

## PHAGE TYPES OF *STAPHYLOCOCCUS AUREUS* IN HOSPITAL INFECTIONS

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### Abstract

Of the 110 isolates of *Staphylococcus aureus* from clinical specimens particularly from osteomyelitis and septic arthritis when subjected to bacteriophage typing using 8 available phages showed that 48 (43.62%) were typable. Among the typable isolates 16 belong to phage type 68, 26 to phage type 44A and 6 to 187 while 62 (56.36%) remained untypable. Phage types 71, 77, 187 and 3A of *S. aureus* were also isolated from skin of healthy normals and hospitalized patients. Phage type 187 showed some relationship with *S. aureus* isolated from clinical specimen and has epidemiological importance.

### Introduction

Phage typing provides a valuable tool to establish the transmission of hospital staphylococci from personnel and patients to newly admitted patients (Arora *et al.*, 1999). Certain phage types appear to spread in the environment and acquire drug resistance more rapidly than others. Mostly drug resistant Staphylococci of hospital origin fall into the series of phage type group III (Wilcox *et al.*, 2000; Jewetz *et al.*, 1987). Phage type III of *S. aureus* (47, 54, 75, 77) and type I (52, 52A, 80) are common in surgical units. Type 52A and 79 have been reported to cause many out breaks in maternity department and type 71 is the most common cause of impetigo (Thomas, 1986). Very little work has been done on phage typing of *S. aureus* isolated from patients of chronic osteomyelitis and septic arthritis. Studies were therefore carried out to study the etiology of osteomyelitis and to determine the phage type/s of *S. aureus* isolates.

### Material and Methods

*S. aureus* cultures were isolated and identified on the basis of their microscopic morphology, cultural characteristics and biochemical reactions. The phage typing of *S. aureus* isolated from osteomyelitis, septic arthritis, wound infections and from the skin of normal individuals and hospitalized patients were performed using following phages:

*S. aureus* (3A, 44A, 68, 71, 77, 187, 285A and Twort).

### Preparation of phage suspension

Two ml of freshly grown standard host culture was infected with 2ml of phage suspension followed by incubation at 37°C. Complete lyses of the culture was observed after 2 hour incubation. Chloroform was added to the broth culture and centrifuged @ 3000rpm for 15minutes to obtain supernate. The supernate that contain the virion was separated out by pipetting avoiding the chloroform sucking. Pure phage suspension was obtained by Millipore filtration (0.45  $\mu$ m Schleicher and Schuell West Germany).

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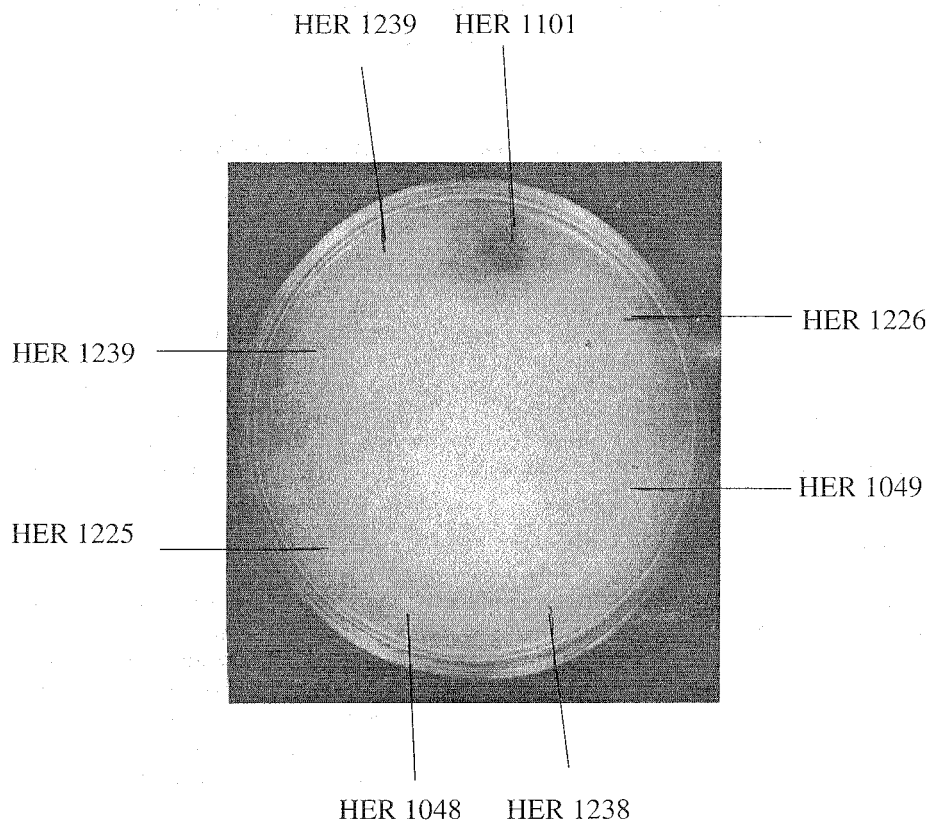


