International Journal of Pathology; 2004; 2(1):10-15

Prevalence and Antimicrobial Susceptibility of Methicillin Resistant Staphylococcus Aureus (MRSA)

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The isolation rate of MRSA from different specimens was determined. Between January 1999 and June 2002, 448 out of 1322 (35.67%) isolates were found to be MRSA. A detailed study of these isolates showed a high prevalence of MRSA in patients in ICU's and special care wards. The yield of MRSA was highest from endotracheal secretions (100%), bronchial washings (70%), and catheter tips (51.85%) followed by sputum (40.54%), ear swabs (40%), fluids (37.25%), pus/wound swabs (34.83%) and blood cultures (28.07%). Vancomycin remains the drug of choice with no resistance detected. The resistance to ciprofloxacin was 86.61%, to erythromycin 85.5% and to gentamicin 81.03%. After vancomycin, fusidic acid was the most sensitive drug with only 21.21% of the isolates exhibiting resistance. In view of the high prevalence of MRSA in our community, authorities must introduce more effective measures to control its spread, otherwise it may seriously disrupt the efficient delivery of the health system in the country.

Key words: Methicillin Resistant Staphylococcus Aureus; MRSA; Antimicrobial Susceptibility.

Introduction

Staphylococcus aureus is perhaps the most notorious of all the bacterial pathogens associated with human infection. The adaptive potential of this highly virulent and ubiquitous pathogen is unique. In 1942, the year penicillin G was introduced, some resistant strains of staphylococcus aureus were found.[1] However the period between 1946 and 1950 was the golden age for the treatment of staphylococcus infections with penicillin. As the problem of drug resistance to penicillins, mediated by the production of beta-lactamase, became prevalent, effective first line therapies were compromised. By 1950, approximately 80% hospital acquired staphylococcal infections were untreatable using penicillins.[2, 3] The introduction of beta-lactamase resistant semi-synthetic penicillins in the early 1960's provided temporary respite which ended with the emergence of Methicillin (oxacillin) Resistant Staphylococcus aureus (MRSA), discovered shortly after methicillin became available for clinical use. Subsequently these resistant Staphylococcus aureus organisms established themselves in hospitals and communities, spreading throughout the world.[4, 5]

MRSA has proven to be one of the more widespread and durable nosocomial pathogens of the late 20th century.[6]

Data from the National Nosocomial Infection Surveillance System reveals MRSA accounts for upto 40% of nosocomial Staphylococcus aureus infections in large hospitals and 25-30% in smaller hospitals. The prevalence of MRSA varies considerably from one region to another and among hospitals in the same city. In some hospitals MRSA accounts for <10% of all staphylococcus aureus isolates whereas in other facilities they account for upto 65%.[7, 8] Richet et al reported an incidence of 1.25% in 27 hospitals of France whereas Berardi et al[9, 10] reported 31.8% from other hospitals in the same area. The vast majority of infections are acquired in hospitals, however, outbreaks of community acquired MRSA have also been reported.[11, 12]

The most common infections caused by MRSA include surgical site infections, bacteraemia, lower respiratory tract infections and urinary tract infections. However, variable incidence has been reported by different researchers.[3, 9, 13-18]

Most MRSA strains are multi-drug resistant. Resistance to erythromycin and clindamycin is very common and many strains are resistant to

gentamicin, tobramycin and ciprofloxacin as well. Recently, strains resistant to methicillin but susceptible to gentamicin have been recovered. Herve et al have reported that the incidence of isolation of Gentamicin sensitive MRSA (GS-MRSA) has steadily increased over a 7 year period to represent in 1998, 46.8% to 94.4 of all strains.[19]

The aim of the present study was to determine the prevalence of MRSA and its antibiotic susceptibility pattern by retrieving data from the past three and a half years and additionally, the percentage of multi-drug resistant isolates was determined for MRSA and MSSA isolates.

Patients and Methods

Source of Bacterial Strains: The prevalence of MRSA was determined retrospectively by studying the data collected over a three and a half year period-from January 1999 to June 2002-from the clinical isolates submitted for culture to Chughtai's Lahore Lab. Specimens were received from different hospitals and included samples from various body sites. Both sexes and all age groups were included.

