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ANTIBIOTIC RESISTANCE AMONG CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS* AND USEFULNESS OF ANTIBIOGRAM

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ABSTRACT

The emerging resistance to antibiotics and the poor pipeline of new antibiotics is creating a major health issue world-wide. The resistance problem demands that a renewed effort be made to seek antibacterial agents effective against pathogenic bacteria resistant to current antibiotics. Cumulative antibiogram is valuable in monitoring the resistance pattern and to improve empirical therapy by increasing knowledge of local outpatient prevalence of antibiotic resistance. In this study, a total of 107 *Staphylococcus aureus* were isolated from pus, blood, throat swab, urine, tracheal aspirates out of which 84 were identified as methicillin sensitive *S. aureus* (MSSA) and 23 were methicillin resistant *S. aureus* (MRSA) by automated identification and susceptibility testing system (Vitek 2 compact) using AST GP-67 card. While testing the *in vitro* activities of 15 antibiotics against 107 *Staphylococcus aureus*, of which 76 were collected from inpatients and 31 from outdoor patient department. The analysis is done on the basis of patient location: whether outpatient and inpatient (non-ICU). It is found that the resistance pattern of these isolates (IPD) were penicillin (91.9%) followed by trimethoprim-sulfamethaxzole (85.3%), ciprofloxacin (84.2%), levofloxacin (83.6%), moxifloxacin (60%), erythromycin (46.7%). Less resistance rate was found against tetracycline (13.2%), gentamycin (11.1%), refampicin (2.7%) and clindamycin (1.4%). None of the isolate was resistant to vancomycin, linezolid, nitrofurantoin and quinpreistin/dafloprístin.

KEYWORDS: ***Staphylococcus aureus,*** **Antibiogram, Antibiotic sensitivity, methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus* (MSSA), minimum inhibitory concentration (MIC).**

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INTRODUCTION

Staphylococcus aureus has long been recognized as an important pathogen in human disease. Staphylococcal infections occur regularly in hospitalized patients and have severe consequences, despite antibiotic therapy[33]. In the hospital environment, *S. aureus* resistant to a variety of classes of antimicrobials, such as β-lactams, quinolones and macrolides, are responsible for many life-threatening infections[8]. Historically, penicillin resistant, methicillin susceptible *S. aureus* first emerged in the hospital and subsequently in the community. The evolution of methicillin-resistant *S. aureus* (MRSA) may follow a similar sequence[26]. Methicillin-sensitive *S. aureus* (MSSA) becomes MRSA by the acquisition of the *mecA* gene, which encodes for PBP2a, a penicillin binding protein, with low-binding affinity to practically all β-lactam antibiotics available[15]. MRSA is defined as an isolate with minimum inhibitory concentration (MIC) of >4µg of oxacillin/ml and MSSA is an isolate with MIC of <2µg of oxacillin/ml. Isolates having MIC of Oxacillin 2-4 µg/ml are defined as borderline resistant *S. aureus* (BRSA)[30]. The spread of these strains may be monitored by antibiograms or by bacteriophage typing. The emergence of MRSA strains and resistance to other antimicrobial agents has become a major concern, especially in the hospital environment, because of increased mortality due to systemic MRSA infection[18]. Therefore, there is a need to monitor the development of resistance and to establish empirical therapy. The antibiogram is a periodic summary of antimicrobial susceptibilities of local bacterial isolates submitted to the microbiology laboratory. Consensus guidelines have been developed by the Clinical and Laboratory Standards Institute (CLSI) to standardise methods used in constructing antibiograms, with the goal of promoting the reporting of reliable and consistent antibiogram data. The antibiogram helps in monitoring antimicrobial resistance trends over different periods: Intensive care unit (ICU) or ward specific data and inpatient versus outpatient data, etc. Different parts of a healthcare institution can have different patterns of antimicrobial use and resistance[17].The cumulative antibiogram is needed capturing the susceptibility data over a period of time either half yearly or annually and analysis of this data is valuable in monitoring the trends in antibiotic resistance. Infections with *S. aureus* are especially difficult to treat because of evolved resistance to antimicrobial drugs. Retrospective hospital based studies conducted suggested no difference in virulence when clinical outcomes between patients with MRSA and MSSA infections were compared[13]. A number of studies have failed to show that the virulence of MRSA differs from that of MSSA. MRSA are more likely to be colonizing bacteria, whereas MSSA are more likely to be associated with infection[7]. This study was undertaken to document the *in vitro* activity of fifteen different antimicrobial agent against *S.aureus* isolates collected from Inpatient (IPD) and outpatient department (OPD) of different hospitals.

