Nontypeable Bacteriophage Patterns of Methicillin-Resistant *Staphylococcus aureus* Involved in a Hospital Outbreak

KHALIFA I. KHALIFA,[1]* ANWAR A. HEIBA,[1] AND GARY HANCOCK[2]

Microbiology Department, Faculty of Medicine, Suez Canal University, Ismailia, Egypt,[1] and Nosocomial Infections Laboratory Branch, Centers for Disease Control, Atlanta, Georgia 30333[2]

Of 93 strains of *Staphylococcus aureus* isolated from inpatient wards of Ismailia General Hospital, 48 (51%) were proven to be methicillin resistant (MR). Of these MR *S. aureus* strains, 44 were isolated from patients and 4 were isolated from healthy carriers, who were newly arrived interns working in the same wards. Bacteriophage patterns of MR *S. aureus* were identified by using routine test dilution (RTD) and 100-fold dilutions (100 RTD) of phages. Of these 48 strains, 37 (75%) (33 from patients and 4 from interns) were nontypeable when using RTD and 100 RTD of phages. Of the other 11 strains, 8 were nontypeable by RTD of phages, but 5 of them had the phage pattern D11/1136 when tested by 100 RTD. Three strains had the phage pattern 3A/3C/55/71, and three strains had different phage patterns, 29/81, 96, and 95/D11. The finding of colonization with virulent MR *S. aureus* strains in interns working on the wards in which these patients were located suggested that new strategies for control of MR *S. aureus* nosocomial infections must be considered and evaluated.

Infections with methicillin-resistant (MR) *Staphylococcus aureus* continue to be problematic in hospitalized patients. Studies (12, 28) have attempted to define the epidemiology of these infections, but many unanswered questions remain. For therapeutic and epidemiological purposes, it is important to identify clinical isolates of *S. aureus* that are resistant to methicillin. The detection of MR *S. aureus* has been a continuing problem for clinical microbiologists since the first reports of resistance to this drug in 1960 (2, 18). Recommendations for improving the detection of these isolates have been reported (3, 5, 7, 26, 29, 30). Thornsberry and McDougal (30) described a reliable and practical microdilution method for detecting resistance to penicillinase-resistant penicillins in *S. aureus*.

S. aureus is responsible for nearly 10% of all nosocomial infections. It is surpassed only by *Escherichia coli* as a cause of bacteremia (8). In the 1950s, a particularly virulent bacteriophage type, 80/81, caused outbreaks of serious nosocomial staphylococcal infection in hospitals throughout the world and was also found to colonize and infect hospital workers (10, 20, 22). The introduction of antistaphylococcal antibiotics in the 1960s coincided with the eclipse of strain 80/81, which was replaced by diverse phage types responsible for nosocomial infection (14–16, 22).

Antibiotic abuse is very common in Egypt, especially oral penicillins (the cheapest antibiotics) (13, 19). Staphylococcal resistance to methicillin develops quickly and is frequently found in clinical isolates, particularly from hospitalized patients with a history of frequent treatment with penicillins (K. I. Khalifa and H. N. Abuelata, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 741, 1987). A high proportion of nosocomial MR staphylococcal infection was noted in our hospitals (13, 19). To better understand the epidemiology of these virulent MR *S. aureus*, we studied their phage type patterns.

In this paper, we report that a significantly high percentage of nontypeable virulent strains of MR *S. aureus* was isolated from patients and nasal carriers (newly arrived interns). The

recovery of strains with these phage patterns from interns and patients in five wards of our hospital over a 4-month period suggests that new strategies for the control of MR *S. aureus* nosocomial infections in our hospitals must be considered.

