Research Article

Emergence of Methicillin Resistant *Staphylococcus aureus* Strains (MRSA) and Multi Drug Resistant (MDR) Strains in Himachal Pradesh

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ABSTRACT

Emergence of Multi drug resistant strains of *Staphylococcus aureus* infections in clinical cases has posed problem in treating such cases and is of great public health concern. The hospital acquired (nosocomial) infections and community acquired *Staph. aureus* infections are on the increase and thus, gaining marked clinical significance. Methicillin resistant *Staphylococcus aureus* (MRSA) strains, which were previously susceptible to vancomycin, have been reported resistant to this antibiotic as well as to other antibiotics used to treat *Staph. aureus* infections. The present study aims at determining the *in vitro* cultural sensitivity of *S.aureus* isolates that originated from urine, blood, pus and catheter associated (CA) infections in patients at Indira Gandhi Medical College (IGMC), Shimla, Himachal Pradesh to the commonly prescribed antibiotics by the clinicians to treat infections due to *Staph. aureus*. "First Catch" isolates recovered by the Department of Microbiology of the Medical College, Shimla were confirmed as *Staph. aureus*. Their identity was established on the basis of their cultural characteristics on Mannitol salt agar medium and other media, Gram's Staining, morphology and biochemical reactions. The isolates were then subjected to *in vitro* antibiotic cultural sensitivity assay by disc diffusion technique using standard antibiotic discs (Hi-midia-IC-002).A total of 135 isolates were confirmed as *Staph. aureus* out of 150 isolates examined. The detail of origin of these strains is as follows: pus (93), urine (21), blood (23) and CA (07). Of these, 62(45.0%) were resistant to Methicillin i.e. (MRSA) strains. These strains were also found resistant to other antibiotics such as clarithromycin, teicoplanin, novobiocin, vancomycin, tetracycline, erythromycin, penicillin, chloramphenicol, linezoid, azithromycin, amoxyclav, ampicillin etc. However, sensitivity to ofloxacin, cephalothin, clindymicin, co-trimozole, gentamicin, oxacillin, amikacin was observed. The study thus, shows the emergence of MRSA strains in the state of Himachal Pradesh and their increased resistance to other antibiotics (MDR) strains. Also, (53.33%) strains were recorded as vancomycin resistant.

Keywords: Antibiotics, Methicillin resistant *Staph. aureus* (MRSA) strains, Multi-drug resistant (MDR) isolates, *Staphylococcus aureus*.

INTRODUCTION

Infections caused by community-associated (CA) and nosocomial methicillin-resistant *Staphylococcus aureus* (MRSA) have become a major public health threat in the developed nations.[1] In developing countries however, the staphylococcal diseases are perceived as trivial in terms of morbidity and mortality in comparison to other infectious diseases such as malaria, tuberculosis and HIV infections. Less attention has therefore been given to *Staph. aureus* in the developing world keeping view the low rates of infections due to this organism.[2] *Staph. aureus* colonizes in healthy individuals and causes several infections in hospitalized patients. These infections are usually treated with β-lactam and related group of antibiotics but due to development of resistance to penicillins, Methicillin resistant and MDR strains have emerged which pose serious therapeutic challenges in the control of these infections.[3,4] The surveillance of different strains circulating in the community as well as among hospital patients and characterization of such strains, therefore, constitutes an important area of investigations in understanding the mechanisms of drug resistance.[5] Since the MRSA and MDR strains have led to the failure of conventional therapy, the knowledge of their prevalence, their antimicrobial susceptibility pattern and molecular characterization become essential for managing such infections. The present study is an attempt in this direction so far as this region of the country is concerned.

MATERIALS AND METHODS

Collection and confirmation of clinical isolates of *Staph. aureus*.

