Methicillin resistant Staphylococcus aureus : a multicentre study

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Abstract

Objective: To determine the frequency of Methicillin resistance Staphylococcus aureus infection in major cities of Pakistan.
Setting: Various laboratones of the country with one as the central Laboratory.
Materials and Methods: Seven hundred and ninety two consecutive clinical isolates of Staphylococcus aureus were collected from 8 laboratories all over Pakistan i.e. Karachi, Peshawar, Lahore, Sukkhur, Islamabad, Quetta, and Mirpur, Azad Kashmir. Antibiotic sensitivity was done by Kirby Bauer disc diffusion method and MIC of Vancomycin was determined by 'E' test.
Result: Forty two percent of the isolates were found to be Methicillin resistant staphylococcus aureus (MRSA) while no Vancomycin resistance was encountered.
Conclusion: Methicillin resistant Staphylococcus aureus (MRSA) are seen in the local population with frequencies varying between 2- 61% highest incidence is seen in the major cities of the country. Fortunately no Vancomycin resistant Staphylococcus has been isolated from any of the major cities (JPMA 52:31 2;2002).

Introduction

For a long time penicillin group of antibiotics have been the main stay for the management of a variety of infections caused by the genus Staphylococcus. During the course of time the genus has gradually been acquiring resistance to antibiotics and a proportion of organisms have become resistant to Methicillin and Cloxacillin. The incidence of methicillin-resistant Staphylococcus aureus (MRSA) has gradually increased, with strains shown to cause up to 21% of skin infections and 59.6% of nosocomial pneumonia[1,2].
There is not much data available regarding the prevalence of MRSA in Pakistan. However in India, MRSA ranges from 27% in Bombay, to 47% in Delhi[3-5]. The research papers read in local forums in Pakistan claimed around 35% MRSA in Pakistan[6,7]. However all of these studies were done in an isolated setting where the overall picture of MRSA in Pakistani population was not available hence a prospective study was planned. Regional laboratories in Pakistan were requested to participate in the

study with the intention of generating local data regarding the prevalence of MRSA in Pakistan.

Materials and Methods

A prospective laboratory based study was designed to ascertain the frequency of Methicillin resistant Staphylococcus aureus in Pakistan. Eight laboratories from Karachi, Peshawar, Lahore, Sukkhur, Islamabad/Rawalpindi, Quetta and Mirpur Azad Kashmir participated in the study. The laboratories chosen were regarded as the busiest laboratories in the cities and received clinical samples from various parts of the city and adjoining areas. These satellite laboratories were requested to collect all Staphylococcus aureus isolates received for testing and send them to the Central Laboratory for confirmation, antibiotic disc sensitivity and MIC determination to Vancomycin by standard international protocol[8-10].

From February - August 2000 clinical samples received at and identified were screened for isolates of Staphylococcus aureus. All consecutive Staphylococcus aureus isolates based on cultural, morphological and biochemical characteristics were collected along with the clinical report form. Each had date of isolation, source, sex and age of the patient. The isolates and forms were shipped through a courier to the central laboratory on a transport/growth medium for further testing.

Upon receiving, the isolates were subcultured, purity checked, rechecked and the identification of the isolates was confirmed by standard protocol, which included Gram's staining, catalase, coagulase, Dnase test and growth on 5% Mannitol salt agar[8,9]. The antibiotic susceptibility pattern was determined by Kirby Bauer disc diffusion method according to NCCLS guidelines[10], against, Erythromycin, Oxacillin, Methicillin, Tetracycline and Vañcomycin. The isolates found to be Methicillin Resistant (MRSA) based on disc sensitivity producing a zone of inhibition < 10 mm with 1ug oxacillin and <9mm with 5ug methicillin were regarded as Oxacillin/ Methicillin resistant Staphylococcus aureus (MRSA). "E" test (AB Biodisk, Solna, Sweden)[11] strips of Vancomycin were used according to the manufacturer's instructions to determine the MIC of all Staphylococcus aureus isolates.

