Effectiveness of simple control measures on methicillin-resistant *Staphylococcus aureus* infection status and characteristics with susceptibility patterns in a teaching hospital in Peshawar

Muhammad Salman Rafiq,[1] Muhammad Imran Rafiq,[2] Taimur Khan,[3] Maria Rafiq,[4] Mah Muneer Khan[5]

Abstract

Objective: To determine the effectiveness of simple control measures on the infection status and characteristics of methicillin-resistant Staphylococcus aureus including susceptibility patterns among health professionals and patients in a teaching hospital.

Methods: The cross-sectional study was conducted from September 2013 to January 2014, and comprised samples collected from healthcare personnel and patients in the various units of Khyber Teaching Hospital, Peshawar. The specimens were collected before and one month after the implementation of simple control measures for outbreak prevention of methicillin-resistant Staphylococcus aureus. These were tested for culture and antimicrobial susceptibility. Data about methicillin-sensitive and methicillin-resistant Staphylococcus aureus infection, wound characteristics and susceptibility patterns was collected and effectiveness of simple control measures was determined. SPSS 20 was used for statistical analysis.

Results: Of the total 390 isolates, 180(46.2%) were Staphylococcus aureus; 77(19.7%) from healthcare personnel and 103(26.4%) from patients. Of these, 164(42.1%) were methicillin-sensitive and 16(4.1%) were methicillin-resistant. Among the patients, 38(15.1%) methicillin-sensitive and 8(3.2%) methicillin-resistant isolates were recovered from wounds or skin and soft tissues. Pus with 33(13.1%) and 4(1.6%) cases respectively was the second most common source. Among methicillin-resistant isolates, resistance to Linezolid was 0%, all were resistant to Oxacillin, Cefoxitin, Amoxicillin, Cefotaxime and Cephradine, and resistance to both Co-Amoxiclav and Ciprofloxacin was 87.5%. After one month of implementation of simple control measures, the number of methicillin-resistant cases among healthcare professionals and patients dropped from 4(2.9%) and 7(10.8%) to 1(0.7%) and 5(2.7%), respectively.

Conclusion: Methicillin-resistant and methicillin-sensitive Staphylococcus aureus differed in their anti-microbial susceptibility profiles. Selection of antibiotics based on susceptibility and culture is needed for prevention of resistance and effective treatment. A decrease was observed in methicillin-resistant cases with implementation of control measures.

Keywords: Staphylococcus aureus, Methicillin resistant, Susceptible, Hospital, Acquired infections. (JPMA 65: 915; 2015)

Introduction

Staphylococcus aureus (S. aureus) is a commensal of the skin and anterior nares in 80% of healthy individuals. It is the most common cause of skin and soft tissue infections (SSTIs), nosocomial infections and can produce pneumonia, septic arthritis, endocarditis and osteomyelitis.[1]

In the pre-antibiotic era, mortality from Haematogenous S. aureus (SA) infections was above 80%.[2] With the discovery of penicillin, this was reduced dramatically.[3] This golden period was soon brought down by the advent of penicillinase-producing SA strains that soon spread into hospitals and later the community.[3] By the early '80s,

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[1,3,5]Surgical D Unit, Khyber Teaching Hospital, [2]Medical B Unit, Lady Reading Hospital, [4]Gynae B Unit, Hayatabad Medical Complex, Peshawar, Pakistan.
Correspondence: Muhammad Salman Rafiq. Email: drsalmanrafiq@hotmail.com

these resistant strains replaced the penicillin-susceptible strains in hospitals and the community to become the prevalent strains.[1] Methicillin introduced in the '60s was effective against these penicillinase-producing strains and became the drug of choice for treating SA infections. These strains are called Methicillin-sensitive Staphylococcus aureus (MSSA). The first documented case of Methicillin-resistant Staphylococcus aureus (MRSA) was observed in 1961 in England.[4] These MRSA strains soon spread throughout the world.[5] The prevalence of healthcare-associated MRSA (HA-MRSA) is now higher than HA-MSSA in some countries.[6] On the other hand, community-associated MRSA (CA-MRSA) is on the rise in many parts of the world[7] and the healthcare burden caused by these infections is higher in the developing world.[8]

