Original Research Article

Study the antimicrobial agents sensitivity of methicillin resistant *Staphylococcus aureus* isolated from patients admitted in RIMS, Ranchi

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes. For this, study of MRSA isolated from admitted patients were carried out. These strains were separately tested for their sensitivity to different antibiotics to know which group of antibiotics are most effective particularly for cases of RIMS, Ranchi. **Material & Methods:** The present study was carried out in the Department of Microbiology, Rajendra Institute of Medical Sciences (RIMS), Ranchi clinical isolates of MRSA strains were obtained from admitted patients of RIMS, Ranchi. The sources of isolate included pus from infected surgical wounds, infected burn wounds, conjunctival swab, aural swab, throat swab, vaginal swab, urine etc for microbiological analysis and antimicrobial sensitivity of MRSA. Disc diffusion method was employed. **Results:** All the 264 cases of staphylococcal species isolated from different clinical specimens were subjected to coagulase test. It was observed that out of 264 strains of staphylococci isolated from different sites 165 strains (62.5%) were coagulase positive and 99 strains (37.5%) were coagulase negative by tube method. It was observed that out of 165 strains of staph. aureus isolated from different clinical samples 64 strains of staph. aureus were resistant to methicillin (38.78%). Maximum isolation of MRSA were from pus 38 (51.35%), followed by throat swab 19 (36.36%), aural swab (14.28%) and conjunctival swab (44.44%). It was observed that out of 165 strains of s. aureus isolated only 64 strains were resistant to methicillin. All strains of MRSA were 100% sensitive to Vancomycin & linezolid. Similarly 92.3% were sensitive to netilmicin, 89.7% to clindamycin, 82.1% to ciprofloxacin, 74.4% to cephotaxime, 69.2% to azithromycin, 56.4% to roxithromycin & clarithromycin, 17.9% to piperacillin/tazobactam. The most effective antibiotic against MRSA was vancomycin, linezolid, netilmicin & clindamycin. **Conclusion:** After comparing the effectiveness of antibiotics against MRSA infection it can be concluded that piperacillin/tazobactam, clarithromycin, roxithromycin azithromycin, cefotaxime & ciprofloxacin are of little value in treating the MRSA infection. They should not be used indiscriminately and in a haphazard manner otherwise increment in emergence of resistant strains may not be checked.

Keywords: Staphylococci, methicillin resistant staphylococcus aureus (MRSA), disc diffusion method,antimicrobial sensitivity.

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Introduction

Methicillin, the first semi-synthetic penicillin derivative resistant to hydrolysis by staphylococcal β-lactamase, was introduced into clinical use for the treatment of infections caused by penicillin-resistant ***Staphylococcus aureus*** in 1960. In 1961 there were reports from the United Kingdom of *S. aureus* isolates that had acquired resistance to methicillin (methicillin-resistant *S. aureus*, MRSA) [1], and MRSA isolates were soon recovered from other European countries, and later from Japan, Australia, and the United States. MRSA is now a problem in hospitals worldwide and is increasingly recovered from nursing homes and the community[2]. The methicillin resistance gene (*mecA*) encodes a methicillin-resistant penicillin-binding protein that is not present in susceptible strains and is believed to have been acquired from a distantly related species [3,4].Isolates of EMRSA-15 and -16 are commonly resistant to erythromycin and ciprofloxacin in addition to β-lactams, and a study at one affected hospital showed a temporal relationship between the rates of MRSA infection and the use of macrolides, third-generation cephalosporins and fluoroquinolones, suggesting that the use of antimicrobials to which an outbreak strain is resistant is an important contributory factor for the persistence of that strain [5]. At present, healthcare-associated methicillin-resistant *S. aureus* (HA-MRSA) is associated with significant mortality and morbidity (longer hospital stays) and imposes a serious economic burden on scarce healthcare resources worldwide compared to methicillin-sensitive *S. aureus* (MSSA) [6].The genetic basis of methicillin resistance in *S. aureus* is associated with carriage of a mobile cassette of genes known as the staphylococcal cassette chromosome mec (SCCmec) [7, 8].