MATERIALS AND METHODS

Bacterial strains. A total of 48 clinical isolates of MR *S. aureus* were collected between June and September 1986 from patients admitted to five inpatient wards and all medical staff working in these wards of Ismailia General Hospital. The hospital is a 400-bed general- and acute-care referral facility. The isolates of MR *S. aureus* were from blood cultures, urine, and septic wounds of patients and from the anterior nares of interns, nurses, and medical students. *S. aureus* was identified by colony characteristics; microscopic morphology; catalase, coagulase, and acetoin production; and mannitol fermentation (17). Methicillin resistance was detected by using oxacillin and methicillin disks (BBL Microbiology Systems) and the Bauer-Kirby disk diffusion test (4, 21). Resistance was confirmed by microdilution testing (30). The tests were performed by using Mueller-Hinton broth (BBL) containing a final density of 5×10^5 CFU/ml. The MICs were determined at 18 to 20 h of incubation at 35°C. Resistance MICs of oxacillin and methicillin were >3.1 and >12.5 µg/ml, respectively (27). MR *S. aureus* obtained from various patients and carriers had variable antibiotic susceptibility patterns.

Phage typing. Strains of MR *S. aureus* were sent to the Centers for Disease Control, Atlanta, Ga. Their phage patterns were determined at the Staphylococcus Laboratory, Nosocomial Infections Laboratory Branch. *S. aureus* propagating strains were subcultured to a blood agar plate. A single colony was picked to a Trypticase soy agar (BBL) slant, incubated overnight at 37°C, transferred from the slant into Trypticase soy broth, and incubated at 37°C for 4 to 6 h. Trypticase soy broth cultures were used to flood Trypticase soy agar plates, and the excess was removed and allowed to dry. A drop of each dilution of the phages was applied on previously designated areas of the dried plates. When the

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* Corresponding author.

TABLE 1. MR and methicillin-susceptible *S. aureus* strains isolated from patients and carriers

S. aureus type	No. (%) of strains isolated		
	Patients	Carriers	Total
MR	44 (64.7)	4 (16.0)	48 (51.6)
MS[a]	24 (35.3)	21 (84.0)	45 (48.4)

[a] MS, Methicillin susceptible.

TABLE 2. Phage patterns of 11 MR *S. aureus* strains

Strain	Phage pattern by:	
	RTD	100 RTD
1	NR[a]	D11[b]/1136[c]
2	NR	D11[b]/1136[c]
3	NR	D11[b]/1136[c]
4	NR	95[d]/D11[e]
5	NR	D11[b]/1136[c]
6	3A/3C[d]	3A/3C/55/71
7	NR	29/81
8	NR	D11/1136[f]
9	NR	96
10	3C[d]	3A/3C/55/71
11	3C[d]	3C/55/71 (weak 3A)

[a] NR, No reaction.
[b] Confluent or semiconfluent lysis.
[c] Over 50 plaques.
[d] 20 to 50 plaques.
[e] Fewer than 20 plaques.
[f] Weak reaction.

phage drops had dried, the plates were covered, inverted, and incubated at 30°C for 18 to 24 hours. The lytic reactions on the plate were read under transmitted light against a black background (see Table 2). The phages used were the international set of 23 typing phages (23) and two experimental phages. The routine test dilutions (RTD) of phages 29, 52, 52A, 79, 80, 3A, 3C, 55, 71, 6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, 81, 94, 95, and 96 and experimental phages D11 and 1136 were used. The procedure was repeated by using 100-fold dilutions (100 RTD) of the phages (6).

RESULTS

Of a total of 93 *S. aureus* strains, 48 (51%) were resistant to methicillin and oxacillin; 44 MR strains were isolated from patients in five inpatient wards of Ismailia General Hospital, and 4 were isolated persistently from healthy hospital workers (carriers) over a period of 4 months. The other 45 strains were methicillin susceptible (24 from patients and 21 from medical staff) (Table 1). A total of 25 *S. aureus* strains were isolated from all medical staff working in the wards where the patients were located; 21 were methicillin susceptible and 4 were MR (Table 1). The four MR *S. aureus* carriers were newly arrived interns working in these wards during the summer semester and were actually responsible for caring for these patients. No MR *S. aureus* strains were isolated from 30 hospital employees with no direct contact with the patients. Patients and employees in the outpatient clinic were not tested.

The frequency of resistance for the isolated strains was 51%. All the resistant strains were uniformly coagulase positive (by both slide and tube methods). Disk diffusion testing, to determine the frequency of resistance, was done under the same conditions for all strains as a function of time, temperature, and source of Mueller-Hinton agar.