Specimen Collection: Specimens were collected using standard collection techniques.[20]

Isolation of Pathogens: Standard commercially available media were used including Blood, MacConkey's & Cled agars. Specimens were inoculated on the required media, incubated aerobically at 37°C for 24 hours and then inspected for growth. Staphylococcus were identified by colonial morphology, catalase testing, tube coagulase testing, DNase reaction and mannitol fermentation.

Antibiotic Susceptibility Test: The in-vitro activity of various antibiotic compounds was tested. Susceptibility testing was performed on Meullar-Hinton Agar by Kirby Bauer method (disk diffusion method) according to the National Committee for Clinical Laboratory Standard (NCCLS) guidelines using standard antibiotic disks (oxoid). Strains were considered resistant to oxacillin if there was a diameter of inhibition around a 1 disk of <10mm dia.[21]

Control and Comparator Strains: Control strains used were Staphylococcus epidermidis ATCC 12228,Staphylococcus aureus ATCC 29213 and MRSA ATCC 49476.

Results

Results over the past three and a half years-from January 1999 to June 2002-were studied retrospectively. Total specimens received for culture were 5821, 5259, 2494, and 3931 for the years 1999, 2000, 2001 and 2002 till June 30th respectively.

Staphylococcus aureus was isolated from 1322 of the specimens. Out of these MRSA was the causative agent in 133 (36.14%), 147 (39.09%), 114 (31.93%) and 54 (24.43%) (Table 1) during the year 1999, 2000, 2001 and 2002 respectively. The overall prevalence over the past years was 36.67% for MRSA and 63.33% for MSSA.

Table I: MRSA Isolation for the Years 1999-2002

	1999	2000	2001	2002
Total Cultures	5821	5259	2494	3931
Staph. aureus	368	376	357	221
MSSA	235	229	243	167
MRSA	133	147	114	54
%MRSA	36.14 %	39.09 %	31.93 %	24.43 %

Isolation rates of MSSA and MRSA from different specimens is highlighted in Table 2. All the specimens isolated from the endotracheal tubes were MRSA. The next highest isolation rate was from bronchial washings (70%) and the catheter tips (51.85%), followed by sputum (40.54%), ear swabs (40%), fluids (37.25%) and wound samples (34.83%).

With the exception of Vancomycin to which MRSA is still 100% susceptible all the other antibiotic compounds showed a variable drug susceptibility pattern (Table 3). Susceptibility of MSSA to these same antibiotics showed a better in vitro activity as compared to MRSA (Table 4).

Discussion

Staphylococcus aureus, in particular MRSA, has been one of the more problematic nosocomial pathogens encountered by the medical profession-

repeatedly acquiring resistance to overcome the challenges presented by the new anti-staphylococcal antibiotics. MRSA infections are of particular concern due to their association with prolonged hospital stays with resultant increased hospital costs. Also , fewer therapeutic options are becoming increasingly available to treat the affected people. The single

Table 2: Isolation Rates of MSSA And MRSA From Different Specimens

Specimen	S. Aureus (n=1322)	MSSA (n= 874)	%MSSA	MRSA (n=448)	%MRSA
Pus/Wound swabs	686	447	65.16	239	34.83
Urine	191	127	66.49	64	33.50
HVS	65	49	75.38	16	24.61
Blood	57	41	71.92	16	28.07
Semen	55	43	78.88	12	21.81
Ear Swabs	55	33	60.00	22	40.00
Fluids *	51	32	62.74	19	37.25
Sputum	37	22	59.45	15	40.54
Nasal Swabs	31	26	83.87	5	16.12
Catheter Tips	27	13	48.14	14	51.85
CSF	17	15	88.23	2	11.76
B. Washings	10	03	30.00	7	70
Tissue	10	07	70.00	3	30.00
Urethral Discharge	9	06	66.66	3	33.33
Endo. Trach, Sec.	8	00	00	8	100

* Includes Pleural, pericardial peritoneal fluids.