The last few decades have seen waves of MRSA outbreaks and therefore it has become one of the most important

and common causes of hospital and community-acquired infections.[9] Unfortunately, data regarding the prevalence of MRSA in Pakistan is scarce.[10] With the lack of resources, such as microbiology laboratories, the unchecked spread of MRSA in developing countries like Pakistan has been devastating.[8] MRSA is mainly transmitted through skin-to-skin contact.[1] In the Indian subcontinent, with high population density, there is excessive abuse of unregulated antibiotics and its misuse in the livestock and poultry industries.[11] This has provided the perfect setting for the development of drug resistance in the community.[2]

MRSA strains that cause the major portion of infections globally include CC5, CC8, CC22, CC30 and CC45.[2] There is a strong association between certain strains and genes for virulence e.g., sea, sek genes in ST239 strains and seg, sei, sem genes in ST5 strain.[12] In the United States, most skin and soft tissue infections (SSTIs) are caused by the MRSA USA300.[13] Prevalence of both HA-MRSA and CA-MRSA in Pakistan has not been documented well and there is limited information about the latest HA-MRSA infections in hospitals in Peshawar. After a recent string of MRSA-positive cases at the hospital, strict adherence to simple control measures, such as universal hand hygiene practices, were implemented. However, no tool existed to assess infection status. This study was planned to assess the infection status and characteristics of SA among the patients and healthcare personnel.

Subjects and Methods

The cross-sectional study was conducted from September 2013 to January 2014, and comprised samples collected from healthcare personnel and patients in the various units of Khyber Teaching Hospital, Peshawar. The samples were collected after approval from the institutional ethics committee and written informed consent from the subjects. The samples were collected before and one month after the implementation of control measures. . Samples were collected through swabs, pus, tissue/wound, urine/catheter and respiratory suction apparatus. These were labelled and screened for MSSA, MRSA and anti-microbial susceptibility.

Isolation of SA, MRSA screening and anti-microbial susceptibility profile were done in the Pathology Department of the hospital according to Centre for Disease Control (CDC) guidelines.[14] Samples were inoculated onto Mannitol-Salt Agar, followed by identification of SA by gram staining and biochemical tests such as catalase, coagulase and deoxyribonuclease (DNase). Methicillin is no longer commercially available and Oxacillin is used instead. CDC standards require that since Oxacillin disk diffusion alone is not reliable, Cefoxitin should be used as a surrogate for disk diffusion testing. Isolates showing growth on this were labelled as MRSA. Kirby-Bauer disc diffusion was used to evaluate susceptibility pattern of the isolates against commonly used antibiotics and the results were interpreted by measuring the zone of inhibitions according to standard guidelines.[15,16]

Data was analysed using SPSS 20 and expressed as mean, percentage and standard deviation. A bar chart of MRSA susceptibility pattern was created using Microsoft Excel 2010.

Results

Overall, a total of 390 samples were collected. Of them 139(35.6%) related to healthcare personnel and 251(64.4%) to patients. Among the healthcare personnel, there were 74(53.2%) doctors, 38(27.3%) nurses and 27(19.4%) staff. There were 63(45.3%) females and 76(54.7%) males. The overall mean age was 29.58±7.326 years (range: 21-58 years). The method most used for specimen collection was nasal swabs 105(75.5%). Bacterial growth was detected in 121(87.1%) isolates. SA was the most frequently observed bacterium in 77(55.4%). Of these, gram +ve bacteria were 121(87.1%) and gram -ve were 18(12.9%). Further, 117(84.2%) were susceptible to Oxacillin, 4(2.9%) were resistant to it and in 18(12.9%) isolates, a pattern could not be established. Likewise, 117(84.2%) were susceptible to Cefoxitin, 4(2.9%) were resistant to it and in 18(12.9%) a pattern could not be established. Only 4(2.9%) isolates were MRSA, and 62(44.6%) were neither MSSA nor MRSA. Nasal carrier status was 4(2.9%) (Table-1).

Table-1: Healthcare personnel characteristics.