The phage typing results for the isolates were restricted to the MR strains only. Methicillin-susceptible strains are not included in these data. Of the 48 MR *S. aureus* strains, 37 (75%) (including the 4 strains isolated from carriers) were nontypeable by RTD and 100 RTD of phages. The phage typing results showed that the other 11 isolates were typeable (Table 2). Of these 11 strains, 8 (73%) were nontypeable by RTD of phages, but typeable by 100 RTD (Table 2). When using 100 RTD of phages, we found that 5 of the 11 typeable strains had the phage pattern D11/1136 and three strains had the phage pattern 3A/3C/55/71 (Table 2). The antibiotic susceptibility patterns of the nontypeable MR *S. aureus* strains were not similar. Our finding that 75% of MR *S. aureus* isolated from patients and carriers were nontypeable by RTD and 100 RTD of phages represents a high percentage. When cultures were retested with the more concentrated phages (100 RTD), the criteria for phage typing may be applied if the reactions are those of strong lysis. Often, the concentrated preparations produce a number of weak reactions (less than 50 plaques) or inhibition reactions which are not reproducible enough to be useful (6). It is advisable

to include only the strong reactions (more than 50 plaques) and to disregard all lesser degrees of reaction (5). If this is done, we can add more nontypeable strains to our results (Table 2).

DISCUSSION

The introduction of phage typing to epidemiological surveys of hospitalized patients for up to 20 years demonstrated that once carriers are colonized with certain strains of *S. aureus*, they generally maintain that strain and become sources of infection (33). Recent studies of the hospital ecology of *S. aureus* did not identify predominant phage types in nosocomially acquired staphylococcal infections (9, 24, 31–33). In contrast, Cross et al. (11) reported that a limited number of *S. aureus* phage types were isolated from the blood of patients over a 1-year period. Egyptian investigators reported that 25% (19) and 40% (13) of *S. aureus* strains isolated from septic wounds were nontypeable. There is the fact that the number of nontypeable *S. aureus* strains is increasing.

Archer and Mayhall (1) reported that an MR *S. aureus* strain may be traced by using four specific epidemiological markers: antibiogram, phage type, production of aminoglycoside-inactivating enzyme, and plasmid pattern. They compared the efficacy of these epidemiological markers and reported that plasmid pattern analysis was superior to both phage typing and the antibiogram and that it should be even more useful for epidemiological investigations of complex MR *S. aureus* outbreaks involving multiple strains in different hospitals (1). However, there are limitations to the plasmid analysis procedure. For instance, two patterns may be very similar but not identical, differing only by a single plasmid. The loss or gain of a single plasmid may be associated with the loss or gain of antibiotic resistance.

Of the 25 *S. aureus* strains isolated from medical staff (responsible for caring for these patients) in our hospital, 4 nontypeable MR strains, responsible for 75% of serious MR staphylococcal infections, were frequently isolated from four interns working in the wards with the patients. This finding raises difficult question about the control of nosocomial staphylococcal infections. There are few data linking staphylococcal carriage by hospital workers to infections in their patient contacts. Rountree and Barbour (25) reported

that nursing students previously identified as noncarriers are at the greatest risk of becoming colonized with organisms containing antibiotic-resistant phage types common to the hospital. In another study, the phage type of the organism carried by medical students who were intermittent carriers frequently changed over a period of months (31).

The presence of these phage patterns in our hospital presents a difficult problem of infection control. Compared with other hospital personnel, interns have a particularly intense and widespread exposure to patients at risk of developing nosocomial infections. If interns did indeed function as a reservoir for virulent staphylococci, their capacity to disseminate and perpetuate these strains must be considered in any attempt to control nosocomial staphylococcal infections. Furthermore, it would be impractical and costly (and probably futile) to treat all carriers with antimicrobial agents, since this would conceivably include many patients. Therefore, the implementation of strict sanitary control measures such as hand washing, using gloves, and observing thorough disinfection practices should be reinforced to prevent MR *S. aureus* nosocomial infections.

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