The Incidence of Methicillin Resistant Staphylococcus Aureus (MRSA) in Lahore.

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Staph. Aureus is the most common gram positive nosocomial pathogen. Strains of Staphylococcus aureus resistant to methicillin are a cause of concern worldwide. To date no data is available on the incidence of MRSA in Lahore. Two hundred randomly selected Staphylococcus aureus isolates from different hospitals of Lahore were tested for sensitivity to methicillin using modified Kirby-Bauer method. Sensitivity plates were incubated at 30°C. Twenty nine percent of Staphylococcus aureus isolates were methicillin resistant. The percentage of MRSA was 20% at Sh. Zayed Hospital, 21.6% at Services Hospital and 32.6% at Jinnah Hospital, Lahore. Majority of MRSA strains were isolated from sputum, pus/wound swabs and blood. All MRSA were betalactamase positive. The present study shows that infection with MRSA is a common occurrence in various hospitals of Lahore, to control these infections it is recommended that a proper infection control program should be instituted.

Key words: Methicillin resistant, Staphylococcus, aureus; MRSA, nosocomial pathogen

A significant feature of medical microbiology in the 1990's has been the increase in concern about antibiotic resistance[1]. Gram positive organisms are responsible for at least one third of the hospital infections[2,3]. Staphylococcus aureus is the most common gram positive nosocomial pathogen[4]. Thus it is important to closely monitor the antibiotic resistance pattern in Staphylococcus aureus.

Methicillin, a penicillinsase resistant penicillin (PRP) was introduced in 1960s for the treatment of infections caused bys trains of Staphylococcus aureus producing penicillinase. Resistance to methicillin was detected very early in 1961[5] but its level remained low[6]. With the continued use of the PRPs there has been an increase in the proportion of infections caused by MRSA worldwide[7,8,9,10]. MRSA is an important pathogen as it is resistant to many other antimicrobials as well[11].

In Pakistan most laboratories do not routinely check for resistance to methicillin. Methicillin resistance in hospital Staphylococcus aureus has been studied in Rawalpindi[12] and Karachi[9]. In these studies methicillin resistance in Staphylococcus aureus was seen to be 13.8% and 43% respectively. To date no data is available on the incidence of MRSA in Lahore.

Present study was planned to assess methicillin resistance among Staphylococcus aureus isolates from patients admitted in different hospitals of Lahore.

Materials and Methods

Two hundred Staphylococcus aureus isolates were collected from Services Hospital, Jinnah Hospital and Sh. Zayed Hospital, Lahore. These strains were isolated from a variety of clinical specimens. The organisms were identified as Staphylococcus aureus by colony morphology on blood agar, Gram's reaction, D Nase test and tube coagulase test[13]. All the strains were kept stored at room temperature as stab cultures in nutrient agar[14].

Methicillin sensitivity was determined suing Kirby-Bauer method. The strains to be tested were sucultured from stock cultures on 5% sheep blood agar. After overnight incubation bacterial suspension from these plates was prepared in nutrient broth equivalent to 0.5. McFarland standard. Sterile cotton swab was dipped in the broth suspension and streaked on whole of the 2% sodium chloride supplemented Mueller-Hinton agar plate. Methicillin disk (5-ug) was placed in the center of the plate. These plates were incubated at 30°C for 24 hours[13,15]. Strains with diameter of inhibition zone equal to or more than 14mmwere labelled as methicillin sensitive. Those with diameter of inhibition zone between 10-13mm were labelled as intermediate sensitive, while strains with diameter of inhibition zone less than or equal to 13mm were considered methicillin resistant.

All the Staphylococcus aureus isolates were also tested for beta-lactamase production for this purpose BBL paper disks (cefinase) prepared by Becton Diskinson and Company, USA were used. These disks are impregnated with chromogenic cephalsporin, nitrocefin. The hydrolysis of the amide bond in the beta-lactam ring of nitrocefin by beta-lactamase leads to a color change from yellow to red. The test was performed according to manufactures instruction.