Source*	n+ (%)	Type++	n (%)	Susceptibility	n (%)	Status#	n (%)
Nasal Swab	105(75.5%)	S. Aureus	77(55.4%)	Oxacillin	117(84.2%)	MSSA	73(52.5%)
Ear Swab	32(23%)	Staph. Epidermidis	44(31.7%)	Cefoxitin	117(84.2%)	MRSA	4(2.9%)
Pus	2(1.4%)	No growth	18(12.9%)				

*Source; Specimen Source, +n; number of cases, ++Type; Bacterial type, #Status; MRSA status,
S: Staphylococcus.
MRSA: Methicillin-resistant Staphylococcus aureus
MSSA: Methicillin-sensitive Staphylococcus aureus

Table-2: Patients characteristics.

Source*	n+(%)	Site	n(%)	Bacteria	n(%)	Status++	n(%)
In-patients	175(69.7%)	Tissue/Wound	106(42.2%)	*S. Aureus*	102(40.6%)	MSSA	91(36.3%)
Surgical ICU	39(15.5%)	Pus	95(37.8%)	*E. Coli*	48(19.1%)	MRSA	12(4.8%)
				Pseudomonas A.	56(22.3%)		
				Strep. Group	6(2.4%)		
				Other bacteria	13(5.2%)		
Post-discharge/follow-up	37(14.7%)	Swabs	22(8.8%)	*No growth*	26(10.4%)		
		Urine/Catheter	20(8%)				
		Suction apparatus	8(3.2%)				

*Source; Specimen source, +n; number of cases, ++Status; Resistance status,
S: Staphylococcus.
MRSA: Methicillin-resistant Staphylococcus aureus
MSSA: Methicillin-sensitive Staphylococcus aureus
ICU: Intensive care unit

Table-3: Percentage resistance of MSSA isolates to various Anti-microbials.

Oxa	Cefo	Amox	Co-Am	Cipro	Erythro	Genta	Clinda	Tetra	TMP-SMX
0%	0%	86%	86%	43.9%	25%	4.3%	10.4%	37.8%	20.7%

OxaL Oxacillin
Cefo; Cefoxitin, Amox;Amoxicillin, Co-Am;Co-Amoxiclav, Cipro;Ciprofloxacin, Eryhtro; Erythromycin, Genta;Gentamycin, Clinda; Clindamycin, Tetra; Tetracycline, TMP-SMX; Tri methoprim Sulpha Methoxazole
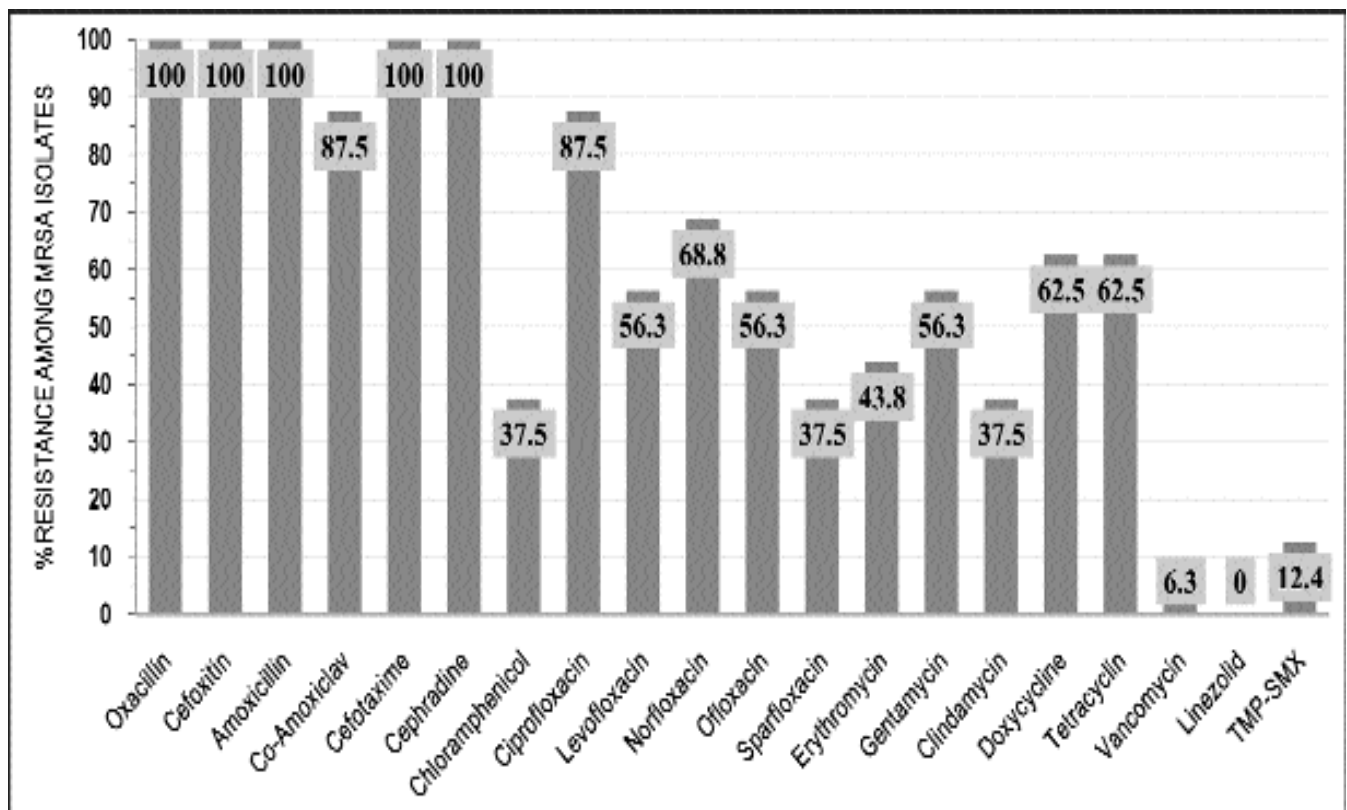MSSA: Methicillin-sensitive Staphylococcus aureus.