Results

A total of 875 isolates of Staphylococcus aureus were received from the collaborating Laboratories out of which 792 were viable. The isolates were sub-cultured purity and identity confirmed by standard criteria disc sensitivity and E test for Vancomycin carried out and the results recorded. The isolates were from Pus, blood, urine, aspirates and ear and eye swabs.

Table 1. Isolation of Staph aureus and MRSA by Location.

SR No.	Laboratory No.	Specimen			Total Staph. Aureus		MRSA	
		Inpatients	Outpatients	Total	No.	%	No.	%
1	Lab 11 Karachi	95	66	161	151	94	87	58
2	Lab 12 Karachi	67	33	100	94	94	54	57
3	Lab 21 Lahore	31	69	100	98	98	60	61
4	Lab 32 Islamabad	51	41	92	85	92	39	46
5	Lab 41 Peshawar	93	94	187	158	84	57	36
6	Lab 51 Quetta	4	38	42	27	64	7	26
7	Lab 61 Azad Kashmir	42	47	89	81	91	26	32
8	Lab 71 Sukkur	37	67	104	98	94	2	2
9	Total	420	455	875	792	91	332	42

Table 1 gives the frequency of Staphylococcus aureus and MRSA isolated by different laboratories of Pakistan and also the breakdown of isolations from in and out patients. Out of a total of 875 isolates received, the Central Laboratory could only isolate 792 (9 1%) viable Staphylococcus aureus, (conforming to the standard criteria of NCCLS for Staphylococcus aureus) out of which 332 (42%) were MRSA. The distribution Of MRSA in different cities varied with highest being from Lahore (61%) followed by Karachi (57.5%), Islamabad/Rawalpindi (46%) and Peshawar (36%), Sukkur hsd only 2% MRSA.

Table 2. Sensitivity Pattern of MRSA (inpatients).

Sr No.	Drug	Sensitive		Resistant		Intermediate		Total
		No.	%	No.	%	No.	%	
1	Tetracycline	88	51	79	45	7	4	174
2	Erythromycin	36	21	134	77	4	2	174
3	Vancomycin	174	100	0	0	0	0	174
4	Oxacillin/ Methicillin	0	0	174	100	0	0	174

Table 3. Sensitivity Pattern of MRSA (outpatients).

Sr No.	Drug	Sensitive		Resistant		Intermediate		Total
		No.	%	No.	%	No.	%	
1	Tetracycline	83	52	66	42	9	6	158
2	Erythromycin	31	20	125	79	2	1	158
3	Vancomycin	158	100	0	0	0	0	158
4	Oxacillin/ Methicillin	0	0	157	99	1	1	158

Table 2 and 3 give the sensitivity pattern of MRSA isolated from in and outpatient respectively. All isolates were resistant to oxacillin/methicillin and 100% sensitive to Vancomycin. Sensitivity pattern to other commonly used drugs other than Penicillin and Cephalosporin group varied between 20-52%. There was no difference between in and out patients, 51% of the isolates were sensitive to Tetracycline and only 20% being sensitive to Erythromycin.

