



## A Study on MRSA Isolates in MCH Microbiology Lab, Medical College Hospital, Thiruvananthapuram

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### Abstract

*Methicillin Resistant Staphylococcus aureus (MRSA) were noticed in 1961 and they developed into a Health-care associated organism throughout the world. Recently the appearance of endemic Community-acquired MRSA has been reported. Infections due to community acquired MRSA are recent world wide phenomenon. This study was undertaken to compare CA-MRSA and HA-MRSA isolates on the basis of their genotyping results, clinical presentations and antibiotic susceptibility pattern.*

### Method

**Study design:** Descriptive study.

**Study Setting:** Central Microbiology laboratory, Govt. Medical College, Thiruvananthapuram.

**Sample size:** 161 isolates of Methicillin Resistant Staphylococcus aureus.

**Study period:** 1 year.

**Results:** The proportion of HA-MRSA is 94(58%) and CA-MRSA 67(42%) among 161 MRSA isolates. A male preponderance is observed in both types of MRSA. The commonest clinical presentation of HA-MRSA is cellulitis ie,32(34.04%) followed by post operative wound infection ie,27 (28.72%). The commonest clinical presentation of CA-MRSA is abscess ie,27(40%), followed by post traumatic wound infection ie,14 (21%) . Extremes of age, history of recent hospitalization in past 1 year, history of surgery in the past and Type 2 Diabetes Mellitus are the risk factors observed in case of HA-MRSA. CA-MRSA isolates show lower resistance rate towards Trimethoprim –Sulfamethoxazole 15(22.3%) than 27,(28.72%) of HA-MRSA. Resistance rates of Gentamicin were high in both CA- MRSA and HA-MRSA, ie 92.5% and 93.6% respectively. CA-MRSA showed more resistance to Amikacin 28(41.79%) and HA-MRSA 0(21.27%).CA-MRSA showed resistance to Ciprofloxacin for 65 strains (97%) and HA- MRSA to 62 strains (65.9%). Both types of MRSA showed a low and similar resistance rate of Tetracycline ie, 10.44% CA and 9.57% HA-MRSA respectively. Lower rate of Clindamycin resistance was shown by both CA-MRSA and HA-MRSA ie, 1 (1.49%) and 6 (6.38%) respectively. All 94 isolates of HA-MRSA and 67 isolates of CA-MRSA were uniformly sensitive to Vancomycin , Linezolid and Rifampicin.

**Keywords:** CA-MRSA,HA-MRSA.

## INTRODUCTION

*Staphylococcus aureus* is a significant human pathogen that it produces a wide range of infections and intoxications. Infections are being ranging from skin lesions to life-threatening systemic infections.. It is a major pathogen responsible for both nosocomial and community-acquired infections. Genetic plasticity and diversity, especially regarding antibiotic resistance, are hallmarks of *S. aureus*. With the wide spread use of Penicillin worldwide, Penicillinase or Beta-lactamase producing strains of *Staphylococcus aureus* began to emerge within hospitalized patients.

This phenomenon necessitated the development of beta-lactamase-resistant antibiotics, including methicillin, nafcillin and the cephalosporins in the late 1950s. In 1961, first isolates of Methicillin Resistant *Staphylococcus aureus* (MRSA) were noticed and they developed into a health-care associated organism throughout the world . Vancomycin intermediate *S.aureus* (VISA) first reported in Japan in 1996, later in many countries. Nine Vancomycin-resistant *S.aureus* (VRSA) isolates have now been reported in the United States. The emergence of these strains represents an ominous threat. So a steady increase in the level of resistance to Vancomycin among unselected *S.aureus* strains that can occur with Vancomycin therapy. Recently the appearance of endemic community- acquired MRSA has been reported. Infections due to community acquired MRSA are recent world wide phenomenon. MRSA prevalence ranges from 23.3% to 73%. Across the globe, it was found to be the most common cause of bacteremia, respiratory, and skin infections<sup>1</sup>. Its risk factors remain constant. In Malaysia, MRSA was most frequently isolated from orthopedic and surgical wards, also there is evidence of its association with invasive procedures<sup>2</sup>. In 1996, an international multicenter study showed that among the countries evaluated, S. Africa and Malaysia showed some of the highest rates of MRSA<sup>3</sup>.

