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Prevalence of Methicillin-Resistant *Staphylococcus aureus* and the Role of Disinfectants in Infection control

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ABSTRACT- Two hundred fifty samples were collected from Khartoum teaching hospital (KTH) by swabs from units' surfaces including walls, seats, tables, floor, medical devices, doors, and windows. Air samples were also investigated by using the settle plate method. The samples were cultured on blood agar for primary isolation. The identification of MRSA was carried out according to the standard method. Resistance to methicillin and vancomycin was done for each isolate. The disc diffusion method and In-Use test were used to evaluate the effectiveness of the four disinfectants (Clorox (sodium hypochlorite) + Water, Phenol + liquid soap + Chloroxylenol "Dettol", Formalin + Water, and Dettol (Chloroxylenol solution) + Liquid soap + Water) against MRSA. Data were analyzed by the statistical analysis program Statistical Package for the Social Science (SPSS) using the One-Way Analysis of Variance (ANOVA) and Least Significant Difference (L.S.D) test. The results revealed that the prevalence of MRSA was 66 (25%). Among these 11(16%) were vancomycin-resistant. Moreover, the study on the role of disinfectants in controlling infection showed that two of these disinfectants (Formalin + Water, Dettol (Chloroxylenol solution) + Liquid soap + Water) were significantly effective on MRSA ($P<0.05$), while the other two disinfectants (Clorox (sodium hypochlorite) + Water, Phenol + liquid soap + Chloroxylenol "Dettol", Formalin + Water) were insignificantly effective ($P>0.05$) on the same organisms. It is concluded that the prevalence of MRSA in KTH was high and the rate of Vancomycin resistant *S. aureus* (VRSA) is increasing. The disinfectants used routinely in KTH were not equal in their efficiency and there was failure in the actions of two of them.

Key words: MRSA, Hospital, Disinfectants, Infection Control, Sudan.

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INTRODUCTION

Microorganisms on hospital surfaces can be transmitted to the hands of healthcare workers, patients, co-patients and visitors, resulting in cross-infections.

Despite the performance of routine cleaning and precautionary measures in most hospitals, effective environmental decontamination methods are still in demand [1]. Disinfectants are commonly used to minimize the risk of Methicillin-resistant *Staphylococcus aureus* (MRSA) [2]. This organism first reported from England in 1961 as a leading patho-

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gen of nosocomial infections [3]. The later in hospital due to MRSA is increasing.

Recently, emergence of resistance strains of *S. aureus* to vancomycin has made MRSA more difficult to treat than before. Patients who infected with hospital strain have increased mortality risk and expanded hospital stay, resulting in increased treatment costs, compared with patients who do not have hospital strain infections [3].

Moreover, the increased risk of a new patient with antibiotic resistant bacterium when admitted to a room previously occupied by another patient with the same bacterium has also been reported. Disinfection of the high-touch areas reduces the load of antibiotic resistant bacteria in the hospital environment [4].

Treatment of Multi-Drug Resistant (MDR) strains is very difficult due to limited alternative to select effective antibiotics. The MDR strains exist in the hospital environment can infect patients through health care devices. Therefore, it is very important to eliminate MDR strains from health care devices by using highly efficient disinfectant [5].

Thus, hospital surfaces disinfection rates remain a true problem, despite a growing body of research [6].

The objectives of this study were to determine the prevalence of MRSA in Khartoum Teaching Hospital (KTH), evaluate the efficiency of disinfectants currently in use, and provide data on the level of bacterial contamination.

MATERIALS AND METHODS

This study was carried out in Khartoum Teaching Hospital (KTH), Sudan during the period October 2007-August 2008. This hospital is a leading hospital in Sudan. The experimental work was carried out in the Research Laboratory, College of Medical Laboratories Science, Sudan University of Science and Technology.