Fig. 1. Phage typing of *Staphylococcus aureus* by eight available phages. The number with HER indicate the Staphylococcal phages. The Plaque formation by HER1101 against *S. aureus* indicates the *S. aureus* phage type 44A.

Spot method was used for the typing of *S. aureus* isolates using the phage suspension. Tryptone Soya agar (Oxoid) plates were overlaid by 3 ml of soft agar containing 0.1 ml of fresh test culture. Undiluted phage suspension of 8 different phage types were placed by special wire loop at 8 different points on the agar surface. Phage suspension drops were allowed to absorb completely. Plates were incubated overnight at 37°C for spot lysis (Fig. 1) (Tortora *et al.*, 1998; Bradley, 1967; Douglas 1975; Gratia, 1936).

### Results and Discussion

All the *Staphylococcus aureus* cultures isolated from the osteomyelitis and infectious arthritis patients when subjected to phage typing by available 8 phages showed that 16 isolates belong to phage type 68; 26 to phage type 44A; 6 to phage type 187 while 62 remained untypable (Table 1).

Table 1. Phage types of *Staphylococcus aureus* isolates.

Source	Phage types									
	No. of isolates	Twort	3A	44A	68	71	77	187	2854	Non-typable
<b>From osteomyelitis and infectious arthritis patients</b>										
Osteomyelitis	96	-	-	23	14	-	-	03	-	56
Infectious bacterial arthritis	04	-	-	01	-	-	-	01	-	02
Wound infections	10	-	-	02	02	-	-	02	-	04
<b>Total</b>	<b>110</b>	<b>-</b>	<b>-</b>	<b>26</b>	<b>16</b>	<b>-</b>	<b>-</b>	<b>06</b>	<b>-</b>	<b>62</b>
%	-	-	-	23.63	14.54	-	-	5.45	-	56.36
<b>From normal healthy and hospitalized persons</b>										
Normal healthy individuals	15	-	01	-	-	03	01	02	-	08
Hospitalized persons	27	-	04	-	-	05	01	03	-	14
<b>Total</b>	<b>42</b>	<b>-</b>	<b>05</b>	<b>-</b>	<b>-</b>	<b>08</b>	<b>02</b>	<b>05</b>	<b>-</b>	<b>22</b>
%	-	-	11.9	-	-	19.04	4.77	11.9	-	52.39

Most of the isolates of *S. aureus* from clinical samples belong to type 44A and 68. It is interesting to note that phage types 44A and 68 were not isolated from skin of healthy and hospitalized individuals (Table 1). Bhattacharya *et al.*, (1972) reported that 120 *S. aureus* isolates from osteomyelitis belong to phage types 29, 25, 52, 3A, 55, 75, 54, 83, 47/42E and 42D while 39.3% isolates were untypable. Similarly Soureck *et al.* (1979) isolated *S. aureus* phage types 29, 52, 79, 3C, 55, 71, 53, 75, 77, 84 and 85 from cases of osteomyelitis. None of the phage types were isolated from the osteomyelitis and arthritis patients in this study, which may be due to unavailability of complete set of phages or the isolates may be untypable. However, on the contrary phage type 3A, 71, 77 and 187 of *S. aureus* have been isolated from normal skin of normal healthy and hospitalized persons. Of these phage type 187 shows some relationship with the present isolates of osteomyelitis. Although we have isolated phage type 44A (24%) from osteomyelitis, but we could not find any relationship between the skin isolates of *S. aureus* with type 44A. This type may have entered from endogenous source like blood or from exogenous source like air and soil in case of open fracture. Phage types 71, 77 and 3A from osteomyelitis have also been reported by Bhattacharya *et al.*, (1972) and Sourek *et al.*, (1979). It may be assumed that as these types are present on the normal skin, there is probability that through a cut on the skin/wound or by fracture they may reach to the bone and joints and may cause osteomyelitis and bacterial arthritis.

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