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Prevalence and Antibiogram of Hospital Acquired Methicllin Resistant *Stapylococcusaureus* (HA-MRSA) from a Tertiary Care Hospital in Peshawar, Pakistan

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Abstract: This survey was implemented to isolate, identify and analyze the susceptibility of HA-MRSA against commonly applied antibiotics as well as to detect its prevalence. Total 1394 samples of various natures were collected from different wards of *Government Lady Reading Hospital* Peshawar (Khyber Pakhtunkhwa) Pakistan. *Staphylococcus aureus* was isolated and identified by using selective media and biochemical tests including catalase, and coagulase tests, followed by detection of MRSA against methicillin. Further, susceptibility of MRSA was investigated against 14 antibiotics using standard disc-diffusion protocol. Demographic analyses revealed that out of 1394 samples, pus were the highest-rated (540) samples followed by urine (381) and blood (354) while the lowest number were semen samples (7). From total number of samples, highest incidences of *Staphylococcus aureus* were detected in pus (71.48%), blood (68.08%) and urine (54.35%) while lowest count was 42.86% in semen samples. Among *Staphylococcus aureus*-positive samples, MRSA rated 100%, 66.67%, 62.95%, 44.44%, 29.68%, 21.16%, 20.93% and 0.00% in semen, sputum, pus, throat-swab, urine, blood HVS and stools samples, respectively. All MRSA was found to possess average resistance of 64.08% (while 35.92% susceptibility) against all antibiotics collectively. Individually, highly resistant MRSA were recorded 98.71% against gentamicin followed by norfloxacin (82.14%) and ciprofloxacin (79.34%) while mostly susceptible to vancomycin (92.09%), chloramphenicol (67.09%) and sparfloxacin (57.40%). Overall, most adult female were found infected than male and children and vancomycin showed highest bactericidal property.

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Key words: Nosocomial Infection, *Staphylococcus aureus*, MRSA, HA-MRSA, Antibiotic Sensitivity Pattern

Introduction

A great damage to health of animals, plants and humans is endemic in population with high ratio due to infectious diseases caused by vast number and types of bacteria. Some infectious diseases are as fatal as leading to death, reported worldwide (Wilson et al., 2002). From the list of dangerous pathogens of health-concern, one is a well-known bacterium: *Staphylococcus aureus* (Persoons et al., 2009), which attains bad effects on health of humans and other animals (Milyani and Ashy, 2012).

Staphylococcus aureus, when finds its way into an organism (human or other animals) through nasal cavity or skin punctures or other possible routes, it results in various infections running from minor cases (abscesses and skin infections) till life endangered cases including toxic shock syndrome (TSS), meningitis, pneumonia, septicemia and endocarditis **(**Enright et al., 2002; Ho et al., 1989).Many more infections can be caused by *Staphylococcusaureus* and their resistance pattern against various antimicrobial agents also varies. One of such *Staphylococcusaureus* that shows resistance against methicillin, is known as '*Methicillin Resistant Staphylococcus aureus (MRSA)*' while other types include *Staphylococcusaureus* resistant to other antibiotics as gentamycin, fucidic acid and clindamycin (Shai et al., 2004).

"Hospital Associated Methicillin Resistant Staphylococcus aureus" (HA-MRSA) and "*Community Associated Methicillin Resistant Staphylococcus aureus"* (CA-MRSA) are two broad categories of MRSA reported till date. Strains of MRSA developed from a community (population or habitat) are known as CA-MRSA while if the incidence of strains of MRSA are related to hospital environment it is known as HA-MRSA which also include the development and emergence of MRSA from the patient having current or recent hospitalization, receives dialysis or resides in long term care service (Milyani and Ashy, 2012). Other problems associated with MRSA include septic arthritides, sepsis, endocarditis, endovascular infections, osteomyelitis and soft tissue infections (SSTIs) (Lowy, 1998).

After its first isolation in Europe in 1960s, MRSA was known as leading agent for nosocomial infections, globally (Lowy, 1998; Tristan et al., 2007) and its prevalence from various parts of world have been reported. However, much less has been known about the prevalence of HA-MRSA in Khyber Pakhtunkhwa province of Pakistan. The purpose of this report was to investigate incidence of MRSA from Lady Reading Hospital, Peshawar and further to investigate their susceptibility profiles against various commonly used antibiotics.