Table 3: MRSA Antibiotic Susceptibility Pattern

Antibiotic	1999 (n=133	%	2000 n=47)	%	2001 (N=114)	%	2002 (n=54)	% Suscept.
Ciprofloxacin	23	17.29	21	14.28	14	12.28	2	3.7
Clindamycin	71	53.83	43	29.25	28	24.56	11	20.37
Doxycycline	42	31.57	27	18.36	17	14.91	6	11.11
Erythromycin	32	36.22	22	14.96	10	8.77	3	5.55
Fusidic acid	112	84.12	123	83.67	75	65.78	43	79.62
Gentamicin	12	9.02	23	15.64	27	23.68	13	24.07

Ofloxacin	97	72.93	76	51.70	31	27.19	15	27.77
Sulphamethoxazole/Trimethoprim	34	25.56	29	19.72	14	12.28	8	14.81
Vancomycin	133	100	147	100	114	100	54	100

Table 4: MSSA Antibiotic Susceptibility Pattern.

Antibiotic	1999 (n=235)	%	2000 (n=229)	%	2001 (n=243)	%	2002 (n=167)	% Suscept.
Ciprofloxacin	162	68.93	153	66.81	144	59.25	152	91.01
Clindmycin	119	50.63	96	41.92	160	65.84	136	81.43
Doxycycline	78	33.19	59	25.76	80	32.92	76	45.50
Erythromycin	88	37.44	79	34.49	144	59.25	118	70.65
Fusidic acid	154	65.53	111	48.47	163	67.07	152	91.01
Gentamicin	73	31.06	62	27.07	59	24.27	67	40.11
Ofloxacin	165	70.21	99	43.23	170	69.95	150	89.82
Sulphamethozole/Trimethoprim	66	28.08	55	24.01	58	23.86	64	38.32
Vancomycin	235	100	229	100	243	100	167	100

Table V: Cumulative Percentage Susceptibility /Resistance of MRSA to Various Antibiotics (n=448)

Antibiotic	% Susceptibility	% Resistance
Ciprofloxacin	13.39	86.61
Clindamycin	34.15	65.85
Doxycycline	20.53	79.47
Erythromycin	14.95	85.05
Fusidic acid	78.79	21.21
Gentamicin	16.74	83.26
Ofloxacin	47.32	52.68
Sulphamethozole / Trimethoprim	18.97	81.03
Vancomycin	100	00

notable exception to resistance development are the glycopeptides-teicoplanin and vancomycin. Although, at present, vancomycin remains the only drug of choice for treating multi-drug resistant MRSA, occasional reports of intermediate resistance emerging are making headlines.[22] In our study, 36.67% of the isolates were methicillin resistant. The prevalence of MRSA is comparable to that found in recent studies.[15, 23-26] The percentage of MRSA isolates is less than half of the percentage reported from that of a Japanese and an American study.[27, 28] The reason for the low prevalence in these studies may be related to the rapid identification and strict policies of isolation of patients with MRSA colonization or infection, combined with the restricted use of antibiotics.

The distributions of both MSSA and MRSA among different age groups were similar. However, with the exception of newborns, S: aureus infections were more often found with increasing age, but their prevalence declined after 75 years of age. Compared to the age distribution for all infections with other

organisms, no significant differences in the age distributions of individuals with MRSA infections were observed.

Considerable differences were observed when the distributions of MRSA isolates in different specimens were compared (Table 2). The prevalence of methicillin resistance was highest among S. aureus isolates responsible for endotracheal intubation associated nosocomial pneumonias-100%; fluids gave a yield of 70% and indwelling catheters 51.85%; sputum and ear swabs showed a similar positivity rate of approximately 40% each; the prevalence of methicillin resistance associated with skin and soft tissue infections was 34.83%, 33.5% among urinary tract infection isolates and 28.07% among blood isolates. These differences might be due to prolonged antibiotic treatment of severely sick patients, who generally have longer hospital stays, resulting in enhanced selection pressure. This reflects the fact that critically ill patients have a greater chance of becoming colonized or infected. The results of our study are similar to those seen in other studies.[29-31]

(Table 6): Cumulative Percentage Susceptibility/Resistance of MSSA to Various Antibiotics (n=874)

Antibiotic	% Susceptibility	% Resistance
Ciprofloxacin	69.91	30.09
Clindamycin	58.46	41.54
Doxycycline	33.52	66.48
Erythromycin	49.08	50.92
Fusidic acid	66.36	33.64
Gentamicin	29.86	70.14
Ofloxacin	66.82	33.18
Sulphametoxazole/ Trimethoprim	27.80	72.20
Vancomycin	100	00