Within this cassette is the mecA **gene that is responsible for resistance to β-lactams including** methicillin. The product of **mecA** is the peptidoglycan synthesis enzyme penicillin binding protein (PBP) 2a involved in cross-linking of peptidoglycan in the bacterial cell wall PBP2a has a lower binding affinity for β-lactam antibiotics than the native PBP proteins encoded in the core genome of *S. aureus*. The subsequent combination of reduced penicillin-binding affinity and increased production of PBP2a accounts for the observed resistance to β-lactam antibiotics [9]. In India, limited information exists on prevalence and drug susceptibility patterns of methicillin-resistant *S. aureus* isolated from clinical samples. The incidence of MRSA varies from 25 per cent in western part of India to 50 per cent in South India. Community acquired MRSA (CA-MRSA) has been increasingly reported from India [10]. Since methicillin resistant staphylococcus aureus strains are resistant to multiple antibiotics, there is possibility of extensive outbreaks which may be difficult to control. Early detection of methicillin resistant staphylococcus is important from patients and hospitals point of view. So knowledge of methicillin resistant staphylococcus aureus strain and their antimicrobial profile is necessary in selection of appropriate treatment for methicillin resistant staphylococcus aureus infection. The main objective of this study was therefore to determine the prevalence of MRSA in patients from selected hospitals in RIMS, Ranchi. An evaluation of the susceptibility patterns of *S. aureus* isolates from the selected specimen to specific antibiotics was also undertaken.

Material and Methods

The present study was carried out in the department of microbiology, RIMS, Ranchi clinical isolates of methicillin resistant staphylococcus aureus strains were obtained from admitted patients of RIMS, Ranchi. The sources of isolate included pus from infected surgical wounds, infected burn wounds, conjunctival swab, aural swab, throat swab, vaginal swab, urine etc. The patients were at first explained the object of the study and the method of obtaining the specimen so that their full co-operation could be obtained and written informed consent were taken.

Collection of Specimen

Pus, conjuctival, aural throat and vaginal swab were collected by means of sterile cotton swab sticks. The sterile cotton swab sticks were moistened with normal saline and rubbed over the infected area taking care not to touch anything outside so as to prevent contamination. Swabs were then aseptically replaced in sterilized test tubes to avoid drying of the material. Efforts were made to inoculate the specimen within two hours of collection. Primary inoculation was done on blood agar. The plates after inoculation were incubated at 37^0C for 24 hours. Midstream samples of urine were received in a sterilized vial and inoculated on MacConkey agar. The plates were incubated at 37^0C for 24 hours.

Test for Determining Methicillin Resistance

Methicillin resistant testing was performed by Kirby Bauer's disc diffusion method using methicillin (5μg) or oxacillin (1μg) disc. The suspensions for inoculation were prepared from isolated colonies from an overnight

growth on nutrient agar plates[11-14]. The growth was suspended in 0.5ml of sterile saline. A sterile swab was dipped into this suspension and excess if inoculum were removed by pressing it against the sides of the tube. These swabs were used to inoculate one quarter of a Mueller Hinton agar plate supplemented with 5% sodium chloride. Methicillin or oxacillin disc were applied within 15 min after inoculation. The plates were incubated at 35^0C for 24 hours. The diameter of the clear zone around the disc was measured and result interpreted as susceptible, moderately susceptible or resistant as per recommendations[15-19]Zone of inhibition less than 10 mm or any growth within the zone of inhibition were indicative of methicillin resistance. There are three conventional susceptibility testing methods like broth dilution, agar dilution and disc diffusion method. In this study disc diffusion method using commercially available discs were used. Lawn cultures were prepared by flooding the surface of the plate with a broth culture of the bacterium. Excess inoculum was pipette off. Antibiotic discs were placed on the inoculated plates by a fine pointed pair of forceps (alcohol flamed and cooled). The discs were firmly pressed onto the agar to ensure complete contact. The discs were distributed so that they were no closer than 15mm from the edge of the Petri dish and no two discs were closer than 24 mm from centre to centre. Plates were placed in the incubator within 15 minutes after placing the discs and incubated at 37^0C for 16-18 hours. Following incubation, the diameters of the zones of inhibition were measured (including the 6mm diameter of the disc itself) by a ruler. Results were seen according to the zone of inhibition (In accordance to performance standards for antimicrobial susceptibility Tests, NCCLS) [20,21].