This study was undertaken to compare CA-MRSA and HA-MRSA isolates on the basis of their genotyping results , clinical presentations and antibiotic susceptibility patterns.

## MATERIALS AND METHODS

**Study design:** Descriptive study

**Study Setting:** Central Microbiology laboratory, Govt. Medical College, Thiruvananthapuram.

### Inclusion criteria

MRSA strains isolated from various clinical specimens received in the Central Microbiology Laboratory, Govt. Medical College Hospital Thiruvananthapuram

### Exclusion criteria

Clinically insignificant isolates

**Sample size:** 161 isolates of Methicillin Resistant *Staphylococcus aureus*.

**Study period:** 1 year ie, 2013 May to 2014 April Isolate were collected from various clinical specimens Pus, Blood and Urine.

All the strains of MRSA obtained from various clinical specimens were subcultured to Blood agar and Mac conkey agar. Colonies grown were subjected to gram staining, catalase test, coagulase test. Culture was also done on MRSA HI Chrome agar. On HICrome agar, spontaneous green colouration of  $\alpha$  glucosidase producing colonies were obtained in the presence of Cefoxitin (4mg/dl). All isolates were catalase positive, coagulase positive, urea hydrolysed, mannitol fermented and showed fermentative pattern in oxidation fermentation media. Antibiotic susceptibility tests were performed by standard disc diffusion using Kirby-Bauer method on Mueller – Hinton agar.

Antibiotic discs used were as follows: Penicillin (10 U), Erythromycin (15  $\mu$ g) , Trimethoprim +sulfamethoxazole(1.25/23.75  $\mu$ g), Gentamicin (10 $\mu$ g), Cefoxitin(30  $\mu$ g), Amikacin(30  $\mu$ g) Ciprofloxacin (5  $\mu$ g ),Vancomycin(30  $\mu$ g ) and E test , Tetracycline (30  $\mu$ g), Rifampicin (5  $\mu$ g), Clindamycin(2  $\mu$ g) , Linezolid(30  $\mu$ g)

MRSA screening methods used were 1) Cefoxitin disk diffusion method,2)Inoculation to MRSA

Chrome Agar plates and 3) PCR Study for detection of *mec A* gene.

The *mec A* detection studies were carried out at the National Institute of Epidemiology (NIE), Ayyapakkam, Chennai. *Pvl gene* detection was also done for strains which were *mec A* positive. Out of 161 *mecA* positive MRSA strains, 67 were found to be *pvl* gene positive and 94 were *pvl* negative. All PCR studies were carried out adhering to standard precautions to avoid contamination which includes the preparation of reaction mixtures and clinical specimens in separate places, the use of gloves, laboratory coats and negative PCR control.

## RESULTS

A total of 196 MRSA isolates were collected during the study period ie, 2013 May to 2014 April in Govt Medical College, Thiruvananthapuram. All the isolates were sent for Genotyping in NIE, Ayyapakkom, Chennai. Among 196 isolates, 35 didn't survive. So remaining 161 isolates were included in this study. All 161 MRSA isolates were positive for *mec A*. Out of 161, *pvl* gene detection positive for 67 isolates and negative for 94 isolates. This has been led to the conclusion that 67 MRSA isolates were taken as CA-MRSAs and the rest 94 isolates were taken as HA-MRSAs. Analysis of results is primarily based on *mec A* and *pvl* gene detection results. 161 clinically significant isolates of Methicillin Resistant Staphylococci were studied. The results of the study are set in the tables below.

**Table 1 - Distribution of cases according to age**

Age Group	CA-MRSA	Percentage	HA-MRSA	Percentage
<10	1	1.5	2	2.12
11-20	1	1.5	8	8.51
21-30	14	20.89	10	10.63
31-40	9	13.43	9	9.57
41-50	16	23.88	24	25.53
51-60	15	22.38	22	23.40
61-70	7	10.44	11	11.71
71-80	4	5.98	5	5.33
>80	0	0	3	3.19
Total	67	100	94	100

**Table 2 - Distribution of cases according to gender**

Nature of specimen	CA-MRSA	Percentage	HA-MRSA	Percentage
Male	43	64.17	67	71.27
Female	24	35.82	27	28.72
Total	67	100	94	100