MATERIALS AND METHODS

Bacterial Isolates

One hundred seven isolates of *S. aureus* were recovered from pus, blood, tracheal aspirates, throat swab, urine and wound. These were collected from patients in different patient care areas including out-patient departments (OPDs), in-patient departments (IPDs) in tertiary care hospitals. We evaluated the *in vitro* activity of fifteen different antimicrobial agents against 84 MSSA and 23 MRSA of which 76 were collected from IPD and 31 from OPD.

Laboratory Methods

Specimens were screened by preliminary Gram's stain and were inoculated on 10% sheep blood agar (β-haemolysis) and Mannitol salt agar (mannitol fermentation). *S. aureus* was identified by standard microbiologic methods like catalase, slide and tube coagulase test. Susceptibility testing on *S.aureus* isolates was initiated on the automated identification and susceptibility

(ID/AST) system, Vitek 2 compact system (bioMerieux, France) in a clinical microbiology laboratory. Briefly, three to five colonies of 18-24 hours young culture was inoculated onto a gram positive panel (AST GP-67) containing 0.45% sodium chloride and placed into the Vitek instrument for incubation and reading. MICs were determined for each isolate and further evaluated by disc diffusion method using cifoxitin disc when Vitek revealed it as MRSA.

Interpretation of Susceptibility Data

Advanced expert system (AES) validates and interprets susceptibility test results in accordance with the CLSI guidelines. After antibiotic susceptibility testing as noted above, antibiogram patterns were determined by characterizing isolate susceptibilities to ciprofloxacin, levofloxacin, moxifloxacin, penicillin, vancomycin, quinpristin/dafloprístin, gentamycin, tetracycline, trimethoprim-sulfamethaxzole, erythromycin, refampicin, nitofurantoin, linezolid, Clindamycin and Oxacillin.

Statistical Analysis

Statistical analysis was carried out using WHONET software program. The MIC50 and MIC90 values (the minimum inhibitory concentration that can prevent bacterial growth by 50 and 90%, respectively) were calculated using W HONET

RESULTS

A total of 107 isolates of *S. aureus* were obtained from different clinical samples out of which 76 were obtained from IPD and 31 from OPD. Maximum isolates of *S. aureus* were from pus (69.1%), followed by blood (13.8%), throat swab (11.2%), urine (3.7%) and tracheal aspirates (2.8%). The analysis was done on the basis of patient location: whether outpatient and inpatient (non-ICU). Of the 107 *S. aureus* isolates tested, 21.3% from IPD and 25% from OPD were resistant to oxacillin.

