely distributed in our environment and can cause a wide variety of infections hence phage typing is an important method to trace the sauce of such infections.[1′2′3′4]

Methodology : Each specimens collected as swabs from the s0urces was subjected to well known established microbiological method for isolation and identification of staph aureus All isolates ,were tested for the presence of phage employing heat method [3] and detected by spotting method [1] which based on resistance on sensitivity of each isolate to a given phage lysate

Results: Thirty staph aureus were obtained from 60 specimen collected from surgical theater (instrument, walls, floor, and masks), nurses and Inpatients.

The collected isolates were distributed into 3 groups 1, 2 and 3 according to their source Spotting method [3′4] revealed that group A phage lysate originally from isalatn1and 6 were able to lyse all group isolates except 1 and 6 and those in other groups which were unlysed.

Group B phage which were originally from isolates 15 and 16 was able to lyse all group 2 isolates except 15and 16 and the remaining isolates in other groups which were unlysed.

Group C phage which was originally from isolates 23 and 26 was able to lyse all group 3 isolates except 23and 26 and the remaining isolates in other groups.

Conclusion: It is concluded that 3 local phage groups of staph. aureus could be applied in phage-typing of staph.oureus for epidemiological purposes to trace the source of an epidemic caused by staph.aurous.

Key words: staph aureus, phage-typing, epidemiology.

INTRODUCTION

Staphylococcus aureus can survive on dry surfaces, increasing the chance of transmission and it is implicated in toxic schock syndrome in which some tampons allowed the rapid growth of this bacteria to release toxins that were absorbed into the blood stream[5,6,7,8,9]. Any staph.oureus Infection can cause the staphylococcus scalded skin syndrom,a cutaneus reaction to exotoxin absorbed into the blood stream, it can cause also a type of infection called pyaemia[10,11,12] Staph.aureus is one of the moast common agents causing infection of a wide spectrum of clinical conditions ranging from simple to life-threatening cases.[13]

Problematically , Methicillin-resistant staph.aureus (MRSA) and Vancomycin-resistant staph.aurous(VRSA) has become a major cause of hospital-acquired infection and is being recognized with increasing frequency in community acquired infections[14,15,16,17] . This necessitate the need for programs to prevent the spread of antimicrobial - resistant microorganisms and control the use of antimicrobial drugs in health-care settings.[18,19]

phage.typing for tracing the source of an epidemic caused by staph.aureus is a reliable epidemiological way for controlling the epidemics caused by such microorgarigins[1,2,3,4] it also provide a useful information about other genera which has been particularly significant for staphylococcus which are difficult to distinguish in any otherbasis[3,4,18,19]

MATERIALS AND METHODS

Sources of specimen: three sites were the sources of the specimen including, surgical theater, nurses and Inpatiens at Sulaimanyia Teaching Hospital.

Processing of specimen: All specimen were collected carefully as swabs from each of the different sources and subjected to well known established microbiological methods.[3]

Methodology: Each sample was cultured on trypticase soy broth and on blood agar plates.

Diagnosis and identification of an isolate as staph. aureus depends on coagulase and catalase tests in addition to production of golden pigment on milk agar and yellow pigment on Mannitol Salt Agar(MSA).in addition to performing Grams-stain looking for typical microscopic morphology of each isolate.

Phage induction: phages were induced from each isolate using the heat method in which 10 ml of an overnight culture of nutrient broth of each isolate at 37˚c, held in a water bath for 2h at 56˚c. centrifuged at 3000 RPM for 15 minutes and filter sterilized.

Supernatants were collected to represent the phage lysates.

Each phage lysate was labeled to correspond the number of the original isolate from which it was induced.

Phage detection: Spotting method [1,2] was followed in which a loopfuIl of the phage supernatal was spotted on the surface of specific bacterial lawns. Several phage spots could be dropped on the same bacterial lawn. All plate were then incubated in the standard manner for 6-8 at 37˚c ,the presence of clearings or turbid plaque in the spotted areas indicated the presence of phage.