**Table 3 - Distribution of cases based on nature of specimen**

Nature of specimen	CA-MRSA	Percentage	HA-MRSA	Percentage
Pus	64	95.5	90	95.74
Blood	1	1.49	2	2.12
Urine	3	4.4	2	2.12
Total	67	100	94	100

**Table 4 - Distribution of cases according to Clinical presentation**

Cinical presentation	CA MRSA	%	HA MRSA	%
Abscess	27	40%	24	25.53%
Cellulitis	10	15%	32	34.04%
Furuncle	4	6%	-	-
Wound infection				
PostTraumaticWI	14	21%	-	-
Post operativeWI	-	-	27	28.72%
Inflammatory	8	12%	6	6.38%
UTI-urine	3	4.5%	2	2.12%
Sepsis-blood	1	1.5%	3	3.19%
Total	67	100	94	100

**Table 5 – Distribution of cases acco to risk factor for HA-MRSA**

Risk factor	Incidents	%
Infected implant in Orthopedics	7	7.44
History of surgery in recent past	15	15.95
History of hospitalization in past 1 yr	30	31.91
>45 yrs	56	59.57

**Table 6- Distribution of cases according to co-morbid conditions**

Co-morbid conditions	CA-MRSA	%	HA MRSA	%
Type II Diabetes Mellitus	10	14.9	27	28.72
Immunosuppression	5	7.51	17	18.1
CLD	1	1.49	3	3.19
Renal disease	-	-	2	2.12
Healthy	51	76.11	45	47.87
Total	67	100	94	100

**Table 7** -Antibiotic resistance pattern of MRSA isolates

	P	E	CO	Cep	CN	G	Amk	Cip
CA MRSA	67 100%	63 94%	15 22.3%	67 100%	67 100%	62 92.5	28 41.79	65 97%
HA MRSA	94 100%	85 90.4%	27 28.72	94 100%	94 100%	88 93.6	20 21.27	62 65.9%

	Van	TC	Rif	Clin	Lin
CA MRSA	0	7 10.44	0	1 1.49	0
HA MRSA	0	9 9.57	1 1.06%	6 6.38%	0

(P-Penicillin, G-Gentamicin, Ak-Amikacin, Ce-Cephalosporin first generation, CN-Cefoxitin,Co-Cotrimoxazole,E-Erythromycin,Cip-Ciprofloxacin,TC Tetracycline, Lin-Linezolid ,Rif- Rifampicin, Cl-Clindamycin )

**Table 8** – D test results of MRSA isolates

Ery- resis Clind- sensi isolates	D test Pos	D test Neg
CA-MRSA 62	12	50
HA-MRSA 85	20	65

All MRSA strains could be able to grow luxuriantly in MRSA Hichrome agar and produced green coloured colonies in all 161 isolates .All the 161 isolates were *mec A* gene positive.Out of 161 MRSA isolates,67 were *pvl* gene positive. Genotyping was done in National Institute of Epidemiology, (NIE), Ayyapakkom. Chennai.

**DISCUSSION**

Methicillin resistant *Staphylococcus aureus* has emerged as one of the most important nosocomial pathogens especially in the last two decades<sup>4</sup>. It has long been a common pathogen in health care facilities confined largely to hospitals, other health care environments and patients frequenting these facilities. It has emerged as a problematic pathogen in community setting also.The health practioner must be aware of signs, symptoms, clinical presentations and risk factors of this infection.

Among the 161 strains studied, 94 were HA-MRSA (58%) and 67 were CA-MRSA(42%). This data is comparable to the prevalence of 54.9% HA-MRSA obtained in a study conducted by Habeeb Khadri in et al in 2010<sup>5</sup>. Also comparable with another study done by Anupurba.S et al in

2003<sup>6</sup>. There may be variations in patient populations, biological charactateristics of organism and infection control measures. According to the documents published CDC 2004, approximately 76% of the purulent SSTI in adults seen in emergency departments were caused by S.aureus,78 % of which were caused by CA-MRSA and overall MRSA caused 59% of all SSTIs in the USA.<sup>7</sup> The prevalence of CA-MRSA have risen in outpatient clinic to 61% depicted in a study by Forcade et al.<sup>8</sup>

In the present study, most of the patients were males in both types of MRSA's ie, 43 nos (64.17%) CA-MRSA and 67 nos (71.27%) HA-MRSA. Females constitute 24(35.82%) CA-MRSA and 27(28.72%) HA-MRSA. Male predominate in HA-MRSA being found in a study by Baddour et al in 2006.<sup>9</sup> and Khanal et al (2010)<sup>10</sup>. This may be due to increased mobility and outdoor activities and greater outdoor exposure in the male population.