Results

Table 1: Number of isolation of staphylococcus sp. from different clinical specimens

Specimens	Number
Pus & wound	84
Throat swab	78
Aural swab	39
Conjunctival swab	32
Urine	18
Vaginal swab	13

Table 2: Results of coagulase test of 264 strains of staphylococci isolated from different clinical specimens

	Coagulase +VE staph.	Coagulase -Ve staph.
Pus & wound	74	1 0
Throat swab	55	23
Aural swab	21	1 8
Conjunctival swab	9	23
Urine	2	16
Vaginal swab	4	9

All the 264 cases of staphylococcal species isolated from different clinical specimens were subjected to coagulase test [Table 1]. It was observed that out of 264 strains of staphylococci isolated from different sites 165 strains (62.5%) were coagulase positive and 99 strains (37.5%) were coagulase negative by tube method. Out of the 165 strains of coagulase positive staphylococci maximum isolation was obtained from pus 74 followed by throat swab 55, aural swab 21, vaginal 4, conjunctival swab 9 and urine 2 [Table 2].

Table 3: Showing drug resistance pattern of MRSA isolated from clinical specimens

Antimicrobial agent (S)	% Susceptible	% Intermediate	% Resistance
Netilmicin	92.3	2.6	5.1
Vancomycin	100	-	-
Clindamycin	89.7	-	10.3
Linezolid	100	-	-

Piperacillin/ Tazobactam	17.9	10.3	71.8
Roxithromycin	56.4	23.1	20.5
Cephotaxime	74.4	7.7	17.9
Ciprofloxacin	82.1	5.1	12.8
Azithromycin	69.2	7.7	23.1
Clarithromycin	56.4	23.1	20.5

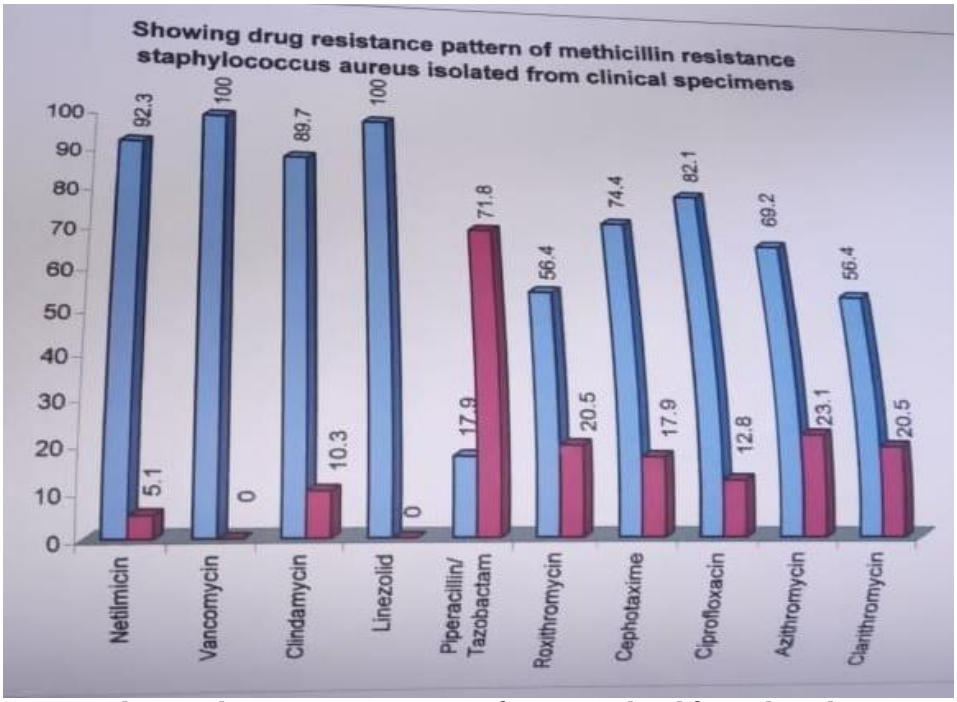

Figure 1: Showing drug resistance pattern of MRSA isolated from clinical specimens

Table 4: Susceptibility of clinical isolates of MRSA from pus (n=38)