Rate of antimicrobial resistance of MRSA and MSSA isolated from different wards

The resistance pattern of 76 isolates of *S. aureus* recovered from IPD was detailed in Table 1. These isolates were highly resistant to penicillin (91.9%) followed by trimethoprim-sulfamethaxzole (85.3%), ciprofloxacin (84.2%), levofloxacin (83.6%), moxifloxacin (60%) and erythromycin (46.7%). The less resistance rate was found against tetracycline (13.2%), gentamycin (11.1%), refampicin (2.7%) and clindamycin (1.4%). None of the isolate was resistant to vancomycin and linezolid, nitrofurantoin and quinpreistin/dafloprístin. Higher percentage of intermediate resistance was noted against antibiotics such as moxifloxacin (25.3%), levofloxacin (1.4%) and nitrofurantoin (1.3%). The resistance pattern of 31 isolates recovered from OPD were shown in Table 2. Majority of the isolate were resistant to penicillin (88.8%), ciprofloxacin (87.1%) and levofloxacin (87.1%). The resistance pattern of these isolates to trimethoprim-sulfamethaxzole, moxifloxacin and erythromycin were (71%), (48.3%) and (40%), respectively. Isolates were least resistant to gentamycin (7.1 %), refampicin (3.2%) and clindamycin (6.9%). Some isolates were intermediately resistant to moxifloxacin (37.9%) and erythromycin (10%) and all were sensitive to linezolid, nitrofurantoin, quinpristin/dafloprístin and vancomycin. Resistance of *S. aureus* against different antibiotics tested was higher for inpatient as opposed to outpatient isolates for many antibiotics as shown in Graph 1.Overall, Vancomycin MICs ranged from 0.5–2 µg/ml for both MSSA and MRSA. The number of *S. aureus* isolates that had a vancomycin MIC of ≤ 0.5 µg/ml was extremely high and all *S. aureus* isolates remained susceptible to vancomycin when breakpoints were applied according to CLSI guidelines (*S. aureus* isolates with vancomycin MICs of ≤2 µg/ml were considered as susceptible. Vancomycin Intermediate *S. aureus (*VISA) was considered as MICs of 4–8 µg/ml, and vancomycin resistant *S. aureus* (VRSA) as MICs of ≥16 µg/ml).

Table 1
Antibiotic sensitivity pattern of Staphylococcus aureus isolates recovered from Inpatient Department (IPD) (n=76)

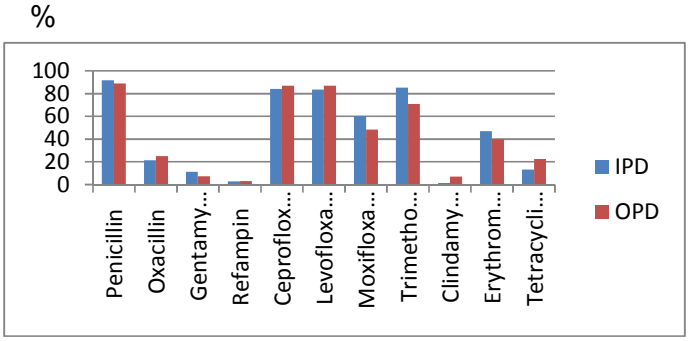
ANTIBIOTIC	R BREAKPOINT	NUMBER	R%	I%	S%	?%	%R95%CI	MIC 50 µg/ml	MIC 90 µg/ml	GEOM. MEAN	MIC RANGE µg/ml
Penicillin-G	S<=0.125 , R>0.25	62	91.9	0	8.1		81.4-97.0	0.5	0.5	0.403	0.03-0.5
Oxacillin	S<= 2 R>=4	75	21.3	0	78.7		13.0-32.6	0.25	4	0.529	0.25-4
Gentamycin	S<=4 R>=16	72	11.1	0	87.5	1.4	5.3-21.2	0.5	16	0.935	0.5-16
Refampicin	S<=1 R>= 4	73	2.7	0	97.3		0.5-10.4	0.5	0.5	0.555	0.125-32
Ceprofloxacin	S<=1 R>= 4	76	84.2	0	15.8		73.6-91.2	8	8	4.714	0.25-8
Levofloxacin	S<=1 R>= 4	73	83.6	1.4	15.1		72.7-90.9	4	8	2.859	0.12-8
Moxifloxacin	S<=0.5 R>=2	75	60	25.3	14.7		48.0-70.9	2	2	1.307	0.25-8
Trimethoprim	S<=2 R>= 4	75	85.3	0	0	14.7	74.8-92.1	192	384	91.896	10-320
Clindamycin	S<=0.5 R>=4	73	1.4	0	98.6		0.1-8.5	0.25	0.25	0.262	0.25-8
Erythromycin	S<=0.5 R>=8	75	46.7	8	45.3		35.2-58.5	1	8	1.408	0.25-8
Nitrofurantoin	S<=32 R>=128	75	0	1.3	98.7		0.0-6.1	32	32	24.477	16-64
Linezolid	S<= 4 R>= 8	74	0	0	100		0.0-6.1	2	2	1.945	01-002
Vancomycin	S<2 R>= 16	72	0	0	100		0.0-12.5	0.5	1	0.7	0.5- 1
Quinupristin/ Dafloprístin	S=1 R>= 4	75	0	0	98.7	1.3	0.0-6.1	0.25	0.25	0.257	001-16
Tetracycline	<=4 R>=16	76	13.2	0	86.8		6.9-23.4	1	16	1.453	0.12-0.25