Material and Methods

Total 1394 samples of different types for this study were collected from Government Lady Reading Hospital, Peshawar (Khyber Pakhtunkhwa), Pakistan within the period from July 2012 to December 2012. These samples were collected aseptically from patients of different wards (OPD, ENT, Medical, Burns, Orthopedic, Surgical, Children, Guiney and Main Operation Theater) which included blood, pus, sputum, HVS, semen, stool, and urine samples. All these samples were subjected for screening of *S. aureus* in Microbiology Laboratory at Lady Reading

Hospital, Peshawar on Mannitol Salt Agar (MSA). The resulted colonies of *S. aureus*, identified from its morphological attributes, were sub-cultured on selective media MSA to obtain pure isolates. Biochemical tests like gram staining, catalase and coagulase tests were performed using the standard protocols to confirm the presence of *S. aureus*. Samples that contained *S. aureus* were noted as *S. aureus* positive and were subjected to check its resistance against antibiotic methicillin. The *S. aureus* samples, which showed resistance against methicillin were marked as MRSA.

The Kirby-Bauer disc diffusion protocol was adopted in order to evaluate antimicrobial susceptibility pattern of MRSA against 14 commonly used antibiotics on Muller-Hilten Agar by following the standard protocols (Bauer et al., 1966).

Results

Demographic data revealed that; out of total 1394 samples, 711 (51.00%) were female while remaining 683 (49.00%) were male. One the basis of type of samples, the highest ratio (38.74%) samples were pus (540 samples) followed by urine (27.33%) and blood (25.39%) having 381 and 354 samples, respectively, while lowest number of samples counted 7 (semen), 10 (stool) and 15 (throat swab).

Results from cultured samples on Mannitol Salt Agar (MSA) revealed two types of colonies; one that turned the media color to yellow and the other without any alteration in color of media. The former colonies were recognized as *Staphylococcus aureus*due to its ability to ferment mannitol available in media and become yellow. From all, 952 samples were *S. aureus* positive and pure colonies obtained from these cultures were identified as *Staphylococcus aureus* from the results of biochemical tests because all were gram, catalase and coagulase positive. Distribution of *S. aureus* positive samples identified, according to gender and sampling ward, are enlisted in Table 1.

Analysis from all the *S. aureus* positive samples tested for the presence of MRSA, revealed that samples included MRSA in various ratios such as all the Semen samples were MRSA positive, HVS (20.93%), Blood (21.16%), Urine (29.69%), Throat swab (44.44%), Pus (62.95%), Sputum (66.67%) and no MRSA were detected in Stool samples as shown in Table 2. Detailed demographic results of MRSA positive samples are shown in Table 3.

Table 4 shows antibiotic sensitivity pattern of MRSA isolates to different antibiotics in various samples, where MRSA showed high resistance to various antibiotics used. MRSA was found highly resistant to gentamicin (98.72%) followed by norfloxacin (82.14%), ciprofloxacin (79.34%) while highest susceptibility was found against vancomycin with 7.91% susceptibility.

Table 1. Gender-wise distribution of *S. aureus* positive samples according to its type from respective wards

Samples		OPD	ENT	Medical	Burns	Orthopedic	Surgical	Children	Guiney	Main OT	Total
Blood	Male	10	7	20	32	4	33	12	0	9	127
	Female	5	8	13	9	12	24	14	20	9	114
	Total	15	15	33	41	16	57	26	20	18	241
Urine	Male	58	9	4	11	5	49	10	0	3	149
	Female	30	3	6	14	2	22	19	9	2	107
	Total	88	12	10	25	7	71	29	9	5	256
Pus	Male	69	7	11	9	14	65	5	8	17	205
	Female	50	14	12	10	21	37	8	6	23	181
	Total	119	21	23	19	35	102	13	14	40	386
Sputum	Male	0	3	0	0	0	1	0	0	0	4
	Female	3	1	0	0	0	1	0	0	0	5
	Total	3	4	0	0	0	2	0	0	0	9
HVS	Male	0	0	0	0	0	0	0	0	0	0
	Female	0	0	0	0	0	0	0	43	0	43
	Total	0	0	0	0	0	0	0	43	0	43
Semen	Male	2	0	1	0	0	0	0	0	0	3
	Female	0	0	0	0	0	0	0	0	0	0
	Total	2	0	1	0	0	0	0	0	0	3
Stool	Male	1	0	2	0	0	0	0	0	0	3
	Female	0	0	2	0	0	0	0	0	0	2
	Total	1	0	4	0	0	0	0	0	0	5
Throat	Male	1	1	0	0	0	0	1	0	0	3
	Female	1	3	1	0	0	1	0	0	0	6
	Total	2	4	1	0	0	1	1	0	0	9
Total		**229**	**56**	**72**	**85**	**58**	**234**	**69**	**86**	**63**	**952**

Table 2.*S. aureus* and MRSA positive samples detected among total and *S. aureus* positive samples, respectively