Antimicrobial agent (S)	% Susceptible	% Resistance
Netilmicin	95.7	4.3
Vancomycin	100	-
Clindamycin	90.5	9.5
Linezolid	100	-
Piperacillin/ Tazobactam	20.2	79.80
Roxithromycin	50.8	49.2
Cephotaxime	71.1	28.9
Ciprofloxacin	79.9	20.1
Azithromycin	63.2	36.8
Clarithromycin	50.8	49.2

Table 5: Susceptibility of clinical isolates of MRSA from throat swab (n=19)

Antimicrobial agent (S)	% Susceptible	% Resistance
Netilmicin	97.4	2.6
Vancomycin	100	-
Clindamycin	92.7	7.3
Linezolid	100	-

Piperacillin/ Tazobactam	23.2	76.8
Roxithromycin	48.5	51.5
Cephotaxime	72	28
Ciprofloxacin	80.1	19.9
Azithromycin	59.3	40.7
Clarithromycin	48.5	51.5

Table 6: Susceptibility of clinical isolates of MRSA from conjunctival swab (n=4)

Antimicrobial agent (S)	% Susceptible	% Resistance
Netilmicin	62	38
Vancomycin	100	-
Clindamycin	97.6	2.4
Linezolid	100	-
Piperacillin/ Tazobactam	6.8	93.2
Roxithromycin	14.8	85.2
Cephotaxime	38	62
Ciprofloxacin	43.2	56.8
Azithromycin	19.9	80.1
Clarithromycin	14.8	85.2

Maximum isolation of MRSA were from pus 38 (51.35%), followed by throat swab 19 (36.36%), aural swab (14.28%) and conjunctival swab (44.44%). It was observed that out of 165 strains of staph. aureus isolated only 64 strains were resistant to methicillin. All strains of MRSA were 100% sensitive to vancomycin & linezolid. Similarly 92.3% were sensitive to netilmicin, 89.7% to clindamycin, 82.1% to ciprofloxacin, 74.4% to cephotaxime, 69.2% to azithromycin, 56.4% to roxithromycin & clarithromycin, 17.9% to piperacillin/tazobactam. The most effective antibiotic against MRSA was vancomycin, linezolid, netilmicin & clindamycin. In the present study all 64 strains of MRSA showed 100% sensitivity to vancomycin & linezolid, followed by 92.3% to netilmicin and 89.7% to clindamycin. All MRSA strains were 71.8% resistant to piperacillin/tazobactam, followed by 23.1% to azithromycin, 20.5% to clarithromycin and roxithromycin, 17.9% to cephotaxime & 12.8% to ciprofloxacin [Table 3-6/Fig.1].

Discussion

The present work is "study of methicillin resistance staphylococcus aureus isolated from patient admitted in RIMS and testing their sensitivity to antimicrobial drugs". Samples were collected from different sources such as pus, throat, ear, conjunctiva, vagina, urine etc. The pathogenic strains of staphylococcus were studied for their resistance to methicillin on Mueller Hinton agar supplemented with 5 percent sodium chloride using oxacillin or methicillin disc. Recent sensitivity pattern of methicillin resistance staphylococcus aureus was studied against the available newer antibiotics. So knowledge of the methicillin resistant staphylococcus aureus strains and their sensitivity pattern will help in proper treatment of such patients. In the present study, 264 strains of staphylococci isolated from different clinical samples were subjected to coagulase test. Out of which 165 strains (62.5%) were coagulase positive staphylococci. Study of coagulase positive staphylococci is being compared here. From the above observation, it is apparent that in the present study (165 strains (62.5%) produced coagulase enzyme and remaining 99 strains (37.5%) were coagulase negative by tube method 160 strains (60.6%) were coagulase positive by slide method). This figure correlated will with the positive staphylocci and 39.64% coagulase negative staphylococci were observed.