The comparative in vitro activities of the antimicrobial agents tested against MSSA and MRSA isolates are listed in Tables 3 and 4 respectively. 89.3% of the MSSA isolates tested were resistant to penicillin, The percentage of MSSA isolates which were sensitive to erythromycin (49.08%) (Table 6) was considerably higher than the percentage of MRSA isolates susceptible to erythromycin (14.95%-Table 5). While 58.46% of the MSSA isolates were susceptible to clindamycin, only 34.15% of the MRSA isolates exhibited susceptibility. The percentage of MRSA isolates showing susceptibility to gentamicin (16.74%) was lower than that of MSSA isolates (29.86%). Susceptibility to doxycycline fell from 33.52% among MSSA isolates to 20.53% among MRSA isolates. About 70% of all MSSA isolates were susceptible whereas less than 15% of all MRSA isolates tested were susceptible to ciprofloxacin.

Vancocomycin was the only antibiotic which showed a 100% susceptibility to all the isolates tested. 70.14% of the MRSA and 83.26% of MSSA isolates were gentamicin resistant. Erythromycin resistance was seen in 85.05% of MRSA and 50.92% of the MSSA isolates. The resistance seen with gentamicin and erythromycin in our study is higher than the incidences reported by Nishijima and Kurokawa and in other studies.[32-34] Isolates were considered to be multidrug resistant when they displayed resistance to five (or more) of the following antibiotics representing different antibiotic classes: oxacillin, penicillin, erythromycin, clindamycin, gentamicin, ciprofloxacin, doxycycline, and fusidic acid. MRSA is by definition, also resistant to penicillin.[3] Thus , all MRSA isolates were resistant to at least two classes of antibiotics. The results are shown in Fig 1. The percentage of MRSA and MSSA isolates which were resistant to all the antibiotics with the exception of vancomycin was 3.34% and 3.20% respectively.

Vancomycin is still the only glycopeptide which is the drug of choice for life threatening infections caused by multi drug resistant MRSA. However, reports of intermediate resistance to this drug nessecitates careful monitoring of the prevalence of MRSA and its antibiotic susceptibility pattern.[22, 34, 35]

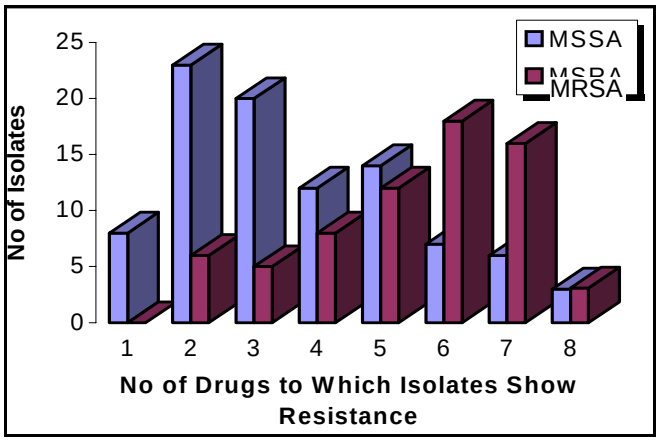

Figure 1: No Of Drugs To Which Staph. Aureus Isolates Were Resistant

The links between antimicrobial use and MRSA are complex; interventions aimed at eradicating MRSA should be aimed to promote more rational prescribing patterns, supported by adequate experimental and epidemiological evidence. This study shows a high prevalence of MRSA in our community with 66% of these isolates being multi-drug resistance. Although this percentage is lower than that reported by Fluit et al (87%), it reflects the need for more prudent use of antibiotics, in addition to the implementation of better infection control and hygiene measures for reducing infections.[3]

An urgent need exists for more appropriate selection and use of antimicrobial drugs in the developed as well as in developing countries. The focus in developing countries should be on the availability of safe and effective drugs and on the enforcement of more responsible national drug policies. These issues must be addressed by the collective action of governments, the pharmaceutical industry, health care providers, and consumers. The developed countries have an important stake in the ways in which antibiotics are used in developing countries because resistant microorganisms do not recognize national boundaries.