Maximum number of cases of HA MRSA are in the age group 41-50 yrs ie, 24 (25.53%) followed by 51-60 yrs, 22 (23.38%).Maximum no. of cases of CA- MRSA are in the age group, 41-50 ie, 16(23.38%) followed by age 51-60 yrs, 15 (22.38%). Majority of the patients of HA-MRSA were in the age >45 yrs age group. But the age group ranged from < 10 yrs to 80 yrs. The finding correlate with many previous study by Huang 2006 that HA-MRSA are more prevalent in extremes of age as elderly and neonatal population.<sup>11</sup>In contrast, majority of CA MRSA patients were predominant in age group 41 -50 yrs,. followed by 21-30 age group 14 (20.89%).

Table 3 shows specimen wise distribution of cases. Most of the specimens were pus samples in both types of MRSA's followed by urine and blood. This may be due to the ease of getting the patients specimen in hospitals. Table 4 shows distribution of cases according to clinical presentation. In this study most of the CA-MRSA cases presented as abscesses ie, 27 (40%) ,followed by post traumatic wound infections ie,14 (21%). This can be comparable in a study by



Forcade et al where the common clinical presentation was abscesses<sup>8</sup>. Most of the HA-MRSA infections presented as cellulitis, 32(34.04%) followed by post operative wound infections, 27(28.72%), followed by abscess, 24(25.53%). Zervos et al (2012) reported that health care associated completed SST were more likely to present as surgical site infections and ulcer.<sup>12</sup>

Since children less than 10 yrs are not much involved among CA-MRSA patients, the risk factors for acquisition of CA-MRSA were not statistically significant in the present study. HA-MRSA infections definitely possess many risk factors which poses a hazard to hospitalized patients. Among this most common risk factor being >45 yrs age followed by history of recent hospitalisation within past 1 yr, 30 (31.91%) and or recent surgery in past, 15(15.95%). Aparna et al (2012) has described that 68.88% of patients acquiring HA-MRSA infection had history of hospitalization in the past 1 yr.<sup>13</sup> Mukesh patel et al (2006) has noted in his study that history of recent surgery is an independent predictor of HA MRSA skin infections.<sup>14</sup> Infected implant in Orthopedics has been also observed as a risk factor in our study 7(7.44%). This can be considered as a recent surgery in the past.

Table 5 shows the distribution of co-morbid conditions. In the case of CA-MRSA infections 10 (14.9%) were suffering from Type II Diabetes Mellitus. A mild immuno suppression has been noted in 5 cases. (7.51%). Most of CA MRSA patients were healthy 51 (76.11%) and not suffered co-morbid conditions.

Co-morbidities associated with HA-MRSA revealed that 27 cases (28.72%) had Type II DM, and 17 cases (18%) were immunocompromised, 3(3.19%) with chronic liver disease and 2 with end stage renal disease on dialysis (2.12%). Aparna et al (2012) pointed out similar findings in her study where Type 2 DM was found to be the most common underlying chronic disease<sup>13</sup>. Mel - Hatra Arakamat et al observed in their study that DM and chronic kidney disease were the most

common underlying conditions identified<sup>14</sup>. Rest of HA -MRSA patients were healthy prior to hospitalisations and had no associated commorbidities.

Antimicrobial resistance pattern of 67 isolates of CA-MRSA and 94 isolates of HA- MRSA were analysed. All 161 isolates were uniformly resistant to penicillins and first generation cephalosporins (100%). Similar results obtained in studies by Anupurba et al in 2003.<sup>6</sup> In the present study, antimicrobial resistance pattern have been used to distinguish between CA-MRSA & HA-MRSA strains. CA-MRSA strains have been shown a greater susceptibility to several antimicrobial agents namely Gentamicin, Clindamycin and Trimethoprim - Sulfamethoxazole than HA-MRSA isolates. CA-MRSA isolates show lower resistance rate towards Trimethoprim - Sulfamethoxazole 15(22.3%) than 27,(28.72%) of HA-MRSA.