Table 7:Study of coagulase positive staphylococcus aureus in different clinical samples

Year	1999	2008	2009
Specimen	Deepak et al [13]	Anuradha et al [14]	Present study
Pus	88.19%	72%	88%
Throat Swab	70.5%	73.12%	70%
Aural Swab	78%	55.56%	54%

Conjunctival Swab	33%	27.27%	28%
Urine	12.5%	15.6%	11%
Vaginal Swab	33%	28.57%	31%

In the present study, the rate of occurrence of staph. aureus in pus was (88%), in urine (11%) and in vaginal swab (31%) this figure correlated will with the study of pathogenic staphylococci by Deepak et al (1999) [13] showing rate of occurrence of staph. aureus in pus (88.19%), in urine (12.5%) and in vaginal swab (33%). It was observed that out of 264 strains of staphylococci isolated from different clinical samples, 165 strains of staphylococci were coagulase positive (62.5%) and 99 strains (37.5%) were coagulase negative. Out of 165 strains of staph. aureus isolates, 64 strains of staph. aureus were resistant to methicillin (38.78%).

Table 8: high incidence of MRSA among *Staph. aureus*

Year	Author	Incidence of MRSA
1998	Mehta et al [15]	31.8%-36.5%
1997	C. Udaya Shanker [16]	20%
2001	Majumdar et al [17]	23.6%
2003	Anuradha et al [14]	54.8%
2004	Quereshi [18]	35.3%
2006	Rajadurai pandi [19]	31.1%
2006	Srinivasan [20]	33.3%
2009	Present study	38.78%

The above study correlated well with the study of Mehta et al [15] who observed incidence of MRSA to range from 31.8% to 36.5%, followed by Quereshi et al [18] who observed incidence of MRSA to be 35.3%, and study of Srinivasan [20] who observed incidence of MRSA to be 33.3%. In the present study, maximum isolation of MRSA were from pus (51.35%) which correlated well with study of Anuradha et al [14] showing MRSA isolation in pus (52.5%) followed by study of Rajadurai pandi et al [19] who observed MRSA isolation in pus (33.6%). In the present study, isolation of MRSA from throat swab were (36.36%) which correlated well with study of Rajadurai pandi [19] showing MRSA isolation in throat swab (35.7%) followed by study of Mehta [15] who observed MRSA isolation in throat swab (28.36%). In the present study, isolation on MRSA from conjunctival swab were (44.44%) which correlated well with study of Rajadurai pandi [19] showing isolation of MRSA from conjunctival swab (40%). In the present study, isolation of MRSA from aural swab were (14.28%) which correlated well with study of Rajadurai pandi [19] showing isolation of MRSA from aural swab (14%). Study by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India showed that antibiotic susceptibility testing data for erythromycin, clindamycin, co-trimoxazole, genta-micin, vancomycin and linezolid were compiled.

There was no resistance documented against vancomycin and linezolid. Resistance to antibiotics amongst the MRSA isolates was more than that in methicillin sensitive **S. aureus** (MSSA) (**P**<0.001) [10].

Antibiotic Sensitivity Pattern of MRSA

In the present work out of 165 strains of pathogenic staphylococci isolated form different clinical samples 64 strains of staph. aureus were resistant to methicillin. These 64 strains of MRSA were studied for their susceptibility to following drugs - netilmicin, vancomycin,clindamycin,linezolid,piperacillin/tazobactam, roxithromycin, cephotaxime, ciprofloxacin, azithromycin and clarithromycin.

Table 9: Comparative study of sensitivity pattern of MRSA by various workers

	Netilmicin			Vancomycin			Clindamycin			Linezolid			Piperacillin/ Tazobactam			Roxithromycin			Cephotaxime			Ciprofloxacin			Azithromycin			Clarithromycin		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
C. Udaya Shankar et al 1997																						95.80%								
Mehta et al 1998	57%		43%	100%																		32%		68%						
Majumder et al 2004																								22.80%						
Anupurba et al 2003	52.50%		47.50%	100%																		15.90%		84.10%						
Srinivasan et al 2006				100%			94.60%		5.40%	100%																				
Rajadurai Pandi et al 2006	92.30%		7.60%	100%						100%									74.40%		25.60%	82.10%		17.90%						
Qureshi et al 2009				100%			34.70%		31.80%	100%												65%		35.40%						
Present Work	92.30%	2.60%	5.10%	100%			89.70%		10.30%	100%			17.90%		71.80%	56.40%		20.50%	74.40%		17.90%	82.10%		12.80%	69.20%		23.10%	56.40%		20.50%