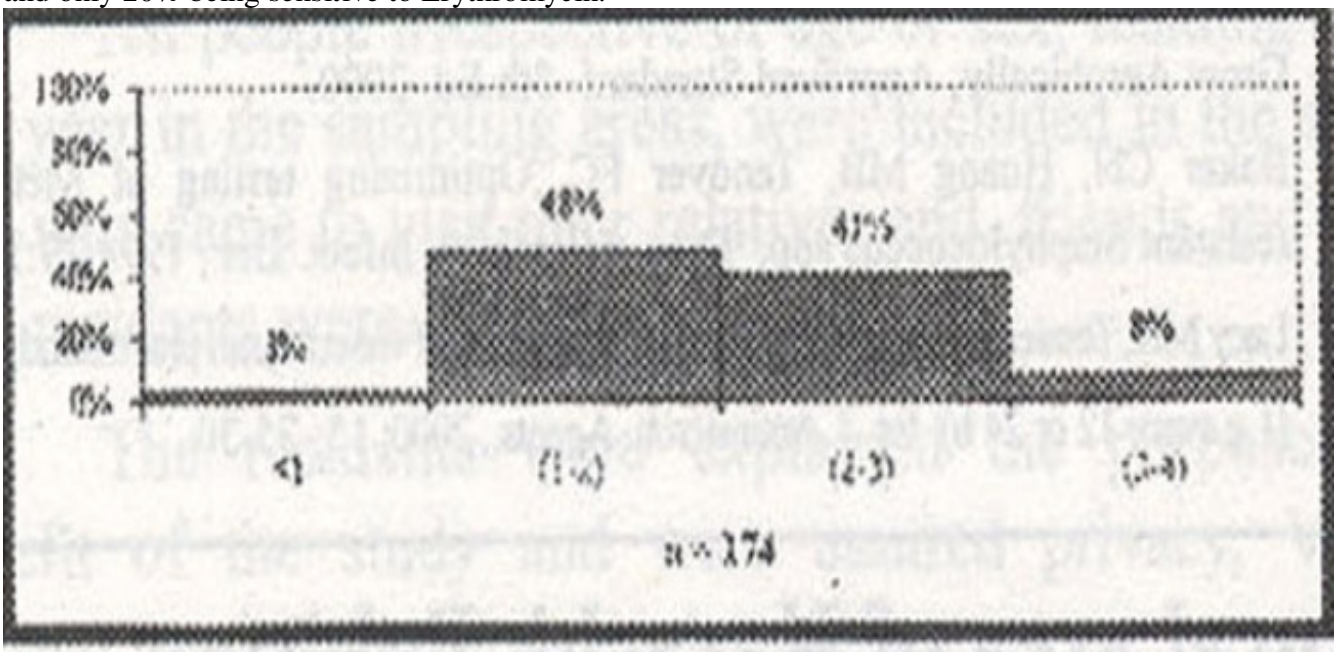

Figure 1. Vancomycin MIC Readings for MRSA (In-patients).