In the present study, CA- MRSA isolates were found to be more resistant to Erythromycin 63 no (94%) when compared to HA-MRSA 85 no (90.4%). CA-MRSA showed more resistance to Amikacin 28(41.79%) and HA-MRSA 20(21.27%). The observation of Amikacin susceptibility pattern in various authors showed that a 20-50 % in hospital strains and 85% community isolates.<sup>6,15</sup>

CA-MRSA showed resistance to Ciprofloxacin for 65 strains (97%) and HA- MRSA to 62 strains (65.9%). A higher resistance rate has been shown by CA- MRSA in present study. This is in concordance with the observations by Anupurba et al in her study in 2003 (84.1%)<sup>6</sup> When Ciprofloxacin was first introduced, it was recommended as the first oral drug effective against MRSA. But a dramatic increase in resistance rate of Ciprofloxacin was developed soon.<sup>16</sup> Currently, none of the fluoroquinolones are FDA- approved for treatment of MRSA infections. Usage of Fluoroquinolones causes development of resistant mutants leading to relapse and treatment failure. Of all the community MRSA clones, non beta -lactam

resistance has been described predominantly in USA300. The increased and inadvertent use of such antibiotic might have contributed to the changing resistance pattern of CA-MRSA.

Both types of MRSA showed a low and similar resistance rate of Tetracycline ie, 10.44% CA and 9.57% HA-MRSA respectively. In my study, Tetracycline didn't exhibit 100% susceptibility in all isolates of MRSA. It showed an average of 10 % resistance in both types of MRSA. Same observation was pointed out in Huang study 2006.<sup>11</sup>

Resistance rates of Gentamicin were high in both CA-MRSA and HA-MRSA ,ie 92.5% and 93.6% respectively. So it is to be noted that the epidemiologic and molecular features of MRSA are evolving such that characteristics that initially distinguished CA-MRSA from HA-MRSA appear to be changing. In addition , the prevalence of invitro resistance to non beta-lactam antimicrobial agents may be increasing among CA-MRSA strains<sup>17</sup>. So CDC recommends that CA-MRSA should not be distinguished from HA-MRSA by antibiotic sensibility tests alone, but should be primarily based on patient's history rather than characteristics of the organism.<sup>18</sup>

In our study, Clindamycin resistance shown by both CA-MRSA and HA-MRSA is very low ie, 1 (1.49%) and 6 (6.38%) respectively. Walraven C J et al 2012 pointed out similar findings in their study<sup>19</sup>. Clindamycin has been used widely in the treatment of SSTI and there are reports of clindamycin being used successfully to treat CA-MRSA infection .CDC recomended that a D-Zone test should be performed to identify inducible clindamycin resistance in Erythromycin -resistant clindamycin susceptible isolates . Table 7 shows the D test results. Among 62 isolates of CA-MRSA isolates , 12,(19.35%) showed inducible resistance to Clindamycin . Among 85 HA-MRSA isolates, 20 , (23.5%) showed inducible resistance to Clindamycin . Gadepalli et al (2006) All India Institute of MS, New Delhi observed in their study that 30% HA-MRSA are D Test positive<sup>20</sup>.

All 94 isolates of HA-MRSA and 67 isolates of CA-MRSA were uniformly sensitive to Vancomycin , Linezold and Rifampicin. Huang et al (2006) demonstrated similar susceptibility to Vancomycin, Linezolid, Tetracycline and Rifamicin among HA-MRSA and CA-MRSA isolates.<sup>11</sup> In my study, Tetracycline didn't exhibit 100% susceptibility in all isolates of MRSA. It showed an average of 10 % resistance in both types of MRSA. All the 161 isolates were subjected to Vancomycin MIC Epsilometer .All the strains were susceptible to Vancomycin and MIC being less than 2µg/ml while performing E test.

PCR detection of *mec A* gene is the gold standard for detection of Methicillin resistance as recommended by CLSI April 2012 . The PCR technique has many added advantages over the conventional techniques. The Multiplex PCR can furnish results within 24 hrs as compared to a minimum of 48 hrs required by the conventional techniques. Detection of *mec A* gene confirm the MRSA status of the isolate and no further test need to be done. PCR is very expensive and restricted to reference centres.