Results

Out of 200 Staphylococcus aureus isolates maximum number were from JinnahHospital[1,38] followed by Services Hospital[37] and Sh. Zayed Hospital[25], Lahore. MRSA strains were present in all the hospitals studied. The lowest percentage of methicillin resistance was observed in Sh. Zayed Hospital (20%) followed by Services Hospital and Jinnah Hospital, Lahore (21.6% & 32.6%) respectively. However, statistically the difference in the number of MRSA isolates was not significant ($p>0.05$) among the different hospitals (Table 1).

Table 1. Distribution of MRSA according to hospitals.

Hospitals	No. of isolates	Methicillin sensitive	Methicillin resistant
Jinnah Hospital	138	93(67.4%	45(32.6%)
Services Hospital	37	29(78.4%)	8(21.6%)
Sh. Zayed Hospital	25	20(80%	5(20%

The distribution of isolates according to clinical specimens revealed that majority of Staphylococcus aureus isolates were from pus/wound swabs[1,35] followed by blood[25], ear/eye/nasal swabs[14], sputum[13] and urine[9]. While four isolates were from different other sources i.e. three from high vaginal swabs and one from ascitic fluid MRSA were isolated from all types of clinical specimens studied. There was no significant difference between the different groups. The highest percentage of methicillin resistance was observed in isolates from sputum (38.5%) followed by pus/wound swabs (29.6%), blood (28%), urine (22.2%) and ear/eye/nasal swabs (21.4%) the rest were recovered from the miscellaneous group (Table 2).

Table 2. Distribution of MRSA according to clinical specimens (n=200)

Clinical specimens	Methicillin Resistant	
	No.	%age
Pus/wound swabs (n=135)	40	29.6
Blood (n=25)	7	28
Sputum (n=9)	5	38.5
Urine (n=9)	2	22.2
Ear/eye/nasal swabs (n=14)	3	21.4
Miscellaneous (n=4)	1	25

Among the 200 Staphylococcus aureus 142(71%) were found to be methicillin sensitive while 58(29%) were found to be methicillin resistant as determine by modified Kirby-Bauer technique. Beta lactimase production by Staphylococcus aureus was determined by nitrocefin test. It was observed that significantly higher ($P<0.001$) number of isolates were beta-lactramase positive as compared to beta lactamase negative isolates. It was also observed that significantly higher number of beta-lactamase producing isolates were methicillin resistant ($P<0.05$) as compared to beta-lactamase negative isolates (Table 3&4).

Table 3.

Tests		Staphylococcus aureus isolates (n=200)	
		No.	%age
Methicillin ensitivity	Sensitive	142	71
	Resistant	58	29
Beta-lactamase production	Positive	188	94
	Negative	12	6

Table 4. Beta-lactamase production and methicillin sensitivity of Staphylococcus aureus isolated (n=200)

Staphylococcus aureus	Results of methicillin sensitivity testing	
Beta-lactamase +ve (n=18)	Sensitive	Resistant
	130	58
Beta-lactamase -ve (n=12)	12	-

Discussion

Staphylococcus aureus remained sensitive to penicillins and cephalosporins after their introduction in the 1940s and 1950s respectively[16,17]. However with continued use of these beta-lactam drugs Staph aureus acquired resistance to these drugs by development of beta-lactamase[3,18].Soon afterwards in 1960s methicillin and other PRPs were introduced[19].For a short time they were the miracle drugs for the treatment of beta-lactam resistant Staphylococcus aureus infections[19,20] Low level resistance to these drugs was noted soon after their introduction in U.K.[5,6] and these resistant isolates were labelled as MRSA.

Mechanism of methicillin resistance is through mutation in the genome of Staph. Aureus[21].As a result these strains acquire the capacity to synthesize an extra penicillin binding protein called PBP2a. This protein carries on the function of synthesizing the cell wall when other PBPs are inhibited[21,22,23].