A total of 150 isolates recovered from suspected cases of *Staph. aureus* infections by Himachal Pradesh (H.P.) were collected in batches over a period of 15 months i.e. during January 2011 to April, 2012. The material in transport medium was brought on ice to the clinical diagnostic laboratory of the Department of Microbiology at Shoolini University, Bajhol, Solan (H.P.) for further studies. The research proposal was cleared by the Institute Ethics Committee (IEC) of the university as communicated through letter no. (SUIEC/12/03 dated 19-03-2012).

On arrival in the laboratory, the bacterial isolates were cultured on selective media i.e. Mannitol salt agar (Hi-media, Mumbai), incubated at $37°C$ overnight. The organisms were indentified on the basis of colony characteristics, pigment production (golden yellow), Gram's staining, hemolysis on blood agar and other biochemical tests such as, Indole, MR-VP, Mannitol fermentation, coagulase, catalase and oxidase tests.[6]

In Vitro antibiotic culture sensitivity assay

The culture of *Staph. aureus* (each isolates containing about 10^6cfu/ml) were spread uniformly on Mueller Hinton Agar (Hi-Media, Mumbai).[7] The standard antibiotics discs were then aseptically placed on bacterial lawns in the plates with the help of forceps and allowed to stand for one hour. The plates were then incubated at 37°C for 24hours.The activity of each antibiotic against different strains was determined by measuring the diameter of the zone of inhibition around each disc as per standard protocol of NCCLS. (M7-44).[8]

Screening of MRSA strains by MRSA detection Kit

In addition to the resistance observed against methicillin by *in vitro* antibiotic culture sensitivity assay, screening of MRSA strains was done with MRSA identification Kit (Hi-media K-058).

RESULTS

Confirmation of Staph*. aureus* isolates

Of the 150 isolates screened, 135 were confirmed as *Staph. aureus,* origin wise detail of the isolates is presented in Table 1. The confirmation was based on growth, cultural characteristics, gram's staining, heamolysis on blood agar.

In vitro antibiotic culture sensitivity assay

All the 135 *Staph. aureus* isolates were tested for their *in vitro* susceptibility to different antibiotics by disc diffusion method. The sensitivity pattern is given in Table 2.

Table 1: Confirmation of *Staph. aureus* isolates (origin wise)

Name of Isolates	Pus	Urine	Blood	C.A.
Staphylococcus aureus	93	19	16	07
Other Bacteria	6	2	07	00
Total	99	21	23	07

It is evident from the table that most of the isolates were resistant to conventionally used drugs against *Staph. aurues* viz. penicillin (86.6%), ampicillin (69.6%), co-trimoxazole (70.0%), and the new generation antibiotics azithromycin (68.14%), clarithromycin and linezoid (65.18% each) etc. The drug resistance pattern against different groups of antibiotics used in the present study is given in table 3. The results reflect that majority of the isolates were resistant to β lactam and macrolide groups (69.62% each) followed by oxazolidinone (62.22%) and sulfonamide group (60.00%), tetracycline (51%). However, the percentage of isolates found resistant to other groups of antibiotics (Glycopeptides, Chloramphinicol and Licosamide) (ranged from 33.3% to 45.9%), Of the resistant isolates, three isolates (two from blood and one from pus) were resistant to all the 20 antibiotic used in the study, and nine isolates all from pus were resistant to 15 to 18 antibiotics.

Screening for Methicillin resistance by MRSA kit and *In vitro* antibiotic culture sensitivity assay

All MRSA strains produced color change from pink to yellow with agglutination by MRSA detection kit out of a total of 135 isolates, 62 (45.92%) isolates could be detected as MRSA. Of these, 32% MRSA were detected positive by the MRSA kit as well as antibiotic sensitivity assay. In this manner, the detection kit appeared more sensitive because it could additionally detect 13%MRSA strains which otherwise could not have been detected.