RESULTS

In this study 60 samples were collected from 3 different sources in Sulaimaniya teaching Hospital, yielded 30s.aureus isolates grouped in to 3 groups according to their sources and distributed as in table 1

Table 1: Distribution of staph aureus according to their sources.

Groups	Number of samples	source
1	1,2,3,4,5,6,7,8,9,10	Surgical theaters
2	11,12,13,14,15,16,17,18,19,20	Nurses
3	21,22,23,24,25,26,27,28,29,30	Inpatients

Table 1 reveals that :-

Group1 isolates contains 10 isolates (1-10) from surgical theater

Group2 isolates contains 10 isolates (11-20) from nurses

Group3 isolates contains 10 isolates (21-30) from Inpatients

Based on sensitivity or resistance of the isolates to a certain phage group, it is seen from table 2 that these phages which have been obtained from all isolates can be grouped into 3 groups in which isolates 1 and 6 represent phage group A which is able to lyse all isolates in group I except isolate 1and 6. Lyses is indicated by plaque formation in the spotting areas .It has been found also that all other isolates in the other groups are resistant to this phage group.

Phage group B which represents the isolates 15 and 16 has been found to lyse all group2 isolates except 15 and 16 which were resistant in addition to all isolates of the other group which were resistant also. Phage group C which represents 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 in the remaining groups of isolates which were resistant to this phage group of staph. aureus.

Table 2:sensitivity or resistance of staph.aureus to the phage groups.

Isolato groups	Phage groups	Sensitive isolates	Resistant isolates
1	A(1,6)	2,3,4,5,7,8,9,10	1,6,11,12,13,14,15,16,17,18,19,20,21,22,23,24,25,26, 27,28,29,30
2	B(15,16)	11,12,13,14,17, 18,19,20	1,2,3,4,5,6,7,8,9,10,15,16,21,22,23,24,25,26,27, 28,29, 3O
3	C(23,26)	21,22,24,25,27,28, 29,30	1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18,19, 20 ,23,26

From this study it whose possible to obtain 3 groups of phage (A, B, C) from the isolates which are of value in phage typing of staph. aureus

DISCUSSION

The significant importance of phage typing of staph.aureus is to control the hospital and community-acquired infection since staph.aureus is a cause of most infection in those environment [14,15,16].

Epidemiological and laboratory investigation are under way to assess the risk of transmission of Vancomycin resistant staph.aureus to other patients health care workers and close family contacts .[15,16] for this special precausions includes, using gloves, gowns, and masks for all contacts with patients. Methicillin and Vancomycin resistant staph.aureus are now widely distributed among hospital and community-acquired infections[16]

Our study is the 1st attempt in our country to present 3 phage groups of staph.aureus which can be used locally for epidemiological purposes.

Our results disagree with other [19] who found 4 types of phages and others who[20] found 5 types .it is possible that some variation could occur due to changes in phage lytic patterns which can be affected by geographical distribution and the emergence of drug resistance by staph.aureus which in turn lead for changing the behavior of both the staph. aureus isolates and their phages

The reason behind taking equal numbers of isolates from each source is to reduce any chance of variation in lytic pattern between phage lysates. A bacterium is resistant to its own phage [1,2] this can be observed in our results in which isolates 1, 6,15,16,23 and 26 are resistant to their own phage i.e no plaque have been observed, this could be due to either that the specific locus on the DNA of the recipient bacterium is already occupied by another phage particle or the receptors are missed.

Lysis as shown by the agar spotting method[1,2,3,4] of supernatant materials on lawns can be caused by (1) high phage multiplicities , leading to lysis from without ,(ii)cell wall enzymes sometimes found in phage – containing crude supernatant fluids (iii) infection by low phage multiplicities followed by lytic cycle and release of phage which can be subsequently propagated in the same strain or (iv) release of phage progeny that are mutated , defective or otherwise unable to propagate in the same strain [20,21] .

It is concluded from this study that 3 local phage groups (A, B, C) from staph.aureus are presented to be used for epidemiological purposes in case of staph.aureus epidemics

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