Figure: Methicillin-resistant Staphylococcus aureus resistance profile against anti-microbial drugs.

Among the 251 specimens related to patients, 65(26%) were collected before and 186(74%) one month after the implementation of control measures. There were 129(51.4%) females and 122(48.6%) males. Mean age was 40.5±14.39 years (range: 16-70 years). Most common sample source was in-patients 175(69.7%). Tissue\wound 106(42.2%) was the most common method for collection. Bacterial growth was detected in 225(89.6%) isolates. SA 102(40.6%) was the most common bacterium observed. Of these, gram+ve bacteria were 113(45%) and Gram-ve were 112(44.6%) and in 26(10.4%), the gram status was not relevant; no growth. Further, 115(45.8%) were susceptible to Oxacillin, 69(27.5%) were resistant to it and in 67(26.7%) a pattern could not be established or did not apply. Likewise, 90(35.9%) were susceptible to Cefoxitin, 100(39.8%) were resistant to it and in 61(24.3%) a pattern could not be established or it did not apply. Within the group, 91(36.3%) isolates were MSSA, 12(4.8%) MRSA and 148(59%) were neither MSSA nor MRSA. Nasal carrier status was 4(1.6%) (Table-2).

Anti-microbial susceptibility profile was generated. Of the total 390 specimens, 180(46.2%) were SA isolates. These included 77(19.7%) from healthcare personnel and 103(26.4%) from patients. A total of 164(42.1%) isolates were MSSA (Table 3). None of the isolates was resistant to Oxacillin and Cefoxitin. Highest resistance was for Amoxicillin and Co-Amoxiclav 141(86%) each.

Besides, 16(4.1%) isolates were MRSA resistant (Figure). All (100%) isolates were resistant against Oxacillin/Methicillin, Cefoxitin, Amoxicillin, Cefotaxime and Cephradine. Lowest resistance was against Linezolid 0%. Resistance to Co-Amoxiclav was 14(87.5%). Resistance against Vancomycin was 1(6.3%) and Trimethoprim Sulpha Methoxazole (TMP-SMX) was 2(12.5%).