The samples were collected from KTH sections and units, including (emergency, surgery, blood bank, pediatrics and obstetrics-gynecology). A total of two-hundred fifty swab samples were collected and eighteen air samples were investigated.

The data were collected primarily from emergency unit 80 samples, general surgery 80, pediatric 40, obstetrics-gynecology 30 and blood bank 20.

Air samples were collected from emergency unit 6 samples, general surgery 3, pediatric 3, obstetrics-gynecology 3 and blood bank 3.

The samples were collected from the sections and unit's environments by using sterile swabs to cover the surfaces of floor, tables, windows, doors, walls, seats and medical devices. Air samples were examined by using settle plate technique [7].

Disinfectants

In KTH several types of disinfectants have been used, some of which in a daily basis to clean floors and surfaces in the units and rooms and others were use to disinfect surgical theatres. Four disinfectants from KTH were used in this study, included Clorox (sodium hypochlorite) + Water, Phenol + liquid soap + Chloroxylenol "Dettol", Formalin + Water, and Dettol (Chloroxylenol solution) + Liquid soap + Water.

Antibiotic discs included methicillin (M), 10µg, vancomycin (Va), 30 µg. Standard strains *S. aureus* ATCC 25923. Obtained from National Health Laboratory in Khartoum. The sterile filter paper was obtained from Micro Master Lab. Pvt. Ltd., India. All media were obtained from Hi Media Lab. Pvt. Ltd. Mumbai, India.

Under aseptic conditions the swabs were inoculated on blood agar and incubated at 37°C overnight.

Only the growth that showed characteristic colonial morphology of golden, yellow and white colonies of 1-2 mm, like staphylococci were selected. For further investigations, the colonies isolated were sub-cultured on Nutrient agar and incubated at 37°C overnight.

S. aureus isolates were identified by their colonial

morphology, Gram's stain, and biochemical tests including catalase, coagulase, mannitol fermentation, DNase.

Examination of air (Settle plates)

An examination of air using settle plate technique, uncovered blood agar plates were exposed to air 1 meter above the ground for 30 minutes, and the plates were incubated at 37°C for 48 hours.

Only plates showing colonies count between "30–300" were considered.

Antimicrobial susceptibility tests

Sensitivity test was performed using Kirby-Bauer disc diffusion method [8]. Briefly, 1-3 isolated colonies were emulsified in 5 ml of sterile physiological saline, and then the turbidity of the suspension was adjusted to McFarland standard by adding normal saline or more bacterial colonies. A swab was dipped into the suspension and the excess fluids was removed by pressing and rotating the swab against the inner side of wall tube, and then streaked the swab over the surface of Muller-Hinton agar three times, rotated the plate through an angle of 60° each time to ensure the distribution of inoculums over the surface of the agar plate. The discs were placed on the surface of the inoculated plate. Each disc was pressed down gently to ensure its contact with the agar. The plates were incubated at 37°C for 24 hr. inverted aerobically for overnight. The diameter of zone of inhibition was measured in mm and diameters of inhibition zone were compared to the standard inhibition zone in the chart (Chart given with the antibiotic).

Disinfectant efficacy

This test was used to determine the potency of disinfectants used in KTH. The same steps in Kirby-Bauer diffusion disc were followed in this test. Sterile filter paper discs were soaked in the disinfectant solution and left to dry for few minutes. The impregnated discs were placed on the Muller-Hinton agar plates and incubated overnight at 37°C. After incubation, the clear zone surrounding the disc was measured the effects of disinfectants were used against the MRSA organisms. The zone inhibitions were measured by a ruler. The last was carried out in replicated. The efficiency of these chemicals was judged by the diameter of the inhibition zone.