R% = resistance percentage , I% = intermediate percentage, S% = Sensitive percentage, ?% = Error, 95% C.I = 95% Confidence Interval,Geom. Mean= Geometric mean, MIC = minimum inhibitory concentration

Table 2
Antibiotic sensitivity pattern of Staphylococcus aureus isolates recovered from outpatient Department (OPD) (n=31)

ANTIBIOTIC	R BREAKPOINT	NUMBER	%R	%I	%S	%?	%R95%CI	MIC 50 µg/ml	MIC 90 µg/ml	GEOM. MEAN	MIC RANGE µg/ml
Penicillin-G	S<=0.125 R>0.25	27	88.9		11.1		69.7-97.1	0.5	0.5	0.39	0.03-0.5
Oxacillin	S<= 2 R>=4	28	25		75		11.4-45.2	0.5	4	0.61	0.25-4
Gentamycin	S<=4 R>=16	28	7.1		92.9		12-24.9	0.5	4	0.8	0.5-16
Refampicin	S<=1 R>= 4	31	3.2		96.8		0.2-18.5	0.5	0.5	0.54	0.25-8
Ceprofloxacin	S<=1 R>= 4	31	87.1		12.9		69.2-95.8	8	8	4.89	0.5-8
Levofloxacin	S<=1 R>= 4	31	87.1		12.9		69.2-95.8	4	8	3.05	0.12-8
Moxifloxacin	S<=0.5 R>=2	29	48.3	37.9	13.8		29.9-67.1	1	2	1.15	0.25-2
Trimethoprim	S<=2 R>= 4	31	71		3.2	25.8	51.8-85.1	192	384	60.7	1-320
Clindamycin	S<=0.5 R>=4	29	6.9		93.1		1.2-24.2	0.25	0.25	0.32	0.25-8
Erythromycin	S<=0.5 R>=8	30	40	10	50		23.2-59.3	0.25	8	1.12	0.125-8
Nitrofurantoin	S<=32 R>=128	31	0		100		0.0-13.7	16	32	20.9	16-32
Linezolid	S<=4 R>=8	30	0		100		00.14.1	2	2	1.87	01-002
Vancomycin	S<=2 R>= 16	29	0		100		0.0-19.6	0.5	1	0.72	0.5- 1
Quinupristin/ Dafloprístin	S<=1 R>=4	30	0		100		0.0-14.1	0.25	0.25	0.25	0.25-0.25
Tetracycline	S<=4 R>=16	31	22.6		77.4		10.3-41.6	1	16	1.87	001-16

Graph 1
Comparison of resistance pattern of Staphylococcus aureus recovered from Inpatient and outpatient department.