In terms of effectiveness of the control measures, MRSA screening before the implementation of control measures revealed an MRSA prevalence of 4(2.9%) among healthcare personnel with 73(52.5%) subjects being MSSA. One month after the control measures, MRSA infection status dropped to 1(0.7%) with MSSA subjects increasing to 76(54.7%).

In patients, before the implementation of control measures, MRSA prevalence was 7(10.8%) 23(35.4%) MSSA, while 35(53.8%) patients had other bacterial infections. One month after the control measures, MRSA infection status dropped to 5(2.7%) with MSSA subjects increasing to 68(36.6%) and other bacterial infections also rising to 113(60.8%).

Discussion

S. aureus is responsible for a major portion of the disease burden in modern healthcare with rising incidence and prevalence. The first case of MRSA in Pakistan was reported in 1989.[14] One study in 2013 reported MRSA prevalence to be 68.1%.[2] In Pakistan, MRSA prevalence was reported to be from as high as 42% to as low as 7.5%.[15] One study showed that MRSA isolates subjected to 3 consecutive tests for their MRSA status had the prevalence reduced from 41.9% with disc diffusion to 38.1% with minimum inhibitory concentration (MIC) to only 27.9% with mecA gene detection.[15] In our study only 16(4.1%) of the total 390 healthcare personnel and patients were MRSA-positive. In Pakistan its incidence and prevalence is steadily increasing due to absence of control measures, misuse of antibiotics and lack of guidelines.

In our teaching hospital, a recent MRSA-positive case was followed by an MRSA outbreak. Subsequently, MRSA outbreak measures were implemented, including simple control measures like strict universal hand hygiene practices, isolation and cohorting from September 2013 to January 2014. During this period for an aggregate of 122 days in the studied units, 180(46.2%) SA infections were identified that included 77(19.7%) from healthcare personnel and 103(26.4%) from patients.

Hospital-acquired SA infection is a leading cause of nosocomial infections. A study reported it to be 20%.[16] In our study, it attributed to 153(39.23%) cases. These were patients infected after their admissions with culture-positive SA. Of the MRSA patients, 8(2.1%) were male and 8(2.1%) female. Gender was not a statistically significant factor as also reported by previous studies.[17] Both MSSA and MRSA isolates had no specificity for any age group. Most of the cases occurred in patients aged 40 years or below, with a peak at 40 years. Therefore in our study old age was not a statistically significant factor as also described earlier.[17]

Isolates were tested for coagulase status. In a previous study, 70% of the isolates were coagulase +ve and 30% coagulase -ve. In our study, among Staphylococci we found this to be 77(55.4%) and 44(31.7%) among healthcare personnel, respectively. Among the patients this was found to be 102(40.6%) and 1(0.4%), respectively. The majority of bacteria among patients that were not coagulase +ve were non-Staphylococci species. Majority of both MSSA 38(15.1%) and MRSA 8(3.2%) isolates were recovered from the wound site, while pus, 33(13.1%) was the second most common source. This is the same as as reported by a study where it was wound swabs 39.18%, followed by pus 20.94%.[18] The majority of the MRSA isolates in our study were from patients with SSTIs, as also demonstrated earlier where it was 46(24.08%).[17] However,

one study showed respiratory tract infections to be the most common source (28%)[15] and another study showed urinary tract infections to be the most common MRSA source at 25.14%.[18]

Different Strains are associated with different virulence genes. A study identified SasX, the surface-anchored protein, in promoting nasal colonisation. ST59-SCC was shown to be responsible for SSTIs.[2] The study suggested that the external community was a significant reservoir of MRSA/MSSA strains causing SSTIs that find their way into hospitals.[2] Therefore, the traditional control strategies aimed solely at prevention of hospital-acquired transmission may be ineffective. In order to adequately control MRSA outbreaks, new health measures that target the community need to be applied.

Antibiotic susceptibility patterns were established for both MSSA and MRSA. Among MSSA isolates, highest resistance was found against Amoxicillin (86%) and Co-Amoxiclav (86%), as also described in an earlier study where it was 80.7% for Amoxicillin and 87.7% for Penicillin.[19] Resistance against Erythromycin, Gentamycin, Clindamycin, Tetracycline and Co-Trimoxazole was shown to be 30.5%, 1.56%, 13.9%, 31.25% and 27.7% respectively.[19] Resistance against these drugs in our study was 25%, 4.3%, 10.4%, 37.8%, and 20.7%, respectively. In our study, no resistance was found against Oxacillin and Cefoxitin.