DISCUSSION

We report high prevalence (32%) of multi drug resistant (MDR) strains of *Staph. aureus* among hospital patients Himachal Pradesh. The methicillin resistance however was alarmingly high as we detected 55% strains as MRSA strains. In this study, all the MRSA strains were multi drug resistant (MDR). Only 31/75 (41%) MRSA did not show multiple resistances. In other words, multiple drug resistance correlated well to the methicillin resistance. It therefore, calls our attention towards further characterizing these strains at molecular level for better understanding of the prevalent strains with particular reference to antibiotics sensitivity patterns of drug resistance. The work in this regard is underway in our laboratory.

Infections due to MRSA are well documented and have been reported from various parts of country. Different prevalence rates of MRSA have been reported by various workers viz. 40.60%[3], 54%[9], 59%.[10] However, low prevalence of MRSA (31.1% and 23.6%) has been reported by other workers; Rajaduraipandi *et al.*, 2006[11] and Majumber *et al.*, 2001[12] respectively. Such prevalence may be attributed to several factors viz. efficiency of infection control practices, healthcare facilities and the trend of antibiotics currently in use in different hospitals and settings. We report 45% MRSA as MDR based on the resistance of the isolates to different group of antibiotics such as Sulphonamides and related antibiotics, β-lactam, macrolide, glycopeptides, cephalosporin, chloramphenicol, licosamide, oxazolidine and tetracycline, aminogycosides, quninones etc. Majority of the isolates were resistant to β lactam and macrolide (69.62% each) groups followed by oxazolidinone (62.22%), sulfonamide group (60.00%) and tetracyclines (51%). However, the percentage of isolates found resistant to other group (glycopeptides, chloramphinicol and licosamide) ranged from 33.3% to 45.9% and the proportion of isolates resistant to qunolones, lesser than 33% (Table 3).

It is interesting to note that of all the resistant isolates, three (two from blood and one from pus) were resistant to all the 20 antibiotic used with study and nine isolates all from pus were resistant to 15 to 18 antibiotics. Whereas 47were resistant to 10 to 14 antibiotics (Table 4). All the 58 resistant isolates can thus, be clearly regarded as MDR.

Table 2: Antibiotic susceptibility and resistance pattern of *Staph.* aureus strains from clinical cases

No. of Strains examined	Antibiotic used	Sensitive		Intermediate		Resistance	
		No. of strains	Sensitive isolates (%)	No. of strains with intermediate sensitivity	Intermediate isolates (%)	Strains found resistant (nos).	Strains found resistant (%).
135	CEP (30mcg)	103	76.29	4	2.96	28	20
135	CD (2mcg)	87	64.44	5	3.70	43	31
135	COT (25mcg)	36	26.66	4	2.96	95	70
135	E(15mcg)	60	44.44	1	0.7	74	54.81
135	OF(5mcg)	94	69.62	2	1.48	39	28.88
135	GEN(10mcg)	83	61	8	5.8	44	32.59
135	P(10unit)	18	13.33	-	0	117	86.66
135	VA(30mcg)	44	32.5	21	15.55	72	53.33
135	AMP(10mcg)	40	29.62	1	0.74	94	69.66
135	C(30mcg)	75	55	8	3.7	55	40
135	OX(1mcg)	88	65.18	2	1.48	45	33.33
135	LZ(30mcg)	45	33.33	2	1.48	88	65.18
135	AZM(15mcg)	41	30.37	2	1.48	92	68.14
135	AK(30mcg)	88	65.18	1	0.7	55	40.74
135	CLR(5mcg)	45	33.33	2	1.48	88	65.18
135	Tei(10mcg)	99	73.33	8	5.92	28	20.74
135	MET (5mcg)	78	57.77	7	5.18	50	37.03
135	AMC(30mcg)	97	71.85	3	2.22	35	25.92
135	NV(5mcg)	107	79.25	2	1.48	26	19.25
135	TE (30mcg)	60	44.44	4	2.98	70	51.85

The isolates have been regarded as sensitive, intermediate or resistant on the basis of specification given by the manufacturer of antibiotic discs (Hi-media).