In the present study all 64 strains of MRSA showed 100% sensitivity to vancomycin & linezolid, followed by 92.3% to netilmicin and 89.7% to clindamycin. All MRSA strains were 71.8% resistant to piperacillin/ tazobactam, followed by 23.1% to azithromycin, 20.5% to clarithromycin and roxithromycin, 17.9% to cephotaxime & 12.8% to ciprofloxacin. In the present study all MRSA strains showed 100% sensitivity to vancomycin and linezolid which correlated well with the study of Mehta et al (1996) [15] followed by Anupurba et al (2003) [21],Rajaduraipandi (2006) [19] and Qureshi et al (2004)[18] showing 100% sensitivity to above drugs [Table 9].In the present study MRSA strains showed 92.3% sensitivity to netilmicin which correlated well with the study of Rajaduraipandi [19] showing 92% sensitivity to netilmicin, followed by study of Mehta et al showing 57% sensitivity to netilmicin and study of Anupurba et al [21] showing 52.5% sensitivity to netilmicin. In the present study all MRSA strains showed 89.7% sensitivity to clindamycin which correlates well with the study of Srinivasan et al [20] who observed 94.6% sensitivity to clindamycin. In the present study MRSA strains showed 82.1% sensitivity to ciprofloxacin which correlated well with the study of C. Udayashankar [16] showing 95.8% sensitivity to ciprofloxacin, followed by study of Rajaduraipandi [18] showing 82% sensitivity to ciprofloxacin. In the present study MRSA strains showed 74.4% sensitivity to cephotaxime which correlated well with the study of Rajaduraipandi [19] showing 74% sensitivity to cephotaxime. In the present study all MRSA strains showed 17.9% sensitivity to piperacillin/tazobactam, followed 56.4% to roxithromycin, 69.2% to azithromycin and 56.4% to clarithromycin. From the above discussions it is clear that most potent antistaphylococcal agent used in MRSA is vancomycin and linezolid. Though clindamycin, netilmicin, ciprofloxacin and cephotaxime is also effective. Piperacillin/tazobactam, roxithromycin, azithromycin & clarithromycin are less effective in cases of MRSA. Unscientific and random use of antibiotics has led to emergence of resistant strains of pathogenic staphylococci to multiple antibiotics commonly used in the hospital. So, for the early recovery of the patients, the easiest way is to know the most virulent strains of staphylococci occurring in the hospital. For this, ideal way is to do the bacteriophage typing, there by knowing which phage type is most frequent. Since phage typing is not possible in most of the institution in our country hence isolated stains are subjected for a relative study of the pathogenecity. Later on sensitivity test of the strains to commonly used antibiotic in the

hospital is done. Thus the most effective antibiotic against MRSA is vancomycin. Here in this study we see that vancomycin is most potent and effective drug against MRSA and sensitive to 100% strains but as the drug is costly and associated with toxicity it is out of reach for the poor people who come to government hospital. So, use of vancomycin is limited to the treatment of serious life threatening MRSA infection. As an alternative to vancomycin, linezolid, netilmicin, clindamycin, ciprofloxacin & cephotaxime can be used for treating MRSA infection. Thus from foregoing discussions it is obvious that in the treatment of MRSA infection the proper way is to have the sensitivity test and then to give antibiotics. Vancomycin should be given only when other antibiotics have proven to be ineffective to a great extent.

Conclusion

It was observed that out of 165 strains of s. aureus isolated only 64 strains were resistant to methicillin. All strains of MRSA were 100% sensitive to vancomycin & linezolid. Similarly 92.3% were sensitive to netilmicin, 89.7% to clindamycin, 82.1% to ciprofloxacin, 74.4% to cephotaxime, 69.2% to azithromycin, 56.4% to roxithromycin & clarithromycin, 17.9% to Piperacillin/Tazobactam. The most effective antibiotic against MRSA was vancomycin, linezolid, netilmicin & clindamycin. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been identified as one of the major risk pathogens associated with the development of antimicrobial resistance (AMR). The emergence of AMR in **S. aureus** is well documented and the species has proven particularly adept at evolving resistance in the face of new antibiotic challenges.