In-use test

The test was done according [9]. Briefly, by using this test we can test the disinfectants were used in hospital. In this test the disinfectants were tested twice one in the normal conditions and in the real environment and could see the ability of disinfectants against normal organisms which already exist normally in the environment. In the second part of test were. The disinfectant is tested in a small place and in stable temperature and stable conditions, so we can see the results according to the test rules. With a sterile pipette, transfer 1 ml of the used disinfectant in 9 ml of nutrient broth in sterile tubes. 0.02 ml drops of this mixture placed onto ten different areas of each of two well dried nutrient agar plates. One of the plates was incubated for 3 days at 37°C and the other for 7 days at room temperature. The presence of growth in more than five drops on either plate indicates failure of disinfectant.

Statistical analysis

The statistical analysis was done using SPSS program. The data obtained from the zone diameter of the disinfectant activity against MRSA were analyzed using One-Way ANOVA (Analysis of Variances) and Post Hoc Test (L.S.D test) to know the variances between the results. For each dependent variable the descriptive output gives the sample size, mean, standard deviation, minimum, maximum, standard error, and confidence interval for each level of the (quasi) independent variable. The ANOVA output gives us the analysis of variance summary table. There are six columns in the output:

The hypothesis in this test is:

H0: d1=d2=d3=d4 (the means are equal)

H1:d1≠d2≠d3≠d4 (the means is not equal)

Where (d)= the means of disinfectant activity

The Table shows the mean of reading between and within groups, also show the test of linearity and the deviation from linearity "linear trend" (this describe if the mean of data for all disinfectants being on right line or if they are equal).

In this test we calculate the F-value, the difference between F-calculated and F-tabulated (sig.); if the F-calculated is bigger than F-tabulated (sig.) we refuse the first hypothesis. That means there's variance between the means.

In this table we see the F-value > sig. so we refused the hypothesis of equality between means, so we go to use the Post Hoc Test (L.S.D) to know the value of variances between the means.

The variances in the means between disinfectants shown in the second column and it's appear that disinfectant number 3 is more effective than the other disinfectants, also show that disinfectant number 1 is the most weak based on the mean of diameter zone. Hence the disinfectants used in KTH were not equal in efficiency while some were not effective.

RESULTS

In this study, 250 samples were collected from different units: Emergency unit including the units of I.C.U, Refreshment Surgery unit, internal unit and X ray unit, General Surgery unit, Blood bank, Pediatrics unit and Obstetrics-Gynecology unit in the Khartoum Teaching Hospital (KTH). The samples were collected by means of cotton swabs from floor, walls, tables, doors, seats, windows and medical devices. Air samples were collected directly on an agar plate using settle plate technique.

Distribution of samples

The distribution of samples according to units is shown in Table (1). Samples were collected from the Emergency unit, General Surgery, Obstetrics-Gynecology, Blood bank and Pediatrics are shown in Table (2). These samples were distributed and covered seven different sites in this unit with an exception of four sites in blood bank. The numbers of samples were collected from the KTH sites (Doors, Windows, Seats, Floor, Medical Devices, Walls and Tables).

Settle plate technique

This was done to examine the unit's air and to know the number of the Colony Forming Unit (CFU). Three Plates were exposed to the air in every unit were covered in this study, and after incubation with the CFU was counted for three plates and was used to calculate the mean of CFU in every unit Table (3).

Isolation and identification of *S. aureus* and MRSA

Of 250 samples and 18 air samples, 265 (99%) showed significant growth on blood agar. Sixty-one 250 of swab samples collected in this study were identified as *S. aureus* and distributed on the units as: Emergency unit (22), General surgery unit (21), Pediatrics unit (9), Blood Bank (3) and Obstetrics-Gynecology unit (6).

After the sensitivity test to methicillin antibiotic fifty-seven of 61 were identified as MRSA. Emergency unit (20), General surgery unit (21), Pediatrics unit (8), Blood Bank (3) and Obstetrics-Gynecology unit (5), Table (4).