DISCUSSION

Bacterial resistance threatens our ability to treat both common and serious infections. In many cases glycopeptides antibiotics such as vancomycine and teicoplanine are the only therapeutic alternatives and there is no doubt that glycopeptides resistance is expected to become an important problem in future[5,1]. Although new antibiotics can effectively treat some resistant pathogens and more research is needed to develop novel antimicrobials than also bacteria will eventually develop resistance to any antibiotic with time. The misuse and overuse of antibiotics drive the emergence and spread of resistance. Eliminating inappropriate antibiotic use and promoting more judicious use are essential parts of the solution. The Antibiogram can be used to select the optimal empiric therapy in an individual patient, as specific patient factors need to be considered, including the type and severity of infection and the patient's medical history and past antibiotic use. Binkley et al. (2006) compared a unit specific antibiogram versus that of the hospital wide antibiogram and found that the ICUs harboured organisms that were 5-25% more resistant than that otherwise predicted by the antibiogram[3]. Daxboeck et al., (2004) found that isolates from outpatients and inpatients showed lower resistance rates in contrast to isolates from ICU.[10].

Baddour et al. (2006) claimed the prevalence of MRSA to be 22.5% (115/512) from outpatients which corroborated well with our findings whereas higher prevalence rate of 77.5% (397/512) was recorded in case of inpatient as compared to our study (21.3%)[2]. Another study (Fluit et al., 2001) showed considerable differences when the distributions of MRSA isolates in IPD and OPD were compared [12]. Almost 38% of the *S. aureus* isolates from (ICUs) and 22.6% of the isolates from internal medicine wards were MRSA whereas 0% of the isolates from emergency rooms and 1% of the isolates from OPD were MRSA which is quite different from our findings [6]. In the present study, this high prevalence of MRSA in OPD suggests that there have been increasing reports of MRSA infections in the community and in the patients with and without risk factors for MRSA infection. This substantial increase in the prevalence of MRSA has increased the challenge of selecting empirical antimicrobial treatments in outpatient settings whereas 21.3% of MRSA in case of IPD partially reflects the fact that some patients, e.g. critically ill patients, have a greater chance of becoming colonized or infected. MRSA has become one of the most important nosocomial pathogens worldwide poses a major problem due to resistance to multiple antibiotics. Isolates were considered to be multidrug resistant when they displayed resistance to five (or more) of the following antibiotics: oxacillin, penicillin, erythromycin, clindamycin, gentamycin, ciprofloxacin, tetracycline and refampicin. MRSA is, by definition also resistant to β-lactam antibiotic. Thus all MRSA

isolates were resistant to at least two classes of antibiotics.

Ciprofloxacin, a fluorinated quinolone, has been very useful in treatment of *S. aureus* infections, especially those caused by MRSA[23,29]. Ciprofloxacin- resistant, MSSA strains and MRSA strains have been reported infrequently[16,22]. In 1989, Schaefler reported that 5.2% of 2,833 *S. aureus* isolates, most of which were MRSA, from hospitals and nursing homes in New York City had high level resistance to ciprofloxacin[28]. However, in the present study *S. aureus* was found to be 84.2% (IPD) and 87.2% (OPD)resistant to ciprofloxacin which is indifferent from previous findings. Blumberg et al. (1991) have shown this data to be as high as 70%. Plumood et al. (1996) from Vellore, India have shown that 90% of 1382 isolates were resistant to ciprofloxacin which is comparable to our findings. Studies have shown that 30% of *S. aureus* were found to be resistant to erythromycin which is almost same compared with our studies [24]. Isolates were classified as to whether they were acquired in the community or nosocomially. A community acquired MRSA isolate was defined as one isolated from a specimen obtained within 72 hours of admission. A nosocomially acquired isolate was one isolated from a specimen obtained beyond that time[14]. Community acquired MRSA infections can be a source for nosocomial MRSA. As more and more patients infected with the community acquired organism entered the hospital there was an increase in nosocomial *S.aureus* infections and specifically infections with methicillin resistant strains paralleled the increase in cases of the community acquired infection [26]. The principal mode of MRSA transmission within an institution is from patient to patient via hospital personnel who transiently acquire the organism after direct contact with infected patients or in certain settings, via inanimate environmental reservoirs[21]. The hospital infection control practices advisory committee (HICPAC) and communicable disease centre recommend that hospitalized children colonized or infected with MRSA should be kept in contact hospitalization.[27]. It is also recommended that patients with similar organisms should be kept either in single room or such patients should be cohorted [11]. In conclusion, knowledge about MRSA and carrier status needs to be raised among the health staff of the hospital and control measures need to be implemented consistently in order to reduce the burden of MRSA infection in the hospital environment.