Sample	Gender	Total		*S. aureus*		MRSA	
		Number	*Percentage (%)*	Number	*Percentage (%)*	Number	*Percentage (%)*
Blood	Male	164	*46.33*	127	*52.70*	32	*62.75*
	Female	190	*53.67*	114	*47.30*	19	*37.25*
	Total	354	-	241	*68.08*	51	*21.16*
Urine	Male	202	*53.01*	149	*58.20*	46	*60.53*
	Female	179	*46.99*	107	*41.80*	30	*39.47*
	Total	381	-	256	*54.35*	76	*29.68*
Pus	Male	292	*54.07*	205	*53.11*	151	*62.14*
	Female	248	*45.93*	181	*46.89*	92	*37.86*
	Total	540	-	386	*71.48*	243	*62.95*
Sputum	Male	9	*52.94*	4	*44.44*	3	*50.00*
	Female	8	*47.06*	5	*55.56*	3	*50.00*
	Total	17	-	9	*52.94*	6	*66.67*
HVS	Male	0	*0.00*	0	*0.00*	0	*0.00*
	Female	70	*100*	43	*100*	9	*100*
	Total	70	-	43	*61.43*	9	*20.93*
Semen	Male	7	*100*	3	*100*	3	*100*
	Female	0	*0.00*	0	*0.00*	0	*0.00*
	Total	7	-	3	*42.86*	3	*100*
Stool	Male	5	*50.00*	3	*60.00*	0	*0.00*
	Female	5	*50.00*	2	*40.00*	0	*0.00*
	Total	10	-	5	*50.00*	0	*0.00*
Throat Swab	Male	4	*26.67*	3	*33.33*	1	*25.00*
	Female	11	*73.33*	6	*66.67*	3	*75.00*
	Total	15	-	9	*60.00*	4	*44.44*
Total		**1394**	**-**	**952**	***68.29***	**392**	***41.18***

Table 3. Prevalence of MRSA positive samples according to its type from respective wards

Samples		OPD	ENT	Medical	Burns	Orthopedic	Surgical	Children	Guiney	Main OT	Total
Blood	Male	3	1	5	10	1	6	4	0	2	32
	Female	0	1	1	1	3	5	3	5	0	19
	Total	3	2	6	11	4	11	7	5	2	51
Urine	Male	17	2	3	3	0	14	6	0	1	46
	Female	13	0	2	3	0	5	7	0	0	30
	Total	30	2	5	6	0	19	13	0	1	76
Pus	Male	55	3	9	5	10	49	5	4	11	151
	Female	38	13	6	7	9	14	0	0	5	92
	Total	93	16	15	12	19	63	5	4	16	243
Sputum	Male	0	2	0	0	0	1	0	0	0	3
	Female	1	1	0	0	0	1	0	0	0	3
	Total	1	3	0	0	0	2	0	0	0	6
HVS	Male	0	0	0	0	0	0	0	0	0	0
	Female	0	0	0	0	0	0	0	9	0	9
	Total	0	0	0	0	0	0	0	9	0	9
Semen	Male	2	0	1	0	0	0	0	0	0	3
	Female	0	0	0	0	0	0	0	0	0	0
	Total	2	0	1	0	0	0	0	0	0	3
Stool	Male	0	0	0	0	0	0	0	0	0	0
	Female	0	0	0	0	0	0	0	0	0	0
	Total	0	0	0	0	0	0	0	0	0	0
Throat Swab	Male	0	0	0	0	0	0	1	0	0	1
	Female	0	2	0	0	0	1	0	0	0	3
	Total	0	2	0	0	0	1	1	0	0	4
Total		**129**	**25**	**27**	**29**	**23**	**96**	**26**	**18**	**19**	**392**

Table 4. Antibiogram pattern of MRSA

Antibiotic	Blood		Urine		Pus		Sputum		HVS		Semen		Stool		Throat Swab		Average Percentage (%)	
	R[1]	**S[2]**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**
Amoxicillin	42	9	63	13	130	113	4	2	6	3	2	1	0	0	2	2	**63.52**	**36.48**
Cefotaxime	47	4	76	0	170	73	5	1	5	4	2	1	0	0	1	3	**78.06**	**21.94**
Cephradin	46	5	71	5	168	75	4	2	9	0	3	0	0	0	3	1	**77.55**	**22.45**
Cephtriaxone	35	16	73	3	176	67	3	3	7	2	2	1	0	0	2	2	**76.02**	**23.98**
Chloramphenicol	3	48	74	2	42	201	1	5	3	6	3	0	0	0	3	1	**32.91**	**67.09**
Ciprofloxacin	50	1	65	11	180	63	4	2	6	3	3	0	0	0	3	1	**79.34**	**20.66**
Coamoxiclave	49	2	70	6	167	76	5	1	5	4	1	2	0	0	3	1	**76.53**	**23.47**
Doxycyclin	13	38	71	5	161	82	2	4	8	1	2	1	0	0	0	4	**65.56**	**34.44**
Gentamicin	51	0	76	0	243	0	6	0	7	2	1	2	0	0	3	1	**98.72**	**1.28**
Levofloxacin	21	30	40	36	191	52	5	1	5	4	3	0	0	0	2	2	**68.11**	**31.89**
Norfloxacin	41	10	69	7	197	46	4	2	5	4	2	1	0	0	4	0	**82.14**	**17.86**
Ofloxacin	22	29	33	43	127	116	2	4	1	8	2	1	0	0	2	2	**48.21**	**51.79**
Sparfloxacin	18	33	40	36	99	144	3	3	2	7	3	0	0	0	2	2	**42.60**	**57.40**
Vancomycin	0	51	6	70	24	219	1	5	0	9	0	3	0	0	0	4	**7.91**	**92.09**
Average Percentage (%)	**61.34**	**38.66**	**77.73**	**22.27**	**61.00**	**39.00**	**58.33**	**41.67**	**54.76**	**45.24**	**69.05**	**30.95**	**0.00**	**0.00**	**53.57**	**46.43**	**64.08** / **54.47**	**35.92** / **45.53**