References

1. Jevons M P. To-day's Drugs. Br Med J 1961;1:124–125.
2. Hussain F M, Boyle-Vavra S, Bethel C D, Daum R S. Current trends in community-acquired methicillin-resistant Staphylococcus aureus at a tertiary care pediatric facility. Pediatr Infect Dis J 2000; 19:1163–1166.
3. Hiramatsu K, Cui L, Kuroda M, Ito T. The emergence and evolution of methicillin-resistant *Staphylococcus aureus*. Trends Microbiol 2001;9:486–493.
4. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H, Spratt BG. The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). Proceedings of the National Academy of Sciences. 2002; 99 (11):7687-7692.
5. Monnet DL, MacKenzie FM, López-Lozano JM, et al. Antimicrobial drug use and methicillin-resistant *Staphylococcus aureus*, Aberdeen, 1996-2000. Emerg Infect Dis. 2004;10(8):1432-1441.
6. Kong EF, Johnson JK, Jabra-Rizk MA. Community-associated methicillin-resistant *Staphylococcus aureus*: an enemy amidst us. PLOS Pathogens 2016;12(10):e1005837.
7. Hartman BJ, Tomasz A. Low-affinity penicillin-binding protein associated with beta-lactam resistance in *Staphylococcus aureus*. J Bacteriol. 1984;158:513–6.
8. Matthews P, Tomasz A. Insertional inactivation of the *mec* gene in a transposon mutant of a methicillin-resistant clinical isolate of *Staphylococcus aureus*. Antimicrob Agents Chemother. 1990;34:1777–9.
9. Harkins CP, Pichon B, Doumith M, et al. Methicillin-resistant *Staphylococcus aureus* emerged long before the introduction of methicillin into clinical practice. Genome Biol. 2017;18(1):130.
10. Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India. Methicillin resistant *Staphylococcus aureus* (MRSA) in India: prevalence & susceptibility pattern. Indian J Med Res. 2013;137(2):363-369.
11. Saffari N, Salmanzadeh-Ahrabi S, Abdi-Ali A, Rezaei-Hemami M. A comparison of antibiotic disks from different sources on Quicolor and Mueller-Hinton agar media in evaluation of antibacterial susceptibility testing. Iran J Microbiol. 2016;8(5):307-311.
12. Bauer AW, Kirby WM, Sherris JC, Turck M. Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol 1966;45: 493– 496 .
13. Deepak S, Samant SA, Urhekar AD. Study of coagulase positive and negative Staphylococci in clinical samples. Indian J Med Sci 1999;53:425-8.
14. Rajput A, Singh KP, Kumar V, Sexena R, Singh RK. Antibacterial resistance pattern of aerobic bacteria isolates from burn patients in tertiary care hospital. Biomedical research. 2008;19(1):1-4.
15. Mehta AA, Rodrigues CC, Kumar RR, Rattan AR, Sridhar HH, Mattoo VV, Ginde VV. A pilot programme of MRSA surveillance in India. (MRSA Surveillance study Group). J Post Grad Med 1996; 42(1):1-3
16. Udaya Shanker C, Harish BN, Navneeth BV. Prevalence of methicillin-resistant Staphylococcus

aureus. Indian J Med Microbiol, 1997; 15: 137-138

17. Majumder D, Bordoloi JS, Phukan AC, Mahanta J. Antimicrobial susceptibility pattern among methicillin resistant staphylococcus isolates in Assam. Indian J Med Microbiol. 2001;19:138–40.

18. Quereshi AH, Rafi S, Qureshi SM, Ali AM. The current susceptibility patterns of methicillin resistant Staphylococcus aureus to conventional anti Staphylococcus antimicrobials at Rawalpindi. Pak J Med Sci 2004; 20: 361-64.

19. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan M. Prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus: A multicentre study. Indian J Med Microbiol. 2006;24:34–8.

20. Srinivasan S, Sheela D, Shashikala, Mathew R, Bazroy J, Kanungo R. Risk factors and associated problems in the management of infections with methicillin resistant Staphylococcus aureus. Indian J Med Microbiol. 2006;24:182–5.

21. Anupurba S, Sen MR, Nath G, Sharma BM, Gulati AK, Mohapatra TM. Prevalence of methicillin resistant Staphylococcus aureus in a Tertiary care Referral Hospital in Eastern Utter Pradesh. IJMM 2003; 21:49-51.

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