Among the MRSA isolates, 100% resistance was shown against Amoxicillin[17] and against Penicillin and Oxacillin.[18] We found 100% MRSA resistance against Oxacillin, Cefoxitin, Amoxicillin, Cefotaxime and Cephradine. The least resistance was demonstrated against Vancomycin.[17-19] This was similar to findings in our study where resistance to Vancomycin was 6.3%. However, in our study, the least resistance 0% was found against Linezolid. MRSA resistance was demonstrated for Ciprofloxacin, Levofloxacin, Ofloxacin, Erythromycin and Gentamycin as 59.16%, 70.15%, 74%, 69.10%, 67.01% by one study and 44.59%, 80.40%, 70%, 85.81% and 76.35% respectively by another.[17,18] In our study it was 87.5%, 56.3%, 56.3%, 43.8% and 56.3% respectively. Another study[19] showed that MRSA resistance to Co-Trimoxazole was 86.48% which in our study was 11%.

The implemented control measures included liquid hand sanitizers and wipes with surface-active antiseptics such as Chlorhexidine. However, resistance emerged. This could account for the 2 MRSA cases among the healthcare personnel i.e. paramedics in our study. Antiseptic overuse has also been responsible for the origin of MRSA strains with decreased antiseptic susceptibility. Hospital-

acquired colonisation and infection can also be controlled adequately with Mupirocin.[20] However, even Mupirocin resistance in SA and MRSA has been reported.[21]

In our study, the MRSA infection status among healthcare personnel dropped from 4(2.9%) before to 1(0.7%) one month after the control measures. The single subject with MRSA persisting despite the simple control measures was advised further treatment. Among the healthcare personnel this decrease in MRSA infection was replaced with colonisation of MSSA among the subjects. As a result, the MSSA colonisation rose from 73(52.5%) to 76(54.7%). Among patients, the MRSA infection dropped from 7(10.8%) before to 5(2.7%) one month after the control measures. It is noteworthy that the pre control measures figure of 7(10.8%) was from 65 patients whereas the one month after control measures figure of 5(2.7%) was from 186 patients. This shows a much greater decrease in the incidence of MRSA among patients after the implementation of simple control measures.

This study demonstrated that MRSA infections among healthcare personnel and patients were therefore infrequent due to the control measures, representing only 0.7% and 2.7% of the total subjects in their respective groups compared to 2.9% and 10.8% without the control measures, respectively. These MRSA isolates were susceptible to Linezolid, Vancomycin and Co-Trimoxazole, but resistant to Co-Amoxiclav and quinolones. This underlines the need for proper drug selection based on culture and sensitivity so as to reduce the pool of drug-resistant bacteria. Lastly, to preserve the effectiveness of Mupirocin in the control of MRSA and Staphylococcus aureus, detection of resistance against it needs to be determined by further studies.

Conclusion

Control measures implemented were largely adequate against MRSA infections. Further studies need to be carried out to determine the effectiveness of these control measures and to determine the extent of colonisation and dissemination of MRSA to hospitalised patients and healthcare personnel.

References

1. David MZ, Daum RS. Community-associated methicillin-resistant Staph aureus: epidemiology and clinical consequences of an emerging epidemic. Clin Microbiol Rev 2010; 23: 616-87.
2. Li T, Song Y, Zhu Y, Du X, Li M. Current status of Staphylococcus aureus infection in a central teaching hospital in Shanghai, China. BMC Microbiol 2013; 13: 153-63.
3. Plorde JJ, Sherris JC. Staphylococcal resistance to antibiotics: origin, measurement and epidemiology. Ann N Y Acad Sci 1974; 236: 413-34.
4. Jevons M. "Celbenin"-resistant staphylococci. Br Med J 1961; 1: 124-5.