All the isolates MRSA were sent for genotyping in National Institute of Epidemiology (NIE), Chennai. Among 196 isolates sent, PCR could be performed for 161 MRSA isolates .Of this, 67 strains were found to have *pvl* gene. Taking PCR as a gold standard assay, an evaluation has been done for the performance of Cefoxitin disc diffusion method and CHROME agar for detection of MRSA resistance. The Cefoxitin disc (30µg) detected Methicillin resistance in all 161 isolates with *mecA* positivity by PCR. ie 100 % sensitivity. Thus it can be concluded that Cefoxitin, as recommended by CLSI is an excellent predictor of *mec A* gene. This can be comparable to many studies<sup>21,22</sup>. All MRSA strains could be able to grow luxuriantly in MRSA Hichrome agar and produced green coloured colonies in all 161 isolates with *mec A* positivity by PCR ie, 100 % sensitivity. Cefoxitin Disc diffusion method can be combined with

CHROME agar for detection of MRSA in routine diagnostic laboratories.

## CONCLUSIONS

The proportion of HA-MRSA is 94(58%) and CA-MRSA 67 (42%) among 161 MRSA isolates. A male preponderance is observed in both types of MRSA. The commonest clinical presentation of HA-MRSA is cellulitis ie, 32(34.04%), followed by post operative wound infection ie, 27 (28.72%). The commonest clinical presentation of CA-MRSA is abscess ie, 27(40%), followed by post traumatic wound infection ie, 14 (21%). Extremes of age, history of recent hospitalization in past 1 year, history of surgery in the past and Type 2 Diabetes Mellitus are the risk factors observed in case of HA-MRSA.

MRSA Hichrome agar was performed well for all the isolates. So Cefoxitin disc diffusion method may be combined with CHROME agar for the detection of MRSA. Genotyping is usually done in research institutions and costs too high. So it cannot be routinely done in diagnostic laboratories. All the strains were Vancomycin susceptible (MIC was less than 2 microgram per ml). Vancomycin MIC should be done routinely in Microbiology laboratories as VRSA and VISA are emerging as evidenced in studies.

ABST CA-MRSA isolates show lower resistance rate towards Trimethoprim –Sulfamethoxazole 15(22.3%) than 27,(28.72%) of HA-MRSA. Resistance rates of Gentamicin were high in both CA- MRSA and HA-MRSA, ie 92.5% and 93.6% respectively. CA-MRSA showed more resistance to Amikacin 28(41.79%) and HA-MRSA 20(21.27%). CA-MRSA showed resistance to Ciprofloxacin for 65 strains (97%) and HA-MRSA to 62 strains (65.9%). Both types of MRSA showed a low and similar resistance rate of Tetracycline ie, 10.44% CA and 9.57% HA-MRSA respectively. Clindamycin resistance shown by both CA-MRSA and HA-MRSA is very low ie, 1 (1.49%) and 6 (6.38%) respectively. Inducible Clindamycin resistance shown by D –

Test results is 19.35% in case of CA-MRSA and 23.5% in case of HA-MRSA.

All 94 isolates of HA-MRSA and 67 isolates of CA-MRSA were uniformly sensitive to Vancomycin, Linezolid and Rifampicin. (100%). The sensitivity to Clindamycin can only be judged after performing D test on the Erythromycin resistant isolates. Performance of D Test in a routine Microbiology Laboratory will help in guiding the clinician regarding the injudicious use of Clindamycin in SSTI.

Analysis of risk factors for development of HA-MRSA and CA-MRSA should be studied so that treatment approach can be modified. Continuous surveillance of antibiotic susceptibility pattern of MRSA is essential for differentiating HA-MRSA and CA-MRSA. For routine detection of MRSA in laboratories, Cefoxitin disc diffusion method may be combined with inoculation on to MRSA Hichrome agar. Genotyping is usually done in research institutions and costs too high. So it cannot be routinely done in diagnostic laboratories. Analysis of risk factors for development of HA-MRSA and CA-MRSA should be studied so that treatment approach can be modified. Continuous surveillance of antibiotic susceptibility pattern of MRSA is essential for differentiating HA-MRSA and CA-MRSA. The sensitivity pattern of isolates and clinical presentation of patients will definitely help the clinicians in implementing appropriate treatment of the patients. MRSA control and preventive measures should be implemented in hospitals especially standard infection control procedures among doctors, nurses, paramedical staff and cleaners.

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