CONCLUSION

To reduce the incidence of Staphylococcal infection, making antibiogram for monitoring the trends in antimicrobial resistance, the regular surveillance of hospital acquired infection, isolation nursing of patients who carry MRSA and the formulation of a definite antibiotic policy may be helpful. Furthermore, the future of antibiograms would be the incorporation of patient related data to make it more informative. The antibiogram could be useful in predicting outbreaks in a healthcare institution

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REFERENCES

1. Appelbaum PC. The emergence of vancomycin-intermediate and vancomycin resistant *Staphylococcus aureus*. Clin. Microbiol. Infect, 1:16-23, (2006).
2. Baddour MM, Abuelkheir MM, Fatani AJ. Trends in antibiotic susceptibility patterns and epidemiology of MRSA isolates from several hospitals in Riyadh, Saudi Arabia Ann. Clin. Microbiol. Antimicrob, 5: 30, (2006).
3. Binkley S, Fishman NO, Larosa LA, Marr AM et al. Comparison of unit-specific and hospital-wide antibiograms: potential implications for selection of empirical

antimicrobial therapy. Infect. Control Hosp. Epidemiol, 27: 682-687, (2006).
4. Blumberg HM, Rimland D, Carroll DJ, Terry P. Rapid development of ciprofloxacin resistance in methicillin susceptible and resistant *Staphylococcus aureus.* J. Infect. Dis, 163: 1278-1285, (1991).
5. Brown DF, Edwards DI, Hawkey PM et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin resistant *Staphylococcus aureus.* J. Antimicrob. Chemother, 56: 1000-1018, (2005).
6. Centres for Disease Control Prevention. Four pediatric deaths from community acquired methicillin resistant *Staphylococcus aureus* – Minnesota and North Dakota,1991-1999. JAMA, 282: 1123-1125, (1999).
7. Collopy BT, Dalton M, Wright C. Comparison of the clinical presentation of methicillin resistant and methicillin sensitive *Staphylococcus aureus*. Med. J. Australia, 140: 211-214, (1984).
8. Cormican MG and Jones RN. Emerging resistance to antimicrobial agents in gram-positive bacteria. Enterococci, Staphylococci and non pneumococcal streptococci. Drugs, 51: 6-12, (1996).
9. Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Susceptibility Testing; Twenty-First Informational Supplement. CLSI document M100-S21.Wayne, PA: Clinical and Laboratory Standards Institute; 2011: 30 -1 and 15, (2011).
10. Daxboeck F, Assadian O, Apfalter P. Resistance rates of *Staphylococcus aureus* in relation to patient status and type of specimen. J. Antimicrob. Chemother, 54: 163-167, (2004).
11. Fisher MC. Control of methicillin resistant *Staphylococcus aureus* and vancomycin resistant enterococcus in hospitalized children. Pediatr. Infect. Dis. J, 17: 823-826, (1998).
12. Fluit AC, Wielders CL, Verhoef J et al. Epidemiology and susceptibility of 3,051 *Staphylococcus aureus* isolates from 25 university hospitals participating in the European SENTRY study. J. Clin. Microbiol, 39: 3727-3732, (2001).
13. French GL, Cheng AF, Ling JM. Hong Kong strains of methicillin resistant and methicillin sensitive *Staphylococcus aureus* have similar virulence. J. Hosp. Infect, 15: 117-125, (1990).
14. Herold BC, Immergluck LC, Maranan MC et al. Community-acquired methicillin resistant *Staphylococcus aureus* in children with no identified predisposing risk. JAMA, 279: 593-597, (1998).
15. Hiramatsu K. Molecular evolution of MRSA. Microbiol. Immunol, 39: 531-543, (1995).
16. Humphreys H, Mulvihill E. Ciprofloxacin resistant *Staphylococcus aureus*. Lancet, 2: 383, (1985).
17. Joshi S. Hospital Antibiogram : a necessity. Ind. J. med. Microbial, 28: 277-280, (2010).
18. Klein E, Smith DL, Laxminarayan R. Hospitalizations and deaths caused by methicillin-resistant *Staphylococcus aureus*, United States, 1999–2005. Emerg. Infect. Dis, 13: 1840–1846, (2007).
19. Kluytmans JA, Verbrush HA, Mounton JW. Nasal carriage of *S. aureus* as a major risk factor for wound infection after cardiac surgery. J. Infect. Dis, 171: 216-219, (1995).
20. Lotus D, Saravolatz MD, Donald J et al. Community acquired Methicillin resistant *S. aureus* infection : a new source for Nosocomial Outbreaks. Ann. Intern. Med, 97: 325-329, (1982).
21. Mulligan ME, Murray-Leisure KA, Standiford HC et al. Methicillin resistant *Staphylococcus aureus*–Consensus review of microbiology, pathogenesis and epidemiology with implications for prevention and management. Am. J. Med, 94: 313-328, (1993).
22. Mulligam ME, Ruane PJ, Johnston L et al. Ciprofloxacin for eradication of methicillin-resistant *Staphylococcus aureus* colonization. Am. J. Med, 82(Suppl.4A): 215-219, (1987).
23. Neu HC. Ciprofloxacin: an overview and prospective appraisal. Am. J. Med, (Suppl. 5A): 12-16, (1987).