Table 3: Antibiotic group specific resistance patterns

Class of antibiotic	No of resistant isolates	% resistance
Sulfonamides and other drugs	81	60.0%
Quinolones	31	22.96%
B-lactum group	94	69.62%
Tetracycline	69	51.11%
Aminoglycosides	26	19.25%
Macrolides	94	69.62%
Glycopeptides	62	45.92%
Cephalosporin	16	11.85%
Chloramphenicol	54	40.0%
Licosamide	45	33.33%
Oxazolidinone	84	62.22%

*The no of isolates resistant are shown within the brackets and sample nos are mentioned outside the brackets

Srinivasan *et al.*, 2009[13] reported very high prevalence (80%) from surgical units and Jhon *et.al* 2002 reported 28% MRSA isolates from operative infections in orthopedic surgery. The association of MDR with MRSA adds to the therapeutics problem as it is rightly said that hospital dust is more dangerous than roadside dust. MRSA strains are more resistant to other antibiotics than methicillin sensitive *Staph. aureus* (MSSA) strains. Such marked difference in the sensitivity patteren of MRSA and MSSA isolates has also been reported by Vidhani *et al.*, 2001.[14]

The heterogeneity among the population of *Staph. aureus* presents difficulty in the detection of MRSA. Keeping this fact in view, we have used Hi-media MRSA detection kit which appears more sensitive in light of the observation that we could record 45% MRSA with this kit as against 32% detected by *in vitro* antibiotic sensitivity assay. Thus, we could record 13% more isolates as MRSA with the detection kit as all strains 32% MRSA could be detected by both the tests. Most isolates used in the study were sensitive to novobiocin (79.25%), cephalothin (76.29%), teicoplanin (73.3%), amoxyclave (71.85%) followed by ofloxacin (69.62%), oxicillin and amikacin (65.18% each), clindymycin (64.4%) and gentamicin (61.0%). The sensitivity in respect of other antibiotics used in the study was up to 44.0%. However, most isolates were resistant to penicillin (86.6%), co-trimoxazole(70.0%), ampicillin (69.6%), azithromycin, (68.14%), linezoid and clarithromycin (65.18% each) and vancomycin (53.33%). The methicillin resistance correlated well with the vancomycin. The increasing trend of vancomycin and

methicillin resistance might be due to acquisition of resistance determining gene such as *van-A, B, C* and *mec-A* respectively in *Enterococcus* and *Staphylococcus aureus*.[15] The study involving the amplification of *van-A, mec-A, pvl* and other genes such as *coagulase (coa), spa* and *tsst are* underway in our laboratory for determining sequence variations which may correlate to the acquisition of resistance and virulence of *Staph. aureus* isolates under study.

Table 4: Details of multidrug resistant strains of *Staph.aureus*

Resistance to antibiotics	Origin pus Sample no (No. of resistant strains)	Origin blood Sample no (No. of resistant strains)	Origin urine (No of resistant strains)	Origin CA (No of resistant strains)
Resistant to all 20 antibiotics used	Isolate no 37 (1)*	64, 148 (2)*	---	---
Resistant to 18 antibiotics	Isolate no 36,72,73, 86(4)*	---	---	---
Resistant to 17 antibiotics	29,108 (2)*	---	---	---
Resistant to 16 antibiotics	30,45(2)*	---	---	---
Resistant to 15 antibiotics	48,50,61,76,87(5)*	---	---	---
Resistant to 10-15 antibiotics	29 isolates	4 isolates	11	3
Resistant to 05-10 antibiotics	22 isolates	5 isolates	4	---

CONCLUSION

The surveillance of MDR-MRSA strains and their characterization will enhance the ability of the public health and health care system to rapidly recognize and aggressively contain infection and colonization due to these antibiotics resistant *Staph. aureus* in the healthy population.

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