From (18) plates were exposure to air in the units and after incubation in Emergency unit (3), General Surgery unit (2), Pediatrics unit (1), Blood Bank (1) and Obstetrics-Gynecology unit (2) colonies identified as *S. aureus* all of these organisms identified as MRSA Table (4). Of the isolated *S. aureus* 3 (4%) were sensitive to methicillin, 1 (1%) with intermediate activity and 66 (94%) were resistant to methicillin Table (5).

Vancomycin sensitivity was done to all *S. aureus* isolated and showed that 59 (84%) were vancomycin sensitive *S. aureus* (VSSA), and 11 (16%) were vancomycin resistant *S. aureus* (VRSA) Table (5).

Efficiency of disinfectants

All 66 MRSA isolated were tested for susceptibility to the disinfectants used in KTH by using the Kirby-Bauer method (disc diffusion), The Petri dish was streaked by the organisms and by using sterile filter paper soaked by the disinfectant was placed on the agar and after incubation the against MRSA organisms isolated and there is significant variance among them.

In-Use test was done to judge on the disinfectants used in diameter zone inhibition was measured, this test repeated three times to every MRSA isolated. The SPSS statistical analysis was done to analysis the out put data and by using One-Way ANOVA test and L.S.D test shows that the four disinfectants were not equal in strength of efficiency KTH and as the results the test shows us, if the disinfectants were

tested in this study were passed or failure Table (6).

Table 1: Distribution of swabs samples (n=250) according to units

Unit	No. of samples (%)
Emergency	80 (32)
General Surgery	80 (32)
Pediatrics	40 (16)
Blood bank	20 (8)
Obstetrics-Gynecology	30 (12)
Total	250 (100)

Table 2: Distribution of samples according to hospital units

Hospital Unit	Site of Collection						
	Door No. of Samples (%)	Seat No. of Samples (%)	Wall No. of Samples (%)	Table No. of Samples (%)	Med. Device No. of Samples (%)	Floor No. of Samples (%)	Window No. of Samples (%)
Emergency	7 (9)	11 (14)	15 (19)	16 (20)	14 (18)	10 (13)	7 (9)
General Surgery	10 (13)	10 (13)	10 (13)	12 (15)	15 (19)	15 (19)	8 (10)
Pediatrics	6 (15)	7 (18)	6 (15)	6 (15)	4 (10)	6 (15)	5 (13)
Blood bank	–	–	4 (20)	4 (20)	8 (40)	4 (20)	–
Obstetrics-Gynecology	4 (13)	5 (17)	4 (13)	4 (13)	5 (17)	4 (13)	4 (13)

Table 3: Distribution of air samples (n=18) according to unit and number of CFU/plate

Unit	Number of plates	CFU/plate
Operation room in EMR.	3	42
Emergency unit	3	111
Pediatrics	3	104
General Surgery	3	205
Blood bank	3	87

Obstetrics-Gynecology	3	84

CFU= Colony forming unit

Table 4: *S. aureus* and MRSA isolated from hospital's unit (swab & air samples)

Sample Type	**Swab**		**Air**	
Unit	*S. aureus*	MRSA	*S. aureus*	MRSA
Emergency	22 (28)	20 (91)	3 (50)	3 (100)
General Surgery	21 (27)	21 (100)	2 (67)	2 (100)
Pediatrics	9 (23)	8 (89)	1 (33)	1 (100)
Blood bank	3 (15)	3 (100)	1 (33)	1 (100)
Obstetrics-Gynecology	6 (20)	5 (83)	2 (67)	2 (100)
Total	61	57	9 (50)	9 (50)

Table 5: Susceptibility of *S. aureus* to methicillin and vancomycin

Activity of antibiotic	**Methicillin**	**Vancomycin**
Sensitive	3 (4)	59(84)
Intermediate	1 (1)	0
Resistant	66 (94)	11(16)
Total	70 (100)	70(100)

Table 6: Efficiency of disinfectant by using In-use test

Disinfectant	**Result**
Sodium Hypochlorite (Clorox) + Water	Failure
Phenol + Liquid soap + Chloroxylenol "Dettol"	Failure
Formalin + Water	Pass
Liquid soap + Chloroxylenol (Dettol) + Water	Pass

DISCUSSION

Environmental surfaces may become contaminated by human pathogens. Extensive environmental contamination with MRSA has been demonstrated in room housing patients. Although most nosocomial infections result from a patient's endogenous flora or person-to-person transmission, contaminated surfaces have been linked to nosocomial infections [10]. This study was conducted to evaluate the effectiveness of disinfectants commonly used in KTH against MRSA.