[1]Number of samples showed resistance against respective antibiotic,

[2]Number of samples that showed susceptibility against respective antibiotic

Discussion

In the present study *Staphylococcus aureus* was isolated from various types of samples obtained from different wards of a tertiary care hospital. Similar studies have been conducted by various researchers at different locations globally.

In Pakistan Iyad et al., (2006)isolated*Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus*and *Escherichia coli* as nosocomial pathogens. Similarly in IndiaManjula et al., (2007) found *Pseudomonasspp*., *Staphylococcus aureus,Klebsiellaspp.* and *Proteusspp.*as nosocomial pathogens. Chikere et al., (2008)from Nigeria isolated *Staphylococcus aureus, E. coli, K. pneumonia,* and *Proteus spp.* as nosocomial pathogens.

Methecillin resistant *Staphylococcus aureus* (MRSA) have been isolated with high ratio from clinical samples of different wards. Similar study conducted by Siddique et al. (1999) at Sargodha, Pakistan isolated 23% MRSA, Samia et al. (2007) at Karachi, Pakistan, isolated 43% MRSA, and then by Khatoon et al. (2002) at Karachi, Pakistan isolated 38.5% MRSA. Similar study was also conducted in India by Shagufta and Jayaraj (2010) isolated 77.9% MRSA, while Helena et al. (2010) in Brazil isolate 61% MRSA. This shows an increase in MRSA percentage with time. This increase is due to transfer of resistance genes among bacterial cell and persistence of bacteria in hospital environment due to antibiotic resistance. Another factor which facilitates MRSA to increase in concentration is absences of control program for antibiotics usage pattern (Hacek et al., 1999).

Our findings are in correspondence with Saima et al. (2007) and Hare et al., (2009) who observed methicillin resistant *Staphylococcus aureus*(MRSA)with high resistance to the various antibiotics including Gentamicin, Ciprofloxacin, Doxycycillin. MRSA were reported to be resistant to Penicillin, Amoxicillin, Ampicillin, rimethoprim/Sulfamethoxazole, Cephaloxin, Amikacin, and Ciprofloxacin at a resistant rate of 110%, 91.9%, 87.6%, 77%, 55.5%, 19%, and 26.5%, respectively (Hare *et al.* 2009). Similar results were also reported by Samia et al. (2007) which showed resistance with similar patterns.

Our findings revealed that highest percent of MRSA were detected in semen samples (100%) while the lowest MRSA ratio was in stool samples with no MRSA. However, it is crucial to mention that total number of samples may be the cause for such diverse results because total numbers of semen and stool samples were 3 and 5, respectively. Such a low sample number may not yield a clear depiction about MRSA while increased number of samples may give statistically more valid results. Same is the case with throat swab and sputum samples each counted only 9 samples. The notable results from high number of samples included pus (386), Urine (256), Blood (241) and Urine (43) samples which included 62.95%, 29.69%, 21.16% and 20.93% MRSA positive samples, respectively.

Conclusion

Methicillin resistant *Staphylococcus aureus* (MRSA) was isolated in high number from hospital, which showed a high risk to the patients and staff working in the hospital. The over-crowding on hospital, lack of facilities and lack of knowledge about hospital acquired methicillin resistant *Staphylococcus aureus* (HA-MRSA) leads to the problems caused by nosocomial pathogen with high frequency. Results showed an increase of MRSA population in hospital environment comparable with time. MRSAalso showed an increase in resistance to different antibiotics. High percentage of ESBL production from gram negative rods

and resistance to other antibiotics shows an increasing rate of acquiring antibiotics resistance. Due to such high risks, extensive care and proper guidelines and/or legislation along with controlling strategies are required.

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