5. Griffiths C, Lamagni TL, Crowcroft NS, Duckworth G, Rooney C. Trends in MRSA in England and Wales: analysis of morbidity and mortality data for 1993-2002. Health Stat Q 2004; 21: 15-22.

6. Stefani S, Chung DR, Lindsay JA, Friedrich AW, Kearns AM, Westh H et al. Meticillin-resistant Staph aureus (MRSA): global epidemiology and harmonisation of typing methods. Int J Antimicrob Agents 2012; 39: 273-82.

7. Skov R, Christiansen K, Dancer SJ, Daum RS, Dryden M, Huang YC et al. Update on the prevention and control of community-acquired meticillin-resistant Staph aureus (CA-MRSA). Int J Antimicrob Agents 2012; 39: 193-200.

8. World Health Organization. Antimicrobial Resistance. WHO Media Centre; 2014. [online] [cited 2014 Jan 2]. Available from: URL: http://www.who.int/mediacentre/factsheets/fs194/en/.

9. Chambers HF, Deleo FR. Waves of resistance: staph aureus in the antibiotic era. Nat Rev Microbiol 2009; 7: 629-41.

10. World Health Organization. The evolving threat of antimicrobial resistance-options for action. Geneva: WHO Press; 2012.

11. Ganguly NK, Arora NK, Chandy SJ, Fairoze MN, Gill JP, Gupta U et al. Rationalizing antibiotic use to limit antibiotic resistance in India. Indian J Med Res 2011; 134: 281-94.

12. Alvarez-Uria, Uvummala P, Praveen KN, Manoranjan M, Raghuprakash R. Mortality associated with community-acquired cephalosporin-resistant Escherichia coli in patients admitted to a district hospital in a resource-limited setting. Clin Pract 2012; e76: 189-91.

13. Chen H, Liu Y, Jiang X, Chen M, Wang H. Rapid change of methicillin-resistant Staph aureus clones in a Chinese tertiary care hospital over a 15-year period. J Antimicrob Agents Chemother 2010; 54: 1842-7.

14. Ashiq B, Tareen AK. Methicillin resistant Staphylococcus aureus in a teaching hospital of Karachi- a laboratory study. J Pak Med Assoc 1989; 39: 6-9.

15. Bukhari SZ, Safia A, Naheed Z. Antimicrobial susceptibility pattern of Staphylococcus Aureus on clinical isolates and efficacy of laboratory tests to diagnose MRSA: a multi-centre study. J Ayub Med Coll Abbottabad 2011; 23: 139-41.

16. Shagufta H, Rabia S, Khurshid A, Roshan P, Bushra R. Prevalence of Methicillin Resistant Staphylococcus Aureus (MRSA) in Surgical Site Infections in a Tertiary Care Hospital. Int J Patho 2005; 3: 81-5.

17. Hussain M, Abdul B, Abdullah K, Kashif R, Asad J, Asif J, et al. Antimicrobial sensitivity pattern of methicillin resistant Staphylococcus aureus isolated from hospitals of Kohat district, Pakistan. J Inf Mol Biol 2013; 1: 13-6.

18. Irum P, Abdul M, Sobia K, Iffat N, Shama S, Safia A, et al. Prevalence and Antimicrobial Susceptibility Pattern of Methicillin-Resistant Staphylococcus aureus and Coagulase-Negative Staphylococci in Rawalpindi, Pakistan. Br J Med Med Res 2013; 3: 198-209.

19. Nitish KS, Raina G, Shrikala B, Gopalkrishna BK. Nosocomial Infections and Drug Susceptibility Patterns in Methicillin Sensitive and Methicillin Resistant Staphylococcus aureus. J Clin Diagn Res 2013; 7: 2178-80.

20. Tacconelli E, Johnson AP. National guidelines for decolonization of methicillin-resistant Staphylococcus aureus carriers: the implications of recent experience in the Netherlands. J Antimicrob Agents Chemother 2011; 66: 2195-8.

21. McNeil JC, Hulten KG, Kaplan SL, Mason EO. Mupirocin resistance in Staphylococcus aureus causing recurrent skin and soft tissue infections in children. J Antimicrob Agents Chemother 2011; 55: 2431-3.