References

1. Kunin CM. . Resistance to anti-microbial drugs-a worldwide calamity-Ann Intern Med.1;118(7):557-611.
2. Lowy FD. Staphylococcus aureus infections. NEJM, 1998; 339(8):520-32.
3. Estrada B. Methicillin-Resistant Staphylococcus aureus in the Community. Infect Med 2001, 18(10):452.
4. Miall LS,McGinley NT, Bronlee KG Conway SP. Methicillin-Resistant Staphylococcus aureus (MRSA) infection in cystic fibrosis. Arch Dis Child, 2001; 84:160-162.
5. Fluit AC, Wielders CLC, Verhoef J, Schmitz FJ. Epidemiology and Susceptibility of 3,051 Staphylococcus aureus Isolates from 25 University Hospitals participating in the European SENTRY Study. J of Clin Microbiology 2001; 39:10:3727-3732.
6. Nimmo GR, Schooneveldt J, O'Kane G, McCall B, Vickery A. Community Acquisition of Gentamicin-Sensitive Methicillin Resistant Staphyloccus aureus in Southeast Queeensland, Australia J of Clin Microbiology 2000, 38:11: 3926-3931.
7. Boyce JM, Diagnosis and treatment of serious anti-microbial resistant Staphylococcus aureus infection. Infectious diseases-clinical updates, 1998; 4:4
8. Struelens MJ, Ronveaus O, Jans B, Mertens R. Methicillin Resistant Staphylococcus aureus epidemiology and control in Belgian hospitals, 1991 to 1995. Infect Control Hosp Epidemiol 1996, 17 (8): 503-8.
9. Richet H, Wiesel M, Gallou FL, Richet BA, Espaze E. Methicillin Resistant Staphylococcus aureus control in hospitals. Infection Control and Epidemiology, 1996;17 (8).
10. Beardi-Grassias L, Kahla N, Richardin F, Boisivon A, Ghnassia JC, Holler C, Leneveu M, Martelli M, Rast G. Staphylocccus aureus resistant to methicliin. Survey of prevalence in Yvelines hospitals. Pathol Biol 1996; 44(5):411-415.
11. Groom AV, Wolsey DH, Naimi TS, Kristine A Cheek JE. Community Acquired Methicillin Resistant Staphylococcus aureus in a Rural American Indian Community. JAMA,2001; 286(10)1201-1205.
12. O,Brien FG, Pearman JW, Gracey M, Riley TV, Grubb WB. Community strain of Methicillin Resistant Staphyloccus aureus involved in a hospital outbreak. J of Clin Microbiol, 1999,37(9)2858-2862.
13. Gang RK Sanyal RL, Mokaddas E, Lari AR.. Staphylococcal Septicaemia in burns. Burns 2000, 2(64):359-66.
14. Blumberg LH, Klugman KP. Control of Methicillin Resistant Staphylococcus aureus bacteraemia in high risk areas. Eur J clin Microbiol Infect Dis, 1994, 13(1) 82-5.
15. Cheong I, Tan SC, Wong YH, Zainudin BM. Rahman MZ. Methicillin Resistant Staphyloccus aureus (MRSA) in a Malaysian hospital Med J Malaysia, 1994,49(1): 24-8.
16. Mashita K, Shinagawa N, Ishikawa S, et al. Bacteria isolated from surgical infections and their susceptibilities to antimicrobial agents. Special references to bacteria isolated between April 1998 and March 1999. Jpn J Antibiot 2001, 54(10): 497-530.
17. Soriano A, Martinez JA, Mensa J, et al. Pathogenic significance of methicillin-resistance for patients with Staphyloccus aureus bacteraemia. Clin Infect Dis, 2000; 30(2)368-73.
18. Maezawa H, Skamoto M, Nakazawa Y, Shindo N, Yoshikawa K, Yoshida M, Shiba K.. A clinical investigation of bacteremia for the past ten years at the Second Department of Internal Medicine, Jikei University Hospital. Kansenshogaku Zasshi, 1998; 72(8): 820-6.
19. Lelievre H, Lina G, Jones ME, Olive C Forey F Rousel-Delvallez M, Nicolas-Chanione M-H Bebear CM Jarlier V Andremont A, Vandenesch F, Etienne J. Staphyloccus aureus with increasing susceptibility to gentamicin and other antibiotics. J of Clin Microbiol, 1999; 37(11): 3452-7.
20. Cheesebrough M. District Laboratory Practice in tropical Countries: Microbiological test 2000; 63-124.
21. National Committee for clinical Laboratory Standards. Methods for dilution antibicrobial susceptibility tests for bacteria that grow aerobically; Approved standard Fifth Edition 2000; 20(2).
22. Tallent SM Bischoff T, Climo M. Ostrowsky B, Wenzel RP, Edmond MB. Vancomycin Susceptibility of Oxacillin Resistant Staphylococcus aureus Isolates Causing Nosocomial Bloodstream Infections. Journal of Clin Microbiol 2002; 40(6)2249-2250.
23. Pfaller MA Jones, RN Doern, Kugler K. and the SENTRY participants Group. Bacterial pathogens isolated from patients with blood stream infection: frequencies of occurrence and antimicrobial susceptibility patterns, from the SENTRY antimicrobial surveillance Program (United States and Canada, 1997). Antimicrob agends chermother, 1998; 42: 1762-1777.
24. Marshall SA Wilke WW Pfaller MA Jones, RN. Staphylococcus aureus and coagulase-negative staphylococci from blood stream infections: frequency and occurrence, antimicrobial susceptibility, and molecular (mecA) characterization of oxacillin resistance in the SCOPE program. Diagn Microbial Infect Dis 1998; 30: 205-214.
25. Simor AE Ofner-Agostini M Bryce E Green K, McGeer A, Mulvey M. The evolution of methicillin resistant Sta;phyloccus aureus in Canadian hospitals : 5 years of national surveillance. CMAJ, 2001; 10; 165(1): 21-26.
26. Blane DS Pittet D Ruef C Widmer AF Muhelemann K Petignat C. Harbarth S Auckenthaler R Bille J Frei R Zbinden R, Peduzzi R Gaia V,