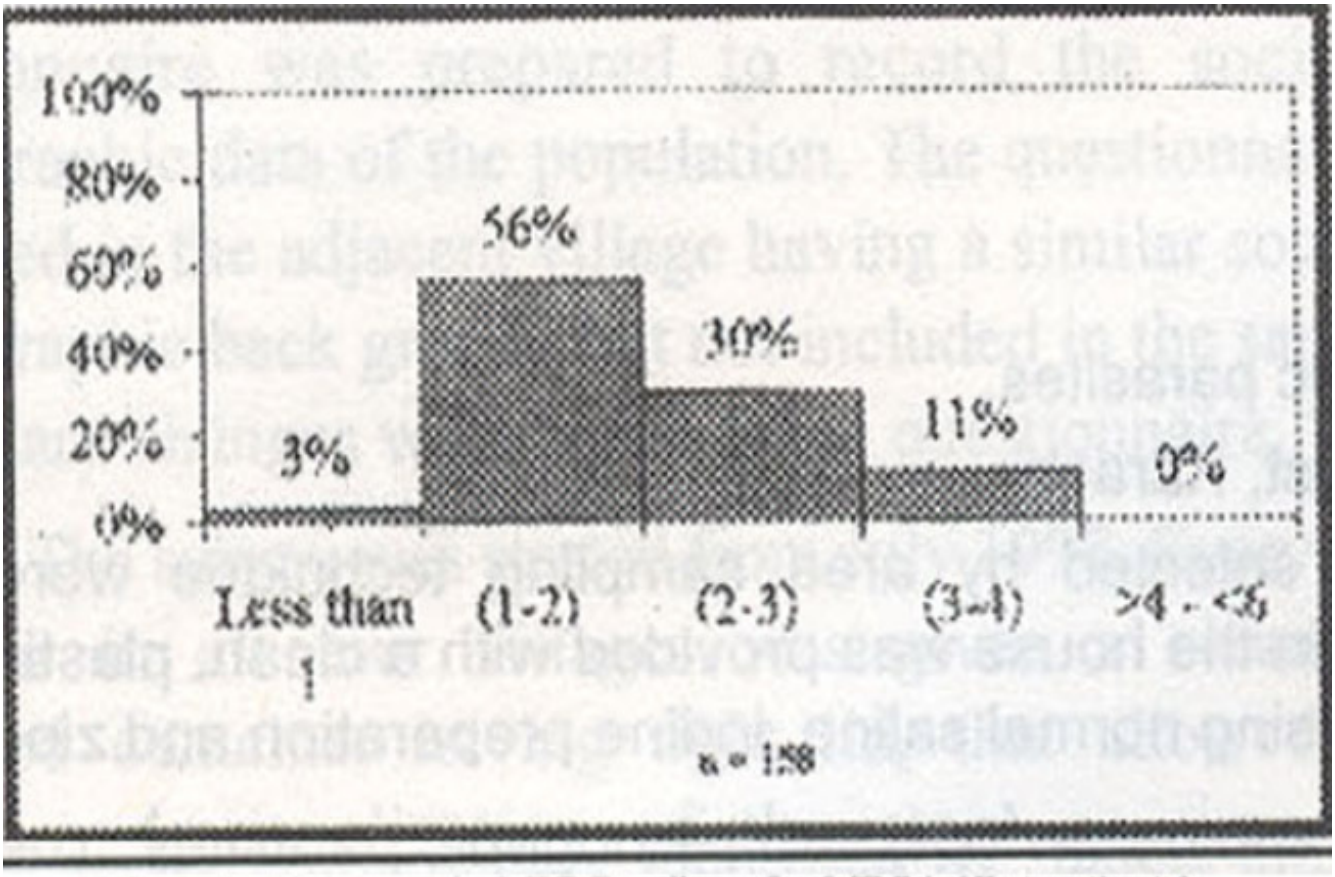

Figure 2. Vancomycin MIC Readings for MRSA (Out-patients).

Figure 1 and 2 gives the Vancomycin MIC ranges of 174 and 158 MRSA isolates from in and out patient respectively. All isolates had MIC less then 4ug/ml, 54.5% of the isolates had MIC <2ug/ml 35.8% between 2-3ug/ml and 9. 6% between 3-4 ug/ml, which is reflected in Table 4.

Table 4. Distribution of Vancomycin MIC Readings for MRSA (Total).

S. No.	Vancomycin (µg/ml)	Frequency	Percentage	Cumulative Percentage
1	Less than 1	9	2.71	2.71
2	1 - 2	172	51.80	54.51
3	2 - 3	119	35.84	90.35
4	3 - 4	32	9.64	99.99
5	Total	332	100	99.99

Discussion

In this study the prevalence of MRSA strains in various cities of Pakistan over 10 months period was found to be 42%. There was a difference in the MRSA frequency in different parts of the country; highest seen in Lahore (61%), closely followed by Karachi (57%), Rawalpindi Islamabad (46%),

Peshawar (36%), Azad Kashmir (32%) and Quetta (26%) while minimum resistance were seen in Sukkur (2%). Current NCCLS guidelines suggest that Oxacillin resistant Staphylococcus must be reported as cephems and other B-lactams. Inspite of the fact that they appear sensitive in vitro but are not effective in vivo as seen in this study, our laboratories generally report them without any warning. So far no Vancomycin resistant or intermediate resistant Staphylococcus aureus has been isolated from any province of Pakistan. The efficacy of Vancomycin against methicillin — resistant staphylococci may decrease if more cases of VISA emerge, thus requiring monitoring of the change in sensitivity pattern.

Several new antibiotics showing promising activity may be used against these multidrug-resistant bacteria. However, as the history of bacterial resistance has taught us, it will be a matter of time until these organisms adopt mechanisms of resistance to these new drugs. The key then lies in preventive measures. Surgeons and physicians must adhere to the precautionary guidelines recently set forth by the CDC and HICPAC: chief among these guidelines being the elimination of inappropriate antibiotic usage, especially inappropriate vancornycin use.

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