24. Pal N, Ayyagari A. Drug resistance pattern of methicillin resistant *Staphylococcus aureus*. Indian Paediatr, 1991; 28: 725-729, (1991).
25. Pulmood TB, Lalitha MK, Jesudason MV, Pandian R, Selwayn J, John TJ (1996). The spectrum of antimicrobial resistance among methicillin resistant *Staphylococcus aureus* (MRSA) in a tertiary care center in India. Indian J. Med. Res, 103: 212-215.
26. Saravolatz LD, Pohlod DJ, Arking LM. Community-acquired MRSA infections: a new source of nosocomial outbreaks. Ann. Intern. Med, 97: 325-329, (1982).
27. Rosenberg J. Methicillin resistant *Staphylococcus aureus* (MRSA) in the community: Who's watching. Lancet, 346: 132-133, (1995).
28. Schaefler S, Jones D, Perry T et al. Methicillin-resistant strains of *Staphylococcus aureus* resistant to quinolones York City hospitals: inter-hospital spread of resistant strains of type 88. J. Clin. Microbiol, 27: 335-336, (1984).
29. Smith SM, Berman E. The effect of ciprofloxacin on methicillin resistant *Staphylococcus aureus*. J. Antimicrob. Chemother, 17: 287-295, (1987).
30. Sugg AH, Maranan MC, Varam SB et al. Methicillin resistant and borderline resistant asymptomatic *Staphylococcus aureus* colonization in children without identifiable risk factors. Pediatr. Infect. Dis. J, 18: 410-414, (1999).
31. Wenzel RP, Osterman CA, Hunting KJ et al. Hospital-acquired infections: surveillance in a university hospital. Am. J. Epidemiol, 103: 251-260, (1976).
32. Winstein RA, Kabins SA. Strategies for prevention and control of multiple drug resistant nosocomial infection. Am. J. Med, 70: 49-54, (1981).
33. Yzerman E. APACH for predicting course and outcome of nosocomial *S. aureus* bacteremia and its relation to host defence. J. Infect. Dis, 173: 914-919, (1996).