Khamis H., Bernasconi E, Francioli P. Epidemiology of methicillin resistant Staphyloccus aureus: results of a nation wide survey in Switzerland. Swiss Med Wkly, 2002; 132: 201-206.

27. Hashimoto H, Inoue M, Hayashi 1 A survey of Staphylococcus aureus for typing and drug-resistance in various areas of Japan during 1992 and 1993. Jpn J Antibiot, 1994; 47(6): 618-26.
28. Panlilio AL, Culver DH, Gaynes RP, Banerjee S, Henderson, TS. Tolson JS, Martone, WJ, Methicillin Resistant Staphyloccus aureus in US hospitals Infect Control Hosp Epidemiol 1992; 13(10): 582-6.
29. Matsui T, Tanaka K, Chokyu H, Miyazaki S, Okada H. Arakawa S, Kamidono S. Bacteraemia of in patients of the department of urology. Kansenshogaku Zasshi, 1994; 68 (1) 34-41.
30. Wang FD Chen YY, Liu, CY. Prevalence of nosocomial respiratory tract infections in the surgical intensive care units of a medical center. Zhonghua Yi Zue Za Zhi (Taipei) 1998; 61 (10): 589-95.
31. Nishijima S, and Kurokawa I Antimicrobial resistance of Staphylococcus aureus isolated from skin infections. Int J Antimicrob Agents 2002; 19(3): 241-3.
32. Roghmann MC. Predicting methicillin resistance and the effect of inadequate empiric therapy on survival in patients with Staphyloccus areus bacteraemia. Arch Intern Med, 2000; 10: 160(7):1001-4.
33. Tosaka M, Yamane N, and Okabe H. Isolation and antimicrobial susceptibility of methicillin resistant Staphyloccus aureus (MRSA) at Kumamoto University Hospital. Nippon Rinsho, 1992; 50(5) :975-80.
34. Geisel R, Schmitz F-J, Thomas L, Berns G, Zetasche O, Ulrich B. Fluit AC, Labischinsky H, Witte W. Emergence of heterogeneous intermediate vancomycin resistance in Staphyloccus aureus isolates in the Dusseldorf area. J Antimicrob Chemother, 1999; 43: 846-848.
35. Srinivasan A, Dick JD Perl TM. Vancomycin Resistance in Staphylococci. Clinical Microbiology Reviews, 2002; 15(3):430-438.