MRSA has frequently been reported as a major hospital acquired pathogen [11]. In U.S. Hospitals the incidence of MRSA has increased from 2.4% in 1975 to 29% in 1991 [12].

In this study, from 248 environmental samples, 57 (23%) were identified as MRSA. This is a high rate indicating high contamination. This ratio is lower than that reported by Sexton [13], who found that (54%) were identified as MRSA. Tanaka [14] isolated a comparable (22%) of MRSA from environmental swab samples taken from the entrance hall of the dispensary at Tottori university hospital in Japan. Airborne transmission has been known to be the route of infection for diseases such as MRSA, *Acinetobacter spp.* and *Pseudomonas* sp. [15]. Airborne transmission is general-

ly considered to occur at lower frequency than transmitted via direct contact, but MRSA in the form of a bioaerosol can contaminate air and cause airborne infection [11].

In this study air investigation was carried out by settle plate's technique. It has shown that MRSA represents 9 (50%) of airborne contaminants. This poses a high risk to immunocompromised patients, in whom it leads to nosocomial infections. However, India a similar study conducted by Kaur and Hans [16] revealed a lower rate of 16%.

Over the past two decades, vancomycin has been considered the antibiotic of choice for MRSA infections. However recent reports revealed the therapeutic failure of vancomycin for MRSA infections [17]. In 1997, VRSA was isolated in Japan's hospitals [18].

In this study the vancomycin sensitivity test showed that 11(16%) of *S. aureus* isolates were resistant to vancomycin (VRSA). This rate shows that the spread of VRSA is in progress, which may be attributed to the wide use of antibiotics in the Sudan [19]. Lower number (1%) of VRSA has been isolated from various hospitals in Khartoum State. In Brazil a study conducted by Oliveria [18] revealed a 5% of VRSA isolated from one Brazilian hospital. These results concluded that a reduced susceptibility to vancomycin in *S aureus* seemed to be an uncommon phenomenon and that it is difficult to identify occult VRSA strains that may be present in other Brazilian hospitals as well.

The presence of MRSA in hospitals poses a significant challenge to hospital infection control. The use of disinfectants for both surfaces and hand cleaning is an essential part of the infection control measures [20]. The sensitivity test to disinfectants used in KTH was carried out against MRSA isolates using Kirby-Bauer Disc Diffusion Method. The statistical analysis showed that the efficiency of these disinfectants was not equal in strength against MRSA.

The statistical calculation showed that the Formalin+Water disinfectant was more effective than other disinfectants, while the Sodium Hypochlorite disinfectant less effective. In a study conducted by Murtough *et al.* [21] to test the disinfectants commonly used in UK hospitals they found that some disinfectants were more effective if used on clean floor but not active against nosocomial microorganisms if used to clean other surfaces. However, standards need to be maintained and regular monitoring may help to achieve this. Comparable results have been found by Suzuki *et al* [22], who recommended adjusting the concentrations of some disinfectants.

In-Use test was done to test the disinfectants used in this study and found that two of them (Formalin+Water and Chloroxynelol+Water+Liquid Soap) passed while; the other two (Sodium Hypochlorite and Chloroxylenol+Liquid Soap) disinfectants failed in this test. This finding is comparable to Khapoor *et al.* [23]. This study proved there is a prevalence of MRSA in KTH units' environment. It is also shown that the disinfectant used is satisfactory.

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