In the present study, of the 200 hospital Staphylococcus aureus strins 29% were found to be methicillin resistant. In a study carried out in Rawalpindi, 481 strains of Staphylococcus aureus isolates from different clinical specimens were examined for their antibiotic susceptibility pattern[12]. They found methicillin resistance in 13.8% of Staphylococcus aureus isolates. In Karachi methicillin resistance among hospital Staphylococcus aureus isolates has been studied by different workers.. In a study carried out at Jinnah Postgraduate Medical Centre out of 100 Staphylococcus aureus isolates from hospitalised patients 43% were found to be methicillin resistant[9]. While in another institution in Karachi[24] resistance to cloxacillin in Staphylococcus aureus isolates from hospitalised patients was 12%. These reports indicate that percentage of MRSA tends to vary not only from place to place but also in different institutions of the same city. Such a variation has also been reported by Roberts and co-workers[25] from USA. They report that MRSA in ten hospitals of New York city varied from 10 to 38%.

Use of cloxacillin disks to determine methicillin resistance among Staphylococcus aureus is not recommended. This is because it fails to detect all methicillin resistant staphylococci[7,18]. This is due to the presence of fewer cloxacillin resistant organisms in a population of these strains than there are organisms resistant to other members of the group (Methicillin, Oxacillin, Nafonitcillin)

All the three hospitals included in the study were facing the problem of infection/colonization with MRSA strain (Table 1). This could be due to the fact that there are no patients isolation or antibiotic policies in he hospitals[27,28]. Therefore, MRSA strains once introduced in the hospitals tend to become endemic.

In the present study 38.5% of MRSA isolates were recovered from sputum and 29.6% from pus/wound swabs (Table 2). These findings are in agreement with those of other workers. In a study carried out in Zurich, Switzerland[29] 38% of MRSA were recovered from sputum

and 30% from pus/wound swabs. While in another study done in New York, USA[25] 43.7% of MRSA were recovered from sputum and 29.6% from pus/wound swabs. However percentage of MRSA isolates from blood in the present study is higher than that of 10% and 17.4% observed by these workers respectively.

Ninety four percent of Staphylococcus aureus isolates produced beta-lactamase (Table3). According to WHO[18] upto 90% of Staphylococcus aureus isolates are resistant to penicillin G due to production of beta-lactamase. In Pakistan figures slightly lower than that in the present study have been reported by different workers. Iqbal et al[31] collected 400 Staphylococcus aureus isolates from different hospitals in Faisalabad. They reported penicillin resistance due to beta-lactamase production in 77% of Staphylococcus aureus isolates.Qureshi[31] studied 481 strains of Staphylococcus aureus obtained from hospitalized patients at AFIP, Rawalpindi. He reported beta lactamase production in 82.1% of isolates. Based on these findings, it is concluded that drug resistance among Staphylococcus aureus due to beta-lactamase production is an enormous problem in Pakistan as it is throughout the world.

In the present study all the MRSA strains produced beta-lactamase. This finding is similar to that reported in a number of studies carried out in different countries of the world. Richard et al[15] studied 61 MRSA isolates obtained from 15 different French hospitals. All these strains produced beta-lactamase when tested with nitrocefin disks. Similar results have been reported in England[32] Switzerland[29],USA[33,34] and Italy[35]. In Karachi, Hafiz[9] tested antibiotic susceptibility pattern of 100 strains of hospital Staphylococcus aureus. She observed that all MRSA were also resistant to penicillin.

In view of non availability of effective antibiotics, prevention of nosocomial infections due to MRSA requires adoption of infection control programmes. The general principles of an infection control programme include accurate identification of patients with MRSA infections, formulation of antibiotic policies, isolation of infected patients and temporary closure of the affected wards[27,36,37].

Isolation of infected patients and temporary closure of the affected wards are important components of infection control programme[36]. However, in the under-developed countries isolation of all infected patients and closure of wards may not be feasible. In such a situation the most effective and easily resourced approach is education of the health care personnel about principles of hygiene and general cleanliness of the hospital environment[38,39]. Along with this contact isolation policies have been found to be effective in reducing the transmission of resistant strains ofbacteria[40]. Contact isolation policies consist of wearing a mask when within 5 feet of the patient, a gown for close contact with the patient and gloves for manual contact with the patients or any other potentially contaminated surfaces. Contact isolation is to be maintained untill the time of discharge from the hospital or eradication of colonisation is documented.

For accurate detection of methicillin resistance in Staphylococcus aureus strains in Pakistan we recommend the use of 5% salt supplemented sensitivity agar and incubation temperature of 37°C.

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